The *Medicago truncatula* sucrose transporter family: sugar transport from plant source leaves towards the arbuscular mycorrhizal fungus

Dissertation
zur Erlangung des Doktorgrades der Naturwissenschaften
an der Fakultät für Biologie der Ludwig-Maximilians-Universität München

vorgelegt von

Joan Doidy

München, April 2012

The *Medicago truncatula* sucrose transporter family: sugar transport from plant source leaves towards the arbuscular mycorrhizal fungus

Date of oral examination : 23 May 2012

Name of the two doctor father's : Dr A. Schüssler (LMU Munich) and Prof. D. Wipf (Burgundy University, France)

Eidesstattliche Erklärung

Ich versichere hiermit an Eides statt, dass die vorgelegte Dissertation von mir selbständig und ohne unerlaubte Hilfe angefertigt ist.

München, den 27.03.2012 .....

(Unterschrift)

Erklärung

Hiermit erkläre ich, *

☒ dass die Dissertation nicht ganz oder in wesentlichen Teilen einer anderen Prüfungskommission vorgelegt worden ist.

☒ dass ich mich anderweitig einer Doktorprüfung ohne Erfolg **nicht** unterzogen habe.

☐ dass ich mich mit Erfolg der Doktorprüfung im Hauptfach .....

und in den Nebenfächern .....

bei der Fakultät für ..... der .....

(Hochschule/Universität)

unterzogen habe.

☐ dass ich ohne Erfolg versucht habe, eine Dissertation einzureichen oder mich der Doktorprüfung zu unterziehen.

München, den 27.03.2012 .....

(Unterschrift)

*) Nichtzutreffendes streichen

Acknowledgement

The accomplishment of this thesis was made possible due to the agreement about joint postgraduate scientific work supervision between the Université de Bourgogne and the Ludwig Maximilians Universität of Munich due to the agreement between Prof. Daniel Wipf, co-head of the équipe Mycorhizes: Mécanismes et Gestion in the UMR Agroécologie, Dijon and Prof. Arthur Schüßler, Genetics Institute, LMU, Munich. This work was supported by the Conseil Régional de Bourgogne [Joan Doidy thesis grant FABER 2008_9201AAO036S00220 and PARI 20100112095254682-1] and the Agence National de la Recherche [project TRANSMUT, ANR- 10-BLAN-1604-01]. Travel expenses for scientific communications and collaborations were supported in part by the Ecole Doctorale Environnements - Santé - STIC - E2S Bourgogne [Bourse de Mobilité E2S].

I would like to thanks the PhD comity, Philipp Franken, Rémi Lemoine, Thomas Ott, David Wendehenne for accepting the evaluation of this work; Nathalie Sejalon-Delmas and Roland Marmeisse for following my work through “comités de suivi de thèse”. I am deeply grateful for the time spent on following my work and reading manuscripts.

The authors are grateful for technical assistance to A. Colombet, V. Monfort, Hoai-Nam Truong, Thibaud Adam, Leonardo Casieri, Odile Chataignier, Matthias Ellerbeck, Trudl Strobl, Charles Schneider, Olivier Lamotte, Gregory Aubert, Gérard Alcaraz, Marion Corneillat; sorry and thanks to the “forgotten” ones…

I would like to thanks all the people from the Genetics Institute at the Ludwig Maximilians Universität of Munich especially the AG of Arthur Schüßler. Andreas Brachman for helpful discussions and Matthias Ellerbeck my bench yeast mate! I really enjoyed the atmosphere in the lab and would like to thank you all very much for this memorable stay.

Daniel, MON encadrant ! Je voulais te remercier pour ta confiance et ta patience à toute épreuve (surtout face à l’humour et autres censures). Tu resteras pour moi, un ami.

Merci beaucoup Diederik, THE co-encadrant ! Pour ta disponibilité, ton aide (les ^p, Artémis dont tu détiens seul le secret…).

A mon directeur de Master Benoît Schoefs pour le soutien dans les bons et mauvais moments.

Je remercie l'ensemble du personnel de l'UMR Agroécologie à Dijon (et ex-PME) pour m'avoir soutenu dans ce travail. Je remercie tout particulièrement la génération Y pour toutes les soirées, mousses, repas etc… ENORMES ! Merci Leo, Loïc, Carolina, Nico, David, Ivan, Herbert, Nass, Tommaso, Franck, Lucka, Iulia, Magda, Ian, Maz, Zhipeng, Manu, Sou, Vedette, Thib, Marine, la mère a Tom, Verge, Jéjé… Vous allez tous me manquer !

Pouliche ! que dire ! Les UP (les bonjours madames), les DOWN (les larmichettes), Mon Alter ego ! Vedette un jour, vedette toujours ! Sou je te kiffe, t'es ma petite brune préférée! Verge, tu fais vraiment trop du bien ! et la cousine Marine. Je décline toute responsabilité pour votre powerpoint (photos non-contractuelles).

A tous les collègues de travail qui sont devenus mes amis.

Une pensée particulière à tout mes amis surtout pour ceux à qui « je ne venais plus aux soirées».

Mes amis d'ailleurs : Christine, Franck, Samir, Angie, Mila, Sabine, Elen, Nico, the Symonds family.

Mes amis d'enfance et de toujours : GTM, PTM, Marie H, Côme, Mick, Bali, Jérome, Djix, Tola, Greg, Soum.

Ma seconde famille !

Enfin, je remercie toute la famille. Merci à tous pour le soutien moral (et financier -pince), vos encouragements durant ces longues années d'études à domicile et à l'étranger ! Un énorme bisou à Zik, Thierry, aux Badji, mes grands-parents.

Et malgré le patrimoine génétique, je dédie l'ensemble de ce travail à ma famille : à ma maman, mon papa et mon frère, merci d'avoir toujours été la.

Abstract

In plants, long distance transport of sugars from photosynthetic source leaves to sink organs comprises different crucial steps depending on the species and organ types. Sucrose, the main carbohydrate for long distance transport is synthesized in the mesophyll and then loaded into the phloem. After long distance transport through the phloem vessels, sucrose is finally unloaded towards sink organs. Alternatively, sugar can also be transferred to non-plant sinks and plant colonization by heterotrophic organisms increases the sink strength and creates an additional sugar demand for the host plant. These sugar fluxes are coordinated by transport systems. Main sugar transporters in plants comprise sucrose (SUTs) and monosaccharide (MSTs) transporters which constitute key components for carbon partitioning at the whole plant level and in interactions with fungi. Although complete SUTs and MSTs gene families have been identified from the reference Dicot *Arabidopsis thaliana* and Monocot rice (*Oriza sativa*), sugar transporter families of the leguminous plant *Medicago truncatula*, which represents a widely used model for studying plant-fungal interactions in arbuscular mycorrhiza (AM), have not yet been investigated.

With the recent completion of the *M. truncatula* genome sequencing as well as the release of transcriptomic databases, monosaccharide and sucrose transporter families of *M. truncatula* were identified and now comprise 62 MtMSTs and 6 MtSUTs. I focused on the study of the newly identified MtSUTs at a full family scale; phylogenetic analyses showed that the 6 members of the MtSUT family distributed in all three Dicotyledonous SUT clades; they were named upon phylogenetic grouping into particular clades: MtSUT1-1, MtSUT1-2, MtSUT1-3, MtSUT2, MtSUT4-1 and MtSUT4-2. Functional analyses by yeast complementation and expression profiles obtained by quantitative RT-PCR revealed that MtSUT1-1 and MtSUT4-1 are H^+/sucrose symporters and represent key members of the MtSUT family. Conservation of transport capacity between orthologous leguminous proteins, expression profiles and subcellular localization compared to previously characterized plant SUTs indicate that MtSUT1-1 is the main protein involved in phloem loading in source leaves whilst MtSUT4-1 mediates vacuolar sucrose export for remobilization of intracellular reserve.

The AM symbiosis between plants and fungi from the phylum Glomeromycota is characterized by trophic exchanges between the two partners. The fungus supplies the autotrophic host with nutrients and thereby promotes plant growth. In return, the host plant provides photosynthate (sugars) to the heterotrophic symbiont. Here, sugar fluxes from plant source leaves towards colonized sink roots in the association between the model leguminous plant *M. truncatula* and the arbuscular mycorrhizal fungus (AMF) *Glomus intraradices* were investigated. Sugar transporter candidates from both the plant and fungal partners presenting differential expression profiles using available transcriptomic tools were pinpointed. Gene expression profiles of MtSUTs and sugar quantification analyses upon high and low phosphorus nutrient supply and inoculation by the AMF suggest a mycorrhiza-driven stronger sink in AM roots with a fine-tuning regulation of MtSUT gene expression. Conserved regulation patterns were observed for orthologous SUTs in response to colonization by glomeromycotan fungi.

In parallel, a non-targeted strategy consisting in the development of a *M. truncatula* - *G. intraradices* expression library suitable for yeast functional complementation and screening of symbiotic marker genes, similar to the approach that led to the identification of the first glomeromycotan hexose transporter (GpMST1), has been developed in this study.

Taken together, with the identification, functional characterization and gene expression pattern of sugar transporter families, a more complete picture of sugar fluxes in the AM symbiosis has begun to emerge. This study opens new perspectives by identifying interesting candidate genes involved in sugar partitioning at both the plant and fungal levels and at the symbiotic interface in the widely used AM symbiosis model between *M. truncatula* and *G. intraradices.*

Key words: sugar transport; sucrose transporter, SUT, monosaccharide transporter, MST, sugar partitioning, *Medicago truncatula*, *Glomus intraradices*, arbuscular mycorrhizal symbiosis.

Abbreviation list

ABA	ABscisic Acid
ABRE	ABscisic acid Responsive Element
ADH	Alcohol-DeHydrogenase
AM	Arbuscular Mycorrhiza
AMF	Arbuscular Mycorrhizal Fungi
AMT	AMmonium Transporter
ARE	Auxin Responsive Elements
ATP	Adenosine TriPhosphate
ATPase	ATP-synthase
BACs	Bacteria Artificial Chromosomes
BCP	Blue Copper-binding Protein
BD	Bimboim and Doly buffer
BEG/IBG	Banque Européenne des Glomeromycètes/International Bank for Glomeromycota
CCCP	Carbonyl Cyanide 3-ChloroPhenylhydrazone
CC	Companion Cell
CC-SE	Companion Cell - Sieve Element complex
cDNA	complementary DNA
CDS	Coding DNA Sequence
CINs	Cytosolic INvertases
CWIN	Cell Wall-bound INvertase
DEPC	DiEthylPyroCarbonate
DES	DiEthylStilbestrol
DFCI	Dana Farber Cancer Institute
DNA	DeoxyriboNucleic Acid
DNP	2,4-DiNitroPhenol
DRM	Detergent-Resistant Membrane
dsDNA	double-stranded DNA
ERE	Ethylene Responsive Element
ERM	Extra-Radicular Mycelium
ESL	Early-responsive to dehydration Six-Like
EST	Expressed Sequence Tag
FRET	Fluorescence Resonance Energy Transfer
Fru	Fructose
GFP	Green Fluorescent Protein
Glu	Glucose
GPCR	G-Protein Coupled Receptor
GPH	Glycoside-Pentoside-Hexuronide
GSSG	Oxidized glutathione
GUS	Beta-glucuronidase
HALC	High-Affinity/Low-Capacity

HIGS	Host-Induced Gene Silencing
HP	High Phosphate
HPAE-PAD	High-Performance Anion Exchange-Pulsed Amperometric Detection
HXK	HeXoKinase
HXT	HeXose Transporter
INT	INositol Transporter
IRM	Intra-Radicular Mycelium
LAHC	Low-Affinity/High-Capacity
LB	Luria-Bertani medium
LD PCR	Long Distance PCR
LP	Low Phosphate
M/NM	Mycorrhizal/Non-Mycorrhizal
MAMP	Microbe Associated Molecular Pattern
MENS	*Medicago* EST Navigation System
MFS	Major Facilitator Superfamily
mRNA	messenger RiboNucleic Acid
MST	MonoSaccharide Transporter
MtGEA	*Medicago truncatula* GEne Atlas
MtMST	*Medicago truncatula* MonoSaccharide Transporter
MtSUT	*Medicago truncatula* SUcrose Transporter
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
NCBI	National Center for Biotechnology Information
NMR	Nuclear Magnetic Resonance
OD	Optical Density
PAM	Peri-Arbuscular Membrane
PCR	Polymerase Chain Reaction
PDI	Protein Disulfide Isomerase
pDR	plasmid Doris Rentsch
pGlcT	plastidic Glucose Transporter
PM	Plasma Membrane
PMA	Plasma Membrane ATPase 1
PMT	Polyol/Monosaccharide Transporter
PPA	PrePenetration Apparatus
PT	Phosphate Transporter
q-RT-PCR	Quantitative Reverse Transcription Polymerase Chain Reaction
RACE	Rapid Amplification of cDNA Ends
RFO	Raffinose Family Oligosaccharide
RNA	RiboNucleic Acid
RNAi	RNA interference
SD	Synthetic medium with Dextrose
SE	Sieve Element
SGB1	Suppressor of G protein Beta1
SIRT	Sucrose-Induced Repression of Translation

SM	Synthetic medium with Maltose
SPP	Sucrose-Phosphate Phosphatase
SPS	Sucrose-Phosphate Synthase
STP	Sugar Transport Protein
Suc	Sucrose
SUF	SUcrose Facilitator
SURE	SUcrose-Responsive cis-Element
SuS or SUSY	Sucrose Synthase
SUT or SUC	SUcrose Transporter
TAG	TriAcylGlycerol
TEF	Translation Elongation Factor
TIPs	Tonoplast Intrinsic Proteins
TM	TransMembrane domains
TMT	Tonoplast Membrane Transporter
TOR	Target Of Rapamycin
UDP	Uridine DiPhosphate
ULD	UnLoading Domain
ura	uracil
UTR	UnTranslated Region
VGT	Vacuolar Glucose Transporter
VINs	Vacuolar INvertases
YFP	Yellow Fluorescent Protein
YPD	Yeast extract Peptone Dextrose medium
YPM	Yeast extract Peptone Maltose medium
YSL2-1	Yeast Sylvie Lalonde 2-1

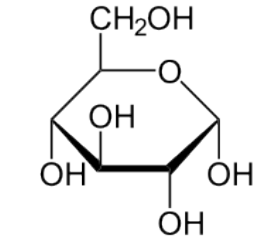
Preamble

The arbuscular mycorrhizal fungus used in the present study is *Glomus intraradices* BEG 141 which represents a study model in our laboratory and has been widely mentioned in the literature (Rivera-Becerril et al. 2005; Seddas et al. 2009b; Tollot et al. 2009; Kuznetsova et al. 2010; Seddas-Dozolme et al. 2010; Hao et al. 2012). In 2009, the isolate *Glomus intraradices* DAOM 197198 was reassigned to *Glomus irregulare* (Stockinger et al. 2009). More recently the taxonomy of Glomeromyceta was deeply redefined (Schüßler and Walker 2010) and *Glomus irregulare* was renamed *Rhizophagus irregularis.* The BEG141 isolate was not included in these studies but should now be reassigned to *Rhizophagus sp.* according to its phylogenetic position (D. Redecker, unpublished data). Nevertheless in the present thesis manuscript we decided to keep using the name *Glomus intraradices* BEG141 for the following reasons:

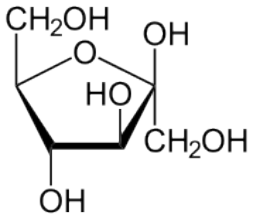
- BEG 141 from the IBG database was not yet phylogenetically studied in depth, so it cannot yet be claimed whether it is *R. irregularis* or potentially another *Rhizophagus* species.
- Papers published by our group (including those present in the annexes) have used the name *Glomus intraradices* and keeping the same name will avoid confusion.

Glossary

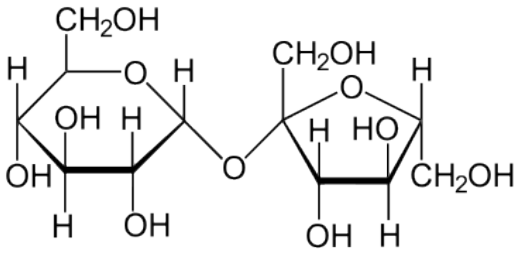
Introduction and literature review



α-D-glucopyranose



β-D-fructofuranose



sucrose

Fig 1. Chemical structure of α-D-glucopyranose, β-D-fructofuranose and sucrose
Backbone structures are represented according to Haworth projection.

Introduction and literature review

1 Sugars as energy rich and signaling metabolites

Life on earth relies on photosynthesis, which provides atmospheric oxygen vital to respiration and fixes gaseous CO_2 into sugar molecules. Indeed, plants are photoautotrophic organisms producing soluble sugars that are centrally embedded in primary metabolism and serve as the primary energy source. Sugars are taken up by all cells for transient or long-term storage, as structural components, as carbon skeletons for biosynthesis of most other metabolites, and as molecules involved in signal transduction. In animals, glucose is the most important energy source and transport form. In plant metabolism, main soluble sugars are sucrose, glucose and fructose (Fig 1).

Sugar production through photosynthesis occurs in two stages and takes place in the chloroplast of plant leaf cells. In the first stage (light-dependent reactions), photons are captured by chlorophyll pigments resulting in an electron transport chain and a proton gradient across the chloroplast membrane leading to the production of chemical energy molecules (NADPH and ATP). Then, light-independent reactions use these products to capture and reduce CO_2 through the Calvin cycle that generates photosynthates, which are exported mainly as triose-phosphates. Finally, conversion to glucose-6-phosphate precedes the production of glucose units that are linked together to form starch or are joined with a fructose unit to form sucrose (Fig 2).

1.1 Sucrose, a plant specific disaccharide

Sucrose (Wind et al. 2010) is a disaccharide composed of a fructosyl and a glucosyl moiety bound by an α-β glycosidic linkage between the reducing end C2 of the fructose and C1 of the glucose (Fig 1; β-D-fructofuranosyl-(2,1)-α-D-glucopyranoside). Sucrose is a non-reducing disaccharide that can only be cleaved by two enzymes and therefore is very stable; such properties enable both its long distance transport and vacuolar storage. Sucrose is by far the most abundant sugar in almost all plant tissues, but industrially, there are only two major sucrose accumulating crops, the temperate dicotyledonous sugar beet (*Beta vulgaris*) and the tropical monocotyledonous sugar cane (*Saccharum* spp). Both provide 35 and 135 million tons, respectively, of sucrose per year, representing approximately 25% and 75% of global sucrose production (Halford et al. 2011).

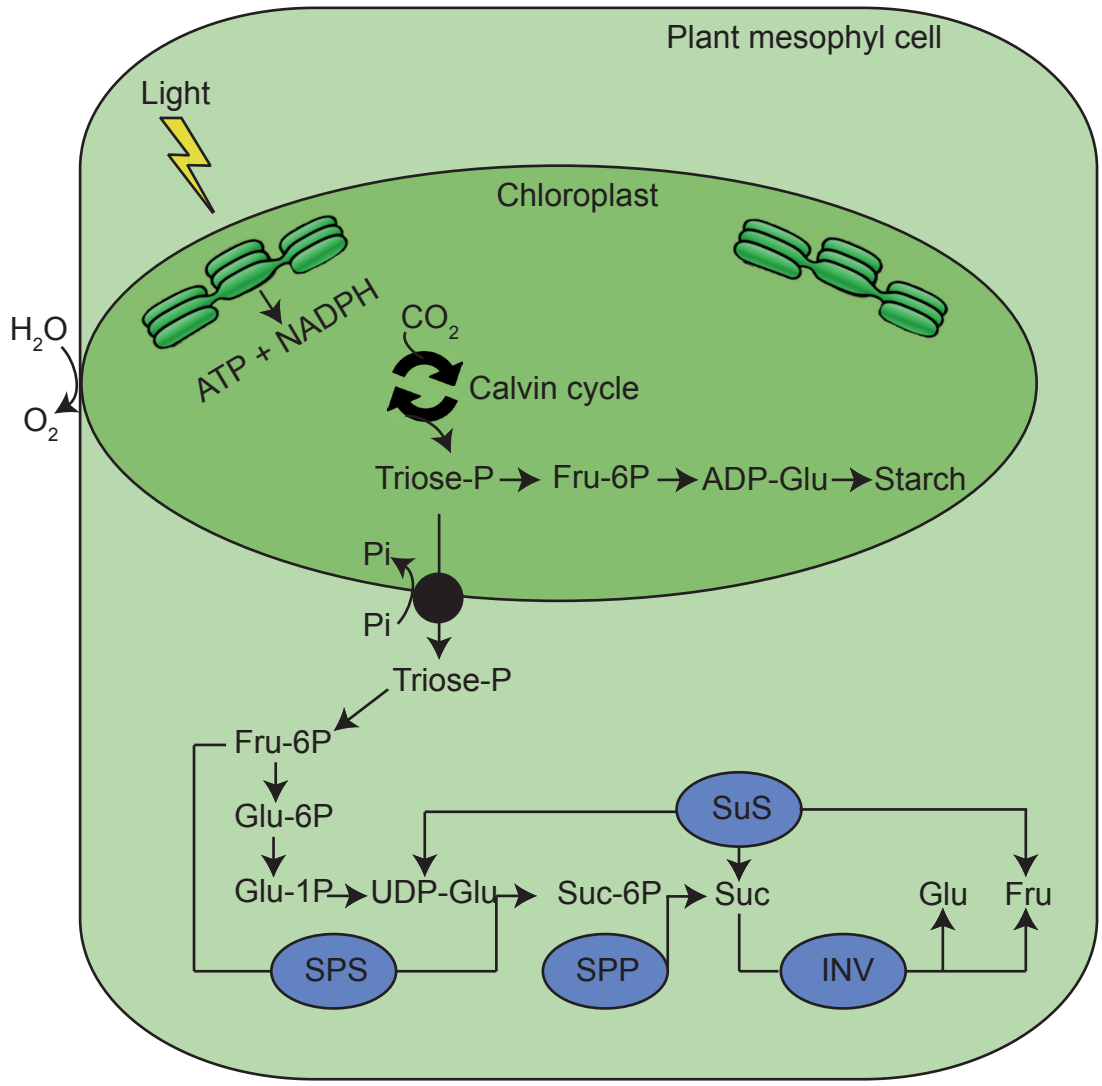


Fig 2. Simplified scheme of photosynthate production in mesophyl cell with a highlight on sucrose metabolism

For full name designation, please refer to the abbreviation list.

1.1.1 Sucrose synthesis

Sucrose is found solely in plants and other photoautotrophic organisms. The origin of sucrose synthesis comes from proteobacteria or cyanobacteria (Lunn 2002). Indeed, chloroplast evolved from these bacteria and this explains how the first plant cells gained the capacity of sucrose synthesis (Salerno and Curatti 2003). In higher plants, sucrose is mainly synthesized in the cytosol from photosynthetically fixed carbon and to a minor extent from the breakdown of starch and lipid reserves. Sucrose is produced by a two-step enzymatic reaction that catalyzes the substrates (fructose 6-phosphate and UDP glucose) involving sucrose-phosphate synthase (SPS) and sucrose-phosphate phosphatase (SPP; Fig 2). Sucrose synthesis is highly regulated with main control on the first metabolic step operated by SPS (Lunn and MacRae 2003). Indeed, a "fine" regulation by allosteric effectors glucose6-phosphate (activator) and phosphate (inhibitor) as well as a "coarse" control through protein phosphorylation coexist (Huber and Huber 1992). This tight regulation of sucrose synthesis responds to sugar production related to plant photosynthesis rate, sugar demand at the whole plant level, light conditions and diurnal changes.

1.1.2 Sucrose catabolism

Sucrose catabolism involves two types of sucrose cleaving enzymes (Fig 2 and 3; Koch 2004). Firstly, the cytosolic sucrose synthase (SuS) catalyzes a reversible reaction and therefore is involved in both sucrose synthesis and catabolism. Nevertheless, SuS role is principally assigned to sucrose cleavage under most physiological conditions. Secondly, key enzymes for sucrose catabolism are invertases which can be classified into 3 groups based on their subcellular localization, solubility and optimal pH (Fig 3; Roitsch and González 2004). Alkaline (also called neutral) soluble cytosolic invertases (CINs) are minimally active in most systems. Indeed, cytoplasmic sucrose is frequently transported into vacuoles where acidic soluble vacuolar invertases (VINs) regulate vacuolar sucrose storage and produce considerable hexose flux across the tonoplast of expanding tissues (Koch 2004). Acidic cell wall-bound invertase (CWIN) is involved in apoplasmic sucrose cleavage for subsequent transport via monosaccharide transporters. In contrast to SuS, which produces UDP-glucose, invertases directly split sucrose into glucose and fructose (Fig 3). Consequently, two-fold more hexoses are produced and thus, hexose sensing mechanism and resulting signal is enhanced. Sugar splitting enzymes are regulated at numerous levels (Koch 2004) and are involved in carbohydrate partitioning, developmental processes, hormone responses as well as biotic and abiotic interactions (Roitsch and González 2004).

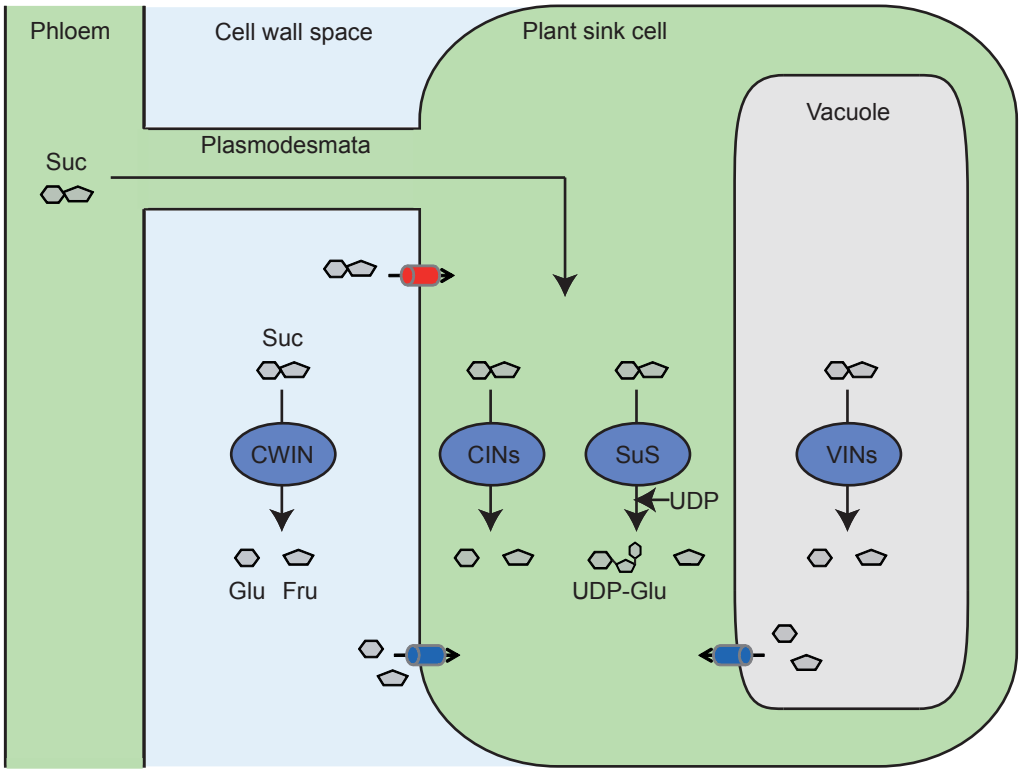


Fig 3. Sucrose cleaving enzymes in a plant cell

Sucrose reaches sink tissues where it is stored in vacuoles or cleaved by sucrose splitting enzymes (blue circles) for subsequent usage. Sucrose transporters are represented in red and monosaccharide transporters in blue**.**

1.2 Glucose and fructose

Glucose (or D-glucose, dextrose) is a simple sugar that is by far the preferred energy source of organisms ranging from unicellular microbes to plants and animals. Indeed, this monosaccharide is embedded in primary metabolism as an energy source of glycolysis and cellular respiration and as substrate for trehalose and starch synthesis as well as the pentose phosphate pathway. The key enzyme for glucose utilization and sensing is the hexokinase (HXK), which catalyzes its phosphorylation into glucose-6-phosphate. In plant, glucose is one of the main products of photosynthesis (Fig 2) but also represents a major signaling metabolite. Fructose (or levulose) is also a simple monosaccharide and its name originates from fruit sugar where it is most abundant. A key enzyme for fructose-6-phosphate production is the fructose-1,6-bisphosphatase, which is regulated by fructose-2,6-bisphosphate to coordinate a key junction in the highways of carbon flux into sucrose and starch biosynthesis (Nielsen et al. 2004). In addition, after starch, fructose polymers known as fructan represent a major storage polysaccharide. The fructose moiety from sucrose is assembled to fructan chains by fructosyl transferases which are related to invertase (Ritsema and Smeekens 2003).

Glucose possesses a free aldehyde group (aldohexose) whereas fructose has a keto group. These carbonyl groups make them reducing sugars. Therefore, their presence can be detected by a Benedict's test, which relies on the reduction of cupric ions (Cu^{2+}) to cuprous ions (Cu^{+}) and the formation of a brick red precipitate of cuprous oxide (Cu_2O).

1.3 Sugars act as signaling molecules

In unicellular model organism like yeast, sugar sensing and signaling pathways are well elucidated; however, sugar regulation is necessarily far more complex in plants (Rolland et al. 2006). Indeed, plants are multicellular organisms that need both long distance and cell specific signaling events and are made up of sugar exporting (source) and sugar importing (sink) organs with signals generated from both locations. In general, source activities like photosynthesis, nutrient mobilization, and sugar export are upregulated under low sugar conditions, whereas sink activities like growth and storage are upregulated when carbon sources are abundant. Moreover, sugar signaling pathways are intimately connected to plant hormones such as abscisic acid (ABA), auxin, cytokinin and ethylene (León and Sheen 2003; Ramon et al. 2008).

In *Arabidopsis thaliana*, mutant characterization showed that hexokinase isoenzyme 1 (HXK1) is the central component for glucose sensing in the cytoplasm and can be targeted to

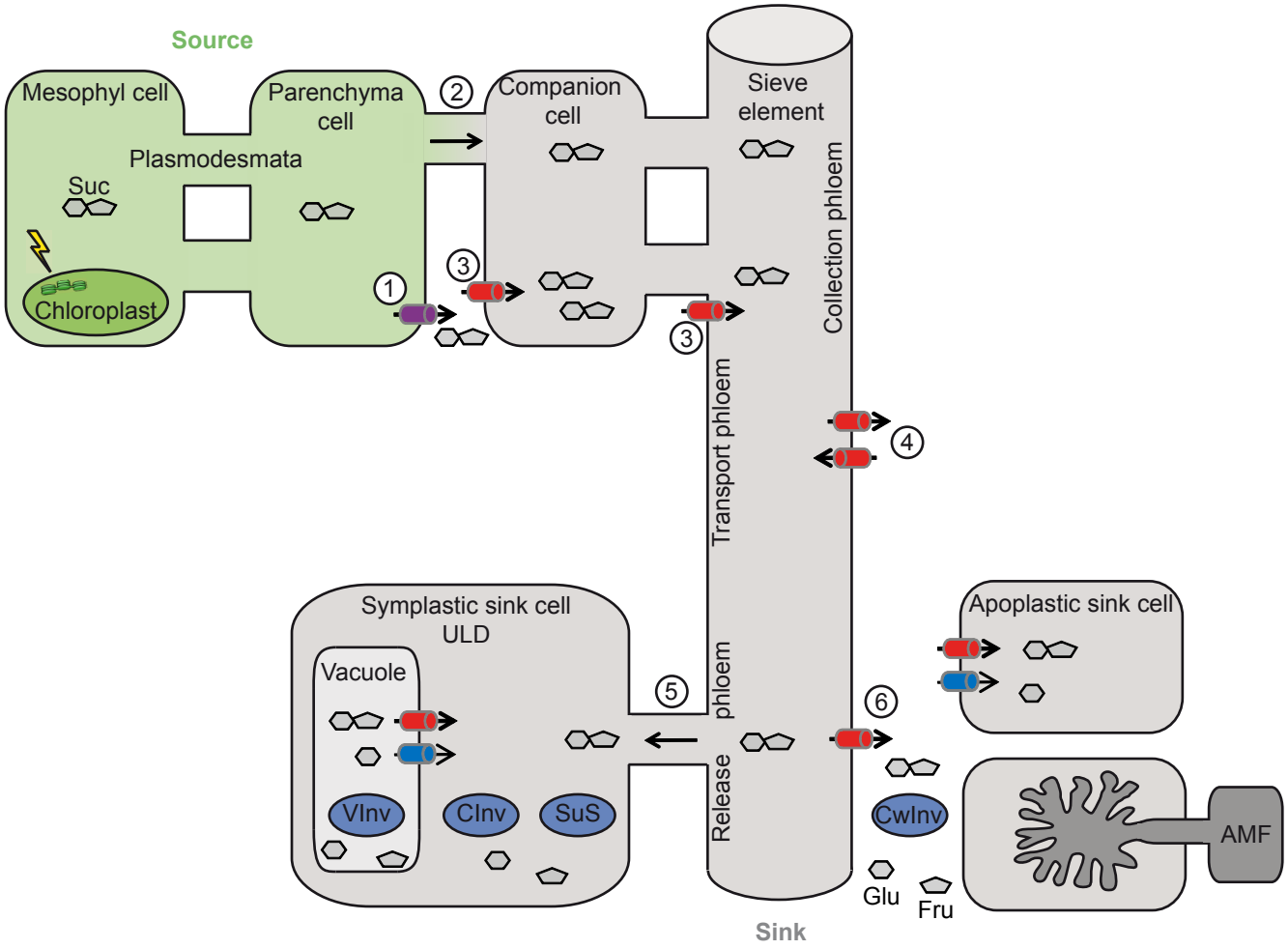


Fig 4. Long distance transport of sugars from source to sink organs

In plants, sucrose (Suc) is synthesized in source leaves and constitutes the main carbohydrate form for long-distance transport; the first step consisting in sucrose export from mesophyll cells is still under debate, however recent findings suggest that SWEET (in purple) are key components for sucrose export likely from parenchyma cells ①. Following export, sucrose is either passively loaded in the companion cell sieve element complex via plasmodesmata in symplastic species ② or actively loaded by a single SUT1 (*e.g.* AtSUC2 in *Arabidopsis*) phloem loading protein in apoplastic species ③ where trafficking of SUT1 is proposed between companion cell and enucleate sieve elements. Once in the phloem sap, sucrose follows the flow of transport phloem where it can be unloaded and retrieved to supply flanking tissues ④. Upon release from the phloem, sucrose is either unloaded to symplasticly connected sink organs, such as unloading domains (ULD) through plasmodesmata ⑤ or via SUTs (in red) to apoplasticly connected sink cells ⑥ and cleaved by sucrose-cleaving enzymes (blue circles) to yield glucose (Glu) and fructose (Fru) that are taken up by MSTs (in blue). Alternatively, sugar can also be transferred to fungal sinks and plant colonization by heterotrophic fungi increases sugar demand (Doidy et al. 2012).

the nucleus for transcription regulation (Jang et al. 1997). Another key regulating pathway rises from G-protein coupled receptor signaling (GPCRs, Grigston et al. 2008). In addition, SUT2 sucrose transporters resemble the yeast sugar sensors SNF3 and RGT2 (Barker et al. 2000), and the monosaccharide transporter AtSTP13 complemented yeast *snf* mutants (Kleinow et al. 2000). Such transporters might have putative sugar sensing function (see 3.2.2.4).

Sugars act as core modulators from gene expression to enzyme activity. Several global gene expression studies revealed large number of plant genes to be transcriptionally regulated by sugars (Price et al. 2004; Rolland et al. 2006). Consistent with this, sucrose-responsive *cis*-elements such as SURE were found to interact with WRKY sugar-induced transcription factors (Sun et al. 2003). In addition, sugars can have effect on 3' UTR regions and on polyA tail length and thereby influence mRNA stability (Chan and Yu 1998; Prieto et al. 2000). At a translational level, sucrose inhibits mRNA translation of the transcription factor AtB2/bZIP11; this specific sucrose-induced repression of translation (SIRT) is due to AtB2/bZIP11 long 5'UTR (Rook et al. 1998). At a metabolic level, sugars can modulate enzyme activity via kinase proteins like SnRK1 (SNF1-Related Kinase, Toroser et al. 2000) and target of rapamycin (TOR) involved in highly conserved signaling network that regulates cell growth in response to nutrients (Soulard et al. 2009).

In conclusion, sugars possess metabolic pathways tightly regulated to meet physiological plant demands but are not just energy rich metabolites considered as fuel for cellular machinery; they also constitute key signaling molecules for coordination of developmental, physiological and environmental changes.

2 Long distance transport of sugar: from source to sinks

Higher plants have developed specialized organs presenting distinct functions. Indeed, aerial parts absorb light and fix CO_2 into sugar photosynthates, while root organs take up water and nutrients from the soil. An essential prerequisite for such specialization is the long distance sap fluxes that occur through xylem and phloem conducting tissues. Xylem sap consists primarily of water and mineral nutrients transported towards aerial parts, whereas phloem sap contains carbohydrates exported from photosynthetic source organs towards demanding sink organs including roots, seeds, flowers, fruits, and newly emerging leaves. Sucrose constitutes the main carbohydrate for this long distance transport through phloem tissues. Sucrose synthesized in the mesophyll is first loaded into the collection phloem, then, long distance

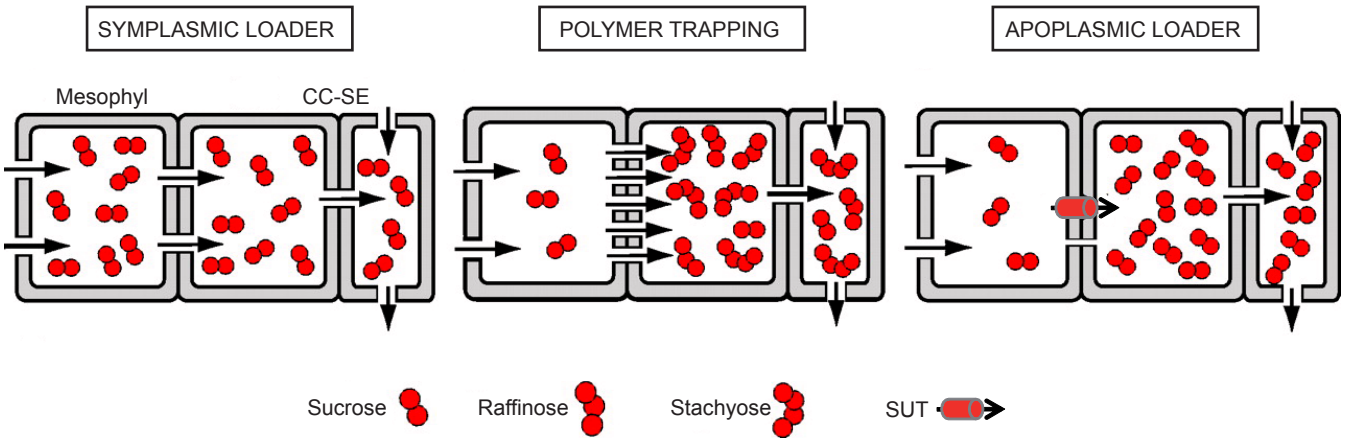


Fig 5. Scheme representing different phloem loading strategies

In symplasmic loading species, sucrose is loaded passively to the CC-SE (companion cell - sieve element) complex via plasmodesmata according to the concentration gradient. Some species use a polymer trapping mechanism to load sucrose. In such species, the polymerization of sucrose into RFOs maintains a concentration gradient suitable for passive loading of sucrose through plasmodesmata. Therefore, they can also be considered as symplasmic loaders (Zhang and Turgeon 2009). In apolplasmic loading species, sucrose is loaded actively to the CC-SE complex by SUT proteins against the concentration gradient. Adapted from Rennie and Turgeon 2009.

transport occurs in the transport phloem and finally sucrose is unloaded in the release phloem (Fig 4).

2.1 Sugar export from mesophyll cells, a black box

The first step consisting in the sucrose export from the mesophyll cell is still under debate (Fig 4; Lalonde et al. 2004). Indeed, this initial step is the least understood and so far no sucrose transporters presented efflux of sucrose *in vivo*. Nevertheless, Carpaneto et al. 2005 showed a sucrose transporter that was able to mediate both influx and efflux of sucrose *in vitro* but this transporter rather seem to be involved in the phloem unloading mechanism (Geiger 2011). Another hypothesis emerged from a vesicular efflux pathway and that vesicles could mediate solute transport by exocytosis to the apoplastic space (Echeverría 2000). Very recently, Chen et al. 2012 identified a novel category of SWEET transporters (see 3.3.3) that catalyze both glucose and sucrose effluxes and mutant phenotypes are consistent with a role in sucrose efflux from parenchymatic cells adjacent to mesophyll source cells (Fig 4.1; Chen et al. 2012).

2.2 Phloem loading

Phloem vessels are composed of companion cell-sieve element complex (CC-SE). Sieve tube is an elongated rank of individual cells arranged end to end to form a conducting vessel. Since sieve elements lack nucleus, vacuoles, ribosomes and Golgi apparatus, their survival depends on a close association with the companion cells. Thereby most cellular functions of a sieve-tube element are carried out by companion cells, which are largely connected by plasmodesmata. In contrast to the sugar export from mesophyll cells, the step consisting in sucrose loading into the phloem vessels is well characterized.

2.2.1 Plants employ different phloem loading strategies

The classification of sugar loading strategies is currently made according to anatomical features and hydrostatic pressure gradients (Pollock et al. 1992). On one hand, there is the "apoplasmic loaders" which transport sucrose actively from cell to cell across the apoplast via sucrose transporter proteins (Fig 4.3 and Fig 5). Plant species using this strategy present a closed minor vein configuration with few plasmodesmata and a high concentration gradient between sucrose production sites and phloem saps. The plant model species *A. thaliana* and *Medicago truncatula* use this apoplasmic pathway. In apoplasmic species, the disruption of the phloem loading protein, for instance the knockout of *AtSUC2* led to sugar accumulation in source leaves and a blockage of sugar export towards sink organs resulting in a stunted growth (Gottwald et al. 2000). On the other hand, "symplasmic loaders" such as *Fragaria* sp.

and *Quercus rubrum* (Rennie and Turgeon 2009) load sugar passively thanks to abundant plasmodesmata (Fig 4.2) in their open minor vein configuration and a high sucrose concentration in the mesophyll cell (Fig 5). Thereby, symplasmic species do not rely on transport protein for sucrose loading and the downregulation of *VpSUT1* had minor effect on sugar partitioning (Zhang and Turgeon 2009; see also Fig 9g).

2.2.2 Plants use different sugar for phloem loading

Recently, van Bel and Hess 2008) showed that plants from the *Ranunculaceae* and *Papaveracea* families contained substantial amount of glucose in their phloem sap. With the presence of reducing hexoses in phloem sap, van Bel and Hess 2008) announced the end of the sucrose "dogma". However, mechanisms of hexose loading and transport strategies in such species have not yet been studied.

Nevertheless and as previously stated, sucrose is by far the major form for long distance transport in plants. Some plant species also transport sugar from the raffinose family oligosaccharides (RFOs; Knop et al. 2001) or sugar alcohol (Reidel et al. 2009) in addition to sucrose. In such species, RFOs (raffinose and stachyose) are synthesized from sucrose polymerization in companion cells and accumulate in the phloem. RFOs are larger than sucrose and cannot diffuse back to the mesophyll through plasmodesmata. This accumulation of RFOs maintains a low sucrose content in the CC-SE complex and a concentration gradient favorable for passive loading through plasmodesmata. In this case, sucrose loading into the phloem vessels occurs by a process known as "polymer trapping" (Fig 5; Turgeon 1991; Rennie and Turgeon 2009).

2.3 Long distance phloem flow

Once sucrose is loaded into the phloem sap, the disaccharide follows its route through the transport phloem where solutes are translocated by mass flux in accordance with Münch's pressure flow hypothesis (Münch 1930). This reviewed hypothesis states that high turgor values resulting from massive photosynthate accumulation in collection phloem at the source ends propel the sieve sap toward sites of low turgor values caused by the escape of photosynthates at the sink ends (van Bel 1995; Knoblauch and Peters 2010). Moreover in the transport phloem conduct, sucrose can be unloaded and retrieved to supply flanking tissues and mobilize sugar to and from short- and long-term storage reserves (Fig 4.3; van Bel 2003; Thompson 2006).

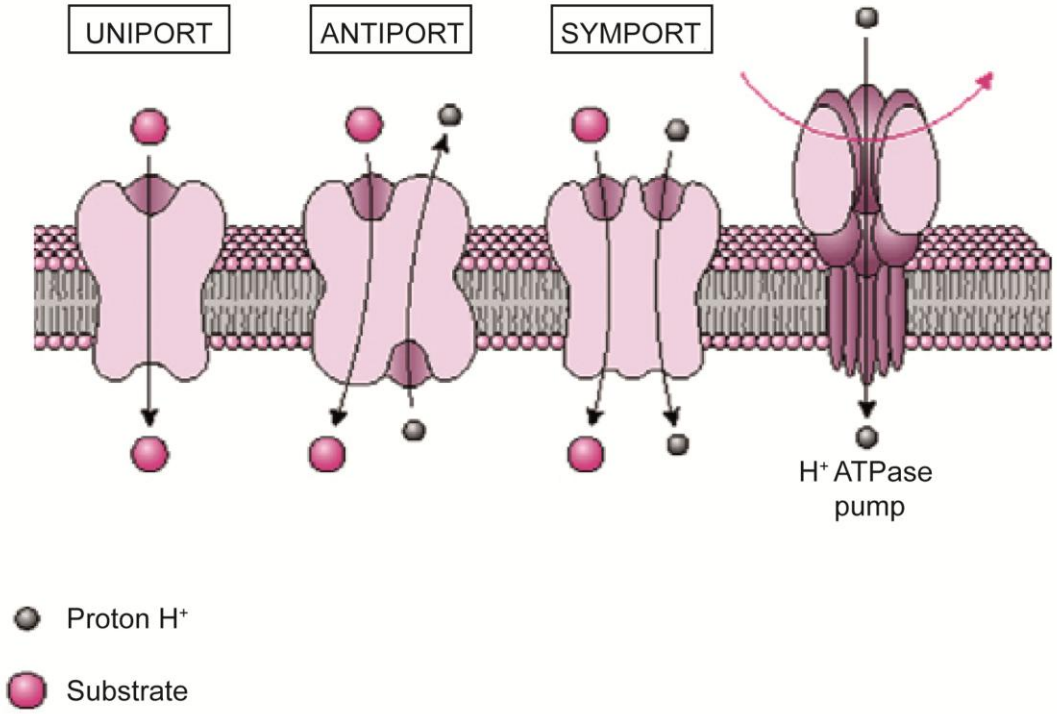


Fig 6. Kinetics mechanisms of major facilitator superfamily transporters
Uniporters transport a substrate according to the substrate gradient; symporters translocate substrates in the same direction; antiporters transport substrates in opposite directions. Coupled symporters and antiporters use the electrochemical gradient of one substrate; for example the secondary active transport uses the driving force generated by the H^+ ATPase pump. Adapted from Afoufa-Bastien 2010.

2.4 Phloem unloading

Finally sucrose reaches the release phloem and is exported to supply sink organs. In contrast to phloem loading, unloading strategies can consist of both apoplasmic and symplasmic pathways in the same individuals (Patrick 1997). Indeed, it seems that unloading pathway rather depends on the type of sink organ (Turgeon and Wolf 2009). For instance, pollen grains are suggested as apoplasmic sink that use active SUT (Fig 4.6) whereas root tips transport their sucrose symplasmically via plasmodesmata in unloading domains (Fig 4.5; Stadler et al. 2005; Sauer 2007). In addition, phloem unloading strategies also depend on the stage of development of sink organs. Indeed, a shift of phloem unloading from symplasmic to apoplasmic pathway occurs during ripening of grape berries (Zhang et al. 2006).

Alternatively, sugar can also be transferred to non-plant sinks. Indeed, plant colonization by heterotrophic organisms represents an increased sugar demand (Fig 4; Doidy et al. 2012a). However, mechanisms of transport and transporter proteins involved in carbon partitioning between symbiotic organisms are still poorly understood (Pollock et al. 1992). The role and regulation of transporters in such interaction will be discussed in 4.3.3.3 and in the third chapter of this thesis.

3 Sugar transporters: state of the art

With the completion of the genome of *A. thaliana* (The *Arabidopsis* Genome Initiative 2000), we learned that hexose and sucrose transport proteins belong to large multigenic families. Indeed, main sugar transporters comprising disaccharide (Williams et al. 2000) and monosaccharide (Buttner 2007) transporters are members of the major facilitator superfamily (MFS, Marger and Saier 1993). More recently and differing from previously identified MFS transporters: SWEETs (Chen et al. 2010), a new sugar transporter family, was defined. Sugar transport system are necessary for coordination of carbon partitioning, plant development, cell to cell communication, environmental adaptation and thereby play pivotal roles upon optimal plant growth and crop yield.

3.1 The major facilitator superfamily

MFS present over 15000 gene members (Law et al. 2008; http://www.membranetransport.org/) and are found in all kingdoms of life with functions ranging from accumulating nutrients in bacteria to the cycling of neurotransmitters in human. Consequently the MFS is one of the largest classes of channel and transport proteins (Hirai et al. 2003b). MFS members show binding capacity for a broad substrate spectrum: sugars, polyols, drugs, phosphorylated glycolytic intermediates, amino acids, peptides, osmolites,

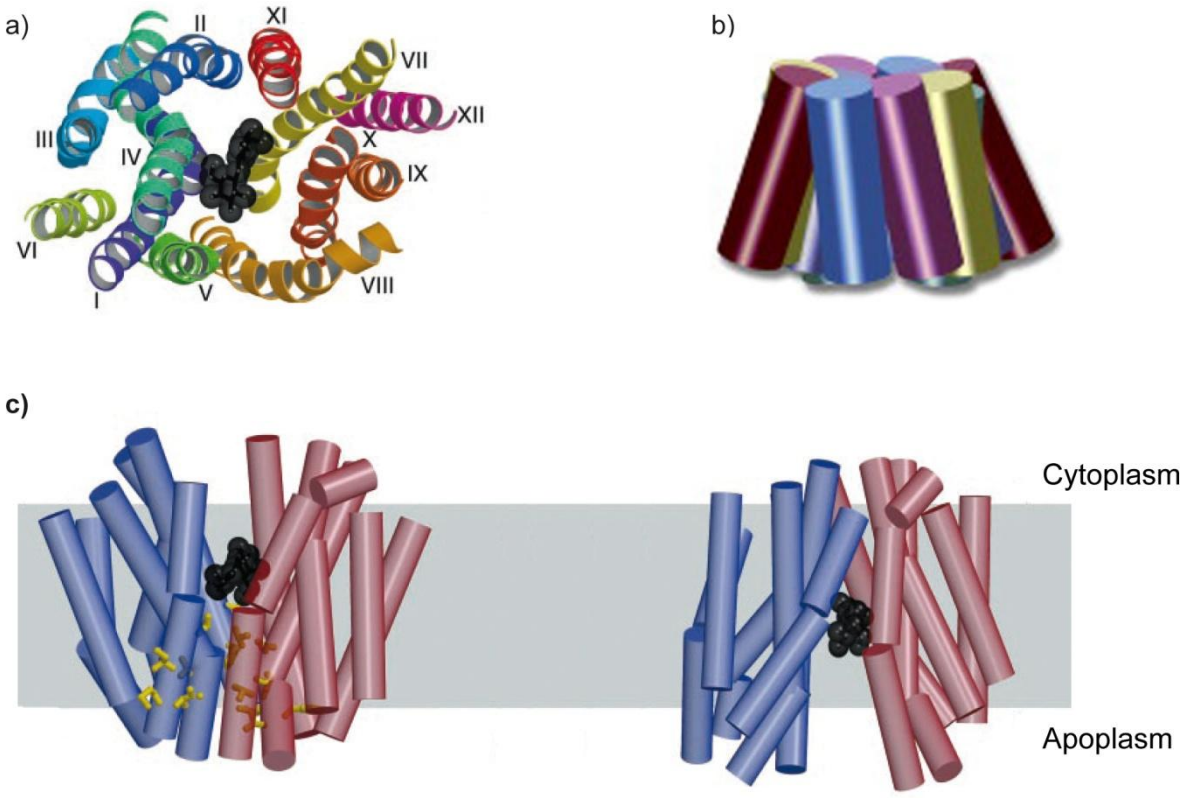


Fig 7. Three-dimensional structure of major facilitator superfamily transporters

a) Ribbon representation of the LAcY with substrate (in black) at the binding site. For clarity, loop regions have been omitted (Abramson et al. 2003).

b) Prediction of the tridimensional arrangement of SUTs drawn in analogy to the LacY crystal structure. Adapted from Sauer 2007.

c) The inward-facing conformation of the LacY turns into the outward-facing conformation by a rocker-switch mechanism. TM symetry between the N- and C-terminal helices is highlighted as blue and red cylinders respectively. Adapted from Abramson et al. 2003.

siderophores, nucleosides, organic anions and inorganic anions (http://www.tcdb.org/). Mechanistically, MFS transporters display three kinetic systems (Fig 6).

Most MFS proteins are composed of 400-600 amino acids and possess 12 transmembrane domains (TMs) originating from the duplication and fusion event of an ancestral 6 TMs motif (Fig 7 and 10; Henderson and Maiden 1990; Rubin et al. 1990; Saier 1994). The tri-dimensional structural analysis of *Escherichia coli* lactose permease (LacY; Abramson et al. 2003) and the glycerol-3-phosphate transporter (GlpT; Huang et al. 2003) confirmed the dual symmetry of TM I-VI and TM VII-XII. However, structural resolution of the bacterial oxalate transporter (OxlT; Heymann et al. 2001) indicated a four-fold symmetry configuration evolving from a 3 TMs element by two successive duplication and fusion events (Hirai et al. 2003a). These structural studies led to the observation of molecular processes involved in substrate translocation, MFS transporters possess a single substrate binding site that operates upon an alternating access comparable to a rocker-switch mechanism (Fig 7c; Abramson et al. 2003; Law et al. 2007).

Up to now, no plant MFS structure has been resolved. However, valuable information obtained from crystal structure of bacterial MFS proteins allowed tridimensional prediction of SUT structure. Comparative studies predicted that TM helices I, IV, VII and X form the central part of the substrate pore surrounded by the other eight TMs (Fig 7b).

3.2 Sucrose transporters

SUcrose Transporters (SUT), also called SUC (for SUcrose Carriers) are members of the glycoside-pentoside-hexuronide (GPH) family within the MFS. All described SUTs have a common structure, with 12 TM domains connected by hydrophilic loops and are assumed to form a single pore for sucrose, N- and C-termini extremities in the cytosolic side (Fig 7b and Fig 10). SUTs have been reviewed extensively over the last decade (Lemoine 2000; Lalonde et al. 2004; Lim et al. 2006; Sauer 2007; Shiratake 2007; Kühn and Grof 2010; Ayre 2011; Geiger 2011).

3.2.1 History

Buchel et al. 1980 sequenced the bacterial LacY permease, the first gene coding for a transport protein. In the 90's, first SUTs were identified from cDNA library of spinach and potato leaves (SoSUT1, *Spinacia oleracea* and StSUT1, *Solanum tuberosum*) using suppression cloning by the mean of a *Saccharomyces cerevisiae* mutant (Riesmeier et al.

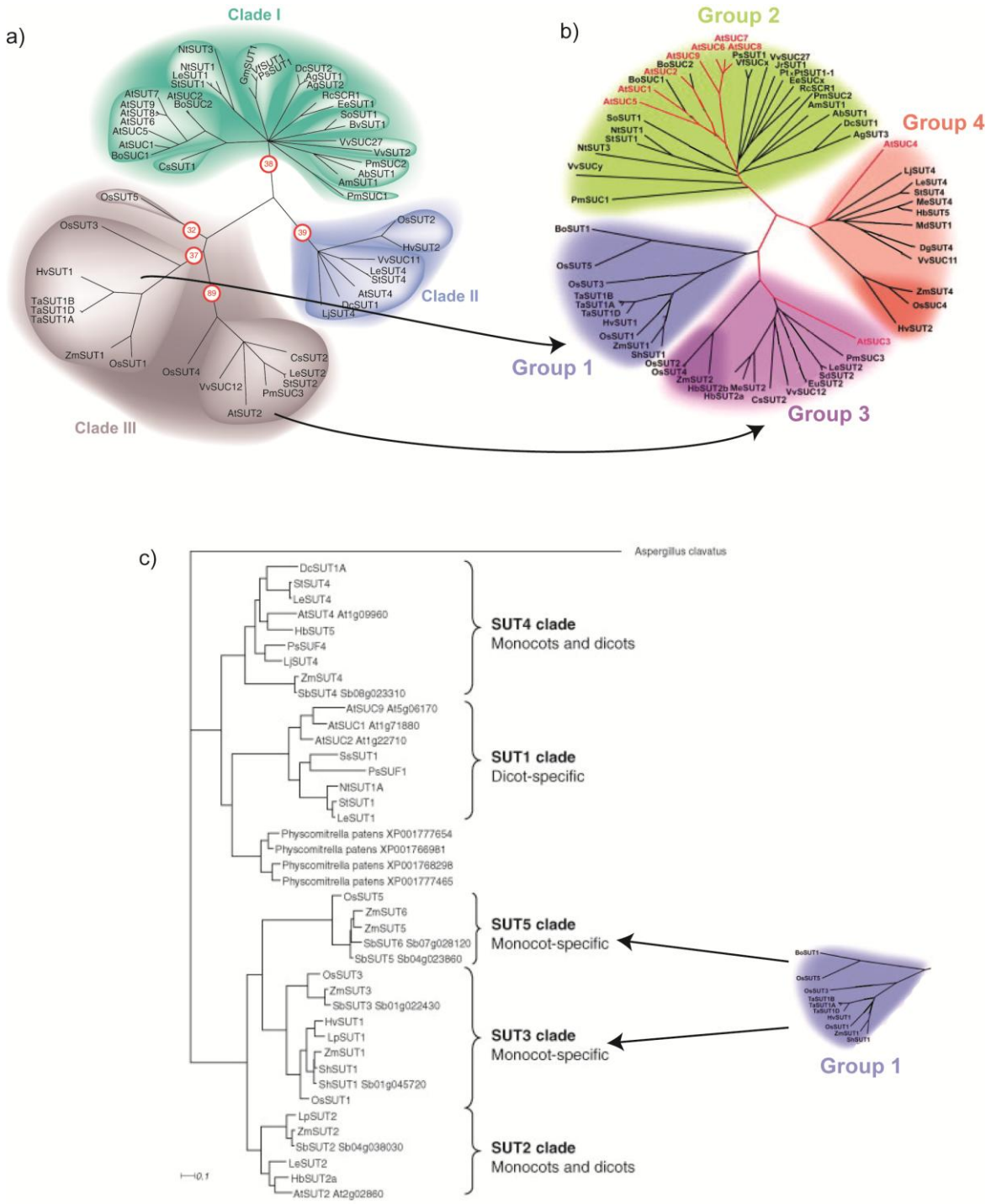


Fig 8. Evolution of SUT classification over the last decade

a) Phylogenetic tree adapted from Lalonde et al. 2004. Red circles give the average amino acid length of the central loop for respective clades. b) Phylogenetic tree adapted from Sauer 2007. The nine AtSUTs are highlighted in red. c) Phylogenetic tree adapted from Kuhn and Grof 2010, distant SUT sequences from the mosses *Physcomitrella patens* are included. For details on sequence accessions, please refer to the respective reviews. Arrows highlight successive division of SUT clades.

1992; Riesmeier et al. 1993). This yeast mutant strain (SUSY7) is impaired for sucrose utilization and therefore is suitable for sucrose uptake characterization (Fig 27); by this mean, SoSUT1 was first determined as a H^+/sucrose importer (Riesmeier et al. 1992). Thereafter, SUT orthologs were identified by sequence homology in *A. thaliana* using radiolabeled *SoSUT1* probes (AtSUC1 and AtSUC2; Sauer and Stolz 1994) and in *Plantago major* using *AtSUC1* probes (PmSUC2; Gahrtz et al. 1994) or for *Oriza sativa* with a degenerated primer approach (OsSUT1, Hirose et al. 1997). More recently, plant genome sequencing projects led to the identification of SUTs in numerous plant species (OsSUTs, Aoki et al. 2003; AtSUTs, Sauer et al. 2004; PtaSUTs of *Populus tremula* x *alba*, Payyavula et al. 2011) and it is now well recognized that all plants have a small-sized family of SUT genes. For instance, the SUT family comprises 9 AtSUTs in *A. thaliana*, 6 ZmSUTs in maize (*Zea mays*) and 5 OsSUTs in rice.

Together with the increasing number of newly isolated sequences, the SUT classification evolved over the last decade. Indeed, first phylogenetic analysis separated SUTs among 3 types or clades with a highlight on central cytosolic loop (Fig 8a; Aoki et al. 2003; Lalonde et al. 2004). Later, Sauer 2007 separated the third clade assembling SUT classification into 4 groups (Fig 8b). Recently, the monocotyledonous specific group 1 was split to build the latest classification composed of 5 distinct clades (Fig 8c; Braun and Slewinski 2009; Kühn and Grof 2010). Despite the classification evolution which led to distinct numbered clades, inconsistency in nomenclature remains with transporters named using different conventions (SUT/SUC). To avoid gene confusion and synonymy, the latest and consistent classification from Kühn and Grof 2010 will be used in this manuscript.

3.2.2 SUTs by clade

3.2.2.1 The SUT1 clade

Initially named SUT1/SUC2 clade (Lalonde et al. 2004) according to *A. thaliana AtSUC2* gene synonymy, which is also called *AtSUT1* in some publications (Feuerstein et al. 2010), the SUT1 clade is the largest and most studied clade and is dicotyledonous specific (Fig 8). It seems that this clade is composed of paralogs that have evolved through gene duplication and that each gene variant acquired different functions (Lynch and Force 2000; Ibraheem et al. 2010). H^+/sucrose importers (Fig 12) from the SUT1 clade show high affinity for sucrose with Km values ranging from 0.07 to 2.0 mM.

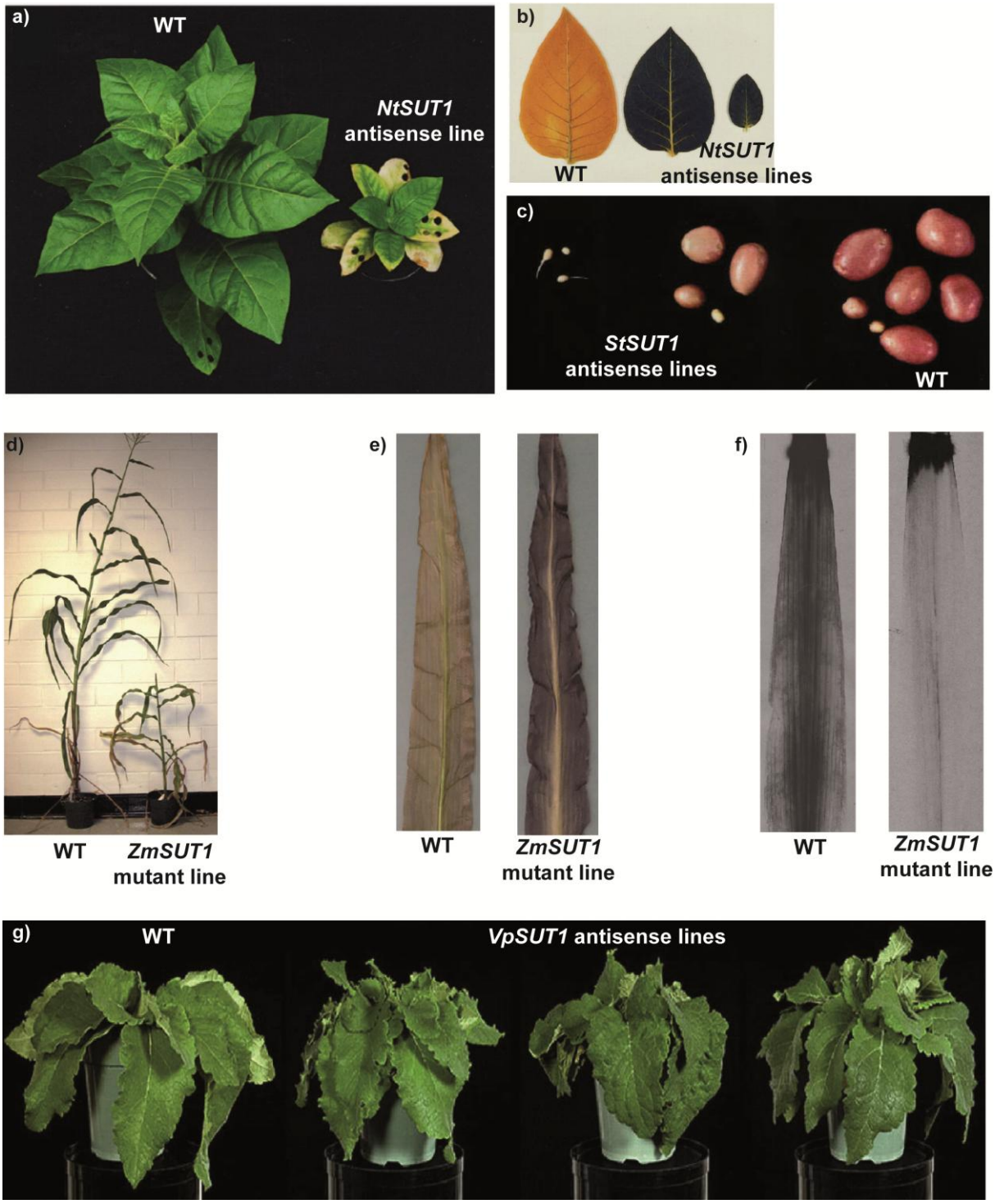


Fig 9. A single SUT protein is necessary for phloem loading in apoplasmic loader plants

a) Phenotype of wild type and *NtSUT1* antisense tobacco plants (Burkle et al. 1998). b) Starch accumulation in leaves of wild type and *NtSUT1* antisense tobacco plants shown by Lugol's iodine staining. c) Reduction of tuber yield in potato tubers in *StSUT1* antisense line αSUT43 and αSUT13 (Riesmeier et al. 1994). d) Phenotype of wild type and *ZmSUT1* insertional mutant maize plants (Slewinski et al. 2009). e) Starch accumulation in leaves of wild type and *ZmSUT1* mutant plants shown by Lugol's iodine staining. f) Autoradiographs showing the blockage of [^{14}C]sucrose export from *ZmSUT1* mutant leaves. g) No phenotype differences were observed in the symplasmic loader (*Verbascum phoeniceum*) between wild type and *VpSUT1* antisense lines (Zhang and Turgeon 2009).

3.2.2.1.1 A single SUT1 member necessary for phloem loading

A single member of the SUT1 clade is responsible for sucrose phloem loading for apoplasmic loaders (Fig 4.3, see 2.2.1, Zhang and Turgeon 2009); for instance AtSUC2 in *A. thaliana*, StSUT1 in potato, SlSUT1 in tomato or NtSUT1 in tobacco. The downregulation (Riesmeier et al. 1994; Kühn et al. 1996; Bürkle et al. 1998; Schulz et al. 1998; Hackel et al. 2006b) or total gene disruption (Gottwald et al. 2000) of the phloem loading protein caused sugar accumulation, reduced photosynthesis and caused chlorotic lesions in source leaves as well as reduced growth of sink organs and thereby an overall stunted plant growth (Fig 9a-c). However, contradictive results were published about the fertility of *AtSUC2* KO plants upon their ability to complete a life cycle and produce viable seeds (Gottwald et al. 2000; Srivastava et al. 2009b). In symplasmic loader species (Fig 4.2) such as *Verbascum phoeniceum*, the disruption of *VpSUT1* the phloem loader orthologs only had minor effect on sugar partitioning and plant morphology was not affected (Fig 9g).

Consistent with their loading functions, all loading proteins are high affinity sucrose importers and were tagged at the plasma membrane of SE (Kühn et al. 1997; Reinders et al. 2002a; Kühn et al. 2003; Hackel et al. 2006a; Weise et al. 2008) or of both CC and SE (Knop et al. 2004; Scofield et al. 2007b). As the expression of antisense *StSUT1* under the control of a CC-specific promoter prevented phloem loading (Lemoine et al. 1996) and as enucleate SE mostly depend on CC's molecular machinery, transcription and translation of the SUT1 loading member are likely to occur in CC. Moreover, various GFP fusion proteins were shown to traffic towards SE with a large exclusion limit (>67kDa) between plasmodesmata connecting SEs and CCs (Stadler et al. 2005; Thompson and Wolniak 2008). However, the presence of *StSUT1* mRNAs in SE (Kühn et al. 1997) together with the discovery of a translational machinery in angiosperm SE (Lin et al. 2009) led to an alternative model for protein synthesis in phloem (Kühn and Grof 2010).

In addition to this source localization, several phloem loader transporter orthologs were also shown to be expressed in sink tissues where their roles remain obscure. Indeed, *AtSUC2* expression was detected in roots and pods (Truernit and Sauer 1995), *StSUT1* in potato tubers (Kühn et al. 2003), *NtSUT1* in roots, buds, sink leaves and flowers (Bürkle et al. 1998) and *SlSUT1* in roots, fruits and flowers (Hackel et al. 2006a; Boldt et al. 2011). Although Srivastava et al. 2008 pointed a major role for AtSUC2 in sugar retrieval but not in sucrose unloading other authors suggest a dual role of such members in both loading and unloading mechanisms (Kühn 2003; Carpaneto et al. 2005; Doidy et al. 2012a).

3.2.2.1.2 The other SUT1 clade members

In addition to the single phloem loading orthologs, the SUT1 clade is also represented by numerous multicopy SUT1 paralogs in all dicotyledonous species. For instance, in addition to AtSUC2, the genome of *A. thaliana* possesses 6 other SUT1 members originating from gene duplication including 2 pseudogenes (*AtSUC6* and *AtSUC7*, Sauer et al. 2004).

These other SUT1 clade members are not as well characterized as the single loading member but are thought to have a role in carbon partitioning of sink organs, namely in flowers and seeds where they are mostly expressed. Indeed, *AtSUC1* expression was shown in anther connective tissue, in funiculi, in fully developed pollen grains and in female gametophyte (Stadler et al. 1999; Feuerstein et al. 2010), *AtSUC8* along the transmitting tissue and at the funiculi (Sauer et al. 2004), *AtSUC9* in sepals, in style and in the filament vasculature (Sivitz et al. 2007), *PmSUC1* in pollen grains, inside the anthers and in pollen tubes (Lauterbach et al. 2007) and *NtSUT3* is pollen specific (Lemoine et al. 1999). Consequently, plant containing a mutation of *AtSUC1* showed impaired pollen germination (Sivitz et al. 2008) and *AtSUC9* mutant showed an early flowering phenotype (Sivitz et al. 2007). In addition to its floral localization, *PmSUC1* was shown to be expressed in the innermost layer of the inner integument (Lauterbach et al. 2007) and AtSUC1 in vascular tissues of siliques (Sivitz et al. 2007). *AtSUC5* expression is endosperm specific and antisense lines showed reduction in fatty acid composition of their seeds (Baud et al. 2005).

Moreover, members of the SUT1 clade were also present in other sink organs. *RhSUC2* is expressed in young bursting rose buds (Henry et al. 2011). *AtSUC1* is expressed in trichomes (Sivitz et al. 2007), in roots and in hypocotyls of young seedling (Sivitz et al. 2008; Hoth et al. 2010) and mutation of an *AtSUC1* key promoter motif revealed an important role for seedling development (Hoth et al. 2010).

In conclusion, SUT1 Dicot clade is large and well studied, with a well characterized SUT1 protein that function as the main phloem loader in apoplasmic loading species. Numerous other SUT1 members are mostly present in sink organs but their exact physiological roles are still not deciphered.

3.2.2.2 SUT3 clade

Initially grouped with the SUT5 clade to form the large Monocot specific group1 (Sauer 2007), it was recently split into 2 distinct monocotyledonous clades (Fig 8; Braun and Slewinski 2009). Despite the phylogenetic distance with the previously described SUT1 clade, both clades present similar characteristics. Indeed, both clades are strictly Dicot (SUT1) or

Monocot (SUT3) specific and possess paralogs originating from gene duplication. However, *TaSUT1A*, *TaSUT1B* and *TaSUT1D* rather seem to originate from respective A, B, and D progenitor genomes of the hexaploid wheat cultivar (Aoki et al. 2002). In addition, it seems that the SUT3 clade also includes the single SUT protein necessary for sucrose phloem loading of monocotyledonous apoplastic loaders (Fig 9d). Indeed, transposable element insertional mutant of *ZmSUT1* showed impaired loading phenotype similar to the one observed in Dicot SUT1 mutants (Fig 9d-f). Applied [^{14}C]sucrose stood literally blocked in the tip of source leaves of the *ZmSUT1* mutant affected in sugar export (Fig 9f ; Slewinski et al. 2009). This raises questions about the phylogenetic origins of SUTs and the use of different Monocot and Dicot SUTs for essential aspects of carbon partitioning (Ayre 2011).

Although the downregulation of *OsSUT1* (closely related to ZmSUT1) had no effect on plant morphology (Ishimaru et al. 2001), Hirose et al. 2010 pointed out that they did not succeed in recovering a double homozygous *OsSUT1* mutant which could suggest a major role for this putative phloem loading protein. Consistent with this function, many SUT3 clade proteins were found in source leaves. OsSUT1 as well as the 3 TaSUT1 proteins were tagged in SE (Aoki et al. 2004; Scofield et al. 2007a) and *HvSUT1* is likely to be expressed in CC-SE since it was amplified from the sap of aphid stylectomy (Doering-Saad et al. 2002). Moreover, those proteins are also present in sink organs, especially in grains of *Poaceae*. Indeed, *HvSUT1* is strongly expressed in developing seeds for the allocation of sucrose required for starch synthesis in the endosperm (Weschke et al. 2000). Subsequently, TaSUTs also seem to be involved in sucrose utilization from previously stored starch in the endosperm when the seeds germinate (Aoki et al. 2006). *OsSUT1* is also expressed in developing grain (Aoki et al. 2003) and germinating seeds where it may play a role in sucrose allocation towards developing shoots and roots of seedlings (Matsukura et al. 2000; Scofield et al. 2007a).

Biochemically, all characterized proteins show a high affinity for sucrose with Km values ranging between 2 and 8 mM and a common trait of the SUT3 clade is their narrow substrate spectrum with the highest specificity for sucrose among all plant SUTs (see 3.2.3.3; Sivitz et al. 2005; Reinders et al. 2006; Sun et al. 2010).

3.2.2.3 SUT5 clade

Newly separated into a distinct clade (Braun and Slewinski 2009), this monocotyledonous specific SUT5 clade is the least studied so far with only a single protein which has yet been characterized. OsSUT5 is a sucrose importer with a Km for sucrose of 2.3 mM and its transport capacity shows a low dependence upon pH condition (Sun et al. 2010).

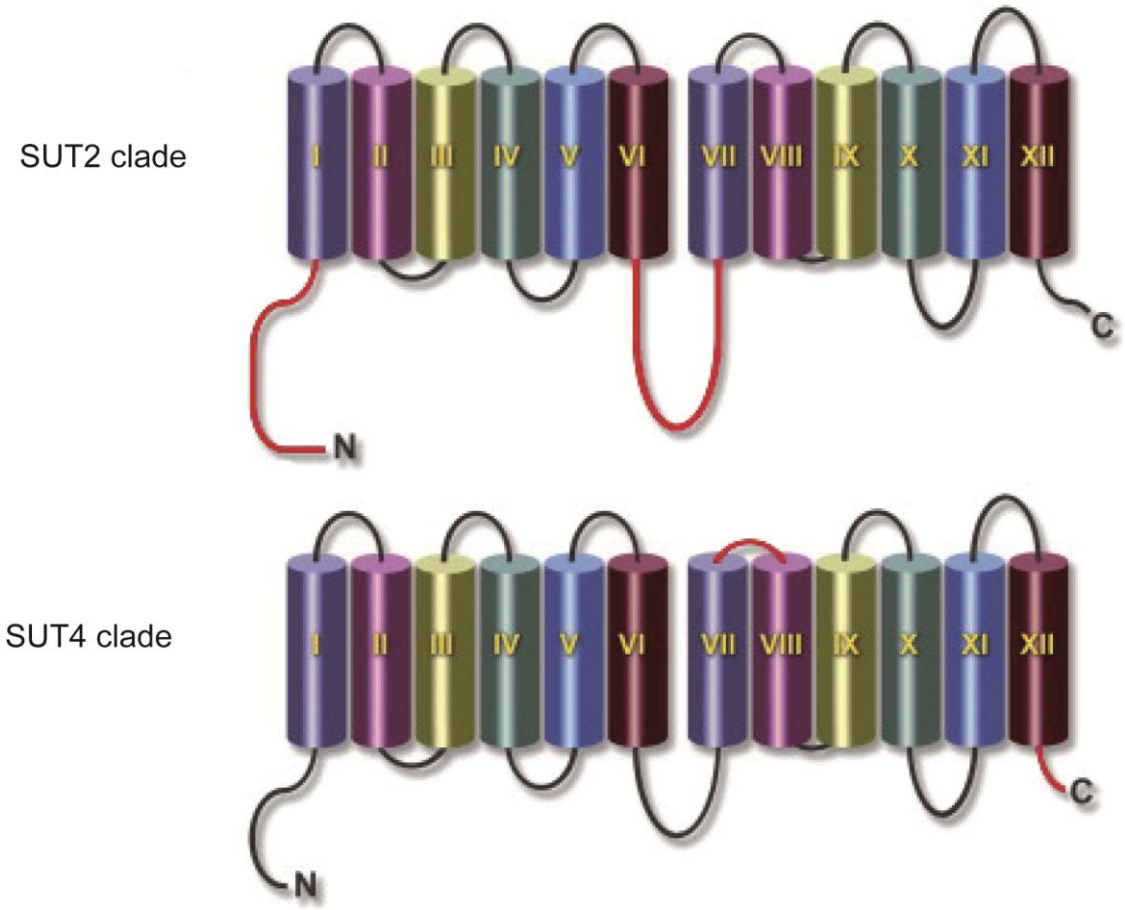


Fig 10. Two-dimensional structure of plant SUTs

Identical colors were used to highlight intra-molecular sequence conservations between the first and the second halves of the protein. Particular features (discussed in the text) of SUT2 and SUT4 clades are highlighted in red. Adapted from Sauer 2007.

In conclusion, the cleavage of the Monocot specific SUT group into 2 different clades (SUT3 and SUT5 clades) has not yet been accepted by all authors (Payyavula et al. 2011) and more research is needed on Monocot SUTs to establish the exact function of this growing family. Indeed, numerous sequences have been identified from newly sequenced species such as *Brachypodium distachyon* and *Sorghum bicolor* and have not yet been studied (Braun and Slewinski 2009).

3.2.2.4 SUT2 clade

Also called SUT2/SUC3 clade according to *A. thaliana AtSUT2*/*AtSUC3* gene synonymy (Barker et al. 2000; Meyer et al. 2000), the SUT2 clade was originally attached to the Monocot clades (Fig 8a; Lalonde et al. 2004). The SUT2 clade comprises a single gene copy in the higher plant genome studied, except in *Hevea brasiliensis*, *Populus tremula* x *alba* and *Glycine max*. Proteins from the SUT2 clade show special features (Fig 10); they are larger (around 600 amino acids) than other SUTs because they have additional amino acids at the N-terminal domains (about 30 amino acids) and an extended central cytoplasmic loop (about 50 amino acids). The chimeric addition of the N-terminal domains of AtSUT2 to the high affinity transporter StSUT1 (Km=1.7 mM) led to lower affinity for sucrose (Km=8.1 mM) while the addition of the central loop of AtSUT2 had no effect (Km=1.5 mM). This suggests an important role of the N-terminal regions of SUTs for substrate affinity (Schulze et al. 2000; Reinders et al. 2002b). As SlSUT2 and StSUT2 were not able to complement SUSY7 deficiency (Barker et al. 2000; Hackel et al. 2006a) and as SUT2 clade members possess a long conserved central loop comparable to the structure of the yeast sensors SNF3 and RGT2 (Ozcan et al. 1996), SUT2 clade members were initially described as sucrose sensors (Lalonde et al. 1999; Barker et al. 2000). Subsequently, AtSUT2 (Km= 11.7 mM; Schulze et al. 2000) and PmSUC3 (Km=5.5 mM; Barth et al. 2003) were shown as active sucrose transporter but such functional SUT2 clade members show a low affinity for sucrose. Proteins were detected in sieve element and in sink organs (Barker et al. 2000; Barth et al. 2003; Meyer et al. 2004). To decipher their physiological role, antisense *SlSUT2* lines have been constructed. Those lines showed impaired tomato fruit and seed development and the authors suggested a role in pollination and/or apoplastic phloem unloading mechanisms (Hackel et al. 2006a). Although the exact functions of SUT2 members remain unknown, their putative sugar sensing function does not seem to be likely any longer.

3.2.2.5 SUT4 clade

Initially called SUT4/SUC4 clade (Lalonde et al. 2004) according to *A. thaliana* gene synonymy (Weise et al. 2000; Endler et al. 2006), as the previously described clade, the SUT4 clade possesses a single gene copy (except in *Hevea brasiliensis* and *Glycine max*) among

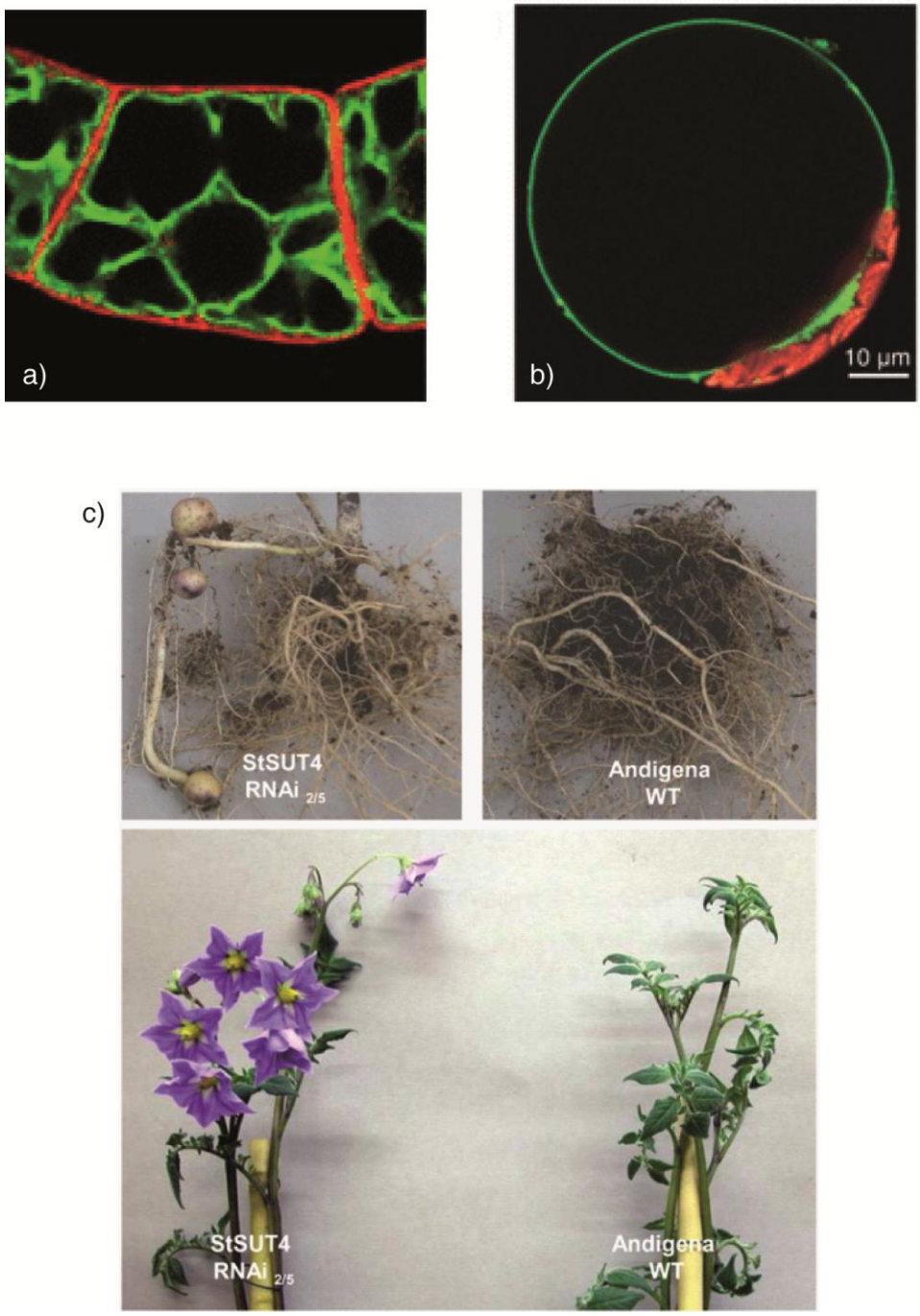


Fig 11. SUT4 clade proteins

a) Subcellular localization of NtSUT4 in BY2 cells. NtSUT4 was fused to GFP while red marker corresponds to FM4-64 which primarily stains the plasma membrane (Okubo-Kurihara et al. 2011).

b) Subcellular localization of a PtaSUT4-GFP fusion in *Nicotiana benthamiana* mesophyll protoplasts. Red chloroplast autofluorescence is localized outside of the PtaSUT4-GFP fusion targeted at the tonoplast (Payyavula et al. 2011). c) Early flowering phenotype and tuber morphology of the *StSUT4* antisense line compared to the Andigena wild type variety (Chincinska et al. 2008).

Monocots and Dicots (Fig 8, Payyavula et al. 2011). SUT4 proteins are characterized as the lowest affinity SUTs with Km around 10 mM and a high capacity for sucrose; however the only characterized SUT4 protein in Monocots shows a high affinity (OsSUC4 Km=1.86 mM; Eom et al. 2011). All SUT4 proteins present particular features comprising a short C-terminal and a small linker sequence between TMs VII and VIII (Fig 10). Consequently, they possess the shortest amino acid sequences among SUTs.

In contrast with all plant SUTs previously cited, SUT4 clade proteins are not solely found at the plasma membrane. Although AtSUT4 and StSUT4 proteins were first characterized to mediate active uptake at the yeast plasma membrane (Weise et al. 2000), a tonoplast proteomic approach screened AtSUT4 and HvSUC2 in vacuolar fractions (Endler et al. 2006) and another proteomics study purified AtSUT4 in chloroplast fractions (Ferro et al. 2003). Now, numerous studies showed that SUT4-GFP protein fusions are targeted to the tonoplast (Fig 11a,b; Endler et al. 2006; Reinders et al. 2008; Eom et al. 2011; Okubo-Kurihara et al. 2011; Schneider et al. 2011). It has been postulated that their initial localization to the plasma membrane was due to mistargeting in heterologous yeast and *Xenopus laevis* systems (Sauer 2007). Since the uptake kinetics of characterized SUT4 transporters were curbed upon the addition of protonophores and H^+-ATPase inhibitors, SUT4 H^+ co-transporters are likely to be involved in sucrose efflux from acidic vacuoles (Fig 12; Neuhaus 2007; Reinders et al. 2008). The study of sucrose induced proton currents in vacuoles overexpressing *AtSUT4* confirmed that AtSUT4 releases sucrose from *A. thaliana* vacuoles (Schneider et al. 2011). However, using specific antibodies, StSUT4 showed a preferential localization in plasma membrane fractions while a lower weight fragment was detected in endosomal fractions (Chincinska et al. 2008). Therefore, the hypothesis of a dual targeting to the vacuolar and plasma membranes already encountered for *A. thaliana* TIP aquaporins (Gattolin et al. 2011) can also be postulated for SUT4 proteins (Doidy et al. 2012a).

SUT4 proteins were detected in sieve elements (Weise et al. 2000) and genes are expressed in numerous source tissues: in the mesophyll, in minor veins, in phloem and also in sink organs: roots, seeds and young flowers (Weise et al. 2000; Chincinska et al. 2008; Eom et al. 2011; Payyavula et al. 2011). In tobacco bright yellow cells, the overexpression of *NtSUT4* led to spherical cell shape and a role in sucrose homeostasis was suggested (Okubo-Kurihara et al. 2011). SUT4 RNAi approaches in rice and poplar led to increase level of soluble sugars, especially sucrose in leaves compared to wild type plant, suggesting a role in intracellular sucrose partitioning (Eom et al. 2011; Payyavula et al. 2011). Moreover, the growth retardation phenotype of *ossut2* mutant indicates the potential role of SUT4 proteins in sucrose efflux from source parts (Eom et al. 2011) and could represent the low-affinity/high-

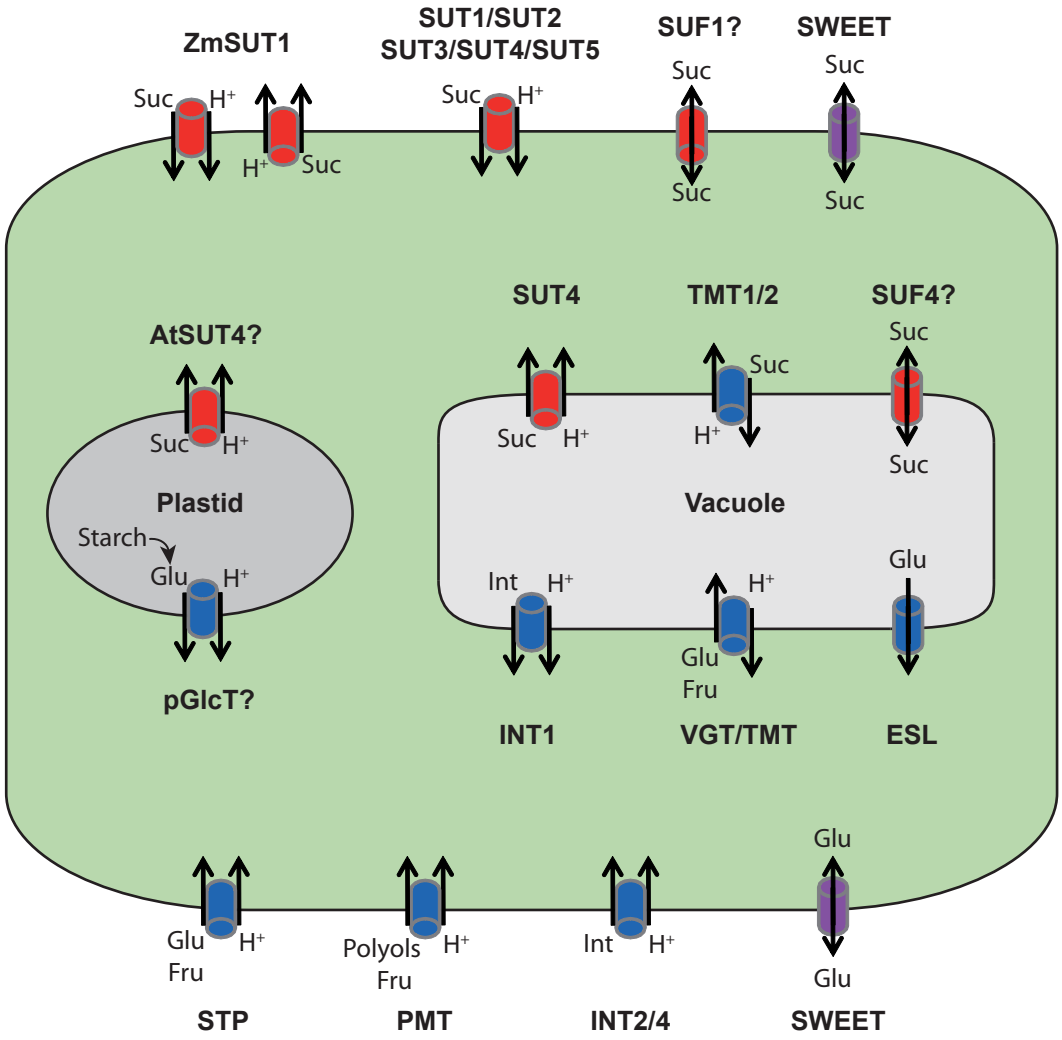


Fig 12. Subcellular distribution of sugar transporters supported by biochemical analysis
Three families of transporters are implicated in the distribution of sugars within the plant cell; sucrose transporters (in red), monosaccharide transporters (in blue) and SWEETs (in purple). At the plasma membrane, most SUTs and MSTs have been characterized as H^+/sugar importers, although recently ZmSUT1 was shown to also mediate active efflux of sucrose (Carpaneto et al. 2005). In contrast, SWEETs and SUFs function as energy-independent uniporters that mediate sugar influx and/or efflux (Zhou et al. 2007; Chen et al. 2010). Localization of SUF1 and SUF4 has not been studied and their membrane localization in this figure is based upon phylogenetic grouping into particular clades.

capacity system encountered in leaves (Delrot and Bonnemain 1981; Weise et al. 2000; Ayre 2011). Nevertheless, anti sense lines in potato gave different phenotypes. Indeed, the downregulation of *StSUT4* led to early flowering and higher tuber yields (Chincinska et al. 2008). Surprisingly, such phenotype is opposite to the *StSUT1* (phloem loader) mutant phenotype (Fig 11c).

3.2.3 SUT transport properties

Most SUTs have been characterized as H^+/sucrose co-importers with a 1:1 proton/sucrose stoichiometry (Bush 1990) and uptake mechanism composed of two different systems with a saturable high-affinity/low-capacity (HALC) and a linear low-affinity/high capacity (LAHC) types (Delrot and Bonnemain 1981; Ayre 2011). The biochemical properties of SUTs were studied using different heterologous systems, often in mutant yeast cells or in *Xenopus* oocytes and sometimes in plant protoplast or isolated vacuoles. So far, SUT characteristics demonstrated that they are able to function properly in different lipid environment.

3.2.3.1 SUTs as efflux carriers

So far all SUTs were characterized to mediate the import of sucrose but recently the study of sucrose induced proton currents of ZmSUT1 in the giant inside-out patch of *X. laevis* oocytes revealed an alternative transport mode for SUT proteins (Carpaneto et al. 2005). Indeed, in physiological condition ZmSUT1 was able to mediate sucrose import with a Km of 2 mM at pH 5.6. However, a rise of cytosolic sucrose concentration above 300 mM with variation of pH conditions inverted the transport mode of ZmSUT1 that was able to mediate sucrose efflux with a 100-fold lower affinity (Km for efflux of 278 mM). Therefore, ZmSUT1 transport mode is reversible (Fig 12) in oocytes and dependent on the direction of the sucrose and pH gradient as well as the membrane potential. *In planta*, the anti sense inhibition of StSUT1 under the control of the class I patatin promoter B33 primarily active in developing tuber led to lower tuber yield when phloem unloading towards tuber is apoplasmic (Viola et al. 2001; Kühn et al. 2003) indicating a major role for StSUT1 in sugar efflux towards sink organs. Very recently Geiger 2011 reviewed the role of the major SUT1 protein necessary for phloem loading. In the release phloem, apoplastic concentrations of sucrose are reduced by the cleavage of sucrose due to the activity of CWIN and the membrane potential mainly depends on the potassium conductance therefore the proton motive force is decreased. Such condition directs SUT1 into the inverse transport mode and sucrose is released from the phloem. Thereby, the SUT1 candidate seems to mediate both the influx of sucrose for phloem loading and the efflux of sucrose for unloading of apoplasmic sink (Fig 4 and 12, Doidy et al. 2012a).

Treatment	PsSUT1	PsSUF1	PsSUF4	PvSUT1	PvSUF1
Estimated C_i/C_o ratio[a]	1.81	1.03	0.73	8.28	1.18
Inhibitor (% control)[b]					
10 μM Antimycin A	40*	110	110	66*	94
50 μM CCCP	69*	105	92	39*	102
1.5 mM DEPC	29*	106	92	59*	100
Sucrose remaining (% of preloaded ^{14}C)[c]	92	52*	39*	90	42*
Competing sugar (% control)[d]					
10 mM glucose	127*	103	95	124*	59*
10 mM fructose	128*	98	86	137*	66*
10 mM maltose	72*	102	90	58*	94
10 mM mannitol	96	111	97	95	90
10 mM raffinose	101	104	104	93	91
10 mM palatinose	99	100	88	98	70*

Table 1. Biochemical uptake characterization of *P. sativum* and *P. vulgaris* SUT and SUF proteins in the SUSY7/ura3- yeast strain Zhou et al. 2007

Green rectangles indicate unimpaired transport properties of SUFs upon inhibitor treatment. Red rectangles highlight efflux capacity of preoladed yeast expressing SUFs.

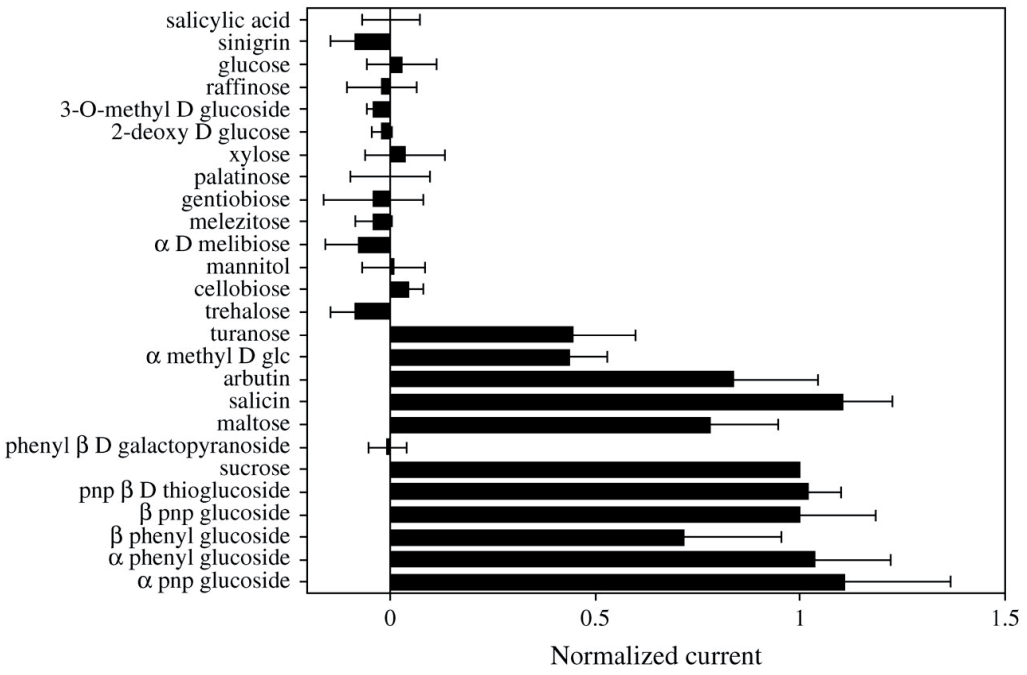


Fig 13. Substrate specificity of AtSUC9

Mean substrate dependent currents recorded from *Xenopus* oocytes expressing *AtSUC9* (Sivitz et al. 2007).

3.2.3.2 Sucrose facilitators

Unlike previously cited SUTs which mediate secondary active transport of sucrose, three leguminous proteins in pea (Fig 8; PsSUF1 and PsSUF4) and in common bean (PvSUF1) were shown to be sucrose facilitators (SUF; Zhou et al. 2007). When expressed in yeast, such facilitators supported bidirectional diffusion of sucrose. Indeed, the addition of respiratory chain inhibitor (antimycin A), protonophore (CCCP, carbonyl cyanide 3-chlorophenylhydrazone) or H^+ flux inhibitor (DEPC, diethylpyrocarbonate) had no effect on SUF transport capacity while the addition of the same agents curbed transport property of the H^+/sucrose co-transporters PsSUT1 and PvSUT1 (Table 1; Zhou et al. 2007). Moreover, when yeast cells expressing respective SUFs and SUTs were preloaded with [^{14}C]sucrose, significant sucrose efflux could only be observed for SUF candidates whereas yeast expressing strict importers kept the radiolabeled sucrose within cells (Table 1; Zhou et al. 2007). Thereby, leguminous SUFs mediate passive sucrose efflux and influx according to the concentration gradient but so far, no other published work has reported the identification of additional SUF proteins.

3.2.3.3 SUTs have a broad substrate spectrum

In addition to sucrose transport capacity, SUTs are also able to bind a large range of other naturally occurring or synthetic sugars. In general, α-glucosides and β-glucosides are well accepted as substrates of SUTs (Fig 13). For instance, the ability to transport maltose seems to be a common trait of all plant SUTs (Sauer 2007). Nevertheless, the uptake of sucrose for leguminous SUFs was not inhibited by the addition of maltose (Table 1; Zhou et al. 2007). Moreover, *A. thaliana* SUT1 clade proteins (AtSUC2 and AtSUC9) mediate the transport of both α- and β-linked glucosides with a higher affinity than the original sucrose substrate (Fig 13; Chandran et al. 2003; Sivitz et al. 2007). In contrast, HvSUT1, ShSUT1 as well as OsSUT1 and OsSUT5 could only transport α-phenylglucose and α-pnp-glucose (Sivitz et al. 2005; Reinders et al. 2006; Sun et al. 2010). These results highlight the importance of characterizing additional transporters from various species rather than relying only on data from the model species *A. thaliana*. In addition, most SUTs are also able to transport salicin and arbutin which are naturally occurring glucosides (Sauer 2007). Protein from the SUT3 clade have the narrowest substrate spectrum and OsSUT1 showed low salicin and helicin transport rate and no arbutin transport capacity (Sun et al. 2010).

Despite the broad spectrum of SUTs, sucralose, a chlorinated analog of sucrose was not transported by ShSUT1. However, sucralose bound to ShSUT1 binding site since the addition

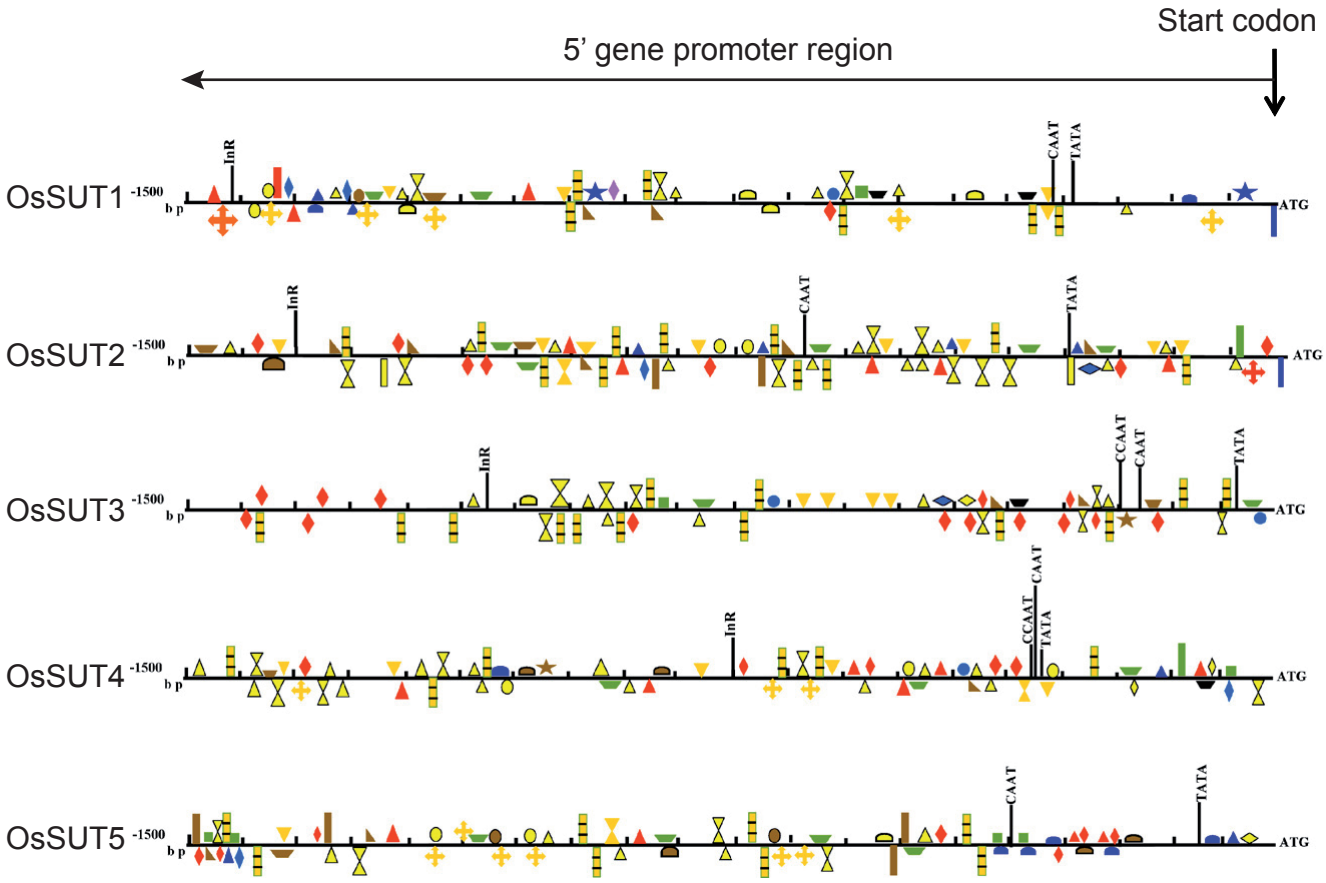


Fig 14. Map of the 5' *cis*-regulatory sequences of the rice OsSUT family
The 1.5 kb of 5' *cis*-regulatory region was analyzed using PLACE, PlantCARE and Genomatix Matinspector professional databases. Adapted from Ibraheem et. al., 2010. For schematic representation of 5' *cis*-regulatory elements, see the legend below:

Salt/drought responsive element; Abscisic acid responsive element; Sugar repression; Antioxidant responsive element; Element involved in early response to drought and abscisic acid induction; Gibberellin responsive element; Element involved in regulation of drought inducible gene expression; Element involved in salicylic acid responsiveness; Module involved in light responsiveness; Element involved in direct fungal elicitor stimulated transcription of defense genes and activation of genes involved in response to wounding; Element involved in methyl jasmonates responsiveness; Element involved in light responsiveness; Part of light responsive element; Element involved in seed-specific regulation; Element required for early response to dehydration; Elicitor responsive element; Core of GCC-box found in many pathogen-responsive and in ethylene responsive genes; Element required for rapid response to pathogen attack, salinity and salicylic acid inducible gene expression; Light box Element; Element required for high level light regulated and tissue specific expression; Element related to meristem expression; Ethylene responsive element; Copper responsive element, also involved in oxygen response; Pyrimidine box partially involved in sugar repression (requires Gibberellin); Sulphur responsive element; Light responsive element; Element required for sugar responsive gene expression; Heat stress element; Element involved in light responsiveness; Low temperature response element; Element involved in auxin responsiveness; Element involved in endosperm expression.

of this analog clearly inhibits ShSUT1 affinity for sucrose (Reinders et al. 2006). In addition, several disaccharides (such as trehalose, cellobiose and melibiose) and all tested trisaccharides (raffinose and melezitose) were not accepted as potential SUT substrates (Fig 13; Sivitz et al. 2005; Reinders et al. 2006; Sivitz et al. 2007; Reinders et al. 2008; Sun et al. 2010). Such results suggest that binding specificity and transport ability of SUTs are tightly modulated among oligosaccharides.

Interestingly, in addition to sugar and sugar-linked substrates, SUT are also able to mediate the transport of a broader spectrum of molecules. Indeed, AtSUC5 was screened from a yeast complementation assay using a biotin uptake deficient strain (Ludwig et al. 2000). The transport of biotin (also called vitamin H) may be a general property of all SUTs as PmSUC2 was also able to transport this vitamin (Ludwig et al. 2000).

3.2.4 Regulation of SUTs

3.2.4.1 Transcriptional control of SUT expression

Organisms have devoted a significant fraction of their DNA to encrypt *cis*-acting regulatory elements to control and coordinate gene expression and much of these regulatory regions consist of transcription factor binding sites generally present in the 5' gene promoter. Recently, Ibraheem et al. 2010 analyzed the 5'cis-acting regulatory elements in the promoter region of the 9 *AtSUT*s of *A. thaliana* and the 5 *OsSUTs* of rice. Interestingly, this *in silico* approach screened numerous *cis*-elements in all analyzed SUTs including A-box, RY, CAT, Pyrimidine-box, Sucrose-box, ABRE, ARF, ERE, GARE, Me-JA, ARE, DRE, GA-motif, GATA, GT-1, MYC, MYB, W-box, and I-box (Fig 14; Ibraheem et al. 2010). In addition to similar promoter patterns screened in the SUT genes of tomato, *SlSUTs* also contain regulatory elements located in intronic sequences (He et al. 2008). Indeed, auxin responsive elements (ARE) were tagged in the 3rd intron of *SlSUT1* and in the 10th intron of *SlSUT2*. These intronic regions were found to be involved in the specific expression of *SlSUT1* in trichomes, guard cells and phloem cells (Weise et al. 2008). In *A. thaliana* plants transformed with the promoter of *AtSUC9* fused to the GUS, no expression was detected (Sauer et al. 2004) but GUS activity could be observed when the full gene locus of *AtSUC9* was inserted (Sivitz et al. 2007). In parallel, *AtSUT1* expression is also dependent on intronic sequence but not *AtSUC2* (Stadler et al. 1999; Sivitz et al. 2007). Thus, SUTs contain numerous intergenic and intronic motifs and each locus shows a unique combination of these regulatory sequences that determine the temporal and spatial expression of the gene.

These patterns are mostly associated with plant hormonal and developmental regulation as well as stress response processes (Fig 14; Ibraheem et al. 2010; Kühn 2011; Liesche et al. 2011). Numerous studies confirm the transcriptional regulation of these crucial processes in regard to the *cis*-element pattern. Indeed; *SlSUT1*and *StSUT1* promoter activity are auxin

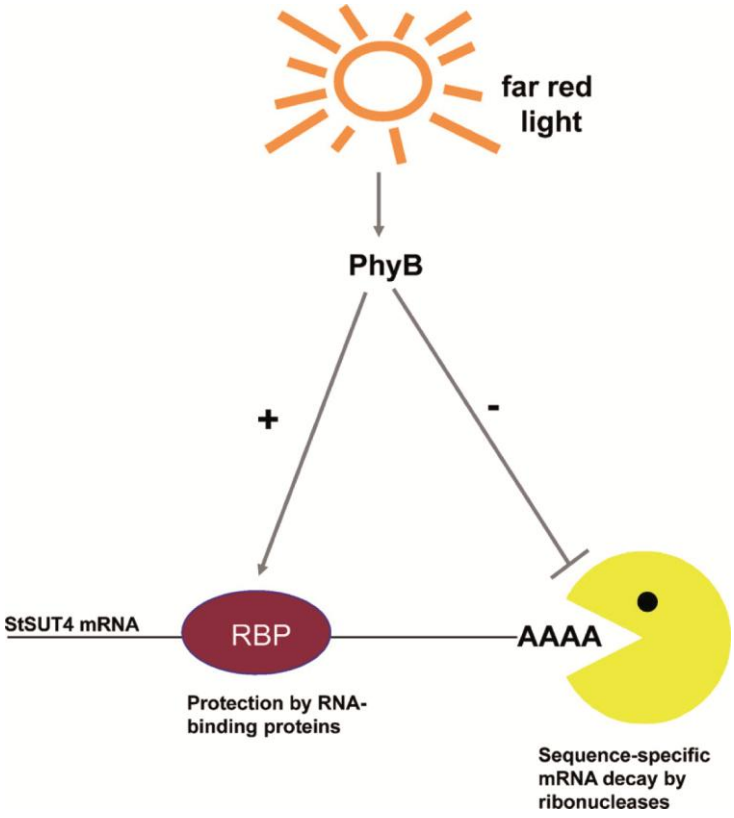


Fig 15. Regulation of the stability of *StSUT4* mRNA according to Liesche et al. 2011

responsive (Harms et al. 1994; He et al. 2008). In addition, *SlSUT1* which contain an imperfect evening element of circadian genes is preferentially expressed at the end of the light period (Harmer and Kay 2005; He et al. 2008) while the regulation of SUT genes upon sucrose addition suggests a transcriptional control via the sugar repressive A-box (Osuna et al. 2007; Kühn 2011). In *A. thaliana*, a 126-bp promoter fragment was sufficient to drive *AtSUC2*-typical expression in source leaves (Schneidereit et al. 2008) and wounding increased the expression of *AtSUC3* which contains several W-boxes in its promoter region (Meyer et al. 2004; Ibraheem et al. 2008; Ibraheem et al. 2010).

3.2.4.2 Post-transcriptional regulation of SUTs

Messenger RNAs (mRNAs) of transporters are often short lived and SUT show a high turn-over rate with an half life for mRNAs estimated between 60 and 130 min (Vaughn et al. 2002; He et al. 2008). In addition, experimental approaches on mRNA stability using transcriptional and translational inhibitors revealed a *StSUT2* and *StSUT4* mRNAs protective mechanism but it was not the case for *StSUT1* mRNAs (He et al. 2008). Indeed, *SUT2* and *SUT4* mRNAs stability seems to be regulated by RNA interacting proteins to ensure steady-state mRNA levels in plant cell. This is ensured either by degradation of ribonucleases or by protection via RNA-binding proteins in a phytochrome B dependent manner in response to reduced-red:far-red light ratio (Fig 15; Liesche et al. 2011).

3.2.4.3 Post-translational control of SUT expression

SUT proteins were also shown to be short lived. Indeed, the half life of BvSUT1 was estimated around 2.7 hours upon the addition of cycloheximide which inhibits *de novo* protein synthesis (Vaughn et al. 2002). At the protein level, SUTs are regulated through several post-translational control steps ranging from phosphorylation of protein residues to preferential association with membrane microdomain (Kühn 2011).

3.2.4.3.1 Phosphorylation

The first evidence of regulatory control via protein phosphorylation came from infiltration with okadaic acid (a toxin affecting protein phosphatases) which inhibited proton-motive force-driven uptake of sucrose into plasma membrane vesicles (Roblin et al. 1998). Thus, it was postulated that when SUTs are in a phosphorylated form, their sucrose import activity is decreased. A supplemental study confirmed the inhibitory effect when phosphatase are inhibited but contradictive results were obtained upon the addition of kinase inhibitors that could increase sucrose transport (Ransom-Hodgkins et al. 2003). Thereafter, investigation of protein residues representing putative phosphorylation site was carried out and

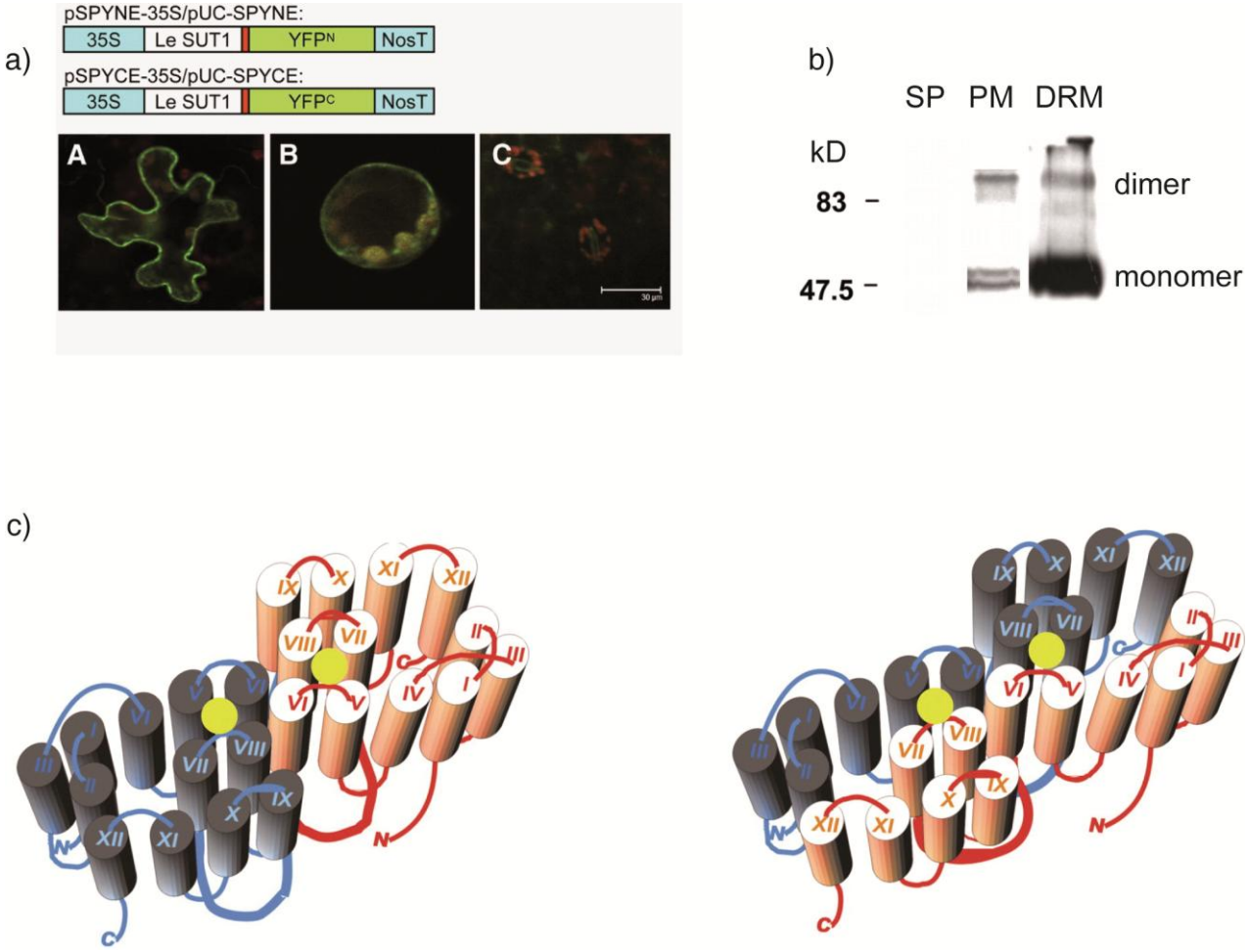


Fig 16. SUT oligomerization

a) Homodimerization of SlSUT1 visualized via split YFP; *SlSUT1* constructs with the N-terminal or C-terminal part of YFP was used to transform tobacco leaves (A) and protoplast (B) with *Agrobacterium*; as a negative control, the potassium channel *AtTPK4* in SPYCE was coinfiltrated with *LeSUT1*-SPYNE (C). YFP fluorescence is visible as green and chlorophyll autofluorescence as red (Krügel et al. 2008).

b) StSUT1 specific immunodetection in soluble proteins fraction (SP), in detergent-resistant fraction (DRM) and in solubilized plasma membrane (PM) treated with o-Phtalaldehyde 0.5%. Adapted from Krügel et al. 2008.

c) Hypothetical model of the intra- and intermolecular interactions between two transporters forming a homo- or heterodimer. SUTs can form their own pore via intramolecular interactions of the first and second halves of the protein or transport pores can be formed by contributions of two different transporters through intermolecular interactions. Substrate are represented at the transporting pore by yellow dots (Reinders et al. 2002).

phosphoproteomics approaches screened a conserved serine in the N-terminal extremities of AtSUC1 and AtSUT5 (Nühse et al. 2004; Niittylä et al. 2007). Furthermore, bionformatics tools predicted threonine 393 as a second phosphorylation site in AtSUC1 (Kühn 2011). However, this later residue seems to be located in an extracellular loop between TM 9 and 10.

3.2.4.3.2 Redox environment

Regarding protein regulation on the redox status, first experiments showed that SUTs were not regulated by cysteine reduction/oxidation as the application of oxidized (GGSG) and reduced glutathione (GSH) did not affect Monocot SUT activity (Sun et al. 2010). However, another analysis showed that the application of GGSG increased the dimeric targeting to the plasma membrane in yeast as well as the transport activity of StSUT1 in oocytes (Krügel et al. 2008). This sucrose transport activity is directly dependent on the redox environment and not due to secondary effect of either the delta pH nor the membrane potential (Krügel et al. 2008). In conclusion, oxidation is thought to have an effect on plasma membrane targeting, activity and dimerization of SUT proteins (Fig 17; Liesche et al. 2008).

3.2.4.3.3 SUT oligomerization and interaction with other proteins

Numerous studies rely on bioinformatics tools that predict number, position and the orientation of putative moieties of SUTs but only few experimental analyses were carried on intra- and intermolecular interactions and therefore such structural information are valuable. As the yeast two-hybrid approach is not suitable for the study of integral membrane protein interactions, first studies of SUT oligomerization were performed using the split-ubiquitin system. By this mean, it was shown that SUT1, SUT2 and SUT4 clade members, which co-localized in CC-SE complexes, have the potential to interact with each other (Reinders et al. 2002a; Schulze et al. 2003). StSUT1, SlSUT1 and SlSUT2 could also form homooligomers in the heterologous yeast system (Reinders et al. 2002a; Krügel et al. 2008). Recently, the first evidence of SUTs interacting *in planta* came from a split-YFP approach of SlSUT1in tobacco cells and supplemental proof of StSUT1 dimerization was shown by immunodetection in potatoes membrane fractions (Fig 16a-b; Krügel et al. 2008). These SUT1 dimers mediate the uptake of sucrose in yeast (Reinders et al. 2002a) and in plasma membrane vesicles (Leggewie et al. 2003; Liesche et al. 2008). In addition, when the 6 first TMs of the low affinity SlSUT2 interacts with the 6 terminal TMs of the high affinity StSUT1 (Km=0.9 mM), the resulting functional transporter showed lower affinity (Km=7.4 mM; Reinders et al. 2002b). In conclusion, SUTs can form functional homo- and heterodimers, their transport capacity and affinity being regulated by intra- and inter-protein oligomerization (Fig 16c).

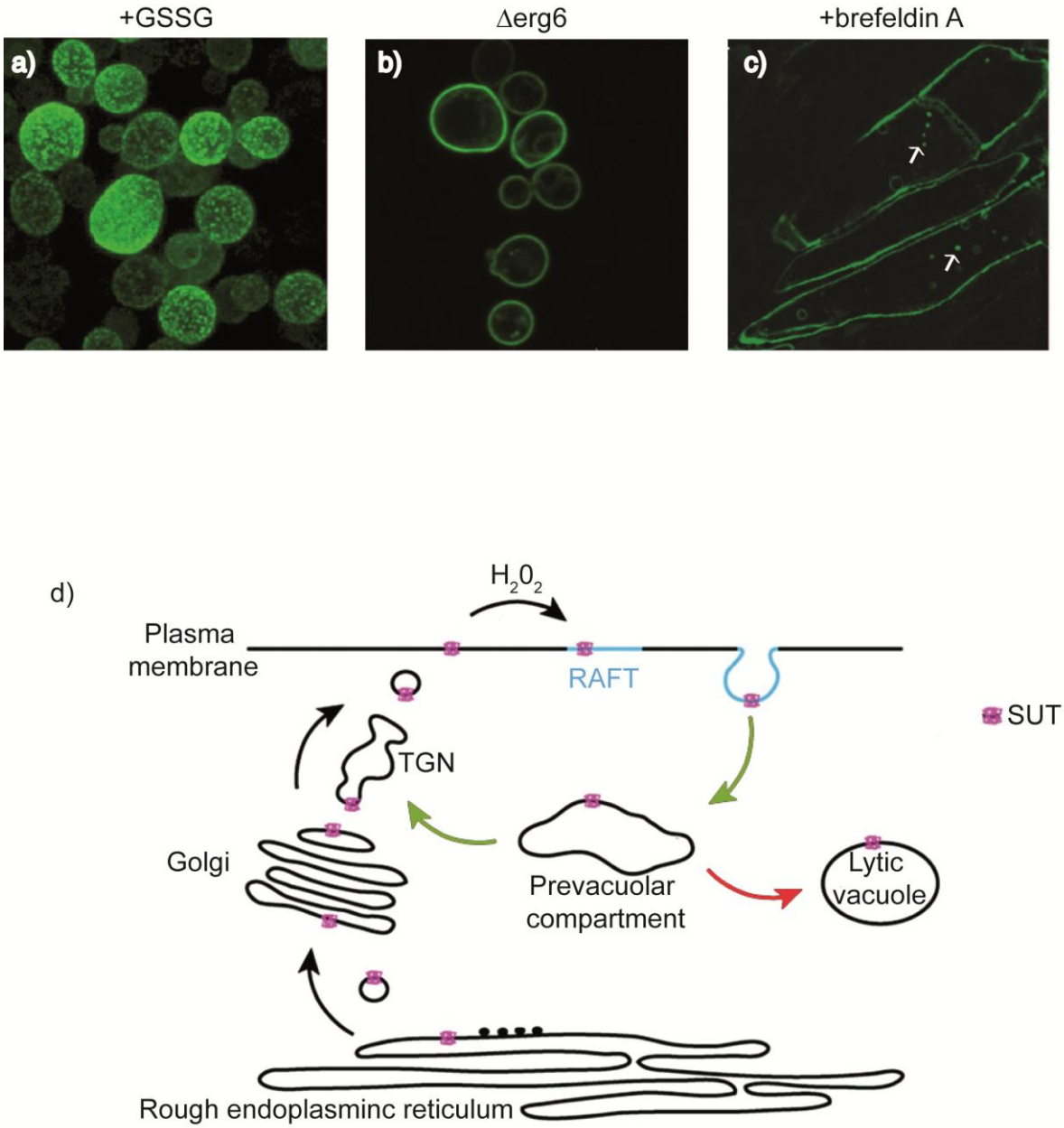


Fig 17. Association to membrane raft and cycling of SUTs

a) Sucrose uptake deficient yeast (SUSY7) transformed with a *LeSUT1*-GFP construct and treated with oxidized glutathione (GGSG) (Krügel et al. 2008).

b) Ergosterol mutant yeast (Δerg6) transformed with a *LeSUT1*-GFP construct (Krügel et al. 2008).

c) Immunohistochemical detection of StSUT1 on samples treated with brefeldin A. Arrows represent vesicle formation in SE (Liesche et al. 2010).

d) Hypothetical model of cycling of solanaceous SUT1. Green arrows represent the recycling and red arrow represents degradation of SUTs. Adapted from Liesche et al. 2010.

Recent findings reveal that SUT regulation is also dependent on their association with other protein and complex. Using a combination of the split-ubiquitin yeast two hybrid, immuno-coprecipitation and bimolecular fluorescence complementation assays, MdSUT1 from apple was shown to interact physically with an apple ER-anchored cytochrome b5 *in vitro* and *in vivo* (Fan et al. 2009). In addition, when this transporter is coexpressed with the cytochrome in a sucrose uptake deficient yeast, the affinity of MdSUT1 for sucrose clearly increased. Furthermore, StSUT1 seems to interact with numerous proteins in raft domains of the plasma membrane and more precisely with a protein disulfide isomerase (PDI), which also interacts with StSUT4 and SlSUT2. PDI is proposed to function as an escort protein for the correct oligomerization and targeting of SUTs to membrane microdomains (Krügel et al. 2011)

3.2.4.3.4 Association to membrane rafts and cycling of SUTs

Plasma membranes contain particular domains (rafts) that exhibit a specific molecular composition. Indeed, membrane rafts are defined as "small (10-200 nm), heterogeneous, highly dynamic, sterol- and sphingolipid-enriched domains that compartmentalize cellular processes" (Pike 2006). Proteomic analyses have indicated that a high proportion of proteins associated with detergent-resistant membranes (DRMs) considered as the biochemical counterpart of membrane raft might be involved in signaling, protein activity, endocytosis, dimerization, degradation and transport pathways (Mongrand et al. 2010).

First evidences of the presence of SUTs in membrane raft came from the isolation of StSUT1 from DRM fractions of plant plasma membrane mostly in its monomeric form but also as homodimers (Fig 16b; Krügel et al. 2008; Krügel et al. 2011). It was also observed in raft-like membrane microdomains when SlSUT1-GFP construct was expressed in yeast upon treatment with oxidizing agent (H_2O_2 and GSSG; Fig 17a; Krügel et al. 2008). In contrast, in the Δ*erg6* yeast strain deficient in ergosterol synthesis (sterol enrichment being a key characteristic of membrane raft), SlSUT1 was homogenously distributed at the plasma membrane (Fig 17b; Krügel et al. 2008).

This association to raft-like domains seems to be essential to the endocytic cycling and the polar distribution of transporters and was previously well described for the *A. thaliana* auxin transporter AUX1 (Kleine-Vehn et al. 2006). Recently, the internalization of solanaceous SUT1 proteins was investigated by the mean of Brefeldin A which inhibit exocytosis and led to the formation of cytosolic vesicles containing StSUT1-GFP protein suggesting a constant recycling of SUTs (Fig 17c; Liesche et al. 2010). In addition, the use of cytoskeleton inhibitors revealed that internalization of SUTs via vesicles progress along actin strands but is independent of the tubulin cytoskeleton (Liesche et al. 2010). Thereby, the constant turnover

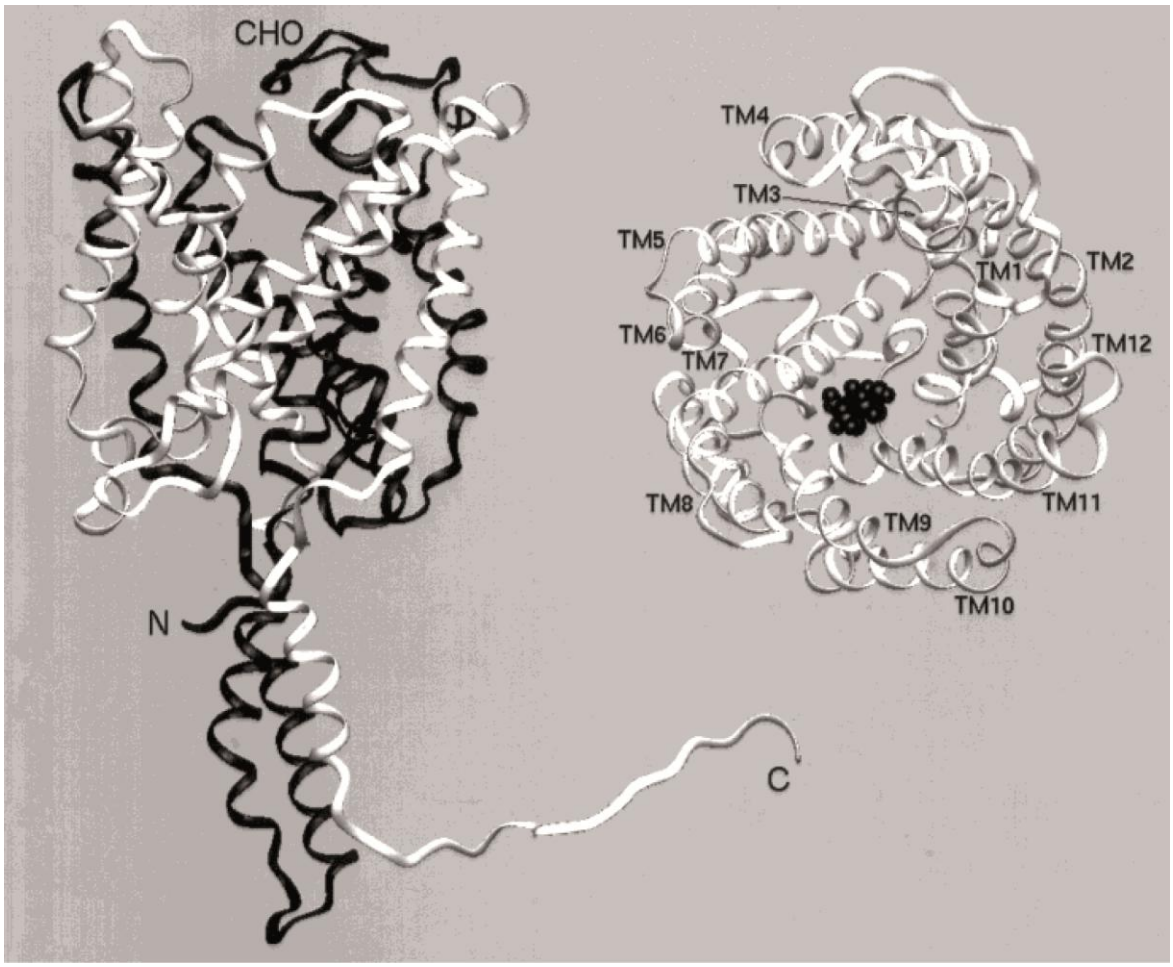


Fig 18. Three-dimensional model of the human glucose transport protein (HsGLUT3) Peptide backbone of HsGLUT3 is represented as a ribbon structure (left). View of the pore with D-glucose (in black) in the center of the pore (right). Intracellular segments have been omitted from this view. Dwyer 2001.

of SUTs via recycling or degradation in lytic vacuoles is dependent on association with membrane microdomains and cytoskeleton in plant cell (Fig 17d).

3.3 Monosaccharide Transporters

Upon release from the phloem, sucrose is either directly transported via SUT importers in apoplasmic connected sink cells or cleaved by CWIN to yield the hexoses glucose and fructose that are taken up by monosaccharide transporters (MSTs; Fig 3). While sucrose is the main long distance transport sugar, glucose and fructose are mostly transferred from cell to cell and between organelles at a single cell level; thereby most MSTs do not directly participate in long-distance transport, still their indirect roles greatly impact on carbon allocation and transport flux at the whole plant level. Monosaccharides are either transported by passive diffusion or by active pathways via MSTs. Unlike the small-sized SUT family which has been intensively reviewed, the current knowledge of the MST family is not so well acknowledged in the literature (Buttner and Sauer 2000; Williams et al. 2000; Buttner 2007; Slewinski 2011).

3.3.1 A large sized family with a “vague” nomenclature

MSTs belong to the sugar porter (SP) family which is the largest family within the MFS. A three dimensional model of the human glucose transporter GLUT3 was designed from the MscL protein (mechanosensitive ion channel) and general insights of aquaporins (Schulz et al. 1998; Dwyer 2001). From the proposed structure of GLUT3, MSTs seem to present 12 TMs helices connected by loops with intracellular N- and C-termini: a similar structure with SUTs (Fig 7 and 18). In contrast with SUT, plant species comprise a large-sized family of MSTs genes; indeed, *A. thaliana* possesses 53 *AtMSTs* (Fig 19), grape has 59 *VvMSTs* and monocotyledonous genome seems to present a larger family with 65 rice *OsMSTs* (Buttner 2007; Johnson and Thomas 2007; Afoufa-Bastien et al. 2010). MSTs are currently distributed among 7 subfamilies including gene homologs already present in land plants at least 400 million years ago (Johnson et al. 2006). In addition, numerous MST homologs are found in bacterial (*e.g.,* EcXylE), yeast (*e.g.,* ScHXTs) and mammal (*e.g.,* HsGLUTs) genomes. However, the current phylogenetic classification of plant MSTs and especially the clade nomenclature remains ambiguous (Fig 19). Indeed, clades are either named according to substrate specificity (sugar transport protein [STP], polyol/monosaccharide transporter [PMT], inositol transporter [INT]), subcellular localization (vacuolar glucose transporter [VGT], tonoplast membrane transporter [TMT], plastidic glucose transporter [pGlcT]), mutant-recovering phenotype (suppressor of G Protein Beta1 [SGB1]) or even to a stress condition that induces gene expression (early-responsive to dehydration six-like [ESL]). For

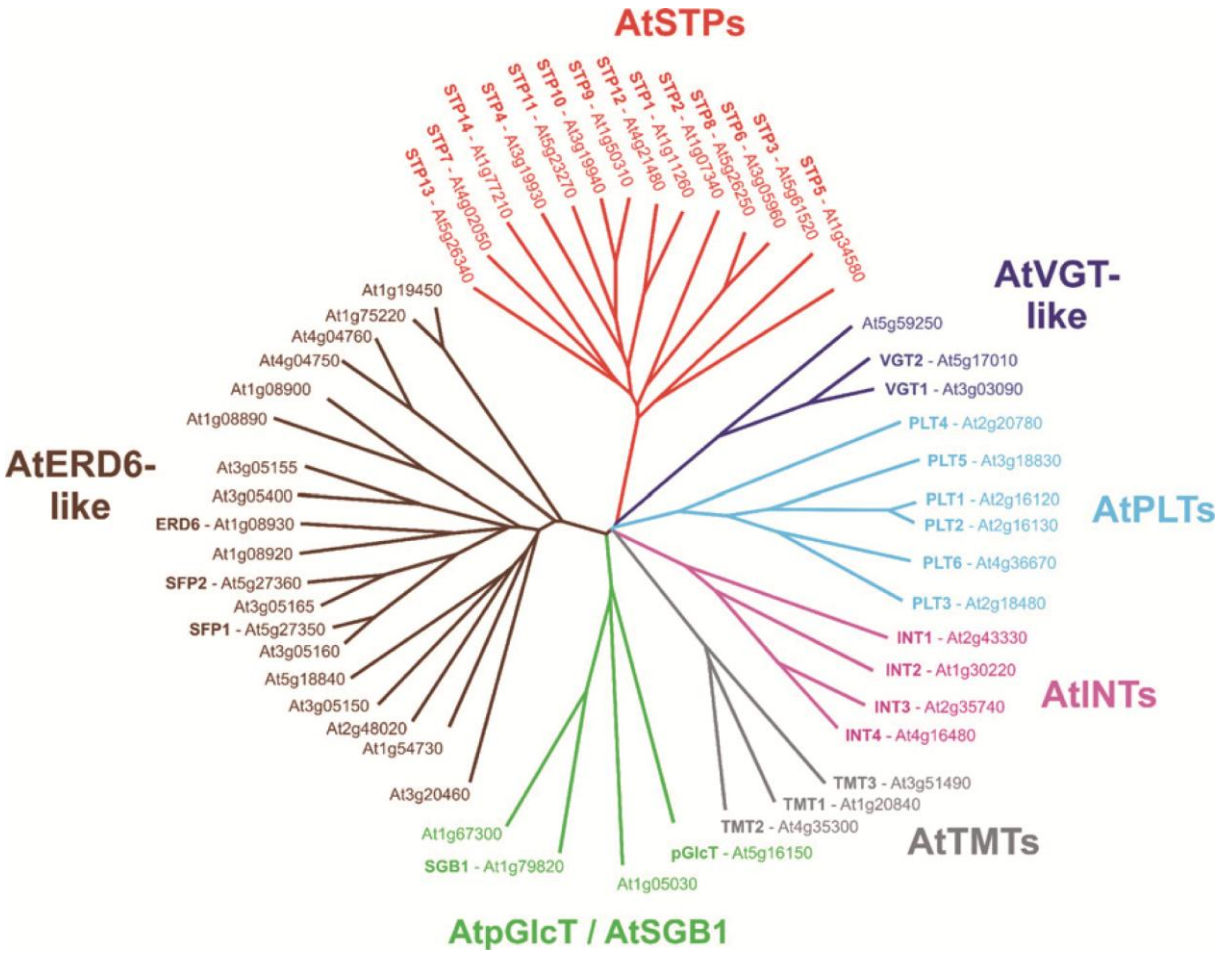


Fig 19. Phylogenetic tree of the MST family from *A. thaliana*

The genome of *A. thaliana* contains 53 AtMSTs that fall into 7 subfamilies: Sugar Transport Protein (STP), Vacuolar Glucose Transporter (VGT), Tonoplast Monosaccharide Transporter (TMT), Plastidic Glucose Transporter/Suppressor of G Protein Beta1 (pGlcT /SGB1), Polyol / Monosaccharide Transporter (PMT or PLT), Inositol Transporter (INT), Early-responsive to dehydration Six-Like (ESL or ERD6-like). Buttner 2007.

instance, the best described clade is still assigned as STP which stands for the general designation “sugar transporter proteins”.

3.3.2 MSTs by clade

3.3.2.1 The STP subfamily

The STP subfamily (Sugar Transport Protein, originally clade1 from Lalonde et al. 2004) is the largest MSTs clade in monocotyledonous species with rice possessing 29 OsSTPs and the second largest clade in *A. thaliana* with 14 AtSTPs, primarily resulting from multiple tandem gene duplications (Fig 19; Johnson and Thomas 2007). To date, most characterized STPs are plasma-membrane localized H^+/hexose symporters identified in the model species *A. thaliana* and have been recently presented in a comprehensive review (Fig 12; Büttner 2010). Many of the transporters show broad substrate specificity; AtSTP1 (Sherson et al. 2000), AtSTP2 (Truernit et al. 1999), AtSTP3 (Buttner et al. 2000b), AtSTP4 (Truernit et al. 1996), AtSTP11 (Schneidereit et al. 2005) and OsMST6 (Wang et al. 2008) transport to differing degrees glucose, xylose, mannose, and galactose but not fructose. In contrast, AtSTP6 (Scholz-Starke et al. 2003), AtSTP13 (Norholm et al. 2006) and OsMST4 (Wang et al. 2007) transport hexoses including fructose but do not transport the pentose sugars, xylose and ribose. Two transporters show high substrate specificity; AtSTP9 is a glucose-specific transporter (Schneidereit et al. 2003) while AtSTP14 is a galactose-specific transporter (Poschet et al. 2010). In most cases, substrate affinity are at the µM ranges except for AtSTP3 which is a low-affinity transporter with a Km for D-glucose of 2 mM (Buttner et al. 2000b).

In general, members of the STP subfamily localize to symplastically isolated sink tissues where they are involved in loading of hexose derived from apoplastic sucrose hydrolysis. However the individual spatial, developmental and environmental expression patterns vary considerably. AtSTP1 expression is reported in germinating seed and young seedlings as well as guard cells (Sherson et al. 2000; Stadler et al. 2003), AtSTP4 is expressed in roots and pollen (Truernit et al. 1996) while four transporters, *AtSTP2*, *AtSTP6*, *AtSTP9* and *AtSTP11* are expressed exclusively in pollen at different developmental stages. Three AtSTPs are expressed in source tissues; *AtSTP14* promoter activity was detected both in the seed endosperm and in source leaves (Poschet et al. 2010), AtSTP13 localized to the vasculature and leaf mesophyll cells while the low-affinity *AtSTP3* gene is also expressed in source leaves. It is postulated that AtSTP14 plays a role in the recycling of galactose derived from cell wall degradation while AtSTP3 and AtSTP13 may be involved in the retrieval of sugars leaked from the cytoplasm. In rice, *OsMST2* and *OsMST3* are strongly expressed in roots and

callus, weakly expressed in source leaf blades and leaf sheaths, but not expressed in sink leaf blades (Toyofuku et al. 2000), while *OsMST5* and *OsMST8* are only specifically expressed in panicles before heading and in anthers, respectively (Ngampanya et al. 2003, Wang et al. 2008), and *OsMST1* is not expressed in any tested organs (Toyofuku et al. 2000). *OsMST3* is mostly expressed in the root xylem and is suggested to be involved in the accumulation of monosaccharides required for cell wall synthesis at the stage of cell thickening (Toyofuku et al. 2000) while *OsMST6* is strongly expressed in various seed tissues, suggested it participates in unloading and sugar supply during grain filling (Wang et al. 2008). AtSTPs have also been shown to respond to wounding (Truernit et al. 1996; Buttner et al. 2000b) and are induced during nematode infection (Hofmann et al. 2009), programmed cell death and pathogen attack (Truernit et al. 1996; Fotopoulos et al. 2003; Norholm et al. 2006). The large numbers of STPs together with their diverse and overlapping expression profiles enable flexible response to developmental and environmental cues.

3.3.2.2 The VGT subfamily

The VGT subfamily (Vacuolar Glucose Transporter, originally clade7 human GLUT 10/12-Like Transporters from Lalonde et al. 2004) comprises 3 members in *A. thaliana* (Fig 19) and 2 in grape and rice. This clade was also designated as "Xylose TP homologs" in regard to their relation with a bacterial D-xylose/H^+ transporter from *Lactobacillus brevis* (Johnson and Thomas 2007). However, AtVGT1 the only characterized VGT was shown to function in the ATP-dependent uptake of glucose (Km= 3.7 mM) and fructose but not xylose when expressed in yeast. Both AtVGT1 and AtVGT2 localize to tonoplast where they are suggested to function as H^+/glucose antiporters to collect monosaccharide storage within the vacuole (Fig 12; Aluri and Buttner 2007; Buttner 2007). The downregulation of AtVGT1 by T-DNA insertion led to delayed flowering and reduced seed viability indicating an important role in plant developmental processes. In addition to AtVGT1 and AtVGT2, the third member from *A. thaliana* (product of the gene At5g5925) possesses a N-terminal extension carrying a potential plastid targeting signal (Fig 19; Buttner 2007), however the function and localization of this protein have to be confirmed experimentally.

3.3.2.3 The TMT subfamily

The TMT subfamily (Tonoplast Monosaccharide Transporter, originally named clade3 MSSP-Like for Monosacharide Sensing Protein in Lalonde et al. 2004 and also AZT for Allgemeine Zucker Transporter, Johnson and Thomas 2007 and N. Sauer personal communication) comprises 3 members in *A. thaliana* and grape, 6 in rice with all characterized proteins localized to the vacuolar membrane (Wormit et al. 2006; Cho et al. 2010). A unique feature of all TMTs proteins is their extended cytoplasmic central loop

between TM 6 and 7 comparable to the one observed in the SUT2 clade (see 3.2.2.4). This huge loop measures around 170 amino acids and contains several phosphorylation sites (Whiteman et al. 2008; Endler et al. 2009; Schulze et al. 2011). Transport assays using isolated vacuoles from AtTMT1, 2 and 3 triple knockout mutants showed reduced glucose uptake activity (Wormit et al. 2006) and OsTMT1 complemented the KO vacuole deficiency and was characterized as a functional glucose importer (Cho et al. 2010). Therefore, TMTs are likely to function as H^+ glucose antiporters that load vacuolar glucose storage (Fig 12). Interestingly, electrophysiological characterization of sucrose transport in vacuoles isolated from *Attmt1/tmt2* knockout mutants indicate that sucrose is also transported by AtTMT1 and AtTMT2 (Schulz et al. 2011); therefore TMTs are suggested as sucrose/H^+ antiporters involved in sucrose loading of vacuolar sucrose storage (Fig 12) *AtTMTs* seem to be expressed preferentially in aerial parts with both *AtTMT1* and *AtTMT2* exhibiting a tissue- and cell type–specific expression pattern while *AtTMT3* is only weakly expressed. In contrast, expression profile of *OsTMT1* and *OsTMT2* overlapped and was higher in bundle sheath cells, and in vascular parenchyma and companion cells of leaves respectively. Overexpression lines of *AtTMT1* demonstrated increased early seedling growth rates and increased seed biomass and it is hypothesized that these physiological changes are the result of down-stream responses to altered sugar sensing and assimilate partitioning (Wingenter et al. 2010). In addition, cold treatment induces changes in protein abundance and activity of numerous vacuolar related proteins and altered the phosphorylation of AtTMT1 and AtTMT2 (Wormit et al. 2006; Schulze et al. 2011). These findings indicate that during cold acclimation, some of the compatible solutes (glucose and fructose) are mainly stored in the vacuole.

3.3.2.4 The pGlcT / SGB1 subfamily

The pGlcT / SGB1 subfamily (Plastidic Glucose Transporter / Suppressor of G Protein Beta1, originally clade 6 GlcT-like transporters in Lalonde et al. 2004) includes 4 homologues in both *A. thaliana* and rice (Fig 19). Phylogenetic analysis separates these homologues into three groups, two of which have putative functions.

One subgroup shows homology to a low affinity glucose transporter (Km=20 mM) from spinach, *SopGlcT*, which is located at the chloroplast inner envelope membrane (Weber et al. 2000). Although the single KO mutant of *AtPGlcT* did not present visible growth phenotype, the double mutant *Atpglct* together with the maltose transporter *mex1* increased the severe growth retardation phenotype of the single *mex1* mutant. In addition, *pglct/mex* mutant line exhibited reduced photosynthetic activities as well as extreme chloroplast abnormalities (Cho et al. 2011). It has been proposed that these chloroplast localized pGlcTs will function in the efflux of glucose derived from the amylolytic breakdown of transitory starch (Fig 12).

The second subgroup include AtSGB1 which restored to wild type an *agb1* mutant (G protein beta1) and was characterized as a functional hexose transporter (Wang et al. 2006). Furthermore, AtSGB was demonstrated as a Golgi-localized hexose transporter by the mean of Brefeldin A which disrupt the integrity of Golgi stacks (Wang et al. 2006). Nevertheless, these results may not be a direct evidence of a function in glucose transport in the Golgi apparatus; in regard to solanaceous SUT1 (see 3.2.4.3.4), this could be the first insight into a cycling mechanism for MST proteins.

3.3.2.5 The PMT subfamily

The PMT subfamily (Polyol / Monosaccharide Transporter, initially named clade2 Sugar Alcohol Transporters in Lalonde et al. 2004) was still recently called PLT (Fig 19; Polyol Transporter) but was renamed PMT (Klepek et al. 2010) in regard to the substrate specificity of these MSTs that do not only transport polyols but also diverse monosaccharides. PMTs were first identified in plants which transport polyols (mannitol or sorbitol) as well as sucrose in their phloem (Noiraud et al. 2001b; Rennie and Turgeon 2009). In celery, AgMaT1 was characterized as a high affinity H^+/mannitol transporter (Km=275 µM) and its expression in mature leaves is consistent with a role in apoplastic phloem loading of mannitol (Noiraud et al. 2001ab). *A. thaliana* has six PMT family members but does not transport polyols in its phloem and as such a physiological function has been more difficult to assign to the PMTs in this species. To date three AtPMTs have been investigated. All characterized AtPMTs localize to the plasma membrane (Fig 12; Klepek et al. 2005; Klepek et al. 2010). AtPMT1, AtPMT2 and AtPMT5 present low substrate specificity and mediate the transport of a range of substrates including polyols (sorbitol, xylitol, glycerol) as well as monosaccharides (hexoses and pentoses) (Klepek et al. 2005; Klepek et al. 2010). Expression of *AtPMT5* was detected in sink tissues being highest in the root elongation zone and was induced in leaves in response to wounding. In the latter case, it may play a role in re-assimilation of sugars from ruptured cells. Truncation of AtPMT5 in a T-DNA insertion mutant did not reveal a phenotype in response to salt or drought stress or in the presence of a range of transported solutes (Klepek et al. 2005). In addition, *AtPMT1* and *AtPMT2* were expressed in immature or germinating pollen as well as pollen tubes (Klepek et al. 2010). This expression profile together with the high fructose transport capacity raise possibility that PMTs may be involved in loading of fructose resulting from CWIN activity in pollen grains. Indeed, the only AtMSTs characterized up to date that are able to mediate the transport of fructose (AtSTP6 and AtSTP13) are only weakly expressed in pollen tissues (Büttner 2010).

3.3.2.6 The INT subfamily

The INT subfamily (Inositol Transporter, initially called clade5 HMITs H^+-Myo-Inositol Transporters in Lalonde et al. 2004) comprises four members in *A. thaliana* and three in rice and grape. Of the four *A. thaliana AtINT* genes only three encode functional transporters; *AtINT3* is incorrectly spliced and encodes a truncated protein (Schneider *et al.*, 2007). Both AtINT4 and AtINT2 have been localized to the plasma membrane and function as H^+/myo-inositol symporters (Fig 12; Schneider et al. 2006; Schneider et al. 2007). They show a high specificity for transport of myo-inositol and a limited number of epimeric forms (Schneider et al. 2007). *AtINT4* expression localizes to pollen grains and phloem companion cells of source leaves whilst the *AtINT2* promoter drives weak expression in the anther tapetum and leaf vasculature. Uptake studies in yeast suggest that AtINT1 also functions as a myo-inositol transporter, however AtINT1 localizes to the tonoplast and the *AtINT1* promoter drives a low and ubiquitous expression in all tissues and organs, therefore Schneider et al. 2008 postulated that AtINT1 is a vacuolar exporter involved in general metabolism of myoinositol (Fig 12). In addition, only AtINT2 and AtINT4 possess a long extracellular loop between TM 9 and 10 which contains a novel PSI (plexins / semaphoring / integrin) domain. The deletion of the extended loop of AtINT2 did not alter the plasma membrane targeting but increased the Vmax of inositol uptake (Dotzauer et al. 2010). This loop region is suggested to be a target for the binding of Ni^{2+} ions which regulates transport function. Due to the absence of a phenotypic difference between single *atint2.1* or double *atint2.1/atint4.*2 mutants and wild-type plants; the physiological role of AtINT2 and AtINT4 remains to be elucidated (Schneider et al. 2006; Schneider et al. 2007).

3.3.2.7 The ESL subfamily

The ESL gene subfamily (Early-responsive to dehydration Six-Like, initially called clade4 Human GLUT6/8-Like Transporters in regard to their homology with HsGLUT6 and 8; Lalonde et al. 2004) is the largest clade of MSTs for dicotyledonous species with 19 members in *A. thaliana* (Fig 19) and 22 in grape; however, in the monocotyledonous rice species the ESL clade is limited to 6 members. The first identified members (AtERD6) gave its name to the subfamily in relation to the high gene expression observed in response to dehydratation (and also low temperature) condition (Kiyosue et al. 1998). Thereafter, two SFPs (Sugar-porter Family Protein) duplicates were identified; *AtSFP1* and *AtSFP2* are differentially regulated both temporally and spatially (Quirino et al. 2001). It is only recently that the first member of the large ESL clade was characterized by Yamada et al. 2010. ESL1 (ERD six-like 1) is a low affinity glucose transporter (Km=102.2 mM) targeted to the vacuolar membrane via a N-terminal L*XXX*LL motifs conserved amongst most members of the *A. thaliana* ESL clade. In addition, ESL1 was characterized as a monosaccharide facilitator since the addition

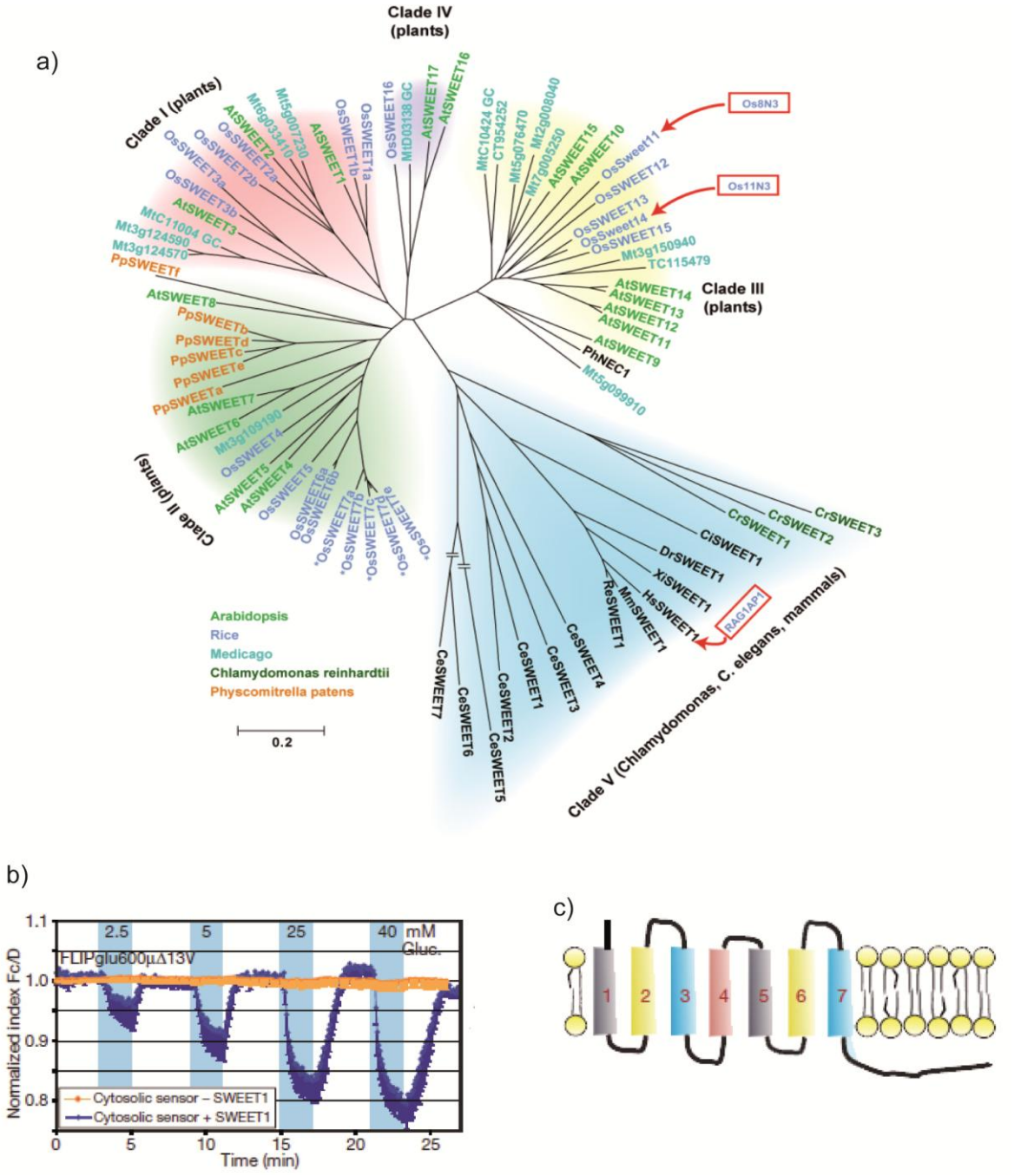


Fig 20. SWEETs, a novel sugar transporter superfamily

a) Phylogenetic tree of the SWEET superfamily of *A. thaliana*, rice, *M. truncatula*, *C. reinhardtii* and *P. patens*.

b) Glucose transport uptake for AtSWEET1 by co-expression with cytosolic FRET glucose nanosensor in HEK293T cells. Orange line indicates cells expressing sensor alone; blue line indicates cells co-expressing sensor and AtSWEET1.

c) Structural model of SWEETs based on hydrophobicity plots. Identical colors were used to highlight intra-molecular sequence conservations between TM 1–3 and 5–7. Adapted from Chen et al., 2010.

of CCCP did not curb the uptake capacity and *Aterdl6* mutant showed elevated vacuolar glucose levels (Yamada et al. 2010; Poschet et al. 2011). Taken together, these results indicate that ESL members mediate an energy independent efflux of glucose for remobilization of vacuolar carbohydrate storage (Fig 12).

Expression profiles of ESL clade members of *A. thaliana* have been identified in response to a number of stresses. Indeed, *AtERD6* expression decreased in response to salinity or treatment with ABA and showed a 3.7 fold change of expression in response to chitin, a plant-defense elicitor (Kiyosue et al. 1998; Libault et al. 2007). In contrast, *ESL1* expression was induced by drought, salinity and ABA treatment (Yamada et al. 2010) and *AtSFP1* expression localized to leaves and increased in parallel with progression of leaf senescence (Quirino et al. 2001). In addition, a tomato homologue of the ESL clade, *LeST3*, was shown to be inducible in tomato leaves during infection with either arbuscular mycorrhizal fungi (see 4.3.3) or the root pathogen *Phytopthora parasitica* (Garcia-Rodriguez et al. 2005). Taken together these data suggest a role for ESL genes in the remobilization of stored sugars from the vacuole in response to environmental and biotic stresses.

3.3.3 **SWEETs, a novel sugar transporter superfamily**

Unlike MSTs and SUTs, SWEETs belong to a distinct superfamily (previously named MtN3/saliva) detached from the MFS superfamily and presenting a different structural model (Fig 20a,c). Indeed, SWEETs are smaller than other sugar carriers (<300AA) since they only possess 7 TMs resulting from an ancient duplication of a 3-transmembrane-helix-domain polypeptide fused via a central TM helix. The N- and C-termini are located in the extracellular and cytosolic side, respectively (Fig 20c). The SWEET superfamily splits into four subclades and comprises 17 members in *A. thaliana* and 21 in rice (Fig 20a; Chen et al. 2010). AtSWEET1 was characterized in yeasts, oocytes as well as in low glucose uptake human cells by the mean of high-sensitivity fluorescence resonance energy transfer (FRET) nanosensors which represent the latest technique to pinpoint intra- and intercellular sugar fluxes (Fig 20b; Takanaga et al. 2008; Chen et al. 2010; Takanaga and Frommer 2010). Thus, AtSWEET1 was characterized as a low affinity uniporter (Km=9 mM), which functions as a facilitator that mediates both influx and efflux of glucose at the plasma membrane (Fig 12). Five additional candidates, AtSWEET4, AtSWEET5, AtSWEET7, AtSWEET8 and AtSWEET13 were also described as functional glucose transporters whereas AtSWEET2 and AtSWEET12 failed to complement the glucose uptake yeast mutant. Interestingly, SWEET homologs are also found in numerous organisms ranging from *Chlamydomonas reinhardtii* to *Caenorhabditis elegans* and even a single copy in the human genome (HsSWEET1).

In addition to hexose transport, AtSWEET10 to 15 and OsSWEET11 and 14 members belonging to the subgroup III (Fig 20a) were shown to transport sucrose by means of FRET nanosensors and oocytes (Chen et al. 2012). This study focused on AtSWEET11 and 12, which present a high expression profile in source leaves of *A. thaliana*, and AtSWEET12 was characterized as a low affinity sucrose uniporter (Fig12; Km for efflux >10 mM). The double *atsweet11;12* KO mutant was affected in respect to photosynthetic capacity, leaf size, reduced root length compared to the wild type and accumulated higher amount of sugars (glucose, sucrose and starch) in rosettes; this mutant is reminiscent of the SUT1 phloem loading mutant phenotype (Fig 9). Indeed, [$^{14}CO_2$]-labeling experiments indicated that under low light conditions, the double mutant only exported half of the fixed ^{14}C relative to wild type controls. In addition AtSWEET11 and AtSWEET12 GFP fusions were tagged at the plasma membrane in *A. thaliana* with localization in cell files along the leaf veins; most likely, these cells correspond to phloem parenchyma. Taken together, Chen et al. 2012 suggested that both SWEETs are involved in a two-step mechanism of SWEET-mediated export from parenchyma cells feeding H^+-SUT1 import into the SE-CC complex (Fig 4.1 and 4.2). The efflux of sucrose to the apoplasm could theoretically occur directly at the site of production in mesophyll cells, from bundle sheath cells, or from phloem parenchyma cells.

Furthermore, it seems that SWEET paralogs play diverse roles in plant. Indeed, the high expression in gametophytes together with the male sterility observed for numerous mutants revealed that AtSWEET1, AtSWEET5, AtSWEET8, OsSWEET11 and the NEC1 homolog from petunia are involved in sugar efflux for pollen nutrition (Ge et al. 2001; Engel et al. 2005; Yang et al. 2006; Guan et al. 2008; Song et al. 2009). In addition, it seems that SWEET play a pivotal role for the competition of sugar fluxes between plant and numerous pathogens, which acquire glucose from their hosts. Indeed, several *AtSWEETs* are induced upon infection by the fungal powdery mildew *Golovinomyces cichoracearum* and the grey mould agent *Botrytis cinerea* (Ferrari et al. 2007; Chen et al. 2010) while OsSWEET11 is involved in bacterial pathogen and its disruption confers resistance to the rice blast agent *Xanthomonas oryzae* (Chu et al. 2006; Yang et al. 2006). The first known plant SWEET (MtN3, nodulin3) was identified in *M. truncatula* from a cDNA library of root nodules and was postulated as a new marker of the symbiotic *Rhizobium*-plant interaction (Gamas et al. 1996). In conclusion, SWEETs represent a novel and widespread type of uniporters, which are able to mediate import and efflux of sugars from cells and are specifically involved in diverting nutritional resources from plant in their interaction with microorganisms.

4 Arbuscular mycorrhiza, an ubiquitous symbiosis

Arbuscular mycorrhizal fungi (AMF) are ubiquitous soil microorganisms living in symbiotic association with plant roots and arbuscular mycorrhiza (AM) is probably the most widespread

Fig 21. Photograph of a *M. truncatula* plant harvested at 4 weeks old.
For culture conditions, see Materials and methods 2.

beneficial interaction between plants and microorganisms (Parniske 2008; Smith and Read 2008). This association between plant and fungi from the phylum Glomeromycota is ancient (Schüßler et al. 2001), dating back to the first appearance of land plants (oldest fossil record around 400 million years ago; Remy et al. 1994). AMF are thus considered as having co-evolved with land plants and to have played a key role as symbiotic component of plant evolution. Today, the AM symbiosis affects the vast majority (80%) of plant species, living in a wide range of terrestrial environments (Wang and Qiu 2006). This mutualistic interaction is based on biotrophic nutrient exchanges between the plant and the fungal partners. Indeed, the fungus supplies the autotrophic host with water and nutrients, mainly phosphate and thereby enhances plant growth. In return, the plant provides sugar photosynthates to the heterotrophic symbiont. The importance of sugar transfer as the sole carbon source for AMF is emphasized by the fact that the fungus is unable to grow and complete its life cycle in absence of the host plant (obligate biotrophy).

AMF also contribute to plant fitness and resistance against diverse biotic (pathogens) and abiotic (water deficiency, polluted soil) stresses. The agronomic and ecological importance of this unique beneficial plant fungal association is largely recognized (Gianinazzi et al. 2010; Fitter et al. 2011).

4.1 The Medicago truncatula – Glomus intraradices symbiosis model

4.1.1 *Medicago truncatula*, the leguminous plant model

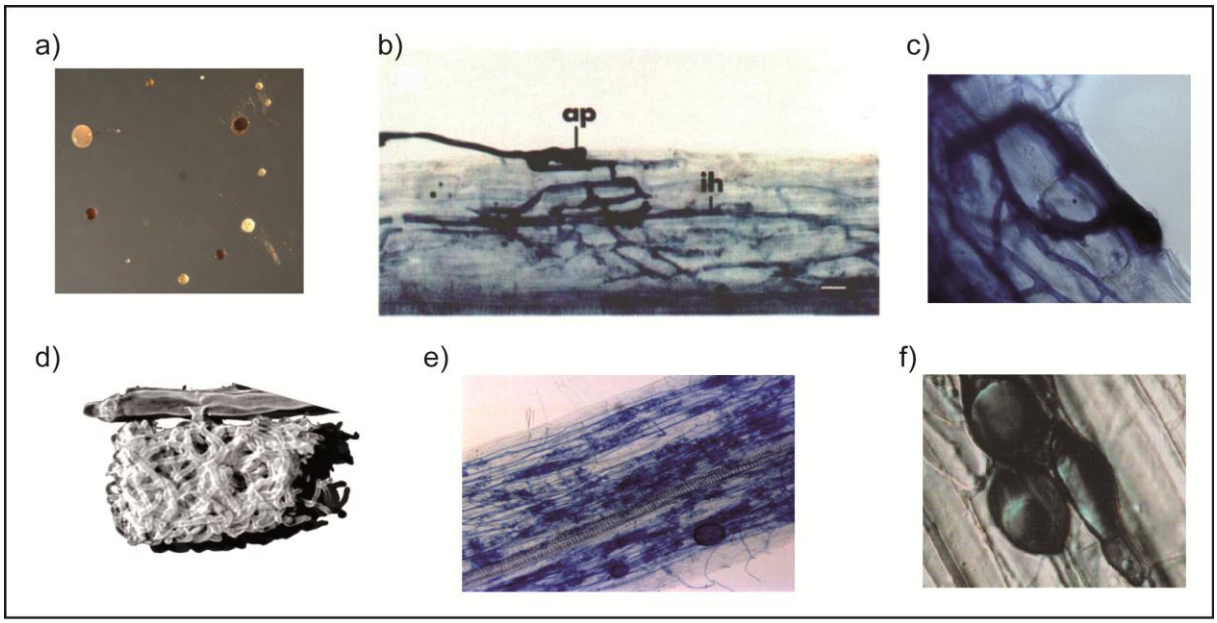
The *Fabaceae* or leguminous family represents the third largest family of flowering plants and the second in importance to humans as a source of food, feed for livestock, and raw materials for industry. Key agronomical and industrial leguminous species include common bean (*Phaseolus vulgaris*), soybean (*Glycine max*), pea (*Pisum sativum*), chickpea (*Cicer arietinum*), broad bean (*Vicia faba*) and important forage species include alfalfa (*Medicago sativa*), clover (*Trifolium* sp.) as well as *Lotus* species. Among the nearly 18000 leguminous species, barrel medic (Fig 21; *M. truncatula*), because of its small size, self-fertility, prolific seed production, rapid generation time and its close relation to the majority of crop and pasture legumes, has been adopted internationally as the primary model and reference legume species (http://www.noble.org/medicago/; Cook 1999). Moreover, *M. truncatula* possesses a small diploid genome and a broad range of available molecular tools. Indeed, the *M. truncatula* genome has a total size of 314 Mb, contain eight chromosomes (2n=16) and 46,016 predicted proteins (Young et al. 2011). The current annotation is the publicly available version 3.5 (Mt3.5; http://medicagohapmap.org/index.php). In addition, a detailed transcriptomic microarray "atlas" of gene expression profiles has been carried out for the

majority of genes covering all plant organs in various conditions (Benedito et al. 2008; http://mtgea.noble.org/v2/).

In contrast with the plant reference species *A. thaliana*, legumes offer a unique model in the area of plant-microbe interactions. Indeed, legumes are unique in their ability to acquire nitrogen thanks to their mutual beneficial association with bacteria mostly from the *Rhizobium* genus. Within root nodules in which these symbiotic bacteria are housed, atmospheric nitrogen is fixed to a free and renewable source of usable nitrogen. Around 40 to 60 million tonnes of nitrogen are fixed annually by legumes, which is equivalent to about 10 billion dollars of chemical fertilizers (Graham and Vance 2003). In addition, legumes also have the capability to establish endomycorrhiza with AMF (Smith and Read 2008). This mutualistic interaction is characterized by biotrophic exchanges between the two partners and is important to plant mineral nutrition. The symbiotic model between *M. truncatula* and the AMF *Glomus intraradices* is widely used in the scientific community and represent a valuable tool to study beneficial plant-fungal interactions.

4.1.2 **Glomus intraradices, the AMF model**

Rhizophagus irregularis, here used under the species name *Glomus intraradices* (see Preamble) is a widespread AMF species present in various ecosystems throughout the world. Indeed, it readily colonizes many plant species as it is known to be efficient in mobilizing, taking up and transferring mineral nutrients toward a wide range of host plants. In addition, *G. intraradices* has been widely used in fundamental and applied research and is a key component of several commercial inocula (*e.g.,* INOCULUM*plus*; http://www.inoculumplus.eu/). Therefore, among the 230 described species in the glomeromycotan phylum, *G. intraradices* was selected as the model species and chosen to be the first AMF to be sequenced by the international *Glomus* Genome Consortium (Martin et al. 2008). However, the *G. intraradices* isolate DAOM197198 which was selected for the genome initiative was shown to fall into a different clade (Stockinger et al. 2009) and to be conspecific with *Glomus irregulare* and was recently reassigned to *Rhizophagus irregularis* (Schüßler and Walker 2010). Besides DAOM197198 isolate, several *G. intraradices* cultures have been used by the scientific community and the *G. intraradices* BEG141 isolate, representing the model of study in our laboratory, is well described in the literature (Rivera-Becerril et al. 2005; Seddas et al. 2009; Tollot et al. 2009; Kuznetsova et al. 2010; Seddas-Dozolme et al. 2010; Hao et al. 2012). Despite a first estimated genome size of 14-16.5 Mb (Hijri and Sanders 2004), a 4 time coverage (52.5 Mb) obtained mainly by whole-shotgun sequencing was not sufficient to largely assemble the genome probably due to the occurrence of multiple polymorphic nuclei. Hower, in addition the genome size was recently reevaluated to 154.8 Mb by the mean of flow cytometry



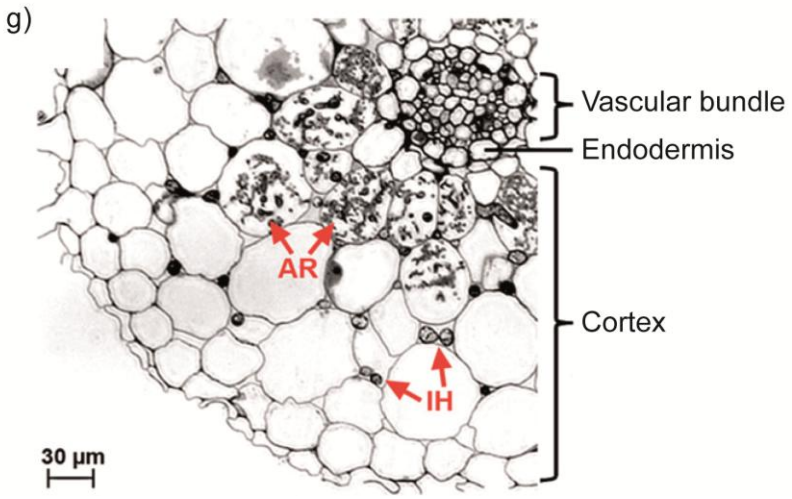


Fig 22. Structural features of arbuscular mycorrhizal fungi

a) Spores of glomeromycotan species including *G. intraradices, G. mosseae, G. caledonium, Gigaspora candida, G. margarita, Acaulospora laevis, Scutellospora calospora, S. pellucida, S. heterogama* (photo by L. Mercy, IPM, Dijon). b) Apressorium (ap) and intercellular hyphae (ih) of *G. mosseae* (Gianinazzi-Pearson 1996). c) Close up of an apressorium in *M. truncatula* roots colonized by *G. intraradices* BEG141. d) Arbuscule (photo by A. Schüßler, LMU, Munich). e) *M. truncatula* root fragment colonized by *G. intraradices* BEG141. f) Vesicles (Peterson et al. 2004). g) Cross-section of a mycorrhizal *M. truncatula* root; arbuscules (AR), intraradical hyphae (IH; Helber et al. 2011).

(Sędzielewska et al. 2011) and exhaustive transcriptomics data have just been released (Tisserant et al. 2011). Sequencing of *G. intraradices* will have a tremendous impact on the scientific community as it will give first access to so far intractable information about processes driving the biology and life cycle of AM fungal symbionts; however, it is still a long hard road to complete the full assembly of the first glomeromycotan genome (Martin et al. 2008; Fitter 2010).

4.2 Structural features of the AM and life cycle of AMF

Spores represent important propagules of AMF, their size ranges from 22 to 1050 µm and they contain abundant storage lipids and carbohydrates (Fig 22a). The root colonization process starts from mutual signal exchanges between host and symbiont. Indeed, root exudates excreted from the plant host act as an initial factor of spore germination; this exudation induces physiological activity stimulating spore germination, hyphal growth and branching (Gomez-Roldan et al. 2008). In response, AMF produce mycorrhizal signals (called "myc factor") that induce calcium oscillations in root epidermal cells, thus activating plant symbiosis-related genes (Fig 23; Maillet et al. 2011). It is important to note that mycorrhizal and rhizobial symbioses use conserved components through a common signaling pathway (Gianinazzi-Pearson 1996; Oldroyd and Downie 2006).

In the second step of the interaction, the developing hyphae enter into physical contact with the root epidermis. With this contact, the fungal hyphae differentiate a special type of apressoria (called hyphopodia) to enter the epidermis (Fig 22b-c and 23). Thereafter, the fungal hyphae penetrate the epidermal cell; the contents of the plant cell undergo important reorganization and form a specialized "transcellular channel": the prepenetration apparatus (PPA; Genre et al. 2005). Once the fungus has entered and fully traversed the epidermal cell, a second PPA forms across the adjacent outer cortical cell in advance of the progressing hyphal tip.

The subsequent colonization of the inner cortical tissues by AMF is dependent on the identity of the host plant. In the case of the *M. truncatula* - *G. intraradices* association (*Arum* AM type), the intra-radicular mycelium (IRM) quickly colonizes the intercellular apoplastic space of the cortical parenchyma and continues their growth until they differentiate into specialized intracellular structures named arbuscules (Fig 22d-e and 23). The term AM comes from the name of these tree-shaped structures. Arbuscules are formed by branching hyphae, which invaginate during penetration of the cortical cell. They are completely surrounded by a periarbuscular membrane (PAM) which is formed *de novo* from the plant plasma membrane (Gianinazzi-Pearson 1996). Arbuscules are key structures of the symbiosis as they are the site of reciprocal nutrient exchanges between the two partners (see 4.3; Fig 24, 25a and 26d) and represent the main symbiotic interface. In addition, vesicles represent a further characteristic

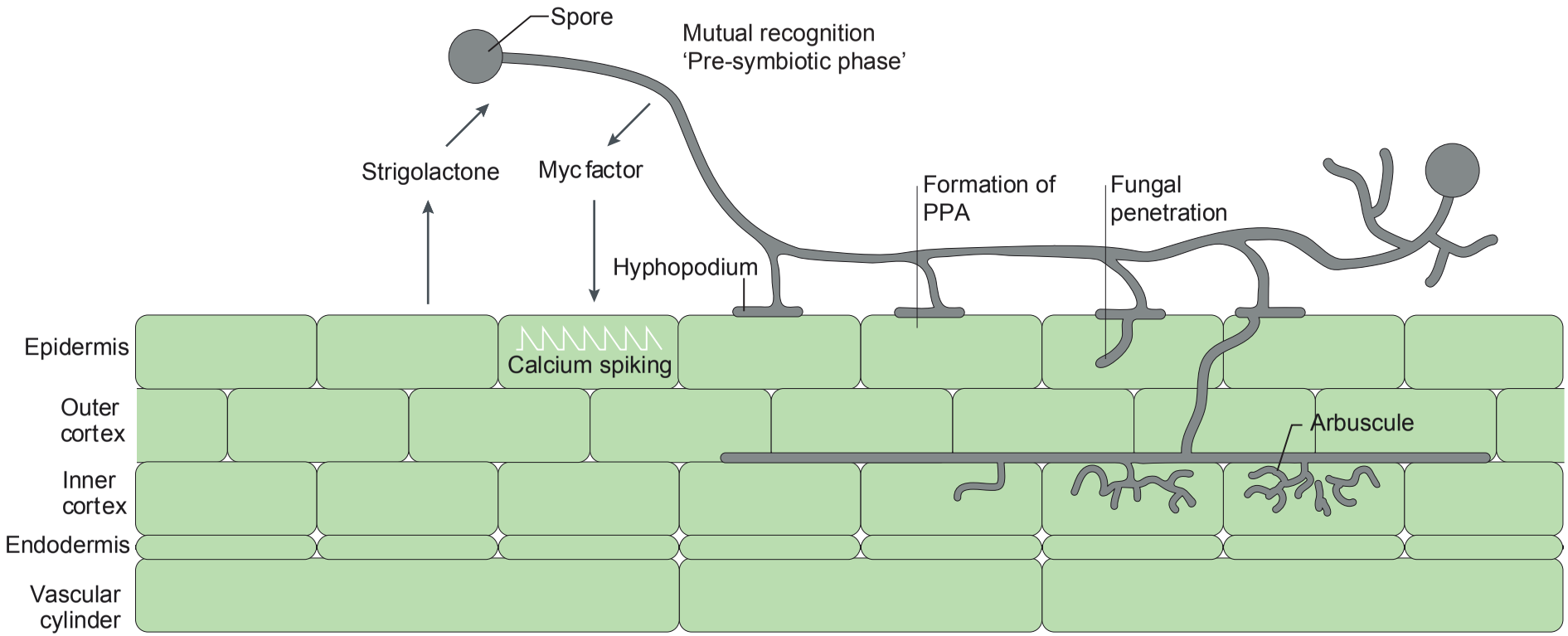


Fig 23. Steps in arbuscular mycorrhiza development
Colonization process is depicted from left to right. Plant and fungal stucture are coloured in green and grey respectively. Adapted from Parniske, 2008.

structure of AMF (Fig 22f). When the plant is well colonized, vesicles are formed inside or between the cortical cells; they are mainly filled with lipids and represent major storage organs of AMF.

AMF grow within plant root tissues and simultaneously develop an extra-radicular mycelium (ERM) in the soil. The ERM form a hyphal network that considerably increases the exploration zone of the soil by plant roots in search of supplemental nutrients (Smith and Read 2008). In addition, the ERM can also serve to the invasion of other roots via the formation of apressoria. Finally, the life cycle of AMF is completed by the formation of new spores by the ERM.

4.3 Biotrophic nutrient exchanges in AM symbiosis

The extra- and intra-radical mycelia of the fungus form a continuum between soil components and root cells (Fig 23 and 24). The ERM recruits mineral nutrients (mainly phosphate and nitrogen) for the plant, greatly improving plant nutrition. In return, the plant supplies AMF with sugar photosynthates. These reciprocal and beneficial exchanges are the basis of the AM symbiosis.

4.3.1 **Phosphate**

In many ecosystems, phosphate represents a limiting factor and as the plant root system takes up and uses available nutrients, depletion zones of phosphate appear around developing roots. In AMF colonized plants, the ERM network increases the potential exploration surface and the small diameter of fungal mycelia can reach soil pores that are inaccessible to roots. Therefore, the most relevant contribution of AMF to plant growth and nutrition is probably the mobilization of phosphate. ERM are able to take up inorganic phosphate (Pi) from the soil; this prerequisites the presence of fungal phosphate transporters at the membrane of ERM. Indeed, the first glomeromycotan transporter characterized in detail encoded a Pi transporter from *G. versiforme* (*GvPT*,; Harrison and Buuren 1995) followed by homologous genes from *G. intraradices* (*GiPT*; Maldonado-Mendoza et al. 2001) and *G. mosseae* (*GmosPT*; Benedetto et al. 2005). These transporters were shown to be expressed in the ERM suggesting a role in initial uptake of Pi from the soil (Fig 24). Thereafter, Pi accumulates in the form of vacuolar polyphosphate and is transferred along hyphae by means of a motile tubular vacuolar network to the IRM (Rasmussen et al. 2000; Perotto et al. 2007). At the symbiotic interface, polyphosphate is hydrolyzed to release Pi ions and subsequent uptake of Pi released at the periarbuscular space is mediated by two types of plant transporters at the PAM (Fig 24). The first type include Pi transporters from potato (*StPT3*; Rausch et al. 2001), maize (*ZEAma*; Nagy et al. 2006) and *L. japonicus* (*LjPT3*; Maeda et al. 2006) which show

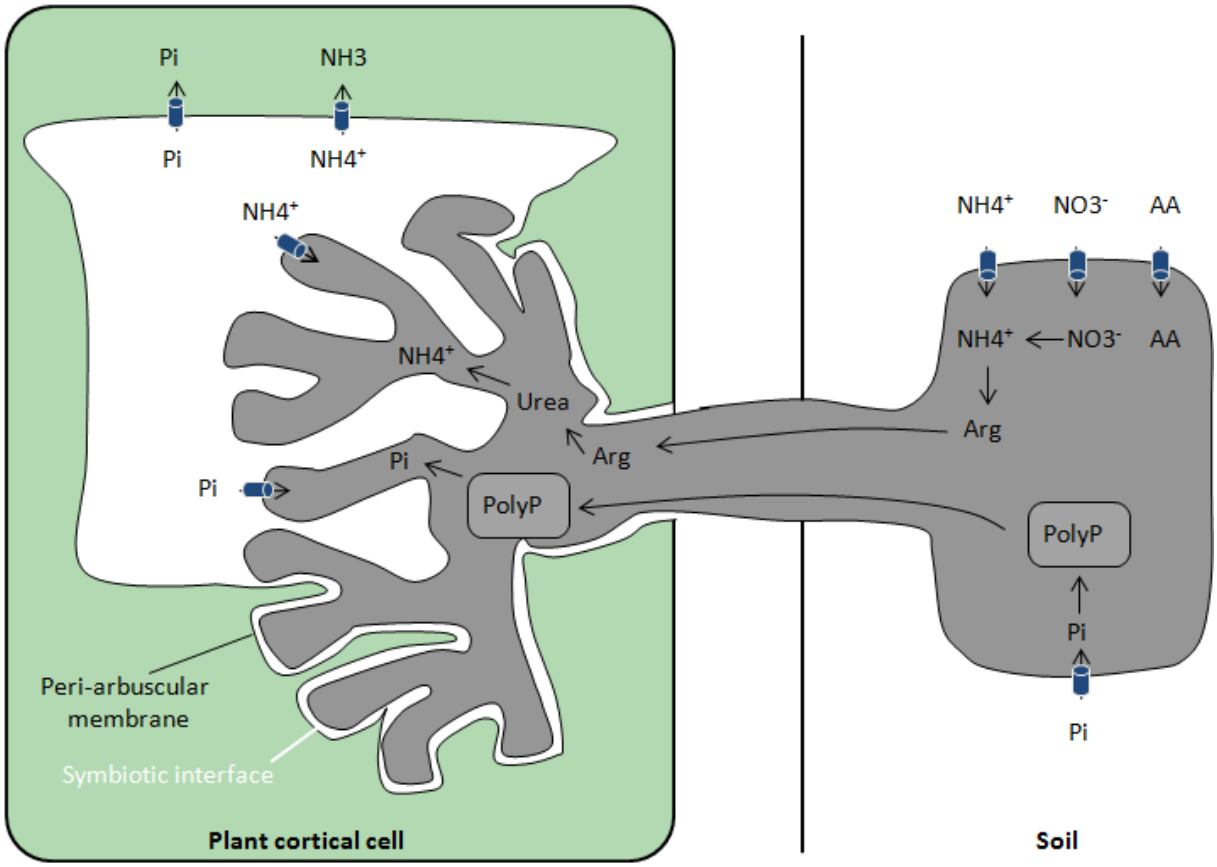


Fig 24. Nutrient transfer of phosphate and nitrogen towards the host plant cell.
The AMF is represented in grey, and the arbusculated cortical plant cell is represented in green. The symbiotic interface (in white) was deliberately enlarged on the upper part of the figure.

increased expression in AM condition, while the second type of transporters are exclusively expressed in response to AM. Therefore, the latter type is AM specific and include Pi transporters from *M. truncatula* (*MtPT4*; Harrison et al. 2002), rice (*OsPT11*; Paszkowski et al. 2002) and tomato (*LePT4*; Nagy et al. 2005). AM specific Pi transporters are thought to be indispensable for the establishment of AM symbiosis as the loss of *MtPT4* resulted in premature death of the arbuscules and arrest of the symbiosis (Javot et al. 2007). In addition to transcripts of plant Pi transporters, Balestrini et al. 2007 also detected the fungal transporter *GmosPT* exclusively in arbusculated cells; these findings suggest that the fungus may regulate nutrient exchange at the symbiotic interface through a phosphate retrieval mechanism (Fig 24).

4.3.2 **Nitrogen**

In addition to Pi, nitrogen is another nutrient commonly supplied through chemical fertilizers and whose availability most commonly limits plant growth. AMF increase access to nitrogen sources from the soil by the transfer of significant amounts to the host plants. Amino acid transporter from *G. mosseae* (*GmosAAP1*, Cappellazzo et al. 2008), as well as ammonium (NH_4^+) transporters (*GintAMT1*, López-Pedrosa et al. 2006; *GintAMT2*,Pérez-Tienda et al. 2011) and a nitrate transporter (*GiNT*, Tian et al. 2010) from *G. intraradices* have been identified in the ERM. Therefore, it seems that AMF are able to take up various sources of nitrogen from the soil (Fig 24). In the ERM, nitrate is reduced by nitrate reductase to ammonium and finally converted into the amino acid arginine, which represents the transport form of nitrogen from the ERM to the IRM (Fig 24). Then, arginine is broken down releasing urea and further catalyzed by urease into NH_4^+ and seems to be the preferential transferred nitrogen source assimilated by the host. However fungal transporters involved in the release of NH_4^+ at the periarbuscular space have not yet been identified. Indeed, *GintAMT1* and *GintAMT2* were detected in arbuscules but they rather seem to be involved in the retrieval of NH_4^+. On the plant side, a single high affinity AMT from *L. japonicus* (LjAMT2;2) is preferentially expressed in arbusculated cells where it is postulated to bind charged NH_4^+ in the periarbuscular space and release uncharged NH_3 into the plant cytoplasm (Fig 24; Guether et al. 2009).

4.3.3 **Sugar transport in AM symbiosis**

In return to this nutrient flow, the plant provides sugar photosynthates to the fungal symbiont. Since AMF are heterotrophic obligate symbionts, they must receive fixed carbon from the autotrophic plant. Therefore, the transfer of carbohydrates is thought to be the main benefit for the fungal partner, as such the root colonization by AMF increase the global sugar sink demand at the whole plant level. Indeed, fungal growth, reproduction and respiration as well

as increased metabolism in several plant tissues (*e.g.*, arbusculated cortical cells) augment the carbon demand in colonized plant roots (Fig 4; Douds et al. 2000). This increased sink strength is balanced by greater CO_2 assimilation and higher photosynthesis rate in source leaves (Wright et al. 1998a; Wright et al. 1998b). However it is still not clear whether this increased photosynthesis is related to the phosphate supply or directly by the increased sink strength caused by AMF colonization (Johnson 1984; Nemec and Vu 1990). Recently, a comprehensive study demonstrated that AMF colonized tomato plants showed increased opening of stomata, assimilated significantly more CO_2 and increased efficiency and yield of the photosystem II as well as higher photochemistry electron transport rate (Boldt et al. 2011). This led to higher sugar production entirely directed toward sink roots independent of the phosphate supply (Boldt et al. 2011). It has been estimated that between 4% to 20% of total photoassimilates are redirected towards AMF colonized roots (Douds et al. 2000; Graham 2000) and transported to the fungus. This represents worldwide, about 5 billion tonnes of transferred carbon from plants to AMF each year (Bago et al. 2000). This high carbon cost can sometimes result in negative effects and may cause plant growth depletion especially in early stages of symbiosis or under high Pi supply (Peng et al. 1993; Dickson et al. 1999; Douds et al. 2000). Even under such conditions, one must not discount the other beneficial effects AMF may have upon the health of their host and upon the environment.

4.3.3.1 Source of transferred sugars

At the cellular level, NMR spectrometry experiments revealed that the IRM can take up hexoses mainly glucose and to a smaller extent fructose, but not sucrose (Shachar-Hill et al. 1995; Solaiman and Saito 1997; Pfeffer et al. 1999). Therefore, before its apoplastic transfer as hexose at the exchange site, sucrose is either cleaved by sucrose invertase or sucrose synthase (see 1.1.2 and Fig 3) then, glucose is mainly transferred to the AMF. Consistent with this, tomato roots colonized by *G. mosseae* showed a higher accumulation of sucrose and fructose (Boldt et al. 2011). Initial experiments showed that ERM was unable to perform this glucose uptake (Pfeffer et al. 1999) which is coherent with the obligate biotrophy and to an exchange site of sugars located in the IRM. Indeed, the scientific community suggests that as for phosphate transport, the exchange site is located at the arbuscular interface (Pumplin and Harrison 2009) and other authors also have pointed intercellular hyphae as the carbon acquisition site (Smith and Smith 1990; Smith et al. 2001). In addition, Helber et al. 2011 recently demonstrated that the ERM was also able to actively take up both glucose and xylose. These recent findings suggest that cell wall monosaccharides may also be a carbon source for AMF; this may give new insights to the axenic culture of the fungus (without the host plant).

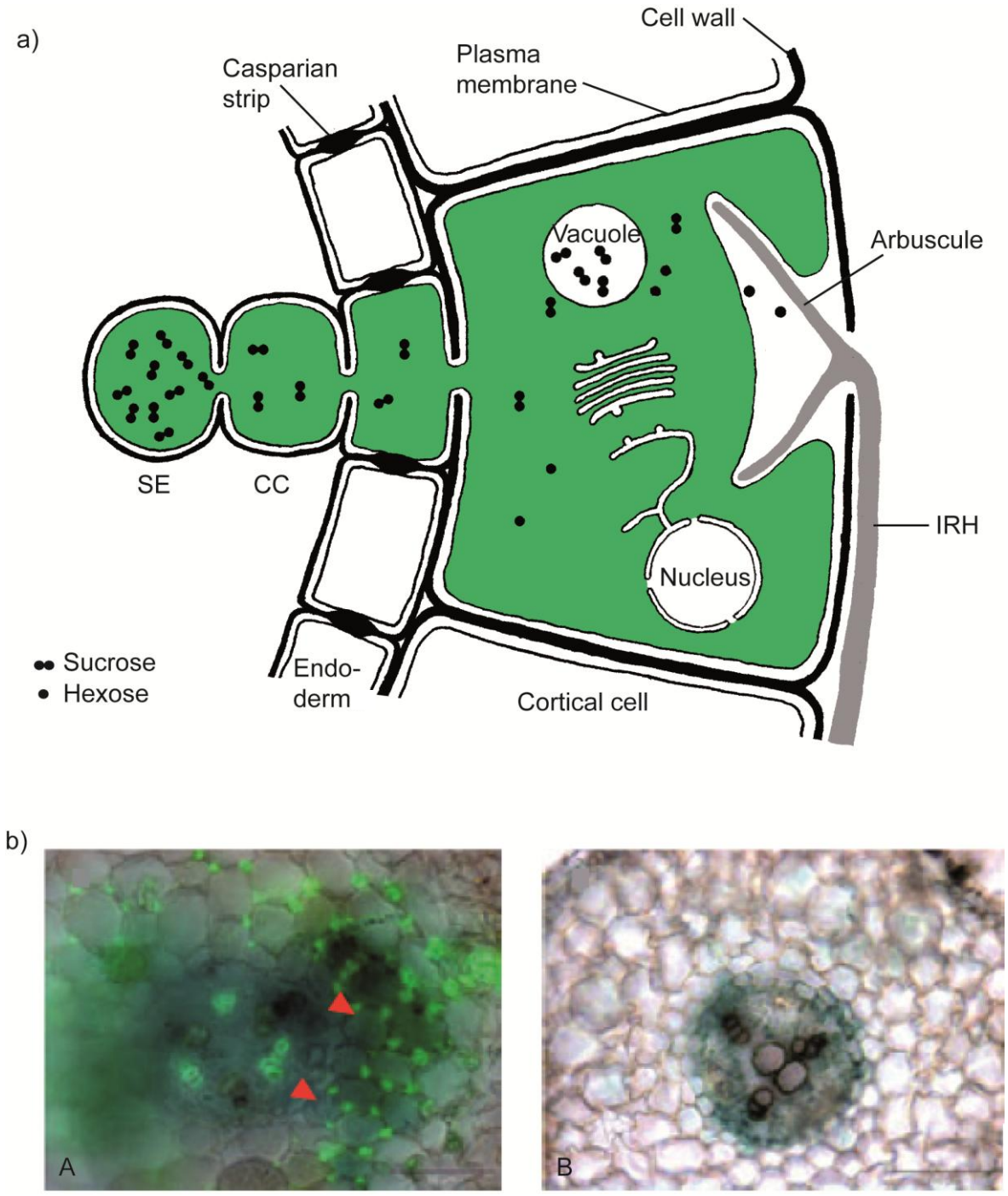


Fig 25. Sucrose partitioning towards AMF

a) Scheme representing a possible direct symplasmic unloading of sugars towards AMF. Modified from Blee and Anderson 1998.

b) The promotor of *MtSut1* was fused to the GUS coding sequence and promotor activity is visualized in blue. Fungal structures are visualized in green by staining with WGA Alexa Fluor 488. The expression of *MtSut1* is induced and located in cortical cells adjacent to fungal structure in mycorrhizal roots (A) whereas the expression is restricted to the central cylinder and pericycle in non-mycorrhizal roots (B). Gaude et al. 2011

On the fungal side, once hexoses are transferred to the IRM, two routes exist for carbon transport to the ERM (Bago et al. 2003). Hexoses are either converted to glycogen (via gluconeogenesis) or to tri-acylglycerol (via lipogenesis); both carbohydrates and lipids are the major transport forms of carbon (Bago et al. 2002a; Bago et al. 2003). Then in the ERM, glycogen and trehalose are the main carbon storage forms whereas chitin is the structural carbohydrate of the fungal cell wall (Fig 60).

4.3.3.2 Role of sucrose cleaving enzymes in sugar transfer

Initial works suggested that both vacuolar invertase and sucrose synthase of cortical cells containing arbuscules maintain a gradient for symplasmic diffusion of sucrose through plasmodesmata and consequently showed an increased accumulation of transcripts in cell containing arbuscules by mRNA hybridization (Blee and Anderson 1998; Blee and Anderson 2002). Consistent with this, Hohnjec et al. 2003 detected the promoter activity of the *M. truncatula* sucrose synthase gene (*MtSucS1*) in root colonized segments especially around arbuscules and internal hyphae by GUS staining. However, recent works also focused on cell wall bound invertase; LIN6, a tomato excreted invertase, showed specific increased expression in colonized plants with a promoter activity specifically localized in colonized cells and to the central cylinder (Schaarschmidt et al. 2006). Furthermore, expression and activity of all types of sucrose cleaving enzymes appeared to be up-regulated in presence of AMF (Wright et al. 1998a; Ravnskov et al. 2003; Garcia-Rodriguez et al. 2007; Tejeda-Sartorius et al. 2008). Taken together, these results suggest that sugars can be loaded to arbusculated cells both symplastically and through intercellular pathway via transporters. This may depend on the position of the colonized cortical cells (Fig 25; Blee and Anderson 1998). Indeed, cortical cells near the endodermis surrounded by the Casparian strip would provide the most direct symplastic access from the phloem while the following cortical layers may be connected apoplastically.

The role of sugar cleaving enzymes was then studied using numerous mutant lines upon AM inoculation. In *M. truncatula*, *MtSucS1* antisense lines showed an overall downregulation of several carbon related genes and were affected during AM colonization, more particularly in the establishment and maintenance of arbuscules (Baier et al. 2010). In parallel, studies using mycorrhized plants transformed to overexpress apoplastic, cytosolic, and vacuolar located yeast invertases did not show an increase in fungal growth, colonization rate nor fungal sugar content, whereas plant with decreased acid invertase activity showed diminished mycorrhization (Schaarschmidt et al. 2007). So far, no sucrose cleaving enzymes have been identified in glomeromycotan genomes and it seems that respective plant invertases and sucrose synthases show a fine modulation upon mychorrhizal conditions.

4.3.3.3 Role of transporters in sugar transfer

As previously stated, the root colonization by AMF greatly improves the sink strength and sugar demand; consequently a greater amount of sucrose is exported from source organs (Wright et al. 1998a; Boldt et al. 2011). In apoplastic loading species, a single protein member of the SUT1 subfamily is principally responsible for the sucrose loading in the phloem (see 3.2.2.1.1; Zhang and Turgeon 2009) and the phloem loading *SlSUT1* gene together with *SlSUT4* showed higher transcript accumulation in leaves of AMF colonized tomato by *G. mosseae* (Boldt et al. 2011). The upregulation of these candidates was also observed in AM sink roots suggesting a role of SUT1 and SUT4 members in sugar allocation towards colonized parts (Boldt et al. 2011). However, studying the same genes by semi-quantitative PCR, Ge et al. 2008 obtained contradictive results showing that only *SlSUT1* was downregulated in roots and leaves of plants mycorrhized by *G. intraradices*, *SlSUT2* and *SlSUT4* expression being stable. Moreover a differential expression pattern was also observed for *SlSUT1* when using *G. caledonium*, suggesting that SUT expression is regulated differently in response to AMF species (Ge et al. 2008). In addition, the antisense inhibition of mRNA coding for the phloem loading protein StSUT1 in potato resulted in sugar accumulation in source leaves and reduced growth of sink organs (Fig 9a-c; Riesmeier et al. 1994) but no differences in AMF colonization were observed for this mutant at low or high phosphate concentration (Gabriel-Neumann et al. 2011). However, potato lines overexpressing *SoSUT1* had a two times higher colonization rate but only in high phosphate conditions (Gabriel-Neumann et al. 2011). Very recently, using laser capture microdissection combined with microarray hybridization, *MtSut1* a *M. truncatula* SUT was shown to be induced in non arbusculated cortical cells of mycorrhized roots (Gaude et al. 2011). In addition, a strong promoter activity was confirmed in cortex cells adjacent to extracellular fungal hyphae (Fig 25b; Gaude et al. 2011). This candidate belong to the SUT4 clade members located at the tonoplast; a role for MtSut1 in the export of sucrose from the vacuole and thus the mobilization of carbohydrate resources in cells adjacent to fungal structures in mycorrhizal roots has been postulated. In addition to *MtSut1*, Gaude et al. 2011 also detected a second gene induced in arbusculated neighboring cells as a SUT; however, this latter candidate (mtr.28814.1.s1) seems to correspond to mtr.28814.1.s1_at which is rather annotated as a nucleobase ascorbate transporter (http://mtgea.noble.org/v2/).

As transferred carbon seems to be mainly taken up by the fungus under the form of glucose, most studies focused on the expression of plant MSTs to pinpoint specifically upregulated glucose transporter candidates involved in this exchange. Although LeST3 (ESL subfamily) was not able to function in the yeast deficient strain, this gene shows a similar upregulation in

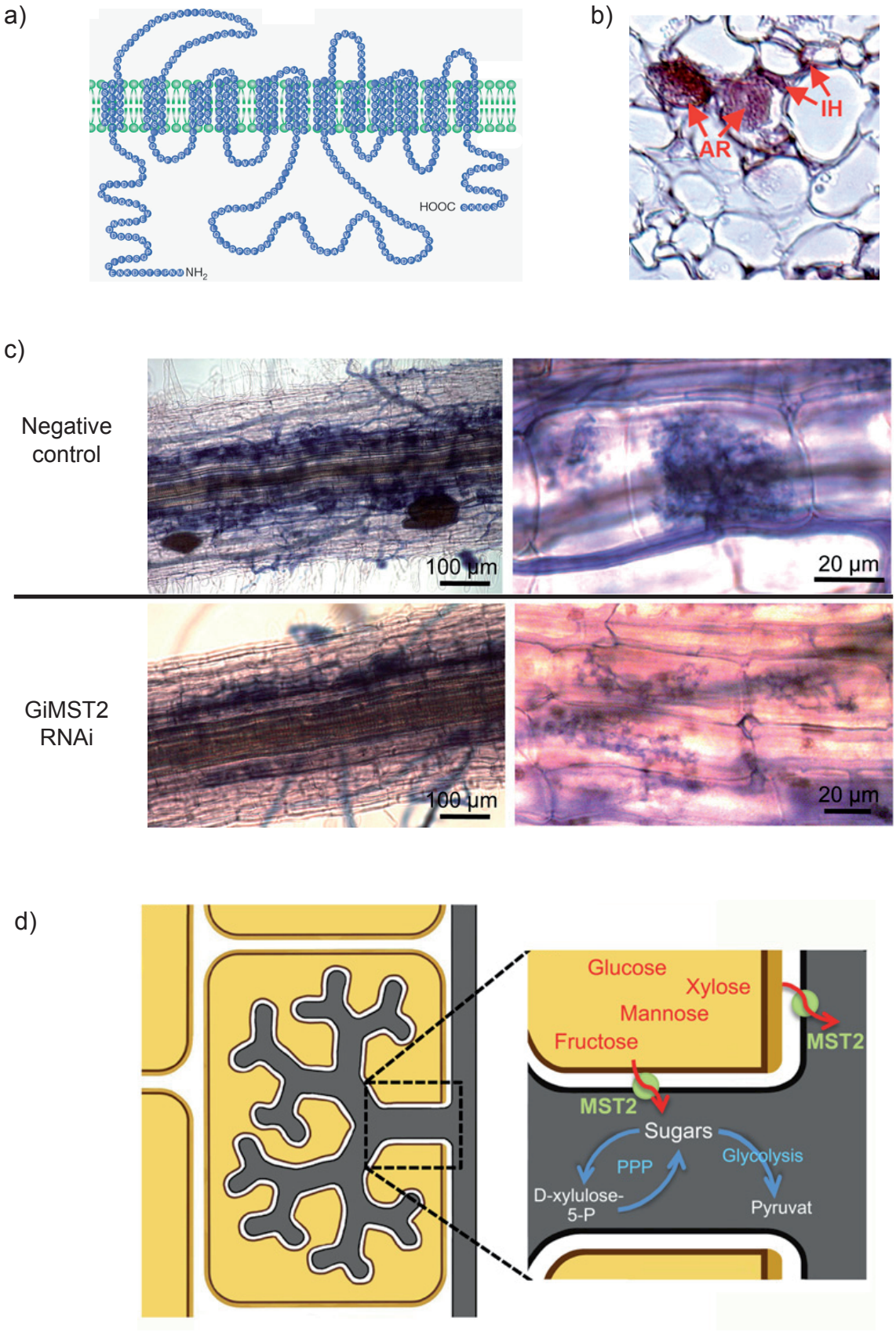


Fig 26. Glomeromycotan sugar transporters (Schüßler et al. 2006; Helber et al. 2011)
a) GpMST1 shows a charcteristic putative topology of MSTs with 12 TMs.
b) Localization of *GiMST2* transcripts in arbucules (AR) and intercellular hyphae (IH) in *M. truncatula* root segments.
c) Reduced expression of *GiMST2* by HIGS results in impaired arbuscules and symbiosis compared with roots transformed with an empty vector control.
d) Model of the sugar transfer for *G. intraradices* at the plant-fungal symbiotic interface.

leaves but not in roots of tomato plants colonized either by *G. intraradices* (BEG121) or *G. mosseae* (Garcia-Rodriguez et al. 2005). In another study, Ge et al. 2008 showed that this gene was differentially regulated (positively or negatively) depending on the AMF species. Indeed, *LeST3* was overexpressed in leaves and roots of tomato plants colonized by *G. intraradices* (unknown isolate) but was downregulated in roots when using *G. caledonium* as inoculum (Ge et al. 2008). In addition, a maize candidate (*ZmMST1*) was shown to be upregulated at submicromolar P concentrations in an African low nutrient adapted cultivar but not in the European cultivar (Wright et al. 2005). Surprisingly, the most promising candidate gene for hexose transfer towards colonized cells is also the first studied, *Mtst1* transcript level increased in AM colonized roots but not in the *myc- M. truncatula* mutant; this gene also showed the highest downregulation over all candidates in AM impaired *MtSucS1* antisense lines (Harrison 1996; Baier et al. 2010). In addition, mRNA hybridization of Mtst1 showed a specific localization to the cortical arbuscule containing cells and in adjacent cells which are frequently in contact with IRM (Harrison 1996).

On the fungal side, the first glomeromycotan glucose transporter was identified in *Geosiphon pyriformis* which forms a symbiosis with *Nostoc punctiforme* and belongs to an ancestral branch within the Glomeromycota (Kluge et al. 1991; Schüßler et al. 2001; Schüßler and Wolf 2005; Schüßler et al. 2006). This unique symbiotic model allowed the isolation of pure fungal mRNA from symbiotic stages to establish a cDNA library (see Chapter IV), which then served to isolate *GpMST1* (Fig 26a; Schüßler et al. 2006; Schüßler et al. 2007). GpMST1 was characterized as a H^+/glucose transporter with a Km of 1.2 mM and highest affinities for glucose and mannose, followed by galactose and fructose; a role for GpMST1 at the *Geosiphon - Nostoc* symbiotic interface was postulated (Schüßler et al. 2006). The information obtained from this unique model together with the available glomeromycotan genomic data recently led to the isolation of three MSTs (MST2, MST3 and MST4) and a putative SUT (SUC1) from *G. intraradices* (Eckardt 2011; Helber et al. 2011). These candidates will be further referred as GiMST2, GiMST3 GiMST4 and GiSUC1 to keep constancy in gene denomination in the manuscript. GiMST2 and GiMST3 fall in a different clade closely related to fungal xylose transporters whereas GiMST4 is the closest homologs of GpMST1. As *GiMST2* is almost only expressed in symbiotic intraradical structures (Fig 26b), Helber et al. 2011 focused on this candidate which was characterized as a high affinity functional H^+/glucose transporter. The active transport system is consistent with H^+ gradient generated by plasma membrane-located H^+-ATPases, which were found to be induced upon mycorrhization (Gianinazzi-Pearson et al. 1991; Ferrol et al. 2000; Krajinski et al. 2002) and the high affinity of GiMST2 favorably position the fungus to efficiently compete with plant MSTs at the plant-fungal interface. Interestingly, glucose uptake of GiMST2 was shown to be outcompeted by xylose, mannose and galactose suggesting that AMF also uses plant cell wall

monosaccharides present at the symbiotic interface (Gianinazzi-Pearson 1996) and the addition of xylose was able to specifically induce the expression of GiMST2 in the ERM (Helber et al. 2011). In addition, mRNA hybridization localized the transcripts of *GiMST2* in arbuscules as well as in intercellular hyphae (Fig 26b) suggesting that sugar transfer from plant to AMF occurs in both structures (Helber et al. 2011). The RNAi silencing of *GiMST2* by Host-Induced Gene Silencing (HIGS) resulted in impaired mycorrhizal formation, malformed arbuscules, and reduced *MtPT4* expression (Fig 26c; Helber et al. 2011). Therefore, it seems that GiMST2 is the major component for sugar uptake by *G. intraradices* and is indispensable for a functional AM symbiosis (Fig 26d).

To sum up, nutrient transport and partitioning between plant hosts and their AM fungal partners is a key question for scientific, economical and environmental matters as this reciprocal transfer is the mainstay of what is arguably the world's most important symbiosis. Regarding sugar fluxes, the form of transferred sugar (glucose) and the sole source (sucrose) are well characterized, but different mechanisms, enzymes and sugar transport proteins involved at both the whole plant level and at the symbiotic interface are still a matter of debate.

5 Research postulate

At the start of my project (November 2008), only a single monosaccharide transporter of *M. truncatula* (MtMST) was published (Harrison 1996), when AtMSTs and OsMSTs comprise 53 and 65 members respectively (Johnson et al. 2006; Buttner 2007) and no genes coding for SUT proteins in *M. truncatula* (MtSUTs) were identified. On the fungal side, the first fungal MST belonging to a species from the Glomeromycota had just been characterized in *G. pyriformis* (GpMST1; Schüßler et al. 2006). However, sucrose cleaving enzyme genes of *M. truncatula* were being studied and their role in AM symbiosis was proposed (Hohnjec et al. 2003; Schaarschmidt et al. 2007; Baier et al. 2010). Sugar transport proteins in *M. truncatula* as well as mechanisms controlling nutrient exchanges in AM symbiosis were not deciphered; this constituted the initial statement of my research postulate. The identification and functional analysis of such transporters would provide key information on mechanisms that underlie nutrient partitioning at both plant and fungal levels for a better comprehension of the cellular and molecular mechanisms in plant-microorganism interactions.

As a consequence, in the first chapter of my PhD, I performed an *in silico* search of *M. truncatula* and *G. intraradices* genomic and expression databases to identify putative SUTs and MSTs from this widely used symbiotic association, the primary model of study of the

host laboratory in Dijon. Detailed phylogenetic analyses of the identified transporters were performed to place candidates in the current classification in order to assign a putative role in regard to previously characterized homologs. In addition, a similar database mining of available transcriptomics tools was carried out to screen candidates that are regulated differentially in response to AM inoculation.

In the second and third chapters, I focused on the study of the newly identified sucrose transporter family from *M. truncatula* (MtSUT). I performed their biochemical characterization in the yeast heterologous system and analyzed their expression profiles in plants cultivated under different phosphorus conditions and in response to arbuscular mycorrhization.

Finally, the fourth chapter consists in a non-targeted approach in regard to the strategy jointly developed by D. Wipf and A. Schüßler that led to the identification of GpMST1 (Schüßler et al. 2006). In that way, a PhD cotutelle has been signed between the Université de Bourgogne and the Ludwig Maximilians Universität of Munich where I screened the *G. pyriformis* expression library for a putative glomeromycotan SUT by functional complementation of deficient yeast strains. In addition, a similar cDNA library was constructed from *M. truncatula* roots mycorrhized with *G. intraradices* for a further screen of plant and fungal genes expressed at symbiotic stages.

The aim of my PhD thesis was to study biotrophic nutritional exchanges and more specifically the transfer of sugar from plant source leaves towards sink roots colonized by the heterotrophic fungal symbiont. In that way, my project focuses on a comprehensive study of the MtSUT family, their identification, characterization and expression of key members from respective clade in response to AMF inoculation to better understand carbon partitioning within and between organisms.

Materials and methods

Bacterial strain	Genotype
One shot® Top10	F⁻ *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) φ80*lac*ZΔM15 Δ*lac*X74 *rec*A1 *ara*D139 Δ(*araleu*)7697 *gal*U *gal*K *rps*L (StrR) *end*A1 *nup*G
OmniMAX™ 2-T1R	F′ [*pro*AB+ *lac*Iq *lac*ZΔM15 *Tn*10(TetR) Δ(*ccd*AB)] *mcr*A Δ(*mrr-hsd*RMS⁻ *mcr*BC) φ80(*lac*Z)ΔM15 Δ(*lac*ZYA-*arg*F) U169 *end*A1 *rec*A1 *sup*E44 *thi*⁻ 1 *gyr*A96 *rel*A1 *ton*A *pan*D
XL10-Gold®	Tet^r D(*mcrA*)*183* D(*mcrCB-hsdSMR-mrr*)*173 endA1 supE44 thi-1 recA1 gyrA96 relA1 lac* Hte [F¢ *proAB lacI* ^{q}*ZDM15* Tn*10* (Tet^r) Amy Cam^r]

Table 2. Genotype of bacterial strains mentioned in this study

Yeast strain	Genotype
EBY.VW4000	Matα *leu2*-3 112 *ura3*-52 *his3*-Δ1 *trp1*-289 *MAL2*-8c *SUC2 Δhxt1-17 gal2*Δ *stl1*Δ::loxP *agt1*Δ::loxP ∆*mph2* ∆*mph*
SUSY7/*ura3*⁻	Matα *leu2*-3 112 *ura3*-52 *trp1 mal0 suc2*::*URA3 ura3 LEU2*::P*ADH1-StSUSY1*
YSL2-1	Matα *ura3*-52 *leu2*-3 112 *his3*- Δ1 *trp1*-289 *mal2*-8c *hxt1-18*::loxP *Gal2*::loxP *Agt1* m2-3::loxP P*suc2*::P*HXT7*

Table 3. Genotype of yeast strains mentioned in this study

Materials and methods

1 Biological materials

1.1 Plant material

The leguminous model plant for studying plant-microbe interactions *Medicago truncatula* (Barrel Medic) belongs to the *Fabaceae* family, the *Faboideae* subfamily and the *Trifolieae* tribe. The *M. truncatula* cultivar Jemalong 5 (J5) was used in this study and seeds were provided by A. Colombet (UMR Agroécologie, Dijon) and came from the 2005 harvest made at the Epoisses INRA domaine.

1.2 Fungal material

The arbuscular mycorrhizal fungus *Glomus intraradices* Schenck & Smith BEG 141 (synonym *Rhizophagus* sp.; D. Redecker, unpublished data) was supplied by the International Bank for Glomeromycota (IBG, http://www.kent.ac.uk/bio/beg/); it was produced on leek (*Allium porrum*) in pot cultures in neutral γ-irradiated Epoisses clay-loam soil by A. Colombet and V. Monfort (UMR Agroécologie, Dijon).

1.3 Bacterial strains

Escherichia coli strain One shot® Top10 and OmniMAX™ 2-T1R (Invitrogen) were used for bacterial transformation and multiplication of plasmids and the ultracompetent strain XL10-Gold® (Stratagene) was used for high efficiency transformation of bacteria with plasmids containing the cDNA of the *M. truncatula* - *G. intraradices* expression library (see 9.5). Respective genotypes of bacterial strains are indicated in Table 2.

E. coli was grown at 37°C on Luria-Bertani (LB, Annex II) or on LB Agar using appropriate antibiotics for selection of resistance conferred by plasmid integration.

1.4 Yeast strains

The glucose deficient yeast (*Saccharomyces cerevisiae*) strain EBY.VW4000 (Fig 27) lacks all 20 transporter genes (*HXT1-17*, *GAL2*, *AGT1*, *MPHs*) required for hexose uptake (Wieczorke et al. 1999).

The sucrose deficient yeast strain SUSY7/*ura3*$^{-}$ (Fig 27) provided by S. Lalonde (Carnegie Institution for Science, Stanford, USA) originate from the mutant strain SUSY7 lacking the

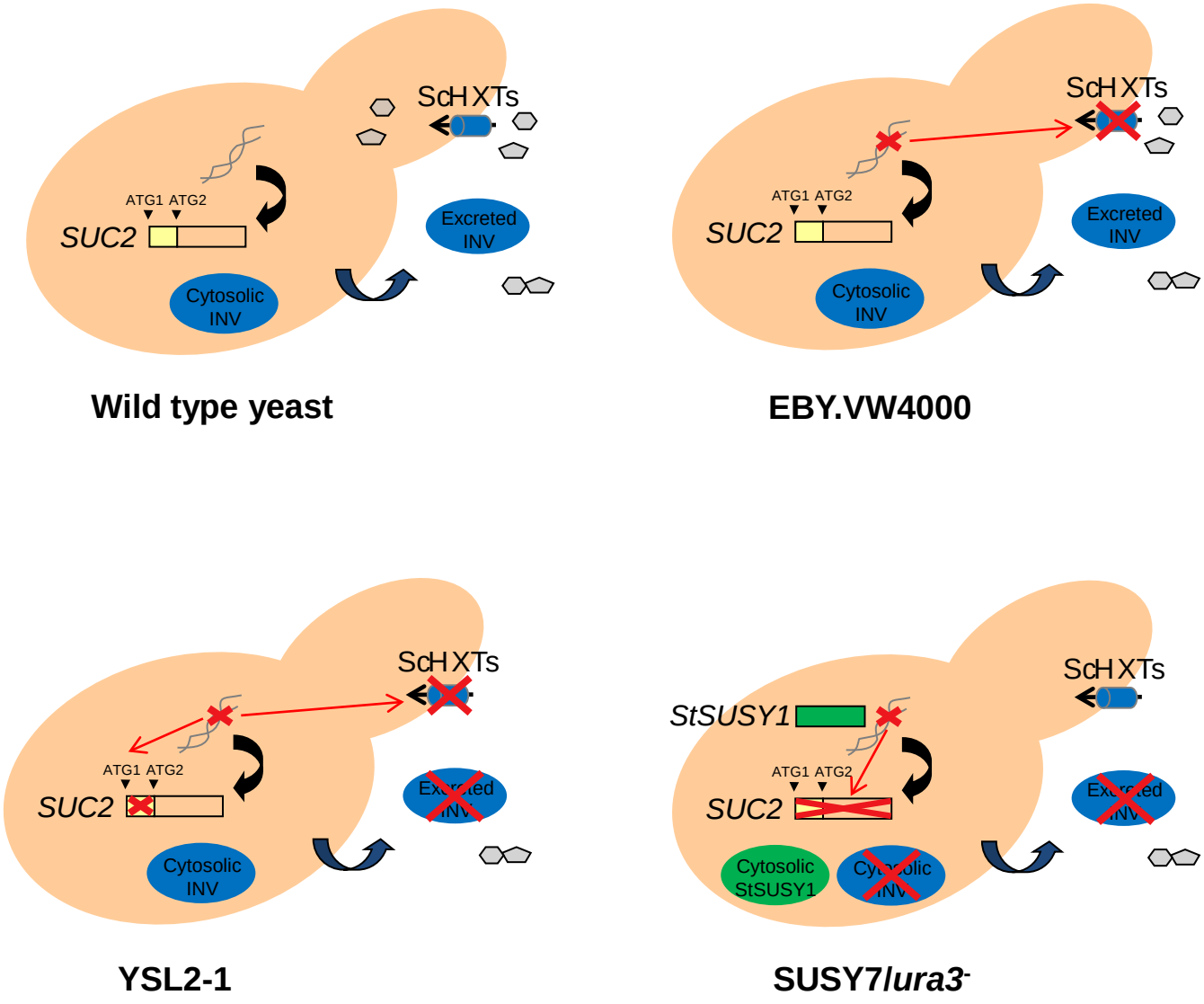


Fig 27. Schematic representation of sugar transport in wild type and mutant yeasts mentioned in this study

Wild type yeasts (*e.g.* S288C and CEN.PK2-1C) cleave extracellular sucrose by the mean of an excreted sucrose invertase (SUC2) and import resulting hexoses via ScHXTs (hexose transporters). The glucose deficient yeast EBYVW4000 (Wieczorke et al. 1999) lacks the 20 sugar transporter genes required for hexose uptake and therefore this strain is suitable to characterize MSTs. The sucrose deficient yeast YSL2-1 (S. Lalonde, unpublished) lacks the 20 sugar transporter genes required for hexose uptake as well as the signal peptide encoded from the 1st initiation start codon of *SUC2* for the excreted invertase form and therefore is not able to use extracellular sucrose in the medium. The sucrose deficient yeast SUSY7/*ura3*- (Riesmeier et al. 1992; Barker et al. 2000) lacks the full *SUC2* locus encoded both excreted and cytosolic invertases, but a plant sucrose synthase gene from potato (*StSUSY1*) was incorporated in the yeast genome. Both YSL2-1 and SUSY7/*ura3*- are suitable to characterize sucrose importers and functional complementation by SUTs targeted at the yeast plasma membrane allow import of extracellular sucrose into yeast cells which is then cleaved by cytosolic invertase (YSL2-1) or potato sucrose synthase (SUSY7/*ura3*-).

entire *SUC2* locus coding for excreted and cytosolic invertases; the gene coding for a cytosolic invertase of potato (*StSUSY1*) was stably integrated in the yeast genome (Riesmeier et al. 1992). The *URA3* gene of the mutant SUSY7 was then deleted to obtain the SUSY7/*ura3*$^{-}$ strain (Barker et al. 2000) for auxotrophic selection on minimun medium without uracil. The sucrose deficient yeast YSL2-1 (unpublished) was provided by S. Lalonde (Fig 27); the original promoter of the yeast invertase SUC2 locus has been substituted with the promoter of the yeast HXT7 (P*suc2*::P*HXT7*) resulting in the deletion of the first initiation codon of the excreted invertase. Respective genotypes of mutant yeast strains are indicated in Table 3.

Yeasts were grown at 30°C using yeast culture medium (Annex I): YPD (yeast extract peptone dextrose for SUSY7/*ura3*$^{-}$), YPM (yeast extract peptone maltose for YSL2-1) or using minimal synthetic medium with dextrose (SD) or maltose (SM), without uracil for SUSY7/*ura3*$^{-}$ and YSL2-1 yeast respectively, when they were transformed with pDR plasmids (Fig 30).

2 Growth conditions and sampling of plant material

Seeds of *M. truncatula* were surface sterilized 6 min in sulfuric acid 98%, 10 min in sodium hypochlorite 3%, washed in sterile water and germinated on 0.7% bactoagar in darkness at 25°C. Then, plantlets were transferred in 75mL pots of sterile mix (2:1, v:v) of Terragreen (OilDri-US special, Mettman) and Epoisses soil (non-mycorrhized plants) or in mix (2:1, v:v) of Terragreen and Epoisses soil base of *G. intraradices* BEG141 inoculum (mycorrhized plants). Plants were grown under controlled conditions (420 µE m^{-2} s^{-1} for 16 h of light; 24 and 19°C day and night temperature, 70% humidity), watered daily with sterile water and supplemented twice a week with 5 mL modified Long Ashton solution (double quantity of nitrate KNO_3, and 1/10 quantity of phosphate NaH_2PO_4 = 0.13 mM phosphate) for low phosphate (LP) control plants and mycorrhized plants (AM) and with 5mL modified Long Ashton solution (double quantity of nitrate and 1.3 mM phosphate) for high phosphate (HP) control plants (Table 4; Hewitt 1966). Plants were harvested every week from first to fourth week post inoculation (wpi); source leaves and root materials were rinsed with distilled water and dried on paper. Materials for RNA extraction and sugar content quantification were frozen in liquid nitrogen upon harvest and stored at -80°C. Plant roots were also stained by the mean of the ink/acid acetic method (see 2.1.4) to determine AM fungal developmental stage on randomly sampled root pieces.

Components	High phosphate (HP) solution $mg.L^{-1}$	Low phosphate (LP) solution $mg.L^{-1}$
Potassium nitrate KNO_3	808	808
Calcium nitrate $Ca(NO_3)_2$, $4H_2O$	1888	1888
Sodium dihydrogen phosphate NaH_2PO_4, $4H_2O$	209	20.9
Magnesium sulphate $MgSO_4$, $7H_2O$	368	368
FeNa-EDTA	22	22
Manganese sulfate $MnSO_4$, $4H_2O$	2.23	2.23
Copper sulphate $CuSO_4$, $5H_2O$	0.25	0.25
Zinc sulfate $ZnSO_4$,$7H_2O$	0.29	0.29
Boric acid H_3BO_3	3.10	3.10
Sodium chloride NaCl	5.90	5.90
Ammonium molybdate $(NH_4)_6Mo_7O_{24,}$ $4H_2O$	0.088	0.088

Table 4. Modified Long Ashton solution (Hewitt 1966) for high and low phosphate conditions

2.1 Plant growth parameter measurements

2.1.1 Fresh and dry weight

At harvest, five plants of each condition (LP, HP, and AM) were separated into shoots and roots and fresh weight was directly monitored. Dry weight of shoots and roots was measured after drying the material in paper bags at 55°C for 3 days.

2.1.2 Total leaf surface

At harvest, digitalized images of the plants have been taken and total leaf surface was measured on 5 individuals for each condition (LP, HP, and AM). The total leaf surface of entire plants was assessed using the ImageJ software (http://rsbweb.nih.gov/ij/) and the image analyses package of Visilog® v6.8 software (Noesis) combined to algorithm script developed by C. Schneider (URLEG, Dijon) allowing analyses of leaf surface.

2.1.3 Chlorophyll content estimation

The chlorophyll content of source leaves can be estimated by the mean of a non-destructive method (Dwyer et al. 1991) with a SPAD chlorophyll meter SPAD-502 (Konica Minolta, Japan); independent measurements were performed on 10 individual trefoils for each condition (LP, HP, AM).

2.1.4 Estimation of mycorrhizal root colonization

To estimate total root colonization, staining with black ink (noir de jais of Shaeffer, Shaeffer Manufacturing Co., USA) was performed. Roots were cleared in 10% potassium hydroxide at 90°C for 80 min, rinsed several times with distilled water, stained for 8 min in 5% ink solution in 8% acetic acid and destained in 0.8% acetic acid for 25 min (Vierheilig et al. 1998). For light microscopy, 30 times 1 cm-long root pieces were mounted on glass slides, observed according to Trouvelot et al. 1986, and colonization estimated using the Mycocalc program (http://www2.dijon.inra.fr/mychintec/Mycocalc-prg/download.html). The parameters used were F% (frequency of mycorrhizal colonization in the root system), M% (intensity of mycorrhizal colonization), m% (intensity of mycorrhizal colonization in mycorrhizal root fragments), A% (arbuscule abundance in the root system), a% (percentage of arbuscules within colonized areas).

3 Standard methods of molecular biology

3.1 Nucleic acid preparation methods

3.1.1 Total RNA extraction

Total RNA from root system and source leaves of *M. truncatula* was extracted with the LiCl method (Franken and Gnadinger 1994). Plant materials stored in liquid nitrogen (see 2) were ground into a fine powder in ceramic mortars using liquid nitrogen and transferred into plastic 50 mL Falcon tubes containing NTES extraction buffer (50 mM Tris/HCl, pH 9; 150 mM NaCl; 5 mM EDTA; 5 % SDS; 1 % β-mercaptoethanol; 1.6 buffer volume (ml) per mg sample). An equal volume of phenol:chloroform:isoamyl alcohol solution (25:24:1, pH 4.5) was added to remove proteins. Tubes were inverted several times to mix, then centrifuged 15 min at 14500 g at room temperature, and the upper aqueous phase transferred into new 1.5 mL Eppendorf tubes. This procedure was repeated twice with the phenol:chloroform:isoamyl alcohol solution and once with pure chloroform. To precipitate RNA and eliminate polysaccharides, 0.7 volume of 96% ethanol (from -20°C) and 0.05 volume of 1 M acetic acid were added to the aqueous phase and left overnight at - 20°C. The next day, tubes were centrifuged for 35 min at 10000 g at 4°C and the liquid phase was discarded. The pellet was resuspended in DEPC water and RNA was specifically precipitated from the solution by 4 M LiCl (1 volume) treatment for 4 hours at 4°C. Tubes were centrifuged for 35 min at 10000 g at 4°C, the aqueous phase was discarded, the pellet was resuspended in DEPC water and another RNA precipitation was performed overnight with 3 M NaAc (0.1 vol) and 96 % ethanol (3 vol) at -20°C. The final pellet was air-dried at room temperature and dissolved in 40 µL RNase-free water. Quantity and quality of RNA were checked with a Nanodrop 1000 (Thermo Scientific) and RNA integrity was checked by electrophoresis in a denaturing agarose gel (see 3.3.2). Finally, a DNase treatment using 2U of RNasin (Promega) and 1U of DNase RQ1 (Promega) per µg of total RNA was performed and RNA quantity, quality and integrity were checked again.

3.1.2 Plasmid DNA mini-preparation from bacteria

Plasmid DNA extraction from bacterial culture was performed from a modified rapid alkaline extraction method (BD, Bimboim and Doly 1979). A 2 mL of LB medium with appropriate antibiotics was inoculated with a single bacterial colony and incubated overnight at 37°C with shaking (180 rpm). Bacterial culture is harvested by centrifugation 5 min, 6000 rpm at 4°C and bacterial pellets is resuspended in BD1 buffer (50 mM glucose, 25 mM Tris-HCl pH 8.0, 10 mM EDTA pH 8.0) with 1µL of RNAse at 10 $\mu g.\mu l^{-1}$ and incubated 20 min at room temperature. During this time, BD2 buffer (0.2 N NaOH and 1% de SDS) is prepared extemporaneously and 200µL is added to the reaction, incubated 5 min at room temperature.

Then, 150µL of BD3 buffer (3 M KAc, pH 5.5) is added to the reaction and incubated 15 min on ice and centrifuged 15 min, 14500 rpm at 4°C. Supernatant is recovered in a new Eppendorf tube with 0.7 volume of isopropanol and incubated 5 min at room temperature, then centrifuged 5 min at 14500 rpm at 4°C. Supernatant is discarded and plasmid DNA pellet is washed with 200µL ethanol (70%), centrifuge 5 min at 14500 rpm at 4°C. Finally, pellet is vacuum-dried and plasmid DNA is eluted in 50 µL sterile water. Plasmid integrity and concentration was checked by appropriate restriction enzyme digestion (New England Biolabs) and water dilutions of the digestion reaction were run on 0.8% Agarose gel along with DNA leader molecular weight (Kalys) marker suitable for DNA quantification.

3.1.3 **Yeast plasmid and genomic DNA preparation**

Total DNA including both gDNA and plasmid can be extracted by the quick DNA preparation from yeast method (Amberg et al. 2005). Yeasts are cultivated overnight in complete (S288C, CEN.PK2-1C, YSL2 -1) or minimal ura$^-$ medium (yeast containing pDRs vectors) and 2mL are transferred in an Eppendorf and centrifuged 2500 rpm, 5 min. Yeast pellet is resuspended in 400 µL yeast lysis buffer (100 mM Tris, 50 mM Na_2EDTA, 1% SDS) with an equal volume of phenol:chloroform:isoamyl alcohol solution (25:24:1, pH 8) and acid wash glass beads and beat into Mixer Mill MM200 (Fischer, Germany) for 30 sec at maximum speed. The total lysate is centrifuged at 14500 rpm, 10 min and supernatant was recovered and transferred into a new Eppendorf mixed with 40µL of 3 M NaAc and 1 mL 96 % ethanol and centrifuged 14000 rpm, 5 min. Supernatant was discarded and pellet was washed with 200 µL 80 % ethanol, vacuum-dried and resuspended in 50µL TE.

3.2 Nucleic acid amplification methods

3.2.1 **DNA amplification by PCR**

Polymerase chain reaction (PCR) is characterized by the ability to generate identical high copy number of dsDNA and was used to amplify sequence of interest from DNA matrix (genomic DNA or plasmid) using the Taq Core kits 25 (Qbiogen MP Biomedicals) with 1X PCR buffer with $MgCl_2$ (1,5mM), dNTPs (125µM each, Invitrogen), forward and reverse specific primers (1µM each, Eurofins MWG) and *Taq* DNA polymerase 15 U/µL (1U, Qbiogen MP Biomedicals) adjusted to a final volume of 20µL with sterile water. PCRs were performed in a T3 Thermocycler (Biometra) with the following protocol parameters: an initial denaturation step at 95°C for 2 min followed by a 30x cycling program each composed of a denaturation step at 95°C for 30 sec, a second step of primer annealing to template DNA for 30 sec with a temperature (53-55°C) adapted to primer pair melting temperatures (Tm) and a

third step of elongation at 72°C with a duration depending of the target size (1 min/kb). PCR reaction is completed by a final elongation step at 72°C for 5 min.

3.2.2 Bacterial colony PCR

Bacterial transformants grown on antibiotic selective LB plates were checked by classic direct colony PCR (Zon et al. 1989). Bacterial colonies were directly recovered in 50 µL sterile water from LB plates with a sterile toothpick. Then, 5 µL of this colony/water dilution was used as the DNA matrix for PCR reactions as described in 3.2.1, with an extended initial denaturation step of 5 min to disrupt bacterial cells and PCR products were run on an electrophoresis gel (see 3.3.1).

3.2.3 Reverse transcription of RNA

Total RNA (1 µg) was mixed with dNTPs (500 µM each, Invitrogen), oligo $d(T)_{15}$ (500 ng, Promega) adjusted to a final volume of 13 µL with sterile DEPC water and heated up at 65°C and immediately placed on ice for 3 min to relax RNA secondary structure. First-strand buffer (1X) and DTT (5 µM), RNaseOut™ (40U) and SuperScript™ III reverse transcriptase (200U, Invitrogen) are mixed to the previous solution in a final volume of 20 µL. RNA are reverse transcribed into cDNA by a performing a cycling program in a T3 Thermocycler (Biometra) at 25°C 5 min, 50°C 60 min, 70°C 15 min, placed immediately on ice for 2 min and stored at -20°C.

3.3 Nucleic acid separation by gel electrophoresis

3.3.1 DNA electrophoresis in native agarose gel

Agarose gel was prepared in 0.5×TAE buffer (Annex III). DNA samples were mixed with 0.1 volume loading buffer (0.25% bromophenol blue, 0.25% xylene cyanol FF, 30% glycerol) (Sambrook et al. 1989), loaded into agarose gel and separated at 100V in 0.5×TAE buffer for 15-60 min (depending on agarose gel concentration and DNA fragment size). After gel staining with ethidium bromide (0.1 µg/µl), DNA was visualized under UV light using a Molecular Imager® Gel Doc XR (Bio-Rad).

3.3.2 RNA separation in denaturing agarose gel

1 µg RNA diluted in 5 µl RNase-free water was denatured in 5 µl denaturing buffer (50% deionized formamide, 20% formaldehyde [37%], 1×MOPS buffer [10×MOPS buffer: 200 mM MOPS pH 7.0, 50 mM sodium acetate, 10 mM EDTA]) and 1 µl loading buffer (50%

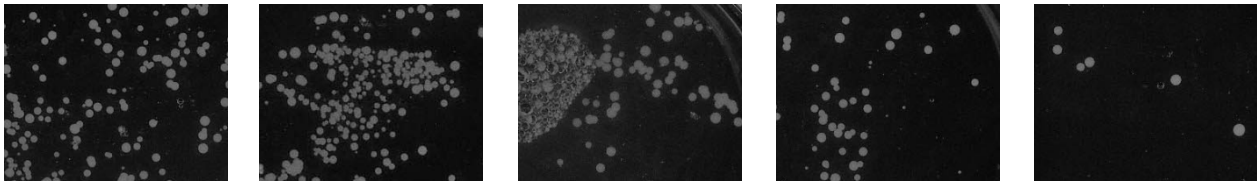


Fig 28. Transformation efficiency and incubation time of the YSL2-1 strain using the high-efficiency yeast transformation method (Gietz and Schiestl 2007)

A 10^{-2} dilution of YSL2-1 yeast transformed with pDR196SfiI (1µg) using different incubation time (42°C) was spread on SM ura^- medium.

sucrose, 1 mM EDTA, 0.25% bromophenol blue, 0.25% xylene cyanol FF, 1% ethidium bromide [10 µg/µl]) (Sambrook et al. 1989) for 10 minutes at 70°C. RNA was separated for 60 min at 50V in a 1.4% denaturing agarose gel prepared with 20% formaldehyde (37%) and 1×MOPS buffer. RNA integrity was visualized under UV light using a Molecular Imager® Gel Doc XR (Bio-Rad).

3.4 Transformation

3.4.1 Bacterial transformation with plasmids

Chemical competent One shot® Top10 and OmniMAX™ 2-T1R (50 µL, Invitrogen) *E. coli* cells were thawn on ice in presence of plasmids (1-100 ng in 4 µL) for 30 min and a heat-shock is performed at 42°C for 30 sec and cells are immediately incubated on ice for 2 min prior the addition of 1 mL of SOC medium, cells are grown for 1 hour at 37°C and shaken horizontally (200 rpm). After this time, 100 µL of cells are spread on LB plates using appropriate antibiotics for selection of resistance and incubated at 37°C overnight. The next day, clones are checked by colony PCR (see 3.2.2) and positive clones are cultivated in liquid LB with antibiotics and glycerol (50%) stocks are stored at -80°C.

3.4.2 Yeast transformation

Saccharomyces cerevisiae were transformed according to Gietz and Schiestl 2007 method suitable to generate sufficient transformants with an efficiency estimated around $3x10^5$ transformants/ µg plasmid/ 10^8 cells (Fig 28), for screening expression libraries but was also used to integrate single plasmid into mutant yeast strains. For this transformation method, a single yeast colony was picked from a fresh culture and cultivated overnight in 5 mL of liquid YPD or YPM depending on the yeast strains (Table 3). The next day, $2.5x10^8$ cells are transferred into 50 mL YPD/YPM in a pre-warmed (30°C) sterile Erlenmeyer flask to give a final OD of 0.5 and yeast is cultivated for 2 generation times when the cell titer reaches at least an OD of 2 (3-5 hours); yeast cells are harvested in a 50 mL Falcon by a 6 min centrifugation 3000g. Yeast pellet is washed with 50 mL sterile water and a second centrifuge step is performed; yeast pellet is resuspended in 1.5 mL sterile water and transferred to an Eppendorf tube and a third centrifugation step is performed. Yeasts are then resuspended in a final volume of 1 mL with sterile water. This yeast suspension is sufficient to perform 10 independent transformations and is divided in 100 µL aliquots per single reaction (for screening library) or per plasmid to be integrated. Each aliquot is centrifuged for 6 min at 3000g and pellet is resuspended in transformation mix (per reaction: 240 µL PEG 3500 50% w/v; 36 µL LiAC 1M, 50µL boiled DNA single-stranded carrier made from salmon testis DNA) and plasmid DNA adjusted to a final volume of 360 µL with sterile water. The

transformation reaction is incubated at 42°C in a water bath during an optimized time of 50 min (Fig 28). After this time, yeasts are harvested by a 6 min centrifugation 3000g and resuspended in 1 mL sterile water. Appropriate dilutions of the cell suspension are spread on synthetic minimal medium plates and incubated at 30°C (5-21 days, Fig 41 and 42). Correct integration of the plasmid DNA is checked by yeast total DNA preparation (see 3.1.3) followed by PCR (see 3.2.1) with PMA forward and ADH close reverse primers.

3.5 Software used for molecular biology analyses

Specific primers were designed (Annex IV) using *Amplif*X 1.5.4 (http://ifrjr.nord.univ-mrs.fr/AmplifX) and Oligo Analyzer 1.0.2 (Teemu Kuulasmaa) software and analyses of DNA sequences retrieved from sequencing of PCR products and plasmids were performed using BioEdit 7.0.9.0 (Hall 1999) and DNAbaser (http://www.dnabaser.com/index.html) software.

3.6 Statistical analyses

All data represent means ± standard deviation of data obtained from biological material of several independently grown plants (the sample number n is indicated in the table and figure legends). The data were statistically checked by the adequate Student t-test upon accomplishing the Fischer F-test.

4 *In silico* search for sugar transporters in *M. truncatula* and *G. intraradices*

Putative *M. truncatula* sugar transporter sequences (MtSUT and MtMST) were retrieved from the *Medicago truncatula* genome version 2.0, 3.0 and 3.5 (http://www.medicagohapmap.org/index.php; Young et al. 2011), from the Dana-Farber cancer institute (DFCI) *Medicago* Gene Index (http://compbio.dfci.harvard.edu/cgi-bin/tgi/gimain.pl?gudb=medicago) and from *Medicago* EST navigation system (MENS, INRA, Toulouse, http://medicago.toulouse.inra.fr/Mt/EST/) using key word searches and BLAST programs of previously published plant sugar transporter sequences. Expression profiles of all available MtSUT were retrieved from the MtGEA (*M. truncatula* gene atlas, http://mtgea.noble.org/v2/) database which archives all publically available gene expression data derived from the use of the *M. truncatula* Affymetrix GeneChip (Benedito et al. 2008) and MtMSTs gene expression profile with a gene expression differentially regulated in

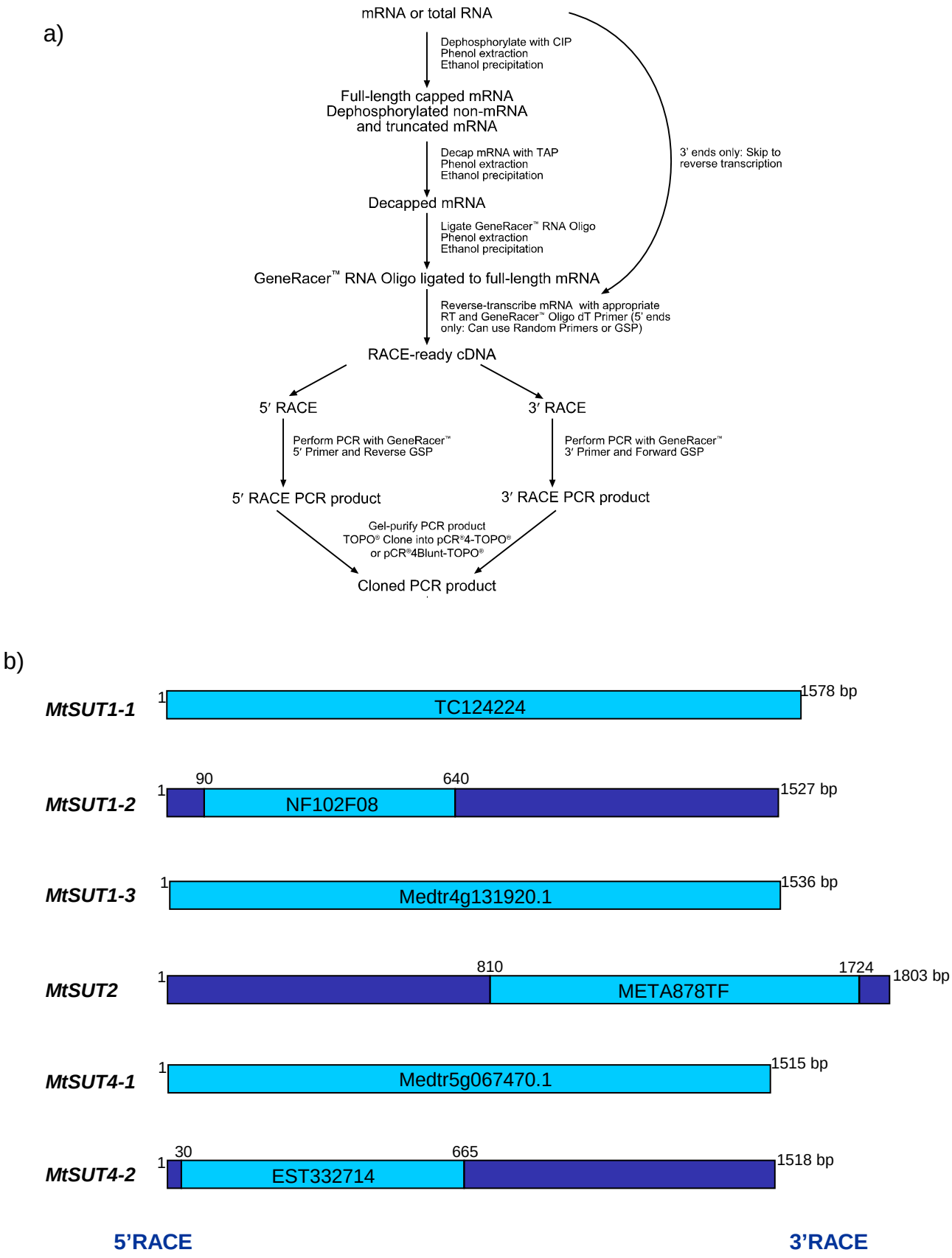


Fig 29. Rapid amplification of cDNA ends of *MtSUT1-2*, *MtSUT2* and *MtSUT4-2*

a) Flow chart of the GeneRacer™ (Invitrogen) procedures used to obtain 5' and 3' cDNA ends of *MtSUT1-2*, *MtSUT2* and *MtSUT4-2*

b) Schematic representation of coding sequences of the MtSUT family. cDNA ends of NF102F08, META878TF, EST332714 were amplified by 5' and 3' RACEs (dark blue) to obtain full length CDS of *MtSUT1-2*, *MtSUT2* and *MtSUT4-2*.

response to fungal colonization by *G. intraradices* were also retrieved from MtGEA (Gomez et al. 2009).

Putative fungal transporter sequences from *G. intraradices* DAOM197198 (synonym *Rhizophagus irregularis*) were retrieved from the available preliminary genomic data (http://mycor.nancy.inra.fr/IMGC/GlomusGenome/index.html) using key word searches and BLAST programs with sequences from the *Geosiphon pyriformis* hexose transporter (GpMST1), the 15 sugar transporter family members of *Laccaria bicolor* (Fajardo Lopez et al. 2008), the fructose transporter *BcFRT1* (*Botrytis cinerea*; Doehlemann et al. 2005) and the sucrose transporter *SpSUT1* (*Schizosaccharomyces pombe*; Reinders and Ward 2001). Expression profile of *G. intraradices* monosaccharide transporter candidates were obtained from the *G. intraradices* expression microarray NimbleGen chip developed by Tisserant et al. 2011.

4.1 Phylogenetic analyses

Alignment of amino acid sequences of sugar transporters was performed with Mafft version 6 (Katoh and Toh 2008) and maximum parsimony analyses were done using PAUP 4.0b10 (Swofford 1998). Heuristic tree searches were executed using 1000 random sequence additions and the tree bisection-reconnection branch-swapping algorithm with random sequence analysis. Unrooted trees (plant and fungal SUT tree, Fig 39 and 55) and consensus unrooted tree (plant MST tree, Fig 36) was displayed on Dendroscope 2.7.4 (Huson et al. 2007).

5 Rapid amplification of cDNA ends (RACE): 5’ and 3’ RACE of *MtSUT1-2*, *MtSUT2* and *MtSUT4-2*

For partial identified MtSUT sequences (Fig 29b; TC124224, META878TF, EST332714), full-length cDNA was obtained with the 5’ and 3’ GeneRacer™ Kit (Invitrogen). Whilst total RNA can be directly reverse transcribed with modified poly(T) oligomers to perform 3’ RACE, mRNA must be dephosphorylated and decapped to yield accessible 5’ cDNA ends to a RNA oligomer (Fig 29a).

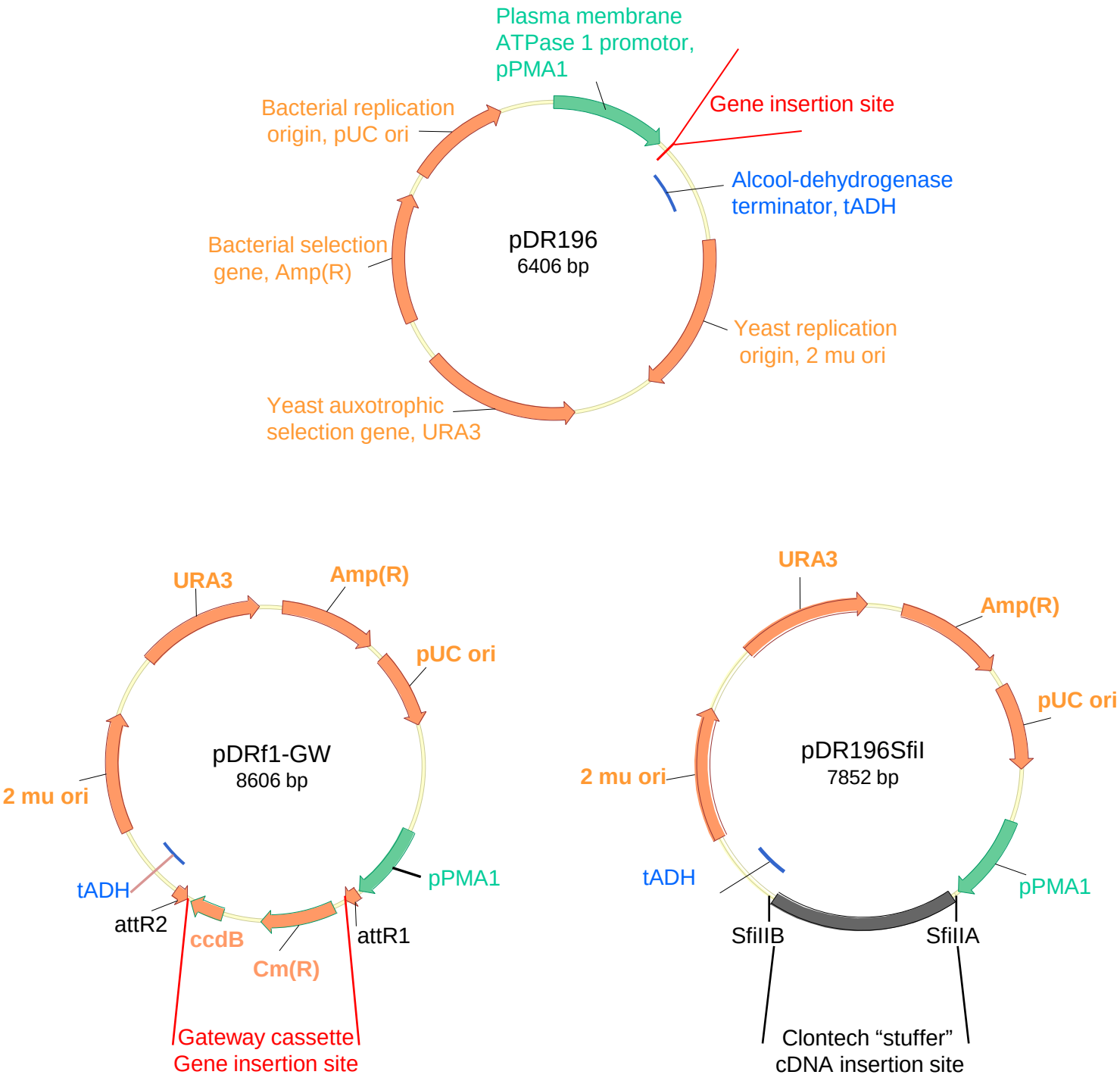


Fig 30. Maps of the pDRs vectors mentioned in this study

Both the pDRf1-GW (Loque et al. 2007) and pDR196SfiI (Martin 2005; Schüßler et al. 2006) were constructed from the original pDR196 vector (Rentsch et al. 1995). attRs recombination sequences and a lethal toxin ccdB cassette were integrated in the pDRf1-GW for recombination with entry plasmids and negative selection after the BP cloning step (Fig 31). The pDR196SfI contains a SfiI Clontech “stuffer” which can be excised by a SfiI restriction digestion resulting in SfiIA and SfiIB sites for directional cloning of cDNA.

5.1 5' RACE: mRNA dephosphorylation, decapping and oligomer ligation

For 5' RACE, starting from roots and leaves total RNA (75µg), mRNA was purified by the mean of the Dynabeads® mRNA Purification Kit (see 9.1). Then mRNA dephosphorylation was performed with 5 µL (250 ng) of the mRNA with provided reagents from the GeneRacer™ Kit: calf intestinal phosphatase (10U), calf intestinal phosphatase buffer (1X) and RNaseOut™ (40U) with DEPC water (qsp 10 µL); this reaction is vortexed briefly, incubated at 50°C for 1 hour, then placed on ice. Then, RNA is precipitated with 90µL phenol:chloroform:isoamyl alcohol (25:24:1, pH 4.5), vortexed 30 sec and centrifuge at 14500 rpm for 30 sec. Top aqueous was transferred in a new Eppendorf with 2 µL mussel glycogen (10 mg/mL), 10 µL NaAc (3M, pH 5,2) and 220 µL ethanol 96%. This reaction is mixed and stored at -20°C for overnight precipitation. The day after, dephosphorylated mRNA is centrifuge 14500 rpm for 20 min at 4°C and the pellet is washed with 500 µL 70% ethanol and recovered by centrifugation 14500 rpm for 2 min at 4°C and resuspended in 7 µL DEPC water. Thereafter mRNA is decapped using tobacco acid pyrophosphatase (0.5U), tobacco acid pyrophosphatase buffer (1X), RNaseOut™ (40U) in a total volume of 10 µL; this reaction is vortexed briefly, incubated at 37°C for 1 hour, then placed on ice. The overnight precipitation step and recovery of RNA was repeated as described in the dephosphorylation step.

After dephosphorylation and decapping the mRNA, a RNA oligomer (0.25 µg) of known sequence provided in the GeneRacer™ Kit is mixed with the decapped RNA heated up at 65°C for 5 min and ligated by the mean of T4 RNA Ligase (5U), Ligase Buffer (1X), ATP (1mM) and RNaseOut™ (40U) in a total volume of 10 µL and incubated at 37°C for 1 hour.

5.2 5' and 3' RACE PCR amplification

Reverse transcription for 5' and 3' RACE was performed using the SuperScript™ III reverse transcriptase (Invitrogen) and a poly(T) adapter oligomer. 1µL of this oligomer and dNTPs (2.5 mM each) are mixed to the 10 µL decapped, dephosphorylated and ligated mRNA (from 5.1) for 5' RACE or directly to pooled total RNA (1µg) from *M. truncatula* roots and leaves for 3' RACE (qsp 13µL). Reaction are heated up to 65°C, 5 min and placed on ice 2 min; then First Strand Buffer (1X), DTT (Dithiothreitol, 10µM), SuperScript™ III reverse transcriptase (200U) and RNaseOut™ (40U) are mixed to the previous solution in a final volume of 20 µL and reverse transcription was performed T3 Thermocycler (Biometra) 25°C 5 min, 50°C 60 min, 70°C 15 min and placed immediately on ice for 2 min. The resulting cDNA are "ready" for 5' and 3' RACE experiments (Fig 29a).

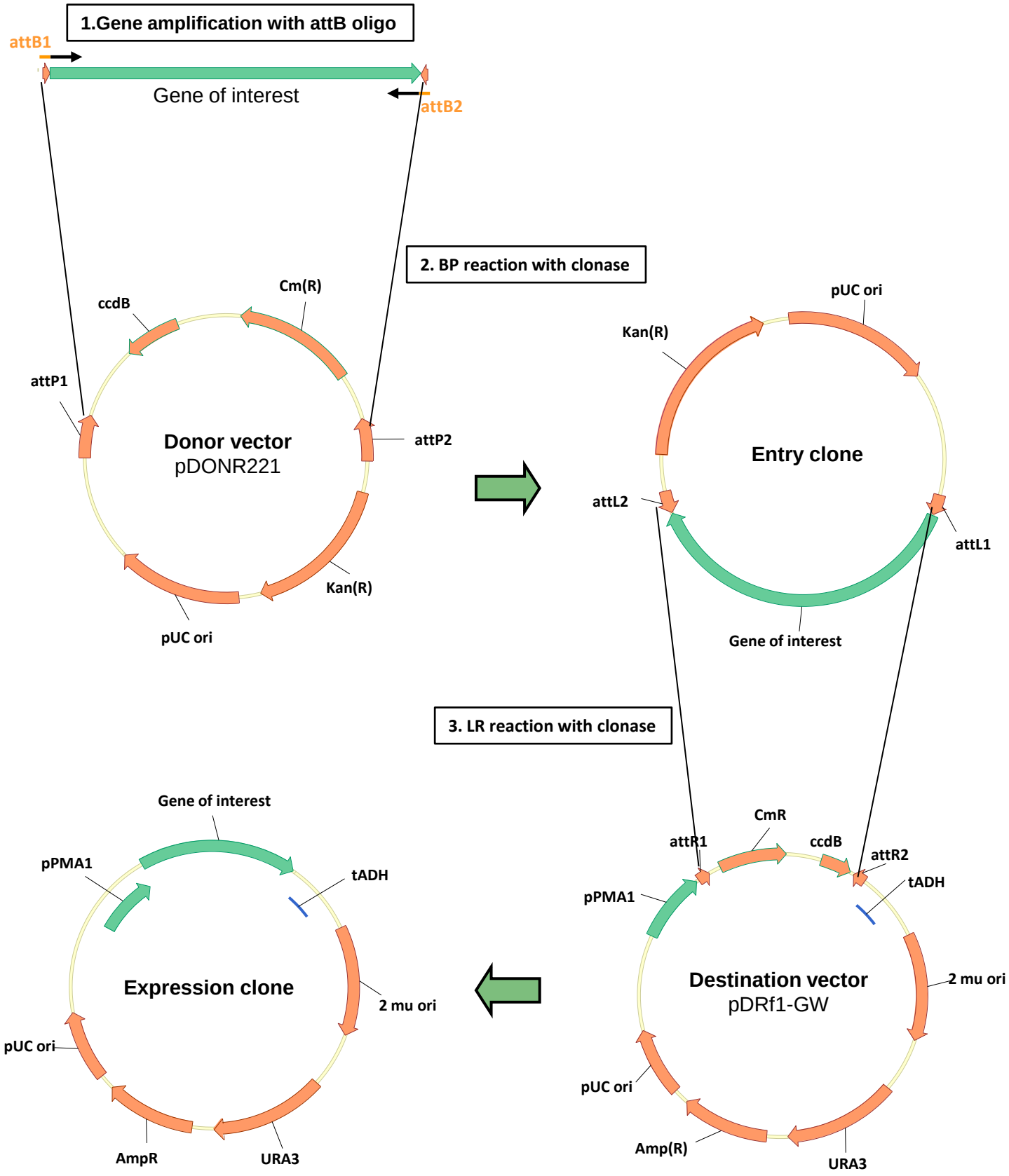


Fig 31. Schematic representation of the Gateway® cloning technology (Invitrogen) used to clone MtSUTs in the yeast expression vector pDRf1-GW

2 mu ori: yeast replication origin; *Amp*(R): ampicillin resistance; atts: Gateway® recombination sequences; *ccdB*: lethal selective gene (Toxin CcdB) for negative selection of entry clone; *Cm*(R): chloramphenicol resistance; *Kan*(R): kanamycin resistance; pPMA1: yeast plasma membrane ATPase 1 promotor; pUC ori: bacterial replication origin; tADH: yeast alcool-dehydrogenase terminator; *URA3*: Orotidine-5'-phosphate (OMP) decarboxylase CDS for auxotrophic selection of *ura3*$^{-}$ yeast.

5' RACE experiment was performed to obtain 5' sequences upstream partial MtSUT sequences by PCR amplification using forward primers complementary to the 5'oligomer adaptor and EST specific reverse primers whilst 3'RACE was performed to obtain 3' sequences downstream partial MtSUT with reverse primers complementary to the 3' modified poly(T) oligomers and EST specific forward primers (Annex IV.1; Fig 29b). For both 5' and 3' RACEs, a first touch down PCR of 35 cycles followed by a second nested PCR of 30 cycles was performed using Platinum® Taq DNA Polymerase High Fidelity (Invitrogen) according to the GeneRacer manufacturers' instruction. PCR products were run on 1% agarose gel by electrophoresis and cloned with TOPO TA cloning® kit in the pCR®2.1-TOPO vectors (Invitrogen, Annex V), correct insertion of PCR products was checked by bacterial colony PCR (see 3.2.2) and positive plasmids were sent to sequence with M13 reverse and forward primers to Eurofins MWG (Ebersberg, Germany).

6 Cloning of *MtSUTs* by Gateway® technology

Specific primers were designed to the full-length coding sequence of the 6 *MtSUTs* adding attB1 and attB2 linkers to the 5' ends of forward and reverse primers (Annex IV.2, Fig 31) and *MtSUTs* were amplified form *M. truncatula* root and leaf cDNA (see 3.2.3) by PCR using Platinum® Pfx DNA Polymerase (Invitrogen). Full-length amplification products were run by electrophoresis and gel-cut purified using the Qiaquick® gel purification kit (Qiagen) according to the manufacturers' instruction. Purified PCR products flanked with attB sequences (75 ng) were cloned in the donor vector by recombination with attP sequences of the pDONR221 (75 ng; Invitrogen) by the mean of the Gateway® technology (Fig 31) using BP Clonase™ II enzyme (1U) adjusting the final volume to 4 µL with TE (pH 8). 2 µL of each reaction was used to transform OmniMAX™ 2-T1R competent *E. coli* cells (see 3.4.1) and bacteria containing entry clones were selected on LB kanamycin medium and checked by bacterial colony PCR (see 3.2.2), positive plasmids were extracted by plasmid preparation (see 3.1.2) and sent to sequence with M13 reverse and forward primers by Eurofins MWG (Ebersberg, Germany).

After having checked the correct integration of *MtSUTs* flanked by attL sequences in entry plasmid (75 ng), recombination with the destination pDRf1-GW vectors (Fig 30; Loqué et al. 2007) containing attR sequences was performed using 1U LR Clonase™ II enzyme (Fig 31) adjusting the final volume to 4 µL with TE (pH 8). 2 µL of each reaction was used to transform OmniMAX™ 2-T1R (see 3.4.1) competent *E. coli* cells and bacteria containing expression clones were selected on LB ampicillin medium and checked by bacterial colony PCR, positive plasmids were extracted by plasmid preparation (see 3.1.2) and sent to

sequence with PMA forward and ADH close reverse primers to Eurofins MWG (Ebersberg, Germany).

7 Functional analysis of MtSUTs

7.1.1 Yeast drop test complementation assays

Respective MtSUTs cloned in the pDRf1-GW vector (see 6) and empty vector control pDRf1 were transformed into *S. cerevisiae* strains SUSY7/*ura3*$^-$ or YSL2-1 (see 3.4.2); single transformant colonies were picked on SDura$^-$ plates and resuspended in 1 mL sterile water. Drop test complementation assays were performed on minimal selective sucrose (2%) medium without uracil by pipetting a 3 µL drop dilutions containing respectively 10^5,10^4,10^3,10^2 and 10 yeast cells per drop which was deposit synthetic on minimal sucrose medium without uracil. A 3µL drop containing 10^3 yeast cells per drop was deposit on SDura$^-$ as a loading control. Plates were incubated at 30°C.

7.1.2 Yeast sucrose uptake experiments

For uptake assays, single yeast colonies were grown in liquid SDura$^-$ to logarithmic phase. Cells were harvested at an OD_{600} of 0.5, washed twice in sterile water, and resuspended in NaPi buffer (0.6 M sorbitol, 50 mM potassium phosphate, pH 5) to a final OD_{600} of 5. Prior to the uptake measurements, cells were energized with 5 µL 10 mM glucose and incubated for 5 min at 30°C. To start the reaction, 100 µL of this cell suspension was added to 100 µL of NaPi buffer containing 7.5 kBq of [^{14}C]sucrose (specific activity 498 mCi/mmol, Perkin Elmer), and unlabeled sucrose to the concentrations used in the experiments. 50 µL aliquots were removed over 3 minutes (at 30 sec, 1, 2 and 3 min) and transferred in 4 mL of ice-cold NaPi buffer. Cells were vacuum-collected onto GF/C microfibre filters (Whatman) and washed twice with 5 mL of NaPi buffer. Inhibitor assays were performed with the addition of inhibitors 30 sec prior the incubation time. Inhibition of 500 µM [^{14}C]sucrose uptake (control) by the addition of 50 µM respective inhibitors: protonophores carbonyl cyanide *m*-chlorophenylhydrazone (CCCP), 2,4-dinitrophenol (DNP) and the plasma membrane H^+-ATPase inhibitors diethylstilbestrol (DES) and vanadate were tested. For sugar specificity, competition for 500 µM [^{14}C]sucrose uptake (control) was assessed by adding a ten-fold molar excess of respective competing sugars: glucose, fructose, trehalose or maltose. For all uptake experiments, [^{14}C]sucrose uptake was radioassayed by vortexing microfibre filters in vials for 30 sec with 6 mL Emulsifier Scintillator Plus™ (Perkin Elmer) and monitored on a Tri-Carb 2100TR Liquid Scintillation Analyzer (Packard) counting 3 biological replicates.

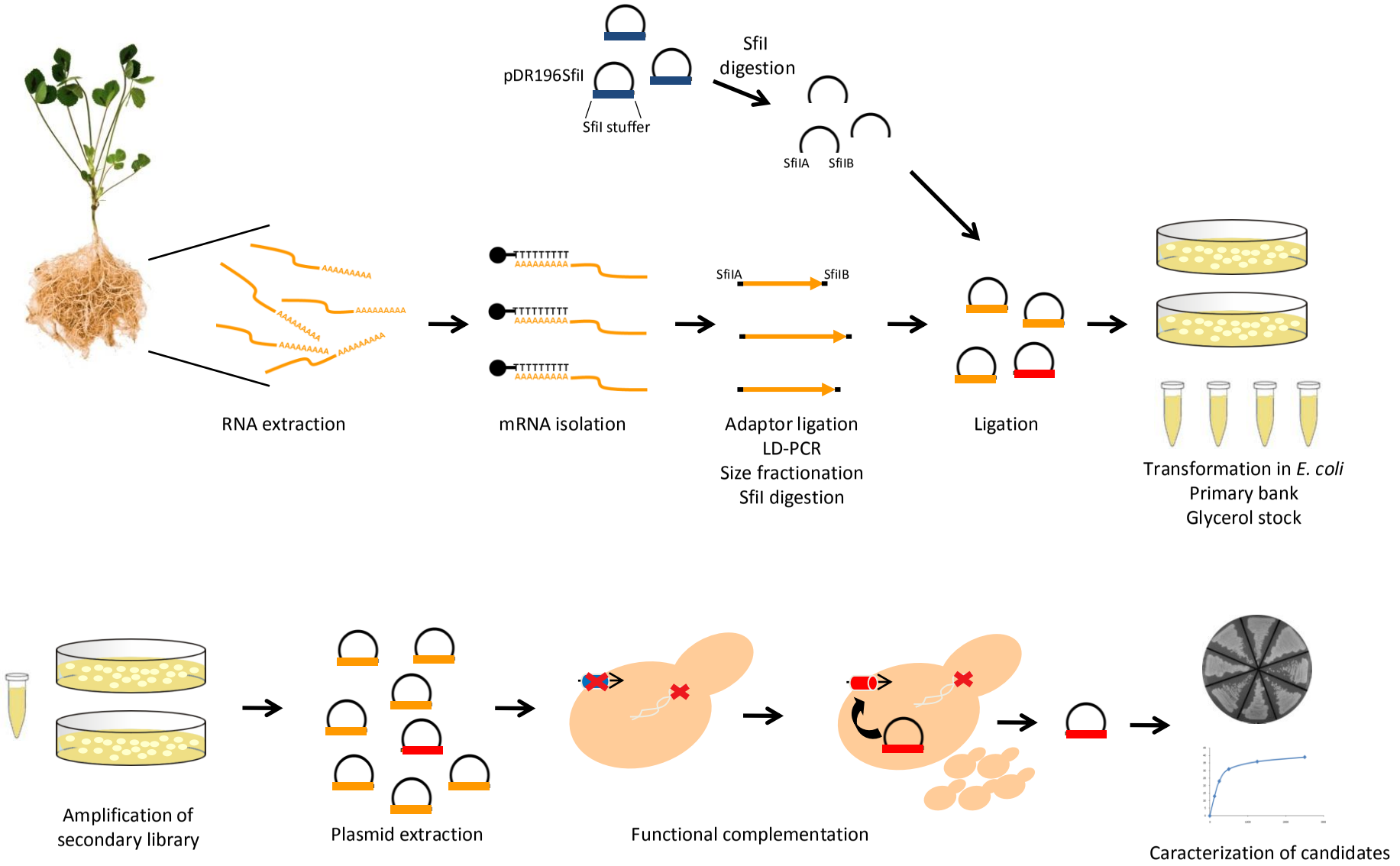


Fig 32. Schematic representation of the construction of the mycorrhized *M. truncatula* - *G. intraradices* expression library and screening for sugar transporter candidates

8 Gene expression analysis

Total RNA was extracted from roots and source leaves (see 2) using SV total RNA isolation system (Promega) according to the manufacturers' instructions. cDNA was produced with SuperScript®III Reverse Transcriptase (see 3.2.3) and cDNA template was diluted 40 times for expression analyses of *MtSUT1-1*, *MtSUT2* and *MtSUT4-1* or 10 times for *MtSUT1-2*, *MtSUT1-3* and *MtSUT4-2*.

Quantitative real-time PCR reactions were performed in a final volume of 15 µL using Absolute SYBR green ROX Mix (Thermo Scientific), 70 nM of gene specific primers (Annex IV.4) and 2 µL of cDNA template in an ABI Prism 7900HT Sequence Detection System (Applied Biosystems). Reaction condition were set as 95°C for 15 min and 40 cycles of 95°C for 15 sec, 60°C for 30 sec, and 72°C for 30 sec. Three biological and three technical repetitions were performed. To check the absence of nonspecific amplicons, dissociation curves were generated at the end of the PCR cycles by heating PCR products from 70 to 95°C and amplicons were cloned in pCR®2.1-TOPO® (Annex V) according to the manufacturers instruction and sequenced (Eurofins MWG, Ebersberg, Germany) to confirm the identity of the amplicon. Primer pair efficiency (90-110%) was estimated for all primer pairs by serial dilutions of the cDNA. Standard PCR (see 3.2.1) was performed on RNA samples to exclude genomic DNA contamination and no-template control (water) were used for each primer pair.

All data were analyzed using the SDS 2.3 software (Applied Biosystems) with a threshold of 0.25 to obtain cycle threshold values. Values were normalized (ΔCt) to the constitutively expressed translation elongation factor (*MtTef1α*, Baier et al. 2010) and expression coefficient were calculated ($2^{-\Delta Ct}$).

9 Construction of a cDNA expression library from *M. truncatula* - *G. intraradices* symbiotic roots

9.1 Isolation of mRNA

Total RNA extracted (for expression analyses in 8) was used to isolate mRNA using the Dynabeads® mRNA Purification Kit (Invitrogen) by the mean of an oligo $(dT)_{25}$ beads that bind polyA tail of mRNA (Fig 32). Bead buffer (400 µL, 2 mg) provided in the kit was placed in an Eppendorf tube and isolated by migration using a MagnaRack™ (Invitrogen, USA). Isolated beads were washed with 200 µL of binding buffer and recovered with the magnet rack. Beads were resuspended in 308 µL of binding Buffer with an equal volume of total RNA (162 µg) coming from 3 biological repetitions for each time of harvest (2, 3 and 4 wpi) and mixed on a roller mixer for 5 min. After this time, beads were recovered by magnet

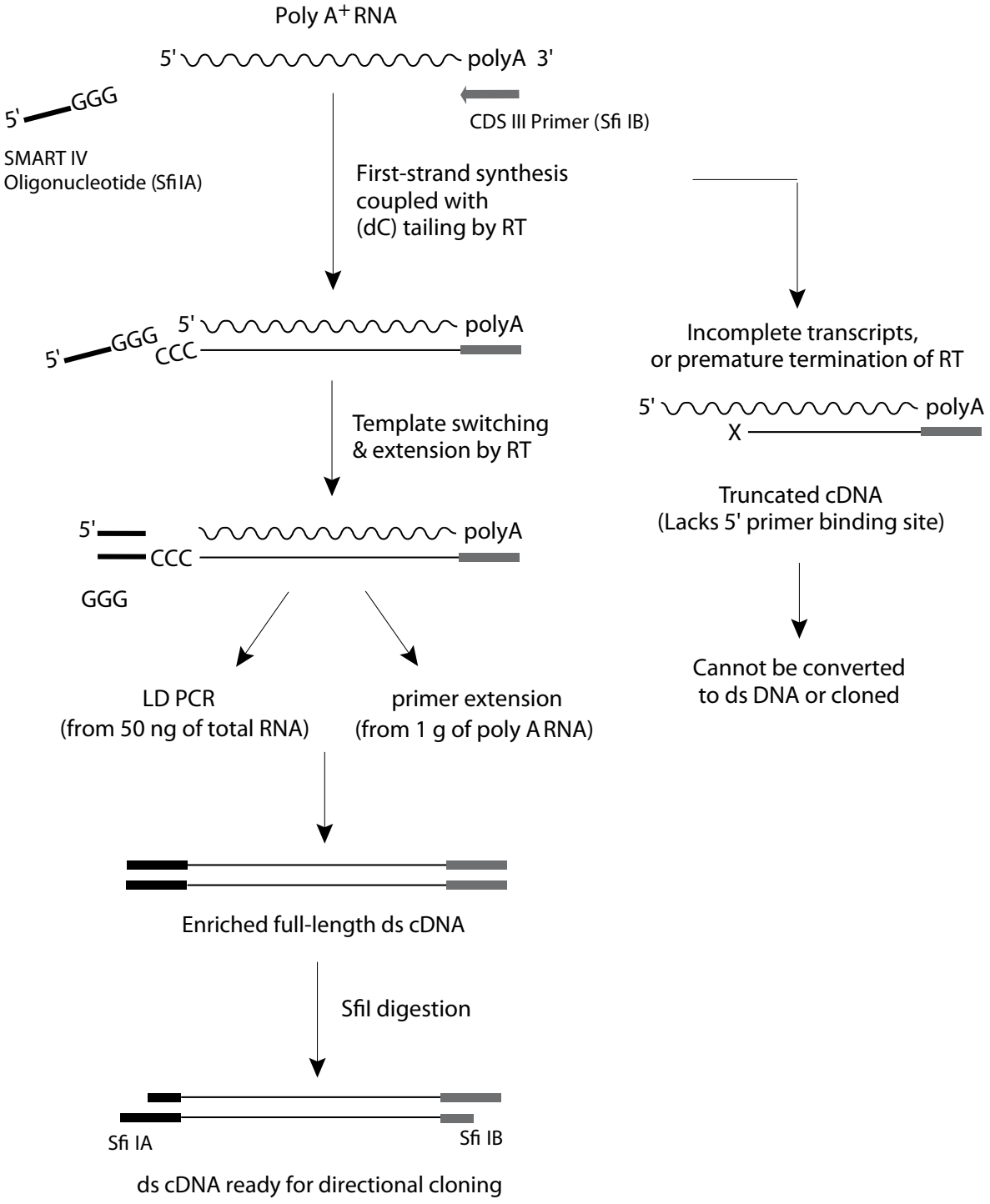


Fig 33. Flow chart of the SMART™ technology (Clontech) procedures to obtain enriched full length cDNA suitable for directional cloning

migration and washed twice with 400 µL of washing buffer. mRNA was then eluted in 30 µL DEPC (50°C) water from the beads bulk on the magnet rack and eluent is transferred to a new Eppendorf tube. mRNA concentration and quality was assessed using a 10^{-2} dilution on a DU® 640 spectrophotometer (Beckman).

9.2 cDNA synthesis by SMART™ technology

cDNA were synthesized from mRNA of mycorrhized roots (see 2) using the creator™ SMART™ cDNA library construction kit (Clontech) which allows production of full-length cDNA ready for directional cloning by the use of SMART primer technology (Fig 33).

First-strand cDNA synthesis was produced starting with the 3µL maximum amount of mRNA (120ng) and 1 µl SMART IV oligonucleotide and 1 µl CDS III/3' PCR primer incubated at 72°C, 2 min and immediately placed on ice. Then, 1X First-Strand Buffer, DTT (2 mM) and dNTP Mix (1 mM) were added and cDNA synthesis was made using SMARTScribe™ reverse transcriptase (100 U, Clontech) at 42°C for 1 hour in a T3 Thermocycler (Biometra). 3 µL of the first-strand cDNA synthesis reaction is transferred to a new Eppendorf tube and cDNA amplification by long distance PCR (LD-PCR) was performed with 1X Advantage 2 PCR Buffer, 1X dNTP Mix, 3 µl 5' PCR Primer, 3 µl CDS III/3' PCR primer and 1 X Advantage 2 Polymerase Mix and adjusted to a final volume at 150 µL with water. The LD-PCR cycling program were carried in a T3 Thermocycler (Biometra) with an initial denaturation step at 95°C, 1 min followed by 24 cycles at 95°C, 15 and 68°C, 6 min. First strand synthesis and LD-PCR (18 cycles) was also carried out with a positive polyA mRNA control from human placenta (1 µg) and was run on an electrophoresis gel alongside the cDNA produced from mycorrhized roots (Fig 53).

The total cDNA reaction (150 µL) is treated by a proteinase K digestion (6 µL, 120 µg) and incubated at 45°C for 20 min. DNA is precipitated with an equal volume of phenol:chloroform:isoamyl alcohol (25:24:1) mixed for 2 min, centrifuged at 14000 rpm for 5 min and top aqueous phase is transferred to a new tube and mixed with 100 µL chloroform:isoamyl alcohol (24:1), centrifuged at 14000 rpm for 5 min. Top aqueous phase is transferred to a new tube and mixed with 10 µL of 3 M sodium acetate, 1.3 µL of glycogen (20 µg/µL) and 260 µL of room-temperature 95% ethanol and centrifuged at 14000 rpm for 20 min; pellet was washed with 100 µL of 80% ethanol prior to a final elution in 50 µL water.

9.3 Digestion and ligation of cDNA into pDR196SfiI vector

In this step, cDNA produced by SMART technology (from 9.2) and the pDR196SfiI vector (Fig 30) are digested by SfiI restriction digestion to release SfiIA and SfiIB sites for directional cloning of cDNA in the yeast expression vector (Fig 32).

The total cDNA reaction produced by SMART technology was digested with 4 µL SfiI (80U, New England Biolabs), 1X NEB buffer 4 and 1X bovine serum albumin in a final volume of 60 µL during 4,5 hours at 50°C. The complete reaction was run on an electrophoresis agarose gel (1%) prepared with Crystal Violet (2 mg/mL) from the S.N.A.P. UV-Free Gel Purification Kit (Invitrogen) alongside 1 KB Plus DNA Ladder (Invitrogen) and cDNA was size fractionated by cutting 5 parallel gel bands between 700 bp to 5000 bp. Gel slices were purified using the Qiaquick® gel purification kit (Qiagen) according to the manufacturers' instruction and eluted in 70 µL water per purification reaction. The total size fractionated cDNA (350 µL) was precipitated with 970 µL ethanol 95% and 35 µL NaAc (3M, pH 5,2), centrifuged at 14000 rpm for 20 min, washed in 150 µL ethanol 80% and pellet was resuspended in 10 µL water. cDNA concentration was estimated on a Nanodrop 1000 (Thermo Scientific) to 196 ng/µL; size and integrity was checked on an Agilent DNAchip 7500 (Agilent) according to the manufacturer instruction (Fig 53b-c).

In parallel, pDR196SfiI plasmid (80 µg) was digested with 12 µL (240 U, New England Biolabs) 1X NEB buffer 4 and 1X bovine serum albumin in a final volume of 500 µL during 3,5 hours at 50°C. Three gel slices corresponding to the linearized vector (6419 bp) were excluded from the SfiI Clontech stuffer (1433 bp, Fig 30) and purified using the Qiaquick® gel purification kit (Qiagen) according to the manufacturers' instruction. The digestion restriction by SfiI restriction was repeated to ensure complete digestion of the plasmid. The digested plasmid (210 µL, 70 µg) was mixed with 8 µL (160 U, New England Biolabs) 1X NEB buffer 4 and 1X bovine serum albumin in a final volume of 250 µL during 3,5 hours at 50°C and gel purification was also repeated to recover linearized plasmid in 50 µL water. Complete linearization of the plasmid was checked on an Agilent DNAchip 7500 (Agilent) according to the manufacturer instruction (Fig 53b) and by transformation of 140ng in One shot® Top10 bacteria.

9.4 Ligation of cDNA into pDR196SfiI vector

After SfiI digestion and having checked cDNA and linearized plasmid integrity (see 9.3). The SMART technology allows directional insertion of full-length cDNA from symbiotic roots into the yeast expression pDR196SfiI vector thanks to SfiIA and SfiIB sites (Fig 32 and 33). Linearized pDR196SfiI (250 ng) was mixed with SfiI digested cDNA (196 ng), 1X T4 ligase buffer, ATP (1mM) and T4 DNA ligase (400U, Clontech) adjusted to a final volume of 10µL with water and incubated at 16°C overnight.

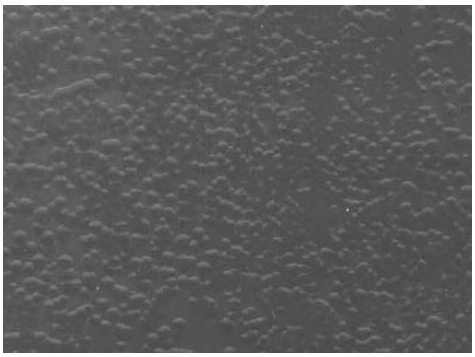


Fig 34. Titration of the primary library

200 µL of a $5x10^{-5}$ dilution of the primary library stock was spread on large LB ampicillin plates and incubated at 37°C, 18 hours to obtain adjacent colonies with 5000-10000 clones/plate.

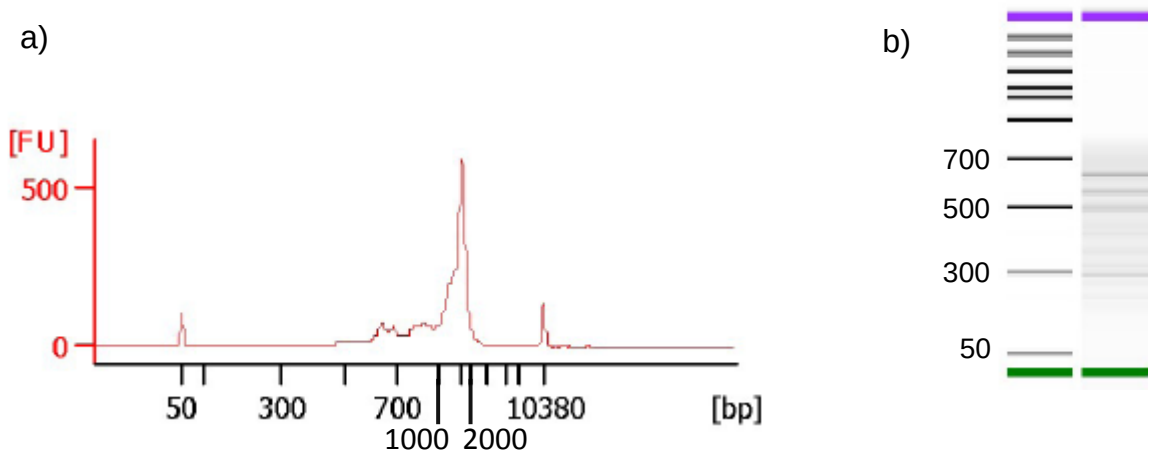


Fig 35. Size fractionation of the expression library inserts for pyrosequencing preparation

a) Chromatogram profile of cDNA inserts after exclusion of the pDR196SfiI vector backbone. No residual presence of the vector backbone (6419 bp) could be detected.

b) Gel profile of cDNA inserts digested (by HaeIII, HpaII, HinfI, MboI, MseI, RsaI and TaqI), pooled and size fractionated between 200-700 bp.

9.5 Generation of the primary library

The resulting ligation reaction was transformed in ultracompetent *E. coli* XL10-Gold® (Fig 32; Stratagene). Three independent transformations containing each 2µL of the ligation is thawed on ice with 100µL aliquots of bacterial cells and 4µL of β-mercaptoethanol mix (provided in the kit), incubated on ice for 30 min. Cells are heat-shocked 30 sec at 42°C and immediately placed on ice for 2 min. The reaction is transferred in 0.9 mL of preheated (42°C) NZY+ broth in 12 mL Falcon and incubated at 37°C for 1 hour with shaking at 225 rpm. 200 µL of transformation reaction was spread on 20 LB ampicillin plates and incubated at 37°C, 18 hours to obtain 5000-10000 clones per large Petri dishes (145 mm). All bacterial clones were harvested with a glass hockey stick in a mix of 50% liquid LB and 50% glycerol to obtain the primary library in 100 tubes of glycerol stock stored at -80°C (Fig 32).

10 Screening of the *Geosiphon pyriformis* cDNA expression library

10.1 Amplification of a secondary library

Primary library constructed from *G. pyriformis* bladder (Martin 2005; Schüßler et al. 2006) was titered to an appropriate dilution (200µL of a $5x10^{-5}$; Fig 34) of the primary library stock which was spread on 20 LB ampicillin plates and incubated at 37°C, 18 hours to obtain 5000-10000 clones per large Petri dishes (145 mm) in order to ensure complete representation of the primary library. This secondary library was harvested with a glass hockey stick in 50 mL of liquid LB and bacterial cells were harvested by centrifugation at 6000 rpm for 5 min. Total plasmid were extracted using HiSpeed Plasmid Maxi Kit (Qiagen) according to the manufacturer's instruction and concentration was estimated on a Nanodrop 1000 (Thermo Scientific)

10.2 Screening of the *G. pyriformis* library for glomeromycotan sucrose transporter

Plasmid extracted from the *G. pyriformis* cDNA expression library (see 10.1) were used to transform the YSL2-1 yeast strain deficient for sucrose uptake according to the high efficiency plasmid transformation described in 3.4.2 with a 20-fold scaled up protocol adapted to transform 20µg of plasmid library. Resulting transformation (150 µL) were spread on 40 plates containing synthetic minimal sucrose without uracil. Transformants isolated from this screen were replated on synthetic minimal sucrose (2%) medium without uracil to confirm the complementation deficiency alongside the YSL2-1 clone transformed with pDRf1-GW harboring MtSUT4-1 as positive control. Plasmid from yeast transformants complementing the sucrose deficiency, were extracted (see 3.1.3) followed by PCR (see 3.2.1)

and sequencing (LMU sequencing service, Munich) with PMA forward and ADH close reverse primers.

10.3 Preparation of *G. pyriformis* library cDNA samples for pyrosequencing

Total plasmid extraction (200 µg) containing cDNA of *G. pyriformis* library (see 10.1) was digested by SfiI digestion, with 20 µL SfiI (400U, New England Biolabs), 1X NEB buffer 4 and 1X bovine serum albumin in a final volume of 750 µL during 3,5 hours at 50°C. The digestion reaction was concentrated using YM-30 Microcon® (Millipore) by centrifugation 15 min at 1000g and microcon reservoir was placed upside-down in a new Eppendorf tube centrifuge 3 min at 1000g to elute DNA in 200 µL water. In order to recover cDNA, the concentrated digestion reaction was run on an electrophoresis agarose gel (1%) prepared with Crystal Violet (2 mg/ml) from the S.N.A.P. UV-Free Gel Purification Kit (Invitrogen) and cDNA were excluded from the digested vector backbone (6419 bp) by gel-cutting 8 parallel gel slices, purified with the Qiaquick® gel purification kit (Qiagen) according to the manufacturers' instruction and eluted in 40 µL water per purification reaction. Resulting eluents (320 µL) were concentrated using YM-30 Microcon® (Millipore) by centrifugation 10 min at 1000g and eluted in 200 µL water. cDNA concentration was estimated on a Nanodrop 1000 (Thermo Scientific) to 97 ng/µL; size and integrity was checked on an Agilent DNAchip 7500 (Agilent) according to the manufacturer instruction (Fig 35a).

To obtain an optimal reading length of 400 to 600 bp recommended for 454 pyrosequencing (Roche), digestion of cDNA samples was carried out using restriction enzymes (2U). Each 4 cutters enzyme (HaeIII, HpaII, HinfI, MboI, MseI, RsaI and TaqI, New England Biolabs) was used in independent digestion reaction with 16 µL library cDNA (1,6 µg), using appropriate NEB buffer (1X) to a final volume of 20 µL and digested during 1 hour with respective enzyme incubation temperature. The digestion was immediately stopped by heating the reaction 10 min at 80°C and digestion was checked on an Agilent DNAchip 7500 (Agilent) according to the manufacturer instruction. Digestion reactions were pooled and run on an electrophoresis agarose gel (1%) prepared with Crystal Violet (2 mg/ml) from the S.N.A.P. UV-Free Gel Purification Kit (Invitrogen) and size fractionated between 200-700 bp by cutting 2 parallel gel slices. Gel slices were purified using the Qiaquick® gel purification kit (Qiagen) according to the manufacturers' instruction and eluted in 15 µL water per purification reaction. Samples were pooled (30 µL), DNA concentration was estimated on a Nanodrop 1000 (Thermo Scientific) to 33 ng/µL and correct size fractionation was checked on an Agilent DNAchip 7500 (Fig 35b, Agilent).

11 Methods for sugar analyses

11.1 HPAE-PAD analysis of soluble sugars

Soluble sugar composition of plant material (see 2) was determined as part of a collaboration with G. Alcaraz (UMR Agroécologie, Dijon). After freezing and grinding *M. truncatula* source leaves, petioles and roots in liquid nitrogen followed by one day of lyophilisation, samples were conserved in a dry cabinet in sealed glass tubes. The lyophilized powder (60 mg) was treated during 30 min by liquid extraction with 1 mL 80% (v/v) ethanolic solution in an ultrasonic bath at 80°C. After centrifugation (10 min at 15000 g), 10 µL of supernatant were injected on an ICS 3000 Dionex instrument (Dionex Corp., Sunnyvale, CA, USA). The elution was made by a gradient of NaAc 100 mM in NaOH 75 mM eluent and soluble sugars contents were determined by high-performance anion exchange-pulsed amperometric detection (HPAE-PAD) using a guard and analytical column CarboPac PA100 (Dionex P/N043055), according to the Dionex instructions [Dionex Application Note 122 (2004)]. Five biological repetitions were performed for each condition.

11.2 Benedict's test

To test the presence of reducing sugars, 3 mL of Benedict's reagent (Na_2CO_3 940mM, sodium citrate 733mM and $CuSO_4$ 80 mM) is placed in a glass test tube with the respective solution (1 mL): sucrose stock solution (50%), synthetic minimal sucrose medium without uracil (Annex I) as solution to be tested and glucose stock solution (40%) and sucrose with the addition of a drop of HCl 0.1M as positive controls. The Benedict's reaction is performed by placing test tube in a boiling water bath for 5 min and positive tests are visualized by the presence of a brick red precipitate of copper oxide while negative test keep the original blue copper color of the Benedict's solution (Fig 52b).

Chapter I

In silico search for putative sugar transporters from both partners of the arbuscular mycorrhizal model association between *Medicago truncatula* and *Glomus intraradices*

Chapter I

In silico search for putative sugar transporters from both partners of the arbuscular mycorrhizal model association between *Medicago truncatula* and *Glomus intraradices*

In plant, sugar fluxes are coordinated by different transport systems with gene expression differentially regulated according to the type of plant organs, the developmental stage and the environmental conditions (see Introduction 3.; Lalonde et al. 2004; Kühn and Grof 2010). With the release of the complete genomes of the *A. thaliana* ecotype Columbia (The *Arabidopsis* Genome Initiative 2000), the rice *japonica* subspecies cultivar Nipponbare (Ouyang et al. 2007) and the grape Pinot Noir variety (Jaillon et al. 2007); complete families of transport proteins have been identified from these model species. Indeed, exhaustive surveys led to the identification of 53 monosaccharide transporters in *A. thaliana* (AtMSTs), 65 in rice (OsMSTs) and 59 in grapevine (VvMSTs) as well as 9 sucrose transporters in *A. thaliana* (AtSUTs), 5 in rice (OsSUTs) and 4 in grapevine (VvSUTs) (Aoki et al. 2003; Buttner 2007; Johnson and Thomas 2007; Sauer 2007; Afoufa-Bastien et al. 2010). Although the soybean and *Lotus japonicus* genomes have been released (Sato et al. 2008; Schmutz et al. 2010) and also, very recently, the version Mt3.5 of the genome of *M. truncatula* cultivar Jemalong A17 (Young et al. 2011), complete sugar transporter families have not yet been identified from any leguminous species. Indeed, at the beginning of my PhD project, a single monosaccharide transporter from *M. truncatula* was published (Mtst1; Harrison 1996). Thereafter, an additional MtMST (MtHext1) and a single MtSUT (Mtsut1) have been reported (Gaude et al. 2011). Therefore, the first part of my research project was to search for sugar transport proteins through available *in silico* databases containing sequences of *M. truncatula* using BLAST program with available transporter accessions from *A. thaliana* (http://aramemnon.botanik.uni-koeln.de/). By this mean, we identified the monosaccharide and sucrose transporter families from the model legume *M. truncatula*. Indeed, phylogenetic analyses revealed that identified candidates fall into 2 distinct groups: the MST and SUT families; respective MtMSTs and MtSUTs have been studied in detail.

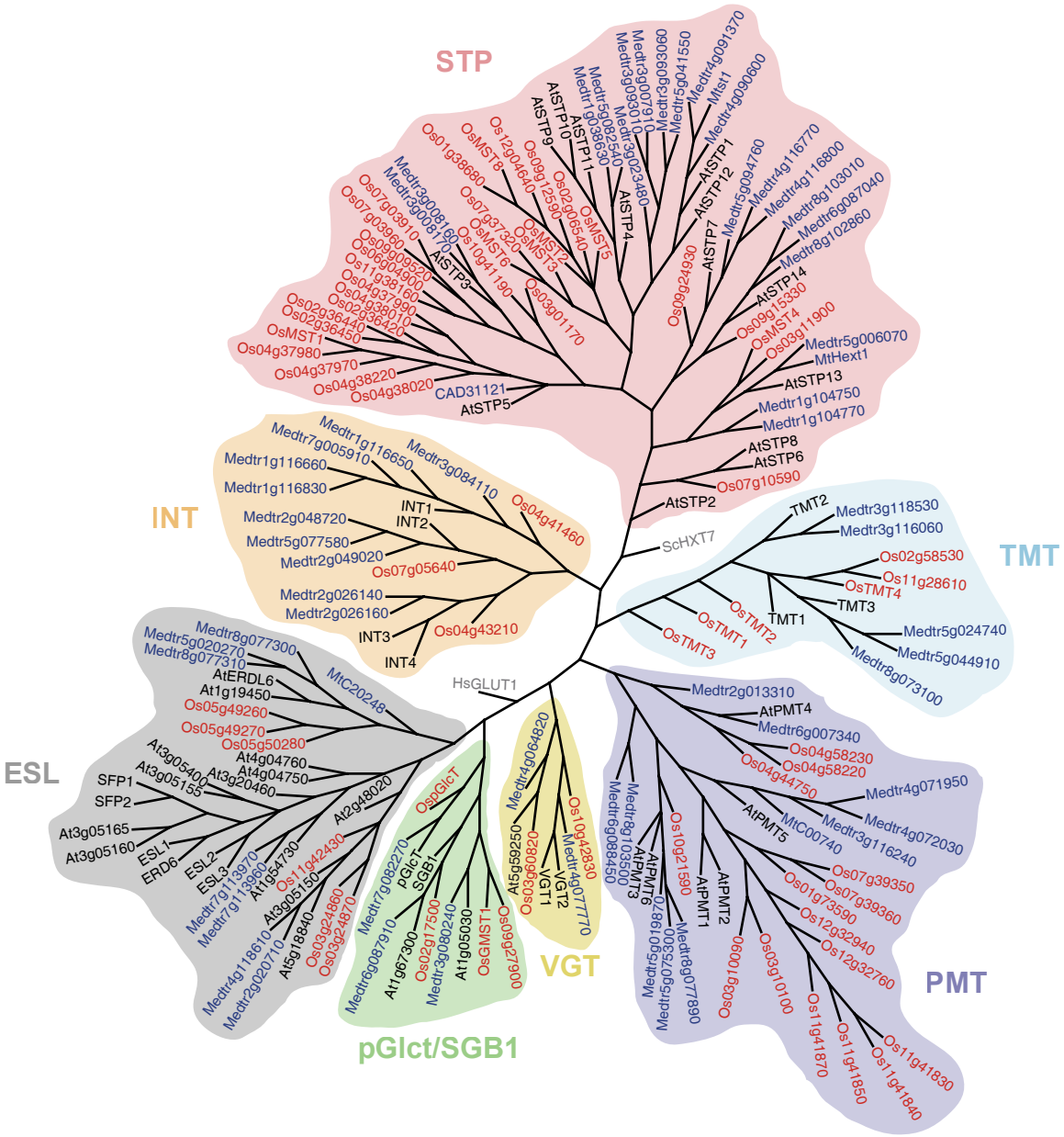


Figure 36. Phylogenetic tree of the MST family in *A. thaliana*, *M. truncatula* and rice

Maximum parsimony consensus tree separates the MST family into 7 sub-families (highlighted in colors) and comprises 183 plant accessions, 53 AtMSTs from *A. thaliana* (black), 62 MtMSTs from *M. truncatula* (blue) and 68 OsMSTs from *Oriza sativa* (red) and the human and yeast transporters (grey). Alignment of amino acid sequences of MSTs was performed and maximum parsimony analyses were done with heuristic tree searches were executed using 1000 random sequence additions and the tree bisection-reconnection branch-swapping algorithm with random sequence analysis. The complete alignment was based on 1638 sites; 869 were phylogenetically informative. Characterized and published proteins are named according to their latest denomination; genomic locus or accession number was used for proteins which have not yet been assigned.

53 AtMSTs were identified in the genome of *A. thaliana* accession Columbia (Buttner 2007); AtSTP1 (At1g11260), AtSTP2 (At1g07340), AtSTP3 (At5g61520), AtSTP4 (At3g19930), AtSTP5 (At1g34580), AtSTP6 (At3g05960), AtSTP7 (At4g02050), AtSTP8 (At5g26250), AtSTP9 (At1g50310), AtSTP10 (At3g19940), AtSTP11 (At5g23270), AtSTP12 (At4g21480), AtSTP13 (At5g26340), AtSTP14 (At1g77210),TMT1 (At1g20840), TMT2 (At4g35300), TMT3 (At3g51490), AtPMT1 (At2g16120), AtPMT2 (At2g16130), AtPMT3 (At2g18480), AtPMT4 (At2g20780), AtPMT5 (At3g18830), AtPMT6 (At4g36670), VGT1 (At3g03090), VGT2 (At5g17010), pGlcT (At5g16150), SGB1 (At1g79820), ERD6 (At1g08930), AtERDL6 (At1g75220), ESL1 (At1g08920), ESL3 (At1g08890), ESL2 (At1g08900), SFP1 (At5g27350), SFP2 (At5g27360), INT1 (At2g43330), INT2 (At1g30220), INT3 (At2g35740), INT4 (At4g16480).

58 MtMSTs were identified in the genome of *M. truncatula* cultivar Jemalong A17; MtHext1 (Medtr1g104780). An additional accession, Mtst1 (AAB06594) was identified from a different subpopulation (2828) of *M. truncatula* Jemalong (Harrison 1996). 3 additional accessions from the A17 line were not found on the current version of the genome; CAD31121 was retrieved from genbank; MtC20248 and MtC00740 were retrieved from MENS.

65 OsMSTs were identified in the genome of rice *japonica* subspecies cultivar Nipponbare (Johnson and Thomas 2007); OsTMT1 (Os10g39440), OsTMT2 (Os02g13560), OsTMT3 (Os03g03680), OsTMT4 (Os11g40540), OsMST2 (Os03g39710), OsMST3 (Os07g01560), OsMST5 (Os08g08070), OsMST8 (Os01g38670), OsGMST1 (Os09g23110), OspGlcT (Os01g04190). An additional accession, OsMST1 (BAB19862) was identified from the cultivar Nipponbare but was not found on the current version of the genome. 2 additional accessions, OsMST4 (AAQ24871) and OsMST6 (AAQ24872) were identified from the rice *indica* subspecies.

Outgroups: HsGLUT1 (NP_006507), ScHXT7 (NP_010629).

1 Database mining for sugar transporter candidates in *M. truncatula*

1.1 Identification of the MST family of *M. truncatula:* MtMST

A total of 62 sequences coding for putative full-length MtMSTs were identified in the model leguminous *M. truncatula*. Forty-six accessions were initially identified from BACs of the version Mt2.0 of the genome and the final search performed on last version (Mt3.5) led to the identification of 12 additional MtMSTs. Therefore, 58 MtMSTs are present in the current annotation version of the genome of *M. truncatula*. Further 4 accessions were identified from other databases (NCBI, MENS) and are absent from the current genome annotation (Mt3.5). This is the case for accessions: Mst1 (Harrison 1996), CAD31121, MtC20248 and MtC00740 (Doidy et al. 2012a). In this study, only sequences with a full-length coding DNA sequence (CDS) have been kept for correct alignment. Indeed, ESTs and partial contigs retrieved from this *in silico* search were not retained. The 62 full-length, sequences were aligned using MAFFT. The protein sequence of Mtst1 (Harrison 1996) showed a 99,8% identity with Medtr4g091370 and a single AA difference; however when nucleic acid sequences were aligned both CDS differed from 4 distant base pairs 3 of which being silent mutations. Since Mtst1 was identified from a different subpopulation (2828 instead of A17) of *M. truncatula* Jemalong (Harrison and Dixon 1993; Harrison 1996), it seems that Mtst1 is an ortholog of Medtr4g091370. For this reason, Mtst1 will not be included from discussion regarding MtMSTs of the sequenced *M. truncatula* cv Jemalong A17. In conclusion, at least 61 MtMSTs are present in the genome of *M. truncatula* cv Jemalong A17.

Including all retrieved full-length MtMSTs, a subsequent alignment with available MST protein sequences from the model species *A. thaliana* and rice was performed and a consensus maximum parsimony tree was constructed from these 183 plant MSTs (Fig 36). As previously shown, MtMSTs fall into 7 clades within the plant MST family and therefore it seems that all plant species possess a large family of MST genes (see Introduction 3.3.1). Out of the 61 identified MtMSTs, 22 belong to the STP family, 2 to the VGT subfamily, 5 to the TMT subfamily, 3 to the pGlcT / SGB1 subfamily, 11 to the PMT subfamily, 10 to the INT subfamily and 8 to the ESL subfamily (Fig 36). In contrast with previous findings from Dycotyledonous species (Buttner 2007; Afoufa-Bastien et al. 2010), the ESL clade is not the largest one in *M. truncatula*. Here, the largest subfamily of MtMST is the STP clade as previously observed for monocotyledonous species (Johnson and Thomas 2007). In this study, we report for the first time an exhaustive search for MSTs from the newly released *M. truncatula* genome, which also represent the first overview of a MST family from a leguminous species. In the near future, identification of sugar transporters from related sequenced leguminous species such as soybean and *L. japonicus* as well as complete

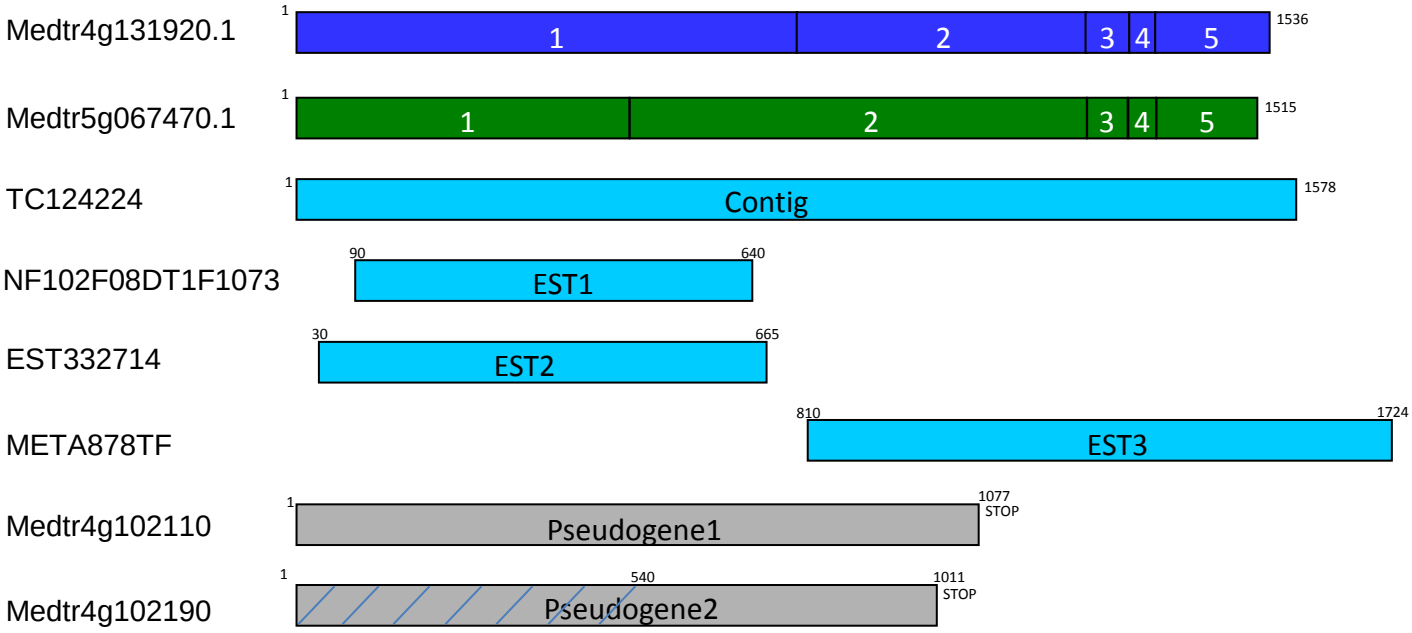


Fig 37. MtSUT candidates retrieved from *in silico* analysis

Genomic loci with exons (in white number) are represented in dark blue and green; the full length contig and 3 ESTs in light blue and the 2 pseudogenes in grey. Pseudogene2 (Medtr4g102190) presents an aberrant sequence in its first half (dash). All sequences are in scale and numbered (bp) to highlight alignment .

characterization of MSTs already identified from model species will help to decipher the role and implication of respective MtMSTs.

1.2 Identification of the SUT family of *M. truncatula:* MtSUT

A similar *in silico* search was performed for putative MtSUTs in databases containing accessions from *M. truncatula* using BLAST program with identified AtSUTs and published leguminous SUTs from soybean (GmSUT1), *L. japonicus* (LjSUT4), pea (PsSUT1, PsSUF1 and PsSUF4), common bean (PvSUF1 and PvSUT3) and broad bean (VfSUT1). By this mean, two genomic loci have been identified from Mt3.5 (Medtr4g131920 and Medtr5g067470); both loci were already annotated in the annotation version 2.0 (Table 7). Two additional tandem genomic loci (Medtr4g102110, Medtr4g102190) have also been identified (Table 7); however, both open reading frames are interrupted by a stop codon and therefore encode for truncated proteins of 358 and 336 respectively (Fig 37). In addition, the second pseudogene seems to present an aberrant pattern when analyzed (blastp) against previously characterized SUTs. Interestingly, *A thaliana* also possesses two pseudogenes (*AtSUC6*, *AtSUC7*) encoding for truncated proteins in its genome (Sauer et al. 2004). In addition, a full-length contig TC124224 as well as 3 partial ESTs (META878TF, EST332714 and NF102F08DT1F1073) were identified in the general database DFCI (Fig 37). In conclusion, 6 non contiguous putative MtSUTs were identified; however, only two of them are being annotated as full-length loci in the current genome annotation (Fig 37). The full MtSUT family will be analyzed in detail and discussed in the second chapter of this manuscript.

2 Database mining for sugar transporter candidates in *G. intraradices*

As the UMR Agroécologie laboratory (Dijon) is member of the international *Glomus* genome consortium, an access to preliminary genomic data was available and a search for sugar transporter candidates was performed by blastn and the use of selected key words. BLAST program was based on gene similarity using the only available glomeromycotan sugar transporter at the time (GpMST1; see Introduction 4.3.3.3) and other fungal sugar transporters: the 15 sugar porter family members of *Laccaria bicolor* (Fajardo Lopez et al. 2008), the fructose transporter *BcFRT1* (*Botrytis cinerea*; Doehlemann et al. 2005) and *SpSUT1* (*Schizosaccharomyces pombe*; Reinders and Ward 2001). By this mean, we identified in total, 12 accessions corresponding to putative sugar transporters from *G. intraradices* (Table 5); consistent with this, all candidates (excepted Gi.7648_C1) present the conserved domains of the MFS superfamily (pfam0083, sugar and other transporter). All

Accession	Synonymous accessions	GC%	Length (bp)	Blastx vs non redundant sequences	Pfam id
Glomus_c6242/GiMST4	CCHU12268, Gi.4727_C1, 11118_1_CACE, Glomus_c1670	32	871	Glucose transporter *Neosartorya fischeri*	pfam00083
CCHU7489.b1		31	737	Similar to MGC84927 protein *Ciona intestinalis*	pfam00083
11118_0_CCHU9778.g1_CACE		27	508	Conserved hypothetical protein *Aspergillus terreus*	pfam00083
Glomus_c4574		29	741	Hypothetical protein *Coprinopsis cinerea*	pfam00083
Gi.7648_C1		34	812	Hypothetical protein *Dictyostelium discoideum*	pfam05631
Gi.8181_C1		34	626	Unnamed protein product *Tetraodon nigroviridis*	pfam00083
CCHU19976.1b		45	595	Hypothetical protein *Sclerotinia sclerotiorum*	pfam00083
Glomus_c10829		57	836	Maltose permease *Aspergillus fumigatus*	pfam00083
CCHU1597.b2	16415_0_CCHU1597.b2_CACE	50	724	Predicted protein *Aspergillus terreus*	pfam00083
Glomus_c7463	10913_1_CACE	49	676	Hexose transporter protein *Aspergillus fumigatus*	pfam00083
Glomus_c8881	9602_1_CACE	53	546	Hypothetical protein *Neurospora crassa*	pfam00083
CCHU18383.b1	16504_0_CCHU18383.b1_CACE	54	773	Monosaccharide transporter *Aspergillus fumigatus*	pfam00083

Table 5. GiMST candidates retrieved from *in silico* analysis

Potential contaminants withdrawn from analysis are highlighted in red.

accessions present a short nucleic sequence (<900bp) and seem to correspond to partial nucleic CDS of transporters since BLAST result indicate incomplete coverage compared to characterized full-length transporters. In addition, we identified numerous sequences closely related to sugar transporters from the phylum Ascomycota. After verification, these sequences seem to originate from an *Aspergillus* sp. contamination present in the initial samples which have been used to generate the DNA sequences (Table 5). As the *G. intraradices* genome presents a very low GC content (~30%), candidates showing a high GC% as well as a BLAST result coherent with bacterial transporters were considered to originate from the *Aspergillus* sp. contamination and were withdrawn from this analysis (Tisserant et al. 2011). Thereby, in the genome of *G. intraradices,* 6 partial sequences coding for new putative GiMSTs were retained for further study (Table 5).

3 Database mining for candidate differentially expressed in response to mycorrhiza

3.1 MtMSTs differentially expressed in AM symbiosis

Once putative sugar transporter sequences were retrieved, I searched for candidates differentially expressed in response to AMF inoculation through microarray database. For *M. truncatula* candidates, this search was performed on the MtGEA web server that archives all publically available gene expression data derived from the use of the Affymetrix GeneChip comprising 61278 probe sets of which 32167 designed on sequences from the *M. truncatula* EST database and 18733 were based on gene predictions from the genome version Mt2.0 (Benedito et al. 2008). *M. truncatula* transcript profiles associated with AM symbiosis were studied using this GeneChip hybridized with RNA from non mycorrhizal roots or mycorrhizal ones, with *G. intraradices* (Gomez et al. 2009). By this mean, 3 MtMST candidates were identified as differentially expressed in response to AM inoculation; 2 of which are downregulated (Medtr3g116060; CAD31121). Medtr3g116060 is a homolog of TMT2 while CAD31121 belongs to the STP subfamily and is homologous to AtSTP5 (Fig 36 and 38). A single candidate belonging to the STP subfamily and ortholog of Mtst1 (Medtr4g091370) is upregulated in response to AM (Fig 38). In addition Medtr4g091370 also showed a high expression level in the *M. truncatula* rhizobial symbiotic samples. However, no transcription profile corresponding to MtMST in the MtGEA showed a mycorrhizal specific expression. A similar search was performed for MtSUT differentially expressed in response to AM and is presented in the third chapter of this manuscript (Fig 49; see Chapter II 2.3 and Chapter III 4.2).

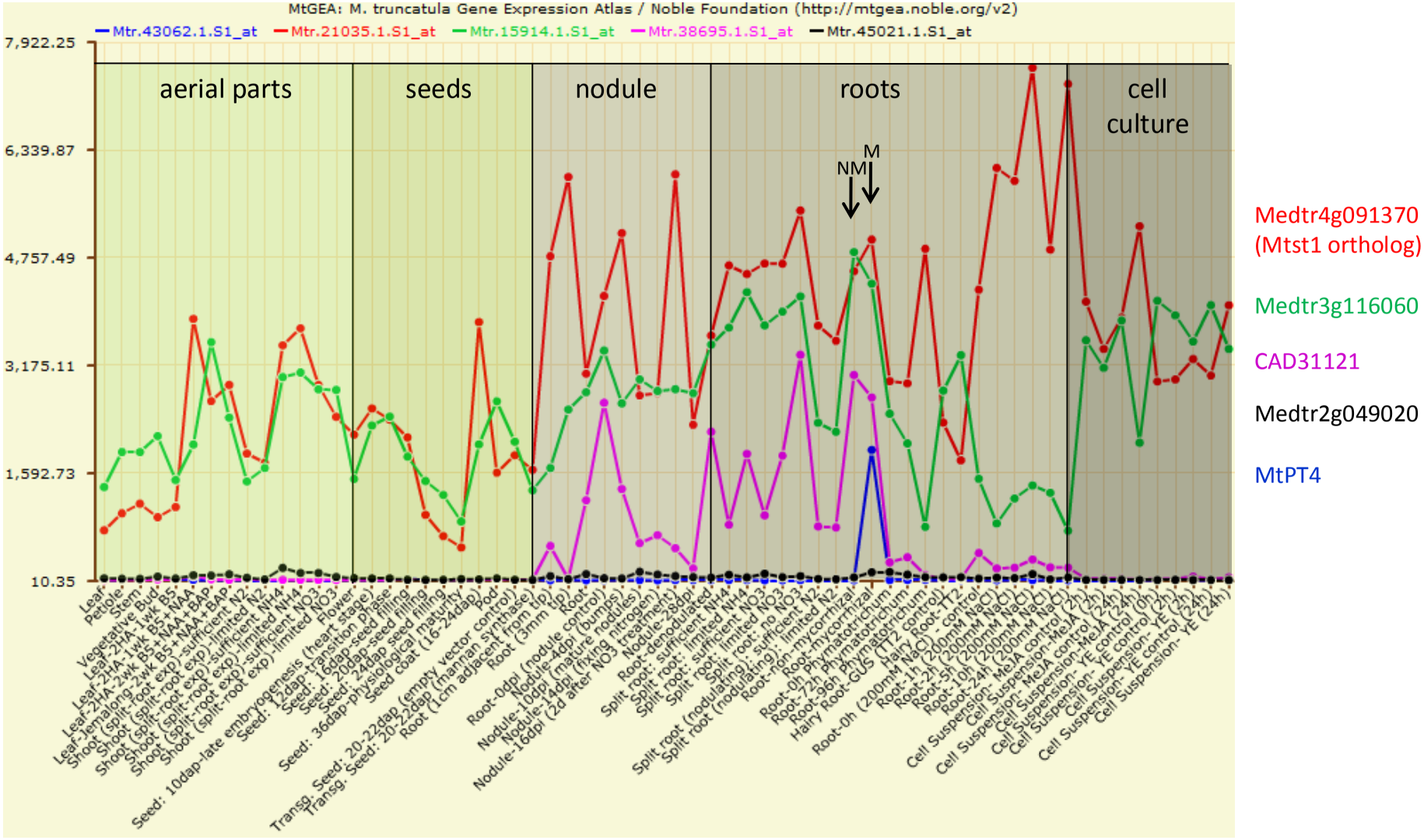


Fig 38. Expression profiles of *MtPT4* and *MtMSTs* differentially regulated in response to AM from MtGEA microarray data

M and NM arrows correspond to mycorrhizal and non-mycorrhizal root samples respectively**.**

3.2 GiMSTs differentially expressed in AM symbiosis

The *G. intraradices* expression microarray (4 x 72K) was designed on the basis of 25 906 non-redundant virtual transcripts using NimbleGen chip with samples from extraradical mycelium, symbiotic roots, germinated and exudates spores (Tisserant et al. 2011). On these data, the 6 accessions considered as contaminants show very low expression signals in analyzed samples confirming their extrinsic origin. However, significant expression signals could be monitored from laser microdissected arbusculated cell samples of *M. truncatula*; therefore, this dataset should be further interpreted with precaution (Table 5 and 6). Regarding the 6 remaining accessions, I searched for GiMST candidates presenting significant differential expression profiles (0.5 < ratio > 2) between available conditions (Table 6). Two candidates showed interesting expression patterns in arbusculated cells. Indeed, *Glomus_c4574* shows a high expression level in both *M. truncatula* and rice arbusculated cells and is not expressed in extraradical structures (Table 6). Therefore, *Glomus_c4574* is only expressed in symbiotic structures that are directly in contact with plant tissues and especially at the arbuscular interface. In contrast, the second candidate (*11118_0_CCHU9778.g1_CACE*) presents a low expression profile in both arbusculated cell types but still shows its highest expression level in intraradical structures (Table 6). In conclusion, both accessions seem to be the most promising candidates likely to be involved in the regulation of sugar transfer at the plant-fungal interface.

4 Conclusion

Sugar is the primary source of energy, sucrose being the main form for plant sugar transport and monosaccharides (mainly glucose) the transferred carbohydrates to AMF at the symbiotic interface. Therefore, we perform database mining for sucrose and monosaccharide transporters (MSTs and SUTs) involved in carbon partitioning from photosynthetic source leaves of *M. truncatula* towards *G. intraradices*, the heterotrophic fungal symbiont which colonizes sink roots. In total, 6 MtSUTs, 62 MtMSTs from *M. truncatula* as well as 6 GiMSTs from *G. intraradices* were identified; candidates presenting differential expression profiles in this model plant-fungal interaction were pinpointed and their respective role in sugar partitioning within and between organisms is discussed (see Discussion). In conclusion, this manuscript presents a first milestone for the study of plant and fungal sugar transporter families in the widely used AM symbiotic model between *M. truncatula* and *G. intraradices*.

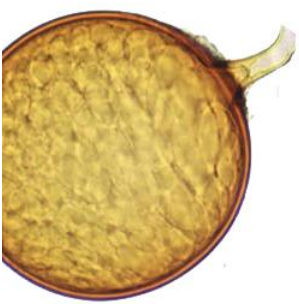
Accession	ERM	IRM	Arbuscule Mt	Arbuscule rice	Spore	ERM/Spore	IRM/ERM	IRM/Spore	Arbuscule Mt/ERM	Arbuscule rice/ERM
Glomus_c6242/GiMST4	11226	12149	5127	10940	13887	0,8	1,1	0,9	0,5	1,0
CCHU7489.b1	5513	6260	4590	732	7926	0,7	1,1	0,8	0,8	0,1
11118_0_CCHU9778.g1_CACE	2726	4812	434	544	2677	1,0	1,8	1,8	0,2	0,2
Glomus_c4574	18	1828	3441	1219	350	0,1	102,3	5,2	192,6	68,2
Gi.7648_C1	9408	13996	1	12087	12359	0,8	1,5	1,1	0,0	1,3
Gi.8181_C1	4733	4538	229	776	5463	0,9	1,0	0,8	0,0	0,2
CCHU19976.1b	1	5	1840	1	1	NC	NC	NC	NC	NC
Glomus_c10829	1	6	387	151	1	NC	NC	NC	NC	NC
CCHU1597.b2	1	10	40	1	1	NC	NC	NC	NC	NC
Glomus_c7463	1	25	1337	1	1	NC	NC	NC	NC	NC
Glomus_c8881	1	7	601	113	1	NC	NC	NC	NC	NC
CCHU18383.b1	5	27	4522	748	1	NC	NC	NC	NC	NC

Table 6. Expression profiles of GiMST candidates from microarray data

Fungal structures in which candidates are up- and down -regulated are highlighted in red and green respectively. Note that high expression signals for contaminants could be detected in laser microdissected arbusculated cells of *M. truncatula* (Arbuscule Mt) but not in similar rice samples (Arbuscule rice). NC: not considered.

Chapter II

The *Medicago truncatula* sucrose transporter family



Proposed name	Length (bp)	Genbank accession	Original name	Genomic locus (Mt3.5)	Affymetrix accesion number (MtGEA)
MtSUT1-1	1578	JN255789	TC124224 (contig, DFCI)	NA	Mtr.43055.1.S1_s_at and Mtr.12339.1.S1_at
MtSUT1-2	1527	JN255790	NF102F08DT1F1073 (EST, DFCI)	NA	Mtr.33446.1.S1_s_at
MtSUT1-3	1536	JN255791	AC152402_6.4 (BAC position, Mt2.0)	Medtr4g131920.1	NA
MtSUT2	1803	JN255792	META878TF (EST, DFCI)	NA	NA
MtSUT4-1	1515	JN255793	AC146866_25.4 (BAC position, Mt2.0)	Medtr5g067470.1	Mtr.21349.1.S1_s_at and Mtr.40688.1.S1_at
MtSUT4-2	1518	JN255794	EST332714 (EST, DFCI)	NA	Mtr.3506.1.S1_s_at
Pseudogene1	1077	JN255795	Medtr4g102110.1	Medtr4g102110.1	NA
Pseudogene2	1011	JN255796	Medtr4g102190.1	Medtr4g102190.1	NA

Table 7. Information about the MtSUTs identified in this study.

MtSUT and pseudogene sequences were retrieved from the *M. truncatula* genome version 2.0 and 3.5 (http://www.medicagohapmap.org/index.php) and DFCI *Medicago* Gene Index (http://compbio.dfci.harvard.edu/cgi-bin/tgi/gimain.pl?gudb=medicago). Accession numbers of the MtSUT sequences reported in this table have been submitted to GenBank (http://www.ncbi.nlm.nih.gov/). Affymetrix probes accessions are from the *Medicago truncatula* gene expression atlas MtGEA (http://bioinfo.noble.org/gene-atlas/v2/).

Chapter II

The *Medicago truncatula* sucrose transporter family

Sucrose transporters (SUTs) represent major components for long distance transport of photosynthates from source leaves to demanding heterotrophic sinks (see Introduction 3.2). SUTs are necessary for coordination/regulation of carbon partitioning, plant development, cell to cell communication, environmental adaptation, and thereby play pivotal roles upon optimal plant growth and crop yield and most probably in determining the outcome of plant fungal interactions (Lalonde et al. 2004; Sauer 2007; Kühn and Grof 2010; Wippel et al. 2010; Boldt et al. 2011). Despite these primordial roles, complete SUT families have only been investigated in a limited number of reference plant species, namely in *Arabidopsis*, grape, poplar, rice and maize which comprise 9 AtSUTs, 4 VvSUTs, 5 PtaSUTs, 5 OsSUTs and 6 ZmSUTs, respectively (Aoki et al. 2003; Sauer 2007; Afoufa-Bastien et al. 2010; Payyavula et al. 2011). Regarding *Fabaceae* species, only few leguminous SUT proteins have been characterized so far (Table 9; Weber et al. 1997; Aldape et al. 2003; Zhou et al. 2007; Reinders et al. 2008). Indeed, at the beginning of my PhD, not a single SUT from the reference leguminous species *M. truncatula* (MtSUT) was identified although it represents a key model species for the study of plant-microorganism interactions (see Introduction 4.1.1). Therefore, in the second part of my work, I chose to focus on MtSUTs in order to investigate sugar transporters and their role in the AM symbiosis (see Chapter III 2.) at a full family scale.

1 Phylogenetic analyses and identification of the *Medicago truncatula* SUT family

Analysis of database led to the identification of 6 putative MtSUTs. Two genomic loci (*Medtr4g131920.1* and *Medtr5g067470.1*) being present respectively on chromosomes 4 and 5, one full-length contig and three ESTs corresponding each to putative MtSUTs (Table 7 and Fig 37; see Chapter I 1.2). For the EST sequences, 5' and 3' specific oligomers were designed in order to obtain the corresponding full-length CDS by RACE experiments (Fig 29b). Thereby, the MtSUT family comprises at least 6 members, for each of which it was possible to obtain a full-length CDS. Proteic sequences of the 6 MtSUTs were aligned, and all 6 members showed common SUT features with 12 predicted TMs, and exhibited amino acid similarities ranging from 35 to 77% (Annex VI). A SUT phylogenetic tree based on the amino

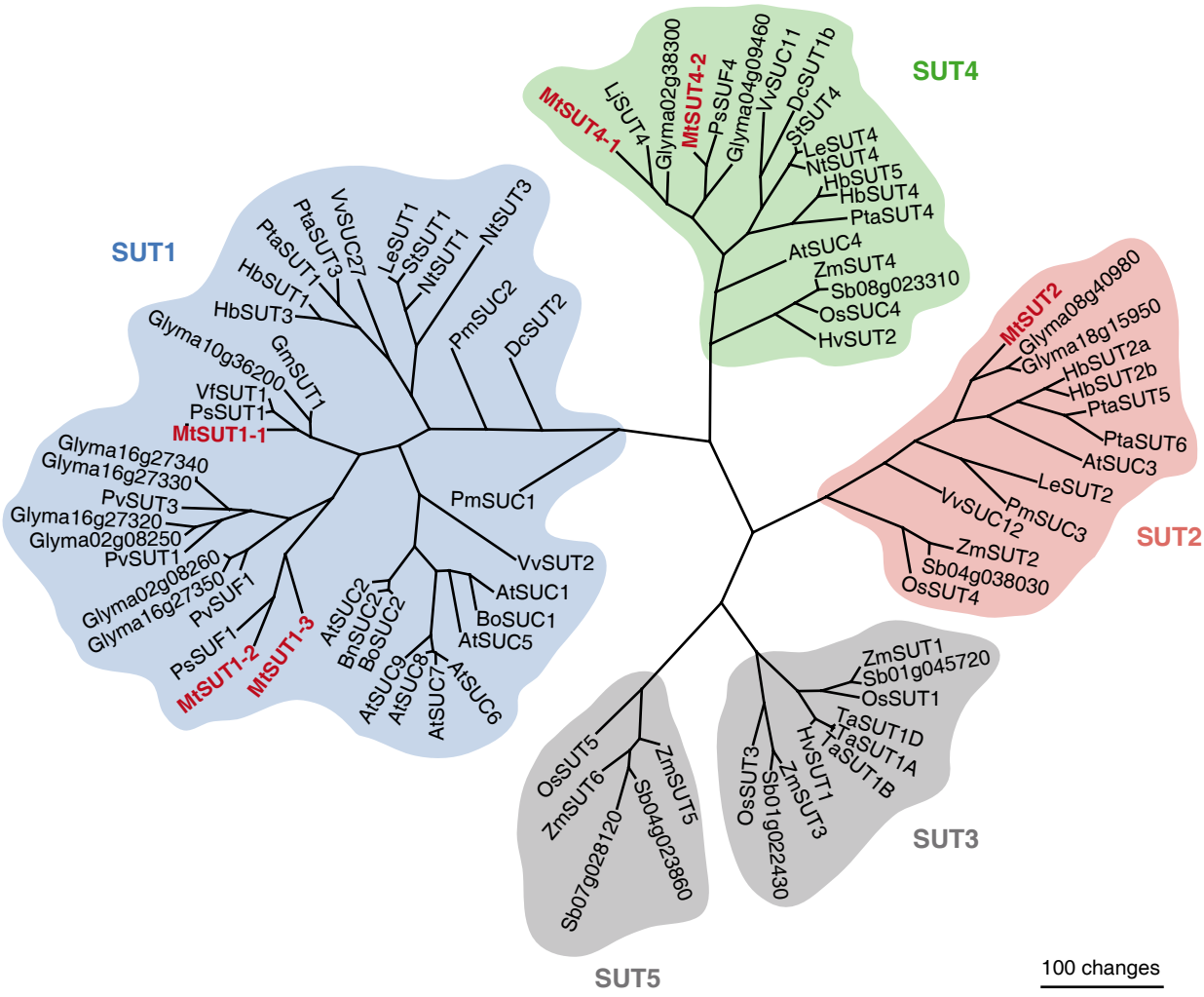


Fig 39. Phylogenetic tree of the plant SUT family

Maximum parsimony tree including 88 plant SUTs. Alignment of amino acid sequences was performed and maximum parsimony analyses were done with heuristic tree searches were executed using 1000 random sequence additions and the tree bisection-reconnection branch-swapping algorithm with random sequence analysis. The complete alignment was based on 730 sites; 513 were phylogenetically informative. The following 88 SUT sequences are included: *Arabidopsis thaliana*, 9 AtSUTs: AtSUC1 (At1g71880), AtSUC2 (At1g22710), AtSUC3 (At2g02860), AtSUC4 (At1g09960), AtSUC5 (At1g71890), AtSUC6 (At5g43610), AtSUC7 (At1g66570), AtSUC8 (At2g14670), AtSUC9 (At5g06170). *Brassica napus*: BnSUC2 (ACB47398). *Brassica oleracea*: BoSUC1 (AAL58071), BoSUC2 (AAL58072). *Daucus carota*: DcSUT2 (O65803), DcSUT1b (O65929). *Glycine max*, 12GmSUTs: GmSUT1 (CAD91334), Glyma10g36200, Glyma02g08250, Glyma02g08260, Glyma02g38300, Glyma04g09460, Glyma08g40980, Glyma16g27320, Glyma16g27330, Glyma16g27340, Glyma16g27350, Glyma18g15950. *Hevea brasiliensis*, 6HbSUTs: HbSUT1 (ABJ51933), HbSUT2a (ABJ51934), HbSUT2b (ABJ51932), HbSUT3 (ABK60190), HbSUT4 (ABK60191), HbSUT5 (EF067333). *Hordeum vulgare*: HvSUT1 (Q9M422), HvSUT2 (Q9M423). *Lotus japonicus*: LjSUT4 (CAD61275). *M. truncatula,* 6MtSUTs: MtSUT1-1 (JN255789), MtSUT1-2 (JN255790), MtSUT1-3 (JN255791), MtSUT2 (JN255792), MtSUT4-1 (JN255793), MtSUT4-2 (JN255794). *Nicotiana tabacum*: NtSUT1 (Q40583), NtSUT3 (Q9XFM1), NtSUT4 (BAI60050). *Oriza sativa,* 5OsSUTs: OsSUT1 (AAF90181), OsSUC4 (BAC67163), OsSUT3 (BAB68368), OsSUT4 (BAC67164), OsSUT5 (BAC67165). *Phaseolus vulgaris*: PvSUT1 (ABB30164), PvSUF1 (DQ221700), PvSUT3 (ABB30166). *Pisum sativum*: PsSUT1 (AAD41024), PsSUF1 (DQ221698), PsSUF4 (DQ221697). *Plantago major*: PmSUC1 (CAI59556), PmSUC2 (CAA53390), PmSUC3 (CAD58887). *Populus tremula x alba,* 5PtaSUTs: PtaSUT1 (HM749898), PtaSUT3 (HM749899), PtaSUT4 (HM749900), PtaSUT5 (HM749901), PtaSUT6 (HM749902). *Solanum lycopersicum*: SlSUT1 (CAA57726), SlSUT2 (AAG12987), SlSUT4 (AAG09270). *Solanum tuberosum*: StSUT1 (CAA48915), StSUT4 (AAG25923.2). *Sorghum bicolor*, 6SbSUTs: Sb01g022430, Sb01g045720, Sb04g023860, Sb04g038030, Sb07g028120, Sb08g023310. *Triticum aestivum*: TaSUT1A (AAM13408), TaSUT1B (AAM13409), TaSUT1D (AAM13410). *Vicia faba*: VfSUT1 (CAB07811). *Vitis vinifera*: VvSUC11 (AF021808), VvSUC12 (AF021809), VvSUC27 (AF021810), VvSUCy (ADP37124). *Zea mays,* 6ZmSUTs: ZmSUT1 (BAA83501), ZmSUT2 (AAS91375), ZmSUT3 (ACF86653), ZmSUT4 (AAT51689), ZmSUT5 (ACF85284), ZmSUT6 (ACF85673).

acid sequence alignment of 88 plant SUTs (including the 6 MtSUTs identified *de novo*) was constructed (Fig 39). The tree confirms the current SUT classification into 5 distinct clades (Braun and Slewinski 2009; Kühn and Grof 2010). The 6 MtSUTs distribute in all three dicotyledonous clades; so, we named each MtSUT upon phylogenetic clustering into particular clades (Fig 39 and Table 7). As proposed by Braun and Slewinski 2009 for the nomenclature of monocotyledonous SUTs, we would suggest in future work all SUTs to be named according to this new annotation version. In that way, upon phylogenetic analysis, we renamed MtSut1 (Gaude et al. 2011) as MtSUT4-1 (Fig 39).

Three MtSUTs belong to the SUT1 clade: MtSUT1-1, MtSUT1-2 and MtSUT1-3. MtSUT1-1 clusters with the leguminous H^+/sucrose importers from pea (PsSUT1), common bean (PvSUT1) and soybean (GmSUT1) whilst MtSUT1-2 and MtSUT1-3 directly cluster with the sucrose facilitator from pea (PsSUF1) which supports bidirectional diffusion of sucrose in yeast (Fig 39; Zhou et al. 2007). Despite this putative differential mode of transport all 3 SUT1 members share an amino acid sequence similarity of around 70% (Annex VIb). MtSUT2 falls into the SUT2 clade; like most plant species, *M. truncatula* comprises a single gene copy in the SUT2 clade (Fig 39). However, 3 species (soybean, rubber tree and poplar) show a gene duplicate in this clade. In addition, MtSUT2 is the most distant member of the MtSUT family, showing the lowest amino acid sequence similarity with the other 5 members of the MtSUT family (around 30%; Annex VIb). Indeed, SUT2 clade proteins are the most phylogenetically distant due to their long N-terminal region and an extended central loop between TMs 6 and 7 (Fig 10); MtSUT2 possesses these characteristic features of SUT2 clade proteins (Annex VIa). MtSUT4-1 (initially named MtSut1 in Gaude et al. 2011) and MtSUT4-2 belong to the SUT4 clade. MtSUT4-1 clusters with the vacuolar H^+/sucrose transporter from *L. japonicus* (LjSUT4) whilst MtSUT4-2 clusters with a facilitator from pea (PsSUF4). Like the SUT2 clade, the SUT4 clade was initially described as comprising a single gene copy in all species (Sauer 2007). Here, we report in *M. truncatula* a SUT4 gene duplicates with protein products sharing a 76% amino acid similarity (Annex VIb). SUT4 gene duplicates are also encountered in soybean, rubber tree but not in poplar. The occurrence of gene duplicates in the SUT2 and SUT4 clades may be the consequence of the genome complexity of these species as for example the partial diploidized tetraploid genome of soybean or the whole-genome duplication event that occurs in *Populus* species (Shultz et al. 2006; Tuskan et al. 2006; Young et al. 2011).

1.1 Identification of *cis*-regulatory elements of MtSUT promoters

Much of the regulatory features of plant genes are primarily located 1 kbp upstream of the transcriptional start site and this is generally referred to as the gene promoter which contains

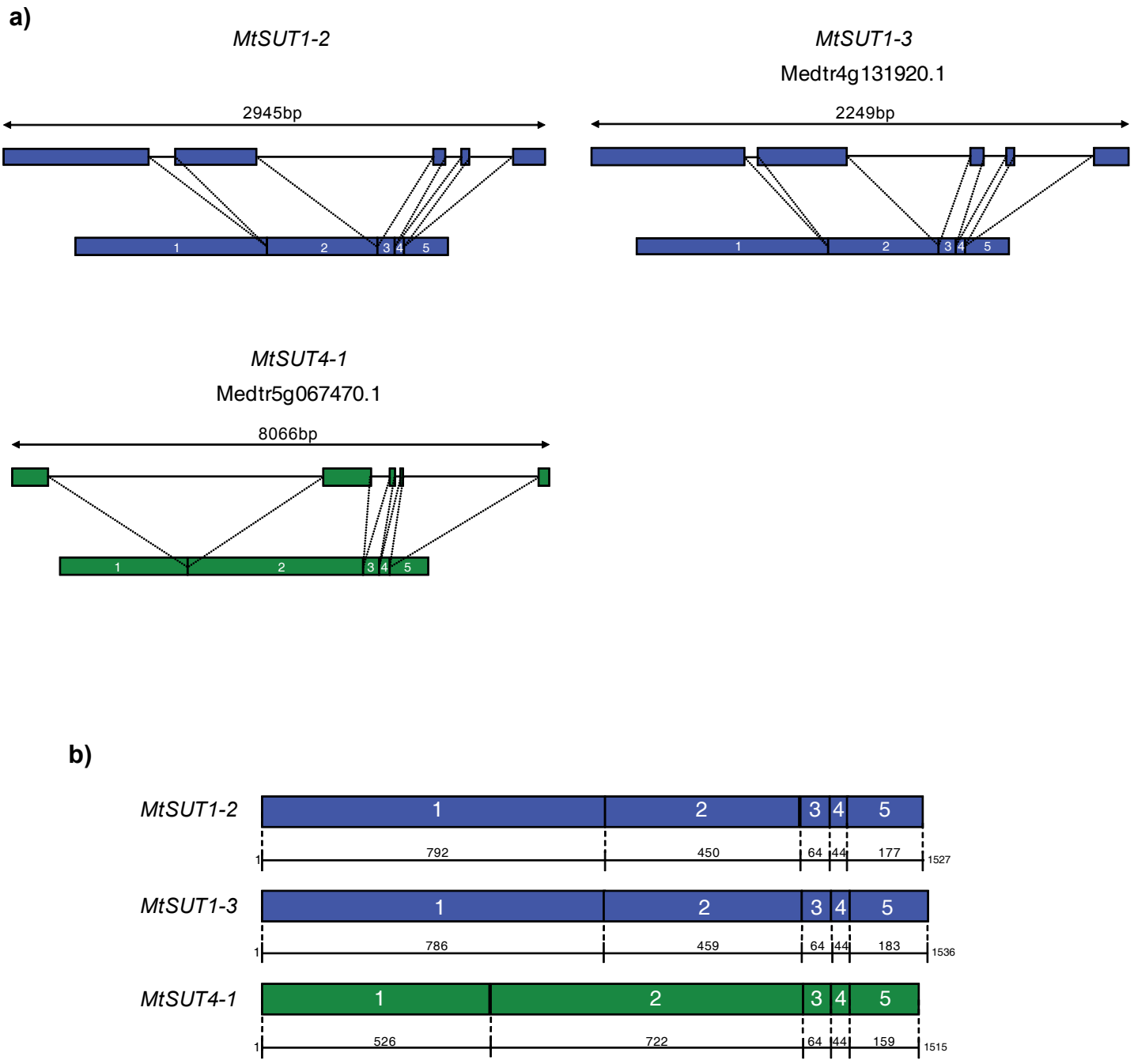


Fig 40. Intron splicing pattern of *MtSUT1-2* and of the two genomic loci *MtSUT1-3* and *MtSUT4-1*

(a) The genomic sequence of *MtSUT1-2* was amplified by PCR. *Medtr4g131920.1* and *Medtr5g067470.1* sequences were retrieved from the *M. truncatula* genome 3.5. All genomic sequences were analyzed with the est2genome tool from the EMBOSS explorer (http://emboss.bioinformatics.nl/) and spliced to their respective coding sequences.

(b) Comparison of the exon distribution of MtSUT1-2, MtSUT1-3 and MtSUT4-1. The length of each exon and of coding sequences is given in base pair.

All schemes in (a) and (b) are in scale.

c*is*-regulatory elements. Therefore, the 1.5 kbp upstream of the CDS of the genomic loci corresponding to promoter regions of *MtSUT1-3* and *MtSUT4-1* were retrieved and possible c*is*-regulatory elements were predicted by *in silico* scanning. Both promoters of *MtSUT1-3* and *MtSUT4-1* contain numerous conserved important regulatory elements for spatial, hormonal and developmental expression pattern as well as elements involved in response to environmental and biotic stresses (Table 8). So far, only two studies report the analysis of c*is*-regulatory elements in plant SUT promoters: an exhaustive and comparative survey of promoter regions of *AtSUTs* and *OsSUTs* (Fig 14; Ibraheem et al. 2010) while a second one reports unique and common 5' regulatory sequences of all sugar transporters from grapevine (*VvMSTs* and *VvSUTs*; Afoufa-Bastien et al. 2010). In total, 24 c*is*-regulatory elements are conserved among *MtSUT1-3* and *MtSUT4-1*, 14 of which were also described in promoter sequences of *AtSUTs*, *OsSUTs* and grapevine sugar transporters (Table 8). This suggests a broad conservation of c*is*-regulatory elements present in promoters of sugar transporters in plant species from monocotyledonous to solanaceous and leguminous Dicots probably due to the pivotal roles of such transporters to adaptation in developmental, physiological and environmental changes.

1.2 Intron splicing and exon patterns of MtSUTs

In addition to genomic loci sequences (*MtSUT1-3* and *MtSUT4-1*) retrieved from the *M. truncatula* genome, the full gene sequence of *MtSUT1-2* was obtained by PCR amplification and intron/exon patterns of these 3 members were analyzed and compared (Fig 40). First, a clear size difference is observed between loci from the SUT1 and SUT4 clades, *MtSUT1-2* and *MtSUT1-3* being respectively 2945 bp and 2249 bp long whereas *MtSUT4-1* presents a large gene size (8066 bp), which is mainly due to *MtSUT4-1* huge first intron (4152 bp). Despite this difference, intron splicing pattern is well conserved between the SUT1 and SUT4 clade members of *M. truncatula* with all 3 genes possessing 5 exons interspersed by 4 introns (Fig 40a). Exonic patterns of *MtSUT1-2* and *MtSUT1-3* are well conserved whilst *MtSUT4-1* presents a shorter first exon and a longer second exon (Fig 40b). Nevertheless, exon patterns is well conserved between genes from the SUT1 and SUT4 clades in *M. truncatula* with *MtSUT1-2*, *MtSUT1-3* and *MtSUT4-1* presenting a very well conserved motif for exons 3, 4 and 5 (Fig 40b).

2 Functional characterization of MtSUTs

The full open reading frames of the 6 MtSUTs were inserted into the pDRf1-GW yeast expression vector (Fig 30 and 31; Loqué et al. 2007) downstream the strong and constitutive

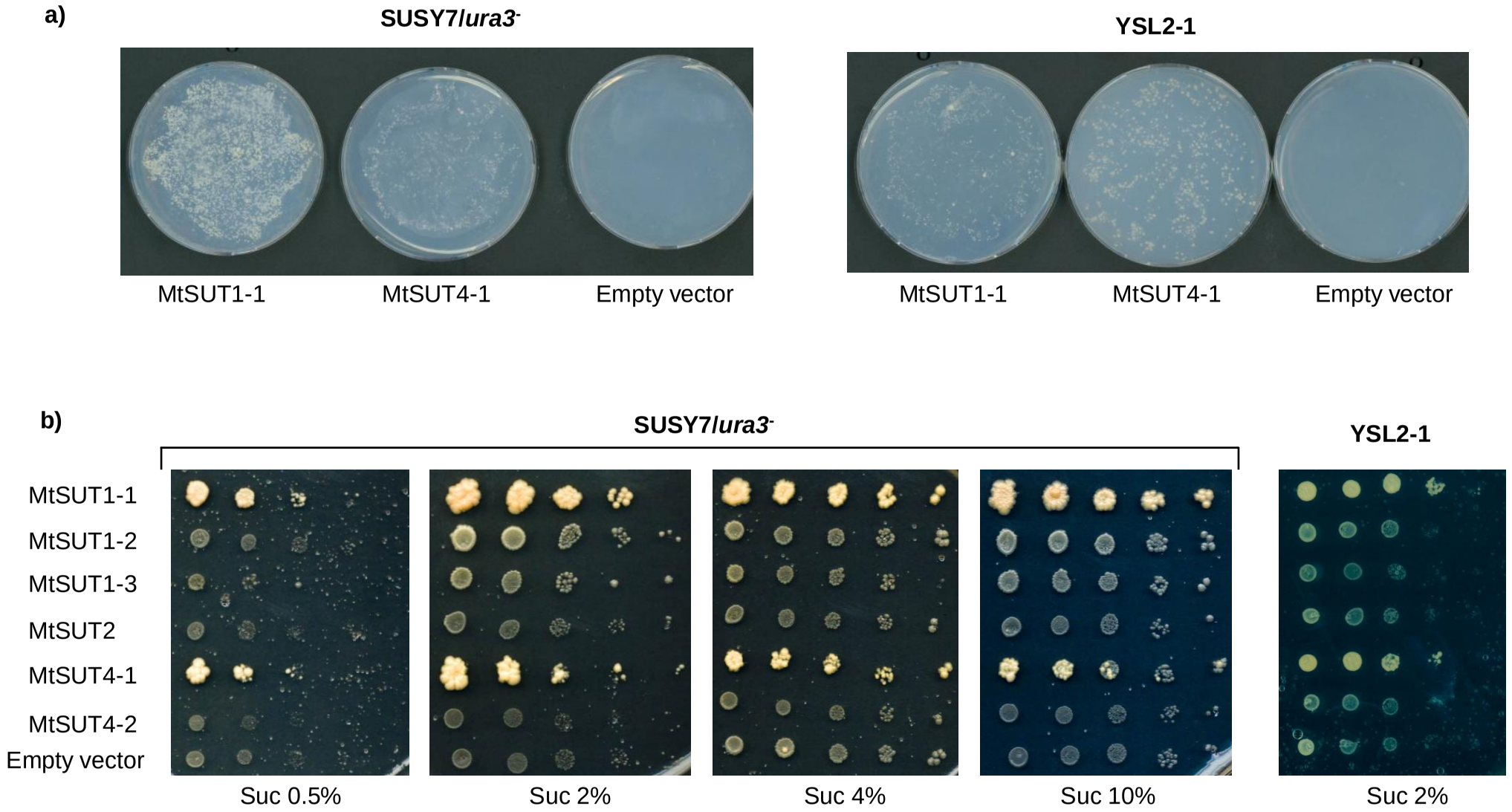


Fig 41. Functional complementation of MtSUTs

a) Direct plating on 2% sucrose minimal medium after transformation of yeast strains SUSY7/*ura3*$^{-}$ and YSL2-1 with vector expressing *MtSUT1-1* or *MtSUT4-1* or with empty vector pDR-f1.

b) Drop test complementation assays of yeast strains transformed with respective *MtSUTs* or with empty vector. Plates were incubated at 30°C, 20 days and 5 days for SUSY7/*ura3*$^{-}$ and YSL2-1 respectively on uracil minus minimal medium with different sucrose concentration as the sole carbon source.

PMA1 promoter and upstream the ADH terminator for correct expression of respective MtSUTs in heterologous system. A corresponding empty vector (pDRf1) containing a short PvuII restriction site inserted downstream the promoter and upstream the terminator was used as a negative control (Loqué et al. 2007). Thereafter, a heterologous model organism deficient in sucrose uptake systems but still able to metabolize this sugar is required to perform the functional and biochemical characterization of each transporters. In that way, the yeast mutant strains SUSY7/*ura3*$^{-}$ and YSL2-1 suitable for sucrose uptake characterization (Fig 27) were transformed with respective MtSUTs cloned in pDRf1-GW and empty vector. After yeast transformation by the "best method" described by Gietz and Schiestl 2007 (http://home.cc.umanitoba.ca/~gietz/) and plating resulting transformation on minimal media, only yeast expressing *MtSUT1-1* and *MtSUT4-1* showed a complementation of sucrose deficiency when the transformants were directly plated on sucrose minimal synthetic medium (Fig 41a). In addition, a similar direct complementation could be observed when transforming independently both deficient yeast strains SUSY7/*ura3*$^{-}$ and YSL2-1 with *MtSUT1-1* and *MtSUT4-1* (Fig 41a). To validate this observation, yeast colonies transformed with respective MtSUTs were selected on non-selective medium for the sugar source but without uracil confirming the correct incorporation of plasmids. Such colonies were then further plated by performing a subsequent drop test complementation, which consist in plating serial dilutions of yeast cells on sugar selective medium (Fig 41b and 42). Drop test assays confirmed that MtSUT1-1 and MtSUT4-1 complement the sucrose uptake deficiency on minimal sucrose synthetic media; this was true on all tested concentrations (Fig 41b). Similar tendency were observed for both deficient strain SUSY7/*ura3*$^{-}$ and YSL2-1. However, a stronger background growth was reported for YSL2-1, after only 5 days of incubation (Fig 41b). Regarding other MtSUT members, yeast expressing *MtSUT1-2* and to a smaller extent *MtSUT1-3* showed improved growth when compared to the control transformed with the empty vector whilst yeast expressing *MtSUT2* and *MtSUT4-2* did not restore the growth of the mutant strain on any of the tested sucrose concentrations. Overall, the best complementation results were obtained with the widely published strain SUSY7/*ura3*$^{-}$ at 2% sucrose concentration, the concentration commonly used to show sucrose complementation of plant SUT by this strain (Fig 42).

2.1 Biochemical characterization of MtSUT1 and MtSUT4-1

Biochemical characterization of MtSUTs was performed through yeast uptake experiments with radiolabeled [^{14}C]sucrose. To do so, yeast must be energized by a sugar source, preferentially glucose, prior to [^{14}C]sucrose uptake measurements. As the YSL2-1 mutants lacks the 20 sugar transporter genes required for hexose uptake (Fig 27; Wieczorke et al. 1999), YSL2-1 is not able to take up glucose and therefore cannot be energized by this sugar. In addition, as a result of mutations in all hexose and sucrose import systems, YSL2-1 can

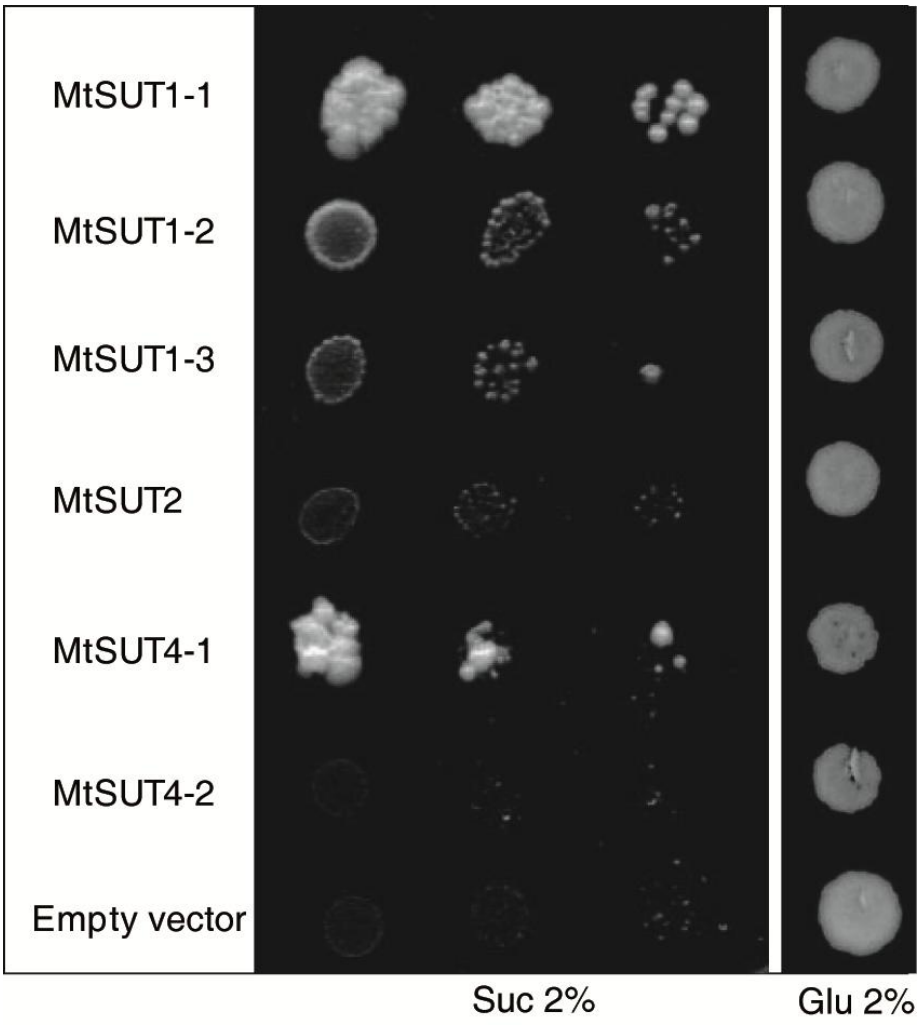


Fig 42. Complementation of the sucrose uptake deficiency of the yeast strain SUSY7/*ura3*$^{-}$

Serial dilutions of yeast cells were grown on minimum medium without uracil with 2% sucrose (Suc) or glucose (Glu, control) as sole carbon source.

only be cultivated on maltose. However, maltose cannot be used as an energizing sugar source since most plant SUTs are also able to transport this disaccharide. Indeed, the high maltose concentration would directly compete with radiolabeled [^{14}C]sucrose used to chase sucrose uptake and to characterize transporter capacities (Fig 43d). In contrast, the second deficient strain SUSY7/*ura3*$^{-}$ (Riesmeier et al. 1992; Barker et al. 2000) possesses the cellular machinery for hexose import and therefore can be energized or grow on glucose (Fig 27 and 42). Despite the mutation of all H^+/hexose transport systems and a narrow sugar spectrum, the YSL2-1 strain shows a higher growth background of yeast expressing MtSUTs or containing the empty vector control (Fig 41b). For all these reasons, we chose to retain the widely published strain SUSY7/*ura3*$^{-}$ for further biochemical characterization of MtSUTs.

The biochemical characterization of the strongly complementing members, MtSUT1-1 and MtSUT4-1 was performed using this model system (SUSY7/*ura3*$^{-}$). Both transporters follow a saturable sucrose uptake typical of the Michaelis-Menten kinetics with a linear Lineweaver-Burk representation (Fig 43a-b). Using linear regression equation, an apparent Km for sucrose of 1.7 mM was calculated for MtSUT1-1 whilst MtSUT4-1 showed a Km of 13.7 mM at ph5. Therefore, MtSUT1-1 presents a higher affinity for sucrose whilst MtSUT4-1 shows a higher transport capacity (Fig 43a-b). However, we were not able to measure significant sucrose uptake for other MtSUT members expressed in yeast as they only showed a slight improved growth compared to yeast transformed with the empty vector (Fig 42). Thereby, it was not possible to biochemically characterize MtSUT1-2, MtSUT1-3, MtSUT2 and MtSUT4-2.

2.2 Transport dependency on membrane potential and substrate specificity of MtSUT1-1 and MtSUT4-1

Transport dependency on membrane potential of MtSUT1-1 and MtSUT4-1 was tested by measuring [^{14}C]sucrose uptake upon the addition of the protonophores carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) and 2,4-dinitrophenol (DNP) and the plasma membrane H^+-ATPase inhibitors diethylstilbestrol (DES) and vanadate. Inhibitor assays revealed that the [^{14}C]sucrose uptake is sensitive to the addition of CCCP, DNP, DES and to a smaller extent vanadate indicating that membrane energization is required for sucrose transport by MtSUT1-1 and MtSUT4-1 (Fig 43c). The lowest inhibitive response upon addition of vanadate is probably due to orthovanadate ion stability at acidic pH. Since transport properties of MtSUT1-1 and MtSUT4-1 are curbed upon addition of protonophores and H^+-ATPase inhibitors, both MtSUT1-1 and MtSUT4-1 are active H^+/sucrose co-transporters.

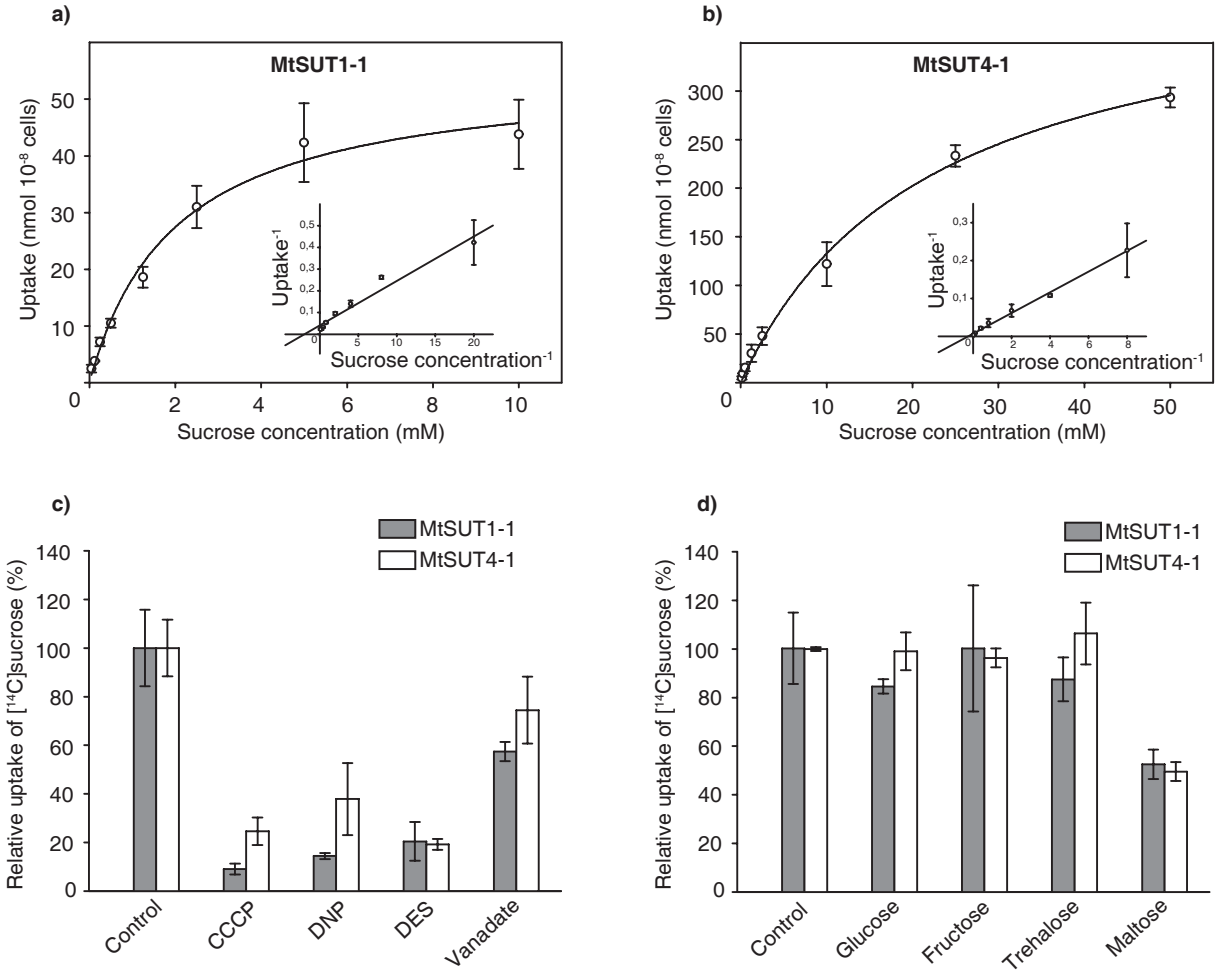


Fig 43. Biochemical characterization of MtSUT1-1 and MtSUT4-1

(a-b) Uptake kinetics of [^{14}C]sucrose by the yeast strain SUSY7/*ura3*$^{-}$ expressing *MtSUT1-1* and *MtSUT4-1* at the indicated sucrose concentration at ph5. Michaelis-Menten and Lineweaver-Burk representation of the data are illustrated.

(c) Influence of plasma membrane energization on the uptake rate of [^{14}C]sucrose in the yeast mutant expressing *MtSUT1-1* or *MtSUT4-1*.

(d) Substrate specificity of MtSUT1-1 and MtSUT4-1. (c-d) Data are expressed as percentage of the sucrose control.

Substrate specificity of both transporters was also tested by the addition of a ten-fold molar excess of competing sugars. In such experiments, the addition of the hexoses, glucose and fructose or the disaccharide, trehalose did not compete with [^{14}C]sucrose uptake by MtSUT1-1 and MtSUT4-1 (Fig 43d). However, the addition of 10 fold maltose inhibited the sucrose uptake to around 50%, indicating that at this concentration half of the sites are bound by the competing disaccharide. Therefore, substrate competition assays showed that, in addition to sucrose, only the disaccharide maltose (α-D-glucopyranosyl-(1,4)-α-D-glucopyranose) is bound by MtSUT1-1 and MtSUT4-1 (Fig 43d). Surprisingly, the other tested disaccharide trehalose (α-D-glucopyranosyl-(1,1)-α-D-glucopyranoside) also made of 2 glucose units did not compete with sucrose and therefore seems not to be a possible substrate for both transporters. The SUT4 clade protein LjSUT4 and the SUT1 clade protein AtSUC9 are also able to conduct transport current in *Xenopus* oocytes in presence of sucrose, maltose but not of trehalose (Sivitz et al. 2007; Reinders et al. 2008). These findings show that substrate specificity seems to be conserved for plant SUTs and is tightly modulated among disaccharides.

2.3 Expression of MtSUTs in plant leaves and roots

Transcript accumulation of each member of the MtSUT family was analyzed by quantitative RT-PCR in plants fertilized with a high phosphate nutrient solution (Table 4). While *MtSUT1-3*, *MtSUT2*, *MtSUT4-1* and *MtSUT4-2* are expressed at relatively similar levels in leaves and roots, *MtSUT1-1* shows a 20 fold higher transcript accumulation in leaves compared to roots (Fig 44). This information suggests a major role for MtSUT1-1 in sucrose fluxes from photosynthetic source leaves to the phloem. Furthermore, SUTs were detected in both sink and source parts. Indeed, transcripts of the 6 MtSUT members were present in leaf and root tissues with the exception of *MtSUT1-2* transcripts only detected in leaves. Regarding different time points, no major differences could be observed between expression levels of MtSUT members with the exception of *MtSUT4-1* presenting a higher transcript accumulation in roots of plants harvested at 21dold (Fig 44b).

In addition, we performed an *in silico* search similar to MtMSTs (Fig 38, see Chapter I 3.1) for expression data of available MtSUT candidates in the *Medicago truncatula* Gene Expression Atlas database (Fig 49). The expression pattern obtained by quantitative RT-PCR (Fig 44) shows differences with available transcriptomic data from the MtGEA, especially for *MtSUT1-1* which shows similar signals in roots and leaves (Fig 49). However, these results confirm that *MtSUT1-1* and *MtSUT4-1* have the highest expression profiles. Moreover, in the microarray database, *MtSUT1-2* transcripts were not detected in leaf samples but only seem present in seed samples and no probes were available for *MtSUT1-3* and *MtSUT2*. Regarding *MtSUT4-2*, probes were designed to the anti-sense sequences and therefore are not able to

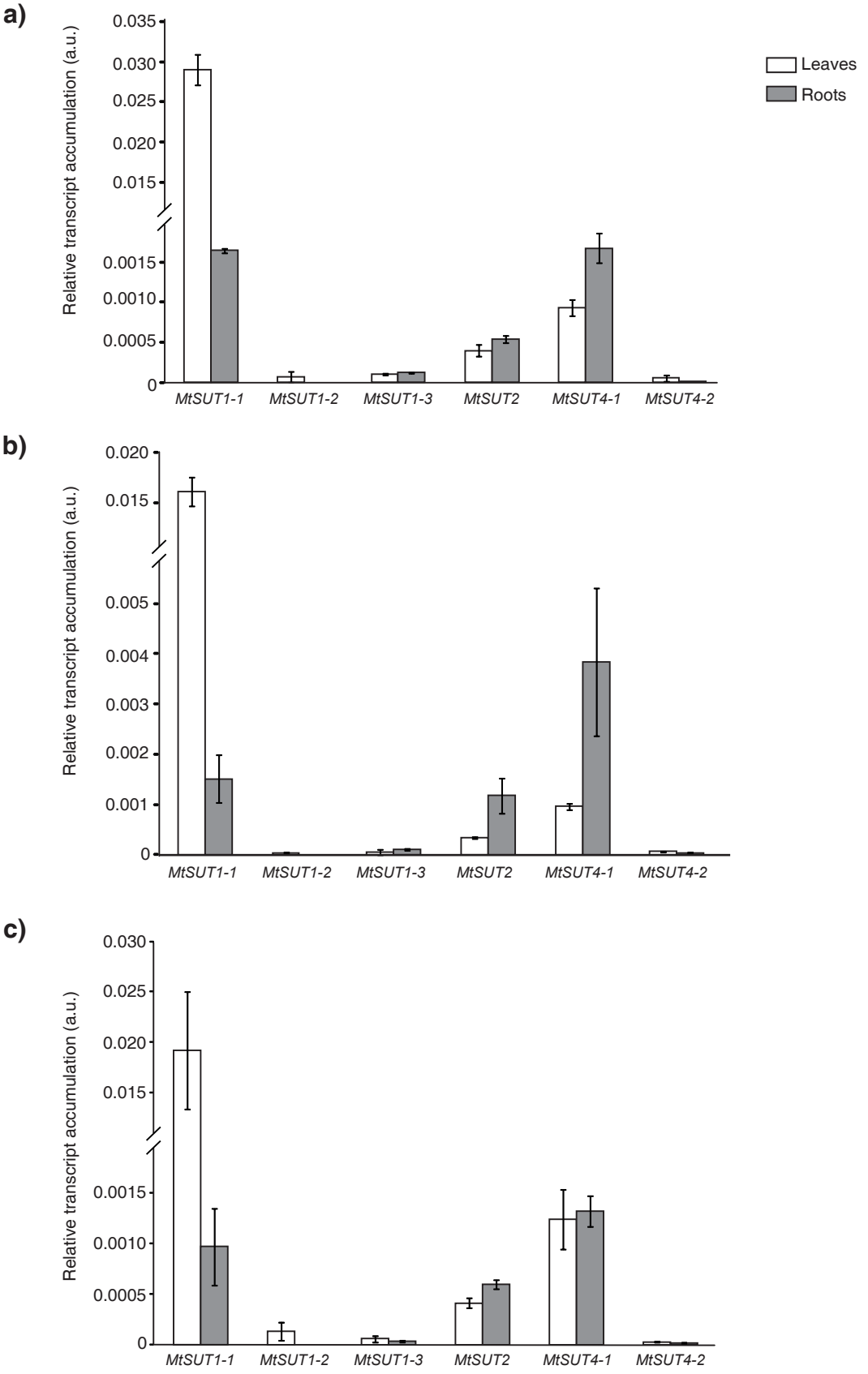


Fig 44. Analysis of transcript levels of *MtSUT* genes

Quantitative reverse transcription polymerase chain reaction (q-RT-PCR) analysis of *MtSUT1-1*, *MtSUT1-2*, *MtSUT1-3*, *MtSUT2*, *MtSUT4-1* and *MtSUT4-2* in leaves (white bar) and roots (tinted bar) of plant treated with high phosphate 1.3mM condition (HP) and harvested at 2 (a), 3 (b) and 4 (c) weeks old. Data are expressed in arbitrary units (a.u.).

hybridize the target cRNA used in microarrays; as a result, no signal is detected for *MtSUT4-2* (Fig 49).

3 Conclusion

Here, we identified and characterized the MtSUT family (Doidy et al. 2012b) which comprises 6 members; phylogenetic, *in silico*, functional and expression analyses were performed to better understand the role of each MtSUT members within particular clades. We report common c*is*-regulatory elements present in both promoters of *MtSUT1-3* and *MtSUT4-1* and more generally also present in sugar transporters of other plant species. In addition, conserved intron splicing and exon patterns were observed for *MtSUT1-2*, *MtSUT1-3* and *MtSUT4-1*. Functional and biochemical characterization revealed that MtSUT1-1 and MtSUT4-1 are active H^+/sucrose importers when expressed in yeast. Transport capacities, affinity for sucrose and specificity to alternative sugar substrates are conserved between MtSUT1-1, MtSUT4-1 and orthologous leguminous proteins which directly cluster in same subclades in the SUT phylogenetic classification. Expression pattern and transport kinetics indicate that MtSUT1-1 is the preferred candidate as the main phloem loading protein whilst MtSUT4-1 cluster with a vacuolar protein (LjSUT4). Altogether, these features suggests a common origin and evolution of plant SUTs probably through gene duplications, adaptive evolution and functional divergence (Johnson and Thomas 2007). The role of respective MtSUTs in plant and in the arbuscular mycorrhizal interaction with *G. intraradices* will be discussed in the next chapter.

Cis -element name	Sequence	Function	Number of copies/promoter	
			MtSUT1-3	*MtSUT4-1*
-300 element	TGHAAARK	Endosperm expression	1	2
AACA motifs	AACAAAC	Endosperm expression	1	1
ABRE*	MACGYGB	Abscisic acid responsive element	1	4
AGL15 binding site	CWWWWWWWWG	Bindin site of AGL15, plant MADS domain protein AGL15	1	2
CACTFTPPCA1	CACT	Mesophyll expression	8	21
CPBCSPOR	TATTAG	Cytokinin dependent protein binding	1	1
CuRE core*	GTAC	Core of copper-response element	1	1
Dof core*	AAAG	Core site required for binding of zinc finger proteins in maize	13	13
Erd1*	ACGT	Regulatory element required for early	3	3
ERE*	AWTTCAAA	Ethylene responsive element	1	1
GATA-box*	GATA	Regulatory element required for high level light regulated and tissue specific expression	19	4
GT-1 binding site*	GAAAAA; GGTTAA	Regulatory element required for rapid response to pathogen attack, salinity and salicylic acid inducible gene expression	14	18
GTGA motif*	GTGA	Motif of the late pollen g10 gene of tobacco	6	2
I-box*	GATAA	Light box element; regulatory element conserved in light-regulated genes	8	2
Initiator elements psaDb	YTCANTYY	light-responsive transcription elements	4	8
MYB*	WAACCA;TAACTG;CN GTTR;	Regulatory element involved in drought inducible gene expression	9	2
Nodulin consensus	CTCTT; AAAGAT	Putative nodulin consensus sequences	4	5
OsBIHD1 binding site	TGTCA	Bindin site of OsBIHD1, BELL homeodomain transcriptional factor, in disease resistance responses.	2	1
POLLEN1LELAT52*	AGAAA	Pollen expression	3	2
ROOTMOTIFTAPOX1*	ATATT	Root expression	11	4
SuRE core*	GAGAC	Core of sulfur-responsive element	1	1
TAAAGSTKST1	TAAAG	Target site for trans-acting StDof1 protein controlling guard cell-specific gene expression	6	4
T-box	ACTTTG	Light-activated gene element	1	1
W-box*	TTGAC; TGACT; TGACY; TGAC	Regulatory element involved in direct fungal elicitor stimulated transcription of defense genes and activation of genes involved in response to wounding	7	1

Table 8. Common *cis*-regulatory elements of *MtSUT1-3* and *MtSUT4-1* promoter regions
Common *cis*-regulatory elements of *MtSUT1-3* and *MtSUT4-1* promoter regions. The 1.5 kbp upstream of the coding regions of genomic loci was retrieved and *cis*-regulatory elements in promoter sequences were scanned using the plant *cis*-acting regulatory DNA elements database (PLACE: http://www.dna.affrc.go.jp/PLACE/). ***** represent regulatory elements also present in promoters of *AtSUTs* and *OsSUTs* and/or sugar transporters of *Vitis vinifera* (Afoufa-Bastien et al., 2010; Ibraheem et al., 2010).

Gene name	Km for sucrose	Transport properties	Reference
MtSUT1-1	1.7mM (pH 5)	active symporter	
GmSUT1	5.6mM (pH4)	active symporter	Aldape *et al.*, 2003
PsSUT1	1.5mM (pH 5.5)	active symporter	Zhou *et al.*, 2007
VfSUT1	ND	ND	Weber *et al.*, 1997
MtSUT4-1	13.7mM (pH 5)	active symporter	
LjSUT4	12.9mM (pH 5)	active symporter	Reinders *et al.*, 2008
PsSUF4	37.8mM (pH 5.5)	facilitator	Zhou *et al.*, 2007

Table 9. Summary of characterized leguminous SUTs and biochemical properties

Chapter III

Sucrose transport, from plant source leaves towards arbuscular mycorrhizal fungus

Leaves	Fresh weight (mg)	Dry weight (mg)	Leaf area (cm²)	SPAD measurement
14 dold				
HP	226.8 ± 72.6 [a]	36.2 ± 17.3 [ab]	4.4 ± 1.4 [a]	50.1 ± 3.9 [a]
LP	238 ± 83.7 [a]	43.8 ± 18.5 [b]	4.6 ± 1.1 [a]	50.6 ± 4.0 [a]
AM	158.6 ± 41.8 [b]	25.6 ± 7.6 [a]	3.1 ± 1.0 [a]	48.6 ± 3.0 [a]
21 dold				
HP	444.4 ± 134.3 [a]	100.8 ± 16.2 [a]	6.2 ± 0.9 [a]	63.2 ± 4.7 [a]
LP	391.8 ± 104.3 [a]	96.4 ± 18.0 [a]	6.1 ± 1.2 [a]	65.3 ± 4.3 [a]
AM	422 ± 67.2 [a]	99 ± 10.2 [a]	5.9 ± 1.4 [a]	61.7 ± 3.5 [a]
28 dold				
HP	492.8 ± 24.6 [b]	191.6 ± 7.6 [b]	9.1 ± 1.5 [a]	71.8 ± 4.9 [a*]
LP	450.8 ± 53.0 [a]	166.2 ± 25.6 [a]	8.0 ± 0.9 [a]	67.2 ± 3.3 [b*]
AM	496.6 ± 12.7 [b]	191.2 ± 8.0 [b]	9.3 ± 1.6 [a]	72.3 ± 3.3 [a*]

Roots	Fresh weight (mg)	Dry weight (mg)
14 dold		
HP	293.4 ± 134.0 [ab]	34.6 ± 9.5 [a*]
LP	363.2 ± 142.7 [a*]	38.2 ± 15.4 [a]
AM	176.2 ± 62.1 [b*]	23.2 ± 5.3 [b*]
21 dold		
HP	630.2 ± 141.9 [a]	99.8 ± 19.7 [ab]
LP	671.6 ± 170.2 [a]	109.6 ± 17.3 [a]
AM	568.6 ± 34.0 [a]	93.6 ± 13.8 [b]
28 dold		
HP	966.0 ± 154.5 [a]	177.0 ± 39.2 [a]
LP	1075.2 ± 208.0 [a]	193.8 ± 24.5 [a]
AM	984.6 ± 48.2 [a]	176.4 ± 18.2 [a]

AM roots	F%	M%	m%	A%	a%
14 dold	60.00 ± 3.33	19.17 ± 0.86	32.05 ± 2.94	17.27 ± 1.93	89.91 ± 6.19
21 dold	73.33 ± 6.67	41.37 ± 6.68	56.21 ± 5.36	39.92 ± 6.15	96.6 ± 0.88
28 dold	78.89 ± 7.07	44.41 ± 7.25	56.88 ± 13.49	46.03 ± 2.26	95.5 ± 4.43

Table 10. Growth parameters of *M. truncatula* plants cultivated under differential phosphate supply and upon inoculation of *G. intraradices*

Medicago truncatula plants were cultivated for 2, 3 and 4 weeks and supplemented with Long Ashton nutrient solution (Table 4) modified for low phosphate conditions both in the presence of *G. intraradices* BEG141 for AM conditions (AM) or in absence of the fungus for control conditions (LP). A second group of control plants was cultivated in high phosphate (HP) conditions, supplemented with a nutrient solution containing 10-fold phosphate. Means ± standard deviations are shown. Significant differences are indicated with a letter ($P<0.1$; $n=5$). Stars* highlight data with a significant differences ($P<0.05$).

Chapter III

Sucrose transport, from plant source leaves towards arbuscular mycorrhizal fungus

Sugar photosynthates produced in plant shoots are loaded into the phloem in the form of sucrose which constitutes the main carbohydrate for long distance transport through phloem vessels; finally sucrose is unloaded in the release phloem towards sink organs; alternatively sugars can also be transferred to non-plant sinks (Fig 4). Root colonization by beneficial symbionts creates an additional sugar demand for the host plant (Doidy et al. 2012a; see Introduction 4.3.3). Indeed, heterotrophic fungal growth, spore formation and respiration as well as increased metabolism in several plant tissues augment the sink strength. The importance of this sugar transfer as the sole carbon source for AMF is emphasized by the fact that the fungus is unable to grow and complete its life cycle in the absence of host plant. Indeed, AMF are obligate biotrophs and rely completely on the host plant for photosynthetically-fixed carbon supply. Thus, in the AM symbiosis, the mutualistic interaction is based on biotrophic exchanges between the plant and the fungal partners. The fungus supplies the autotrophic host with nutrients, mainly phosphate and thereby enhances plant growth. In return, the plant provides sugar photosynthates to the heterotrophic symbiont; sucrose, being the main carbon source transported from photosynthetic leaves towards colonized roots and glucose, the preferred sugar transferred toward the fungus at the symbiotic interface (see Introduction 4.3.3.1). While the phosphate supply towards the host plant was characterized as being mediated by *MtPT4*, a mycorrhizal specific plant phosphate transporter of *M. truncatula* (Harrison et al. 2002; Javot et al. 2007), mechanisms of transport and plant transporters involved in carbon partitioning towards the AMF are still poorly understood.

Indeed, in *M. truncatula,* which represents the most important model plant for studying AM interaction (see Introduction 4.1.1), only a single plant monosaccharide transporter (Mtst1) is characterized and located in AM colonized root parts; and therefore Mtst1 was assumed to play a critical role for a functional symbiosis (Harrison 1996). Furthermore, just few additional monosaccharide transporter (MST) genes from a limited number of plant species were shown to be differentially regulated in response to AM colonization (Garcia-Rodriguez et al. 2005; Wright et al. 2005). Regarding long distance transport of sucrose and transporter proteins (SUT) involved in carbon partitioning at the whole plant level; at the start of my PhD, no plant SUTs had ever been studied in response to AMF inoculation. It is only recently that a comprehensive study reported a complete analysis of available tomato gene members

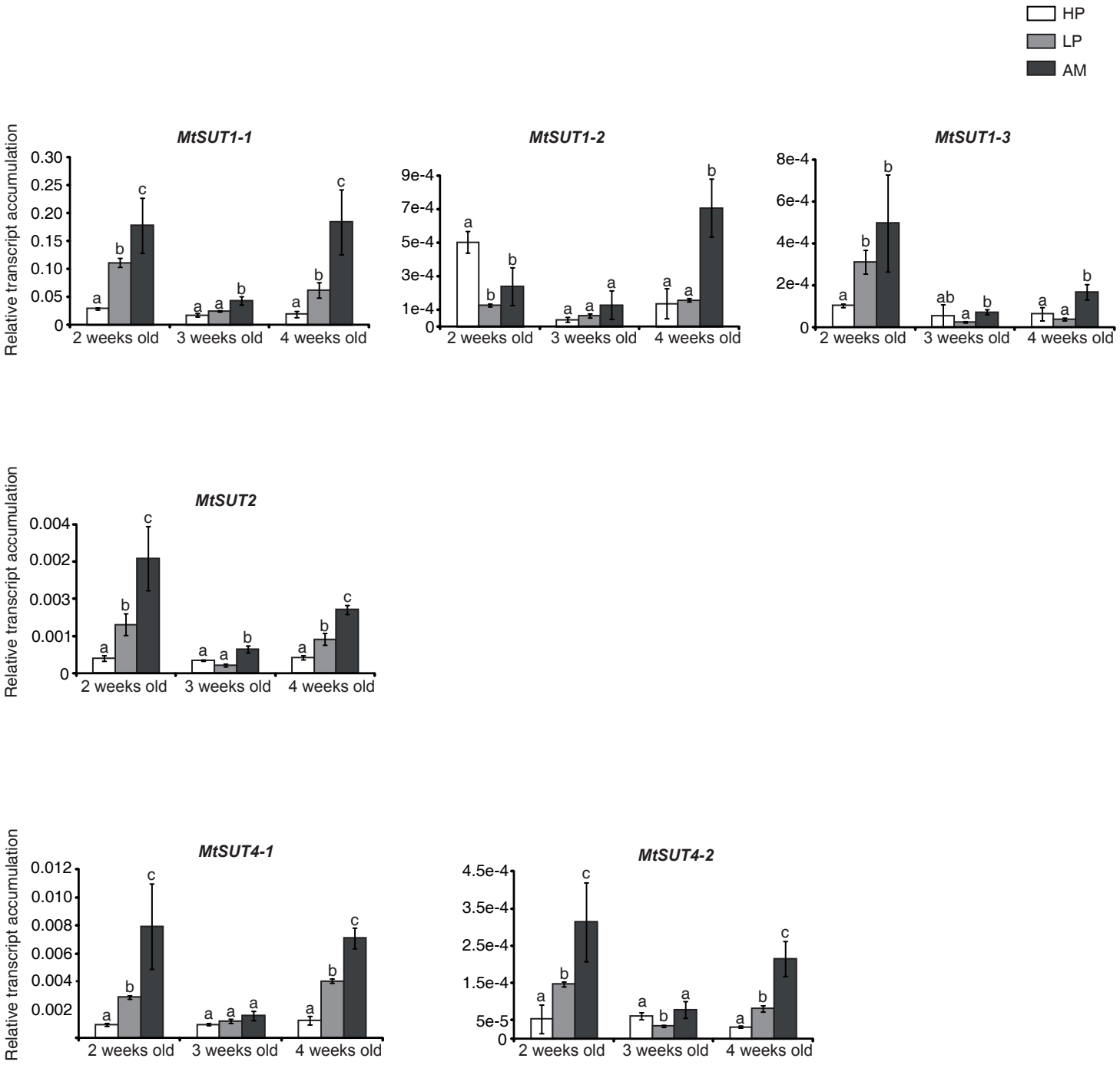


Fig 45. Relative transcript accumulations of *MtSUTs* in source leaves of *M. truncatula*
Quantitative reverse transcription polymerase chain reaction (q-RT-PCR) analysis of source leaves of plants either treated with high phosphate (HP) 1.3mM condition (white bars) or with low phosphate (LP) 0.13mM condition (light tinted bar) or treated with low phosphate and inoculated (AM) with *G. intraradices* (dark tinted bar) and harvested at 2, 3 or 4 weeks time point. The data were statistically checked by the adequate Student t-test upon accomplishing the Fischer F-test and stars* indicate significant differences between independent treatments (P = 0.05; n = 3).

SlSUT1, *SlSUT2* and *SlSUT4* belonging to the known dicotyledonous SUT clades (Fig 39), which presented a differential regulation in sink roots and source leaves of mycorrhized plants (Fig 58; Boldt et al. 2011).

Uptake, exchanges and competition for sugar, at biotrophic interfaces, are controlled by membrane transporters and their regulation patterns are essential in determining the outcome of plant-fungal interactions and in adapting to changes in soil nutrient quantity and/or quality (Doidy et al. 2012a). Thereby, in a third part of my PhD thesis; I chose to focus on plant sugar fluxes from their site of production (leaves) towards AM colonized sink (roots) as well as the expression pattern of the newly identified sucrose transporter family of *M. truncatula* to better understand the cellular and molecular mechanisms controlling nutrient exchanges in AM symbiosis. In that way, we report a complete study of the MtSUT family and discuss the implication of sugar transporters in carbon partitioning towards the fungal symbiont during the AM interaction between *M. truncatula* and *G. intraradices*.

1 Analysis of *M. truncatula* plant development

M. truncatula plants were cultivated for 2, 3 and 4 weeks and supplemented with Long Ashton nutrient solution modified for low phosphate conditions both in the presence of *G. intraradices* BEG141 for AM conditions (AM) or in absence of the fungus for control conditions (LP). In addition, a second group of control plants was cultivated in high phosphate (HP) conditions, supplemented with a nutrient solution containing 10-fold phosphate, to balance the provision of phosphate by AMF plant (Table 4). At each time point, fresh and dry weights of root and aerial parts were monitored as well as total leaf surface. In addition, chlorophyll abundance of source leaves was estimated by SPAD measurements.

At early stage (2 weeks post-inoculation, wpi), AM plants presented a high mycorrhizal rate (Table 10); indeed, the frequency of mycorrhizal colonization in the root system (F%) is of 60% and an arbuscule abundance in the root system close to 20% (A%). In addition, at this time point, AM root system and aerial part showed a significant delay of development compared to non-mycorrhizal control plants (Table 10). This may be due to the strong sink constituted by *G. intraradices* BEG 141 when associated to *M. truncatula* J5, which represents a plant hyper-responsive to mycorrhization with this AMF isolate. However, this delay of development was caught up from the third wpi; indeed, plants cultivated in HP, LP or AM conditions presented similar fresh and dry weights, leaf surface and SPAD measurement for aerial parts; the only significant differences were noticed between dry weight of root system in AM and LP conditions; LP plants developed larger root systems, probably to explore the soil in search of supplemental nutrients. Nevertheless, at 4 wpi, the root system of LP plants was not anymore significantly greater. However, the aerial parts of LP plants

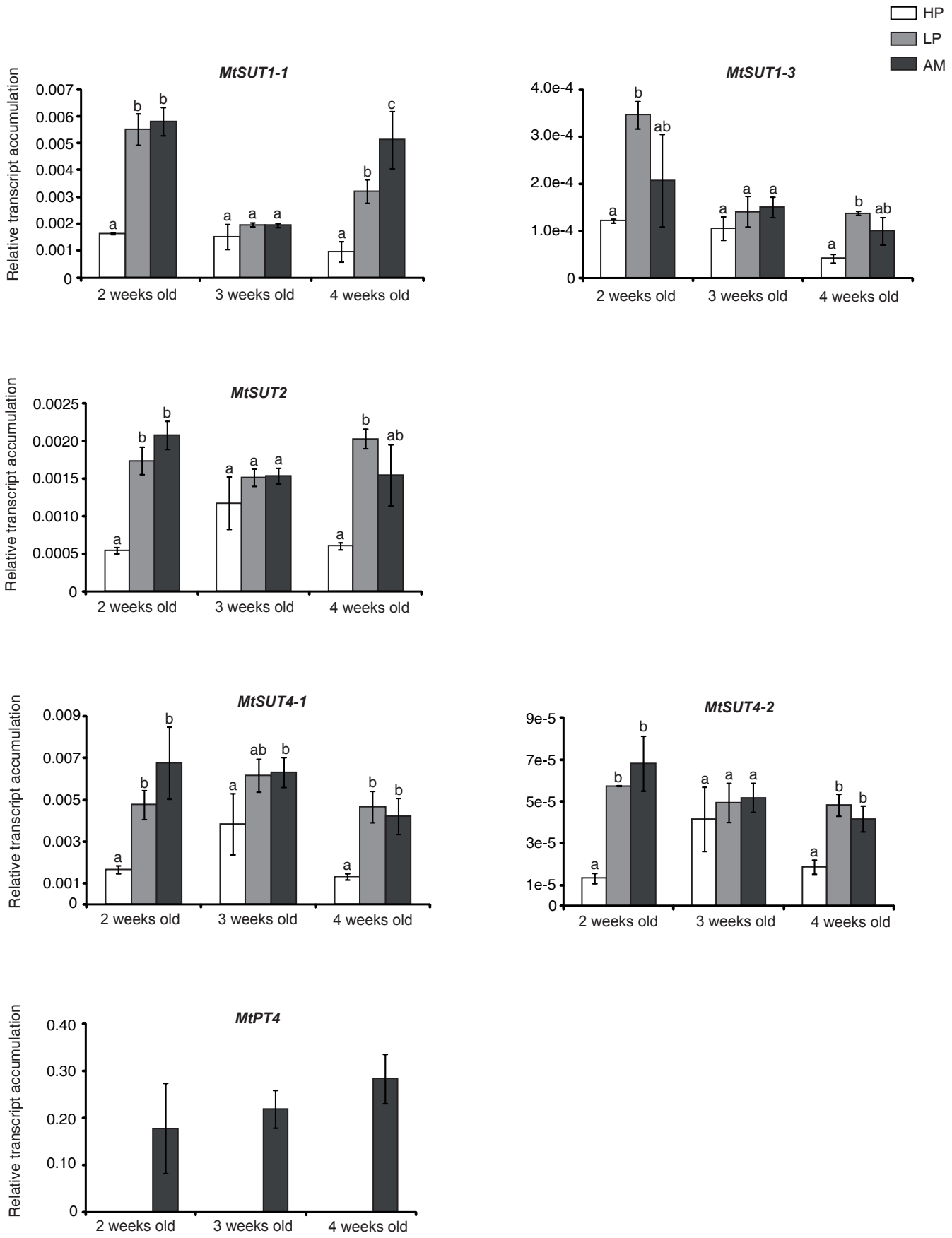


Fig 46. Relative transcript accumulations of *MtSUTs* in roots of *M. truncatula*

Quantitative reverse transcription polymerase chain reaction (q-RT-PCR) analysis of total root system of plants either treated with high phosphate (HP) 1.3mM condition (white bars) or with low phosphate (LP) 0.13mM condition (light tinted bar) or treated with low phosphate and inoculated (AM) with *G. intraradices* (dark tinted bar) and harvested at 2, 3 or 4 weeks time point. The data were statistically checked by the adequate Student t-test upon accomplishing the Fischer F-test and stars* indicate significant differences between independent treatments (P = 0.05; n = 3).

showed a reduced growth compared to the one supplied with HP solution or inoculated with AMF. In addition, at 4 wpi, SPAD measurements indicate a lower chlorophyll content in source leaves of LP plants suggesting a lower photosynthetic activity.

At 3 and 4 wpi, AM plants presented a fully established mycorrhization with high frequency (F%) and intensity (m% and M%) of mycorrhizal colonization. In addition, the arbuscular development, which represents the key fungal structure for symbiotic nutrient exchanges, estimated by the arbuscule abundance (a% and A%) indicates a fully functional symbiosis. Furthermore, at these time points, most differences regarding growth parameters were observed for plants cultivated under LP conditions (Table 10); plants inoculated by AMF or supplied with HP solution to balance the provision of phosphate by AMF presented similar growth parameters. In conclusion, *M. truncatula* plants inoculated with *G. intraradices* presented a delay of development at early stage; but once AM is fully established, beneficial effects of the mycorrhizal symbiosis led to similar growth parameters when compared to plants fertilized with higher P solution.

2 Transcript levels of *MtSUTs* and *MtPT4* in *M. truncatula* leaves and roots in relation to phosphate level and/or mycorrhization

MtSUT transcript accumulations from source leaves and total root system of *M. truncatula* plants harvested at 2, 3 and 4 wpi and cultivated under differential phosphate supply and upon inoculation of *G. intraradices* were analyzed by quantitative RT-PCR (Fig 45 and 46).

MtPT4 showed the highest expression levels of all analyzed genes, with a specific expression in mycorrhizal roots increasing over the time of colonization (Fig 46); this high transcript accumulation of *MtPT4* confirms the functionality of the AM symbiosis (Table 10). However, no *MtSUT* showed a mycorrhizal specific expression. Nevertheless, in contrast to microarray analyses which did not present differential regulation of *MtSUT1-1*, *MtSUT1-2* and *MtSUT4-1* in roots samples in response to AM inoculation (Fig 46), transcript quantification by quantitative RT-PCR shows that *MtSUTs* present a fine-tuning of their expression profiles in root and leaf according to the phosphate supply and upon AM inoculation (Fig 45 and46). All *MtSUT* genes could be quantified in roots and leaves for all tested conditions (HP, LP, AM) with the exception of *MtSUT1-2* transcripts which were not detected in source leaf samples (Fig 45 and 46). Furthermore, all *MtSUTs* showed higher transcript accumulations in root samples in LP and AM compared to HP treatment at 2 and 4 wpi. Although we used similar sampling and harvesting conditions, it seems that *MtSUT* gene expressions monitored at 3 wpi present a divergent expression pattern from the one recorded at 2 and 4 wpi. Only *MtSUT4-1* showed a significant upregulation in LP and AM roots sample at 3 wpi compared to HP. In

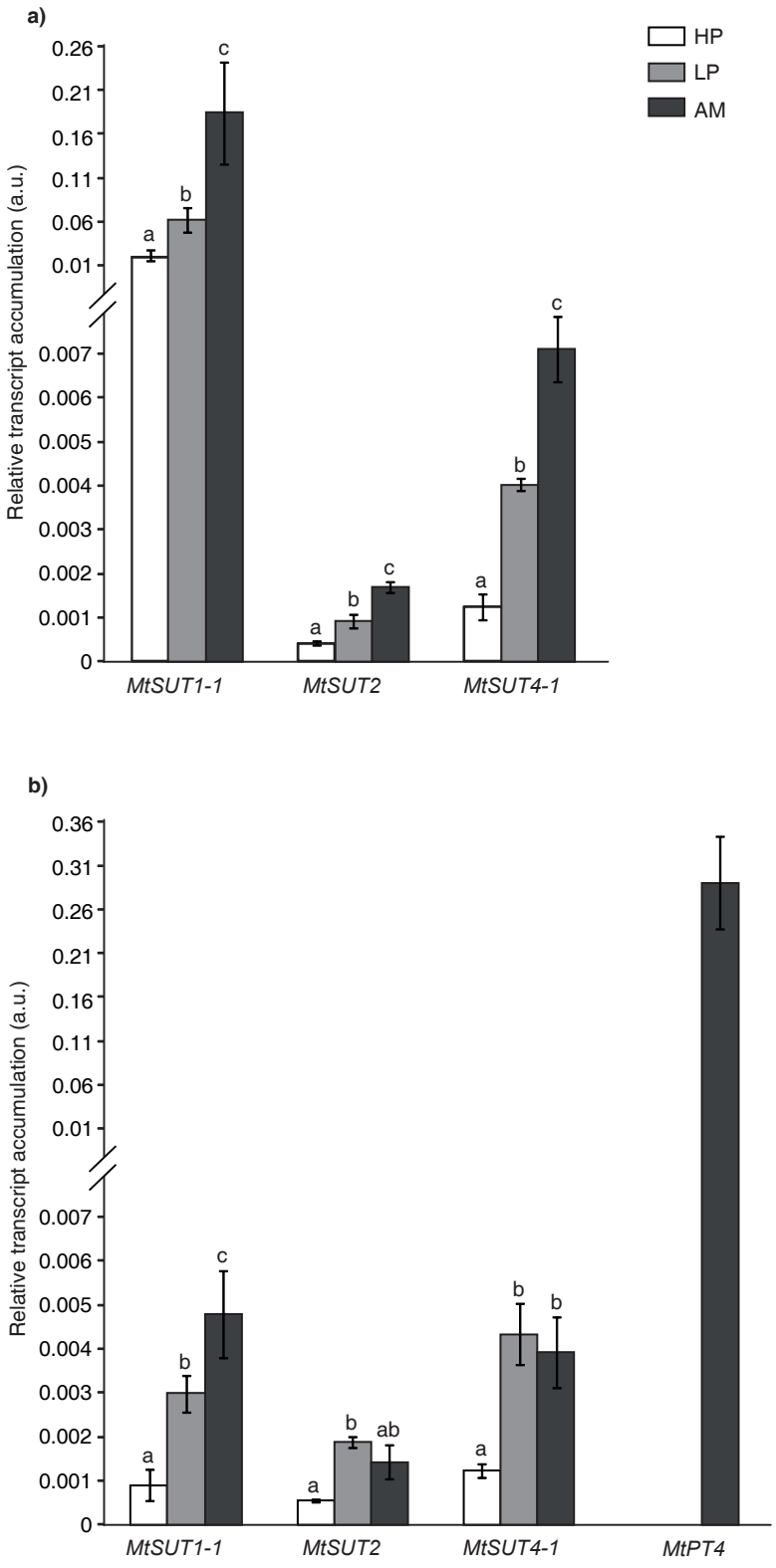


Fig 47. Effect of phosphate nutrition and AMF on transcript level of *MtSUT* genes

a) Quantitative reverse transcription polymerase chain reaction (q-RT-PCR) analysis of *MtSUT1-1*, *MtSUT2* and *MtSUT4-1* in leaves.

b) q-RT-PCR analysis of *MtSUT1-1*, *MtSUT2*, *MtSUT4-1* and *MtPT4* in roots.

Four weeks old plants were either treated with high phosphate (HP) 1.3mM condition (white bars) or with low phosphate (LP) 0,13mM condition (light tinted bar) or treated with low phosphate and inoculated (AM) with *G. intraradices* (dark tinted bar). Data are expressed in arbitrary units (a.u.). Independent statistical analyses were performed for each gene, with letters indicating a statistically significant difference ($P<0.05$, $n=3$).

addition, the expression profiles of *MtSUTs* showed a generally lower expression levels in samples harvested at 3 wpi, especially in source organs (Fig 45).

In that way, we focused the interpretations of the results of mycorrhizal influence on *MtSUT* expression for plant samples harvested at 4 wpi, when the AM structure are fully established creating a higher fungal sink strength (Table 10). In addition, *MtSUT1-2*, *MtSUT1-3* and *MtSUT4-2* transcript levels were close to detection thresholds in all tested conditions (Fig 45 and 46); indeed, for these samples a higher cDNA concentration had to be used to obtain a detectable cycle threshold (Ct). So, we retained the key members *MtSUT1-1* and *MtSUT4-1* which were characterized as functional transporters (Fig 43) and *MtSUT2* which presents a substantial expression level in source and root parts of all tested conditions (Fig 47) for further analysis. Transcript accumulation of *MtSUT1-1*, *MtSUT2* and *MtSUT4-1* decreased significantly in plants fertilized with ten time phosphate (HP; Fig 47). In contrast, all *MtSUTs* showed a higher transcript accumulation in leaves of AM plants (Fig 47a) when compared to plants treated with the same amount of phosphate (LP) or with high phosphate (HP) but in the absence of AMF. Moreover, when compared to the plants treated with HP, to mimic phosphate allocation of AMF, all analyzed *MtSUTs* showed a higher transcript accumulation in source leaves and sink roots of AM plants (Fig 47). These findings indicate a higher sucrose export from source leaves and higher sucrose transfer to the roots when the sink strength is enhanced by the colonization of AMF independently of the phosphate supplied by the fungus. In addition, *MtSUT1-1* the potential candidate for phloem loading (see Chapter II 3.3) showed the highest transcript upregulation in roots and leaves of AM plants while *MtSUT2* is the only candidates not showing significant differences in AM root samples compared to non-mycorrhizal controls. A similar tendency was observed for orthologous genes of tomato when comparing the expression in mycorrhizal and non-mycorrhizal plants fertilized with the same amount of phosphate (Fig 58; Boldt et al. 2011).

Overall, *MtSUTs* generally showed a lower expression in non-mycorrhizal plants treated with HP conditions (Fig 45 and 46); thus being consistent with lower root sink strength in HP conditions due to sufficient nutrients in phosphate fertilized soil. In contrast, at 4 wpi, when the AM fungal sink is fully established, all *MtSUTs* are significantly upregulated in source leaves (Fig 45) suggesting higher long distance transport rates of sucrose towards AM colonized roots when the root sink strength is enhanced by the sugar demand due to the AMF. Thereby, it seems that MtSUT expression in source leaves is directly correlated to the root sink strength in *M. truncatula*.

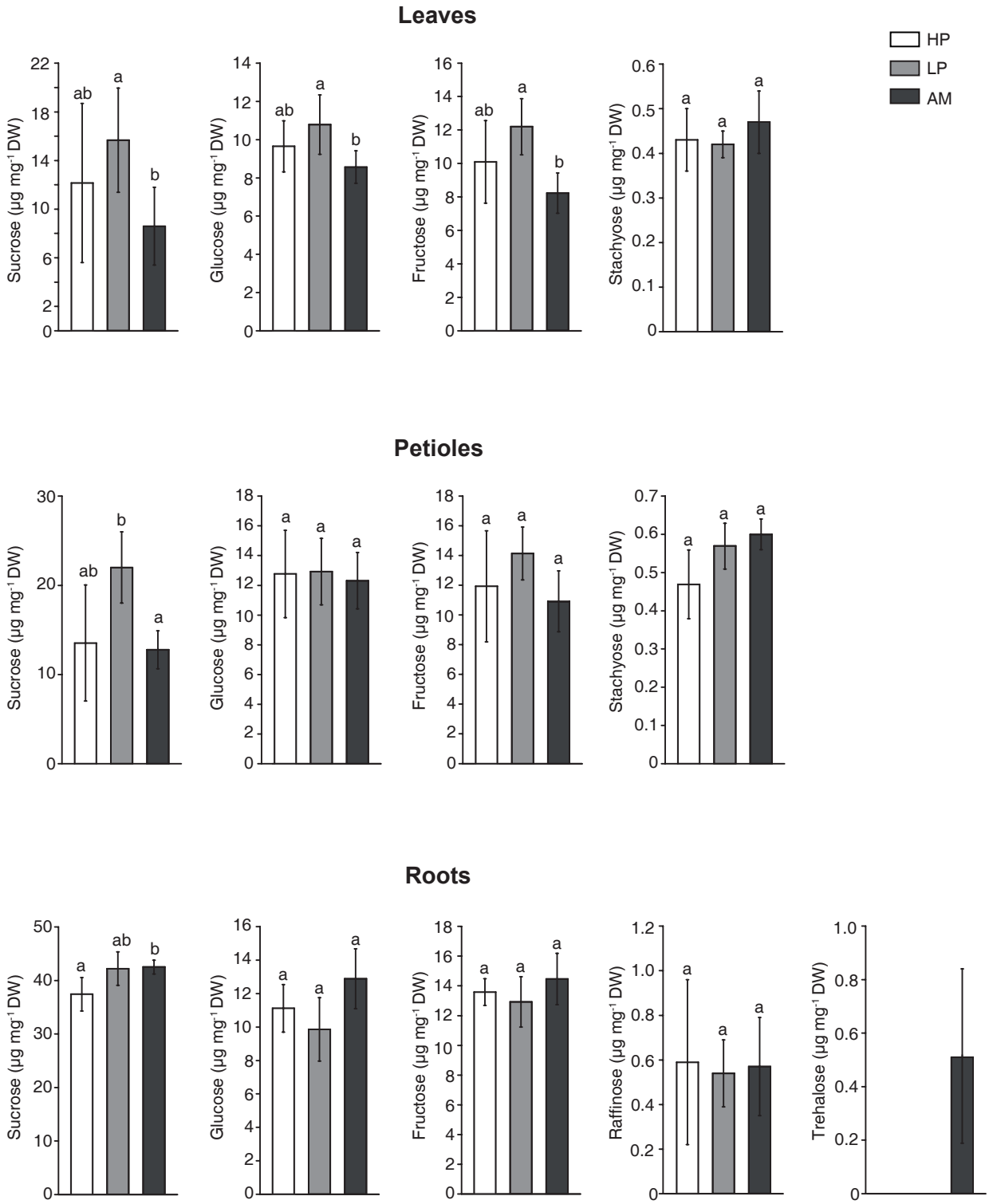


Fig 48. Sugar content of *M. truncatula* leaves, petioles and roots

Quantification of soluble sugars by high-performance anion exchange-pulsed amperometric detection (HPAE-PAD) in plants either treated with high phosphate (HP) 1.3mM condition (white bars) or with low phosphate (LP) 0.13mM condition (light tinted bar) or treated with low phosphate and inoculated (AM) with *G. intraradices* (dark tinted bar) in a) leaves, b) petioles and c) roots.). Mycorrhization rate of AM plants were estimated to F% 73.3 ± 14.5; M% 51.1 ± 14.4; m% 69.0 ± 10.4; a% 92.5 ± 1.3; A% 47.4 ± 13.9. Independent statistical analyses were performed for each sugar, with letters indicating a statistically significant difference ($P<0.05$, n=5).

3 Sugar quantification

Soluble sugar content of source leaves, petioles and root compartments was quantified using high-performance anion exchange-pulsed amperometric detection (HPAE-PAD) for plants harvested at 4 wpi. Sucrose, glucose and fructose were detected at substantial levels in all samples; sucrose being the main sugar in leaves and roots of *M. truncatula* (Fig 48). At a lower level, stachyose and raffinose were respectively detected in aerial parts (leaves and petioles) and root compartments. Trehalose, already known to be a major form of sugar storage for AMF (Bago et al. 2003) was found exclusively in roots of mycorrhizal plants (Fig 48c).

A significant decrease of total soluble sugars was observed in source leaves in response to AMF inoculation compared to non-mycorrhizal plants cultivated in LP conditions (Fig 48a), which is consistent with a higher sucrose loading towards phloem saps of colonized root sink. However, collecting petioles to have an overview of transporting fluids from source leaves to sink roots did not confirm this higher sucrose export towards colonized sinks. In addition, all major soluble sugars (glucose, fructose and sucrose) could be detected in petioles (Fig 48b) and therefore sugar content of petioles seems not to reflect phloem sap contents mainly constituted of sucrose as the long distance transport sugar. Moreover, in all tested conditions, only small variations of sugar content were measured in sink roots. Indeed, only a slight increase of sucrose could be observed in AM colonized roots compared to the HP fertilized non-mycorrhizal plants. However, an overall tendency to accumulate supplemental soluble sugars in AM colonized roots was observed (Fig 48c). In our conditions, we were not able to confirm the substantial sugar export towards AM colonized sinks roots by HPAE-PAD sugar quantification.

4 Conclusion

In conclusion, the recent completion of the *M. truncatula* genome and first genomic data from *G. intraradices* as well as the release of transcriptomic database allowed the generation of an inventory of sugar transporter genes in the leguminous host plant and its model fungal symbiont (see Chapter I). With the identification and functional characterization (see Chapter II) and gene expression pattern of sugar transporter families, a more complete picture of sugar fluxes in the AM symbiosis has begun to emerge (Fig 60).

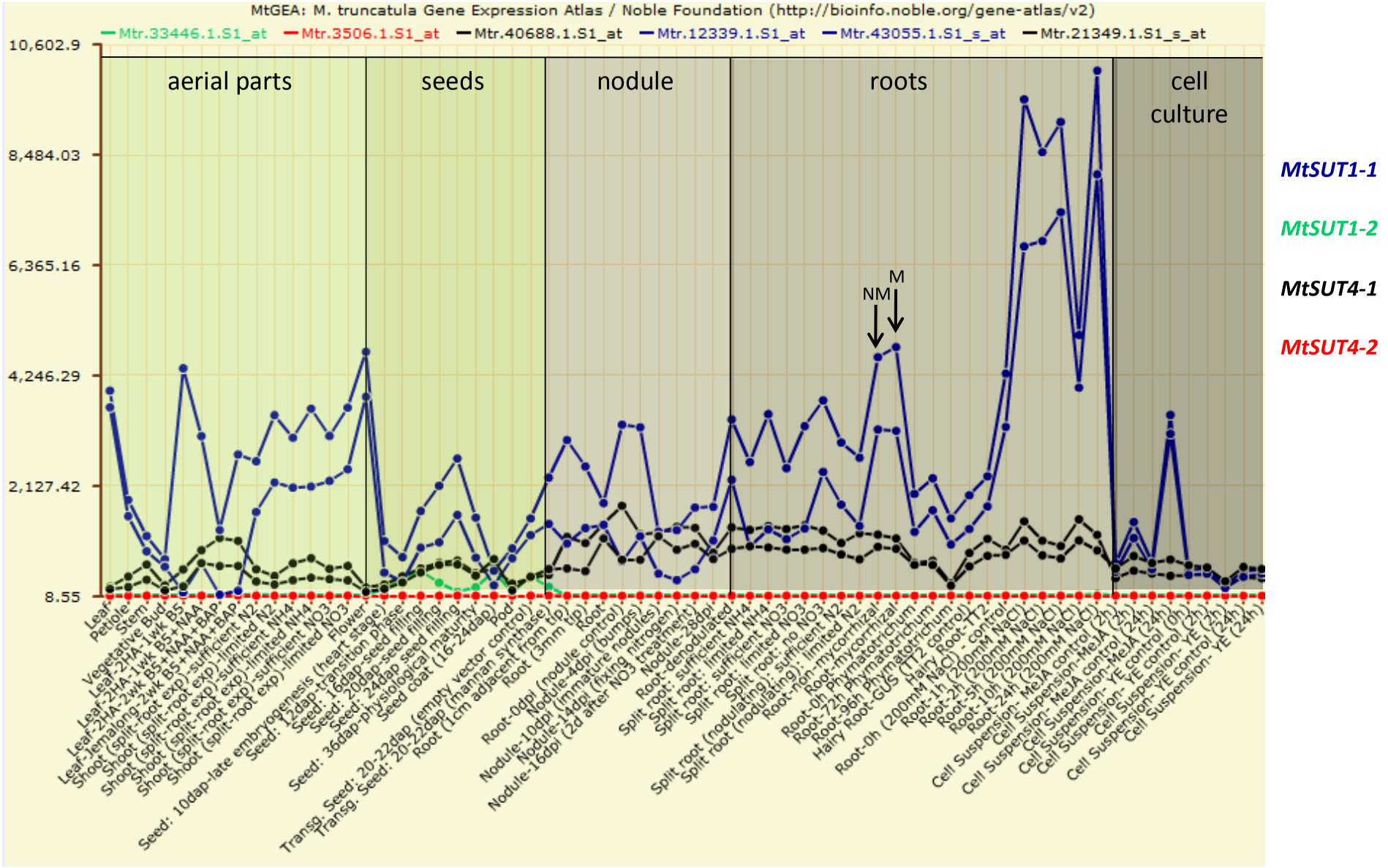


Fig 49. Expression profiles of available MtSUTs from MtGEA microarray data

M and NM arrows correspond to mycorrhizal and non-mycorrhizal root samples respectively.

Chapter IV

Construction of a cDNA expression library from *Medicago truncatula* symbiotic roots and screening for sugar transporter candidates

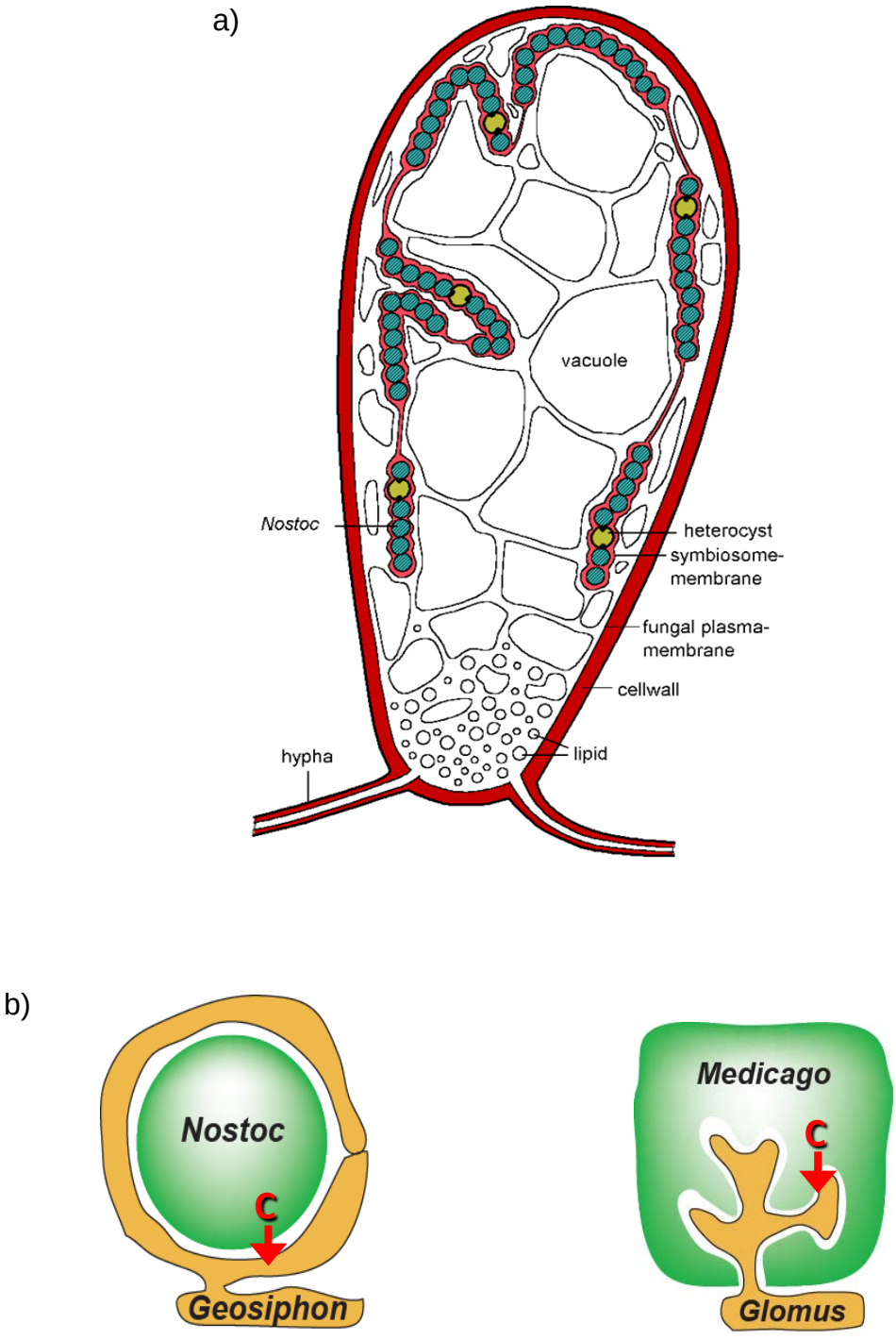


Fig 50. The unique symbiotic model between *G. pyriformis* and *N. punctiforme*

a) Scheme of the compartmentation of the *Geosiphon* symbiosis. Schüßler and Kluge, 2001.

b) Comparative scheme of the symbiotic bladder structure of the *Geosiphon* - *Nostoc* symbiosis and the arbusculated cell in the AM symbiosis between *M. truncatula* and *G. intraradices.* Carbon exchange is represented with a red arrow. Adapted from Schüßler et al., 2006.

Chapter IV

Construction of a cDNA expression library from *Medicago truncatula* symbiotic roots and screening for sugar transporter candidates

In parallel to the targeted approach developed in chapters I-III, I also carried out a non-targeted approach which consists in the development of cDNA libraries to screen for putative symbiotic gene candidates by functional complementation of yeast (*Saccharomyces cerevisiae*) mutant deficient in uptake capacities. The construction of such an expression library led to the isolation of the first glomeromycotan sugar transporter from *Geosiphon pyriformis* (Schüßler et al. 2006; Schüßler et al. 2007). This fungus belongs to an ancestral branch within the phylum Glomeromycota and forms the only known fungal endocyanosis, with the cyanobacteria *Nostoc punctiforme*. In the *Geosiphon* - *Nostoc* model, *Nostoc* contacts the tip of the fungal hyphae that bulges out and surrounds a part of a cyanobacterial filament, thus incorporating the *Nostoc* cells. Then, the unicellular fungus forms a bladder structure that contains 5 to 15 *Nostoc* cells (Fig 50a; Schüßler and Kluge 2001; Schüßler and Wolf 2005). Thereby, in contrast to the conventional AM symbiosis between plants and members of the glomeromycotan, cyanobacteria are incorporated within fungal bladders. Nevertheless, this symbiotic bladder structure of *Geosiphon* is the homologous stage to the arbuscules containing cells in AM and nutrient exchanges take place at the symbiotic interface of bladders (Fig 50b).

This unique *Geosiphon* - *Nostoc* symbiosis model was used as a tool to isolate pure fungal mRNA from symbiotic bladders. Indeed, as *Nostoc* is a prokaryotic organism; its mRNA does not possess poly(A) tail at the 3' end, and therefore pure (eukaryotic) fungal mRNA from symbiotic stages are easily isolated with poly(T) tails. By the mean of a poly(T) bead discrimination methods, a cDNA libraries suitable for yeast functional complementation has been constructed (Fig 32) and random sequencing of >100 cDNA clones did not result in any *Nostoc* sequences, indicating that the library is derived from nearly exclusively fungal transcripts (Schüßler et al. 2007). The screening of this library in EBY.VW4000, a yeast deficient for glucose uptake which lacks the 20 sugar transporter genes required for hexose uptake (Fig 27), led to the isolation of GpMST1. At the beginning of my PhD, GpMST1 was the only sugar transporters identified from a glomeromycotan species. Therefore in a first part of this work, we focused on the screening of the expression library made from *G. pyriformis* bladders in order to screen for glomeromycotan symbiotic genes including additional sugar

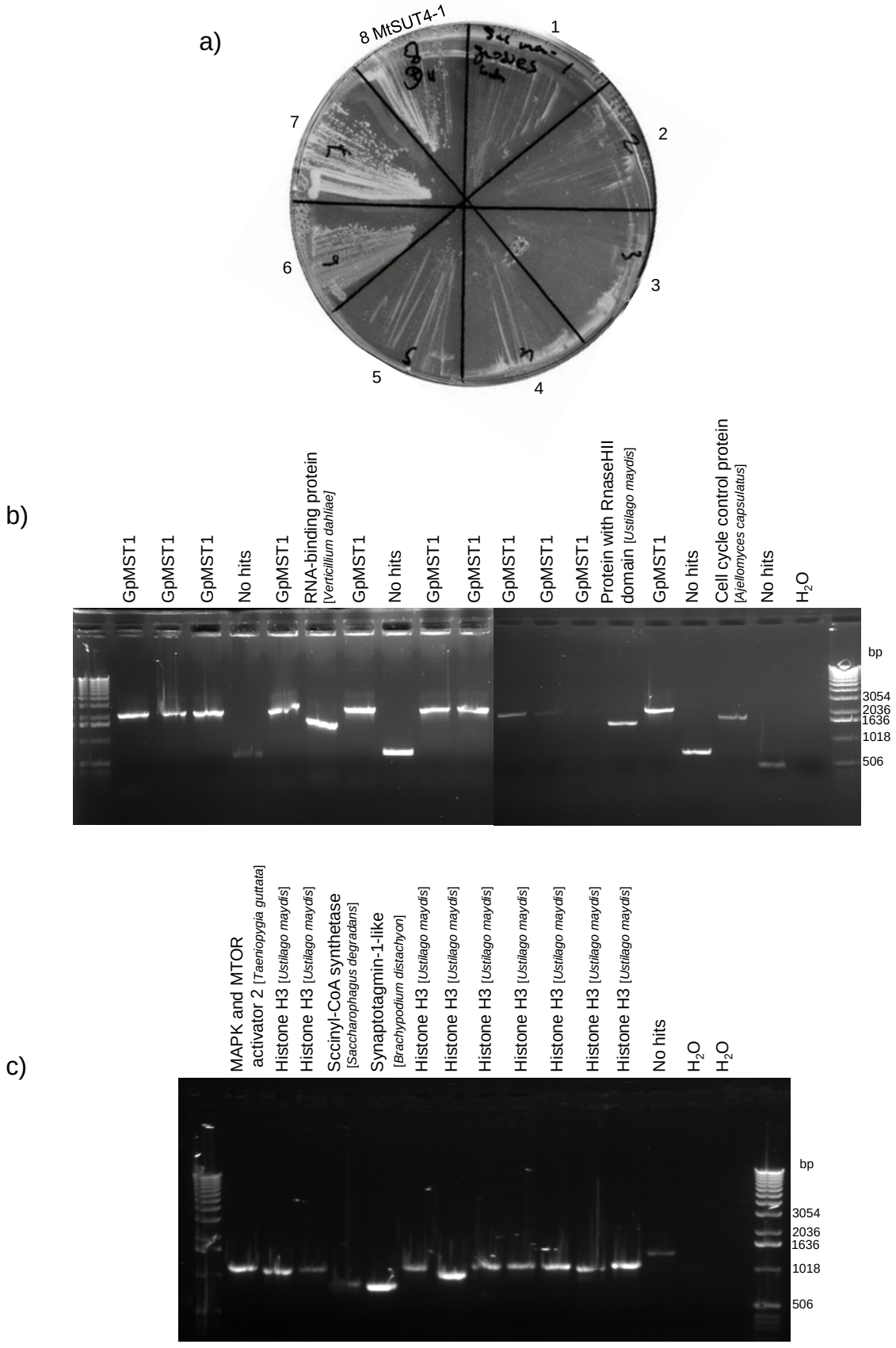


Fig 51. Screening of the *G. pyriformis* expression library by functional complementation of YSL2-1

a) Transformants which complemented the sucrose deficiency of YSL2-1 were replated on sucrose (2%) minimal medium and compared to a positive control yeast YSL2-1 transformed with MtSUT4-1 (line 8). As for example in this plate, we confirm that clones in line 6 and 7 show a functional complementation of the sucrose deficiency of YSL2-1.

b), c) PCR using primers PMAF and ADH closeR flanking the insertional site of plasmids extracted from positive clones after b) 1 week and c) 3 weeks on minimal sucrose medium. Top blastx hits of the sequencing results are given.

transporter candidates. In a second part, we benefited from the knowledge acquired from this unique symbiotic model to develop a similar yeast complementation approach to study the widely used AM symbiosis model between *M. truncatula* and *G. intraradices.*

1 Pyrosequencing of the *G. pyriformis* library

Pyrosequencing is a high-throughput method for large-scale genetic analysis notably used to sequence full genome organism from genomic libraries. Thereby, pyrosequencing would be a valuable tool for the analysis of the expression library from *G. pyriformis* in order to obtain a detailed analysis of fungal gene expressed at symbiotic stages when nutrient transfer occurs between both partners.

Before the pyrosequencing can be performed, fungal cDNA has to be recovered from the pDR196SfiI expression vector in which they are inserted. cDNA was extracted from pDR196SfiI vector by SfiI restriction enzyme digestion and separated from the vector backbone through gel cutting. cDNA inserts extracted from the library showed an average size between 1000 and 2000 bp (Fig 35a). However, an optimal fragment length of 400 to 600 bp is recommended for 454FLX pyrosequencing, providing approximately 400 bp read lengths (Roche). According to the Helmholtz Zentrum in Munich which perform sequencing in collaboration with the Genetics Institute LMU Munich, DNA nebulization is not suitable to size fractionate such samples. Therefore, the alternative method that we first used was the enzyme fragmentase that randomly generates DNA breaks in a time dependent manner. Using this method, we were not able to produce sufficient DNA quantity for the 454 run. We then chose to generate DNA breaks by digestion with 4 cutter restriction enzymes. Independent digestions of the cDNA inserts were pooled and size fractionated from gel cutting to obtain a sample with a size distribution between 200-700 bp suitable for pyrosequencing (Fig 35b). Results from the 454 sequencing run are currently analyzed by an ongoing project at the LMU.

2 Screening of the *G. pyriformis* cDNA expression library for glomeromycotan sucrose transporters by yeast complementation

In parallel, the *G. pyriformis* expression library was screened by yeast functional complementation with a sucrose deficient strain in order to isolate a glomeromycotan sucrose transporter candidate. Yeast expression vectors from the library containing *G. pyriformis* cDNAs were therefore transferred in the YSL2-1 strain lacking all hexose and sucrose import systems (Fig 27). Resulting transformants were replated on sucrose minimal synthetic

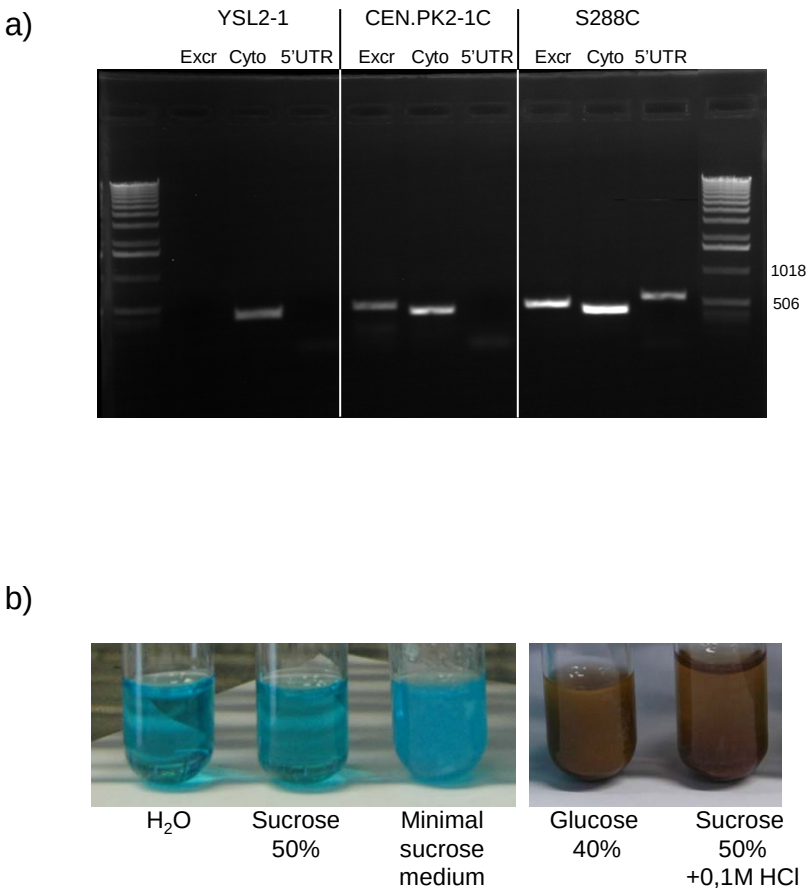


Fig 52. Genomic amplification of the yeast SUC2 excreted and cytosolic invertases and Benedict's test for the presence of reducing sugars in sucrose medium

a) PCR amplification using a specific forward primers of the excreted invertase form (Excr, 449bp) or the cytosolic form (Cyto, 389bp) or in the 5'UTR (531bp) of the SUC2 locus and with a common reverse primer located in the SUC2 CDS for all three reactions (see Annex IV.5), on yeast DNA samples extracted from the YSL2-1 sucrose deficient strain, its parental strain CEN.PK2-1C and the reference yeast strain S288C.

b) Result of the Benedict's test on water (negative control), on sucrose stock solution, on minimal medium use to grow yeast, on glucose and sucrose with the addition of a drop of HCl 0.1M (positive controls).

Benedict's reagent is used as a test for the presence of reducing sugars. Reducing carbohydrates (*e.g.*, glucose and fructose) are oxidized by copper ions present in the Benedict's reagent resulting in the reduction of blue copper and the precipitation of brick red copper oxide. Sucrose is a non reducing sugars and therefore does not react under these conditions. However, the addition of diluted chloric acid to sucrose prior to boiling samples during the Benedict's reaction caused sucrose hydrolysis into reducing glucose and fructose giving a positive Benedict's test.

medium without uracil to confirm the complementation of the sucrose deficiency. After one week of growth, out of the 92 initial transformants, we obtained 18 transformants which positively complemented the sucrose deficiency of YSL2-1 when compared to the positive control containing the *M. truncatula MtSUT4-1* (Fig 51a) and plasmids of positive yeast clones were extracted and checked by PCR. Surprisingly, most transformants (11) contained *GpMST1*, the gene coding for the hexose transporters previously characterized by Schüßler et al. 2006. *GpMST1* sequences inserted in the plasmid showed slightly different sizes corresponding to different sizes of the UTR regions (Fig 51b). In addition, the screen also resulted in the isolation of 4 unknown sequences which resulted in no hits when blastx against NCBI non redundant sequence database and 3 fungal sequences corresponding respectively to a putative RNA binding protein, a protein harboring an RNase HII domain and a cell cycle control protein (Fig 51b). Retransformation of plasmids containing these genes did not complement YSL2-1 (data not shown). Therefore, these candidates were considered as false positives. Furthermore, after 3 weeks of growth, 14 additional yeast transformants were isolated on sucrose minimal medium included several clones containing histone H3 genes were identified (Fig 51c); this could be explained by massive rearrangement in the yeast cell for survival on minimal medium.

The complementation of the sucrose deficiency of the YSL2-1 strain by GpMST1, a hexose transporter which shows high affinity for glucose but is not able to transport sucrose when characterized in yeast (Schüßler et al. 2006), was rather unexpected. The genotype of the sucrose deficiency of the YSL2-1 strain was checked by PCR. The YSL2-1 strain possesses a mutation in its genotype for the excreted sucrose invertase gene and therefore is not able to utilize extracellular sucrose from the minimal medium (Fig 27). In wild type yeasts, both cytosolic and excreted invertases are coded by a single locus (SUC2) but the excreted and the intracellular invertase coding sequences have different initiation codons (Carlson and Botstein 1982; Taussig and Carlson 1983). Indeed, the start codon of the excreted invertase is located 60 bp upstream the start codon of the cytosolic and the additional 20 amino acids encode a signal peptide of glycosylation for the extracellular form. Primers flanking the start codons of the excreted and cytosolic invertase sequences were designed to check the sucrose deficiency of the YSL2-1. The results confirm the absence of the signal sequence coding for the extracellular invertase and the presence of the start codon for the cytosolic form in YSL2-1 (Fig 52a), whilst wild type yeasts possess both invertase sequence initiation sites. In addition, a primer in the 5' UTR of the SUC2 locus was designed to obtain the exact sequence in the mutated strain. However, primers were designed against the SUC2 locus of the sequenced reference yeast strain S288C and did not amplify neither the locus of the YSL2-1 strain nor its parental wild type strain CEN.PK2-1C (Fig 52a; Wieczorke et al. 1999). This may be due to genetic differences in non-coding regions of wild type strains. Although the absence of the

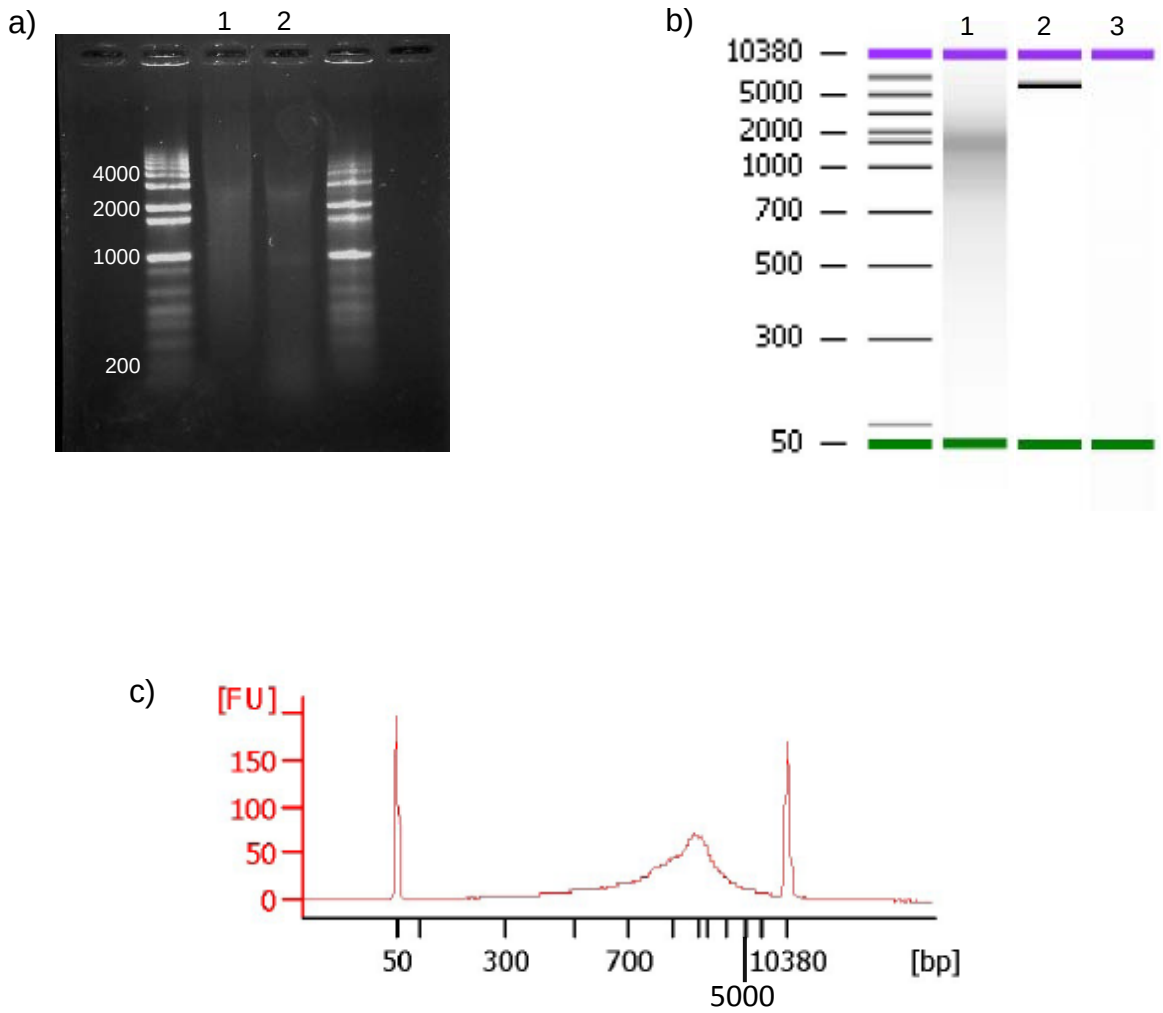


Fig 53. Construction of the expression library from *M. truncatula* symbiotic roots

a) cDNA amplification by "long distance" (LD-PCR) of the mRNA samples from mycorrhized *M. truncatula* roots (line 1) and from the control human placenta polyA RNA provided in the Creator™ SMART™ cDNA library construction kit (line 2). LD-PCR cycling was performed according to Clontech guidelines, 24 cycles was used for the library sample (120ng) and 18 cycles when starting with 1µg human mRNA.

b) Gel from DNA chip 7500 (Agilent) of library cDNA after LD-PCR, proteinase K digestion, SfiI digestion and size fractionation (line 1); pDR196SfiI vector digested with SfiI and gel cut purified twice from its SfiI inserts "stuffer " (line 2). Note that no traces of the SfiI stuffer (1433bp) or of the circular non-cut plasmid (7852bp) could be detected (line 2). Negative water control (line 3)

c) Chromatogram of library cDNA, corresponding to line 1 in b), ready for ligation in the linearized yeast expression vector (line 2 in b).

excreted invertase in the YSL2-1 genome was checked, sugars were apparently imported into the mutant yeast cells containing GpMST1 and subsequent growth on minimal sucrose medium was observed.

Another explanation of the functional complementation by GpMST1 could be the presence of monosaccharides resulting from sucrose hydrolysis in the selective medium. However, we were not able to detect reducing hexoses (glucose and fructose) by performing a Benedict's test neither on minimal sucrose medium use to cultivate the complementing clones nor on the sucrose source solution used to prepare this selective medium (Fig 52b).

In conclusion the screen of the *G. pyriformis* expression library only led to the identification of a single transporter sequence corresponding to the previously identified GpMST1 (Schüßler et al. 2006), with an aberrant functional complementation of the YSL2-1 sucrose deficiency by this hexose transporter.

3 Construction of an expression library from *M. truncatula* roots colonized by *G. intraradices*

A non-targeted approach, similar to the one developed for *G. pyriformis* has been developed by constructing an expression library from AM symbiotic roots (Fig 32). *Medicago truncatula* plants inoculated with *G. intraradices* BEG141 were cultivated under low phosphate condition and harvested at different developmental stages: at 2, 3 and 4 wpi. These RNAs belong to the same samples as the one used for quantitative RT-PCR in AM conditions (see Chapter III.2); equal concentrations for each time point were used (54 µg for each time point resulting from 18 µg of 3 biological repetitions). In order to have large amount of RNA and maximum diversity of expressed genes, total RNA (162 µg) from symbiotic roots was pooled. Thereafter, mRNA was isolated by the means of poly(T) beads and we obtained 1.2 µg of pure mRNA from symbiotic roots. The yield of mRNA extraction by poly(T) bead method from total RNA of *M. truncatula* roots was estimated around 0.74%. A maximum of 120 ng (3 µL) of mRNA can be used for the first strand synthesis and after "long distance" amplification of cDNA according to the SMART DNA synthesis (Clontech), a similar cDNA smear was obtained when compared to the positive control made from human polyA RNA (Fig 53a). Resulting cDNA were size fractionated between 700 bp to 5000 bp (Fig 53b-c) by gel cutting. This fractionation size is consistent with the size of most transporter coding sequences and allows the discrimination of partial sequences. cDNA was then inserted in pDR196SfiI vector modified with SfiI sites to allow directional cloning (Fig 30; Schüßler et al. 2006) and transformed in ultracompetent *E. coli* cells to obtain the primary library. Twenty large Petri dishes containing each 5000-10000 clones were harvested to constitute the primary library, ensuring a large coverage of gene expressed present in the library.

Clone n°	Length (bp)	Top blastx in NCBI	Accession n°	CDS
JD-247	1113	Signal peptide peptidase-like protein [*Medicago truncatula*]	MTR_1g100980	Complete CDS
JD-249	516	Zinc finger A20 and AN1 domain-containing stress-associated [*Medicago truncatula*]	MTR_2g098160	Complete CDS
JD-259	888	Albumin [*Medicago truncatula*]	MTR_3g067430	Complete CDS
JD-261	1433	SfiI "stuffer" Clonetech	NA	NA
JD-262	606	Alpha-amylase/subtilisin inhibitor [*Medicago truncatula*]	MTR_3g014820	Complete CDS
JD-264	834	Lectin [*Medicago truncatula*]	MTR_5g031100	Complete CDS
JD-265	1254	Fructose-bisphosphate aldolase A [*Macaca fascicularis*]	EHH60297.1	Partial CDS
JD-267	1137	No significant hits	NA	NA
JD-268	795	Hypothetical protein [*Medicago truncatula*]	MTR_043s0006	Complete CDS
JD-270	739	No significant hits	NA	NA
JD-273	1288	Nodal modulator [*Medicago truncatula*]	MTR_7g109730	Partial CDS
JD-275	543	Uncharacterized protein [*Glycine max*]	LOC100499863	Complete CDS

Table 11. Sequencing results of cDNA inserts present in the primary library of *M. truncatula* - *G. intraradices* expression library symbiotic roots.

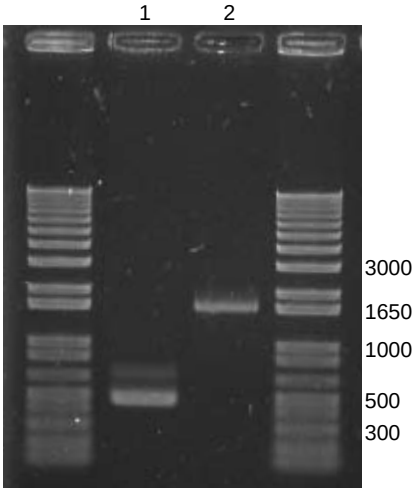


Fig 54. Screening of the secondary library of *M. truncatula* symbiotic roots by PCR amplicication of *MtBCPs* and *MtPT4*

Specific primers (see Annex IV.3) were designed against the full CDS of *MtBCP1a* (line 1) and *MtPT4* (line 2). Note that the 2 amplicons present in line 2 correspond to closely related *MtBCP* isoforms (Paradi et al. 2010) which were both amplified with the primer pairs.

To check quality of the primary library, 32 random clones were picked and cDNA inserts were analyzed by plasmid preparation. Despite the size fractionation, the average size distribution of this library was estimated around 615 bp and plasmids harboring an insert over 500 bp were sequenced to verify the incorporation of full-length coding sequences (Table 11). Out of the 32 randomly picked colonies, a single pDR196SfiI vector without cDNA insert was isolated. Indeed, it contains the SfiI insert "stuffer" which was added to the original pDR196 vector to allow directional cloning with SfiIA and SfiIB sites (Fig 30; Martin 2005). Although pDR196SfiI plasmid was digested and gel cut twice to separate the SfiI stuffer (1433 bp) from the linearized vector (6419 bp; Fig 53b), it seems that pDR196SfiI containing the original "stuffer" is still present in the expression library. In parallel, seven sequences coding for full-length CDS could be identified, and these sequences showed a BLAST result to leguminous sequences coherent with the integration of *M. truncatula* sequences (Table 11). In addition, two partial sequences were also identified. Two sequences present a BLAST results with no significant similarity found when blastx against non redundant protein database of NCBI; it was thus not possible to give a postulate of their origin (*i.e.,* from *G. intraradices* or *M. truncatula*). Thereby, no fungal sequences could be screened from this first analysis of the primary library.

As primary libraries can be unstable, amplification of secondary libraries is recommended immediately (Fig 32). After amplification of the primary library, total plasmid preparation from the secondary library was checked by PCR to confirm the presence of symbiotic expressed genes. In that way, we were able to amplify the full-length sequences of *MtPT4* and two blue copper-binding protein isoforms (*MtBCP1a* and *TC132480*) which are *M. truncatula* genes specifically expressed in mycorrhizal roots and both represent valuable markers of the symbiosis functionality (Fig 54; Harrison et al. 2002; Paradi et al. 2010). The *M. truncatula* - *G. intraradices* cDNA library shows relatively high diversity of full-length genes (Table 11) and contains gene markers specifically expressed at symbiotic stages (Fig 54). The screening of this *M. truncatula* - *G. intraradices* expression library notably for sugar transporters genes and further fungal candidates is currently being carried out by an ongoing PhD project.

4 Conclusion

Numerous genes from species belonging to the phylum Glomeromycota involved in nutrient transport and metabolism have been identified from cDNA libraries (Gianinazzi-Pearson et al. 2012) but no library containing *Glomus* sp. sequences and suitable for functional

complementation of yeast has yet been constructed. The construction of such libraries enabled the rapid identification of functional nutrient transporters expressed in symbiotic structures of ectomycorrhizal fungi (Wipf et al. 2002; Wipf et al. 2003; Lambilliotte et al. 2004; Benjdia et al. 2006) and led to the identification of the first glomeromycotan sugar transporter (Schüßler et al. 2006). Besides transport related genes, the identification and characterization of numerous candidates is also possible using appropriate yeast mutants. The screening of the mycorrhized *M. truncatula* - *G. intraradices* expression library developed in this study by complementation of the *ade2*$^{-}$ mutant yeast (red colored) did not result in the isolation of positive clones with wild type restored phenotypes (white colored) containing the ADE2 gene coding for phosphoribosylaminoimidazole carboxylase (N. Ait Lahmidi personal communication). Additional screens by yeast functional complementation of various nutrient transporters expressed in symbiotic roots are currently carried out as part of N. Ait Lahmidi PhD project. In addition, no fungal sequences could be detected by non-targeted analysis of the mycorrhized *M. truncatula* - *G. intraradices* expression library (Table 11); the detection of fungal markers such as *GiMST2* which is mostly expressed in symbiotic structures (Helber et al. 2011) and the quantification of the fungal representation as for example by quantitative PCR of the ratio of plant and fungal reference genes such as *MtTEF*/*GiTEF* (Seddas et al. 2009; Baier et al. 2010) will also be envisaged.

The screen of the *G. pyriformis* expression library by functional complementation of the sucrose deficient yeast strain YSL2-1 led to the identification of the monosaccharide transporter GpMST1 which was not able to mediate the transport of sucrose when characterized in the glucose deficient yeast strain EBY.VW4000 (Schüßler et al. 2006). It is very unlikely that GpMST1 mediate sucrose transport in the YSL2-1 strains, since both the YSL2-1 and EBY.VW4000 strains originate from the same parental wild type yeast CEN.PK2-1C (Wieczorke et al. 1999). Although sucrose source solution was filter-sterilized and prepared extemporaneously with no residual presence of monosaccharides in the minimal sucrose medium and that the mutation of the excreted invertase of YSL2-1 strain essential for the cleavage of sucrose in the medium was confirmed (Fig 52), no sucrose transporter candidate could be isolated from the *G. pyriformis* expression library. Recently, a putative SUT (GiSUC1) was identified in the model glomeromycotan species *G. intraradices* which belongs to the same phylum as *G. pyriformis* (Schüßler et al. 2001; Helber et al. 2011). Up to now, GiSUC1 is the only identified SUT candidate from mycorrhizal fungi and its role in the AM symbiosis has still to be deciphered (Fig 60). Among the so-called biocontrol fungi which reduce or prevent plant-pathogens occurrence and promote plant growth and stress resistance, two novel fungal sucrose transporters (MRT and TvSUT) have been characterized from *Metarhizium robertsii* and *Trichoderma virens* and shown to be involved in sugar uptake from root exudates (Fang and St. Leger 2010; Vargas et al. 2011). The role of fungal SUTs was also pointed out in the pathogenic relation between maize and *Ustilago maydis*. In this plant-

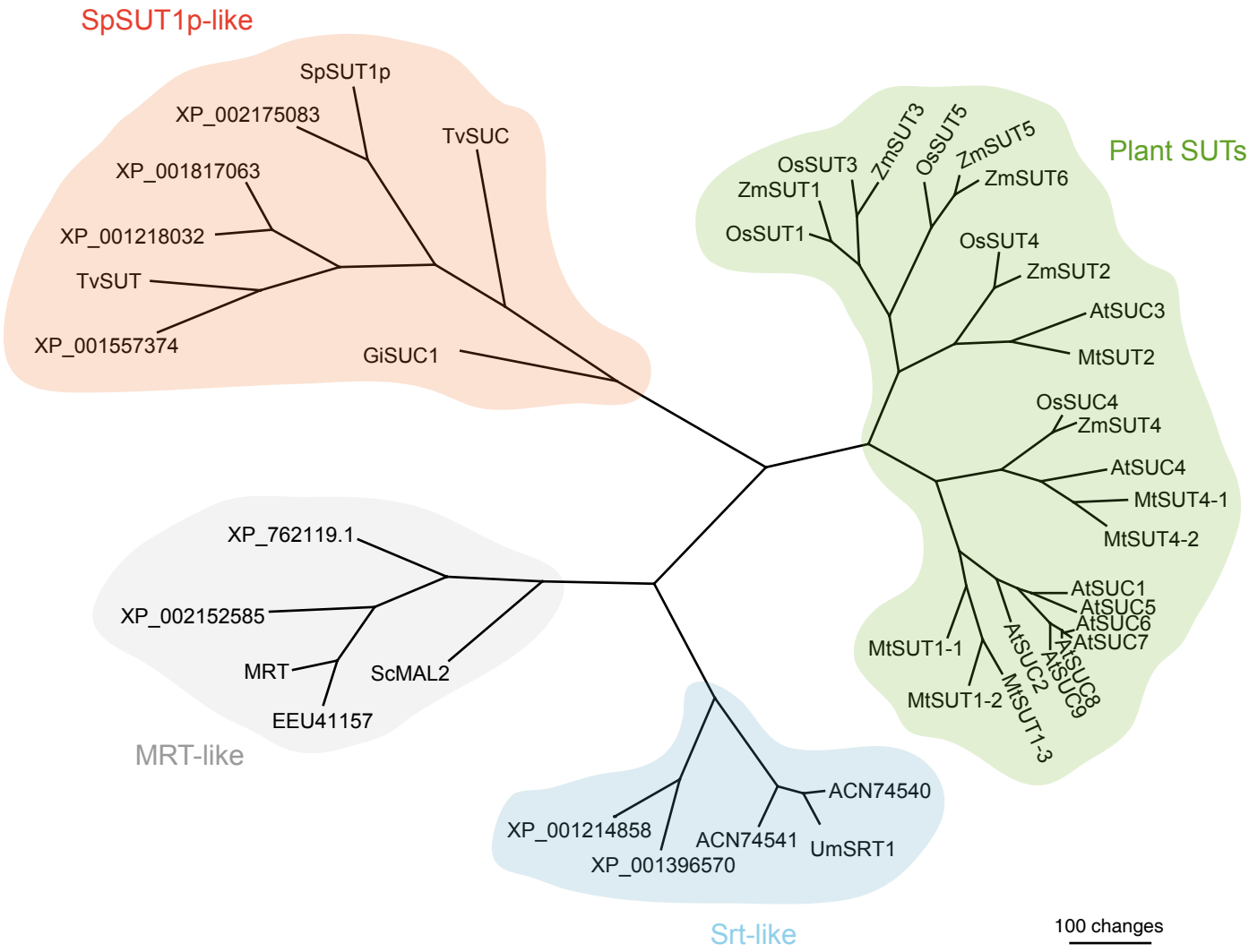


Fig 55. Phylogenetic tree of the plant and fungal SUT family

Alignment of amino acid sequences of 18 fungal and 26 plant SUTs was performed with Mafft version 6 (Katoh and Toh, 2008) and maximum parsimony analyses were done using PAUP 4.0b10 (Swofford, 1998). Heuristic tree searches were executed using 1000 random sequence additions and the tree bisection-reconnection branch-swapping algorithm with random sequence analysis. The complete alignment was based on 947 sites; 689 were phylogenetically informative. Unrooted tree was displayed on Dendroscope2.7.4 (Huson et al., 2007). Characterized and published proteins are named according to their latest denomination; accession number was used for proteins which have not yet been assigned.

SpSUT1p-like: *Aspergillus oryzae* (XP_001817063), *Aspergillus terreus* (XP_001218032), *Botrytis cinerea* (XP_001557374), *Glomus intraradices* (GiSUC1: AEK82125), *Schizosaccharomyces japonicas* (XP_002175083), *Schizosaccharomyces pombe* (SpSUT1p: NP_594387), *Trichoderma virens* (TvSUT: CBH19584; TvSUC: CBK33777).

MRT-like: *Metarhizium robertsii* (MRT: ACS83541), *Nectria haematococca* (EEU41157), *Penicillium marneffei* (XP_002152585), *Saccharomyces cerevisae* (ScMAL2: P15685), *Ustilago maydis* (XP_762119.1)

Srt-like: *Aspergillus niger* (XP_001396570), *Aspergillus terreus* (XP_001214858), *Sporisorium reilianum* (ACN74540), *Ustilago hordei* (ACN74541), *Ustilago maydis* (UmSrt1: XP_758521).

pathogenic interaction model, the very high affinity fungal sucrose transporter UmSrt1 was shown to directly compete with sucrose retrieval of the maize transporter ZmSUT1 at the plant-fungal interface (Wahl et al. 2010, Wippel et al. 2010). As this field opens up, a novel SUT family from fungi is emerging (Fig 55); the study of the evolution of sugar transport genes from mutualistic to pathogenic fungi and their role in carbon partitioning within and between organisms represents a major challenge to better understand the outcome of plant-fungal interactions (Doidy et al. 2012a).

In this study, we screened the expression library constructed from the unique *Geosiphon* - *Nostoc* symbiotic model and developed a similar strategy for the reference AM model between *M. truncatula* and *G. intraradices*. The transfer of knowledge between these two glomeromycotan models led to the construction of an expression library from AM plant root which represents an interesting tool to isolate candidates by yeast heterologous expression and identify functional transporters involved in biotrophic exchanges from both the plant and fungal partners. Besides a large number of putative transporters (around 500) recently identified in the sequenced *G. intraradices* transcriptome (Tisserant et al. 2011), the current knowledge on the transportome in the whole glomeromycotan phylum is still in its infancy with only fifteen transporter genes characterized (Gianinazzi-Pearson et al. 2012). In addition, as the complete assembly of the first glomeromycotan genome is not yet achieved, the construction of expression libraries and sequencing of such libraries as well as the release of post-genomic databases constitute valuable tools for the scientific community working on AM symbiosis.

Discussion

Discussion

1 *In silico* search of sugar transporters in *M. truncatula* and *G. intraradices*

Database mining for sugar transporters from the model legume *M. truncatula* led to the identification of 6 sucrose transporters (MtSUTs) and 61 monosaccharide transporters (MtMSTs). These findings are relatively consistent with previously published families from the plant model species *A. thaliana*, *V. vinifera* and *O. sativa* (Aoki et al. 2003; Buttner 2007; Johnson and Thomas 2007; Sauer 2007; Afoufa-Bastien et al. 2010). Therefore, all plant species seem to possess a small SUT and a large MST family. Out of the 6 MtSUT candidates, only 2 loci are present and annotated in the genome (Table 7) despite a current capture of 94% of all genes in the *M. truncatula* genome (Young et al. 2011). In addition, we also identified 61 MtMSTs including 58 genomic loci. For the consistency of phylogenetic classification study (Fig 36), only sequences presenting full-length coding DNA sequences (CDS) have been kept. Therefore, additional partial contigs and ESTs retrieved from DFCI and MENS databases were not included. Regarding the low percentage of available genomic loci for MtSUTs retrieved from Mt3.5 annotation and that partial accessions of MtMSTs were not retained for this study. In addition, 3 MtMSTs were not retrieved from the genome database (CAD31121, MtC20248 and MtC00740) despite all accessions were identified from the sequenced *M. truncatula* line A17 (Journet et al. 2002; Lefebvre et al. 2007). Taken together, we can postulate that the 61 MtMSTs identified in this project does not constitute the full MtMST family and that supplemental MtMST loci remain to be identified in the genome of *M. truncatula*. Partial sequences coding for putative MtMSTs could be further studied in full-length by RACE or library screening (Fig 29a and 32).

The *in silico* search for MtMSTs candidates differentially regulated in response to AM inoculation led to the identification of 3 MtMST candidates. *Medtr4g091370* is the only sugar transporter candidate to present a higher transcript accumulation from the MtGEA data (Fig 38). This candidate is closely related to Mtst1 (Fig 36) which was reported to present an increase of at least 2 fold transcript levels using northern blot in roots of *M. truncatula* mycorrhized with *G. intraradices* or *G. versiforme* (Harrison 1996). However, according to the MtGEA microarray (Gomez et al. 2009), *Medtr4g091370* only present a faint upregulation in roots colonized by *G. intraradices* (Fig 38). In addition, the STP *MtHext1* (Medtr1g104780) and the INT (Medtr2g049020) candidates previously reported to be AM

upregulated in microarray on characteristic cell-types obtained via laser microdissection (Gaude et al. 2011; Hogekamp et al. 2011) did not show a differential regulation in available data from the MtGEA obtained from mycorrhized whole root samples (Fig 38; Gomez et al. 2009). Furthermore, Hogekamp et al. 2011 identified two additional candidates (*Medtr5g044220* and *Medtr4g131800*) annotated as "carbohydrate transporters" that were coactivated in both the symbiotic interaction with *G. intraradices* or *G. mosseae*; however, they do not belong to the MST family and are rather related to triose phosphate/phosphate and glucose-6-phosphate/phosphate translocators, respectively. In addition, the mycorrhizal specific phosphate transporter MtPT4 clearly shows a specific "switch" signal in mycorrhized root samples (Harrison et al. 2002; Gomez et al. 2009); in contrast, no sugar transporters (MtMSTs and MtSUTs) present a mycorrhizal specific induction of its gene expression (Fig 38 and 49).

On the fungal side, 6 candidates were retained as putative sugar transporters of *G. intraradices* (Table 5). Out of the 3 GiMSTs recently identified by Helber et al. 2011, only partial sequences correspond to GiMST4 but the sequences corresponding to GiMST2 and GiMST3 were not retrieved in this *in silico* search as they rather seem related to putative xylose transporters. Furthermore, an additional candidate (Glomus_c8992) was annotated as MFS glucose transporter (Tisserant et al. 2011) but according to BLAST searches, corresponds to lipopolysaccharide-induced tumor necrosis factor-alpha. The fact that all candidates withdrawn according to high GC% and bacterial relation could not be detected in microarray data, confirm that they originate from extrinsic contamination (Table 5 and 6).

Although *Glomus_c6242* and *Glomus_c1670* are synonymous accessions corresponding to *GiMST4* gene (Table 5; Helber et al. 2011), dissimilar gene expression profiles were obtained according to microarray and quantitative RT-PCR experiments (Helber et al. 2011; Tisserant et al. 2011). Indeed, *Glomus_c6242* seems highly expressed in spore, ERM and IRM but no significant difference of expression could be observed between these different fungal structures whilst *Glomus_c1670* is weakly expressed in all structures but present a significant higher expression ratio in IRM/spore (2.9) and IRM/ERM (3.7) (Tisserant et al. 2011) indicating a potential role in sugar transfer towards the fungus in intraradical structures. Furthermore, *GiMST4* expression assessed by quantitative RT-PCR present its highest expression in ERM but is also expressed at lower level in spores and arbuscule-enriched material (Helber et al. 2011). Therefore, contradictory expression patterns remain for *GiMST4* which is closely related to the functional transporter GpMST1 suggested to play a critical role for sugar transfer at the cyanobacterial-fungal interface (Fig 50; Schüßler et al. 2006; Schüßler et al. 2007). Concerning the 6 retained GiMSTs (Table 5), Glomus_c4574 and 11118_0_CCHU9778.g1_CACE showed the most promising expression profile for candidates to be involved in sugar partitioning towards intraradical structures when AM

symbiosis is well established (Table 6). However, it would also be interesting to study expression patterns of all identified GiMST at different time point of the symbiosis (*i.e.*, at early symbiotic stage) in order to determine if alternative candidates take turns at different stages of the AM symbiosis. To date, only a single glucose transporters from *G. intraradices* (GiMST2) was clearly proved to be essential for sugar transfer towards arbuscules and intercellular hyphae (Helber et al. 2011); more work is needed to identify the complete GiMST family and to decipher the role of all sugar transporter candidates in AM symbiosis.

2 Characterization of the first leguminous SUT family: the MtSUT family

In a second part, I focused on the sucrose transporters of *M. truncatula* (MtSUTs) retrieved from the *in silico* analysis (see Chapter II) and we report, for the first time, the identification of the MtSUT family that comprises at least 6 members in the genome of *M. truncatula* (Doidy et al. 2012b). MtSUTs were annotated, according to the latest classification (*i.e.*, *MtSUT2* when belonging to SUT2 clade; Kühn and Grof 2010). As the SUT1 and SUT4 are multigenic copy clades in *M. truncatula*, we named the corresponding genes upon phylogenetic grouping into particular clades (Fig 39) adding an hyphen for each gene duplicates (*i.e., MtSUT1-1*, *MtSUT1-2* and *MtSUT1-3* as well as *MtSUT4-1* and *MtSUT4-2*) and as Braun and Slewinski 2009 proposed for the nomenclature of monocotyledonous SUTs, we suggest in future work that all SUTs to be named according to this new annotation.

2.1 Intron/exon pattern of plant SUTs

Promoter region and gene locus patterns of MtSUTs were studied, in particular for available gene members from the SUT1 and SUT4 clades (Table 8 and Fig 40). In *Arabidopsis*, rice and grape, a similar number of 5 exons interspersed by 4 introns is also reported for the SUT4 clade members *AtSUC4*, *OsSUC4* (Fig 56a; Aoki et al. 2003) and *VvSUC11* (Afoufa-Bastien et al. 2010). However, it seems that SUT1 clade members present a slightly lower number of exon/intron in these species (Fig 56a; Aoki et al. 2003; Baud et al. 2005; Afoufa-Bastien et al. 2010; Payyavula et al. 2011). In *M. truncatula*, we report a conserved exon/intron patterns for SUT1 and SUT4 clade members *MtSUT1-2*, *MtSUT1-3* and *MtSUT4-1* (Fig 40). Genes from the SUT2 clade seem to present a different gene pattern with a significant higher number of intron/exon (Fig 56b); this gene structure comprises 14 exons interspersed by 13 introns which is very well conserved among species (*SlSUT2* and *AtSUT2*, Barker et al. 2000; *PtaSUT5* and *PtaSUT6*, Payyavula et al. 2011; *OsSUT4*, Aoki et al. 2003; *VvSUC12*, Afoufa-

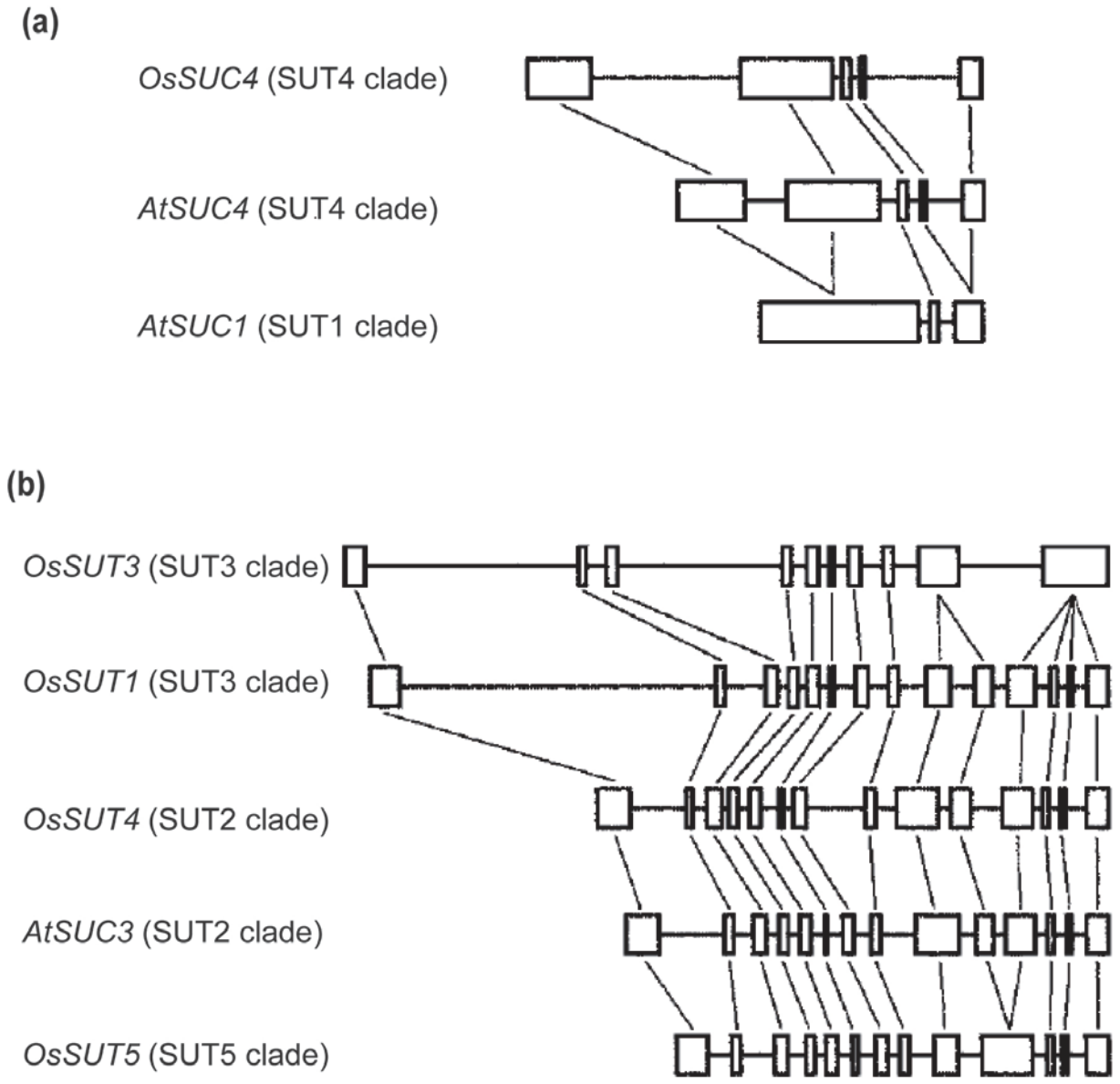


Fig 56. Comparison of the gene structures from (a) the SUT1 and SUT4 clades and (b) the SUT2, SUT3 and SUT5 clades
Adapted from Aoki et al. 2003

Bastien et al. 2010). However, we were not able to obtain genomic sequence of the *MtSUT2* locus by PCR amplification (data not shown). monocotyledonous specific SUT3 and SUT5 clades (Fig 39) which were initially attached to the SUT2 clade (Fig 8) also seem to present a high number of exon/intron comparable to the SUT2 gene structure (Fig 56b). In conclusion, it seems that gene structure is relatively well conserved between the SUT1 and SUT4 clades and between the SUT2, SUT3, SUT5 clades, respectively (Fig 56).

2.2 Functional and biochemical characterization of MtSUTs

Functional characterization using two different deficient yeast strains for sucrose led to similar results: *MtSUT1-1* and *MtSUT4-1* expressing yeast cells show a clear complementation phenotype. Best complementation results were obtained with a 2% sucrose concentration, which is the usual and preferred concentration used to show sucrose complementation of plant SUTs in SUSY7/*ura3*$^{-}$ (Weise et al. 2000; Aoki et al. 2006; Zhou et al. 2007; Berthier et al. 2009; Okubo-Kurihara et al. 2011) and YSL2-1 (S. Lalonde personal communication). At this concentration, SUSY7/*ura3*$^{-}$ yeast expressing *MtSUT1-2* and to a smaller extent *MtSUT1-3* showed improved growth when compared to the control transformed with the empty vector (Fig 42). In the SUT phylogeny, MtSUT1-2 and MtSUT1-3 as well as MtSUT4-2 cluster with the recently characterized SUFs (Fig 39) which present a complementation of the SUSY7/*ura3*$^{-}$ mutant only when grown on minimal medium containing 4% sucrose (Fig 57; Zhou et al., 2007). However in our conditions, the weak growth of strains expressing *MtSUT1-2* and *MtSUT1-3* was not increased when grown on 4% sucrose and MtSUT4-2 showed no transport capacity.

In addition, performing biochemical characterization, I encountered several problems regarding uptake measurements with yeast expressing *MtSUT1-2*, *MtSUT1-3*, *MtSUT2* and *MtSUT4-2*, which showed a weak or no complementation of the sucrose deficiency but resulted in aberrant uptake measurements. These limitations could be the result of the growth background observed for all yeast strains on sucrose minimal medium (Fig 41b). A growth background for yeast containing pDRs empty vector was also observed in previous studies (Fig 57; Barker et al. 2000; Weise et al. 2000; Zhou et al. 2007; Henry 2011) but was not always reported (Aoki et al. 2006). Increasing the amount of radiolabeled [^{14}C]sucrose (up to 120 kBq) in experiments or harvesting cells at longer time points (up to 10 min) did not result in higher uptake rates of yeasts expressing *MtSUT1-2*, *MtSUT1-3*, *MtSUT2* and *MtSUT4-2* compared to the yeast transformed with the empty vector. Another explanation could be the overnight liquid culture in 2% glucose medium lacking uracil prior to uptake experiments. Indeed, such cultures do not present a sugar restriction due to the presence of glucose and sugar reserve may occur in such conditions limiting the sucrose uptake in subsequent uptake

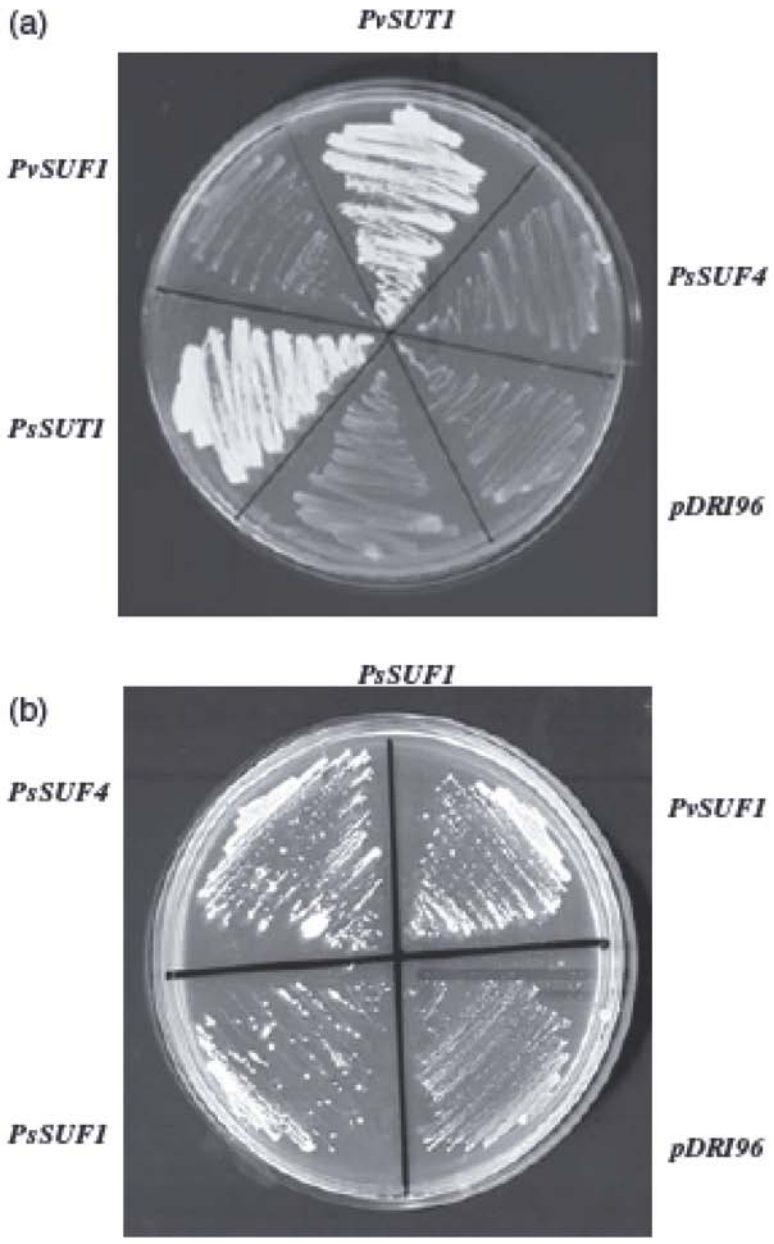


Fig 57. Functional complementation by SUT/SUF from pea and common bean
(a) P sSUT1 a nd P vSUT1 complemented the de ficient yeast s train SUSY7/*ura3*$^{-}$ on 2% sucrose medium w ithout ur acile (b) whilst SUFs showed a c omplementation a t 4% s ucrose concentration. Note that a high growth background is reported for yeast containing the empty vector pDR196 (Zhou et al. 2007).

experiment. However, successful biochemical characterization of MtSUT1-1 and MtSUT4-1 are not consistent with this assumption and trying different sugar substrates (fructose and galactose) for initial culture did not result in sufficient growth of yeasts to perform uptake experiments. As a result, uptake experiments of MtSUT1-2, MtSUT1-3, MtSUT2 and MtSUT4-2 resulted in measurements of aberrant uptake values and therefore, linear kinetics of sucrose uptake and biochemical characterization by expression in the yeast model could not be completed for these members. Although SUFs (Zhou et al. 2007) and the orthologous protein PmSUC3 from the SUT2 clades (Barth et al. 2003) were characterized in the yeast model SUSY7/*ura3*$^{-}$, the heterologous expression in *X. laevis* oocytes system (Reinders et al. 2008) or the use of high-sensitivity FRET nanosensors in human cells (Takanaga et al. 2008; Chen et al. 2010) could be an alternative approach to characterize biochemically MtSUT1-2, MtSUT1-3, MtSUT2 and MtSUT4-2.

Nevertheless, key members MtSUT1-1 and MtSUT4-1, which strongly complement both deficient yeast strains, are functional transporters and present sucrose uptake kinetics similar to previously characterized plant SUTs (see Introduction 3.2.3). MtSUT1-1 presents a Km for sucrose of 1.7 mM consistent with the saturable high-affinity/low-capacity (HALC) system while MtSUT4-1 with a Km of 13.7 mM may represent the low-affinity/high capacity (LAHC) component as already reported for SUT4 clade proteins AtSUT4, StSUT4 (Delrot and Bonnemain 1981; Weise et al. 2000). These transport kinetics are consistent with previously characterized leguminous SUTs (Table 9) relatively to their phylogenetic position (Fig 39).

2.3 Putative function of MtSUT1-1, MtSUT2 and MTSUT4-1

Interestingly, the gene coding for functional H^{+}/sucrose co-transporters *MtSUT1-1* and *MtSUT4-1* were also shown to have the highest expression profile among MtSUTs (Fig 43 and 44) with *MtSUT1-1* showing a 20 fold transcript accumulation in source leaves of all conditions (Fig 45). The high affinity active transport system of MtSUT1-1 together with its expression profile in leaves and its phylogenetic position in the SUT1 clade close to the principal phloem loading orthologs of potato (see Introduction 3.2.2.1.1; StSUT1, Riesmeier et al. 1994), tobacco (NtSUT1, Bürkle et al. 1998) and *A. thaliana* (AtSUC2, Gottwald et al. 2000), strongly suggest a potential role for MtSUT1-1 as the main phloem loader in *M. truncatula*. Two independent mutant Tnt1 insertion lines are available including one with a tnt1 transposon inserted in the CDS of *MtSUT1-1* (Annex VII.1); these plantlets seem to present a retarded phenotype with stunted growth of root organs reminiscent of the phenotype observed in mutants of the gene coding for the SUT1 loading proteins (Fig 9; Riesmeier et al. 1994; Bürkle et al. 1998; Srivastava et al. 2009a). In addition, MtSUT1-1 and MtSUT4-1 show conserved biochemical kinetics with their leguminous orthologs PsSUT1 and LjSUT4

respectively (Table 9; Zhou et al. 2007; Reinders et al. 2008). MtSUT4-1 and LjSUT4 are both H^+/sucrose co-transporters and cluster to a separated branch encompassing MtSUT4-2 and the facilitator protein PsSUF4 (Fig 39; Zhou et al. 2007). Numerous proteins of the SUT4 clade localize at the tonoplast in several species (see Introduction 3.2.2.5 ; AtSUC4 and HvSUT2 Endler et al. 2006; NtSUT4, Okubo-Kurihara et al. 2011; PtaSUT4, Payyavula et al. 2011) where they are involved in vacuolar sucrose efflux to the cytosol (Fig 12; Schneider et al. 2011). The closest related orthologs of MtSUT4-1, LjSUT4 was also detected at the vacuolar membrane (Reinders et al. 2008). No tnt1 mutant lines could be screened for *MtSUT4-1*; however, a protein-GFP fusion could validate the localization of MtSUT4-1.

In contrast, *MtSUT2* allowed no sucrose transport in yeast but was substantially expressed in root and leave tissues (Fig 42 and 44). SUT2 clade members are in general not able to complement mutant yeast strain (Barker et al. 2000; Hackel et al. 2006a) and were first described as sugar sensors (Lalonde et al. 1999). However, AtSUC3 (Meyer et al. 2000) and PmSUC3 (Barth et al. 2003) are characterized as functional low affinity sucrose importers and GFP fusion experiment showed a SUT2 protein localization notably to sieve elements of source leaves and in sink root tips. The exact role of SUT2 clade proteins is still not deciphered.

3 Sugar fluxes and sugar transporters in AM

In a third part (see Chapter III), I focused on biotrophic exchanges in arbuscular mycorrhiza and more particularly on sugar exchanges from the host model plant *M. truncatula* and the fungal symbiont *G. intraradices*. The role of respective plant and fungal sugar transporters is discussed in this chapter.

3.1 Fluxes of soluble sugars towards AMF colonized roots

The quantification of sugar content in *M. truncatula* highlighted the importance of sucrose as the predominant carbohydrate. AM plants showed lower levels of sucrose, glucose and fructose in source leaves, consistent with a higher sugar transfer to sink roots. These results are significantly different from the control plants treated with LP (Fig 48a). However, only small variations of sugar content were measured in sink roots. These results, are in relation with the study of Schliemann et al. 2008 showing similar accumulation of sucrose, glucose and fructose in *M. truncatula* roots colonized by *G. intraradices* and in control plants fertilized with differential amount of phosphate. Consistent with this, in soybean, soluble sugars also accumulated at similar levels in roots of non-mycorrhizal and mycorrhizal plants inoculated with *G. mosseae* (Schubert et al. 2004). In clover, another leguminous species,

sucrose, glucose and fructose accumulated at higher levels in mycorrhizal roots especially at late colonization stage (Wright et al. 1998a). However, in this latter study a fungal inoculum containing unknown isolates as well as rhizobial inoculants has been used to cultivate their plants and therefore sugar content monitored from those plants may not be directly related to the mere presence of the AMF. Also in tomato, contradictory results subsist; similar levels or even a decrease of all main sugars (sucrose, glucose and fructose) was reported in AM roots (Garcia-Rodriguez et al. 2007; Tejeda-Sartorius et al. 2008) while a recent study showed an increase of root sucrose and fructose consistent with a preferential glucose uptake by the AMF (Boldt et al. 2011). In mycorrhizal melon (*Cucumis melo*) and strawberry (*Fragaria* sp.) plants, fruits present a higher fructose content compared to non-mycorrhizal controls, suggesting that the excess of fructose can be redirected towards different sink organs (G. Lingua personal communication; Fig 60). The differences observed in these studies could be explained by different model plants and AMF species combinations as well as different culture conditions, highlighting the complexity of the mechanisms involved in the mycorrhizal association.

3.1.1 **Higher sugar export from source leaves of AM plants**

The root colonization by AMF augment the carbon demand at the whole plant level (Fig 4; Douds et al. 2000). This increased sink strength is balanced by greater CO_2 assimilation and higher photosynthesis rate in source leaves of clover (Wright et al. 1998a; Wright et al. 1998b). In tomato, it was clearly shown that mycorrhizal plants showed increased opening of stomata and assimilated significant more CO_2 with increased efficiency and yield of photosystem II as well as higher electron transport rate. This excess of photosynthates was entirely directed towards mycorrhizal roots likely by the mean of tomato SUTs (SlSUTs) which showed increased transcript accumulation in AM conditions (Fig 58). In *M. truncatula*, we observed similar chlorophyll content in AM and HP plants, as well as lower soluble sugar content together with a higher transcript levels of *MtSUTs* in source leaves of AM plants; including *MtSUT1-1*, the gene likely to code for the protein responsible of phloem loading in *M. truncatula*. Altogether, these findings suggest a higher sucrose export from source leaves of mycorrhizal plants.

In addition, petioles of *M. truncatula* were collected and sugar content of the samples was analyzed (Fig 48b). In our conditions, collecting full petioles did not reflect the actual sugar fluxes in phloem saps but rather represented the sugar content of tissues which composed the petioles. The collection of phloem sap exuding from freshly cut peduncles was sufficient to quantify soluble sugar and estimate sucrose fluxes from source parts to sink seeds in peas (Munier-Jolain and Salon 2003); however in *M. truncatula* plants, the removal of source

leaves to collect phloem fluids exuding from petioles did not result in sufficient amount of saps. In addition, even in cucurbit species which exude large amount of saps upon cutting, resulting exudates contained high amount of hexose contamination from the contents of other cell types (Zhang et al. 2012; Fig 48b). Thereby, other methods such as the collection of phloem saps by the use of aphid stylectomy, which allowed the isolation of *HvSUT1* in barley (Doering-Saad et al. 2002), may be an interesting tool for the detection and quantification of nutrients (*e.g.*, soluble sugars) exported from source leaves towards AM colonized sink roots.

3.1.2 **Higher sugar fluxes towards AMF colonized roots**

It has been estimated between 4% to 20% of total photoassimilates to be redirected towards AMf colonized roots (Douds et al. 2000; Graham 2000); as such, a higher sucrose export towards colonized roots compared to non-mycorrhizal controls is suggested for several plant species (Wright et al. 1998a; Boldt et al. 2011). However, in *M. truncatula,* it was not yet possible to confirm that exported photosynthates are redirected towards AM colonized roots upon sugar quantification analysis of the whole root system (Fig 48c; Schliemann et al. 2008). It has been shown that part of carbon transferred through the form of sugar is then converted to lipids, mostly in the form of triacylglycerols, and structural components such as chitin in the intraradical mycelium (IRM) and in external fungal structures (ERM, Pfeffer et al. 1999; Bago et al. 2000; Bago et al. 2002b; Bago et al. 2003; Délano-Frier and Tejeda-Sartorius 2008; Fig 60). This biochemical conversion of sugar would explain that it was not possible to monitor higher sugar content in AM root samples (Fig 48c). In addition, significant amount of fungal sugar reserve, such as trehalose and glycogen are also mainly allocated to the ERM; indeed we were able to detect the fungus derived metabolite trehalose synthesized from plant hexoses in AM roots (Fig 48c; Bécard et al. 1991; Bago et al. 2000; Schliemann et al. 2008). Large amount of ERM containing those fungal carbohydrate reserves may be lost during washing steps at harvest. The combination of techniques of pure ERM harvest prior to labeling of plant photosynthates may enable to chase carbon transferred from plant tissues towards extraradical fungal structures.

3.2 SUT expression profile in response to inoculation with AMF

From the *in silico* data available in the MtGEA database (Fig 49), no MtSUTs showed a differential regulation in roots sample in response to AMF inoculation. *Medicago truncatula* plants used for transcriptomic microarray analyses were harvested 30 days post inoculation of *G. intraradices* and supplemented with a low phosphate nutrient solution containing 20µM phosphate (Liu et al. 2007; Gomez et al. 2009). This represents around 10 times lower phosphate concentration than the conditions used in our study for quantitative RT-PCR analyses where plants were harvested at similar time points (4 wpi) and supplemented with

low phosphate solutions (LP: 0,13mM). In both microarray and quantitative RT-PCR, *MtPT4* showed a mycorrhizal specific gene expression (Fig 47 and 49; Harrison et al. 2002). Indeed, *MtPT4* is considered to be one of the best indicators reflecting the functionality of the AM symbiosis and presents a gene expression level increasing with AM colonization (Fig 46; Harrison et al. 2002; Helber et al. 2011). In contrast, the upregulation of *MtSUT1-1* and *MtSUT4-1* shown by quantitative RT-PCR in roots was not reported from microarray data (Fig 47 and 49). In addition, the upregulation of the orthologous *SlSUT1* and *SlSUT4* genes of tomato in response to AM was also described in roots supplemented with LP solution and harvested at 6 wpi (Fig 58; Boldt et al. 2011). Using laser capture microdissection combined with microarray hybridization on root samples harvested at 3 wpi and supplied with a nutrient solution containing 20µM phosphate, *MtSUT4-1* was shown to be upregulated in non-arbusculated cortical cells adjacent to arbusculated cells (Fig 25b; Gaude et al. 2011). In our conditions, *MtSUT4-1* was differentially regulated at all time points in AM roots but only compared to the non-mycorrhizal roots sample of plants supplemented with HP (Fig 46). Different expression patterns are obtained for SUTs when using microarray and quantitative PCR approaches on whole root systems and/or microdissected cells.

In this study, we report for the first time the expression profile of the whole MtSUT family by quantitative RT-PCR, and we focused on the plant gene expression upon differential phosphate concentrations and inoculation of *G. intraradices* (Doidy et al. 2012b). *MtSUT* expression was tightly regulated according to the plant nutritional status. Indeed, in HP plants harvested at 4 wpi, all *MtSUT* transcripts in roots and leaves accumulated at lower levels (Fig 45 and 46). In tomato, *SlSUT* expression was not modified after fertilization with a 10 fold higher phosphate concentration (Ge et al. 2008). Despite similar growth parameters observed for HP and AM plants at 3 and 4 wpi indicating that mycorrhizal-enhanced phosphate delivery was used by the plant for biomass production (Boldt et al. 2011); *MtSUT* expression patterns between these 2 conditions were the most divergent observed in our experiment. Indeed, the expression profile in AM conditions and the addition of 10 fold phosphate in HP conditions suggest that *MtSUT* expression patterns are not related to the phosphorus supply by the fungus, but are rather related to the increased sink strength upon AM colonization. It is worthwhile to note that Ge et al. 2008 showed by semi-quantitative PCR assays that the expression of numerous sugar transporters is differentially regulated depending on the *Glomus* species used for inoculation. However, Boldt et al. 2011 reported that all *SlSUT* genes are upregulated in source leaves of tomato plants; while in roots only *SlSUT1* and *SlSUT4* accumulated transcripts at higher levels in roots colonized by *G. mosseae* compared to non mycorrhized control in LP conditions (Fig 58). In our study, AM colonization led to an overall upregulation of orthologous *MtSUTs* in source leaves; in roots, only *MtSUT2* did not present a higher transcript accumulation upon inoculation with *G. intraradices* (Fig 47).

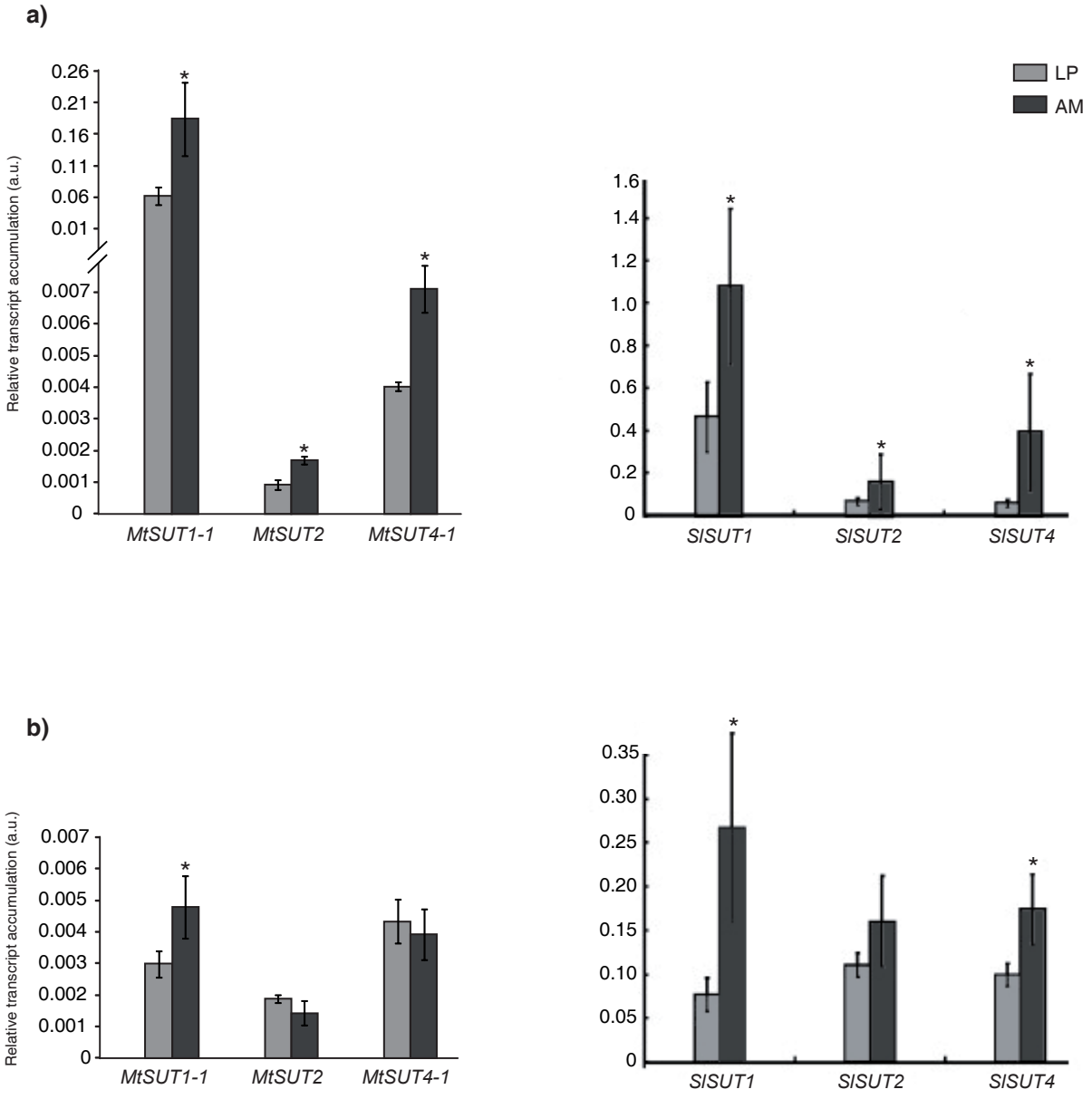


Fig 58. Comparative transcript accumulations a) in leaves and b) roots of respective SUT orthologs from *M. truncatula* and tomato

Medicago truncatula and tomato plants were respectively harvested at 4 and 6 weeks and fertilized with comparable amount of phosphate (0.13mM). *Medicago truncatula* AM plants were inoculated with *G. intraradices* BEG141 and tomato AM plants with *G. mosseae* BEG12. Data represent means ± standard deviation of data obtained from biological material of several independently grown plants. The data were statistically checked by the adequate Student t-test upon accomplishing the Fischer F-test and stars* indicate significant differences between treatments AM versus LP (P = 0.05; n = 4 for SlSUTs; n = 3 for MtSUTs). Colors and font of SlSUTs graphs were modified from Boldt et al. 2011 to allow a better comparison with MtSUT transcript profiles.

Thereby, we confirm a comparable expression profile for *M. truncatula* orthologous genes in agreement with the observation from Boldt et al. 2011 indicating a conserved regulation pattern for respective solanaceous and leguminous SUTs in response to colonization by fungi from the phylum Glomeromycota (Fig 58).

4 Cellular and molecular mechanisms driving sugar fluxes in the symbiotic model association between *M. truncatula* and *G. intraradices*

4.1 Cellular and molecular mechanisms of sucrose fluxes in source leaves

In leguminous leaves, sucrose is actively loaded by SUTs to the companion cell sieve element complex via the apoplast (Fig 4.3; Zhou et al. 2007; Rennie and Turgeon 2009). In such "apoplastic loading species", a single protein member of the SUT1 clade is responsible for the sucrose loading in the phloem (see Introduction 3.2.2.1.1; Zhang and Turgeon 2009). As phloem loading capacity is directly proportional to the rate of transcription of symporter genes in source leaves (Ainsworth and Bush 2010); *MtSUT1-1*, the gene likely to code for the high affinity phloem loading protein of *M. truncatula* (see Chapter II 3.3), which showed the highest upregulation in source leaves of AM plants may constitute the key component for sucrose export from source leaves towards AM colonized sinks. The antisense inhibition of *StSUT1*, coding for the phloem loading protein in potato, led to limiting sugar export toward sink organs (Riesmeier et al. 1994) but did not result in the decrease of AM fungal colonization by *G. intraradices* (Gabriel-Neumann et al. 2011). In parallel, potato lines overexpressing *SoSUT1* had a 2 times higher colonization rate but only when cultivated in HP conditions (Gabriel-Neumann et al. 2011). Thereby, phloem loading proteins may not be the only components responsible of the sugar allocation from source leaves towards AM colonized roots. Indeed, in plants inoculated with *G. intraradices* and *G. mosseae*; both plant SUT4 orthologs, *MtSUT4-1* and *SlSUT4*, showed a high upregulation in source leaves (Fig 58; Boldt et al. 2011) and recently, SUT4 clade proteins were assumed to play a role in the orchestration of the intracellular sucrose partitioning affecting the sucrose efflux from source leaves (Eom et al. 2011; Payyavula et al. 2011). In addition, SWEETs (AtSWEET11 and 12) are suggested to be key sucrose effluxers responsible of sucrose export from photosynthetic mesophyll cells (Fig 4.1); this first step is critical for sugar partitioning towards sink roots (Chen et al. 2012). Although MtSWEETs ortholog genes (Fig 20a; Medtr3g150940 and TC115479) show a strong activation of their gene expression in flowers according to microarray data (Fig 59), their role in sugar partitioning in source leaves has not yet been investigated in *M. truncatula*. In conclusion, sugar export from plant source leaves and

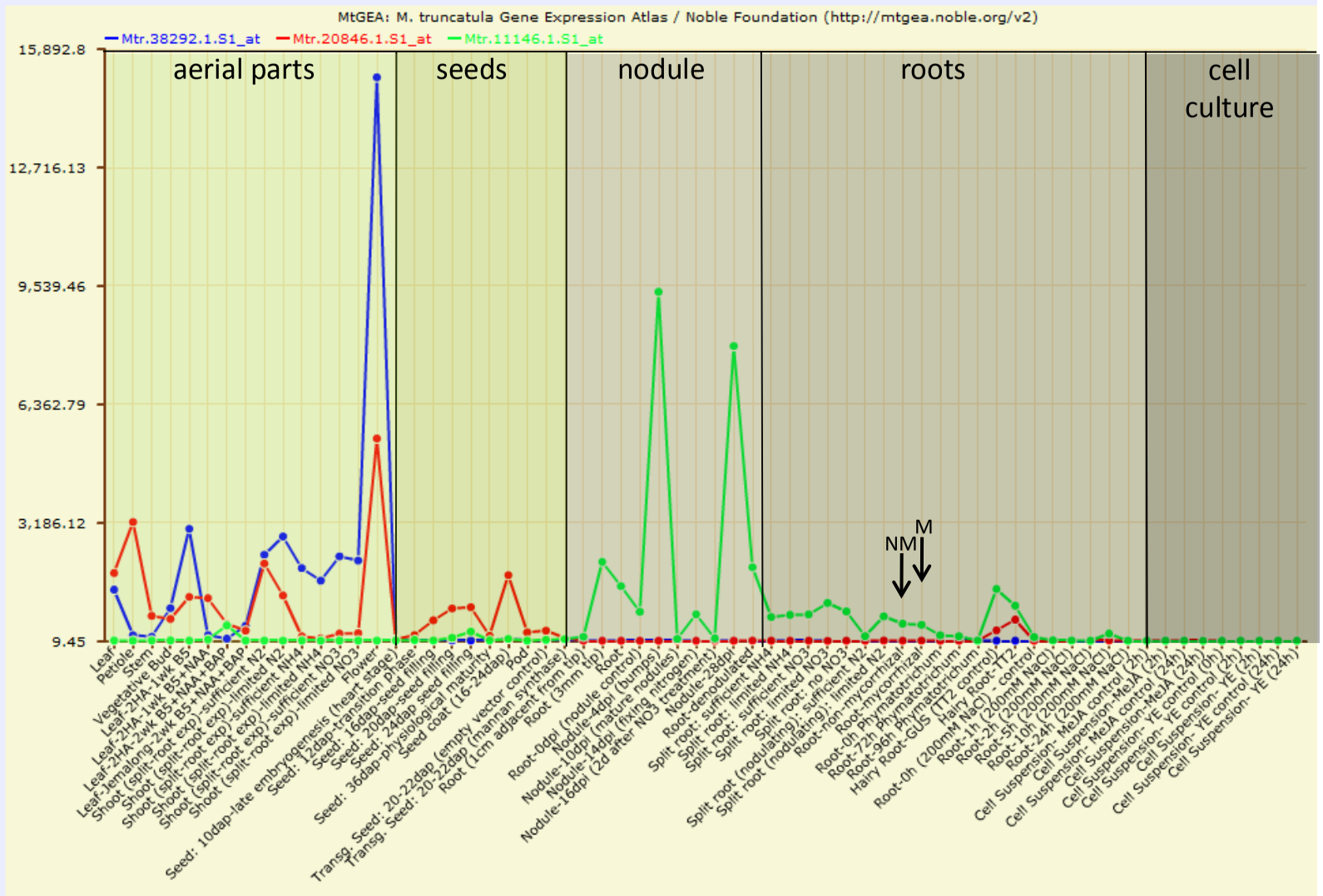


Fig 59. Expression profiles of *M. truncatula* SWEET orthologs to sucrose effluxers from *A. thaliana* (AtSWEET11, 12 and 15) from MtGEA microarray data.

M and NM arrows correspond to mycorrhizal and non-mycorrhizal root samples respectively.

subsequent allocation towards mycorrhized roots may be coordinated by all three main sucrose transport systems of source leaves: MtSWEETs exporting sucrose from production sites, MtSUT1-1 loading sucrose in the phloem vessels and MtSUT4-1 mobilizing intracellular vacuolar reserve.

4.2 Cellular and molecular mechanisms of sucrose fluxes in sink roots

Once sucrose is loaded into the phloem sap, the disaccharide follows its route through the transport phloem and reaches sink organs (Fig 4.3). In contrast to loading mechanism in source leaves, strategies of sucrose unloading in sinks can consist of both apoplasmic unloading via SUTs or symplasmic pathways through plasmodesmata (see Introduction 2.4). Therefore, upon release of sucrose in AM colonized roots both apoplastic and symplasmic unloading can be postulated (Fig 4); this may depend on the position of the arbusculated cortical cells (Fig 25a; Blee and Anderson 1998). Indeed, cortical cells near the endodermis surrounded by the Casparian strip would provide the most direct symplastic access from the phloem while the following cortical layers may be connected apoplastically. Very recently, SUT1 proteins necessary for phloem loading were suggested to also play a role in unloading mechanisms (Geiger 2011; Doidy et al. 2012a). The antisense inhibition of *StSUT1* in potato led to lower tuber yield when phloem unloading towards tuber is apoplastic (Viola et al. 2001; Kühn et al. 2003), indicating a major role for StSUT1 in efflux towards sink organs. In the release phloem of maize, apoplastic sucrose concentration, membrane potential and proton motive force are in favor of sucrose export from the phloem by ZmSUT1 (Carpaneto et al. 2005; Geiger 2011). Thereby, *in planta* ZmSUT1 seems to mediate efflux of sucrose for unloading towards apoplastic sinks. In our study, *MtSUT1-1* showed a higher transcript accumulation in AM roots, this information could indicate a further role for MtSUT1-1 in phloem unloading towards AM colonized sink tissues (Fig 60). Furthermore, SWEETs effluxers are not only expressed in source parts, indeed, in *M. truncatula* the MtSWEET (MtN3, TC129646) orthologs of AtSWEET15 characterized as a sucrose transporters (Chen et al. 2012) was identified from a cDNA library of root nodules (Fig 59; Gamas et al. 1996); such effluxers may also be involved in sucrose partitioning towards symbiotic organisms (Fig 60). In addition, transport mechanism of sucrose retrieval from apoplastic space can also be postulated for a control of photosynthates export towards AMF as for example by conventional MtSUT symporters system or through a similar reversible transport mode as described for ZmSUT1(Fig 60).

4.3 Cellular and molecular mechanisms of hexose fluxes towards the AMF

Before the transfer of sugar under the form of hexoses (mainly glucose and to a smaller extent fructose) at the plant-fungal interface (Solaiman and Saito 1997; Pfeffer et al. 2001; Bago et

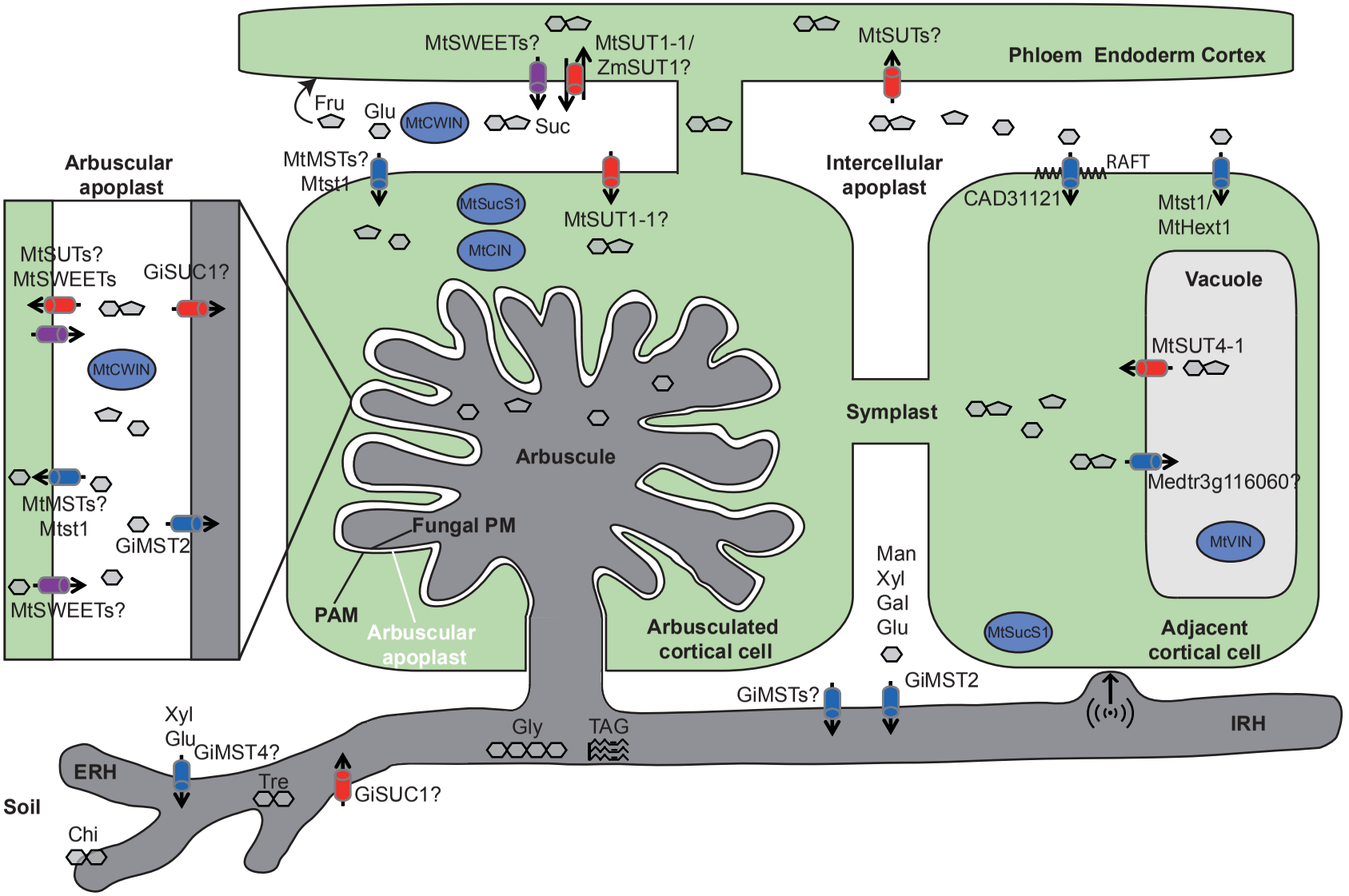


Fig 60. Schematic representation of sugar fluxes from the symbiotic model between *M. truncatula* and *G. intraradices*
Chi: Chitin; ERH: extraradical hyphae; Fru; fructose; Gal; galactose; Glu: glucose; Gly: glycogen; IRH: intraradical hyphae; Man: mannose; PAM: periarbuscular membrane; PM: plasma membrane; Suc: sucrose; TAG: triacylglycerol; Tre: trehalose

al. 2003), sucrose has to be cleaved by plant enzymes, either by sucrose synthase and/or invertases (Fig 3). Most postulates place the site of sucrose cleavage by plant invertase directly at the interfacial arbuscular apoplast located between the periarbuscular membrane of arbusculated plant cells and the fungal arbuscular membrane (Schüßler et al. 2006; Guether et al. 2009; Fellbaum et al. 2012). Nevertheless, sucrose cleavage can also occur within the plant cells (Fig 60); in *M. truncatula*, a study showed promoter activity of the cytosolic sucrose synthase gene (*MtSucS1*) in cells around internal hyphae and arbuscules (Hohnjec et al. 2003). In addition, *MtSucS1* antisense lines showed an overall down regulation of several carbon related genes and were affected during AM colonization, more particularly in the establishment and maintenance of arbuscules (Baier et al. 2010). Cytosolic invertase also presents an increased enzymatic activity in mycorrhizal roots (Wright et al. 1998a; Schubert et al. 2004). In addition, cleavage of sucrose may also take place at intercellular apoplastic space of plant cells. Indeed, the localization of the fungal hexose transporter *GiMST2* mRNA in arbuscules and IRM is consistent with both a glucose uptake by the fungus at arbuscular and intercellular apoplasts (Helber et al. 2011). Thereby, in contrast to phosphate and nitrogen exchange towards the plant which mainly occurs at the arbuscular interface (Fig 24); both arbuscular and intercellular apoplastic spaces constitute the symbiotic interface for sugar exchanges from the host plant towards the AMF (Fig 60; Douds et al. 2000; Hause and Fester 2005; Helber et al. 2011).

In conclusion, increases of photosynthesis and invertase activity coupled with higher SUT transcript levels, as well as accumulation of sucrose and monosaccharides in source and sink organs indicate a mycorrhizal-driven stronger sink and sugar allocation toward roots for fungal symbiont development (Wright et al. 1998a; Boldt et al. 2011). However, the inhibition of sucrose export from leaves by a knock down of *StSUT1,* the gene coding for the phloem loading protein in potato, (Gabriel-Neumann et al. 2011), or the increase of hexose levels in tobacco roots through overexpression of yeast-derived invertases did not affect the fungal colonization by *G. intraradices* (Schaarschmidt et al. 2007). These findings suggest that carbon supply through sucrose breakdown is not the only bottleneck for a functional symbiosis.

4.4 Sugar fluxes in arbusculated cells

Hexoses resulting from the sucrose cleavage are transported by MST transporters (see Introduction 3.3). In *M. truncatula*, Medtr2g049020 was exclusively expressed in cells containing arbuscules (Hogekamp et al. 2011). However, Medtr2g049020 clusters within the INT subfamily (Fig 36) and therefore is likely to transport inositol rather than hexoses. Another MtMST (Mtst1) belonging to the STP subfamily which include plasma membrane

hexose transporters with highest affinity for glucose (Büttner 2010) showed increased transcript level in *M. truncatula* AM colonized roots; and mRNA located in cortical arbuscule containing cells (Harrison 1996). However, the exact intracellular location of such MST transporters in arbusculated cells is not yet known. As no MSTs, up to date, were shown to be exporters, and considering that proton gradient created by plant and fungal ATPase pump at the arbuscular interface are in favor of an import system of MSTs (Gianinazzi-Pearson et al. 1991; Krajinski et al. 2002), it is tempting to speculate that Mtst1 in arbusculated cells is preferentially situated at the plant plasma membrane to recruit hexoses towards arbusculated cells; indeed, if located at the periarbuscular membrane, this hexose importer would mediate glucose and fructose retrieval towards plant cell (Fig 60). In addition, the export of sugar at the periarbuscular membrane may also be mediated by the newly identified glucose and sucrose effluxers of the SWEET family (Fig 60; Chen et al. 2010; Chen et al. 2012). However, the role of SWEETs in sugar partitioning in AM symbiosis has not yet been investigated.

In addition, numerous MSTs of *M. truncatula* present a differential regulation of their transcript levels in response to mycorrhiza (see Chapter I 3.1). One of which (*CAD31121*) presents decreased transcript levels in response to AM inoculation (Fig 38) and its protein product was detected in DRM fraction of *M. truncatula* roots (Lefebvre et al. 2007). The AM differential expression of a nutrient transport gene which product is targeted to raft domains gives new insights that raft associated proteins may be involved in the regulation of trophic exchanges in mycorrhizal symbiosis (Fig 60). However, the exact location of this STP member has not yet been shown in planta (*e.g.,* in arbusculated or adjacent cortical cells).

4.5 Sugar fluxes in non-arbusculated cortical cells

Interestingly, sugar transporters were also shown to be differentially regulated in non-arbusculated cortical cells. Indeed, in addition to localization in arbusculated cortical cells, *Mtst1*gene expression is also activated in adjacent cortical cells which are frequently in contact with IRM. Furthermore, *MtHext1* is solely induced in non-arbusculated neighboring cells. Both STP members are likely to mediate hexose uptake at the plasma membrane of non-arbusculated cortical cells (Fig 60). In parallel, sucrose transport components are also activated in AM roots (see 5.2). *MtSUT4-1* promoter-GUS fusion combined to staining of AM fungal structures showed that this gene is activated in cortical cells adjacent to arbusculated cells (Fig 25b; Gaude et al. 2011). The closest related ortholog of MtSUT4-1, LjSUT4 was detected at the vacuolar membrane and is suggested to play a role in vacuolar sucrose release towards nodules in plant-rhizobial symbioses (Reinders et al. 2008). In addition, Medtr3g116060, an homolog of TMT2 which is involved in sucrose export in *Arabidopsis* vacuole (Schulz et al. 2011), is downregulated in mycorrhizal roots (Fig 38). However, the

exact location of this latter candidate has not yet been reported in *M. truncatula*. Such sucrose transporters (SUT4 and TMTs) could be responsible for carbon reallocation of vacuolar sucrose storage towards colonized parts during plant symbioses (Fig 60).

The activation of monosaccharide import pathway at the plasma membrane of non-arbusculated cells together with the reallocation of vacuolar sugar reserve, result in cytosolic sugar enrichment in non-arbusculated cells. This sugar flow in adjacent cortical cells may be directed to feed arbusculated cells through symplasmic pathways via plasmodesmata. It is also tempting to speculate that this sugar enrichment in non-arbusculated cells is part of a signaling cascade resulting in arbuscular development and colonization of adjacent cortical cells (Fig 60).

4.6 Fungal sugar uptake

On the fungal side, 2 hexose transporters have been characterized up to date from *G. pyriformis* and *G. intraradices* (GpMST1 and GiMST2; see Introduction 4.3.3.3), both transporters show their highest affinity in yeast for glucose and fructose only weakly compete with glucose uptake which is consistent with the preferred glucose uptake by the fungus (Schüßler et al. 2006; Helber et al. 2011). In addition, both transporters also take up xylose indicating that the AMF is also able to import monosaccharides from cell wall degradation (Fig 60). The capacity to take up cell wall monosaccharides may be especially important at early symbiotic stages when arbuscules are not yet formed. In addition, GiMST2 the only characterized sugar transporter from the model AMF *G. intraradices*, is a major component for sugar uptake at the symbiotic interfaces since RNAi silencing of *GiMST2* by HIGS led to lower mycorrhization levels and abnormal arbuscular morphology (Fig 26). However the silencing of *GiMST2* did not completely prevent a functional symbiosis. Therefore, other sugar transporters may also be involved in sugar partitioning towards the fungus. Indeed, a supplemental transporter GiMST3 is also expressed in arbuscules-enriched samples (Helber et al. 2011), and we identified Glomus_c4574a (see Chapter I 3.2), a putative fungal glucose transporters which shows high expression in IRM and arbuscules, the major fungal structures for sugar transfer. Such candidates may also take part in sugar uptake in intraradical structures together with GiMST2 (Fig 60). In contrast to NMR spectrometry and radiorespirometry experiments which revealed carbon uptake by the IRM only (Shachar-Hill et al. 1995; Solaiman and Saito 1997; Pfeffer et al. 1999); very recently, a transfer of glucose and xylose has been suggested to occur in ERM (Helber et al. 2011). Indeed, a putative monosaccharide transporters of *G. intraradices* GiMST4 shows its highest expression in ERM samples (Helber et al. 2011) but microarray experiments did not confirm the preferential ERM expression of this latter candidate (see Glomus_c6242 in Table 6). In addition, GiMST4 does

not seem to be functional in yeast as it failed to complement the glucose deficient yeast mutant (A. Schüßler personal communication).

Finally, a putative sucrose transporter (*GiSUC1*) has also been identified in the genome of *G. intraradices*. Although GiMST2 was transformed into a sucrose deficient yeast strain and was not able to mediate sucrose uptake, Helber et al. 2011 did not report the functional characterization of GiSUC1. GiSUC1 seems constitutively expressed in all fungal structures (arbuscule, ERM and spores). As restricted absorption of sucrose in intraradical structures has only been suggested once (Pfeffer et al. 1999), the role of this sucrose transporters remains to be deciphered.

4.7 Conclusion

In conclusion, the recent completion of the *M. truncatula* genome and first genomic data from *G. intraradices* as well as the release of transcriptomic database allowed the generation of an inventory of sugar transporter genes in the leguminous host plant and its model fungal symbiont (see Chapter I). The identification and functional characterization (see Chapter II and IV) and gene expression pattern of sugar transporter families (see Chapter III) led to a comprehensive model of sugar fluxes in the AM symbiosis (Fig 60).

Concluding remarks and perspectives

Concluding remarks and perspectives

The aim of my PhD thesis was the study of biotrophic nutritional exchanges and more specifically the transfer of sugars from plant source leaves towards sink roots in the arbuscular mycorrhiza (AM) association between the model leguminous plant *M. truncatula* and the reference arbuscular mycorrhizal fungus (AMF) *G. intraradices*. Here, I conclude on a comprehensive study of sugar transport components involved in carbon partitioning at the whole plant level, with a special interest on the *M. truncatula* sucrose transporter (MtSUT) family. The identification, characterization and analysis of the expression of key members of this family in response to AMF inoculation was carried out to better understand the role of the sugar transportome involved in sugar partitioning within plant and the cellular and molecular mechanisms of carbon exchanges between symbiotic organisms during the AM interaction.

First, a targeted approach has been developed to search for putative monosaccharide and sucrose transporters (MST and SUT) by *in silico* mining of *M. truncatula* databases; this leguminous species being a widely used model plant for AM interaction. The sugar transporter families of *M. truncatula* were identified, for the first time, in the frame of this study; they respectively comprise 6 MtSUTs and 62 MtMSTs. A similar *in silico* search was performed for sugar transporters from the reference fungal symbiont *G. intraradices*. Gene candidates presenting differential expression profiles using available transcriptomic tools from both the plant and fungal partners were pinpointed in this model plant-fungal interaction.

As plant SUTs represent major components of the long distance transport of photosynthates from source leaves to sink organs, I focused, in a second part, on the study of the newly identified MtSUTs at a full family scale. The 6 members of the MtSUT family distributed in all three dicotyledonous SUT clades and were named according to their phylogenetic position: MtSUT1-1, MtSUT1-2, MtSUT1-3, MtSUT2, MtSUT4-1 and MtSUT4-2. Functional analyses by yeast complementation and expression profiles by quantitative RT-PCR revealed that MtSUT1-1 and MtSUT4-1 are H^+/sucrose symporters and represent key members of the MtSUT family. Indeed, conservation of transport capacity between orthologous leguminous protein, expression profiles and subcellular localization compared to plant SUTs from respective SUT1 and SUT4 clades suggest that MtSUT1-1 is the main protein involved in phloem loading in source leaves whilst MtSUT4-1 mediate vacuolar sucrose export for remobilization of intracellular reserve.

Thirdly, sugar fluxes from *M. truncatula* source leaves towards AM colonized sink roots were investigated. Gene expression profiles of MtSUTs and sugar quantification analyses upon high and low phosphorus supply and inoculation by the AMF suggest a mycorrhizal-driven stronger sink in AM roots with a fine-tuning regulation of MtSUT gene expression and conserved regulation pattern in leaves and roots for orthologous plant SUTs in response to colonization by glomeromycotan fungi. Along with these new findings, we reviewed the regulation of sugar fluxes in AM plants: sucrose loading in source leaves, with subsequent unloading and cleavage towards colonized roots, and finally hexoses uptake at the plant-fungal interface. We discuss the implication of corresponding transport proteins including SUTs, MSTs and the recently identified SWEETs as well as plant sucrose cleaving enzymes and fungal transporters in the model AM interaction between *M. truncatula* and *G. intraradices* (Fig 60).

In parallel, a non-targeted approach consisting in the development of expression libraries suitable for yeast functional screening has been developed. Such libraries represent valuable tools for the isolation of functional candidates from both the plant and fungal partners involved in biotrophic exchanges. The expression library constructed from symbiotic roots will be analyzed within an ongoing PhD project (N. Ait Lahmidi) supervised by D. Wipf which follows the work that has been performed during my PhD. Besides this detailed work on sugar transporter families and their role in sugar fluxes from source to sink organs, timely perspectives can be addressed regarding the current advances in the field of sucrose transport protein in plants (*e.g.*, interaction with raft domains) and increasing application developed for the study of AM symbiosis (*e.g.,* host-induced gene silencing).

In this study, the characterization of the MtSUT family was performed and MtSUT1-1 and MtSUT4-1 were shown to act as H^+/sucrose importers at the plasma membrane in *S. cerevisiae*. The biochemical characterization of remaining MtSUT members, which exhibited a weak or even no complementation when expressed in this heterologous model, could be envisaged in the *Xenopus laevis* oocyte model, especially for MtSUT1-3, MtSUT1-2 and MtSUT4-2 candidates which cluster with leguminous sucrose facilitators (SUFs) and may be involved in facilitated influx and efflux of sucrose *in planta*. In addition, the analysis of MtSUT1-1 in oocytes would enable to study whether this transporter is able to elicit both inward and outward proton currents through a reversible transport mode for import/export of sucrose, as previously and only shown for ZmSUT1 (Fig 12; Carpaneto et al. 2005). In contrast to most SUTs and all MSTs characterized up to date which are shown to mediate either sucrose or hexose uptake in plant cells, the recently identified SWEETs are uniporters and mediate both glucose and sucrose export in *A. thaliana* (Chen et al. 2010; Chen et al.

2012). Thereby, it would be very interesting to investigate the role of those newly identified SWEETs in the genome of *M. truncatula* and their role in sugar efflux in source (mesophyll/parenchymatous cells) and sink organs.

MtSUT1-1 and MtSUT4-1 have been demonstrated to be key sucrose transporters and seem to be targeted to two different cellular compartments: plasma membrane and tonoplast respectively. However, SUT4 proteins were also shown to be localized at the plasma membrane *in planta* (Weise et al. 2000) and a dual targeting of SUT4 proteins to both plasma and vacuolar membranes was postulated (Doidy et al. 2012a). Plant SUTs have been shown to interact with each other family member; indeed, SUTs can form functional homodimers as well as heterodimers (see Introduction 3.2.4.3.3). The interaction of SUT4 with SUT1 may redirect the vacuolar SUT4 transporter to the plasma membrane. In addition, the mutation of the *SUT1* members coding for phloem loading proteins blocks the sucrose export, thus resulting in reduced growth of sink organs (Fig 9), whereas potato *SUT4* mutant plants show opposite phenotypes with early flowering and higher tuber yields indicating a higher sucrose export toward sink organs (Fig 11c; Chincinska et al. 2008). Therefore, it can be postulated that SUT4 proteins may act as negative regulator of the SUT1 phloem loading protein at the plasma membrane. One could hypothesize that when the high affinity SUT1 protein interacts with the low affinity SUT4 transporter, resulting heteromers show a lower affinity for sucrose than the SUT1 homomers. Key members of the SUT family of *M. truncatula* may also form MtSUT1-1/MtSUT4-1 heteromers regulating the amount of sucrose loaded in the phloem and the subsequent transport of sucrose towards sinks organs. A fine tuning regulation of the *SUT1* and *SUT4* gene expression would balance the amount of SUT1/SUT4 homo/heteromers and adjust the rate of sucrose exported from source organs. Considering that the localization and oligomerization of SUTs is dependent on transporter cycling and membrane dynamics, the recent identification of sugar transporters in raft domains open a new field of research (Lefebvre et al. 2007; Liesche et al. 2008; Krügel et al. 2011). The role of SUT protein oligomerization and interaction with specialized microdomains in regulating sugar uptake has not yet been investigated *in planta* and may play crucial roles in sugar exchanges towards heterotrophic organisms (Doidy et al. 2012a).

Furthermore, the use of *M. truncatula* mutant lines could also be envisaged. Indeed, we identified 6 mutant lines, from large scale Tnt1 transposon insertional mutagenesis database (Tadege et al. 2008), including 2 independent mutant lines for *MtSUT1-1* and *MtSUT1-3* as well as a single mutant line for *MtSUT4-2* (Annex VII Table). Apart from nf3112I7 line that contain the Tnt1 insertion in the promoter of *MtSUT1-1*, all other insertions were tagged in the CDS of *MtSUT* candidate (Annex VII) and are likely to show knock out phenotype since

Tnt1 insertions are 3500 bp long. Backcrosses with wild type plants to clear out additional Tnt1 insertions and ensure a single insertion in *MtSUT* loci with subsequent cross would allow the isolation of heterozygous and homozygous individuals. Such plants will be interesting to confirm that MtSUT1-1 is the main phloem loading protein in *M. truncatula* and to see if leguminous plants with mutation of this SUT1 locus present similar phenotypes to previously described mutants in *Arabidopsis*, potato and maize (Fig 9; Riesmeier et al. 1994; Gottwald et al. 2000; Slewinski et al. 2009). In addition, the study of *MtSUT1-3* and *MtSUT4-2* mutant lines would give new insights for the role of these proteins which may act as sucrose facilitators (Zhou et al. 2007). It would also be interesting to follow AMF colonization in such mutant lines. Up to now, a single mutant plant for the SUT1 gene has been investigated in potato, the antisense inhibition of *StSUT1* coding for the phloem loading protein, had no effect on mycorrhization rate (Gabriel-Neumann et al. 2011). On the fungal side, the development of host-induced gene silencing (HIGS) in obligate biotrophic fungi made possible for the first time the gene antisense silencing in AMF species (Nowara et al. 2010; Helber et al. 2011). The analysis of putative fungal hexose transporters identified in this study (Table 5) or of the glomeromycotan sucrose transporter recently identified (GiSUC1, Helber et al. 2011) by HIGS will allow a better comprehension of molecular and cellular mechanisms involved in sugar uptake by AMF.

The regulatory system for delivering photosynthates towards heterotrophic organisms must be under tight control to ensure functional interactions with symbiotic partners and avoid diversion of such mechanisms by pathogens; a reward strategy by symbiotic fungal and plant partners must guarantee a "fair trade" of nutrients. Consistent with this, Fitter 2006 postulated that first plant detect the increased phosphate supply, resulting in a transfer of sugars to the fungus to a spatially defined location in the root. This C-P exchange model would prevent "cheat fungi", which are unable to provide phosphate, to feed from plant photosynthates. However, a contradictory model postulates that it is carbon which is invested by plants to acquire phosphate from the fungus (Landis and Fraser 2008). In addition to phosphate, recent studies on C-N exchanges report that nitrogen transport to the host plant is stimulated only when carbon is delivered to the fungus (Fellbaum et al. 2012). Altogether, these models highlight that "plant detect, discriminate, and reward the best fungal partners with more carbohydrates; in turn, their fungal partners enforce cooperation by increasing nutrient transfer only to those roots providing more carbohydrates" (Kiers et al. 2011).

Here, we report that MtSUT expression is regulated by different phosphate conditions in soil and upon mycorrhizal inoculation. A similar gene induction in mycorrhizal plants could be observed for solanaceous and leguminous SUTs and common *cis*-regulatory elements were

identified in gene promoters of plant sugar transporters as for example the presence of W-box which is activated in response to biotic and abiotic stresses (Table 8). It would therefore be interesting to investigate if regulation of sugar transporter gene expression is the result of a common signal cascade in plants induced by mycorrhizal-driven sink. Several WRKY transcription factors which bind specifically to the W-box were shown to be induced during AM symbiosis (Gallou et al. 2011). WRKY proteins often act as repressors as well as activators regulating several seemingly disparate processes and most reports on WRKY transcription factors have focused on transcriptional reprogramming particularly associated with the activation of plant defense in response to microbe associated molecular pattern (MAMP; Rushton et al. 2010). In addition, it is suggested that expression of sucrose transport and hydrolysis proteins, including SUTs, is regulated by phytohormones, and that jasmonic acid may be part of the signal cascade regulating sugar partitioning towards the AMF (Tejeda-Sartorius et al. 2008). Phytohormones also contribute to the modulation of plant defense gene expression in bioprotection against pathogens conferred by AM symbiosis (García-Garrido and Ocampo 2002). Finally, it is also suggested that a sensor system based on the flux of sugars regulates plant defense gene expression in plant-fungal interactions (Blee and Anderson 2000; García-Garrido and Ocampo 2002; Doidy et al. 2012a). Whilst pathogenic fungi efficiently divert plant nutrients causing, eventually, host damage and/or death; AMF promote plant growth, contribute to general plant fitness and resistance against diverse biotic stresses. In the actual context, deciphering evolution and regulation of molecular patterns involved in nutrient balance and plant immune response at the frontiers between pathogenic and symbiotic organisms is a major challenge for a sustainable agricultural model for optimal crop production associated to responsible pest management.

In conclusion, this work constitutes a pioneer study of the *M. truncatula* sugar transporter families and a milestone for the study of the transportome in the widely used AM symbiotic model between *M. truncatula* and *G. intraradices*. With the identification, functional characterization and gene expression pattern analysis of sugar transporters, a more complete picture of sugar fluxes in the AM symbiosis has begun to emerge (Fig 60). Nevertheless, the sugar transportome puzzle in AM is still far from complete and major pieces such as the system of cellular efflux and localization of proteins at biotrophic interfaces, proteins oligomerization and interaction with raft microdomains are still missing. Increasing application of molecular (*e.g.,* HIGS) and post-genomic methodologies (*e.g.,* cDNA expression libraries) as well as technological improvements with higher spatial resolution (*e.g.,* laser capture microdissection technology) are now available for the detection of nutrients, transcripts and proteins present at plant-fungal interfaces. There is no doubt that the recent development of such techniques will allow a better comprehension of sugar partitioning within and between organisms.

References

References

Abramson, J., I. Smirnova, V. Kasho, G. Verner, H. R. Kaback and S. Iwata (2003). "Structure and Mechanism of the Lactose Permease of *Escherichia coli*." Science 301(5633): 610-615.

Afoufa-Bastien, D., A. Medici, J. Jeauffre, P. Coutos-Thevenot, R. Lemoine, R. Atanassova and M. Laloi (2010). "The *Vitis vinifera* sugar transporter gene family: phylogenetic overview and macroarray expression profiling." BMC Plant Biology 10(1): 245.

Ainsworth, E. and D. Bush (2010). "Carbohydrate export from the leaf - A highly regulated process and target to enhance photosynthesis and productivity." Plant Physiology 155: 64–69.

Aldape, M. J., A. M. Elmer, W. S. Chao and H. D. Grimes (2003). "Identification and characterization of a sucrose transporter isolated from the developing cotyledons of soybean." Archives of Biochemistry and Biophysics 409(2): 243-250.

Aluri, S. and M. Buttner (2007). "Identification and functional expression of the *Arabidopsis thaliana* vacuolar glucose transporter 1 and its role in seed germination and flowering." Proceedings of the National Academy of Sciences of the United States of America 104(7): 2537-2542.

Amberg, D. C., D. Burke, J. N. Strathern and L. Cold Spring Harbor (2005). Methods in yeast genetics : a Cold Spring Harbor Laboratory course manual. Cold Spring Harbor, N.Y., Cold Spring Harbor Laboratory Press.

Aoki, N., T. Hirose, G. N. Scofield, P. R. Whitfeld and R. T. Furbank (2003). "The sucrose transporter gene family in rice." Plant and Cell Physiology 44(3): 223-232.

Aoki, N., G. N. Scofield, X.-D. Wang, C. E. Offler, J. W. Patrick and R. T. Furbank (2006). "Pathway of Sugar Transport in Germinating Wheat Seeds." Plant Physiology 141(4): 1255-1263.

Aoki, N., G. N. Scofield, X. D. Wang, J. W. Patrick, C. E. Offler and R. T. Furbank (2004). "Expression and localisation analysis of the wheat sucrose transporter *TaSUT1* in vegetative tissues." Planta 219(1): 176-184.

Aoki, N., P. Whitfeld, F. Hoeren, G. Scofield, K. Newell, J. Patrick, C. Offler, B. Clarke, S. Rahman and R. T. Furbank (2002). "Three sucrose transporter genes are expressed in the developing grain of hexaploid wheat." Plant Molecular Biology 50(3): 453-462.

Ayre, B. G. (2011). "Membrane-transport systems for sucrose in relation to whole-plant carbon partitioning." Molecular Plant 4(3): 377-394.

Bago, B., P. E. Pfeffer, J. Abubaker, J. Jun, J. W. Allen, J. Brouillette, D. D. Douds, P. J. Lammers and Y. Shachar-Hill (2003). "Carbon export from arbuscular mycorrhizal roots involves the translocation of carbohydrate as well as lipid." Plant Physiology 131(3): 1496-1507.

Bago, B., P. E. Pfeffer and Y. Shachar-Hill (2000). "Carbon metabolism and transport in arbuscular mycorrhizas." Plant Physiology 124(3): 949-957.

Bago, B., P. E. Pfeffer, W. Zipfel, P. Lammers and Y. Shachar-Hill (2002a). "Tracking metabolism and imaging transport in arbuscular mycorrhizal fungi. Metabolism and transport in AM fungi." Plant and Soil 244(1-2): 189-197.

Bago, B., W. Zipfel, R. M. Williams, J. Jun, R. Arreola, P. J. Lammers, P. E. Pfeffer and Y. Shachar-Hill (2002b). "Translocation and Utilization of Fungal Storage Lipid in the Arbuscular Mycorrhizal Symbiosis." Plant Physiology 128(1): 108-124.

Baier, M. C., M. Keck, V. Gödde, K. Niehaus, H. Küster and N. Hohnjec (2010). "Knockdown of the symbiotic sucrose synthase *MtSucS1* affects arbuscule maturation and maintenance in mycorrhizal roots of *Medicago truncatula*." Plant Physiology 152(2): 1000-1014.

Balestrini, R., J. Gómez-Ariza, L. Lanfranco and P. Bonfante (2007). "Laser microdissection reveals that transcripts for five plant and one fungal phosphate transporter genes are contemporaneously present in arbusculated cells." Molecular Plant Microbe Interactions 20(9): 1055-1062.

Barker, L., C. Kuhn, A. Weise, A. Schulz, C. Gebhardt, B. Hirner, H. Hellmann, W. Schulze, J. M. Ward and W. B. Frommer (2000). "SUT2, a putative sucrose sensor in sieve elements." Plant Cell 12(7): 1153-1164.

Barth, I., S. Meyer and N. Sauer (2003). "PmSUC3: Characterization of a SUT2/SUC3-type sucrose transporter from *Plantago major*." Plant Cell 15(6): 1375-1385.

Baud, S., S. Wuillème, R. Lemoine, J. Kronenberger, M. Caboche, L. Lepiniec and C. Rochat (2005). "The AtSUC5 sucrose transporter specifically expressed in the endosperm is involved in early seed development in *Arabidopsis*." The Plant Journal 43(6): 824-836.

Bécard, G., L. W. Doner, D. B. Rolin, D. D. Douds and P. E. Pfeffer (1991). "Identification and quantification of trehalose in vesicular-arbuscular mycorrhizal fungi by in vivo13C NMR and HPLC analyses*." New Phytologist 118(4): 547-552.

Benedetto, A., F. Magurno, P. Bonfante and L. Lanfranco (2005). "Expression profiles of a phosphate transporter gene (*GmosPT*) from the endomycorrhizal fungus *Glomus mosseae*." Mycorrhiza 15(8): 620-627.

Benedito, V. A., I. Torres-Jerez, J. D. Murray, A. Andriankaja, S. Allen, K. Kakar, M. Wandrey, J. Verdier, H. Zuber, T. Ott, S. Moreau, A. Niebel, T. Frickey, G. Weiller, J. He, X. Dai, P. X. Zhao, Y. Tang and M. K. Udvardi (2008). "A gene expression atlas of the model legume *Medicago truncatula*." The Plant Journal 55(3): 504-513.

Benjdia, M., E. Rikirsch, T. Müller, M. Morel, C. Corratgé, S. Zimmermann, M. Chalot, W. B. Frommer and D. Wipf (2006). "Peptide uptake in the ectomycorrhizal fungus *Hebeloma cylindrosporum*: characterization of two di- and tripeptide transporters (HcPTR2A and B)." New Phytologist 170(2): 401-410.

Berthier, A., M. Desclos, V. Amiard, A. Morvan-Bertrand, B. Demmig-Adams, W. W. Adams, R. Turgeon, M.-P. Prud'homme and N. Noiraud-Romy (2009). "Activation of Sucrose Transport in Defoliated *Lolium perenne* L.: An Example of Apoplastic Phloem Loading Plasticity." Plant and Cell Physiology 50(7): 1329-1344.

Bimboim, H. C. and J. Doly (1979). "A rapid alkaline extraction procedure for screening recombinant plasmid DNA." Nucleic Acids Research 7(6): 1513-1523.

Blee, K. A. and A. J. Anderson (1998). "Regulation of arbuscule formation by carbon in the plant." The Plant Journal 16(5): 523-530.

Blee, K. A. and A. J. Anderson (2000). Defence responses in plants to arbuscular mycorrhizal fungi. Current Advances in Mycorrhizae Research. G. K. Podila and D. D. Douds. St Paul, APS Press.

Blee, K. A. and A. J. Anderson (2002). "Transcripts for genes encoding soluble acid invertase and sucrose synthase accumulate in root tip and cortical cells containing mycorrhizal arbuscules." Plant Molecular Biology 50(2): 197-211.

Boldt, K., Y. Pörs, B. Haupt, M. Bitterlich, C. Kühn, B. Grimm and P. Franken (2011). "Photochemical processes, carbon assimilation and RNA accumulation of sucrose transporter genes in tomato arbuscular mycorrhiza." Journal of Plant Physiology 168(11): 1256-1263.

Braun, D. M. and T. L. Slewinski (2009). "Genetic control of carbon partitioning in grasses: roles of sucrose transporters and Tie-dyed loci in phloem loading." Plant Physiology 149(1): 71-81.

Buchel, D. E., B. Gronenborn and B. Muller-Hill (1980). "Sequence of the lactose permease gene." Nature 283(5747): 541-545.

Bürkle, L., J. M. Hibberd, W. P. Quick, C. Kühn, B. Hirner and W. B. Frommer (1998). "The H+-sucrose cotransporter NtSUT1 is essential for sugar export from tobacco leaves." Plant Physiology 118(1): 59-68.

Bush, D. R. (1990). "Electrogenicity, pH-Dependence, and Stoichiometry of the Proton-Sucrose Symport." Plant Physiology 93(4): 1590-1596.

Buttner, M. (2007). "The monosaccharide transporter(-like) gene family in *Arabidopsis*." FEBS Letters 581(12): 2318-2324.

Büttner, M. (2010). "The *Arabidopsis* sugar transporter (AtSTP) family: an update." Plant Biology 12: 35-41.

Buttner, M. and N. Sauer (2000). "Monosaccharide transporters in plants: structure, function and physiology." Biochimica et Biophysica Acta 1465(1-2): 263 - 274.

Buttner, M., E. Truernit, K. Baier, J. Scholz-Starke, M. Sontheim, C. Lauterbach, V. A. R. Huss and N. Sauer (2000). "AtSTP3, a green leaf-specific, low affinity monosaccharide-H+ symporter of *Arabidopsis thaliana*." Plant Cell and Environment 23(2): 175-184.

Cappellazzo, G., L. Lanfranco, M. Fitz, D. Wipf and P. Bonfante (2008). "Characterization of an Amino Acid Permease from the Endomycorrhizal Fungus *Glomus mosseae*." Plant Physiology 147(1): 429-437.

Carlson, M. and D. Botstein (1982). "Two differentially regulated mRNAs with different 5' ends encode secreted and intracellular forms of yeast invertase." Cell 28(1): 145-154.

Carpaneto, A., D. Geiger, E. Bamberg, N. Sauer, J. Fromm and R. Hedrich (2005). "Phloem-localized, proton-coupled sucrose carrier ZmSUT1 mediates sucrose efflux under the control of the sucrose gradient and the proton motive force." Journal of Biological Chemistry 280(22): 21437-21443.

Chan, M.-T. and S.-M. Yu (1998). "The 3′ untranslated region of a rice α-amylase gene functions as a sugar-dependent mRNA stability determinant." Proceedings of the National Academy of Sciences of the United States of America 95(11): 6543-6547.

Chandran, D., A. Reinders and J. M. Ward (2003). "Substrate Specificity of the *Arabidopsis thaliana* Sucrose Transporter AtSUC2." Journal of Biological Chemistry 278(45): 44320-44325.

Chen, L.-Q., X.-Q. Qu, B.-H. Hou, D. Sosso, S. Osorio, A. R. Fernie and W. B. Frommer (2012). "Sucrose efflux mediated by SWEET proteins as a key step for phloem transport." Science 335(6065): 207-211.

Chen, L. Q., B. H. Hou, S. Lalonde, H. Takanaga, M. L. Hartung, X. Q. Qu, W. J. Guo, J. G. Kim, W. Underwood, B. Chaudhuri, D. Chermak, G. Antony, F. F. White, S. C. Somerville, M. B. Mudgett and W. B. Frommer (2010). "Sugar transporters for intercellular exchange and nutrition of pathogens." Nature 468(7323): 527-532.

Chincinska, I. A., J. Liesche, U. Krugel, J. Michalska, P. Geigenberger, B. Grimm and C. Kuhn (2008). "Sucrose transporter StSUT4 from potato affects flowering, tuberization, and shade avoidance response." Plant Physiology 146(2): 515-528.

Cho, J. I., B. Burla, D. W. Lee, N. Ryoo, S. K. Hong, H. B. Kim, J. S. Eom, S. B. Choi, M. H. Cho, S. H. Bhoo, T. R. Hahn, H. E. Neuhaus, E. Martinoia and J. S. Jeon (2010). "Expression analysis and functional characterization of the monosaccharide transporters, OsTMTs, involving vacuolar sugar transport in rice (*Oryza sativa*)." New Phytologist 186(3): 657-668.

Cho, M.-H., H. Lim, D. H. Shin, J.-S. Jeon, S. H. Bhoo, Y.-I. Park and T.-R. Hahn (2011). "Role of the plastidic glucose translocator in the export of starch degradation products from the chloroplasts in *Arabidopsis thaliana*." New Phytologist 190(1): 101-112.

Chu, Z., M. Yuan, J. Yao, X. Ge, B. Yuan, C. Xu, X. Li, B. Fu, Z. Li, J. L. Bennetzen, Q. Zhang and S. Wang (2006). "Promoter mutations of an essential gene for pollen development result in disease resistance in rice." Genes & Development 20(10): 1250-1255.

Cook, D. R. (1999). "*Medicago truncatula* - a model in the making! Commentary." Current Opinion in Plant Biology 2(4): 301-304.

Délano-Frier, J. P. and M. Tejeda-Sartorius (2008). "Unraveling the network: Novel developments in the understanding of signaling and nutrient exchange mechanisms in the arbuscular mycorrhizal symbiosis." Plant Signaling & Behavior 3(11): 936-944.

Delrot, S. and J.-L. Bonnemain (1981). "Involvement of Protons as a Substrate for the Sucrose Carrier during Phloem Loading in *Vicia faba* Leaves." Plant Physiology 67(3): 560-564.

Dickson, S., S. E. Smith and F. A. Smith (1999). "Characterization of two arbuscular mycorrhizal fungi in symbiosis with Allium porrum: colonization, plant growth and phosphate uptake." New Phytologist 144(1): 163-172.

Doehlemann, G., F. Molitor and M. Hahn (2005). "Molecular and functional characterization of a fructose specific transporter from the gray mold fungus *Botrytis cinerea*." Fungal Genetics and Biology 42(7): 601-610.

Doering-Saad, C., H. J. Newbury, J. S. Bale and J. Pritchard (2002). "Use of aphid stylectomy and RT-PCR for the detection of transporter mRNAs in sieve elements." Journal of Experimental Botany 53(369): 631-637.

Doidy, J., E. Grace, C. Kühn, F. Simon-Plas, L. Casieri and D. Wipf (2012a). "Sugar transporters in plants and in their interactions with fungi." Trends in Plant Science 17(7): 413-422.

Doidy, J., D. van Tuinen, O. Lamotte, M. Corneillat, G. Alcaraz and D. Wipf (2012b). "The *Medicago truncatula* sucrose transporter family. Characterization and implication of key members in carbon partitioning towards arbuscular mycorrhizal fungi." Molecular Plant.

Dotzauer, D., S. Wolfenstetter, D. Eibert, S. Schneider, P. Dietrich and N. Sauer (2010). "Novel PSI domains in plant and animal H^+-inositol symporters." Traffic 11(6): 767-781.

Douds, D. D., P. E. Pfeffer and Y. Shachar-Hill (2000). Carbon Partitioning, cost and metabolism of arbuscular mycorrhizae. Arbuscular mycorrhizas: physiology and function. Y. Kapulnick and D. D. Douds. London, Kluwer Academic Press.

Dwyer, D. S. (2001). "Model of the 3-D structure of the GLUT3 glucose transporter and molecular dynamics simulation of glucose transport." Proteins: Structure, Function, and Bioinformatics 42(4): 531-541.

Dwyer, L. M., M. Tollenaar and L. Houwing (1991). "A nondestructive method to monitor leaf greenness in corn." Canadian Journal of Plant Science 71(2): 505-509.

Echeverría, E. (2000). "Vesicle-Mediated Solute Transport between the Vacuole and the Plasma Membrane." Plant Physiology 123(4): 1217-1226.

Eckardt, N. A. (2011). "A Symbiotic Sugar Transporter in the Arbuscular Mycorrhizal Fungus *Glomus sp*." The Plant Cell 23(10): 3561.

Endler, A., S. Meyer, S. Schelbert, T. Schneider, W. Weschke, S. W. Peters, F. Keller, S. Baginsky, E. Martinoia and U. G. Schmidt (2006). "Identification of a vacuolar sucrose transporter in barley and *Arabidopsis* mesophyll cells by a tonoplast proteomic approach." Plant Physiology 141(1): 196-207.

Endler, A., S. Reiland, B. Gerrits, U. G. Schmidt, S. Baginsky and E. Martinoia (2009). "In vivo phosphorylation sites of barley tonoplast proteins identified by a phosphoproteomic approach." Proteomics 9(2): 310-321.

Engel, M. L., R. Holmes-Davis and S. McCormick (2005). "Green Sperm. Identification of Male Gamete Promoters in *Arabidopsis*." Plant Physiology 138(4): 2124-2133.

Eom, J.-S., J.-I. Cho, A. Reinders, S.-W. Lee, Y. Yoo, P. Q. Tuan, S.-B. Choi, G. Bang, Y.-I. Park, M.-H. Cho, S. H. Bhoo, G. An, T.-R. Hahn, J. M. Ward and J.-S. Jeon (2011). "Impaired function of the tonoplast-localized sucrose transporter in rice (*Oryza sativa*), OsSUT2, limits the transport of vacuolar reserve sucrose and affects plant growth." Plant Physiology 157: 109-119.

Fajardo Lopez, M., S. Dietz, N. Grunze, J. Bloschies, M. Weiss and U. Nehls (2008). "The sugar porter gene family of *Laccaria bicolor*: function in ectomycorrhizal symbiosis and soil-growing hyphae." New Phytologist 180(2): 365-378.

Fan, R.-C., C.-C. Peng, Y.-H. Xu, X.-F. Wang, Y. Li, Y. Shang, S.-Y. Du, R. Zhao, X.-Y. Zhang, L.-Y. Zhang and D.-P. Zhang (2009). "Apple Sucrose Transporter SUT1 and Sorbitol Transporter SOT6 Interact with Cytochrome b5 to Regulate Their Affinity for Substrate Sugars." Plant Physiology 150(4): 1880-1901.

Fang, W. and R. J. St. Leger (2010). "Mrt, a gene unique to fungi, encodes an oligosaccharide transporter and facilitates rhizosphere competency in *Metarhizium robertsii*." Plant Physiology 154(3): 1549-1557.

Fellbaum, C. R., E. W. Gachomo, Y. Beesetty, S. Choudhari, G. D. Strahan, P. E. Pfeffer, E. T. Kiers and H. Bücking (2012). "Carbon availability triggers fungal nitrogen uptake and transport in arbuscular mycorrhizal symbiosis." Proceedings of the National Academy of Sciences of the United States of America 109(7): 2666-2671.

Ferrari, S., R. Galletti, C. Denoux, G. De Lorenzo, F. M. Ausubel and J. Dewdney (2007). "Resistance to Botrytis cinerea Induced in Arabidopsis by Elicitors Is Independent of Salicylic Acid, Ethylene, or Jasmonate Signaling But Requires PHYTOALEXIN DEFICIENT3." Plant Physiology 144(1): 367-379.

Ferro, M., D. Salvi, S. Brugière, S. Miras, S. Kowalski, M. Louwagie, J. Garin, J. Joyard and N. Rolland (2003). "Proteomics of the chloroplast envelope membranes from *Arabidopsis thaliana*." Molecular and Cellular Biology 2(5): 325-345.

Ferrol, N., J. M. Barea and C. Azcón-Aguilar (2000). "The plasma membrane H^+-ATPase gene family in the arbuscular mycorrhizal fungus *Glomus mosseae*." Current Genetics 37(2): 112-118.

Feuerstein, A., M. Niedermeier, K. Bauer, S. Engelmann, S. Hoth, R. Stadler and N. Sauer (2010). "Expression of the AtSUC1 gene in the female gametophyte, and ecotype-specific expression differences in male reproductive organs." Plant Biology 12: 105-114.

Fitter, A. (2010). A forgotten phylum? The 9th International Mycological Congress, IMC9: The Biology of Fungi. Edinburgh.

Fitter, A. H. (2006). "What is the link between carbon and phosphorus fluxes in arbuscular mycorrhizas? A null hypothesis for symbiotic function." New Phytologist 172(1): 3-6.

Fitter, A. H., T. Helgason and A. Hodge (2011). "Nutritional exchanges in the arbuscular mycorrhizal symbiosis: Implications for sustainable agriculture." Fungal Biology Reviews 25(1): 68-72.

Fotopoulos, V., M. J. Gilbert, J. K. Pittman, A. C. Marvier, A. J. Buchanan, N. Sauer, J. L. Hall and L. E. Williams (2003). "The monosaccharide transporter gene, *AtSTP4*, and the cell-wall invertase, *Atβfruct1*, are induced in *Arabidopsis* during infection with the fungal biotroph *Erysiphe cichoracearum*." Plant Physiology 132(2): 821-829.

Franken, P. and F. Gnadinger (1994). "Analysis of parsley arbuscular endomycorrhiza - infection development and messenger-RNA levels of defense-related genes." Molecular Plant-Microbe Interactions 7(5): 612-620.

Gabriel-Neumann, E., G. Neumann, G. Leggewie and E. George (2011). "Constitutive overexpression of the sucrose transporter *SoSUT1* in potato plants increases arbuscular mycorrhiza fungal root colonization under high, but not under low, soil phosphorus availability." Journal of Plant Physiology 168(9): 911-919.

Gahrtz, M., J. Stolz and N. Sauer (1994). "A phloem-specific sucrose-H+ symporter from Plantago major L. supports the model of apoplastic phloem loading." The Plant Journal 6(5): 697-706.

Gallou, A., S. Declerck and S. Cranenbrouck (2011). "Transcriptional regulation of defence genes and involvement of the WRKY transcription factor in arbuscular mycorrhizal potato root colonization." Functional & Integrative Genomics: 1-16.

Gamas, P., F. D. C. Niebel, N. Lescure and J. V. Cullimore (1996). "Use of a subtractive hybridization approach to identify new *Medicago truncatula* genes induced during root nodule development." Molecular Plant-Microbe Interactions 9(4): 233-242.

Garcia-Rodriguez, S., C. Azcón-Aguilar and N. Ferrol (2007). "Transcriptional regulation of host enzymes involved in the cleavage of sucrose during arbuscular mycorrhizal symbiosis." Physiologia Plantarum 129(4): 737-746.

Garcia-Rodriguez, S., M. J. Pozo, C. Azcón-Aguilar and N. Ferrol (2005). "Expression of a tomato sugar transporter is increased in leaves of mycorrhizal or *Phytophthora parasitica*-infected plants." Mycorrhiza 15(7): 489-496.

García-Garrido, J. M. and J. A. Ocampo (2002). "Regulation of the plant defence response in arbuscular mycorrhizal symbiosis." Journal of Experimental Botany 53(373): 1377-1386.

Gattolin, S., M. Sorieul and L. Frigerio (2011). "Mapping of tonoplast intrinsic proteins in maturing and germinating *Arabidopsis* seeds reveals dual localization of embryonic TIPs to the tonoplast and plasma membrane." Molecular Plant 4(1): 180-189.

Gaude, N., S. Bortfeld, N. Duensing, M. Lohse and F. Krajinski (2011). "Arbuscule-containing and non-colonized cortical cells of mycorrhizal roots undergo a massive and specific reprogramming during arbuscular mycorrhizal development." The Plant Journal 69(3): 510–528.

Ge, L., S. B. Sun, A. Q. Chen, Y. Kapulnik and G. H. Xu (2008). "Tomato sugar transporter genes associated with mycorrhiza and phosphate." Plant Growth Regulation 55(2): 115-123.

Ge, Y. X., G. C. Angenent, E. Dahlhaus, J. Franken, J. Peters, G. J. Wullems and J. Creemers-Molenaar (2001). "Partial silencing of the NEC1 gene results in early opening of anthers in Petunia hybrida." Molecular Genetics and Genomics 265(3): 414-423

Geiger, D. (2011). "Plant sucrose transporters from a biophysical point of view." Molecular Plant 4(3): 395-406.

Genre, A., M. Chabaud, T. Timmers, P. Bonfante and D. G. Barker (2005). "Arbuscular Mycorrhizal Fungi Elicit a Novel Intracellular Apparatus in *Medicago truncatula* Root Epidermal Cells before Infection." The Plant Cell 17(12): 3489-3499.

Gianinazzi-Pearson, V. (1996). "Plant Cell Responses to Arbuscular Mycorrhizal Fungi: Getting to the Roots of the Symbiosis." The Plant Cell 8(10): 1871-1883.

Gianinazzi-Pearson, V., S. E. Smith, S. Gianinazzi and F. A. Smith (1991). "Enzymatic studies on the metabolism of vesicular—arbuscular mycorrhizas." New Phytologist 117(1): 61-74.

Gianinazzi-Pearson, V., D. van Tuinen, D. Wipf, E. Dumas-Gaudot, G. Recorbet, Y. Liu, J. Doidy, D. Redecker and N. Ferrol (2012). Exploring the Genome of Glomeromycotan Fungi. The Mycota Vol IX: Fungal associations. B. H. (ed), Springer Verlag, Berlin - Heidelberg - New York**:** in press.

Gianinazzi, S., A. Gollotte, M.-N. Binet, D. van Tuinen, D. Redecker and D. Wipf (2010). "Agroecology: the key role of arbuscular mycorrhizas in ecosystem services." Mycorrhiza 20(8): 519-530.

Gietz, R. D. and R. H. Schiestl (2007). "High-efficiency yeast transformation using the LiAc/SS carrier DNA/PEG method." Nature Protocols 2(1): 31-34.

Gomez-Roldan, V., S. Fermas, P. B. Brewer, V. Puech-Pages, E. A. Dun, J.-P. Pillot, F. Letisse, R. Matusova, S. Danoun, J.-C. Portais, H. Bouwmeester, G. Becard, C. A. Beveridge, C. Rameau and S. F. Rochange (2008). "Strigolactone inhibition of shoot branching." Nature 455(7210): 189-194.

Gomez, S. K., H. Javot, P. Deewatthanawong, I. Torres-Jerez, Y. Tang, E. Blancaflor, M. Udvardi and M. Harrison (2009). "*Medicago truncatula* and *Glomus intraradices* gene expression in

cortical cells harboring arbuscules in the arbuscular mycorrhizal symbiosis." BMC Plant Biology 9(1): 10.

Gottwald, J. R., P. J. Krysan, J. C. Young, R. F. Evert and M. R. Sussman (2000). "Genetic evidence for the in planta role of phloem-specific plasma membrane sucrose transporters." Proceedings of the National Academy of Sciences of the United States of America 97(25): 13979-13984.

Graham, J. H. (2000). Assessing costs of arbuscular mycorrhizal symbiosis agroecosystems fungi. Current Advances in Mycorrhizae Research. G. K. Podila and D. D. Douds. St Paul, APS Press**:** 127-140.

Graham, P. H. and C. P. Vance (2003). "Legumes: Importance and Constraints to Greater Use." Plant Physiology 131(3): 872-877.

Grigston, J. C., D. Osuna, W.-R. Scheible, C. Liu, M. Stitt and A. M. Jones (2008). "d-Glucose sensing by a plasma membrane regulator of G signaling protein, AtRGS1." FEBS Letters 582(25): 3577-3584.

Guan, Y.-F., X.-Y. Huang, J. Zhu, J.-F. Gao, H.-X. Zhang and Z.-N. Yang (2008). "RUPTURED POLLEN GRAIN1, a Member of the MtN3/saliva Gene Family, Is Crucial for Exine Pattern Formation and Cell Integrity of Microspores in *Arabidopsis*." Plant Physiology 147(2): 852-863.

Guether, M., B. Neuhäuser, R. Balestrini, M. Dynowski, U. Ludewig and P. Bonfante (2009). "A Mycorrhizal-Specific Ammonium Transporter from *Lotus japonicus* Acquires Nitrogen Released by Arbuscular Mycorrhizal Fungi." Plant Physiology 150(1): 73-83.

Hackel, A., N. Schauer, F. Carrari, A. Fernie, B. Grimm and C. Kühn (2006a). "Sucrose transporter LeSUT1 and LeSUT2 inhibition affects tomato fruit development in different ways." The Plant Journal 45(2): 180 - 192.

Hackel, A., N. Schauer, F. Carrari, A. R. Fernie, B. Grimm and C. Kühn (2006b). "Sucrose transporter LeSUT1 and LeSUT2 inhibition affects tomato fruit development in different ways." The Plant Journal 45(2): 180-192.

Halford, N. G., T. Y. Curtis, N. Muttucumaru, J. Postles and D. S. Mottram (2011). "Sugars in crop plants." Annals of Applied Biology 158(1): 1-25.

Hall, T. A. (1999). "BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT." Nucleic Acids Symposium Series 41: 95-98.

Hao, Z., L. Fayolle, D. van Tuinen, O. Chatagnier, X. Li, S. Gianinazzi and V. Gianinazzi-Pearson (2012). "Local and systemic mycorrhiza-induced protection against the ectoparasitic nematode *Xiphinema index* involves priming of defence gene responses in grapevine." Journal of Experimental Botany.

Harmer, S. L. and S. A. Kay (2005). "Positive and Negative Factors Confer Phase-Specific Circadian Regulation of Transcription in *Arabidopsis*." The Plant Cell 17(7): 1926-1940.

Harms, K., R. V. Wöhner, B. Schulz and W. B. Frommer (1994). "Isolation and characterization of P-type H+-ATPase genes from potato." Plant Molecular Biology 26(3): 979-988.

Harrison, M. J. (1996). "A sugar transporter from *Medicago truncatula*: Altered expression pattern in roots during vesicular-arbuscular (VA) mycorrhizal associations." The Plant Journal 9(4): 491-503.

Harrison, M. J. and M. L. v. Buuren (1995). "A phosphate transporter from the mycorrhizal fungus *Glomus versiforme*." Nature 378(6557): 626-629.

Harrison, M. J., G. R. Dewbre and J. Liu (2002). "A Phosphate Transporter from *Medicago truncatula* Involved in the Acquisition of Phosphate Released by Arbuscular Mycorrhizal Fungi." The Plant Cell 14: 2413-2429.

Harrison, M. J. and R. A. Dixon (1993). "Isoflavonoid accumulation and expression of defense gene transcripts during the establishment of vesicular-arbuscular mycorrhizal associations in roots of *Medicago truncatula*." Molecular Plant-Microbe Interactions 6(5): 643-654.

Hause, B. and T. Fester (2005). "Molecular and cell biology of arbuscular mycorrhizal symbiosis." Planta 221(2): 184-196.

He, H., I. Chincinska, A. Hackel, B. Grimm and C. Kühn (2008). "Phloem mobility and stability of sucrose transporter transcripts." The Open Plant Science Journal 2: 15-26.

Helber, N., K. Wippel, N. Sauer, S. Schaarschmidt, B. Hause and N. Requena (2011). "A versatile monosaccharide transporter that operates in the arbuscular mycorrhizal fungus *Glomus sp* is Crucial for the symbiotic relationship with plants." The Plant Cell 23: 3812-3823.

Henderson, P. J. F. and M. C. J. Maiden (1990). "Homologous Sugar Transport Proteins in *Escherichia coli* and Their Relatives in Both Prokaryotes and Eukaryotes." Philosophical Transactions of the Royal Society of London. B, Biological Sciences 326(1236): 391-410.

Henry, C. (2011). Etude de l'implication des transporteurs de sucres dans le photocontrôle du débourrement chez le rosier buisson (*Rosa hybrida* L.). Angers, Université d'Angers. PhD thesis.

Henry, C., A. Rabot, M. Laloi, E. Mortreau, M. Sigogne, N. Leduc, R. Lemoine, S. Sakr, A. Vian and S. Pelleschi-Travier (2011). "Regulation of RhSUC2, a sucrose transporter, is correlated with the light control of bud burst in *Rosa sp*." Plant, Cell & Environment 34(10): 1776–1789.

Hewitt, E. (1966). "Sand and water culture methods used in the study of plant nutrition." Technical communications, no. 22. Commonwealth Bureau of Horticulture and Plantation Crops. East Malling, England: 479-534.

Heymann, J. A. W., R. Sarker, T. Hirai, D. Shi, J. L. S. Milne, P. C. Maloney and S. Subramaniam (2001). "Projection structure and molecular architecture of OxlT, a bacterial membrane transporter." EMBO Journal 20(16): 4408-4413.

Hijri, M. and I. R. Sanders (2004). "The arbuscular mycorrhizal fungus *Glomus intraradices* is haploid and has a small genome size in the lower limit of eukaryotes." Fungal Genetics and Biology 41(2): 253-261.

Hirai, T., J. A. W. Heymann, P. C. Maloney and S. Subramaniam (2003a). "Structural Model for 12-Helix Transporters Belonging to the Major Facilitator Superfamily." J. Bacteriol. 185(5): 1712-1718.

Hirai, T., J. A. W. Heymann, P. C. Maloney and S. Subramaniam (2003b). "Structural Model for 12-Helix Transporters Belonging to the Major Facilitator Superfamily." Journal of Bacteriology 185(5): 1712-1718.

Hirose, T., N. Imaizumi, G. N. Scofield, R. T. Furbank and R. Ohsugi (1997). "cDNA Cloning and Tissue Specific Expression of a Gene for Sucrose Transporter from Rice (*Oryza sativa* L.)." Plant and Cell Physiology 38(12): 1389-1396.

Hirose, T., Z. Zhang, A. Miyao, H. Hirochika, R. Ohsugi and T. Terao (2010). "Disruption of a gene for rice sucrose transporter, OsSUT1, impairs pollen function but pollen maturation is unaffected." Journal of Experimental Botany 61(13): 3639-3646.

Hofmann, J., P. H. Hess, D. Szakasits, A. Blochl, K. Wieczorek, S. Daxbock-Horvath, H. Bohlmann, A. J. E. van Bel and F. M. W. Grundler (2009). "Diversity and activity of sugar transporters in nematode-induced root syncytia." Journal of Experimental Botany 60(11): 3085-3095.

Hogekamp, C., D. Arndt, P. Pereira, J. D. Becker, N. Hohnjec and H. Küster (2011). "Laser-microdissection unravels cell-type specific transcription in arbuscular mycorrhizal roots, including CAAT-box TF gene expression correlating with fungal contact and spread." Plant Physiology: published October 26, 2011, doi.org/2010.1104/pp.2111.186635

Hohnjec, N., A. M. Perlick, A. Pühler and H. Küster (2003). "The *Medicago truncatula* sucrose synthase gene *MtSucS1* is activated both in the infected region of root nodules and in the cortex of roots colonized by arbuscular mycorrhizal fungi." Molecular Plant-Microbe Interactions 16(10): 903-915.

Hoth, S., M. Niedermeier, A. Feuerstein, J. Hornig and N. Sauer (2010). "An ABA-responsive element in the *AtSUC1* promoter is involved in the regulation of *AtSUC1* expression." Planta 232(4): 911-923.

Huang, Y., M. J. Lemieux, J. Song, M. Auer and D.-N. Wang (2003). "Structure and Mechanism of the Glycerol-3-Phosphate Transporter from *Escherichia coli*." Science 301(5633): 616-620.

Huber, S. C. and J. L. Huber (1992). "Role of Sucrose-Phosphate Synthase in Sucrose Metabolism in Leaves." Plant Physiology 99(4): 1275-1278.

Huson, D., D. Richter, C. Rausch, T. Dezulian, M. Franz and R. Rupp (2007). "Dendroscope: An interactive viewer for large phylogenetic trees." BMC Bioinformatics 8(1): 460.

Ibraheem, O., C. E. J. Botha and G. Bradley (2010). "In silico analysis of *cis*-acting regulatory elements in 5' regulatory regions of sucrose transporter gene families in rice (*Oryza sativa* Japonica) and *Arabidopsis thaliana*." Computational Biology and Chemistry 34(5-6): 268-283.

Ibraheem, O., R. M. Hove and G. Bradley (2008). "Sucrose assimilation and the role of sucrose transporters in plant wound response." African Journal of Biotechnology 7(25): 4850-4855.

Ishimaru, K., T. Hirose, N. Aoki, S. Takahashi, K. Ono, S. Yamamoto, J. Wu, S. Saji, T. Baba, M. Ugaki, T. Matsumoto and R. Ohsugi (2001). "Antisense Expression of a Rice Sucrose Transporter OsSUT1 in Rice (Oryza sativa L.)." Plant and Cell Physiology 42(10): 1181-1185.

Jaillon, O., J.-M. Aury, B. Noel, A. Policriti, C. Clepet, et al. (2007). "The grapevine genome sequence suggests ancestral hexaploidization in major angiosperm phyla." Nature 449(7161): 463-467.

Jang, J. C., P. Leon, L. Zhou and J. Sheen (1997). "Hexokinase as a Sugar Sensor in Higher Plants." The Plant Cell 9(1): 5-19.

Javot, H., R. V. Penmetsa, N. Terzaghi, D. R. Cook and M. J. Harrison (2007). "A *Medicago truncatula* phosphate transporter indispensable for the arbuscular mycorrhizal symbiosis."

Proceedings of the National Academy of Sciences of the United States of America 104(5): 1720-1725.

Johnson, C. (1984). "Phosphorus nutrition on mycorrhizal colonization, photosynthesis, growth and nutrient composition of *Citrus aurantium*." Plant and Soil 80(1): 35-42.

Johnson, D., J. Hill and M. Thomas (2006). "The monosaccharide transporter gene family in land plants is ancient and shows differential subfamily expression and expansion across lineages." BMC Evolutionary Biology 6: 64.

Johnson, D. A. and M. A. Thomas (2007). "The monosaccharide transporter gene family in *Arabidopsis* and rice: A history of duplications, adaptive evolution, and functional divergence." Molecular Biology and Evolution 24(11): 2412-2423.

Journet, E. P., D. van Tuinen, J. Gouzy, H. Crespeau, V. Carreau, M. J. Farmer, A. Niebel, T. Schiex, O. Jaillon, O. Chatagnier, L. Godiard, F. Micheli, D. Kahn, V. Gianinazzi-Pearson and P. Gamas (2002). "Exploring root symbiotic programs in the model legume *Medicago truncatula* using EST analysis." Nucleic Acids Research 30(24): 5579-5592.

Katoh, K. and H. Toh (2008). "Recent developments in the MAFFT multiple sequence alignment program." Briefings in Bioinformatics 9(4): 286-298.

Kiers, E. T., M. Duhamel, Y. Beesetty, J. A. Mensah, O. Franken, E. Verbruggen, C. R. Fellbaum, G. A. Kowalchuk, M. M. Hart, A. Bago, T. M. Palmer, S. A. West, P. Vandenkoornhuyse, J. Jansa and H. Bücking (2011). "Reciprocal rewards stabilize cooperation in the mycorrhizal symbiosis." Science 333(6044): 880-882.

Kiyosue, T., H. Abe, K. Yamaguchi-Shinozaki and K. Shinozaki (1998). "*ERD6*, a cDNA clone for an early dehydration-induced gene of *Arabidopsis*, encodes a putative sugar transporter." Biochimica et Biophysica Acta 1370(2): 187 - 191.

Kleine-Vehn, J., P. Dhonukshe, R. Swarup, M. Bennett and J. Friml (2006). "Subcellular Trafficking of the Arabidopsis Auxin Influx Carrier AUX1 Uses a Novel Pathway Distinct from PIN1." The Plant Cell 18(11): 3171-3181.

Kleinow, T., R. Bhalerao, F. Breuer, M. Umeda, K. Salchert and C. Koncz (2000). "Functional identification of an *Arabidopsis* Snf4 ortholog by screening for heterologous multicopy suppressors of snf4 deficiency in yeast." The Plant Journal 23(1): 115-122.

Klepek, Y., D. Geiger, R. Stadler, F. Klebl, L. Landouar-Arsivaud, R. Lemoine, R. Hedrich and N. Sauer (2005). "*Arabidopsis* POLYOL TRANSPORTER5, a new member of the monosaccharide transporter-like superfamily, mediates H+-Symport of numerous substrates, including myo-inositol, glycerol, and ribose." The Plant Cell 17(1): 204 - 218.

Klepek, Y., M. Volke, K. Konrad, K. Wippel, S. Hoth, R. Hedrich and N. Sauer (2010). "*Arabidopsis thaliana* POLYOL/MONOSACCHARIDE TRANSPORTERS 1 and 2: fructose and xylitol/H+ symporters in pollen and young xylem cells." Journal of Experimental Botany 61(2): 537 - 550.

Kluge, M., D. Mollenhauer and R. Mollenhauer (1991). "Photosynthetic carbon assimilation in *Geosiphon pyriforme* (Kützing) F. v. Wettstein, an endosymbiotic association of fungus and cyanobacterium." Planta 185(3): 311-315.

Knoblauch, M. and W. S. Peters (2010). "Münch, morphology, microfluidics – our structural problem with the phloem." Plant, Cell & Environment 33(9): 1439-1452.

Knop, C., R. Stadler, N. Sauer and G. Lohaus (2004). "AmSUT1, a Sucrose Transporter in Collection and Transport Phloem of the Putative Symplastic Phloem Loader *Alonsoa meridionalis*." Plant Physiology 134(1): 204-214.

Knop, C., O. Voitsekhovskaja and G. Lohaus (2001). "Sucrose transporters in two members of the *Scrophulariaceae* with different types of transport sugar." Planta 213(1): 80-91.

Koch, K. (2004). "Sucrose metabolism: regulatory mechanisms and pivotal roles in sugar sensing and plant development." Current Opinion in Plant Biology 7(3): 235-246.

Krajinski, F., B. Hause, V. Gianinazzi-Pearson and P. Franken (2002). "Mtha1, a Plasma Membrane H+-ATPase Gene from *Medicago truncatula*, Shows Arbuscule-Specific Induced Expression in Mycorrhizal Tissue." Plant Biology 4(6): 754-761.

Krügel, U., H.-X. He, K. Gier, J. Reins, I. Chincinska, B. Grimm, W. X. Schulze and C. Kühn (2011). "The potato sucrose transporter StSUT1 interacts with a DRM-associated protein disulfide isomerase." Molecular Plant: doi: 10.1093/mp/ssr1048.

Krügel, U., L. M. Veenhoff, J. Langbein, E. Wiederhold, J. Liesche, T. Friedrich, B. Grimm, E. Martinoia, B. Poolman and C. Kühn (2008). "Transport and sorting of the *Solanum tuberosum* sucrose transporter SUT1 is affected by posttranslational modification." The Plant Cell 20(9): 2497-2513.

Kühn, C. (2003). "A comparison of the sucrose transporter systems of different plant species." Plant Biology 5: 215 - 232.

Kühn, C. (2011). Sucrose Transporters and Plant Development. Heidelberg; New York, Springer Verlag.

Kühn, C., V. Franceschi, A. Schulz, R. Lemoine and W. Frommer (1997). "Macromolecular trafficking indicated by localization and turnover of sucrose transporters in enucleate sieve elements." Science 275: 1298 - 1300.

Kühn, C. and C. Grof (2010). "Sucrose transporters of higher plants." Current Opinion in Plant Biology 13: 1 - 11.

Kühn, C., M.-R. Hajirezaei, A. R. Fernie, U. Roessner-Tunali, T. Czechowski, B. Hirner and W. B. Frommer (2003). "The sucrose transporter StSUT1 localizes to sieve elements in potato tuber phloem and influences tuber physiology and development." Plant Physiology 131(1): 102-113.

Kühn, C., W. P. Quick, A. Schulz, J. W. Riesmeier, U. Sonnewald and W. B. Frommer (1996). "Companion cell-specific inhibition of the potato sucrose transporter SUT1." Plant, Cell & Environment 19(10): 1115-1123.

Kuznetsova, E., P. Seddas-Dozolme, C. Arnould, M. Tollot, D. van Tuinen, A. Borisov, S. Gianinazzi and V. Gianinazzi-Pearson (2010). "Symbiosis-related pea genes modulate fungal and plant gene expression during the arbuscule stage of mycorrhiza with *Glomus intraradices*." Mycorrhiza 20(6): 427-443.

Lalonde, S., E. Boles, H. Hellmann, L. Barker, J. W. Patrick, W. B. Frommer and J. M. Ward (1999). "The Dual Function of Sugar Carriers: Transport and Sugar Sensing." The Plant Cell 11(4): 707-726.

Lalonde, S., D. Wipf and W. B. Frommer (2004). "Transport mechanisms for organic forms of carbon and nitrogen between source and sink." Annual Review of Plant Biology 55: 341-372.

Lambilliotte, R., R. Cooke, D. Samson, C. Fizames, F. Gaymard, C. Plassard, M.-V. Tatry, C. Berger, M. Laudié, F. Legeai, E. Karsenty, M. Delseny, S. Zimmermann and H. Sentenac (2004). "Large-scale identification of genes in the fungus *Hebeloma cylindrosporum* paves the way to molecular analyses of ectomycorrhizal symbiosis." New Phytologist 164(3): 505-513.

Landis, F. C. and L. H. Fraser (2008). "A new model of carbon and phosphorus transfers in arbuscular mycorrhizas." New Phytologist 177(2): 466-479.

Lauterbach, C., M. Niedermeier, R. Besenbeck, R. Stadler and N. Sauer (2007). "Immunolocalization of the PmSUC1 Sucrose Transporter in *Plantago major* Flowers and Reporter-Gene Analyses of the *PmSUC1* Promoter Suggest a Role in Sucrose Release from the Inner Integument." Plant Biology 9(3): 357-365.

Law, C. J., P. C. Maloney and D.-N. Wang (2008). "Ins and Outs of Major Facilitator Superfamily Antiporters." Annual Review of Microbiology 62(1): 289-305.

Law, C. J., Q. Yang, C. Soudant, P. C. Maloney and D.-N. Wang (2007). "Kinetic Evidence Is Consistent with the Rocker-Switch Mechanism of Membrane Transport by GlpT†." Biochemistry 46(43): 12190-12197.

Lefebvre, B., F. Furt, M.-A. Hartmann, L. V. Michaelson, J.-P. Carde, F. Sargueil-Boiron, M. Rossignol, J. A. Napier, J. Cullimore, J.-J. Bessoule and S. Mongrand (2007). "Characterization of lipid rafts from *Medicago truncatula* root plasma membranes: a proteomic study reveals the presence of a raft-associated redox system." Plant Physiology 144(1): 402-418.

Leggewie, G., A. Kolbe, R. Lemoine, U. Roessner, A. Lytovchenko, E. Zuther, J. Kehr, W. B. Frommer, J. W. Riesmeier, L. Willmitzer and A. R. Fernie (2003). "Overexpression of the sucrose transporter *SoSUT1* in potato results in alterations in leaf carbon partitioning and in tuber metabolism but has little impact on tuber morphology." Planta 217(1): 158-167.

Lemoine, R. (2000). "Sucrose transporters in plants: update on function and structure." Biochimica Et Biophysica Acta-Biomembranes 1465(1-2): 246-262.

Lemoine, R., L. Bürkle, L. Barker, S. Sakr, C. Kühn, M. Regnacq, C. Gaillard, S. Delrot and W. B. Frommer (1999). "Identification of a pollen-specific sucrose transporter-like protein NtSUT3 from tobacco." FEBS Letters 454(3): 325-330.

Lemoine, R., C. KÜHn, N. Thiele, S. Delrot and W. B. Frommer (1996). "Antisense inhibition of the sucrose transporter in potato: effects on amount and activity." Plant, Cell & Environment 19(10): 1124-1131.

León, P. and J. Sheen (2003). "Sugar and hormone connections." Trends in Plant Science 8(3): 110-116.

Libault, M., J. Wan, T. Czechowski, M. Udvardi and G. Stacey (2007). "Identification of 118 *Arabidopsis* Transcription Factor and 30 Ubiquitin-Ligase Genes Responding to Chitin, a Plant-Defense Elicitor." Molecular Plant-Microbe Interactions 20(8): 900-911.

Liesche, J., H.-X. He, B. Grimm, A. Schulz and C. Kühn (2010). "Recycling of *Solanum* sucrose transporters expressed in yeast, tobacco, and in mature phloem sieve elements." Molecular Plant 3(6): 1064-1074.

Liesche, J., U. Krügel, H. He, I. Chincinska, A. Hackel and C. Kühn (2011). "Sucrose transporter regulation at the transcriptional, post-transcriptional and post-translational level." Journal of Plant Physiology 168(12): 1426-1433.

Liesche, J., A. Schulz, U. Krügel, B. Grimm and C. Kühn (2008). "Dimerization and endocytosis of the sucrose transporter StSUT1 in mature sieve elements." Plant Signaling & Behavior 3(12): 1136-1137.

Lim, J. D., J.-I. Cho, Y.-I. Park, T.-R. Hahn, S.-B. Choi and J.-S. Jeon (2006). "Sucrose transport from source to sink seeds in rice." Physiologia Plantarum 126(4): 572-584.

Lin, M.-K., Y.-J. Lee, T. J. Lough, B. S. Phinney and W. J. Lucas (2009). "Analysis of the Pumpkin Phloem Proteome Provides Insights into Angiosperm Sieve Tube Function." Molecular & Cellular Proteomics 8(2): 343-356.

Liu, J., I. Maldonado-Mendoza, M. Lopez-Meyer, F. Cheung, C. D. Town and M. J. Harrison (2007). "Arbuscular mycorrhizal symbiosis is accompanied by local and systemic alterations in gene expression and an increase in disease resistance in the shoots." The Plant Journal 50(3): 529-544.

López-Pedrosa, A., M. González-Guerrero, A. Valderas, C. Azcón-Aguilar and N. Ferrol (2006). "GintAMT1 encodes a functional high-affinity ammonium transporter that is expressed in the extraradical mycelium of *Glomus intraradices*." Fungal Genetics and Biology 43(2): 102-110.

Loqué, D., S. Lalonde, L. L. Looger, N. von Wirén and W. B. Frommer (2007). "A cytosolic trans-activation domain essential for ammonium uptake." Nature 446(7132): 195-198.

Ludwig, A., J. Stolz and N. Sauer (2000). "Plant sucrose-H+ symporters mediate the transport of vitamin H." The Plant Journal 24(4): 503-509.

Lunn, J. E. (2002). "Evolution of Sucrose Synthesis." Plant Physiology 128(4): 1490-1500.

Lunn, J. E. and E. MacRae (2003). "New complexities in the synthesis of sucrose." Current Opinion in Plant Biology 6(3): 208-214.

Lynch, M. and A. Force (2000). "The Probability of Duplicate Gene Preservation by Subfunctionalization." Genetics 154(1): 459-473.

Maeda, D., K. Ashida, K. Iguchi, S. A. Chechetka, A. Hijikata, Y. Okusako, Y. Deguchi, K. Izui and S. Hata (2006). "Knockdown of an Arbuscular Mycorrhiza-inducible Phosphate Transporter Gene of *Lotus japonicus* Suppresses Mutualistic Symbiosis." Plant and Cell Physiology 47(7): 807-817.

Maillet, F., V. Poinsot, O. Andre, V. Puech-Pages, A. Haouy, M. Gueunier, L. Cromer, D. Giraudet, D. Formey, A. Niebel, E. A. Martinez, H. Driguez, G. Becard and J. Denarie (2011). "Fungal lipochitooligosaccharide symbiotic signals in arbuscular mycorrhiza." Nature 469(7328): 58-63.

Maldonado-Mendoza, I. E., G. R. Dewbre and M. J. Harrison (2001). "A Phosphate Transporter Gene from the Extra-Radical Mycelium of an Arbuscular Mycorrhizal Fungus Glomus intraradices Is Regulated in Response to Phosphate in the Environment." Molecular Plant-Microbe Interactions 14(10): 1140-1148.

Marger, M. D. and M. H. Saier, Jr (1993). "A major superfamily of transmembrane facilitators that catalyse uniport, symport and antiport." Trends in Biochemical Sciences 18(1): 13-20.

Martin, F., V. Gianinazzi-Pearson, M. Hijri, P. Lammers, N. Requena, I. R. Sanders, Y. Shachar-Hill, H. Shapiro, G. A. Tuskan and J. P. W. Young (2008). "The long hard road to a completed *Glomus intraradices* genome." New Phytologist 180(4): 747-750.

Martin, H. (2005). Isolierung symbiosespezifischer Gene aus *Geosiphon pyriformis* und funktionelle Charakterisierung des ersten Glomeromycota-Zuckertransporters. Darmstadt, Technischen Universität Darmstadt. Phd thesis.

Matsukura, C., T. Saitoh, T. Hirose, R. Ohsugi, P. Perata and J. Yamaguchi (2000). "Sugar uptake and transport in rice embryo. Expression of companion cell-specific sucrose transporter (OsSUT1) induced by sugar and light." Plant Physiology 124(1): 85 - 93.

Meyer, S., C. Lauterbach, M. Niedermeier, I. Barth, R. Sjolund and N. Sauer (2004). "Wounding enhances expression of AtSUC3, a sucrose transporter from *Arabidopsis* sieve elements and sink tissues." Plant Physiology 134(2): 684 - 693.

Meyer, S., M. Melzer, E. Truernit, C. Hümmer, R. Besenbeck, R. Stadler and N. Sauer (2000). "AtSUC3, a gene encoding a new *Arabidopsis* sucrose transporter, is expressed in cells adjacent to the vascular tissue and in a carpel cell layer." The Plant Journal 24(6): 869-882.

Mongrand, S., T. Stanislas, E. M. F. Bayer, J. Lherminier and F. Simon-Plas (2010). "Membrane rafts in plant cells." Trends in Plant Science 15(12): 656-663.

Münch, E. (1930). Die stoffbewegungen in der pflanze. Jena, G. Fischer.

Munier-Jolain, N. and C. Salon (2003). "Can sucrose content in the phloem sap reaching field pea seeds (Pisum sativum L.) be an accurate indicator of seed growth potential?" Journal of Experimental Botany 54(392): 2457-2465.

Nagy, R., V. Karandashov, V. Chague, K. Kalinkevich, M. B. Tamasloukht, G. Xu, I. Jakobsen, A. A. Levy, N. Amrhein and M. Bucher (2005). "The characterization of novel mycorrhiza-specific phosphate transporters from *Lycopersicon esculentum* and *Solanum tuberosum* uncovers functional redundancy in symbiotic phosphate transport in solanaceous species." The Plant Journal 42(2): 236-250.

Nagy, R., M. J. V. Vasconcelos, S. Zhao, J. McElver, W. Bruce, N. Amrhein, K. G. Raghothama and M. Bucher (2006). "Differential Regulation of Five Pht1 Phosphate Transporters from Maize (*Zea mays* L.)." Plant Biology 8(2): 186-197.

Nemec, S. and J. C. V. Vu (1990). "Effects of soil phosphorus and *Glomus intraradices* on growth, nonstructural carbohydrates, and photosynthetic activity of *Citrus aurantium*." Plant and Soil 128(2): 257-263.

Neuhaus, H. E. (2007). "Transport of primary metabolites across the plant vacuolar membrane." FEBS Letters 581(12): 2223-2226.

Ngampanya, B., A. Sobolewska, T. Takeda, K. Toyofuku, J. Narangajavana, A. Ikeda and J. Yamaguchi (2003). "Characterization of Rice Functional Monosaccharide Transporter, OsMST5." Bioscience, Biotechnology, and Biochemistry 67(3): 556-562.

Nielsen, T. H., J. H. Rung and D. Villadsen (2004). "Fructose-2,6-bisphosphate: a traffic signal in plant metabolism." Trends in Plant Science 9(11): 556-563.

Niittylä, T., A. T. Fuglsang, M. G. Palmgren, W. B. Frommer and W. X. Schulze (2007). "Temporal Analysis of Sucrose-induced Phosphorylation Changes in Plasma Membrane Proteins of *Arabidopsis*." Molecular & Cellular Proteomics 6(10): 1711-1726.

Noiraud, N., L. Maurousset and R. Lemoine (2001a). "Identification of a mannitol transporter, AgMaT1, in celery phloem." The Plant Cell 13(3): 695 - 705.

Noiraud, N., L. Maurousset and R. Lemoine (2001b). "Transport of polyols in higher plants." Plant Physiology and Biochemistry 39(9): 717-728.

Norholm, M., H. Nour-Eldin, P. Brodersen, J. Mundy and B. Halkier (2006). "Expression of the *Arabidopsis* high-affinity hexose transporter STP13 correlates with programmed cell death." FEBS Letters 580(9): 2381 - 2387.

Nowara, D., A. Gay, C. Lacomme, J. Shaw, C. Ridout, D. Douchkov, G. Hensel, J. Kumlehn and P. Schweizer (2010). "HIGS: Host-Induced Gene Silencing in the obligate biotrophic fungal pathogen *Blumeria graminis*." The Plant Cell.

Nühse, T. S., A. Stensballe, O. N. Jensen and S. C. Peck (2004). "Phosphoproteomics of the *Arabidopsis* Plasma Membrane and a New Phosphorylation Site Database." The Plant Cell 16(9): 2394-2405.

Okubo-Kurihara, E., T. Higaki, Y. Kurihara, N. Kutsuna, J. Yamaguchi and S. Hasezawa (2011). "Sucrose transporter NtSUT4 from tobacco BY-2 involved in plant cell shape during miniprotoplast culture." Journal of Plant Research 124(3): 395-403.

Oldroyd, G. E. D. and J. A. Downie (2006). "Nuclear calcium changes at the core of symbiosis signalling." Current Opinion in Plant Biology 9(4): 351-357.

Osuna, D., B. Usadel, R. Morcuende, Y. Gibon, O. E. Bläsing, M. Höhne, M. Günter, B. Kamlage, R. Trethewey, W.-R. Scheible and M. Stitt (2007). "Temporal responses of transcripts, enzyme activities and metabolites after adding sucrose to carbon-deprived *Arabidopsis* seedlings." The Plant Journal 49(3): 463-491.

Ouyang, S., W. Zhu, J. Hamilton, H. Lin, M. Campbell, K. Childs, F. Thibaud-Nissen, R. L. Malek, Y. Lee, L. Zheng, J. Orvis, B. Haas, J. Wortman and C. R. Buell (2007). "The TIGR Rice Genome Annotation Resource: improvements and new features." Nucleic Acids Research 35(suppl 1): D883-D887.

Ozcan, S., J. Dover, A. G. Rosenwald, S. Wölfl and M. Johnston (1996). "Two glucose transporters in *Saccharomyces cerevisiae* are glucose sensors that generate a signal for induction of gene expression." Proceedings of the National Academy of Sciences of the United States of America 93(22): 12428-12432.

Paradi, I., D. van Tuinen, D. Morandi, S. Ochatt, F. Robert, L. Jacas and E. Dumas-Gaudot (2010). "Transcription of Two Blue Copper-Binding Protein Isogenes Is Highly Correlated with Arbuscular Mycorrhizal Development in *Medicago truncatula*." Molecular Plant-Microbe Interactions 23(9): 1175-1183.

Parniske, M. (2008). "Arbuscular mycorrhiza: the mother of plant root endosymbioses." Nature Reviews Microbiology 6(10): 763-775.

Paszkowski, U., S. Kroken, C. Roux and S. P. Briggs (2002). "Rice phosphate transporters include an evolutionarily divergent gene specifically activated in arbuscular mycorrhizal symbiosis." Proceedings of the National Academy of Sciences of the United States of America 99(20): 13324-13329.

Patrick, J. W. (1997). "PHLOEM UNLOADING: Sieve Element Unloading and Post-Sieve Element Transport." Annual Review of Plant Physiology and Plant Molecular Biology 48(1): 191-222.

Payyavula, R. S., K. H. C. Tay, C.-J. Tsai and S. A. Harding (2011). "The sucrose transporter family in *Populus*: the importance of a tonoplast PtaSUT4 to biomass and carbon partitioning." The Plant Journal 65(5): 757-770.

Peng, S., D. M. Eissenstat, J. H. Graham, K. Williams and N. C. Hodge (1993). "Growth depression in mycorrhizal citrus at high-phosphorus supply: analysis of carbon costs." Plant Physiology 101(3).

Pérez-Tienda, J., P. S. Testillano, R. Balestrini, V. Fiorilli, C. Azcón-Aguilar and N. Ferrol (2011). "GintAMT2, a new member of the ammonium transporter family in the arbuscular mycorrhizal fungus Glomus intraradices." Fungal Genetics and Biology 48(11): 1044-1055.

Perotto, S., F. Martin and P. Bonfante (2007). Mycorrhizal Fungi. The Rhizosphere, CRC Press**:** 201-236.

Pfeffer, P. E., B. Bago and Y. Shachar-Hill (2001). "Exploring mycorrhizal function with NMR spectroscopy." New Phytologist 150(3): 543-553.

Pfeffer, P. E., D. D. Douds, G. Becard and Y. Shachar-Hill (1999). "Carbon uptake and the metabolism and transport of lipids in an arbuscular mycorrhiza." Plant Physiology 120(2): 587-598.

Pike, L. J. (2006). "Rafts defined: a report on the keystone symposium on lipid rafts and cell function." Journal of Lipid Research 47(7): 1597-1598.

Pollock, C. J., J. F. Farrar and A. J. Gordon (1992). Carbon Partitioning: Within and Between Organisms. (Environmental Plant Biology Series.). Oxford, Bios Scientific Publishers

Poschet, G., B. Hannich and M. Buttner (2010). "Identification and characterization of AtSTP14, a novel galactose transporter from *Arabidopsis*." Plant and Cell Physiology 51(9): 1571-1580.

Poschet, G., B. Hannich, S. Raab, I. Jungkunz, P. A. W. Klemens, S. Krueger, S. Wic, H. E. Neuhaus and M. Büttner (2011). "A novel *Arabidopsis* vacuolar glucose exporter is involved in cellular sugar homeostasis and affects the composition of seed storage compounds." Plant Physiology 157(4): 1664-1676.

Price, J., A. Laxmi, S. K. St. Martin and J.-C. Jang (2004). "Global Transcription Profiling Reveals Multiple Sugar Signal Transduction Mechanisms in *Arabidopsis*." The Plant Cell 16(8): 2128-2150.

Prieto, S., B. J. de la Cruz and I. E. Scheffler (2000). "Glucose-regulated Turnover of mRNA and the Influence of Poly(A) Tail Length on Half-life." Journal of Biological Chemistry 275(19): 14155-14166.

Pumplin, N. and M. J. Harrison (2009). "Live-Cell Imaging Reveals Periarbuscular Membrane Domains and Organelle Location in *Medicago truncatula* Roots during Arbuscular Mycorrhizal Symbiosis." Plant Physiology 151(2): 809-819.

Quirino, B., W. Reiter and R. Amasino (2001). "One of two tandem *Arabidopsis* genes homologous to monosaccharide transporters is senescence-associated." Plant Molecular Biology 46(4): 447 - 457.

Ramon, M., F. Rolland and J. Sheen (2008). "Sugar Sensing and Signaling." The Arabidopsis Book 1: 1-22.

Ransom-Hodgkins, W., M. Vaughn and D. Bush (2003). "Protein phosphorylation plays a key role in sucrose-mediated transcriptional regulation of a phloem-specific proton-sucrose symporter." Planta 217(3): 483 - 489.

Rasmussen, N., D. Lloyd, R. Ratcliffe, P. Hansen and I. Jakobsen (2000). "31P NMR for the study of P metabolism and translocation in arbuscular mycorrhizal fungi." Plant and Soil 226(2): 245-253.

Rausch, C., P. Daram, S. Brunner, J. Jansa, M. Laloi, G. Leggewie, N. Amrhein and M. Bucher (2001). "A phosphate transporter expressed in arbuscule-containing cells in potato." Nature 414(6862): 462-470.

Ravnskov, S., Y. Wu and J. H. Graham (2003). "Arbuscular mycorrhizal fungi differentially affect expression of genes coding for sucrose synthases in maize roots." New Phytologist 157(3): 539-545.

Reidel, E. J., E. A. Rennie, V. Amiard, L. Cheng and R. Turgeon (2009). "Phloem Loading Strategies in Three Plant Species That Transport Sugar Alcohols." Plant Physiology 149(3): 1601-1608.

Reinders, A., W. Schulze, C. Kühn, L. Barker, A. Schulz, J. M. Ward and W. B. Frommer (2002a). "Protein-protein interactions between sucrose sransporters of different affinities colocalized in the same enucleate sieve element." The plant Cell 14(7): 1567-1577.

Reinders, A., W. Schulze, S. Thaminy, I. Stagljar, W. B. Frommer and J. M. Ward (2002b). "Intra- and Intermolecular Interactions in Sucrose Transporters at the Plasma Membrane Detected by the Split-Ubiquitin System and Functional Assays." Structure (London, England : 1993) 10(6): 763-772.

Reinders, A., A. Sivitz, C. Starker, J. Gantt and J. Ward (2008). "Functional analysis of LjSUT4, a vacuolar sucrose transporter from *Lotus japonicus*." Plant Molecular Biology 68(3): 289-299.

Reinders, A., A. B. Sivitz, A. Hsi, C. P. L. Grof, J. M. Perroux and J. M. Ward (2006). "Sugarcane ShSUT1: analysis of sucrose transport activity and inhibition by sucralose." Plant, Cell & Environment 29(10): 1871-1880.

Reinders, A. and J. M. Ward (2001). "Functional characterization of the α-glucoside transporter Sut1p from *Schizosaccharomyces pombe*, the first fungal homologue of plant sucrose transporters." Molecular Microbiology 39(2): 445-455.

Remy, W., T. N. Taylor, H. Hass and H. Kerp (1994). "Four hundred-million-year-old vesicular arbuscular mycorrhizae." Proceedings of the National Academy of Sciences 91(25): 11841-11843.

Rennie, E. A. and R. Turgeon (2009). "A comprehensive picture of phloem loading strategies." Proceedings of the National Academy of Sciences of the United States of America 106(33): 14162-14167.

Riesmeier, J. W., B. Hirner and W. B. Frommer (1993). "Potato Sucrose Transporter Expression in Minor Veins Indicates a Role in Phloem Loading." The Plant Cell 5(11): 1591-1598.

Riesmeier, J. W., L. Willmitzer and W. B. Frommer (1992). "Isolation and characterization of a sucrose carrier cDNA from spinach by functional expression in yeast." EMBO Journal 11(13): 4705 - 4713.

Riesmeier, J. W., L. Willmitzer and W. B. Frommer (1994). "Evidence for an essential role of the sucrose transporter in phloem loading and assimilate partitioning." Embo Journal 13(1): 1-7.

Ritsema, T. and S. Smeekens (2003). "Fructans: beneficial for plants and humans." Current Opinion in Plant Biology 6(3): 223-230.

Rivera-Becerril, F., D. van Tuinen, F. Martin-Laurent, A. Metwally, K.-J. Dietz, S. Gianinazzi and V. Gianinazzi-Pearson (2005). "Molecular changes in *Pisum sativum* L. roots during arbuscular mycorrhiza buffering of cadmium stress." Mycorrhiza 16(1): 51-60.

Roblin, G., S. Sakr, J. Bonmort and S. Delrot (1998). "Regulation of a plant plasma membrane sucrose transporter by phosphorylation." FEBS Letters 424(3): 165-168.

Roitsch, T. and M.-C. González (2004). "Function and regulation of plant invertases: sweet sensations." Trends in Plant Science 9(12): 606-613.

Rolland, F., E. Baena-Gonzalez and J. Sheen (2006). "Sugar sensing and signaling in plants: conserved and novel mechanisms." Annual Review of Plant Biology 57: 675 - 709.

Rook, F., N. Gerrits, A. Kortstee, M. Van Kampen, M. Borrias, P. Weisbeek and S. Smeekens (1998). "Sucrose-specific signalling represses translation of the *Arabidopsis* ATB2 bZIP transcription factor gene." The Plant Journal 15(2): 253-263.

Rubin, R. A., S. B. Levy, R. L. Heinrikson and F. J. Kézdy (1990). "Gene duplication in the evolution of the two complementing domains of Gram-negative bacterial tetracycline efflux proteins." Gene 87(1): 7-13.

Rushton, P. J., I. E. Somssich, P. Ringler and Q. J. Shen (2010). "WRKY transcription factors." Trends in Plant Science 15(5): 247-258.

Saier, M. H., Jr (1994). "Computer-aided analyses of transport protein sequences: gleaning evidence concerning function, structure, biogenesis, and evolution." Microbiology and Molecular Biology Reviews 58(1): 71-93.

Salerno, G. L. and L. Curatti (2003). "Origin of sucrose metabolism in higher plants: when, how and why?" Trends in Plant Science 8(2): 63-69.

Sambrook, J., E. F. Fritsch and T. Maniatis (1989). Molecular Cloning. A Laboratory Manual. Cold Spring Harbor, USA, Cold Spring Harbor Laboratory Press.

Sato, S., Y. Nakamura, T. Kaneko, E. Asamizu, T. Kato, et al. (2008). "Genome Structure of the Legume, *Lotus japonicus*." DNA Research 15(4): 227-239.

Sauer, N. (2007). "Molecular physiology of higher plant sucrose transporters." FEBS Letters 581(12): 2309-2317.

Sauer, N., A. Ludwig, A. Knoblauch, P. Rothe, M. Gahrtz and F. Klebl (2004). "AtSUC8 and AtSUC9 encode functional sucrose transporters, but the closely related AtSUC6 and AtSUC7 genes encode aberrant proteins in different *Arabidopsis* ecotypes." The Plant Journal 40(1): 120-130.

Sauer, N. and J. Stolz (1994). "SUC1 and SUC2 - 2 sucrose transporters from *Arabidopsis thaliana* expression and characterization in bakers yeast and identification of the histidine tagged protein." The Plant Journal 6(1): 67-77.

Schaarschmidt, S., M.-C. González, T. Roitsch, D. Strack, U. Sonnewald and B. Hause (2007). "Regulation of arbuscular mycorrhization by carbon. The symbiotic interaction cannot be improved by increased carbon availability accomplished by root-specifically enhanced invertase activity." Plant Physiology 143(4): 1827-1840.

Schaarschmidt, S., T. Roitsch and B. Hause (2006). "Arbuscular mycorrhiza induces gene expression of the apoplastic invertase LIN6 in tomato (*Lycopersicon esculentum*) roots." Journal of Experimental Botany 57(15): 4015-4023.

Schliemann, W., C. Ammer and D. Strack (2008). "Metabolite profiling of mycorrhizal roots of *Medicago truncatula*." Phytochemistry 69(1): 112-146.

Schmutz, J., S. B. Cannon, J. Schlueter, J. Ma, T. Mitros, et al. (2010). "Genome sequence of the palaeopolyploid soybean." Nature 463(7278): 178-183.

Schneider, S., D. Beyhl, R. Hedrich and N. Sauer (2008). "Functional and physiological characterization of Arabidopsis INOSITOL TRANSPORTER1, a novel tonoplast-localized transporter for myo-inositol." Plant Cell 20(4): 1073-1087.

Schneider, S., S. Hulpke, A. Schulz, I. Yaron, J. Höll, A. Imlau, B. Schmitt, S. Batz, S. Wolf, R. Hedrich and N. Sauer (2011). "Vacuoles release sucrose via tonoplast-localised SUC4-type transporters." Plant Biology 14(2): 325–336.

Schneider, S., A. Schneidereit, K. R. Konrad, M. R. Hajirezaei, M. Gramann, R. Hedrich and N. Sauer (2006). "*Arabidopsis* INOSITOL TRANSPORTER4 mediates high-affinity H^+ symport of myoinositol across the plasma membrane." Plant Physiology 141(2): 565-577.

Schneider, S., A. Schneidereit, P. Udvardi, U. Hammes, M. Gramann, P. Dietrich and N. Sauer (2007). "Arabidopsis INOSITOL TRANSPORTER2 mediates H+ symport of different inositol epimers and derivatives across the plasma membrane." Plant Physiology 145(4): 1395-1407.

Schneidereit, A., A. Imlau and N. Sauer (2008). "Conserved cis-regulatory elements for DNA-binding-with-one-finger and homeo-domain-leucine-zipper transcription factors regulate companion cell-specific expression of the *Arabidopsis thaliana* SUCROSE TRANSPORTER 2 gene." Planta 228(4): 651 - 662.

Schneidereit, A., J. Scholz-Starke and M. Buttner (2003). "Functional characterization and expression analyses of the glucose-specific AtSTP9 monosaccharide transporter in pollen of *Arabidopsis*." Plant Physiology 133(1): 182-190.

Schneidereit, A., J. Scholz-Starke, N. Sauer and M. Buttner (2005). "AtSTP11, a pollen tube-specific monosaccharide transporter in *Arabidopsis*." Planta 221(1): 48-55.

Scholz-Starke, J., M. Buttner and N. Sauer (2003). "AtSTP6, a new pollen-specific H^+-monosaccharide symporter from *Arabidopsis*." Plant Physiology 131(1): 70-77.

Schubert, A., P. Allara and A. Morte (2004). "Cleavage of sucrose in roots of soybean (*Glycine max*) colonized by an arbuscular mycorrhizal fungus." New Phytologist 161(2): 495-501.

Schulz, A., D. Beyhl, I. Marten, A. Wormit, E. Neuhaus, G. Poschet, M. Büttner, S. Schneider, N. Sauer and R. Hedrich (2011). "Proton-driven sucrose symport and antiport are provided by the vacuolar transporters SUC4 and TMT1/2." The Plant Journal 68(1): 129-136.

Schulz, A., C. Kühn, J. W. Riesmeier and W. B. Frommer (1998). "Ultrastructural effects in potato leaves due to antisense-inhibition of the sucrose transporter indicate an apoplasmic mode of phloem loading." Planta 206(4): 533-543.

Schulze, W., A. Reinders, J. Ward, S. Lalonde and W. Frommer (2003). "Interactions between co-expressed *Arabidopsis* sucrose transporters in the split-ubiquitin system." BMC Biochemistry 4(1): 3.

Schulze, W., A. Weise, W. B. Frommer and J. M. Ward (2000). "Function of the cytosolic N-terminus of sucrose transporter AtSUT2 in substrate affinity." FEBS Letters 485(2-3): 189-194.

Schulze, W. X., T. Schneider, S. Starck, E. Martinoia and O. Trentmann (2011). "Cold acclimation induces changes in *Arabidopsis* tonoplast protein abundance and activity and alters phosphorylation of tonoplast monosaccharide transporters." The Plant Journal: no-no.

Schüßler, A. and M. Kluge (2001). *Geosiphon pyriformis*, an endocytosymbiosis between fungus and cyanobacteria, and its meaning as a model system for arbuscular mycorrhizal research. The Mycota IX. H. B. (ed), Springer, Berlin Heidelberg New York**:** 151–161.

Schüßler, A., H. Martin, D. Cohen, M. Fitz and D. Wipf (2006). "Characterization of a carbohydrate transporter from symbiotic glomeromycotan fungi." Nature 444(7121): 933-936.

Schüßler, A., H. Martin, D. Cohen, M. Fitz and D. Wipf (2007). "Arbuscular mycorrhiza - Studies on the *Geosiphon* symbiosis lead to the characterization of the first Glomeromycotan sugar transporter." Plant Signaling & Behavior 2(5): 431-434.

Schüßler, A., D. Schwarzott and C. Walker (2001). "A new fungal phylum, the Glomeromycota: phylogeny and evolution." Mycological Research 105: 1413-1421.

Schüßler, A. and C. Walker (2010). "The Glomeromycota. A species list with new families and new genera." Published in libraries at The Royal Botanic Garden Edinburgh, The Royal Botanic Garden Kew, Botanische Staatssammlung Munich, and Oregon State University.

Schüßler, A. and E. Wolf (2005). *Geosiphon pyriformis* - a Glomeromycotan Soil Fungus Forming Endosymbiosis with Cyanobacteria. In Vitro Culture of Mycorrhizas. S. Declerck, J. A. Fortin and D.-G. Strullu, Springer Berlin Heidelberg. 4**:** 271-289.

Scofield, G. N., N. Aoki, T. Hirose, M. Takano, C. L. D. Jenkins and R. T. Furbank (2007a). "The role of the sucrose transporter, OsSUT1, in germination and early seedling growth and development of rice plants." Journal of Experimental Botany 58(3): 483-495.

Scofield, G. N., T. Hirose, N. Aoki and R. T. Furbank (2007b). "Involvement of the sucrose transporter, OsSUT1, in the long-distance pathway for assimilate transport in rice." Journal of Experimental Botany 58(12): 3155-3169.

Seddas-Dozolme, P. M., C. Arnould, M. Tollot, E. Kuznetsova and V. Gianinazzi-Pearson (2010). Expression Profiling of Fungal Genes During Arbuscular Mycorrhiza Symbiosis Establishment Using Direct Fluorescent In Situ RT-PCR. Molecular and Cell Biology Methods for Fungi. A. Sharon, Springer Verlag, Berlin. 638**:** 137-152.

Seddas, P. M. A., C. M. Arias, C. Arnould, D. van Tuinen, O. Godfroy, H. A. Benhassou, J. Gouzy, D. Morandi, F. Dessaint and V. Gianinazzi-Pearson (2009). "Symbiosis-related plant genes modulate molecular responses in an arbuscular mycorrhizal fungus during early root interactions." Molecular Plant-Microbe Interactions 22(3): 341-351.

Sędzielewska, K. A., J. Fuchs, E. M. Temsch, K. Baronian, R. Watzke and G. Kunze (2011). "Estimation of the *Glomus intraradices* nuclear DNA content." New Phytologist: no-no.

Shachar-Hill, Y., P. E. Pfeffer, D. Douds, S. F. Osman, L. W. Doner and R. G. Ratcliffe (1995). "Partitioning of Intermediary Carbon Metabolism in Vesicular-Arbuscular Mycorrhizal Leek." Plant Physiology 108(1): 7-15.

Sherson, S. M., G. Hemmann, G. Wallace, S. Forbes, V. Germain, R. Stadler, N. Bechtold, N. Sauer and S. M. Smith (2000). "Monosaccharide/proton symporter AtSTP1 plays a major role in uptake and response of *Arabidopsis* seeds and seedlings to sugars." The Plant Journal 24(6): 849-857.

Shiratake, K. (2007). "Genetics of Sucrose Transporter in Plants " Genes, Genomes and Genomics 1(1): 73-80.

Shultz, J. L., D. Kurunam, K. Shopinski, M. J. Iqbal, S. Kazi, K. Zobrist, R. Bashir, S. Yaegashi, N. Lavu, A. J. Afzal, C. R. Yesudas, M. A. Kassem, C. Wu, H. B. Zhang, C. D. Town, K. Meksem and D. A. Lightfoot (2006). "The Soybean Genome Database (SoyGD): a browser for display of duplicated, polyploid, regions and sequence tagged sites on the integrated physical and genetic maps of *Glycine max*." Nucleic Acids Research 34(suppl 1): D758-D765.

Sivitz, A. B., A. Reinders, M. E. Johnson, A. D. Krentz, C. P. L. Grof, J. M. Perroux and J. M. Ward (2007). "*Arabidopsis* Sucrose Transporter AtSUC9. High-affinity transport activity, intragenic control of expression, and early flowering mutant phenotype." Plant Physiology 143(1): 188-198.

Sivitz, A. B., A. Reinders and J. M. Ward (2005). "Analysis of the Transport Activity of Barley Sucrose Transporter HvSUT1." Plant and Cell Physiology 46(10): 1666-1673.

Sivitz, A. B., A. Reinders and J. M. Ward (2008). "*Arabidopsis* Sucrose Transporter AtSUC1 Is Important for Pollen Germination and Sucrose-Induced Anthocyanin Accumulation." Plant Physiology 147(1): 92-100.

Slewinski, T. L. (2011). "Diverse Functional Roles of Monosaccharide Transporters and their Homologs in Vascular Plants: A Physiological Perspective." Molecular Plant 4(4): 641-662.

Slewinski, T. L., R. Meeley and D. M. Braun (2009). "Sucrose transporter1 functions in phloem loading in maize leaves." Journal of Experimental Botany 60(3): 881-892.

Smith, S. E., S. Dickson and F. A. Smith (2001). "Nutrient transfer in arbuscular mycorrhizas: how are fungal and plant processes integrated?" Australian Journal of Plant Physiology 28(7): 683-694.

Smith, S. E. and D. J. Read (2008). Mycorrhizal Symbiosis. Amsterdam; Boston, Academic Press.

Smith, S. E. and F. A. Smith (1990). "Structure and Function of the Interfaces in Biotrophic Symbioses as They Relate to Nutrient Transport." New Phytologist 114(1): 1-38.

Solaiman, M. D. Z. and M. Saito (1997). "Use of sugars by intraradical hyphae of arbuscular mycorrhizal fungi revealed by radiorespirometry." New Phytologist 136(3): 533-538.

Song, L.-F., J.-J. Zou, W.-Z. Zhang, W.-H. Wu and Y. Wang (2009). "Ion transporters involved in pollen germination and pollen tube tip-growth." Plant Signaling & Behavior 4(12): 1193-1195.

Soulard, A., A. Cohen and M. N. Hall (2009). "TOR signaling in invertebrates." Current Opinion in Cell Biology 21(6): 825-836.

Srivastava, A., S. Ganesan, I. Ismail and B. Ayre (2008). "Functional characterization of the *Arabidopsis thaliana* AtSUC2 Suc/H+ symporter by tissue-specific complementation reveals an essential role in phloem loading but not in long-distance transport." Plant Physiology 148: 200 - 211.

Srivastava, A., S. Ganesan, I. Ismail and B. Ayre (2009a). "Effective carbon partitioning driven by exotic phloem-specific regulatory elements fused to the *Arabidopsis thaliana* AtSUC2 sucrose-proton symporter gene." BMC Plant Biology 9(1): 7.

Srivastava, A. C., K. Dasgupta, E. Ajieren, G. Costilla, R. C. McGarry and B. G. Ayre (2009b). "*Arabidopsis* plants harbouring a mutation in AtSUC2, encoding the predominant sucrose/proton symporter necessary for efficient phloem transport, are able to complete their life cycle and produce viable seed." Annals of Botany 104(6): 1121-1128.

Stadler, R., M. Buttner, P. Ache, R. Hedrich, N. Ivashikina, M. Melzer, S. M. Shearson, S. M. Smith and N. Sauer (2003). "Diurnal and light-regulated expression of AtSTP1 in guard cells of *Arabidopsis*." Plant Physiology 133(2): 528-537.

Stadler, R., E. Truernit, M. Gahrtz and N. Sauer (1999). "The AtSUC1 sucrose carrier may represent the osmotic driving force for anther dehiscence and pollen tube growth in *Arabidopsis*." The Plant Journal 19(3): 269-278.

Stadler, R., K. M. Wright, C. Lauterbach, G. Amon, M. Gahrtz, A. Feuerstein, K. J. Oparka and N. Sauer (2005). "Expression of GFP-fusions in *Arabidopsis* companion cells reveals non-specific protein trafficking into sieve elements and identifies a novel post-phloem domain in roots." The Plant Journal 41(2): 319-331.

Stockinger, H., C. Walker and A. Schüßler (2009). "'*Glomus intraradices* DAOM197198', a model fungus in arbuscular mycorrhiza research, is not *Glomus intraradices*." New Phytologist 183(4): 1176-1187.

Sun, C., S. Palmqvist, H. Olsson, M. Borén, S. Ahlandsberg and C. Jansson (2003). "A Novel WRKY Transcription Factor, SUSIBA2, Participates in Sugar Signaling in Barley by Binding to the Sugar-Responsive Elements of the iso1 Promoter." The Plant Cell 15(9): 2076-2092.

Sun, Y., A. Reinders, K. R. LaFleur, T. Mori and J. M. Ward (2010). "Transport activity of rice sucrose transporters OsSUT1 and OsSUT5." Plant and Cell Physiology 51(1): 114-122.

Swofford, D. L. (1998). "PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4." Sinauer Associates, Sunderland, Massachusetts.

Tadege, M., J. Q. Wen, J. He, H. D. Tu, Y. Kwak, A. Eschstruth, A. Cayrel, G. Endre, P. X. Zhao, M. Chabaud, P. Ratet and K. S. Mysore (2008). "Large-scale insertional mutagenesis using the Tnt1 retrotransposon in the model legume *Medicago truncatula*." The Plant Journal 54(2): 335-347.

Takanaga, H., B. Chaudhuri and W. B. Frommer (2008). "GLUT1 and GLUT9 as major contributors to glucose influx in HepG2 cells identified by a high sensitivity intramolecular FRET glucose sensor." Biochimica et Biophysica Acta (BBA) - Biomembranes 1778(4): 1091-1099.

Takanaga, H. and W. B. Frommer (2010). "Facilitative plasma membrane transporters function during ER transit." The FASEB Journal 24(8): 2849-2858.

Taussig, R. and M. Carlson (1983). "Nucleotide sequence of The yeast SUC2 gene for invertase." Nucleic Acids Research 11(6): 1943-1954.

Tejeda-Sartorius, M., O. Martínez de la Vega and J. P. Délano-Frier (2008). "Jasmonic acid influences mycorrhizal colonization in tomato plants by modifying the expression of genes involved in carbohydrate partitioning." Physiologia Plantarum 133(2): 339-353.

The *Arabidopsis* Genome Initiative (2000). "Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*." Nature 408(6814): 796-815.

Thompson, M. (2006). "Phloem: the long and the short of it." Trends in Plant Science 11: 26 - 32.

Thompson, M. V. and S. M. Wolniak (2008). "A Plasma Membrane-Anchored Fluorescent Protein Fusion Illuminates Sieve Element Plasma Membranes in *Arabidopsis* and Tobacco." Plant Physiology 146(4): 1599-1610.

Tian, C., B. Kasiborski, R. Koul, P. J. Lammers, H. Bücking and Y. Shachar-Hill (2010). "Regulation of the Nitrogen Transfer Pathway in the Arbuscular Mycorrhizal Symbiosis: Gene Characterization and the Coordination of Expression with Nitrogen Flux." Plant Physiology 153(3): 1175-1187.

Tisserant, E., A. Kohler, P. Dozolme-Seddas, R. Balestrini, K. Benabdellah, et al. (2011). "The transcriptome of the arbuscular mycorrhizal fungus *Glomus intraradices* (DAOM 197198) reveals functional tradeoffs in an obligate symbiont." New Phytologist 193(3): 755–769.

Tollot, M., J. Wong Sak Hoi, D. Van Tuinen, C. Arnould, O. Chatagnier, B. Dumas, V. Gianinazzi-Pearson and P. M. A. Seddas (2009). "An STE12 gene identified in the mycorrhizal fungus *Glomus intraradices* restores infectivity of a hemibiotrophic plant pathogen." New Phytologist 181(3): 693-707.

Toroser, D., Z. Plaut and S. C. Huber (2000). "Regulation of a Plant SNF1-Related Protein Kinase by Glucose-6-Phosphate." Plant Physiology 123(1): 403-412.

Toyofuku, K., M. Kasahara and J. Yamaguchi (2000). "Characterization and Expression of Monosaccharide Transporters (OsMSTs) in Rice." Plant and Cell Physiology 41(8): 940-947.

Trouvelot, A., J. L. Kough and V. Gianinazzi-Pearson (1986). "Mesure du taux de mycorhization VA d'un système radiculaire. Recherche de méthodes d'estimation ayant une signification fonctionnelle." Physiological and Genetical Aspects of Mycorrhizae: 217-221.

Truernit, E. and N. Sauer (1995). "The Promoter of the *Arabidopsis thaliana* Suc2 Sucrose-H+ Symporter Gene Directs Expression of Beta-Glucuronidase to the Phloem - Evidence for Phloem Loading and Unloading by Suc2." Planta 196(3): 564-570.

Truernit, E., J. Schmid, P. Epple, J. Illig and N. Sauer (1996). "The sink-specific and stress-regulated *Arabidopsis STP4* gene: enhanced expression of a gene encoding a monosaccharide transporter by wounding, elicitors, and pathogen challenge." The Plant Cell 8(12): 2169-2182.

Truernit, E., R. Stadler, K. Baier and N. Sauer (1999). "A male gametophyte-specific monosaccharide transporter in *Arabidopsis*." The Plant Journal 17(2): 191-201.

Turgeon, R., Ed. (1991). Symplastic phloem loading and the sink-source transition in leaves: a model. Recent Advances in Phloem Transport and Assimilate Compartmentation, Ouest Editions, Nantes, France.

Turgeon, R. and S. Wolf (2009). "Phloem Transport: Cellular Pathways and Molecular Trafficking." Annual Review of Plant Biology 60(1): 207-221.

Tuskan, G. A., S. DiFazio, S. Jansson, J. Bohlmann, I. Grigoriev, et al. (2006). "The Genome of Black Cottonwood, *Populus trichocarpa* (Torr. & Gray)." Science 313(5793): 1596-1604.

van Bel, A. J. (1995). The low-profile directors of carbon and nitrogen economy in plants: parenchyma cells associated with translocation channels. San Diego [u.a.], Academic Pr.

van Bel, A. J. and P. H. Hess (2008). "Hexoses as phloem transport sugars: the end of a dogma?" Journal of Experimental Botany 59(2): 261-272.

van Bel, A. J. E. (2003). "Transport phloem: Low profile, high impact." Plant Physiology 131(4): 1509-1510.

Vargas, W. A., F. K. Crutcher and C. M. Kenerley (2011). "Functional characterization of a plant-like sucrose transporter from the beneficial fungus *Trichoderma virens*. Regulation of the symbiotic association with plants by sucrose metabolism inside the fungal cells." New Phytologist 189(3): 777-789.

Vaughn, M., G. Harrington and D. Bush (2002). "Sucrose-mediated transcriptional regulation of sucrose symporter activity in the phloem." Proceedings of the National Academy of Sciences of the United States of America 99(16): 10876 - 10880.

Vierheilig, H., A. P. Coughlan, U. Wyss and Y. Piché (1998). "Ink and vinegar, a simple staining technique for arbuscular-mycorrhizal fungi." Applied Environement Microbiology 64(12): 5004-5007.

Viola, R., A. G. Roberts, S. Haupt, S. Gazzani, R. D. Hancock, N. Marmiroli, G. C. Machray and K. J. Oparka (2001). "Tuberization in potato involves a switch from apoplastic to symplastic phloem unloading." The Plant Cell 13(2): 385-398.

Wahl, R., K. Wippel, S. Goos, J. Kämper and N. Sauer (2010). "A novel high-affinity sucrose transporter is required for virulence of the plant pathogen *Ustilago maydis*." PLoS Biology 8(2): e1000303.

Wang, B. and Y. L. Qiu (2006). "Phylogenetic distribution and evolution of mycorrhizas in land plants." Mycorrhiza 16(5): 299-363.

Wang, H. X., R. R. Weerasinghe, T. D. Perdue, N. G. Cakmakci, J. P. Taylor, W. F. Marzluff and A. M. Jones (2006). "A golgi-localized hexose transporter is involved in heterotrimeric G protein-mediated early development in *Arabidopsis*." Molecular Biology of the Cell 17(10): 4257-4269.

Wang, Y., Y. Xiao, Y. Zhang, C. Chai, G. Wei, X. Wei, H. Xu, M. Wang, P. Ouwerkerk and Z. Zhu (2008). "Molecular cloning, functional characterization and expression analysis of a novel monosaccharide transporter gene OsMST6 from rice (*Oryza sativa* L.)." Planta 228(4): 525-535.

Wang, Y., H. Xu, X. Wei, C. Chai, Y. Xiao, Y. Zhang, B. Chen, G. Xiao, P. Ouwerkerk, M. Wang and Z. Zhu (2007). "Molecular cloning and expression analysis of a monosaccharide transporter gene OsMST4 from rice (*Oryza sativa* L.)." Plant Molecular Biology 65(4): 439-451.

Weber, A., J. C. Servaites, D. R. Geiger, H. Kofler, D. Hille, F. Groner, U. Hebbeker and U. I. Flugge (2000). "Identification, purification, and molecular cloning of a putative plastidic glucose translocator." The Plant Cell 12(5): 787-801.

Weber, H., L. Borisjuk, U. Heim, N. Sauer and U. Wobus (1997). "A role for sugar transporters during seed development: molecular characterization of a hexose and a sucrose carrier in fava bean seeds." The Plant Cell 9(6): 895-908.

Weise, A., L. Barker, C. Kühn, S. Lalonde, H. Buschmann, W. B. Frommer and J. M. Ward (2000). "A new subfamily of sucrose transporters, SUT4, with low affinity/high capacity localized in enucleate sieve elements of plants." The Plant Cell 12(8): 1345-1356.

Weise, A., S. Lalonde, C. Kühn, W. Frommer and J. Ward (2008). "Introns control expression of sucrose transporter LeSUT1 in trichomes, companion cells and in guard cells." Plant Molecular Biology 68(3): 251-262.

Weschke, W., R. Panitz, N. Sauer, Q. Wang, B. Neubohn, H. Weber and U. Wobus (2000). "Sucrose transport into barley seeds: molecular characterization of two transporters and implications for seed development and starch accumulation." The Plant Journal 21(5): 455-467.

Whiteman, S.-A., L. Serazetdinova, A. M. E. Jones, D. Sanders, J. Rathjen, S. C. Peck and F. J. M. Maathuis (2008). "Identification of novel proteins and phosphorylation sites in a tonoplast enriched membrane fraction of *Arabidopsis thaliana*." Proteomics 8(17): 3536-3547.

Wieczorke, R., S. Krampe, T. Weierstall, K. Freidel, C. P. Hollenberg and E. Boles (1999). "Concurrent knock-out of at least 20 transporter genes is required to block uptake of hexoses in *Saccharomyces cerevisiae*." FEBS Letters 464(3): 123-128.

Williams, L. E., R. Lemoine and N. Sauer (2000). "Sugar transporters in higher plants - a diversity of roles and complex regulation." Trends in Plant Science 5(7): 283-290.

Wind, J., S. Smeekens and J. Hanson (2010). "Sucrose: Metabolite and signaling molecule." Phytochemistry 71(14-15): 1610-1614.

Wingenter, K., A. Schulz, A. Wormit, S. Wic, O. Trentmann, Hoermiller, II, A. G. Heyer, I. Marten, R. Hedrich and H. E. Neuhaus (2010). "Increased activity of the vacuolar monosaccharide transporter TMT1 alters cellular sugar partitioning, sugar signaling, and seed yield in *Arabidopsis*." Plant Physiology 154(2): 665-677.

Wipf, D., M. Benjdia, E. Rikirsch, S. Zimmermann, M. Tegeder and W. B. Frommer (2003). "An expression cDNA library for suppression cloning in yeast mutants, complementation of a yeast his4 mutant, and EST analysis from the symbiotic basidiomycete *Hebeloma cylindrosporum*." Genome 46(2): 177-181.

Wipf, D., M. Benjdia, M. Tegeder and W. B. Frommer (2002). "Characterization of a general amino acid permease from *Hebeloma cylindrosporum*." FEBS Letters 528(1): 119-124.

Wippel, K., A. Wittek, R. Hedrich and N. Sauer (2010). "inverse pH regulation of plant and fungal sucrose transporters: a mechanism to regulate competition for sucrose at the host/pathogen interface?" Plos One 5(8): e12429.

Wormit, A., O. Trentmann, I. Feifer, C. Lohr, J. Tjaden, S. Meyer, U. Schmidt, E. Martinoia and H. E. Neuhaus (2006). "Molecular identification and physiological characterization of a novel monosaccharide transporter from *Arabidopsis* involved in vacuolar sugar transport." The Plant Cell 18(12): 3476-3490.

Wright, D. P., D. J. Read and J. D. Scholes (1998a). "Mycorrhizal sink strength influences whole plant carbon balance of *Trifolium repens* L." Plant Cell and Environment 21(9): 881-891.

Wright, D. P., J. D. Scholes and D. J. Read (1998b). "Effects of VA mycorrhizal colonization on photosynthesis and biomass production of *Trifolium repens* L." Plant, Cell & Environment 21(2): 209-216.

Wright, D. P., J. D. Scholes, D. J. Read and S. A. Rolfe (2005). "European and African maize cultivars differ in their physiological and molecular responses to mycorrhizal infection." New Phytologist 167(3): 881-896.

Yamada, K., Y. Osakabe, J. Mizoi, K. Nakashima, Y. Fujita, K. Shinozaki and K. Yamaguchi-Shinozaki (2010). "Functional analysis of an *Arabidopsis thaliana* abiotic stress-inducible facilitated diffusion transporter for monosaccharides." Journal of Biological Chemistry 285(2): 1138-1146.

Yang, B., A. Sugio and F. F. White (2006). "Os8N3 is a host disease-susceptibility gene for bacterial blight of rice." Proceedings of the National Academy of Sciences of the United States of America 103(27): 10503-10508.

Young, N. D., F. Debelle, G. E. D. Oldroyd, R. Geurts, S. B. Cannon, et al. (2011). "The *Medicago* genome provides insight into the evolution of rhizobial symbioses." Nature 480: 520-524.

Zhang, C. and R. Turgeon (2009). "Downregulating the sucrose transporter *VpSUT1* in *Verbascum phoeniceum* does not inhibit phloem loading." Proceedings of the National Academy of Sciences of the United States of America 106(44): 18849-18854.

Zhang, C., X. Yu, B. G. Ayre and R. Turgeon (2012). "The origin and composition of Cucurbit phloem exudate." Plant Physiology.

Zhang, X.-Y., X.-L. Wang, X.-F. Wang, G.-H. Xia, Q.-H. Pan, R.-C. Fan, F.-Q. Wu, X.-C. Yu and D.-P. Zhang (2006). "A Shift of Phloem Unloading from Symplasmic to Apoplasmic Pathway Is Involved in Developmental Onset of Ripening in Grape Berry." Plant Physiology 142(1): 220-232.

Zhou, Y. C., H. X. Qu, K. E. Dibley, C. E. Offler and J. W. Patrick (2007). "A suite of sucrose transporters expressed in coats of developing legume seeds includes novel pH-independent facilitators." The Plant Journal 49(4): 750-764.

Zon, L. I., D. M. Dorfman and S. H. Orkin (1989). "The polymerase chain-reaction colony miniprep." Biotechniques 7(7): 696-698.

Annexes

Annex I

Yeast culture medium

YPD	
Yeast extract	10 g
Peptone	20 g
Glucose	20 g
(Agar Oxoid)	(20 g)
H_2O qsp	1 L

YPM	
Yeast extract	10 g
Peptone	20 g
Maltose	20 g
(Agar Oxoid)	(20 g)
H_2O qsp	1 L

Synthethic medium	
Yeast Nitrogen Base with ammonium sulfate w/o amino acids	6,7 g
10X Drop out	100 mL
(Agar Oxoid)	(20 g)
H_2O qsp	1 L

10X drop-out supplement (DO, Clonetech)

Nutriment	concentration
L-Adenine (hémisulfate)	200 mg/L
L-Arginine HCl	200 mg/L
L-histidine HCl monohydrate	200 mg/L
L-Isoleucine	300 mg/L
L-Leucine	1000 mg/L
L-Lysine HCl	300 mg/L
L-Methionine	200 mg/L
L-Phenylalanine	500 mg/L
L-Threonine	2000 mg/L
L-Tryptophane	200 mg/L
L-Tyrosine	300 mg/L
L-Uracil	200 mg/L
L-Valine	1500 mg/L

Synthethic medium is adjusted to ph 5.8, autoclaved and cooled down to 50°C prior the addition of filtered-sterilized drop out without uracil (Clonetech, 630416) and the addition of 20 g/L dextrose (Sigma-aldrich G7528), maltose (M5895) or sucrose (S0389) filtered-sterilized and prepared extemporaneously.

Annex II
Bacterial culture medium

LB	
Yeast extract	5 g
Tryptone	10 g
NaCl	10 g
(Agar)	(20 g)
H_2O qsp	1 L

LB medium is autoclaved and cool down to 50°C prior the addition of appropriate antibiotics (ampicillin 100µg/mL or kanamycin 50µg/mL).

SOC	
Yeast extract	5 g
Tryptone	20 g
NaCl 5M	2 mL
KCl 1M	2.5 mL
MgCl21M	10 mL
MgSO4 1M	10 mL
Glucose 1M	20 mL
H_2O qsp	1 L

NZY+ Broth	
Yeast extract	5g
NZ amine (casein hydrolysate)	10 g
NaCl	5 g
H_2O qsp	1 L

Adjust ph to 7.5, autoclave the NZY+ solution and add the following filter-sterilized supplements prior to use:
12.5 ml of 1 M $MgCl_2$
12.5 ml of 1 M $MgSO_4$
20 ml of 20% (w/v) glucose (or 10 ml of 2 M glucose)

Annex III

Standard solutions for molecular biology

TAE 10X	
Tris	48.4 g g
Acetic acid	11.42 ml
EDTA 0.5 M	20 ml
H_2O qsp	1 L

TE 10X	
Tris 1M	100mL
EDTA 0.5 M	20mL
H_2O qsp	1 L

Annex IV

Primers used in this study

Primer name	Sequence	Tm
RACE experiments		
EST332714 3'F1	CAAGAATCATCGCCATTCTGCTGTCG	65°C
EST332714 3'F2	CATTGCTTGGTGATCTCACTGGTAAGG	65°C
EST332714 5'R1	GGTGGTGATGATAATGAAACCAATGTCC	65°C
EST332714 5'R2	GCAGGGGTAAGTGTGAAAGGAAAGAC	65°C
META878 3'F1	CGTCTTTGTTTGCATGGCTGGCAC	65°C
META878 3'F2	CCACTTGCGATTACCTACAGTGTTCC	65°C
META878 5'R1	CAGGAATATTGCCACCACCAAAAGAGAGC	65°C
META878 5'R2	CGTGAGAGCCATTACAACCAGC	65°C
NF102F08DT1F1073 3'F1	CGTGCAGTGGTGATCTTTGTTTTCG	65°C
NF102F08DT1F1073 3'F2	CTTCATTGGTGACCTCGCTGGTG	65°C
NF102F08 5'R1	CCATCATATTCCCAAAGCACGAAACC	65°C
NF102F08 5'R2	GATTCCGATTCTGATTGTGATTCCGAC	65°C
GeneRacer™ 5'F1	CGACTGGAGCACGAGGACACTGA	65°C
GeneRacer™ 5'F2	GGACACTGACATGGACTGAAGGAGTA	65°C
GeneRacer™ 3'R1	GCTGTCAACGATACGCTACGTAACG	65°C
GeneRacer™ 3'R2	CGCTACGTAACGGCATGACAGTG	65°C

Table IV.1. Primers used for 5’ and 3’ RACE experiments

Primer name	Sequence	Tm
Cloning esperiments		
MtSUT1-1 F GW	GGGGACAAGTTTGTACAAAAAAGCAGGCTATGGAGCCTTTCTCTTCC	55°C
MtSUT1-1 R GW	GGGGACCACTTTGTACAAGAAAGCTGGGTTTAATGAAAGCCTCCTCCTG	55°C
MtSUT1-2 F GW	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATAATGGATAATGCTGCCACC	55°C
MtSUT1-2 R GW	GGGGACCACTTTGTACAAGAAAGCTGGGTCTATGTTAATTAATGGAAACCACC	55°C
MtSUT1-3 F GW	GGGGACAAGTTTGTACAAAAAAGCAGGCTCACTCTTGCACATAAACTTCTCC	55°C
MtSUT1-3 R GW	GGGGACCACTTTGTACAAGAAAGCTGGGTGAGATTTAGTGGGACGTAATATGCC	55°C
MtSUT2 F GW	GGGGACAAGTTTGTACAAAAAAGCAGGCTCGTAATGGCGGGTAAGTCTGACTC	55°C
MtSUT2 R GW	GGGGACCACTTTGTACAAGAAAGCTGGGTACGGATTAGATCTCCCAACAGC	55°C
MtSUT4-1 F GW	GGGGACAAGTTTGTACAAAAAAGCAGGCTATGCCGAATCCCACTACAACAAACC	55°C
MtSUT4-1 R GW	GGGGACCACTTTGTACAAGAAAGCTGGGTTCATATGCGGACTCGAGGCTTTTGTG	55°C
MtSUT4-2 F GW	GGGGACAAGTTTGTACAAAAAAGCAGGCTCACAATACAATCTTCCAAGCCAC	55°C
MtSUT4-2 R GW	GGGGACCACTTTGTACAAGAAAGCTGGGTCCCTCTTCAAAAGAAATCTGTGC	55°C
M13Rev (-29)	CAGGAAACAGCTATGACC	53°C
M13 F	GTAAAACGACGGCCAG	53°C
PMA F(PDRs)	CTCTCTTTTATACACACATTC	53°C
ADH close R(PDRs)	CGTAATACGACTCACTATAGG	53°C

Table IV.2. Primers used for cloning experiments

Primer name	Sequence	Tm
Symbiotic markers		
MtPT4_ATG_F	ATGGGATTAGAAGTCCTTGAG	55°C
MtPT4_Stop_R	TCACATCTTCTCAGTTCTTG	55°C
MtBcp1a_ATG_F	ATGGTTTTACTTTCATCAGTTGC	55°C
MtBcp1a_Stop_R	CTCTCATGCAAAGATGACTGC	55°C

Table IV.3. Primers used for screnning symbiotic markers in the *M. truncatula - G. intraradices* expression library

Primer name	Sequence	Tm
qPCR experiments		
MtSUT1-1 F qPCR	AGTGGCATATTATCCGTAGTC	60°C
MtSUT1-1 R qPCR	TGAAGAAAATGTTCCACACTG	60°C
MtSUT1-2 F qPCR	TTGTTGTACCACAAATGATCG	60°C
MtSUT1-2 R qPCR	TAATTAATGGAAACCACCTCCA	60°C
MtSUT1-3 F qPCR	CCTACTCCAAAGTCTGTTGAT	60°C
MtSUT1-3 R qPCR	TTAGTGGGACGTAATATGCC	60°C
MtSUT2 F qPCR	AACTGCCAAACCTTTCTAGC	60°C
MtSUT2 R qPCR	CACAATCAACGTGCCTACTC	60°C
MtSUT4-1 F qPCR	GCAGATTGTGGTATCACTG	60°C
MtSUT4-1 R qPCR	TAAGTGCCAAAAGAAAACAGC	60°C
MtSUT4-2 F qPCR	CAGTGGACTCATAGCTGTC	60°C
MtSUT4-2 R qPCR	AGAAATCTGTGCCAAAGCAG	60°C
MtPT4-F qPCR	GACACGAGGCGCTTTCATAGCAGC	60°C
MtPT4-R qPCR	GTCATCGCAGCTGGAACAGCACCG	60°C
MtTef1a F qPCR	ACTGTGCAGTAGTACTTGGTG	60°C
MtTef1a R qPCR	AAGCTAGGAGGTATTGACAAG	60°C

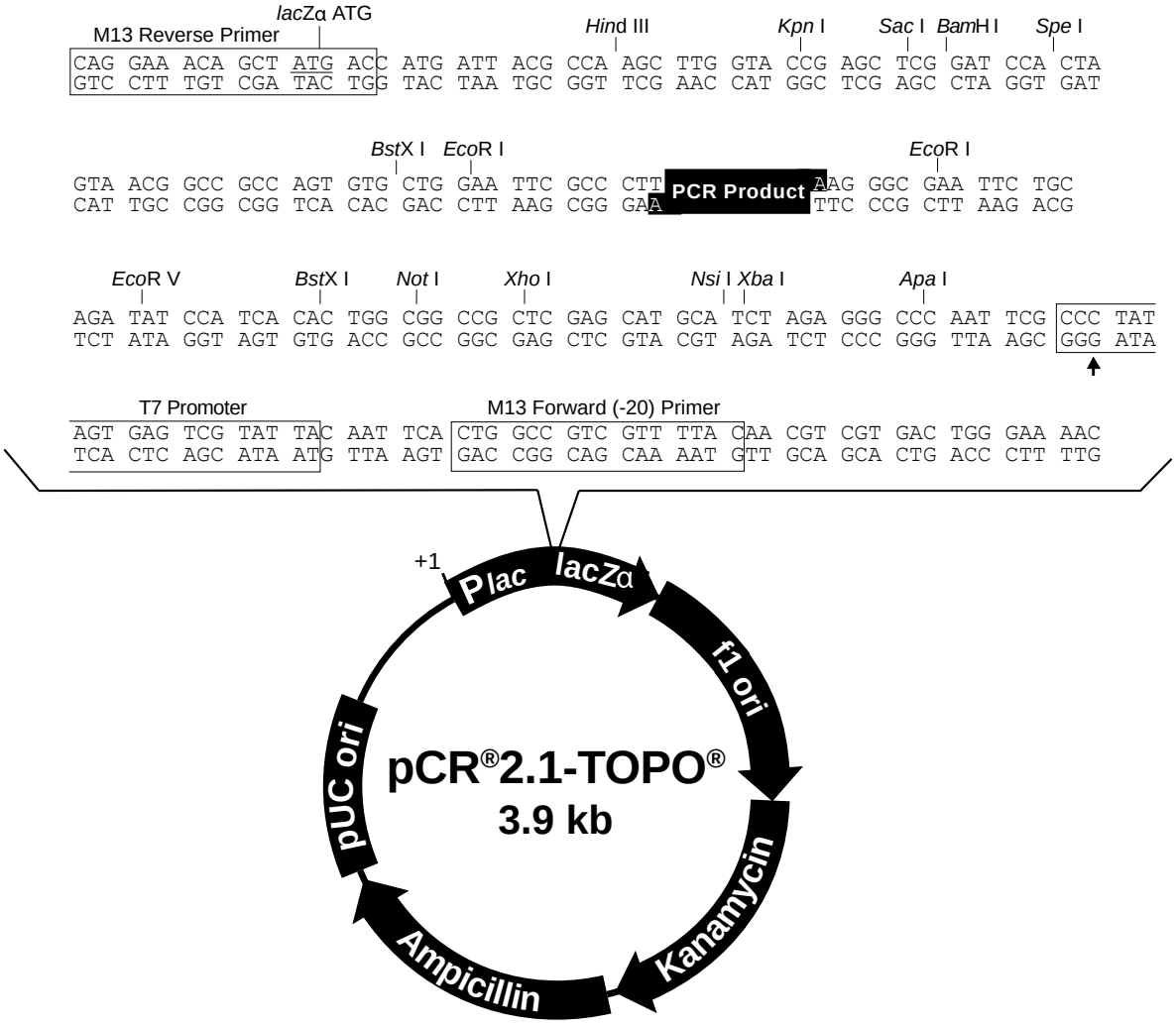
Table IV.4. Primers used for qPCR experiments

Primer name	Sequence	Tm
Yeast SUC2 invertase		
ScSUC2 excreted INV F	ATGCTTTTGCAAGCTTTCC	55°C
ScSUC2 cytosolic INV F	ATGACAAACGAAACTAGCG	55°C
ScSUC2 5'UTR F	ACATTCTCTTGTTCTTGTGC	55°C
ScSUC2 R	CCACCATCAAGAGAATAGC	55°C

Table IV.5 . Primers used for amplification the yeast SUC2 locus

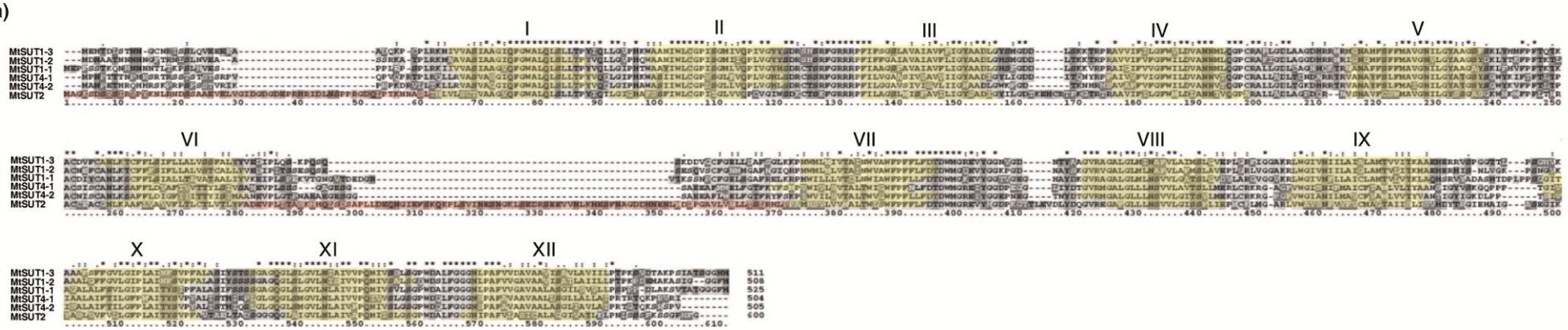
Annex V

Map of the pCR®2.1-TOPO® vector



Annex VI Amino acid alignment of the MtSUT family

(a)



(b)

	MtSUT1-3	MtSUT1-2	MtSUT1-1	MtSUT4-1	MtSUT4-2
MtSUT2	36,7	35,0	38,5	39,4	40,5
MtSUT1-3		77,2	65,6	48,9	48,1
MtSUT1-2			64,5	48,2	48,1
MtSUT1-1				50,7	52,6
MtSUT4-1					76,4

(a) Amino acid alignment of the MtSUT family; sequences were aligned using Mafft (Katoh and Toh 2008). The 12 characteristic TM domains of SUTs are highlighted in yellow with roman numbers and were predicted using the TMHMM Server v.2.0 (http://www.cbs.dtu.dk/services/TMHMM/). The extended N-Terminal and central loop of MtSUT2 are highlighted in red.

(b) Percentage of identity calculated from the amino acid alignment.

Annex VII

Medicago truncatula mutant Tnt1 lines

Gene	Line n°	FST	Insertion in *MtSUT* locus
MtSUT1-1	nf0500 (I19) nf3112 (I7)	18 25	CDS promoter
MtSUT1-3	nf0018 (I1) nf4703 (I8)	12 15	exon 1 exon 2
MtSUT4-2	nf4354 (I9)	19	CDS

Table VII. Available Tnt1 mutant insertional lines
Flanking sequence tags (FST) represent the total number of Tnt1 transposon inserted in each line

nf3112

nf0500

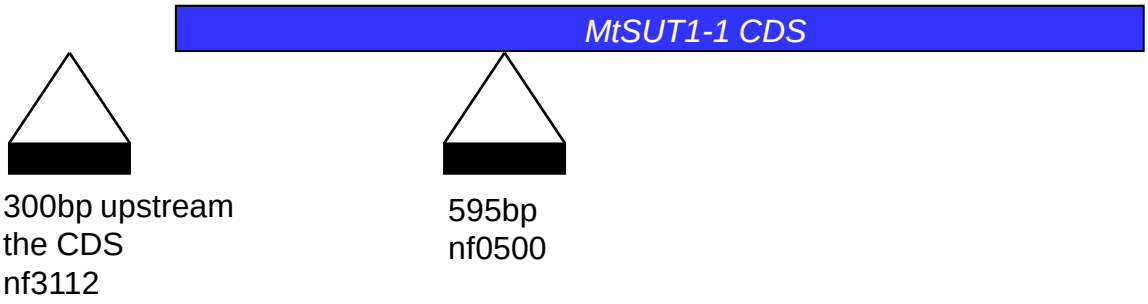


Fig VII.1. Photo and schematic representation of the Tnt1 insertion lines in the *MtSUT1-1* locus

nf4703

nf4354

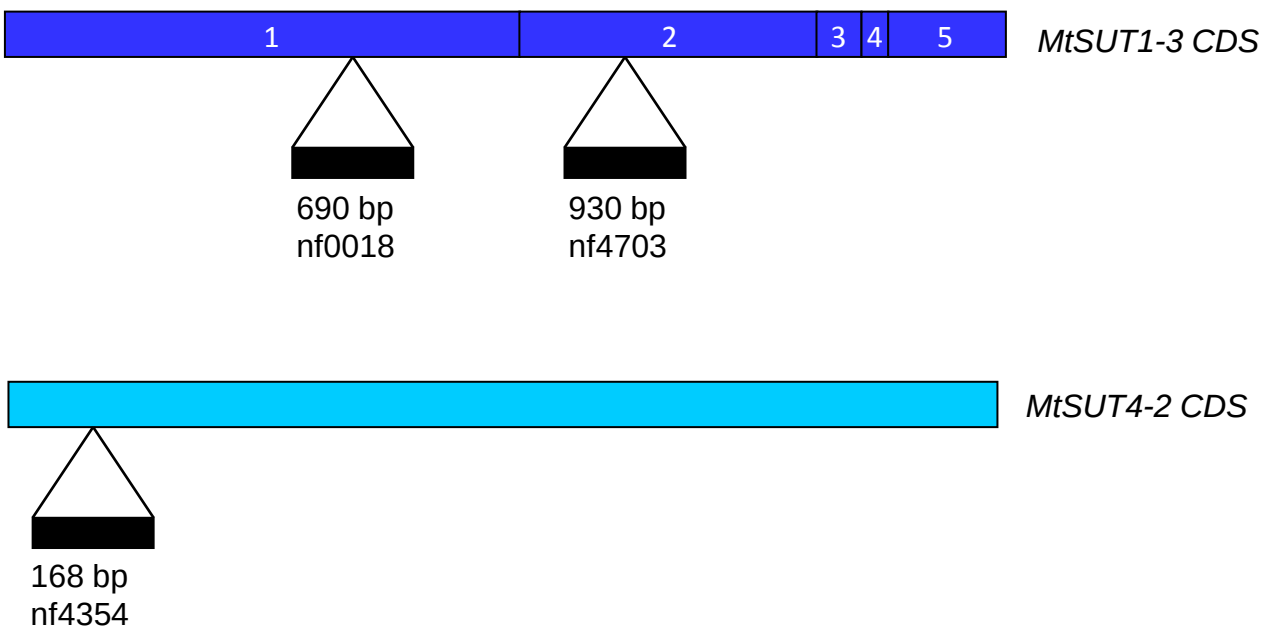


Fig VII.2. Photo and schematic representation of the Tnt1 insertion lines in the *MtSUT1-3* and *MtSUT4-2* CDS. Note that photograph for the nf0018 line is not available.

Publications

List of publications and presentations

Publication in peer reviewed journals

1. Couturier J., Doidy J., Wipf D., Blaudez D., Chalot M. (2010) Glutamine, arginine and the amino acid transporter Pt-CAT11 play important roles during senescence in poplar. Annals of Botany 105(7): 1159-1169.
2. Doidy J., Grace E., Kühn C., Simon-Plas F., Casieri L., Wipf D. (2012). Sugar transporters in plants and in their interactions with fungi. Trends in Plant Science 17(7): 413-422
3. Doidy J., van Tuinen D., Corneillat M., Alcaraz G., Wipf D. (2012) The *Medicago truncatula* sucrose transporter family. Characterization and implication of key members in carbon partitioning towards arbuscular mycorrhizal fungi. Molecular plant doi:10.1093/mp/sss079

Book chapter

1. Gianinazzi-Pearson V., van Tuinen D., Wipf D., Dumas-Gaudot E., Recorbet G., Liu Y., Doidy J., Redecker D., Ferrol N. (2012). Exploring the Genome of Glomeromycotan Fungi. Mycota

Oral communications

1. Doidy J., Schüssler A., van Tuinen D. & Wipf D.* (2009) Importance of fungal transporters in successful plant-fungus collaboration. 6th International Conference on Mycorrhizae, Belo Horizonte (Brazil) 9-14 August 2009.
2. Doidy J.*, van Tuinen D., Schüssler A. & Wipf D. (2010) Sugar transport in abuscular mycorrhiza. First PME-IGZ joint meeting on mycorrhizas, Dijon (France) 18 May 2010.
3. Doidy J.*, van Tuinen D., Schüssler A. & Wipf D. (2010) Transport de sucres au sein de la mycorhize à arbuscules. JFM2, 2nd Journées Francophones Mycorhizes, Brussels (Belgium) 15-17 September 2010.
4. Doidy J.*, van Tuinen D., Schüssler A. & Wipf D. (2011) The *Medicago truncatula* sucrose transporter family: From plant leaves to fungal symbiont. 3ème Journées des doctorants INRA SPE (Santé Plante Environnement), Dijon (France) 8-10 June 2011.

Poster presentation

1. Doidy J.*, van Tuinen D. Schüssler A., & Wipf D. (2009) Sugar transport in arbuscular mycorrhiza: Transfer of knowledge between two glomeromycotan models. XVéme Forum des Jeunes Chercheurs ED E2S Dijon (France) 25,26 June 2009.

2. Doidy J.*, D. van Tuinen, A. Schüssler & Wipf D. (2009) Transport de sucres au sein de la mycorhize à arbuscules : Transfert de connaissances entre deux modèles symbiotiques. 1ère Journée des doctorants INRA SPE (Santé Plante Environnement), Rennes (France) 1,2 September 2009.
3. Doidy J.*, Mercy L.*, & Wipf D*. (2009) Programmes cellulaires des interactions durables entre symbiotes mycorhiziens, et exploitations biotechnologiques. Exposition des champignons du sol à l'assiette, Dijon (France) 10,11 October 2009.
4. Doidy J.*, van Tuinen D. Schüssler A., & Wipf D. (2010) Sugar transport in abuscular mycorrhiza IMC9, 9th International mycorrhiza congress, Edinburgh, (UK) 1-6 August 2010.

* presenting author

Annals of Botany Page 1 of 11
doi:10.1093/aob/mcq047, available online at www.aob.oxfordjournals.org

ANNALS OF BOTANY
Founded 1887

Glutamine, arginine and the amino acid transporter Pt-CAT11 play important roles during senescence in poplar

Jérémy Couturier[1,*], Joan Doidy[2], Frédéric Guinet[1], Daniel Wipf[2], Damien Blaudez[1] and Michel Chalot[1]

[1]*UMR INRA/UHP 1136 'Interactions Arbre-Microorganismes', Faculté des Sciences et Techniques, Nancy-Université, BP 70239, F-54506 Vandoeuvre-les-Nancy Cedex, France and* [2]*UMR INRA 1088/CNRS 5184/Université Bourgogne, Plante-Microbe-Environnement, INRA-CMSE, BP 86510, 21065 Dijon Cedex, France*
** For correspondence. E-mail Jeremy.Couturier@scbiol.uhp-nancy.fr*

Received: 9 October 2009 Returned for revision: 22 December 2009 Accepted: 22 January 2010

• *Background and Aims* Nitrogen (N) availability in the forest soil is extremely low and N economy has a special importance in woody plants that are able to cope with seasonal periods of growth and development over many years. Here we report on the analysis of amino acid pools and expression of key genes in the perennial species *Populus trichocarpa* during autumn senescence.
• *Methods* Amino acid pools were measured throughout senescence. Expression analysis of arginine synthesis genes and cationic amino acid transporter (CAT) genes during senescence was performed. Heterologous expression in yeast mutants was performed to study Pt-CAT11 function in detail.
• *Key Results* Analysis of amino acid pools showed an increase of glutamine in leaves and an accumulation of arginine in stems during senescence. Expression of arginine biosynthesis genes suggests that arginine was preferentially synthesized from glutamine in perennial tissues. *Pt-CAT11* expression increased in senescing leaves and functional characterization demonstrated that Pt-CAT11 transports glutamine.
• *Conclusions* The present study established a relationship between glutamine synthesized in leaves and arginine synthesized in stems during senescence, arginine being accumulated as an N storage compound in perennial tissues such as stems. In this context, Pt-CAT11 may have a key role in N remobilization during senescence in poplar, by facilitating glutamine loading into phloem vessels.

Key words: Nitrogen metabolism, senescence, glutamine, arginine, cationic amino acid transporters, storage protein, *Populus trichocarpa*.

INTRODUCTION

Seasonal nitrogen (N) cycling is an adaptation of plants to winter cold seasonal climates in which nutrients (mostly N) are often considered to be the major growth-limiting factor (Cooke and Weih, 2005). Nitrogen translocation from senescing leaves to over-winter storage sites is a common feature of temperate deciduous trees (Ryan and Bormann, 1982). Poplar is extremely efficient at N conservation since $>80\,\%$ of the whole-tree nitrogen content is conserved during dormancy (Pregitzer *et al.*, 1990). During autumnal leaf senescence, there is a functional shift in leaf metabolism from resource assimilation to resource remobilization and export. N-rich amino acids and other mobile nutrients are transported via the phloem from senescing leaves to perennial tissues where they are used to synthesize proteins (Sauter *et al.*, 1989; Hörtensteiner and Feller, 2002). Proteins represent the major fraction of the stored N, and vegetative storage proteins (VSPs) represent the major form of reduced N storage in vegetative tissues of both annual and perennial plants (Staswick, 1994; Stepien *et al.*, 1994). The bark storage protein (BSP) family comprises the major VSPs in *Populus*. During autumn, BSPs accumulate in the bark parenchyma and xylem cells of the main stem, branches and roots of the tree (Sauter *et al.*, 1989).

Amino acids are the currency of N exchange between source and sink tissues in plants (Bush, 1999). Glutamine is the predominant translocated form for organic N in poplar (Dickson, 1979; Sauter and van Cleve, 1992) and is preferentially transported through the stem to developing leaves via a xylem to phloem transfer facilitated by ray cells (Dickson *et al.*, 1985). Nevertheless, the amino acid composition of xylem sap exhibits seasonal variations. During the wintering phase, arginine is the major amino acid in bark and xylem of poplar, whereas at the time of budding and growing, glutamine and glutamate become dominant (Sagisaka, 1974). These variations in amino acid pools could be associated with variations in expression of amino acid transporter genes not only in storage tissues but also in sieve elements which allow amino acid distribution in the whole plant.

In plants, the majority of genes encoding putative amino acid transporters can be classified into two major groups: the amino acid transporter family (ATF) and the amino acid polyamine choline (APC) superfamily (Wipf *et al.*, 2002). Most of the amino acid transporters from plants that have been characterized functionally belong to the ATF superfamily, with the amino acid permease (AAP) family being the best studied subfamily (Boorer *et al.*, 1995; Fischer *et al.*, 1995, 2002; Boorer and Fischer, 1997; Okumoto *et al.*, 2002, 2004). In plants, APC amino acid transporters are poorly understood and have

been described only in *Arabidopsis thaliana*. APC transporters of the L-type amino acid transporter (LAT) sub-family (five members) have not been characterized and only a few members of the cationic amino acid transporters (CAT) family have been studied (Frommer *et al.*, 1995; Su *et al.*, 2004; Hammes *et al.*, 2006). They contain between 11 and 14 putative transmembrane (TM) domains and they are high-affinity basic amino acid transporters. They are located in the plasma membrane or in the vacuolar membrane (Frommer *et al.*, 1995; Su *et al.*, 2004; Hammes *et al.*, 2006). It has been demonstrated that *At-CAT1* is expressed in leaves, flowers and developing siliques, and transcripts were specifically localized in major veins of leaves and roots (Frommer *et al.*, 1995). It has been suggested that At-CAT1 might play multiple roles in phloem physiology, from phloem loading to providing amino acids for developing embryos. Moreover, At-CAT1 is likely to be a proton-driven high-affinity transporter that transports mainly cationic amino acids (Frommer *et al.*, 1995). At-CAT2 is probably localized to the tonoplast and may be the long-sought vacuolar amino acid transporter (Su *et al.*, 2004). At-CAT5 functions as a high-affinity, basic amino acid transporter at the plasma membrane. Expression profiles suggest that At-CAT5 may function in the re-uptake of leaked amino acids at the leaf margin (Su *et al.*, 2004). *At-CAT8* is expressed in young and rapidly dividing tissues such as young leaves and root apical meristem. At-CAT8 is also localized to the plasma membrane (Su *et al.*, 2004). At-CAT6 has a high affinity for cationic amino acids and is also likely to be energized by protons (Hammes *et al.*, 2006). At-CAT6 transports large, neutral and cationic amino acids in preference to other amino acids and plays a role in supplying amino acids to sink tissues of plants and nematode-induced feeding structures.

As exemplified above, N storage and cycling have traditionally been investigated at the molecular physiology and ecophysiology scales. Taking advantage of the annotated *Populus trichocarpa* (Nisqually 1) genome (Tuskan *et al.*, 2006), we present here the analysis of amino acid pools in different organs of poplar during autumn and winter, combined with the expression analysis of genes encoding enzymes of arginine biosynthesis and genes encoding CAT members. Finally, we also characterize Pt-CAT11 by heterologous expression in yeast and show that it preferentially transports glutamine.

MATERIALS AND METHODS

Plant material

Leaves from 1- and 2-year-old stems were sampled from free-growing *Populus trichocarpa* trees at the University of Nancy campus. About 20 leaves and four stems were sampled at 14 h for every time point, frozen in liquid nitrogen and stored at −80 °C. Leaves were sampled on 27 October, 23 November, 5 December and 12 December. This latter point corresponds to a period just before leaf fall. Stems were also sampled on 8 January and 2 February. These two dates correspond to the wintering phase.

Semi-quantitative RT–PCR

Total RNA extraction was performed with the RNeasy Plant Mini kit (Qiagen, Darmstadt, Germany) from approx. 100 mg of frozen tissues of poplar. To remove contaminating genomic DNA, the samples were treated with DNase I (Qiagen), as recommended by the manufacturer. To obtain cDNA, 500 ng of total RNA were annealed to oligo(dT) primers (Promega, Madison, WI, USA) and reverse transcribed using reverse transcriptase (Eppendorf, Hamburg, Germany) at 42 °C for 90 min. Each reaction was set up in three biological replicates. For each *Pt-CAT*, the PCR program was as follows: 94 °C for 3 min and 35 cycles of 94 °C for 30 s, 58 °C for 45 s and 72 °C for 1 min. The whole set of *Pt-CAT* genes (12 genes) was tested by reverse transcription–PCR (RT–PCR) in every experiment performed, but only *Pt-CAT* genes detected and well expressed are retained in figures for greater clarity. To study the expression of genes involved in the pathway of arginine biosynthesis, cDNA corresponding to argininosuccinate lyase (AL), argininosuccinate synthase (AS), ornithine transcarbamoylase (OTC) and carbamoyl-phosphate synthase (CPS) were also amplified using the same PCR program as described above. The numbers of genes coding for *AL*, *AS*, *OTC* and *CPS* were one, two, one and two, respectively. When two genes were coding for an enzyme, primers were designed for the gene with the highest expressed sequence tag (EST) numbers in poplar databases. Control PCRs were sequenced to ensure that only one gene was amplified.

A cDNA fragment corresponding to the constitutively expressed ubiquitin gene was amplified simultaneously (28 cycles) and used as a control. Cysteine protease (CP) was amplified (28 cycles) and used as control of the senescence state of leaves. The sequences of the gene-specific oligonucleotides, designed in the non-conserved regions of the genes and used for RT–PCR, are listed in Table 1. The ethidium bromide-stained agarose gels were imaged on a Bio-Rad GelDoc 2000 transilluminator, and quantitative data were determined using Quantity One software (Bio-Rad, Hercules, CA, USA). Signal intensities were normalized to the constitutively expressed poplar ubiquitin gene.

Amino acid extraction and analysis

Amino acids were extracted twice from 10–20 mg of freeze-dried plant tissues with 300 μL of 70 % (v/v) cold ethanol. The samples were dried under N_2 using a Reacti-Therm Heating Module (Pierce, Rockford, IL, USA) and resuspended in 400 μL of 0·1 N HCl. Extracts and standards were loaded onto a Dowex 50WX-8 cation ion exchange column (Sigma-Aldrich, St Louis, MO, USA). After two successive washing steps with sterile water, amino acids were eluted with 4·5 N ammonia. Aliquots of purified samples were then transferred to microvials, dried in a Reacti-Therm Heating Module (Pierce) and derivatized according to Javelle *et al.* (2003). Gas chromatography and mass spectrometry (GC-MS) analysis was performed as described previously (Javelle *et al.*, 2003).

TABLE 1. *Primers used for RT–PCR analysis*

Name	Sequence
CAT1 f	ACCATTTATGCCATATGATGTCCG
CAT1 r	GGTTCAACTTGTGATGACACAAC
CAT2 f	TTCCTCTGCATTCGCTGCATAT
CAT2 r	TAGTGACATCTGGGCTACCTGTA
CAT3 f	GTCCTCTTCGTTTTACAACG
CAT3 r	TTTCTCCAGAGCTCCGATAA
CAT4 f	TTTGCATAGGAGAAGGTGCAGCAT
CAT4 r	GACAAAGCAACGCCTATACCT
CAT5 f	ACAGCACTGAATACTGCTGTA
CAT5 r	GCTAGCTTCAAGAGGTTTGTT
CAT6 f	TACATGTGTGTTATCGGACGGTC
CAT6 r	TTACACTTTGAAAGAATTAATATGGTCCTCGC
CAT7 f	CTGTCTTTGCCATAGCACAAAG
CAT7 r	CTGGCCTTTAGTGTGGTCATG
CAT8 f	GCCTCTATTGCTACTGCTTTTATC
CAT8 r	TCCAAGTGATCCAACCATTAAGCT
CAT9 f	CAGCTTTCAATGAGCTTACTGCTT
CAT9 r	ACAAGACTTCCAATGATGCCT
CAT10 f	ACAGCTTCAATTGCACTCTTTACC
CAT10 r	TCATAGCAGCTGAATATCTAGC
CAT11 f	TCATCAAGAAGGTGGAGACCAAGA
CAT11 r	GGCAGCACAAACAAAAACAGAT
CAT12 f	TGATCATCAAGAAGAAGGGCTG
CAT12 r	CACAACACCAACAAGAACAGCA
Ubq f	GCACCTCTGGCAGACTACAA
Ubq r	TAACAGCCGCTCCAAACAGT
CP f	AGTCACTGAGAAAGGCTGTGG
CP r	CCAAATGGATTGTTCTTGCTC
AS f	AGCGGAAATACTTATTGGGGACGT
AS r	ACAAGTTCCTGTCCCTGCTATA
AL f	GTTCCTGGTTACACACATTTGCAA
AL r	ACAGGTTCCTTGTCTTCCTGCAAA
OTC f	ATGGCCTGAACTATAACCATCC
OTC r	CTCGATCTTGCTGATTCCAGC
CPS f	CGGTGTCCTAACCACAGAAGAATT
CPS r	CCTCAGGATGGTATTGTAGAGA

Statistical analysis

The effects of the senescence state on tissue amino acid concentrations, soluble protein concentrations and gene expression were tested with a one-way analysis of variance (ANOVA) using the SYSTAT statistical package (SYSTAT Inc., Evanston, IL, USA). The Tukey test was used for all pairwise comparisons of the mean responses to the different treatment groups.

Protein extraction and analysis

Small pieces (about 50–100 mg) of stems were ground with a mortar and pestle cooled in liquid nitrogen in 2 mL of 50 mM Tris–HCl pH 8·0, 1 mM PMSF (phenylmethylsulfonyl fluoride) and 50 mM mercaptoethanol. Samples were then mixed by vortexing, and held at 4 °C for 30 min. Samples were centrifuged at 13 000 rpm for 15 min and the supernatants collected. Proteins were precipitated with acetone at −20 °C for 2 h. Aliquots of 100 μL were centrifuged at 13 000 rpm for 15 min and proteins were resuspended with 50 μL of 0·2 % SDS. Protein concentration was determined by the bicinchoninic acid (BCA) colorimetric assay kit (Interchim, Montluçon, France; Brown *et al.*, 1989). The BCA procedure followed the manufacturer's recommendations, with bovine serum albumin as a standard and absorbance measured at 562 nm. Protein concentrations were determined for duplicate sub-samples for each replicate.

DNA constructs

The predicted coding sequence corresponding to *Pt-CAT11* (1767 bp) was amplified by PCR using cDNA generated for RT–PCR studies (see above) and the following primers: Pt-CAT11fow (5′-CCCGAATTCATGAGGAGGAGGAGGG GATGT-3′) and Pt-CAT11rev (5′-CCCCTCGAGTCATGAA CCATTCCGGGAAGG-3). The amplification product was cloned into the *Eco*RI/*Xho*I sites of the yeast expression vector pYES2 and sequenced to confirm that no modifications occurred.

Yeast transformation

The yeast strains 22Δ8AA (MATα, *ura3-1, gap1-1, put4-1, uga4-1, can1::HisG, lyp1/alp1::HisG, hip1::HisG, dip5::HisG, ura3-1*) (Fischer *et al.*, 2002) and JA248 (MATα *ura3Δ gap1Δ gnp1Δ agp1Δ*) (Velasco *et al.*, 2004) were transformed with pYES2 harbouring the cDNA sequence of *Pt-CAT11*. Yeast transformants were selected on synthetic dextrose minimal medium. Yeast strain 22Δ8AA complementation tests were performed on N-free medium supplemented with 20 g L^{-1} Gal and either 1, 3 or 6 mM L-proline, L-citrulline, L-aspartate or L-glutamate as sole N source, whereas yeast strain JA248 complementation tests were performed on N-free medium supplemented with 20 g L^{-1} Gal and either 0·5, 1, 2 or 5 mM L-glutamine as sole N source.

Transport measurements

For *Saccharomyces cerevisiae* uptake studies, yeast cells were grown to logarithmic phase. Cells were harvested at an OD_{600} of 0·5, washed twice in water, and resuspended in buffer A (0·6 M sorbitol, 50 mM potassium phosphate, at the desired pH) to a final OD_{600} of 5. Prior to the uptake measurements, the cells (100 μL) were supplemented with 5 μL of 1 M galactose and incubated for 5 min at 30 °C. To start the reaction, 100 μL of this cell suspension was added to 100 μL of the same buffer containing at least 18·5 kBq of [^{3}H]glutamine, and unlabelled glutamine to the concentrations used in the experiments. Sample aliquots of 50 μL were removed after 30, 60 and 120 s, transferred to 4 mL of ice-cold buffer A, filtered on glass fibre filters and washed twice with 4 mL of buffer A. The uptake of tritium was determined by liquid scintillation spectrometry.

Phylogenetic analyses

CAT sequences were retrieved by text and Blast searches from the *P. trichocarpa* whole genome database (version 1·1) at the US Department of Energy Joint Genome Institute (JGI) (http://genome.jgi-psf.org/Poptr1_1/Poptr1_1.home.html). The curated poplar amino acid sequences were used to search against five other genomes from photosynthetic organisms using BLASTP or TBLASTN. The genomes are available at the following websites, for *A. thaliana*

(http://www.arabidopsis.org/), *Oryza sativa* (http://rice.plantbiology.msu.edu/), *Vitis vinifera* (http://www.genoscope.cns.fr/spip/Vitis-vinifera-whole-genome.html) and *Sorghum bicolor* (http://genome.jgi-psf.org/Sorbi1/Sorbi1.home.html). Amino acid sequences were aligned by CLUSTALW and imported into the Molecular Evolutionary Genetics Analysis (MEGA) package version 4·1 (Tamura *et al.*, 2007). Phylogenetic analyses were conducted using the neighbor–joining (NJ) method implemented in MEGA, with the pairwise deletion option for handling alignment gaps, and with the Poisson correction model for distance computation. Bootstrap tests were conducted using 1000 replicates. Branch lengths are proportional to phylogenetic distances. All protein sequences and corresponding accession numbers can be found in the databases mentioned above and as Supplementary Data (available online).

RESULTS

Glutamine–arginine relationships during senescence

Amino acid concentrations were investigated in the lamina (Fig. 1A), central vein (Fig. 1B) and petiole (Fig. 1C) of poplar leaves. In the lamina, the total amino acid concentration did not change much during senescence, varying between 5 and 8 nmol mg^{-1} d. wt. Amino acid profiling indicated that the glutamate (and aspartate; not shown) concentration decreased whereas that of glutamine (and asparagine; not shown) increased during leaf senescence (Fig. 1A). In the central vein and petiole, the total amino acid concentration increased by approx. 6-fold from 27 October to 5 December, thereafter decreasing at the latest sampling date (Fig. 1B, C). Glutamine was the predominant amino acid before leaf fall, representing 23 and 34 % of the total amino acid pool in the central vein and petiole, respectively, followed by leucine, isoleucine and valine.

Amino acid pools were investigated in 1-year-old (Fig. 2A) and 2-year-old (Fig. 2B) stems. In October, total amino acid concentrations were <15 nmol mg^{-1} d. wt and arginine was almost undetectable in 2-year-old stems. During autumnal senescence, total amino acid pools increased by 20- and 37-fold in 1- and 2-year-old stems, respectively, when measured at their maximal level. Noticeably, arginine rapidly became the predominant amino acid accumulated in stems, accounting for 91 and 92 % in 1- and 2-year-old stems, respectively, on 8 January. Arginine accumulation was slightly delayed in 2-year-old stems, peaking on 5 December in 1-year-old stems and on 12 December in 2-year-old stems (Fig. 2).

The amount of total soluble protein was investigated in 1- and 2-year-old stems (Fig. 3). During autumnal senescence, soluble protein content of 2-year-old stems increased by >3-fold between 27 October and 8 January. In contrast, there were no statistically significant changes in soluble protein content of 1-year-old stems during senescence (Fig. 3A). Soluble proteins from 2-year-old stems were analysed by SDS–PAGE (Fig. 3B). Analysis revealed the presence of two major proteins with relative molecular masses of between 30 and 37 kDa. Interestingly, the content of these two proteins increased during autumn and winter. These

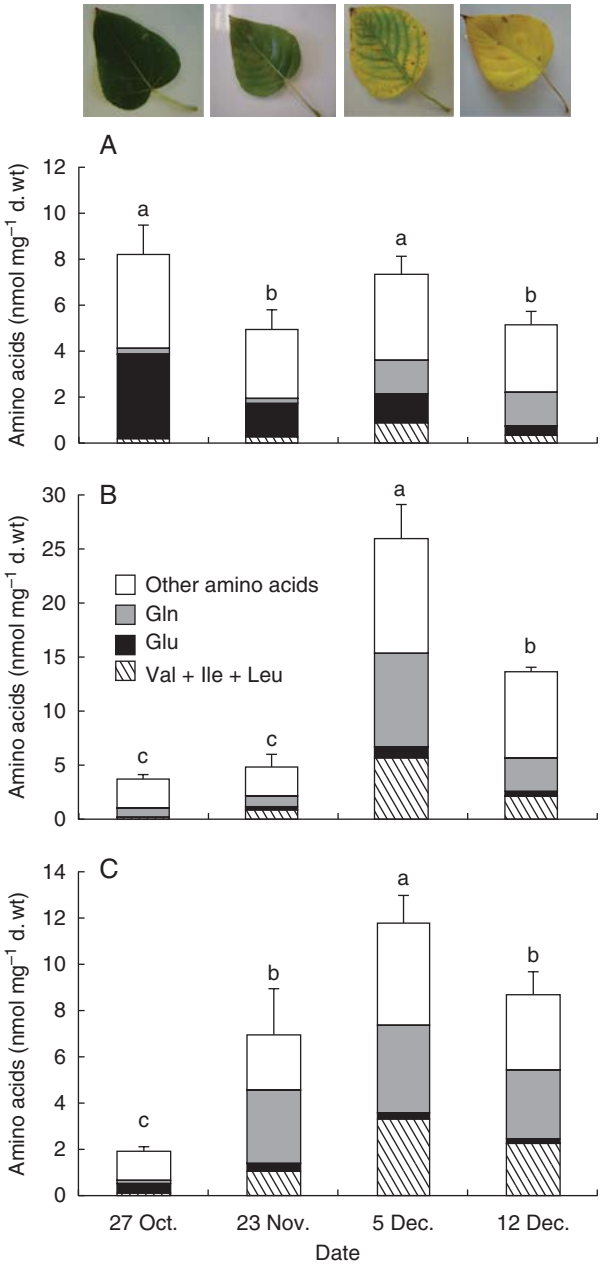


FIG. 1. Quantification of amino acids by gas chromatography–mass spectrometry in (A) laminae, (B) central veins (C) and petioles of poplar during senescence. Values are expressed as the mean ± s.e. of three replicate experiments. For a given tissue, amino acid concentration values with the same letter are not significantly different, according to ANOVA at $P < 0.05$.

accumulating proteins correspond to the well-characterized BSPs of poplar.

The metabolic route to arginine synthesis in plants involves two distinct processes: synthesis of ornithine from glutamate and synthesis of arginine from the ornithine intermediate (Slocum, 2005). Considering the striking accumulation of arginine during senescence, some of the genes involved in its biosynthesis were investigated: *CPS*, *OTC*, *AS* and *AL* genes. In order to investigate their expression during senescence in poplar, total RNAs were extracted from laminae, petioles

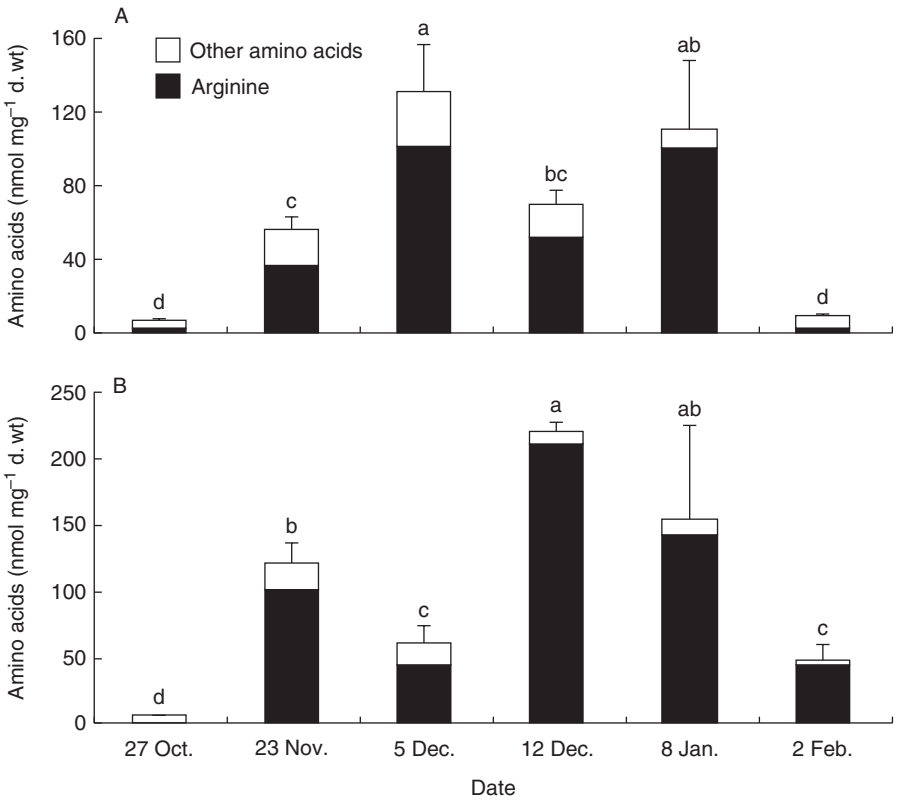


FIG. 2. Quantification of amino acids by gas chromatography–mass spectrometry in (A) 1-year-old and (B) 2-year-old stems of poplar during senescence. Values are expressed as the mean ± s.e. of three replicate experiments. For a given tissue, amino acid concentration values with the same letter are not significantly different, according to ANOVA at $P < 0.05$.

and 2-year-old stems sampled at different times during autumn and winter. *AL* transcripts were undetectable under conditions used in these experiments (not shown). In laminae, *AS*, *CPS* and *OTC* transcripts decreased at the end of the senescence period (Fig. 4A). The senescence state of leaves was confirmed by the parallel amplification of a *CP* transcript (Bhalerao *et al.*, 2003; Andersson *et al.*, 2004), which was highly expressed on 5 December. In petioles, *CPS* and *OTC* transcripts were less abundant than *AS* transcripts during autumnal senescence (Fig. 4B). Moreover, *AS* and *OTC* were maximal on 23 November. In 2-year-old stems, as observed in laminae and petioles, *OTC* was weakly expressed and, as observed for *AS*, its expression decreased after 23 November (Fig. 4C). In contrast, *CPS* expression increased by 11-fold during leaf senescence and was maximal on 5 December (Fig. 4C).

Pt-CAT11 is a glutamine transporter upregulated during senescence

The JGI *P. trichocarpa* gene search mode revealed the existence of 12 CAT gene models. As described for the *Arabidopsis* CAT family, plant CAT members can be phylogenetically grouped into four small sub-groups (Fig. 5). Sub-group 1 contains the members CAT1, CAT5, CAT8, CAT11 and CAT12, whereas sub-group 2 includes the members CAT6, CAT7 and CAT10. Interestingly sub-group 3 only includes CAT9 whereas sub-group 4 contains CAT2, CAT3 and CAT4. Analysis of the assembled genome revealed relatively recent whole-genome duplication shared among all modern taxa in Salicaceae. A second, older duplication appears to be shared with the *Arabidopsis* lineage (Tuskan *et al.*, 2006). These duplicated genes originated through very recent small-scale gene duplications and one relatively recent large-scale gene duplication event (Sterck *et al.*, 2005). A detailed analysis of duplication events for the *Pt-CAT* members revealed that poplar *CAT6*, *CAT7* and *CAT9* derived from a common ancestor through an ancient and a recent duplication event, and that poplar *CAT2* and *CAT3* derived from a common ancestor through a recent duplication event. The same analysis also revealed that poplar *CAT11* and *CAT12* derived from a common ancestor through a recent duplication event.

In *Arabidopsis*, members of the CAT family have been characterized as high affinity basic amino acid transporters. For instance, At-CAT1 and At-CAT5 mediate high-affinity transport of arginine, lysine and histidine (Frommer *et al.*, 1995; Su *et al.*, 2004). To complement previous expression studies, we extracted GENEVESTIGATOR (Zimmermann *et al.*, 2004; www.genevestigator.ethz.ch) data for the *Arabidopsis* CAT gene family, which indicated that *At-CAT2* and *At-CAT5* were mostly upregulated during leaf senescence.

To investigate the potential role of Pt-CAT members during senescence, transcript levels were estimated in laminae, petioles and 2-year-old stems sampled at different times during autumn and winter. *Pt-CAT3* and *Pt-CAT4* transcripts remained high throughout the season and were not affected

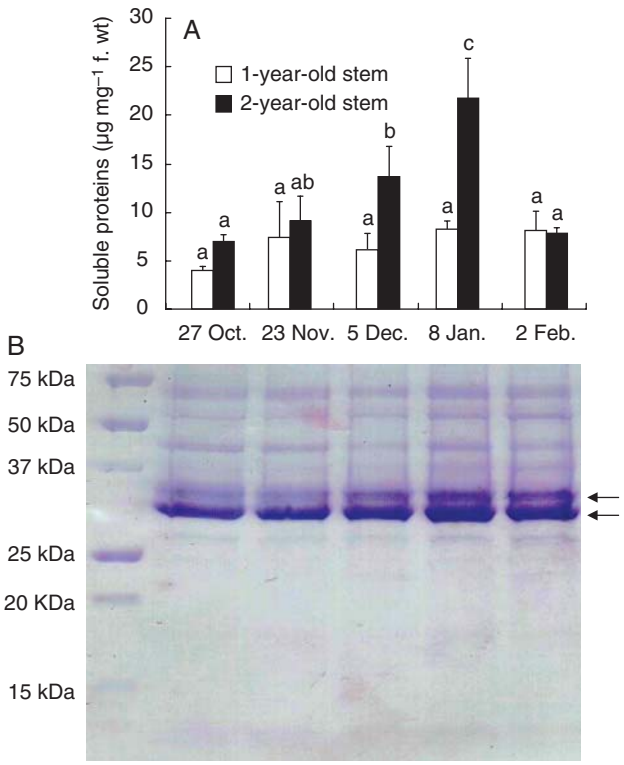


FIG. 3. Quantification of soluble proteins in 1- and 2-year-old stems of poplar during autumnal senescence and winter. Protein concentration (A) was determined for duplicate sub-samples from each replicate. Values are expressed as the mean ± s.e. of three replicate experiments. For a given tissue, protein concentration values with the same letter are not significantly different, according to ANOVA at $P < 0{\cdot}05$. Soluble proteins from 2-year-old (B) stems of poplar were separated by SDS–PAGE. The presence of two major proteins with a relative molecular mass of between 30 and 37 kDa is indicated by arrows.

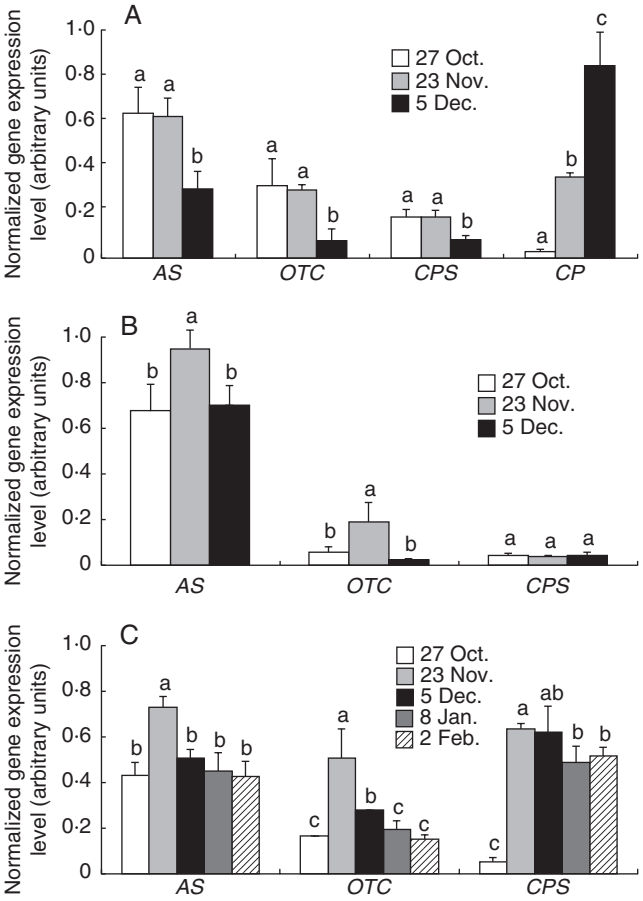


FIG. 4. Expression of arginine biosynthetic genes in various poplar tissues during or after senescence. (A) Expression of *AS*, *OTC*, *CPS* and *CP* genes in laminae. Cysteine protease (CP) was used as a marker of the senescence state (Bhalerao *et al.*, 2003). (B) Expression of *AS*, *OTC* and *CPS* genes in petioles. (C) Expression of *AS*, *OTC* and *CPS* in 2-year-old stems. Total RNAs were extracted at different time periods during autumn and winter and 300 ng of total RNAs were reverse transcribed into cDNA. The ubiquitin (Ubq) gene was amplified and used as internal control. Analyses were performed by RT–PCR in triplicate. Signal intensities were normalized to the constitutively expressed poplar ubiquitin gene. Values are expressed as the mean ± s.e. of three replicate experiments. For a given gene, values with the same letter are not significantly different, according to ANOVA at $P < 0{\cdot}05$.

by leaf senescence (Fig. 6A). Similarly, *Pt-CAT8* was weakly expressed and poorly affected by senescence in these experiments (Fig. 6A). *Pt-CAT1*, *Pt-CAT2* and *Pt-CAT12* were expressed in leaves in October and November but not expressed at the end of the senescing period (Fig. 6A). Conversely, *Pt-CAT10* and *Pt-CAT11* showed increased expression levels in senescing leaves in December compared with leaves collected in October (Fig. 6A). These expression patterns could be related to amino acid concentration and more particularly to glutamine. Indeed, the amino acid concentration of laminae displayed the same variations during autumn (Fig. 2A), and regression analysis between glutamine concentration and *Pt-CAT11* expresssion in laminae revealed a good correlation ($R^2 = 0{\cdot}994$). All other poplar *CAT* members were also analysed but were not detected in these samples.

As observed in laminae, *Pt-CAT3* and *Pt-CAT4* were strongly expressed in petioles but transcript levels of these genes were poorly affected by leaf senescence (Fig. 6B). *Pt-CAT1*, *Pt-CAT9* and *Pt-CAT12* showed a similar expression pattern with more transcripts detected on 23 November (Fig. 6B). *Pt-CAT2* showed increased expression levels in petioles in December compared with petioles collected in October (Fig. 6B).

Interestingly, although not expressed in laminae and only poorly expressed in petioles, *Pt-CAT9* was highly expressed in 2-year-old stems, just before leaf fall. In contrast, *Pt-CAT2* was very weakly expressed in 2-year-old stems in autumn and in winter (Fig. 6C). *Pt-CAT12* presented the same expression pattern in 2-year-old stems, in laminae and in petioles (Fig. 6C). As observed in laminae and petioles, *Pt-CAT3* transcript levels were high and barely affected by senescence in 2-year-old stems (Fig. 6C). Interestingly, *Pt-CAT4* and *Pt-CAT9* transcript levels were high during autumn senescence and decreased in 2-year-old stems after leaf fall (Fig. 6C). More surprising, *Pt-CAT11* expression was subjected to quite high variations (Fig. 6C) which could be related to amino acid concentration. Indeed, the amino acid concentration of 2-year-old stems displayed the same variations as *Pt-CAT11* expression during autumn and winter (Fig. 2B). Regression analysis between total amino acid

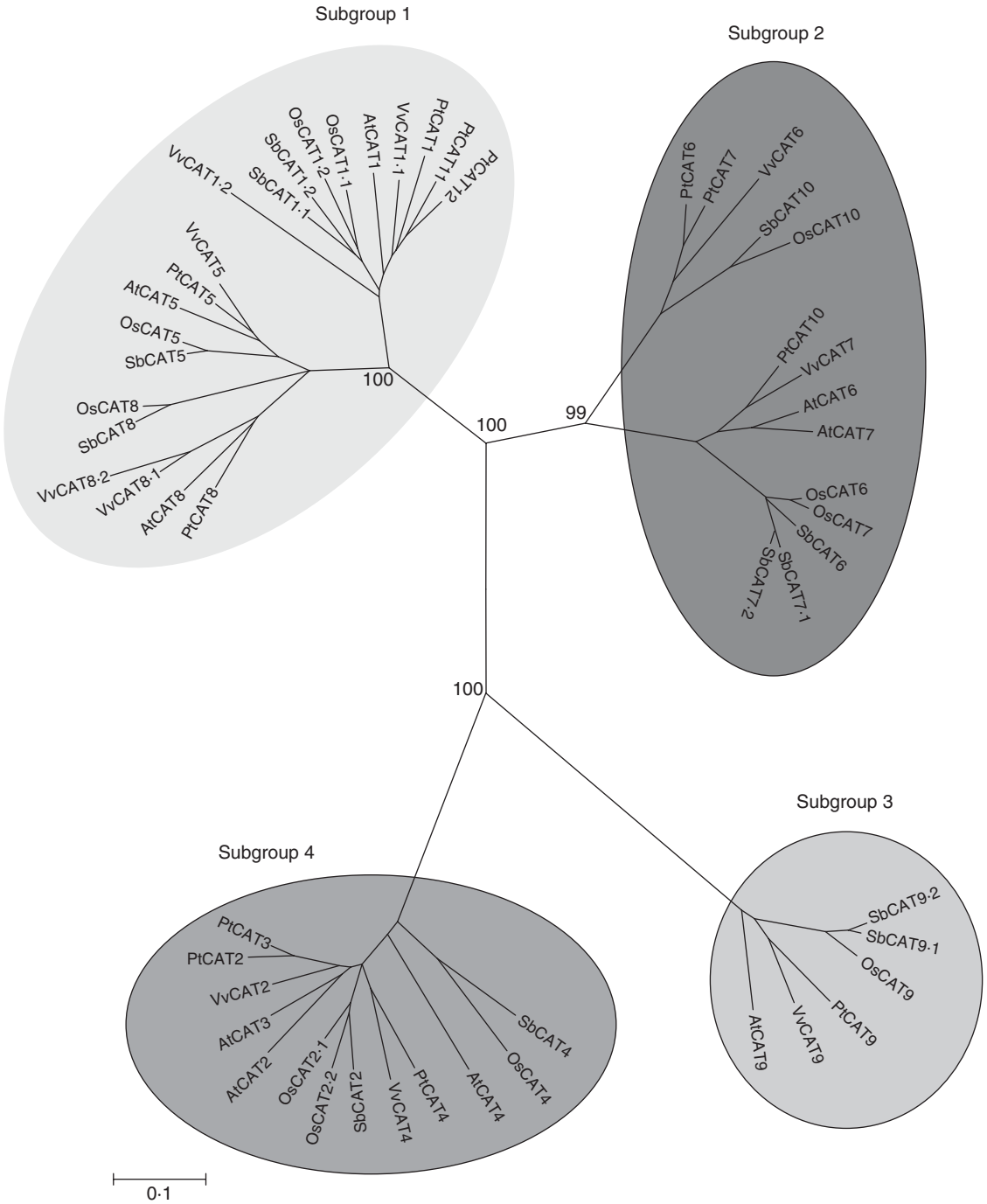


FIG. 5. An unrooted, neighbor–joining (NJ)-based tree of the cationic amino acid transporter (CAT) family. The analysis was performed as described in the Materials and Methods. Branch lengths (drawn in the horizontal dimension only) are proportional to phylogenetic distances. Corresponding gene loci or protein IDs are given in the Supplementary Data (available online).

concentration and *Pt-CAT11* expresssion did not reveal a correlation between these two parameters ($R^2 = 0{\cdot}295$). Regression analysis between glutamine concentration and *Pt-CAT11* expression, however, reveals a better correlation ($R^2 = 0{\cdot}730$). As observed in laminae, *Pt-CAT11* expression in stems seems to be related to glutamine concentration. All other poplar *CAT* genes were also analysed but were not detected in these experiments.

In order to determine the function of Pt-CAT11, yeast complementation experiments were performed with the yeast mutants 22Δ8AA and JA248. The 22Δ8AA strain is unable to use arginine, aspartate, citrulline, γ-aminobutyric acid (GABA), glutamate and proline efficiently as sole N sources (Fischer *et al.*, 2002) and the JA248 strain is unable to use glutamine efficiently as sole N source (Velasco *et al.*, 2004). As controls, strains 22Δ8AA and JA248 were transformed with

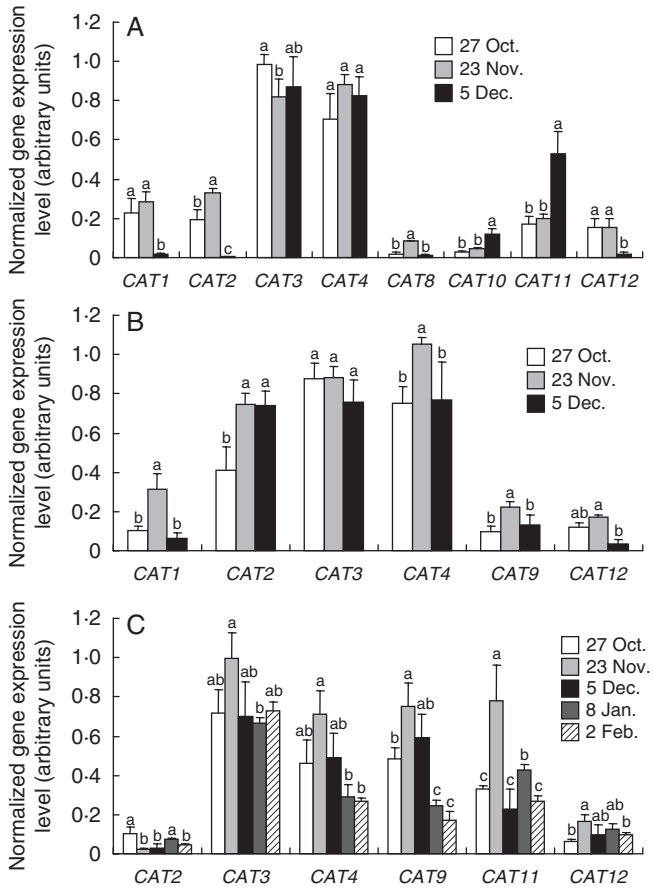


FIG. 6. Expression of *CAT* transporter genes in various poplar tissues during or after senescence. (A) Expression of poplar *CAT1*, *CAT2*, *CAT3*, *CAT4*, *CAT8*, *CAT10*, *CAT11* and *CAT12* genes in laminae. (B) Expression of poplar *CAT1*, *CAT2*, *CAT3*, *CAT4*, *CAT9* and *CAT12* genes in petioles. (C) Expression of poplar *CAT2*, *CAT3*, *CAT4*, *CAT6*, *CAT11* and *CAT12* genes in in 2-year-old stems. Total RNAs were extracted at different time periods during autumn and winter and 300 ng of total RNAs were reverse transcribed into cDNA. The ubiquitin (Ubq) gene was amplified and used as internal control. Analyses were performed by RT–PCR in triplicate. Signal intensities were normalized to the constitutively expressed poplar ubiquitin gene. Values are expressed as the mean ± s.e. of three replicate experiments. For a given gene, values with the same letter are not significantly different, according to ANOVA at $P < 0.05$.

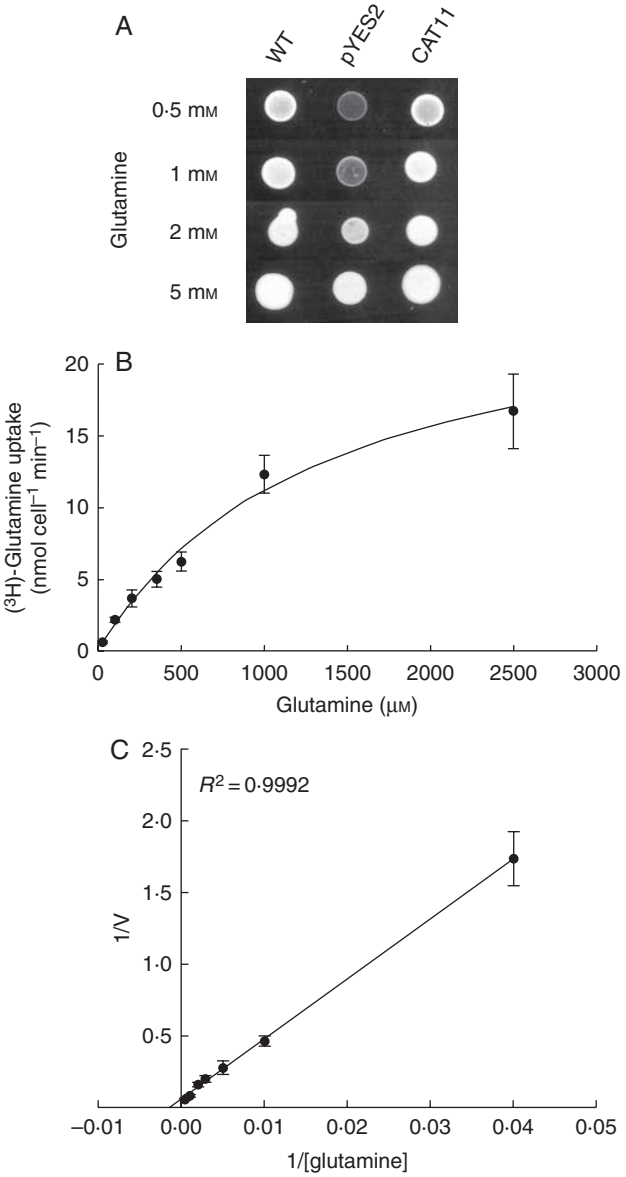


FIG. 7. Functional characterization of poplar CAT11 by heterologus expression in a yeast mutant strain. (A) Yeast strain JA248 was transformed with the yeast expression vector pYES2 or pYES2 harbouring the coding sequence of *Pt-CAT11*. Growth was assayed on N-free medium containing 20 g L^{-1} Gal and either 0·5, 1, 2 or 5 mM L-glutamine as sole N source. Pictures were taken after 2 d of growth at 30 °C and are representative of three replicates. (B) Concentration-dependent kinetics of [^{3}H]glutamine uptake by yeast strain JA248 expressing Pt-CAT11. The Michaelis–Menten constant for glutamine is 690 μM. Values are expressed as the mean ± s.e. of three replicate experiments. (C) Lineweaver–Burk representation of [^{3}H]glutamine uptake by yeast strain JA248 expressing Pt-CAT11. Values are expressed as the mean ± s.e. of three replicate experiments.

the expression vector pYES2. Transformation with the yeast expression vector pYES2 bearing the *Pt-CAT11* coding sequence under the control of the *GAL1* promoter conferred the ability of JA248 to grow in the presence of 0·5 mM glutamine (Fig. 7A). The transport of glutamine by Pt-CAT11 was further confirmed by uptake experiments, which demonstrated that Pt-CAT11-mediated [^{3}H]glutamine uptake was concentration dependent and showed saturable kinetics with an apparent K_m value of 690 μM (Fig. 7B, C). Transformation with the yeast expression vector pYES2 bearing the *Pt-CAT11* coding sequence under the control of the *GAL1* promoter conferred the ability of 22Δ8AA to grow when supplied 3 and 6 mM proline, GABA or citrulline as the sole N source but not when supplied aspartate or glutamate (not shown). Yeast transformed with Pt-CAT11 showed no growth on medium containing 1 mM arginine as sole N source (data not shown).

DISCUSSION

Glutamine is the key metabolite to transfer N from senescing leaf to perennial tissues

Nitrogen economy has a special importance in woody plants that are able to cope with seasonal periods of growth and development over many years. As N availability in the forest

soil is extremely low, efficient mechanisms are required for the assimilation, storage, mobilization and recycling of inorganic and organic forms of N. Seasonal N cycling is an adaptation of plants to winter cold seasonal climates in which nutrients (mostly N) are often considered to be the major growth-limiting factor (Cooke and Weih, 2005). In the N metabolism of conifers, the cyclic interconversion of arginine and the amides glutamine and asparagine plays a central role, and its regulation is critical to maintain the N economy of these long-living plants (Canovas *et al.*, 2007).

At the beginning of autumn, the major amino acids found in laminae were glutamate and glutamine. During senescence the glutamate concentration decreased whereas that of glutamine increased. The same variations were observed for aspartate and asparagine but to a smaller extent (Fig. 1A). It has been demonstrated that N content decreased in autumn leaves of aspen and about 80 % of total leaf N was withdrawn during autumn senescence (Keskitalo *et al.*, 2005). Amino-N pools may contribute only slightly to this decrease, while other N-containing compounds (chlorophyll for instance) may be of more importance in this process.

On the other hand, qualitative changes in amino acid concentration also revealed this N remobilization process. In senescing leaves, a large amount of ammonium is produced as a result of protein hydrolysis (Hörteinsteiner and Feller, 2002). Ammonium is assimilated into the glutamine amide group, and the specific expression of the glutamine synthetase gene *NtGLN1;3* was observed in senescing leaves of *Nicotiana tabacum* (Brugiere *et al.*, 2000). Moreover, it has been demonstrated recently that expression of several ammonium transporter genes in poplar (*PtAMT1;5*, *PtAMT1;6* and *PtAMT3;1*) increased with leaf maturation, suggesting that they are specifically recruited to ensure ammonium assimilation during the process of leaf senescence (Couturier *et al.*, 2007). Whereas glutamate decreased with ageing, the glutamine pool increased, suggesting that glutamine biosynthesis had exhausted the glutamate pool.

In the central vein and petiole, amino acid concentrations increased during senescence until 5 December, before leaf fall (Fig. 1B, C). Glutamine was the major amino acid found in the central vein and petiole, whereas glutamate represented <3 % and 6 %, respectively in these tissues. The increase in glutamine concentration in the central vein and petiole suggests an export from leaves to perennial organs during autumn (Fig. 1). It can be also noted that leucine, isoleucine and valine concentrations increased strongly in the central vein and petiole during senescence, which was not observed in the lamina. Interestingly, pools of leucine and isoleucine also increased with ageing in *Arabidopsis* leaves (Diaz *et al.*, 2005). It has been suggested that isoleucine and leucine biosynthesis exhausted the aspartate pool. The same processes could occur in poplar leaves during senescence.

During autumn leaf senescence, there is a functional shift in leaf metabolism from resource allocation to resource remobilization and export. Rubisco breakdown during autumn leaf senescence in poplar (Brendley and Pell, 1998) accounts for a notable proportion of the N exported from leaves (Titus and Kang, 1982; Millard and Thompson, 1989). N-rich amino acids are transported via the phloem from senescing leaves to perennial tissues, where they are used to synthesize proteins (Sauter *et al.*, 1989). During autumn, BSPs accumulate in perennating tissues such as bark, wood and roots (Sauter and van Cleve, 1990; Langheinrich and Tischner, 1991). Interestingly, arginine and soluble protein contents increased during autumn and were higher after leaf fall (Figs 2 and 3). This was mostly evidenced for arginine, which increased from undetectable levels in October to >200 nmol mg^{-1} d. wt on 12 December in 2-year-old stems (Fig. 2). Arginine concentration decreased after leaf fall. Furthermore, as observed in several *Populus* species (Langheinrich and Tischner, 1991), two major polypeptides accumulated in 1- and 2-year-old stems during autumn and winter (Fig. 3B). Previous studies have demonstrated that during the wintering phase, arginine was the major amino acid in both bark and xylem (Sagisaka, 1974). It can be noted that storage proteins are particularly rich in arginine and in amide-containing amino acids (Müntz, 1998). Arginine accumulation in poplar stems during autumn and winter could therefore be considered as a temporary N storage form that could be used thereafter for storage protein synthesis.

Arginine is preferentially synthesized in perennial tissues

The fact that arginine was not detected in laminae, central veins and petioles during senescence suggests that arginine synthesis may occur in perennial tissues such as stems. The metabolic route to arginine synthesis in plants involves two distinct processes: synthesis of ornithine from glutamate and synthesis of arginine from the ornithine intermediates (Slocum, 2005). The second process requires the carbamoyl-phosphate intermediate, which is generated from glutamine via CPS which also contributes to nucleotide metabolism. The CPS protein is made up of a small and a large subunit (Slocum, 2005). Three others enzymes are involved in arginine synthesis: OTC, AS and AL. A detailed expression analysis of *AS* and *OTC* revealed that these genes were expressed in leaves and petioles during autumn (Fig. 4A, B). However none of these gene expression patterns followed the *CP* marker gene. *AS* transcript levels were preferentially higher in autumn than in winter (Fig. 4). *OTC* transcripts did not show variations during senescence in leaves, petioles and stems (Fig. 4). In contrast, the *CPS* gene expression level strongly increased in stems during senescence (Fig. 4C), whereas it was weakly detected in leaves and petioles (Fig. 4A, B). It also clearly matched the expression of *CP* in leaves. Interestingly, stem glutamine concentration increased from November to January (data not shown) and carbamoyl-phosphate is generated from glutamine via CPS. In stems, glutamine could be used for carbamoyl-phosphate synthesis and consequently for arginine synthesis. In leaves, glutamine could be preferentially used as a transport component from senescing leaves to perennial poplar tissues. Nevertheless, it cannot be ruled out that arginine could also be synthesized in leaf but to a smaller extent. Indeed, in *Arabidopsis* senescing leaves, it has been demonstrated that arginine content increased and represented around 1 % of total amino acid content (Diaz *et al.*, 2005).

Pt-CAT11 is a candidate for glutamine transfer during the senescing process

In senescing leaves, production of glutamine increases and glutamine is further loaded into central veins and petioles to reach perennial tissues where it may be used for arginine synthesis. We therefore looked at the genetic potential for loading glutamine into the phloem, and more specifically we looked at the expression levels of AAP and CAT amino acid transporters. The *AAP* members were either not expressed in senescing tissues or even not expressed in leaves at all. We therefore did not focus much attention on this family.

Expression data for the *Arabidopsis* CAT gene family indicated that *At-CAT2* and *At-CAT5* were upregulated during leaf senescence. At-CAT2 is probably located in the tonoplast and may be the long-sought vacuolar amino acid transporter (Su *et al.*, 2004). At-CAT5 functions as a high-affinity, basic amino acid transporter in the plasma membrane and At-CAT5 may function in reuptake of leaking amino acids at the leaf margin (Su *et al.*, 2004). In contrast to their *Arabidopsis* orthologues (Fig. 5), *Pt-CAT5* transcripts were not detected and *Pt-CAT2* was only expressed at the beginning of senescence (Fig. 6A). However, *Pt-CAT2* was strongly upregulated in the petiole during senescence and very weakly expressed in stems (Fig. 6B, C). Nevertheless, expression of the orthologous genes of amino acid transporters may not be similar because the pool of amino acids available for phloem transport is differentially regulated in different species (Delrot *et al.*, 2001). A detailed analysis of each amino acid transporter gene must be made before conclusions can be drawn about the role of the different orthologues. *Pt-CAT3* was highly expressed in laminae, petioles and stems and was only slightly affected by senescence in leaves (Fig. 6). *Pt-CAT4* was also highly expressed in laminae, petioles and stems but, in contrast to *Pt-CAT3*, it was downregulated in stems during winter (Fig. 6). *Pt-CAT9* transcripts were weakly detected in petioles and strongly in stems (Fig. 6B, C). *Pt-CAT9* seems to be preferentially expressed in organs containing sieve elements. In poplar senescing leaves, *Pt-CAT10* and *Pt-CAT11* were upregulated during senescence (Fig. 6A). *Pt-CAT11* was not expressed in petioles but was expressed in laminae and stems (Fig. 6). *Pt-CAT11* expression was upregulated in senescing leaves (Fig. 6A) and subject to quite high variations in stems (Fig. 6C). Interestingly, regression analyses have shown that in the lamina and stem, *Pt-CAT11* expression and glutamine concentration are related and displayed variations of the same order (Figs 1A and 2B).

Functional analysis demonstrated that Pt-CAT11 restored growth of the yeast mutant JA248 on low glutamine medium (Fig. 7A). Additionally, Pt-CAT11 allowed growth of the yeast mutant 22Δ8AA on medium containing neutral amino acids (proline, citrulline and GABA) but not medium containing acid (aspartate and glutamate) amino acids or arginine. Determination of kinetic parameters for [^{3}H]glutamine uptake by Pt-CAT11 in yeast revealed that it can transport glutamine efficiently, with an apparent K_m value of 690 μM (Fig. 7B, C).

Most importantly, recent analysis of expression data showed that *Pt-CAT11* was highly and preferentially expressed in phloem tissues (Courtois-Moreau *et al.*, 2009). Taken together, these data suggest that the major function of Pt-CAT11 is related to the transport of amino acids, and notably glutamine, from senescing leaves to sink tissues such as stems, thus facilitating N remobilization during senescence in poplar.

Conclusions

The analysis of amino acid pools in different organs showed that N remobilization from leaves to perennial organs occurs in poplar during autumn senescence. N-rich amino acids, such as glutamine, are transported via the phloem from senescing leaves to perennial organs, such as stems, where they are used to synthesize storage proteins. The glutamine pools in late autumn correlate with increased *Pt-CAT11* expression, which may function as a glutamine transporter for amino acid transfer between source and sink tissues during senescence processes in poplar. Arginine was being accumulated, probably as an N storage compound, and would be preferentially synthesized in stems, as indicated by the strong arginine accumulation in stems during autumn and at the beginning of winter and the large increases in *CPS* transcript levels during autumn. Whether arginine would be further metabolized to provide N for protein biosynthesis remains to be demonstrated.

The elucidation of amino acid concentrations and profiles together with the characterization of a new amino acid transporter (Pt-CAT11) may present a comprehensive foundation for future studies on amino acid transport and metabolism during autumn N remobilization in perennial plants.

SUPPLEMENTARY DATA

Supplementary data are available online at www.aob.oxfordjournals.org and provide all protein sequences and corresponding accession numbers that were used for the phylogenetic analyses.

ACKNOWLEDGEMENTS

J.C. was supported by a PhD fellowship from the 'Ministère délégué à l'Enseignement supérieur et à la Recherche'. Aude Migeon is gratefully acknowledged for help in sampling leaves, petioles and stems of poplar and assistance with statistical analysis. We thank Dr Nicolas Rouhier (University Nancy, France) for help with protein extraction.

LITERATURE CITED

Andersson A, Keskitalo J, Sjodin A, *et al*. 2004. A transcriptional timetable of autumn senescence. *Genome Biology* **5**: R24.

Bhalerao R, Keskitalo J, Sterky F, *et al*. 2003. Gene expression in autumn leaves. *Plant Physiology* **131**: 430–442.

Boorer KJ, Fischer WN. 1997. Specificity and stoichiometry of the *Arabidopsis* H^+/amino acid transporter AAP5. *Journal of Biological Chemistry* **29**: 13040–13046.

Boorer KJ, Frommer WB, Bush DR, Loo DDF, Wright EM. 1995. Kinetics and specificity of a H^+/amino acid transporter from *Arabidopsis thaliana*. *Journal of Biological Chemistry* **29**: 2213–2220.

Brendley BW, Pell EJ. 1998. Ozone-induced changes in biosynthesis of Rubisco and associated compensation to stress in foliage of hybrid poplar. *Tree Physiology* **18**: 81–90.

Brown RE, Jarvis KL, Hyland KJ. 1989. Protein measurement using bicinchoninic acid: elimination of interfering substances. *Analytical Biochemistry* **180**: 136–139.

Brugiere N, Dubois F, Masclaux C, Sangwan RS, Hirel B. 2000. Immunolocalization of glutamine synthetase in senescing tobacco (*Nicotiana tabacum L.*) leaves suggests that ammonia assimilation is progressively shifted to the mesophyll cytosol. *Planta* **211**: 519–527.

Bush DR. 1999. Sugar transporters in plant biology. *Current Opinion in Plant Biology* **2**: 187–191.

Canovas FM, Avila C, Canton FR, Canas RA, de la Torre F. 2007. Ammonium assimilation and amino acid metabolism in conifers. *Journal of Experimental Botany* **58**: 2307–2318.

Cooke JE, Weih M. 2005. Nitrogen storage and seasonal nitrogen cycling in *Populus*: bridging molecular physiology and ecophysiology. *New Phytologist* **167**: 19–30.

Courtois-Moreau CL, Pesquet E, Sjödin A, *et al*. 2009. A unique program for cell death in xylem fibers of Populus stem. *Plant Journal* **58**: 260–274.

Couturier J, Montanini B, Martin F, Brun A, Blaudez D, Chalot M. 2007. The expanded family of ammonium transporters in the perennial poplar plant. *New Phytologist* **174**: 137–150.

Delrot S, Rochat C, Tegeder M, Frommer WB. 2001. Amino acid transport. In: Lea P, Gaudry JFM. eds. *Plant nitrogen*. Paris, France: INRA-Springer, 215–235.

Diaz C, Purdy S, Christ A, Morot-Gaudry JF, Wingler A, Masclaux-Daubresse C. 2005. Characterization of markers to determine the extent and variability of leaf senescence in *Arabidopsis*. A metabolic profiling approach. *Plant Physiology* **138**: 898–908.

Dickson RE. 1979. Xylem translocation of amino acids from roots to shoots in cottonwood plants. *Canadian Journal of Forest Research* **9**: 374–378.

Dickson RE, Vogeman TC, Larson PR. 1985. Glutamine transfer from xylem to phloem and translocation to developing leaves of *Populus deltoides*. *Plant Physiology* **77**: 412–417.

Fischer WN, Kwart M, Hummel S, Frommer WB. 1995. Substrate specificity and expression profile of amino acid transporters (AAPs) in *Arabidopsis*. *Journal of Biological Chemistry* **29**: 16315–16320.

Fischer WN, Loo DD, Koch W, *et al*. 2002. Low and high affinity amino acid H^+-cotransporters for cellular import of neutral and charged amino acids. *The Plant Journal* **29**: 717–731.

Frommer WB, Hummel S, Unseld M, Ninnemann O. 1995. Seed and vascular expression of a high affinity transporter for cationic amino acids in *Arabidopsis*. *Proceedings of the National Academy of Sciences, USA* **29**: 12036–12040.

Hammes UZ, Nielsen E, Honaas LA, Taylor CG, Schachtman DP. 2006. AtCAT6, a sink-tissue-localized transporter for essential amino acids in *Arabidopsis*. *The Plant Journal* **48**: 414–426.

Hörtensteiner S, Feller U. 2002. Nitrogen metabolism and remobilization during senescence. *Journal of Experimental Botany* **53**: 927–937.

Javelle A, Morel M, Rodriguez-Pastrana BR, *et al*. 2003. Molecular characterization, function and regulation of ammonium transporters (Amt) and ammonium-metabolizing enzymes (GS, NADP-GDH) in the ectomycorrhizal fungus *Hebeloma cylindrosporum*. *Molecular Microbiology* **47**: 411–430.

Keskitalo J, Bergquist G, Gardestrom P, Jansson S. 2005. A cellular timetable of autumn senescence. *Plant Physiology* **139**: 1635–1648.

Langheinrich U, Tischner R. 1991. Vegetative storage proteins in poplar. Induction and characterization of a 32- and 36-kilodalton polypeptide. *Plant Physiology* **97**: 1017–1025.

Millard P, Thomson CM. 1989. The effect of the autumn senescence of leaves on the internal cycling of nitrogen for the spring growth of apple trees. *Journal of Experimental Botany* **40**: 1285–1289.

Müntz K. 1998. Deposition of storage protein. *Plant Molecular Biology* **38**: 77–99.

Okumoto S, Schmidt R, Tegeder M, *et al*. 2002. High affinity amino acid transporters specifically expressed in xylem parenchyma and developing seeds of *Arabidopsis*. *Journal of Biological Chemistry* **277**: 45338–45346.

Okumoto S, Koch W, Tegeder M, *et al*. 2004. Root phloem-specific expression of the plasma membrane amino acid proton co-transporter AAP3. *Journal of Experimental Botany* **55**: 2155–2168.

Pregitzer KS, Dickmann DI, Hendrick R, Nguyen PV. 1990. Whole-tree carbon and nitrogen partitioning in young hybrid poplars. *Tree Physiology* **7**: 79–93.

Ryan DF, Bormann FH. 1982. Nutrient resorption in northern hardwood forests. *Bioscience* **32**: 29–32.

Sagisaka S. 1974. Effect of low temperature on amino acid metabolism in wintering poplar: arginine–glutamine relationships. *Plant Physiology* **53**: 319–322.

Sauter JJ, van Cleve B, Wellenkamp S. 1989. Ultrastructural and biochemical studies results on the localization and distribution of storage proteins in a poplar tree and in twigs of other tree species. *Holzforschung* **43**: 1–6.

Sauter JJ, van Cleve B. 1990. Biochemical, immunochemical, and ultrastructural studies of protein storage in poplar (*Populus* × *canadensis* '*robusta*') wood. *Planta* **183**: 92–100.

Sauter JJ, van Cleve B. 1992. Seasonal variation of amino acids in the xylem sap of *Populus* × *canadensis* and its relation to protein body remobilisation. *Trees* **7**: 26–32.

Slocum RD. 2005. Genes, enzymes and regulation of arginine biosynthesis in plants. *Plant Physiology and Biochemistry* **43**: 729–745.

Staswick PE. 1994. Storage proteins of vegetative plant tissues. *Annual Review of Plant Physiology and Plant Molecular Biology* **45**: 303–322.

Stepien V, Sauter JJ, Martin F. 1994. Vegetative storage proteins in woody plants. *Plant Physiology and Biochemistry* **32**: 185–192.

Sterck L, Rombauts S, Jansson S, Sterky F, Rouze P, van de Peer Y. 2005. EST data suggest that poplar is an ancient polyploid. *New Phytologist* **167**: 165–170.

Su YH, Frommer WB, Ludewig U. 2004. Molecular and functional characterization of a family of amino acid transporters from *Arabidopsis*. *Plant Physiology* **136**: 3104–3113.

Tamura K, Dudley J, Nei M, Kumar S. 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4·0. *Molecular Biology and Evolution* **24**: 1596–1599.

Titus JS, Kang S-M. 1982. Nitrogen metabolism, translocation, and recycling in apple trees. *Horticultural Reviews* **4**: 204–246.

Tuskan GA, Difazio S, Jansson S, *et al*. 2006. The genome of black cottonwood, *Populus trichocarpa* (Torr. & Gray). *Science* **313**: 1596–1604.

Velasco I, Tenreiro S, Calderon IL, André B. 2004. *Saccharomyces cerevisiae* Aqr1 is an internal-membrane transporter involved in excretion of amino acids. *Eukaryotic Cell* **3**: 1492–1503.

Wipf D, Ludewig U, Tegeder M, Rentsch D, Koch W, Frommer WB. 2002. Conservation of amino acid transporters in fungi, plants and animals. *Trends in Biochemical Sciences* **27**: 139–147.

Zimmermann P, Hirsch-Hoffmann M, Hennig L, Gruissem W. 2004. GENEVESTIGATOR. *Arabidopsis* microarray database and analysis toolbox. *Plant Physiology* **136**: 2621–2632.

Exploring the Genome of Glomeromycotan Fungi

Running title: Genomics of Glomeromycota

Gianinazzi-Pearson et al.

Exploring the Genome of Glomeromycotan Fungi

V. Gianinazzi-Pearson[1], D. van Tuinen[1], D. Wipf[1], E. Dumas-Gaudot[1], G. Recorbet[1], Y. Liu[1], J. Doidy[1], D. Redecker[2], N. Ferrol[3]

[1] UMR INRA 1088/CNRS 5184/Université de Bourgogne, Plante-Microbe-Environnement, BP 86510, 21065 Dijon Cedex, France
[2]UMR INRA 1229/Université de Bourgogne, Microbiologie du Sol et de l'Environnement, BP 86510, 21065 Dijon Cedex, France
[3] Departamento de Microbiología del Suelo y Sistemas Simbióticos, Estación Experimental del Zaidín, Consejo Superior de Investigaciones Científicas, C. Profesor Albareda 1, 18008 Granada, Spain

CONTENTS

Abstract

The Glomeromycota form a complex but extremely successful group of root symbionts that have accompanied land plants through evolution and survived across periods of important environmental change. They form a distinct, heterogeneous and unusual ensemble within the fungal kingdom which is characterized by obligate biotrophy, multinucleate nature, large genomes and asexuality, features which have been obstacles to the analysis of the glomeromycotan genome and its functions. Recent targeted and high throughput sequencing programmes have given access to a better understanding of nuclear and mitochondrial genome complexity, diversity and function in these organisms, but the lack of a stable transformation system remains a major drawback to their manipulation. This review updates on progress during the past decade in knowledge about structural, evolutionary and functional aspects of genomes in the Glomeromycota, and points to avenues of research to more widely explore diversity and symbiotic attributes in this fascinating and unique phylum.

I. Introduction

All fungi forming the mutualistic symbiosis with plant roots called arbuscular mycorrhiza were formerly grouped together in one order, the Glomales, placed in the Zygomycota (Morton 1993). Based on molecular analyses suggesting that arbuscular mycorrhizal fungi should be separated from other fungal taxa, they were transferred a decade ago to the **Glomeromycota**, a new phylum created specifically for them (Schüssler et al. 2001). Whilst members of this monophyletic group originated from the same common ancestor as the Ascomycota and Basidiomycota, they have no **obvious affinity to other major extant phylogenetic groups in the kingdom Fungi** (James et al. 2006) and they probably diverged from the other fungal lineages several hundred million years before plants colonized terrestrial habitats 400-500 mya (Heckman et al. 2001). Glomeromycotan fungi are complex but extremely successful organisms. They establish a compatible interaction with plants by either avoiding or suppressing plant defence reactions whilst redirecting host metabolic flow to their benefit without being detrimental to their host. The mechanisms by which this biotrophy is achieved are largely unknown but the Glomeromycota have accompanied land plants through evolution and survived across periods of important environmental change to become ecologically and agriculturally important symbionts which improve overall fitness of very different plant taxa in terrestrial ecosystems world-wide (Smith and Read 2008). Substantial evidence has accumulated about how rational use of the microsymbiont properties should significantly contribute to decreasing fertilizer and pesticide use in agriculture (Gianinazzi et al. 2010).

Although the Glomeromycota show considerable diversity between or within morphologically recognizable species (Rosendahl 2008), they share some singular biological traits which limit experimental approaches that can be exploited to characterize their complexity. One particularity is their **reproduction through large asexual spores**, each of which is a single cell harbouring several hundreds or thousands of nuclei. The main mechanism for filling a spore with so many nuclei appears to be a massive influx of nuclei from subtending mycelium into the developing spore (Jany and Pawlowska 2010). **No sexual stage is known** for these fungi and although they are assumed to only reproduce asexually, recent transcriptome analyses indicate that they do possess genetic information essential for sexual reproduction and meiosis (Tisserant et al. submitted). Absence of a sexual cycle raises questions about how Glomeromycota deal with deleterious mutations usually eliminated through meiosis and how they have adapted to new hosts or habitats during evolution. High polyploidy, with multiple gene copies in the same genome, has been speculated as a possible mechanism to buffer against mutational events (Pawlowska and Taylor 2004), **whilst nuclear exchange through hyphal anastomosis between different individuals of a same species** (Casana and Bonfante-Fasolo 1988; Giovannetti et al. 2001) provides the possibility for

genetic flux and recombination events (Croll et al. 2009; Angelard and Sanders 2011). However, basic information concerning ploidy, karyosis, number of chromosomes or whether meiosis does occur in the Glomeromycota is still lacking, and evidence for genetic exchange, recombination or segregation is very limited.

Another particularity is that **all glomeromycotan fungi are obligate symbionts** which have so far proved to be incalcitrant to pure culture in the absence of a host root on which they depend as a carbon source. This introduces inherent **limitations in the application of standard techniques like genetic transformation, mutant generation/characterization**, and greatly hinders advances in the knowledge about gene function in these organisms. As previously pointed out (Gianinazzi-Pearson et al. 2001), there is converging evidence that the Glomeromycota are an unusual group of fungi and information about their genome structure, complexity and function is essential to understanding the processes regulating their symbiotic attributes, their reproductive biology and their apparent stability during coevolution in symbiosis with many different plant taxa. Despite the fact that the biology of glomeromycotan fungi makes them extremely difficult to manipulate experimentally, the advent of powerful molecular techniques has considerably furthered research during the past decade. The present chapter updates on the state of the art on Glomeromycota genomics within the last decade (Gianinazzi-Pearson et al. 2001), as well as on progress made through targeted and high throughput sequencing programmes into understanding of their basic biology.

II. Glomeromycota Genome Organization

A. Nuclear genome characteristics

Values for DNA contents of Glomeromycota fungi vary depending on the analytical method and the genome of reference used (see Gianinazzi-Pearson et al. 2001). The **haploid genome size** was previously estimated to range from 128 to 1065 Mb, depending on the fungal species studied which further underlines the **high diversity within this phylum** (Hosny et al. 1998a). Important differences also exist within a same genus; the genome size of *Glomus* species was estimated by similar methods to be, for example, 177 Mb for *G. geosporum* and 375 Mb for *G. caledonium* (Hosny et al. 1998a), and more recently to be 14-16.5 Mb for *G. intraradices* DAOM 197198 (syn. *G. irregulare*) (Hijri and Sanders 2004). The apparently small genome of the latter, together with its cultivability in vitro on root organ cultures has made it an appropriate candidate for first sequencing attempts of a glomeromycotan genome (Lammers et al. 2004). Up to date several genome sequencing programmes, based mainly on extensive whole-shotgun (WGS) sequencing, have generated altogether 345 Mbp that have been assembled in 163,968 contigs in a total of 52.5 Mb (Martin et al. 2008b) which corresponds to about four-fold the expected genome size of *G. intraradices* DAOM 197198. With these data, a sequence depth of coverage of just over 3 has been obtained for an estimated genome space

of >150 Mb. However, correct genome assembly is hindered by the fact that critical genetic information, such as a genetic map, is not available for the Glomeromycota. The discrepancy between the estimated genome size and the sequencing data could be due to technical reasons or biological issues. For example, **multiple gene copies in the same genome** (Pawlowska and Taylor 2004) would lead to different assemblies with the same set of scaffold data, and these alternative assemblies (haplotypes) would increase the genome space. **High polymorphism**, which appears to be characteristic of glomeromycotan fungi (see Sanders and Croll 2010) and which has also been indicated from genome sequencing (Martin et al. 2008b), also raises the question of functional genes versus pseudogenes, which are difficult to separate for most software packages used for whole genome assemblies. Recent information about functional genes is discussed below in the section transcriptomics.

The **very low GC content** (~30%) observed from genome sequencing of *G. intraradices* DAOM 197198 is in agreement with values of 30-35% obtained by other methods for a range of Glomeromycota, and which are relatively low as compared to most other fungal taxa (Hosny et al 1998b). The latter authors proposed that a mutational pressure from GC to AT may exist in the Glomeromycota and that, since these fungi proliferate under light-deprived conditions in soil and roots, their environment has not exerted any significant counter selection against AT-rich sequences so that GC contents have become low. The **relatively high proportion of methylated cytosine residues**, which are frequent in repeated sequences, is another particularity of the glomeromycotan genome (Hosny et al. 1998b). A number of repetitive non-coding DNA sequences have been characterized in several species in the Glomeromycota (Gollotte et al. 2006), and genome sequencing of *G. intraradices* DAOM 197198 has revealed that this fungus is also rich in small repeats. Interestingly, variable tandem repeats are considered to affect the rate of evolution of coding and regulatory sequences in other organisms (Gemayel et al. 2010).

B. Nuclear ribosomal genes

The **number of nuclear ribosomal gene copies** has been estimated to be 71-88 in representative glomeromycotan genomes (Gollotte et al. 2006), which is considerably less than in fungi with smaller genomes like yeast or *Cochliobolus heterostrophus* (see Gianinazzi-Pearson et al. 2001). The polymorphic characteristics of ribosomal genes have made them a choice target for phylogenetic and taxonomic studies over a wide range of eukaryotic organisms. The **three coding regions of ribosomal genes**, the small sub-unit (SSU) or 18S, the 5.8S unit and the large sub-unit (LSU) or 25S, are **separated by two internal transcribed spacers** (ITS1 and ITS2) (Mitchell et al. 1995). The two non coding regions (ITS), being less under functional pressure, are more variable and mutate more frequently (Sanders et al. 1995) than the three conserved coding regions. A comprehensive description of nuclear ribosomal genes in the Glomeromycota and their exploitation in

phylogeny prior to 2001 is given by Gianinazzi-Pearson et al. (2001). **SSU, LSU and ITS regions are used for molecular phylogeny and taxonomy** of fungi in this phylum. The highly conserved SSU was the first region to be selected for phylogenetic analysis of the Glomeromycota, mainly because of the large number of eukaryotic sequences present in public databases (Simon et al. 1992). It enabled the initial dating back of this taxon to an ancestral *Glomus*-like fungus 353-462 mya (Simon et al. 1993). SSU sequences remain the most numerous available for glomeromycotan fungi and they are mainly at the origin of more recent taxonomic reorganisations (Schüssler et al. 2001; Schüssler and Walker 2010).

However, the SSU region does not allow species level resolution in the Glomeromycota. Also, the first primer allowing identification of Glomeromycota in host roots, the SSU-based VANS1, is not well conserved across the phylum (Clapp et al. 1999). Consequently, other ribosomal regions, such as the ITS/5.8S region in combination with the SSU, or the 5' end of the LSU have been exploited to generate more taxon-specific primers for monitoring fungal communities in roots (see for example van Tuinen et al. 1998; Redecker 2000; Pivato et al. 2007). Comparison of the level of polymorphism between the different ribosomal regions of *G. mosseae* BEG12, *G. mosseae* BEG69, *G. coronatum* BEG22 and *G. intraradices* AFTOL ID48, clearly illustrates the **potentiality of the 5' end of the LSU for diversity studies** (Figure 1). Sequence differences between *G. mosseae* BEG12 and *G. coronatum* BEG22 or *G. intraradices* AFTOL ID 48 are greatest for the 5' end of the LSU region and in particular for the variable D2 domain. In spite of this, a general caution applies to the use of nuclear ribosomal sequences for diversity studies in that they have a potentially high inter and intra-sporal heterogeneity which implies that several nuclear ribosomal variants can occur in a same isolate (Sanders et al 1995; Sanders and Croll 2010).

Figure 1

C. The mitochondrial genome

The first **mitochondrially-encoded genes** from the phylum Glomeromycota were identified from an amplified and sequenced region of the mitochondrial gene coding for the LSU of ribosomal RNA (Raab et al. 2005). In contrast to their nuclear-encoded counterparts, these genes do **not show variation within single spores and fungal isolates**. This is of particular importance as it has been proposed that the **nuclei in the coenocytic glomeromycotan mycelium are genetically heterogeneous**, resulting in a population of allelic variants with no fixed overall genotype (Kuhn et al. 2001). The first complete mitochondrial genome of a glomeromycotan fungus was sequenced from *G. intraradices* strain 494 by whole genome amplification and subsequent pyrosequencing (Lee and Young 2009). It has a size of 70.6 Kbp and a GC content of 37.2%, which is higher than the GC content of the nuclear genome.

The genetic code used in the protein-coding genes is the standard code except that UGA is used for tryptophan, which is typical for many fungal mitochondrial genomes. The mitochondrial genome of *G. intraradices* contains a similar set of genes to that of other fungi: three subunits of ATP synthase (*atp6*, *atp8* and *atp9*), three of cytochrome c oxidase (*cox1*, *cox2* and *cox3*), seven of NADH dehydrogenase, and apocytochrome b (cob). Moreover, it contains the standard set of 26 tRNAs and ribosomal small subunit (*rns*) and large subunit (*rnl*) genes. Notably, all genes are encoded on the same strand. However, the *rps3* gene, which is found in the mitochondrial genome of other fungi, was apparently transferred to the nuclear genome in *G. intraradices*, and there is evidence for the presence of nuclear copies of other mitochondrial sequences (Lee and Young 2009). The *G. intraradices* mitochondrial genome bears little resemblance to that of other fungi, but this comes as little surprise since gene order in fungal mitochondrial genomes is not conserved among distantly related taxa.

A large part of the *G. intraradices* mitochondrial genome consists of introns and other non-coding sequences; only 24.6% are coding sequences. A total of 26 introns were identified in strain 494, most of them belonging to type 1. As in other fungi, the mitochondrial genome of this glomeromycotan fungus is rich in **homing endonucleases**, enzymes that are often coded in introns and thought to function **as mobile elements** in the insertion of introns containing an endonuclease open reading frame (ORF) into intron-free-alleles (Dalgaard et al. 1997). Homing endonucleases of the LAGLIDADG family were first reported in introns of the rnl gene (Raab et al. 2005). The complete mitochondrial genome sequence shows that four of these ORFs are within introns and one is attached to the *nad3* gene, whilst two GIY-YIG type homing endonuclease ORFs are in intergenic spacers. As mobile genetic elements, homing endonucleases are presumably transferred laterally. Comparison of different isolates and species of *Glomus* has provided evidence for the existence in glomeromycotan fungi of **horizontal transfer of *rnl* introns and the "homing cycle"** (Chevalier and Stoddard, 2001), involving insertion, degeneration and loss (Thiéry et al. 2010). Due to this activity, introns containing homing endonuclease ORFs seem to show higher degrees of polymorphism. This polymorphism has been used to distinguish between isolates of *G. intraradices* in field studies (Börstler et al. 2010) and to track an inoculated isolate over several years in a field site (Sýkorová et al. 2011).

The involvement of fungal mitochondria in spore germination and early signal exchange with host root colonization (Besserer et al. 2006) highlights the need to study the coding sequences of mitochondrial genomes. On the other hand, non-coding regions are of direct importance as molecular markers of these fungi in ecological studies and in biotechnological applications. In addition, mitochondrial markers to study inheritance of these organelles could provide important baseline data to help to elucidate the genetics of the Glomeromycota and possible previously unrecognized sexual processes.

III. Nuclear Genome Evolution in the Glomeromycota

The **Glomeromycota are traditionally thought to be ancient asexuals**. This assumption is based on the fact that no morphological structures conclusively indicative of sexual reproduction have been observed in the whole phylum; a study reporting zygosporangia in *Gigaspora* (Tommerup and Sivasithamparam 1990) has never been confirmed. The lack of basic knowledge about their genetics leaves the evolutionary biology of the Glomeromycota open to a lot of speculation. The inability to culture these fungi separately from their host plants and to obtain stable transformants has contributed to this situation.

Considering the well-known **benefits of sexual reproduction** in avoiding the accumulation of deleterious mutations in the genome, which should inevitably lead to evolutionary meltdown processes such as Mullers's ratchet (Muller 1932), it seems hard to understand how these fungi could persist as important actors of terrestrial ecology for more than 400 million years. Morphological stasis resulting in a relatively low number of described morphospecies and striking similarities of extant Glomeromycota with 400-million-year old fossils have been cited as other lines of evidence for the lack of diversification caused by long term clonal propagation in conjunction with the asexual lifestyle (Sanders and Croll 2010). Ancient asexuals are often considered as "scandalous" exceptions of the rule as they challenge current theories of sex (Judson and Normark 1996). The bdelloid rotifers, a lineage of invertebrates solely consisting of parthenogenetically-reproducing females for at least 40 million years, are often cited as a striking example where clonal organisms have evolved into more than 300 species in a similar way as sexual organisms (Fontaneto *et al.* 2007). Alternative **mechanisms to purge deleterious mutations from the genome** have therefore to be considered for Glomeromycota as they have been for bdelloid rotifers (Gladyshev and Arkhipova 2010).

When the nuclear rDNA genes were characterized as the first genetic components in the Glomeromycota, an **unusually high level of intra-organism polymorphism** was noted (see Gianinazzi-Pearson et al. 2001). In sexual organisms, this polymorphism is prevented by a mechanism known as "concerted evolution" that is thought to homogenize rDNA copies (Gandolfi et al. 2001). Intra-organismal polymorphism of nuclear rDNA or other genes has been demonstrated in many organisms, including fungi (O'Donnell 1992), but it seems to be exceptionally large in the Glomeromycota (Stockinger et al. 2009). Here, it was postulated that rDNA variation occurs between different genomes that are present in genetically different nuclei in the coenocytic mycelium (Hijri and Sanders 2005; Kuhn *et al.* 2001). **Heterokaryosis** was hypothesized to have arisen by hyphal anastomosis and accumulation of mutations (Bever and Wang 2005) and evoked as a possible mechanism to compensate for the absence of sexual processes in glomeromycotan species, although other authors have

presented data for homokaryosis (Pawlowska and Taylor 2004). Different likely scenarios in this context were recently reviewed by Young (2008).

It is helpful to note in this context that numerous fungal lineages, though none as ancient as the Glomeromycota, were once thought to be asexual based on the absence of the expected morphological structures. In many cases, analyses of the population biology using molecular markers revealed evidence for recombination (Burt et al. 1996). Many possibilities may exist in fungi for cryptic sexuality or non meiotic recombination and low levels of recombination can be sufficient to prevent the accumulation of deleterious mutations. In contrast to other fungal groups there does not seem to be a uninucleate stage in the Glomeromycota, which would give the opportunity to reduce genetic variation to a single haploid genome but, as mentioned previously, a large number of nuclei are instead migrating into newly formed spores (Jany and Pawlowska 2010). However, some evidence for recombination in the genome of *G. intraradices* has been detected using molecular markers (Croll and Sanders 2009), whereas the genome of *Scutellospora castanea* was previously found to be predominantly clonal (Kuhn *et al.* 2001) and the life history of the cosmopolitan *G. etunicatum* was shown to be dominated by clonality with rare recombination, if at all (den Bakker et al. 2010).

A **parasexual mechanism for the exchange of genetic information** in fungi is through the formation of hyphal networks by **anastomoses** (hyphal bridges). This phenomenon has been well documented in the genus *Glomus* but the extent appears to differ between isolates. For example, it seems to be limited to within-isolate connections for *G. mosseae* originating from different geographic regions (Giovannetti et al. 2003), whilst genetically distinguishable isolates of *G. intraradices* from one field site in Switzerland formed anastomoses at a frequence dependent on their genetic relatedness (Croll et al. 2009). The *G. intraradices* isolates exchanged genetic markers, resulting in recombinant genotypes in offspring strains which showed altered symbiotic capabilities, indicating that this kind of genetic exchange may have some relevance for host plant fitness (Angelard et al. 2010).

However, basic parameters can differ substantially between members of the Glomeromycota. Their genome sizes vary greatly (see section IIA), not much is known about genome structure outside the model species *G. intraradices*, and retrotransposons have been suggested to play an important role in the genome of at least one species (*S. castanea*; Gollotte et al. 2006). In addition, anastomosis formation has not been observed in the Gigasporaceae, and other lineages, resulting in a completely different architecture of the mycelium and ruling out hyphal cross-bridges as a means to redistribute nuclei (Purin and Morton 2011). Also, in contrast to *G. intraradices*, *G. mosseae* was shown to have a rather uniform worldwide population structure, suggesting a different genetic disposition (Rosendahl et al. 2009). Due to these apparent differences across the Glomeromycota, it seems difficult to generalize questions of evolution or genetic exchange from any findings obtained with one or

another fungus. Even though first steps towards understanding genome evolution have been taken in the model species *G. intraradices* (Sanders and Croll 2010), there remains much more to be explored for the rest of the phylum.

IV. The Symbiotic Genome of Glomeromycota

The **symbiotic genome** comprises those glomeromycotan **genes that are associated with development and functioning of the fungi in arbuscular mycorrhiza interactions**. Initial steps require genes permitting a switch from asymbiotic spore germination to pre-symbiotic stages of hyphal branching and appressorium formation at the root surface under the influence of host plant signals (Gianinazzi-Pearson et al. 2007). Once within roots, morphogenetic processes in the Glomeromycota lead to differentiation of characteristic intracellular haustoria-like structures, termed arbuscules, and the establishment of a symbiotic interface boardered by fungal and plant membranes thought to be the main site of nutrient and signal flow between the symbionts (Smith and Read 2008). Hyphae subsequently develop out from the mycorrhizal roots to form the extraradical mycelium (ERM), and provide extensive pathways for nutrient fluxes through the soil into mycorrhizal roots (Gianinazzi et al. 2010) which presumably rely on the regulation of fungal genes related to nutrient sensing, production of specific enzymes and resource partitioning between the fungal symbionts and host roots (Leake et al. 2004).

A. Transcriptome features

In the absence of a full genome sequence, functional genomic studies of symbiotic traits in the Glomeromycota have so far primarily relied on **gene expression profiling**. Initial studies using targeted approaches, based on the assumption that a gene or gene product plays a role in developmental or metabolic processes, identified a certain number of genes encoding proteins with important nutritional and morphogenetic functions, such as phosphate transporters, H^+-ATPases, ammonium and amino acid transporters, carbohydrate metabolism, chitin synthases and β-tubulin (see Ferrol et al. 2004; Gianinazzi-Pearson et al. 2004). For example, as discussed below in the transportome section, the characterization of a high-affinity phosphate transporter in *G. versiforme* by heterologous screening of a cDNA library (Harrison and van Buuren 1995) provided a breakthrough in the understanding of fungal functioning in phosphate uptake by mycorrhizal plants.

Identification of a more comprehensive collection of fungal genes became possible during the last decade with the emergence of transcriptome technologies that allow analysis of the mRNA pool of a cell at any one moment. **Transcriptome studies** of glomeromycotan fungi initially focused on small collections of **expressed sequence tags (ESTs)** in cDNA

libraries generated exclusively from activated spores (Stommel et al. 2001), germinated spores (Lammers at al. 2001; Lanfranco et al. 2002) or extraradical hyphae (Sawaki and Saito 2001; Jun et al. 2002). Clone sequencing revealed several interesting similarities to known genes which are consistent with postulated fungal activities in the symbiotic state. For example, evidence that **arginine** is probably the **preferred molecule for long-distance fungal transport of nitrogen to the host plant** was obtained by identifying a glutamine synthase gene from a *G. intraradices* cDNA library which is preferentially expressed in extraradical hyphae and a gene associated with arginine breakdown which is more highly expressed in the intraradical mycelium (Govindarajulu et al. 2005). Also, expression profiling of an acyl-CoA-dehydrogenase gene from the same library indicated mechanisms of lipid utilization in germinating spores and in extraradical mycelium, and expression analysis of genes coding for a malate synthase and an isocitrate lyase gene containing, motifs responsible for glyoxisomal targeting, reinforced the hypothesis that **substantial carbon fluxes within symbiotic hyphae involve the glyoxylate cycle** (Bago et al. 2002).

With the advent of techniques like **differential RNA display (DDRT)** and **suppression subtractive hybridization (SSH)**, snapshots of the glomeromycotan genome active in fungal structures associated with different stages in the mycorrhizal symbiosis became more easily accessible. Differential expression of numerous genes was revealed in a first SSH-based comparison of transcript profiles between germination hyphae and extraradical mycelium of *G. mosseae*, with the identification of a gene (*GmGin1*) probably involved in signalling during spore germination before symbiosis formation (Requena et al. 2002). Subsequent exploration, using DDRT, SSH and EST screening, of genetic determinants controlling the developmental switch from asymbiotic spore germination to presymbiotic hyphal branching of *Gigaspora rosea* and *Gig. gigantea*, stimulated by **a root exuded factor**, showed activation of genes encoding proteins involved in mitochondrial function, signal transduction, gene expression, DNA synthesis and cell cycle regulation (Tamasloukht et al. 2003, 2007). Induction of genes encoding mitochondrial proteins occurred before increases in respiratory activity, reorganization of the mitochondrial system and stimulation of fungal ramification, indicating that this **branching response is the result of a metabolic switch** (Tamasloukht et al. 2003). Strigolactones were later discovered to be the root exudate components which induce hyphal branching (Akiyama et al. 2005) and which provoke the responses of the mitochondrial apparatus in the fungus, leading to the conclusion that mitochondrial activation is a key event in the switch from asymbiotic to pre-symbiotic stages (Besserer et al. 2006; 2008).

Analysis of the fungal transcriptome during colonization of host plant tissues has been hampered by a low abundance of fungal transcripts (Maldonado-Mendoza et al. 2002). However, using SSH it was possible to detect transcriptome modifications in *G. mosseae* sporocarps triggered in synchrony with appressoria formation linked to recognition of a host

root surface by fungal hyphae, and show induction of genes with functions in signalling, transduction, general cell metabolism, defence or stress responses, or of unknown function during this early morphogenetic event (Breuninger and Requena, 2004). Several of the identified genes code for proteins that have a potential role in **calcium-based signalling pathways**, indicating that **Ca^{2+}** could be involved as a **second messenger in the perception and transmission of a plant signal leading to appressorium formation**. Transcript profiling studies undertaken to understand molecular changes that accompany arbuscular mycorrhiza development have also led to the identification of a few fungal genes in symbiotic tissues. For example, DDRT analyses of mycorrhizal and non-mycorrhizal tomato gave a cDNA fragment with similarity to a phosphoglycerate kinase gene from *G. mosseae* that accumulates in higher amounts in colonized roots than in germinated spores (Harrier et al. 1998), and the same approach identified three differentially displayed cDNA fragments of *G. intraradices* in barley mycorrhiza which code for peptide sequences with similarities to proteins involved in gene regulation (Delp et al. 2000). A further six ESTs of *G. mosseae* up-regulated in mycorrhizal roots were identified in a SSH library of *M. truncatula*, two of which showed similarity to a thioredoxin homolog and to a peptidylprolyl cis-trans isomerase (Brechenmacher et al. 2004).

Studies aimed at understanding the molecular response of the ERM of Glomeromycota to various growth conditions, including nitrogen starvation (Capellazzo et al. 2007) and heavy metal stress (Waschke et al. 2006; Ouziad et al. 2005) and interaction with other microorganisms (Hildebrandt et al. 2006), have given access to more fungal genes. Among the ESTs of *G. intraradices* induced by heavy metals, for example, several stress-responsive genes were identified, particularly genes encoding enzymes involved in oxidative protection, such as CuZnSOD, thioredoxins and glutathione-S-transferases, supporting the hypothesis that a primary strategy of the fungus to survive in heavy metal-polluted soils is to cope with the heavy metal-induced oxidative stress (González-Guerrero et al. 2007; Benabdellah et al. 2009a).

Glomeromycotan genes have also been searched for amongst ESTs **in mycorrhizal root cDNA libraries**. In one approach, tblastx analysis of the Affymetrix GeneChip® Medicago Genome Array identified 49 putative fungal genes, all present exclusively in *Medicago truncatula*/*G. intraradices* root cDNA libraries (Gomez et al. 2009). Further analysis of 10 of these genes, associated with the urea cycle, amino acid biosynthesis and cellular autophagy, showed they were expressed in laser-microdissected cortical cells containing arbuscules. These data confirm previous predictions by Govindarajulu et al. (2005) that the **urea cycle is active in the arbuscules** and provide the first molecular hint that **arbuscule turnover might involve autophagy**. In another analysis, a blastn search against 3,034 ESTs contigs from a *M. truncatula*/*G. intraradices* cDNA library (Journet et al. 2002) was performed and 42 clones putatively corresponding to fungal genes were obtained. On the

basis of their annotation, they were distributed in 12 different functional groups (van Tuinen et al. unpublished; Figure 2); none of these fungal genes correspond to those reported by Gomez et al. (2009). The largest functional category, with 30% of the sequences, encodes proteins involved in protein synthesis and degradation and 14% are related to primary metabolism, suggesting a high metabolic activity of the mycorrhizal fungus during root colonization. The high number of orphan genes (19%), with unknown function or no homology to database sequences, could be an indication of the presence mycorrhiza-specific genes.

Figure 2

Altogether, these different approaches have generated about 5200 ESTs from Glomeromycota that are publicly available in databases. In the absence of a full genome sequence, these ESTs have represented a valuable resource to identify candidate genes for targeted studies of, for example, glomeromycotan carbon metabolism (Lammers et al. 2001; Bago et al. 2002, 2003), nitrogen metabolism (Govindarajulu et al. 2005; Tian et al. 2010), sulfur metabolism (Allen and Shachar-Hill 2009), heavy metal homeostasis (Lanfranco et al. 2002; González-Guerrero et al. 2005, 2007) and redox homeostasis (Lanfranco et al. 2005; Benabdellah et al. 2009a, b; González-Guerrero et al. 2010a, b). Genes selected from these databases have also been exploited to show the impact of symbiosis-related plant genes on fungal activity during root interactions (Seddas et al. 2009; Kuznetsova et al. 2010).

Recently, the international *Glomus* consortium has expanded the EST repository of *G. intraradices* by generating cDNA libraries from different fungal structures that have been sequenced using high-throughput technologies. A robust set of non-redundant virtual transcripts (25906, about 20 Mb) transcribed in quiescent and activated spores, ERM and symbiotic roots have been generated (Tisserant et al. submitted). This comprehensive *G. intraradices* transcriptome has provided evidence that **obligate biotrophy** in this fungus **cannot be explained by loss of metabolic complexity** and, as mentioned previously, **genetic information essential for sexual reproduction and meiosis is present** although a known sexual stage is absent from the glomeromycotan life-cycle. The virtual transcriptome of *G. intraradices* (based on >430,000 reads) has served to construct an oligoarray as a basis to underpinning the peculiar biological traits of these organisms and to examine the functional responses of *G. intraradices* genes to symbiosis development. Gene expression profiling in ERM and symbiotic root tissues has, for example, shown that **pathways of amino acid and polyphosphate biosynthesis are highly expressed** in the fungus, which concords with **nutrient fluxes through mycelium during symbiotic interactions**. Up-regulation of genes encoding for membrane transporters, signal transduction pathways and small secreted proteins in intraradical mycelium and arbuscules, together with the lack of expression of hydrolytic

enzymes acting on plant cell wall polysaccharides, are characteristics of *G. intraradices* that are shared with the ectomycorrhizal fungi *Laccaria bicolor* (Martin et al. 2008a) and *Tuber melanosporum* (Martin et al. 2010), and with the biotrophic pathogen *Blumeria graminis* (Spanu et al. 2010). In addition, data sets support biological traits identified in previous gene expression studies; for example, a number of *G. intraradices* genes encoding proteins putatively involved in Ca^{2+} homeostasis/signalling are upregulated with mycorrhiza development (Figure 3). Moreover, *G. intraradices* transcriptome features highlight the existence of *Glomus*-specific genes, including those coding for small secreted proteins, which are amongst the most highly up-regulated in the mycorrhizal association and which could be specific to the symbiotic state (Tisserant et al. submitted). The recent characterization of a secreted fungal effector (SP7) from *G. intraradices* points to a role of secreted proteins in managing the accommodation process of the fungus within plant roots (Kloppholz et al. 2011).

Figure 3

B. Proteome insights

Much of the early studies of proteins in the Glomeromycota focussed on polypeptides or enzyme-active gene products, separated by gel electrophoresis, to investigate expressed functions in the fungal genome during the arbuscular mycorrhizal symbiosis or as polymorphic genetic markers in diagnostic taxonomy (see Gianinazzi-Pearson et al. 2001). However, with advances in bioinformatics and the development of mass spectrometry (MS) (Oeljeklaus et al. 2008; Rohrbough et al. 2007), efficient **large-scale profiling of the fungal proteome** has become possible. Although initial proteomic studies conducted on the extra- and intra-radical stages of the glomeromycotan life cycle experimented difficulties in revealing the accumulation of fungal gene products (Bestel-Corre et al. 2002; Dumas-Gaudot et al. 2004), systematic nanoscale capillary liquid chromatography–tandem mass spectrometry (LC–MS/MS) has since proved successful in enlarging the coverage of the fungal proteins.

Proteomics performed on the ERM from *in vitro* mycorrhizal root organ cultures, using large-scale protein-profiling based on two-dimensional electrophoresis (2-DE) and MS-based identification analyses, gave the first extraradical glomeromycotan 2-DE reference map (438 spots from *G. intraradices* DAOM 181602), but only a limited number of fungal proteins could be identified (Dumas-Gaudot et al. 2004). A subsequent shotgun proteome analysis of *in vitro*-grown ERM, involving one-dimensional (1D)-PAGE-nanoscale capillary liquid chromatography-MS/MS (GeLC-MS/MS), led to the confident identification of 158 phenol-extracted fungal proteins, corresponding to 92 different (distinct or differentiable) isoforms after parsimony analysis, thus representing the most **comprehensive list of glomeromycotan proteins** so far identified (Recorbet et al. 2009). Over half (88) of the

proteins retrieved from *G. intraradices* ERM were not previously indexed as related to mycorrhizal fungi in protein databases, and consequently represent **new protein candidates of the glomeromycotan life cycle**.

Biological process grouping of the GeLC-MS/MS-identified ERM proteins from *G. intraradices* indicates that the **majority of identified proteins have putative functions in sustaining energetic metabolism, protein synthesis, folding, transport and catabolism**, which suggests **an important protein turn-over and trafficking in the extra-radical phase** of the symbiotic fungus (Recorbet et al. 2009). Among these proteins are enzymes involved in dark CO_2 fixation, glycolysis/gluconeogenesis, pentose phosphate and glutamine biosynthesis-related pathways. Likewise, several proteins related to vesicular trafficking, including the GTP-binding protein Ypt1, the small GTPase SAR1, and three members of the Rab GTPase subfamily were identified, together with the signal-transducing proteins calcineurin, Rho1, Cpc2 and Bmh2, for which a role has been demonstrated in fungal morphogenesis. Bmh proteins are also involved in cell cycle regulation as being necessary for the initiation of DNA replication. Among proteins playing roles in the cell division cycle, the DNA damage checkpoint protein rad25, two isoforms of the AAA ATPase Cdc48, and a putative prohibitin were concomitantly identified in the ERM of *G. intraradices*. Current hypotheses on the mechanisms underlying the glomeromycotan cell cycle mostly refer to the switch from G0/G1 to S/M during root colonisation and to DNA replication occurring during the production of mycelium from germinating spores (Bianciotto and Bonfante 1993; Bianciotto et al. 1995). Additionally, GeLC-MS/MS data have also pointed to two modules of enzymes related to cell redox homeostasis that accumulate in the ERM of glomeromycotan fungi, corresponding to the *trans*-sulphuration pathway/glutamyl cycle and the glutathione/thioredoxin system. Overall, this strategy has opened the way towards large-scale analyses of fungal genome responses and metabolic adjustments to environmental cues, including nutrient supply, host recognition and stress–related stimuli.

Although arbuscules have been microdissected from mycorrrhizal roots for transcriptome analyses (Balestrini et al. 2007; Gomez et al. 2009; Kuznetsova et al. 2010), these fungal structures have not been isolated in sufficient amount and purity for proteomic analysis so **that intra-radical fungal gene products** have not yet been directly profiled on a large scale. To enlarge the coverage of intraradical fungal proteins, Recorbet et al. (2010) compared protein profiles of *G. intraradices* and *G. mosseae* in roots of *M. truncatula* using LC-MS/MS based on an enlarged pH gradient and two-dimensional gels coupled to quantitative analysis by Progenesis workstation. Over two thousand protein spots were detected from mycorrhizal roots, of which confident identifications encompassed 21 fungal proteins. Homology-inferred functions were found to complement the working models so far proposed for functioning of the intra-radical mycelium with regards to carbon utilization, energy generation, redox homeostasis and protein turnover-related processes, thus

representing **the largest set of *in planta*-expressed glomeromycotan proteins so far identified**. The proteins appear to belong to pathways active in other stages of the fungal life cycle, including the ERM (Recorbet et al. 2009, 2010).

Overall, this comparative 2-DE-based analysis provided evidence for the existence at the protein level of a conserved set of expressed genome functions associated with the mycorrhizal state of the symbiotic fungus. In relation to proteolytic processing, for example, a fungal subtilase was detected among the induced mycorrhiza-related proteins. Subtilisin-like proteases have been shown to be virulence factors in fungal pathogens of insects, nematodes and plants, including *Magnaporthe poae* (Bidochka and Khachatourians 1990; Tunlid et al. 1994; Sreedhar et al. 1999), and a serine proteinase was proposed to be a potential general feature of leaf infection by the mutalistic fungal plant endophyte *Acremonium typhinum* (Reddy et al. 1996). Some of the proteins identified in glomeromycotan symbionts may thus point to candidate genes required for mycorrhiza formation. In this context, it has been demonstrated that the **Glomeromycota and pathogenic fungi share some genetic components required for colonisation of plant tissues** (Tollot et al. 2009; Heupel et al. 2009).

Because identification of a large majority of proteins from the Glomeromycota has been achieved by homology searches in databases for non mycorrhizal fungi, they are unlikely to be specialized for the arbuscular mycorrhizal symbiosis. Nonetheless, high-throughput identification of protein orthologs in other biotrophic fungal species, even lacking complete genome coverage, remains a pertinent approach for deciphering some conserved key actors of biotrophy in glomeromycotan fungi. Regarding features and processes supposed to be unique to mycorrhizal fungi, which nowadays cannot be approached *via* homology searches in other species, broad insight into the genome and transcriptome of *G. intraradices* (see sections IIA and IIIA) will undoubtedly boost knowledge on the glomeromycotan proteome. Besides limited available genomic resources for Glomeromycota, global proteomic approaches also suffer from a restricted dynamic range resolution in that house-keeping proteins usually hinder the detection of low abundant polypeptides, which are regarded as important effectors in cell regulation pathways. A wide variety of fractionation tools are now available to cope with this issue in non-model organisms (Carpentier et al. 2008), and which could be adapted to proteomics of glomeromycotan fungi in mycorrhizal associations. Furthermore, although laser-capture micro-dissection (LCM)-based techniques require optimization of tissue preparation prior to LCM and GeLC-MS/MS, they represent a promising approach for broad spectrum profiling of glomeromycotan proteins and identification of corresponding genes active in mycorrhizal roots.

C. Transportome genes

Current knowledge on the **transportome** in the Glomeromycota, that is, the complete repertoire of fungal genes encoding membrane transporters, ion exchangers and ion channels, is still in its infancy. In fact, only fourteen transporter genes (for 9 different substrates) have been characterized up to now in the glomeromycotan genome. This number is low compared to the number of putative transporters (around 500) that have recently been identified in the

sequenced *G. intraradices* transcriptome (Tisserant et al. submitted; L. Casieri, personal communication).

1. Ion and water transporter genes

In order to gain insight into mechanisms underlying the role of Glomeromycota in plant nutrient acquisition, studies have focussed on **fungal genes encoding transporters** potentially involved in the uptake from soil of solutes by the fungal hyphae, their transport through the mycelium and their transfer to the plant. Given the central role **phosphate (Pi) transport** plays in the arbuscular mycorrhizal symbiosis (Smith and Read 2008), the first fungal genes to be identified encoded proteins mediating Pi transport across the fungal membranes. The first gene characterized in detail encoded a Pi transporter of *G. versiforme* (*GvPT*, Harrison and van Buuren 1995), followed by homologous genes from *G. intraradices* (*GiPT*, Maldonado-Mendoza et al. 2001) and *G. mosseae* (*GmosPT*, Benedetto et al. 2005). *GvPT* codes for a high-affinity proton-coupled transporter and shares structural as well as sequence similarity with other plant and fungal high affinity phosphate transporters. The apparent Km of GvPT, evaluated in a heterologous system, is in the micromolar range which is a value comparable to free Pi concentrations generally found in soil solution. *GvPT* and *GiPT* transcripts are in fact predominantly detected in the ERM, thus indicating a role in Pi acquisition from the soil. Moreover, *GiPT* expression appears to be regulated by phosphate as it responds to external Pi concentrations and to overall mycorrhiza Pi content (Maldonado-Mendoza et al. 2001). In contrast, *GmosPT* is highly expressed also in intraradical mycelium and in particular in arbuscules (Balestrini et al. 2007). This finding provides a new scenario for the plant–fungus nutrient exchanges suggesting that, at least when the plant is actively growing, the **fungus may regulate nutrient exchange at the symbiotic interface** (Balestrini et al. 2007).

Figure 4

Until now four different **genes encoding membrane transporters of nitrogen compounds** (Figure 4) have been identified from Glomeromycota genomes: **one amino acid transporter** in *G. mosseae* (*GmosAAP1*, Cappellazzo et al. 2008), and two **ammonium transporters** (*GintAMT1*, Lopez-Pedrosa et al. 2006; *GintAMT2*, Pérez-Tienda et al. submitted) and one nitrate transporter (*GiNT*, Tian et al. 2010) in *G. intraradices*. GmosAAP1 is able to transport proline through a proton-coupled, pH- and energy-dependent process but it can bind non polar and hydrophobic amino acids, thus indicating a relatively specific substrate spectrum. *GmosAAP1* expression is detected in the ERM developing out from mycorrhizal roots, where transcript abundance could be increased by exposure to organic nitrogen, in particular when supplied at 2 mM concentrations (Cappellazzo et al. 2008). These

findings suggest that the GmosAAP1 transporter plays a role in the first steps of amino acid acquisition by hyphae, allowing direct amino acid uptake from the soil and facilitating exploitation of soil nitrogen resources. *GintAMT1* and *GintAMT2* code for the ammonium transporter/methylamine permease/rhesus (AMT/Mep/Rh) protein family (TC#1.A.11) that mediate transport of NH_4^+ across biological membranes (Lopez-Pedrosa et al. 2006; Pérez-Tienda et al. submitted). Both genes functionally complement corresponding mutant yeast strains, and the apparent Km of GintAMT1 has been evaluated in yeast to be in the micromolar range characteristic of a high-affinity and low capacity NH_4^+ transporter. *GintAMT1* and *GintAMT2* are differentially expressed during the fungal life cycle and in response to N, suggesting that the encoded proteins play different roles in the symbiosis. Whilst transcripts of both genes were detected in arbuscules and spores, *GintAMT1* was more highly expressed than *GintAMT2* in the ERM. Gene activity in these extraradical hyphae indicates an involvement in fungal nutrient uptake from the soil, but their expression in arbuscules and spores suggests a role for these transporters in processes other than N uptake for nutrition, such as retrieval of NH_4^+ that leaks out during metabolism and, therefore, in fungal NH_4^+ retention. For the **nitrate transporter gene** *GiNT*, only a partial cDNA sequence encoding a putative high-affinity nitrate transporter has so far been obtained (Tian et al. 2010). Nitrate availability stimulates the expression of *GiNT* so that it may play a role in transporting NO_3^- into the ERM.

Heavy metal membrane transporter genes have also been identified in the glomeromycotan genome. In *G. intraradices*, a member of the cation diffusion facilitator (CDF) family *GinZnT1* was shown to decrease cytosolic Zn levels in yeast, although not to completely restore the phenotype of a *zrc1cot1* yeast mutant affected in two vacuolar CDF transporters (González-Guerrero et al. 2005). In yeast, CDF family members are involved in Zn, Fe and Co homeostasis by playing a role in metal efflux from the cytosol, either outside the cell or into intracellular organelles. The expression pattern of *GintZnT1* in response to short-time exposure to Zn and when *G. intraradices* is grown in the presence of high Zn concentrations, reveals a role for GintZnT1 in Zn detoxification and in the protection of the mycorrhizal fungus against Zn stress. Recently, a gene member of the ATP-binding cassette (ABC) family of transporters that may also play a role in heavy metal detoxification has been reported in *G. intraradices* (*GintABC1*; González-Guerrero et al. 2010a). The fact that *GintABC1* is up-regulated by Cu and Cd, and that it has a high homology to the yeast Cd factor gene that transports bis-glutathione-Cd complexes across the vacuolar membranes (Li et al. 1997), suggests a putative role for GintABC1 in heavy metal tolerance by transporting the excess of metal into vacuoles in *G. intraradices*.

In addition to these ion transporters, **transmembrane water channels** or **aquaporins** can mediate the flux of small solutes including glycerol or ions, and in the fungal kingdom, five kinds of aquaporins have been described which are subdivided into orthodox aquaporins

and aquaglyceroporins (Pettersson et al. 2005). Only one putative aquaporin gene has so far been described from the Glomeromycota (Aroca et al. 2009) although the beneficial effects of the symbiotic fungi include transport of water from soil to host plant roots (Smith and Read 1998). The function of the fungal gene *GintAQP1* (Figure 4), identified in *G. intraradices*, has not yet been demonstrated. However, *GintAQP1* expression shows a variable response to different environmental stress and host plant, and it has been inferred that a certain compensatory mechanism exists between *GintAQP1* and host aquaporin gene expression pointing to communication mechanisms between mycelium and host root (Aroca et al. 2009).

2. Sugar transporter genes

Although in arbuscular mycorrhiza, potential carbon compounds delivered to the fungal symbiont by the plant partner are soluble sugars, carboxylic acids, and amino acid, **hexoses are considered to be the main source of carbon for the fungus** (Bago et al. 2000). In spite of this, only one gene encoding a **monosaccharide transporter** (*GpMST1*, Figure 4) has been characterized from a glomeromycotan species (Schüssler et al. 2006, 2007). The gene was isolated from the unusual and culturable glomeromycotan fungus *Geosiphon pyriformis* which forms a unique symbiosis with cyanobacteria. The encoded protein, GpMST1, is a membrane domain transporter, which phylogenetically belongs to a new, not yet characterized, monosaccharide transporter (MST) clade. It functions as proton co-transporter (Figure 4) with highest affinity for glucose, followed by mannose, galactose, and fructose. The *GpMST1* gene has a very low GC content (32%), typical of the Glomeromycota (see Section IIb) and contains 6 introns with unusual boundaries. *G. pyriformis* is not able to take up glucose, as is the case of 'typical' arbuscular mycorrhizal Glomeromycota *via* the non-symbiotic plasma membrane (spores, germination hyphae, extraradical hyphae). It is supposed that GpMST1 may represent the type of MST that is responsible for the uptake of plant carbohydrates by 'typical' glomeromycotan fungi at the symbiotic interface in mycorrhizal interactions. As such, it should enable the isolation and characterization of orthologues in mycorrhiza-forming Glomeromycota, and so lead to a much better understanding symbiotic carbon fluxes.

3. Plasma membrane H^+-ATPase encoding genes

The activity of **proton-coupled membrane transporters** like phosphate or hexose transporters is dependent on electrochemical gradients which correspond to the H^+ gradient created by H^+-ATPases in the membranes. Plasma membrane H^+-ATPases are generally found in higher concentrations or activities in cell types that are specialised for intensive active transport, as is the case of the fungal and plant membranes at the symbiotic interface of arbuscular mycorrhiza (Gianinazzi-Pearson et al. 1991, 2000; Harrison 2005). In the Glomeromycota, two **H^+-ATPase genes**, *GmPMA1* (Requena et al. 2003) and *GmHA5* (Ferrol et al. 2000; Requena et al. 2003), have been isolated from the genome of *G. mosseae* (Figure

4). *GmPMA1* is highly expressed during asymbiotic development, but its expression is not modified during symbiotic interactions with host roots, whereas *GmHA5* is induced upon plant recognition at the appressorium stage and up-regulated in the arbuscule (Balestrini et al. 2007). Both genes are highly expressed during intraradical development, but their expression is reduced in the ERM. Phosphate, the key nutrient transferred from fungus to plant in the symbiosis, induces *GmHA5* expression during asymbiotic growth, whereas sucrose has a negative effect. It has been suggested that different fungal H^+-ATPases isoforms might be recruited at different developmental stages of the mycorrhizal fungus, possibly in response to the different requirements during its life cycle (Requena et al. 2003).

With wider coverage of the transcriptome of *G. intraradices*, it is expected that many more glomeromycotan transporters will be characterized. Deciphering the function of individual proteins of the entire transportome, as well as understanding how transporters from intracellular organelles work together with those from the plasma membrane in different fungal structures and how they are regulated to ensure homeostasis, will give more insight into the symbiotic life style and physiology of these obligate biotrophs.

V. Concluding Remarks

Although obligate biotrophy, multinucleate nature, large genomes and asexuality of glomeromycotan fungi forming root symbiosis still remain challenges to understanding genome complexity, diversity and function in these organisms, the past decade has seen considerable advances in knowledge about their genomic make-up. Characterisation of nuclear and mitochondrial genomes in the Glomeromycota has reinforced conclusions that they form a distinct, heterogeneous (Schussler et al. 2001, James et al. 2006) and unusual (Gianinazzi-Pearson et al. 2001, 2004; Sanders and Croll 2010) ensemble within the fungal kingdom. However, much of the information generated by more recent research focuses on *G. intraradices* (syn *G. irregulare*) as a model species and although symbiotic attributes of the genome may be broadly based across the Glomeromycota, basic parameters need to be more widely explored in order to draw conclusions for other members of the phylum. Also, whilst strategies like wide genome sequencing and comparative genomics have become amenable to research on glomeromycotan fungi, the lack of a stable transformation system remains a major drawback to manipulating their genome for functional studies of genes (Gianinazzi-Pearson et al. 2004, Helber and Requena 2008). The magnitude of intraspecific diversity within the genome still needs to be clearly defined for a large number of glomeromycotan species. This implies exploring target genes as well as rDNA in a larger spectrum of isolates and establishing more extensive sequence data sets to evaluate allele frequencies within individuals.

Acknowledgements

Part of the work reported in this review article was supported by the Burgundy Regional Council and the Agence National de Recherche (project TRANSMUT, ANR- 10-BLAN-1604-01), France, the Education Ministry of China (scholarship to YL) and the Spanish Ministry of Science and Innovation (Project AGL2009-08868).

References

Akiyama K, Matsuzaki K, Hayashi H (2005) Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi. Nature 435:824-827

Allen JW, Shachar-Hill Y (2009) Sulfur transfer through an arbuscular mycorrhiza. Plant Physiol 149:549-560

Angelard C, Sanders IR (2011) Effect of segregation and genetic exchange on arbuscular mycorrhizal fungi in colonization of roots. New Phytol 189:652-657

Angelard C, Colard A, Niculita-Hirzel H, Croll D, Sanders IR (2010) Segregation in a mycorrhizal fungus alters rice growth and symbiosis-specific gene transcription. Current Biol 20:1216-1221

Aroca R, Bago A, Sutka M, Paz JA, Cano C, Amodeo G, Ruiz-Lozano JM (2009) Expression analysis of the first arbuscular mycorrhizal fungi aquaporin described reveals concerted gene expression between salt-stressed and nonstressed mycelium. Mol Plant Microbe Interact 9:1169-1178

Bago B, Pfeffer PE, Shachar-Hill Y (2000) Carbon metabolism and transport in arbuscular mycorrhizas. Plant Physiol 124:949-95

Bago B, Zipfel W, Williams RM, Jun J, Arreola R, Lammers PJ, Pfeffer PE, Shachar-Hill Y (2002) Translocation and utilization of fungal storage lipid in the arbuscular mycorrhizal symbiosis. Plant Physiol 128:108-124

Bago B, Pfeffer PE, Abubaker J, Jun J, Allen JW, Brouillette J, Douds DD, Lammers PJ, Shachar-Hill Y (2003) Carbon export from arbuscular mycorrhizal roots involves the translocation of carbohydrate as well as lipid. Plant Physiol 131:1496-1507

Balestrini R, Gómez-Ariza J, Lanfranco L, Bonfante P (2007) Laser microdissection reveals that transcripts for five plant and one fungal phosphate transporter genes are contemporaneously present in arbusculated cells. Mol Plant Microbe Interact 9:1055-1062

Benabdellah K, Azcón-Aguilar C, Ferrol N (2009a) GintGRX1, the first characterized glomeromycotan glutaredoxin, is a multifunctional enzyme that responds to oxidative stress. Fungal Gen Biol 46:94-104

Benabdellah K, Valderas A, Azcón-Aguilar C, Ferrol N (2009b) PDX1, a protein involved in vitamin B6 biosynthesis, is transcriptionally up-regulated by oxidative stress in the arbuscular mycorrhizal fungus *Glomus intraradices*. New Phytol 184:682-693

Benedetto A, Magurno F, Bonfante P, Lanfranco L (2005) Expression profiles of a phosphate transporter gene (*GmosPT*) from the endomycorrhizal fungus *Glomus mosseae*. Mycorrhiza 15:620–627

Besserer A, Puech-Pages V, Kiefer P, Gomez-Roldan V, Jauneau A, Roy S, Portais JC, Roux C, Bécard G, Séjalon-Delmas N (2006) Strigolactones stimulate arbuscular mycorrhizal fungi by activating mitochondria. PLoS Biol 4:e226

Besserer A, Bécard G, Jauneau A, Roux C, Séjalon-Delmas N (2008) GR24, a synthetic analog of strigolactones, stimulates the mitosis and growth of the arbuscular mycorrhizal fungus *Gigaspora rosea* by boosting its energy metabolism. Plant Physiol 148:402-413

Bestel-Corre G, Dumas-Gaudot E, Poinsot V, Dieu M, van Tuinen D, Remacle J, Gianinazzi-Pearson V Gianinazzi S (2002) Proteome analysis and identification of symbiosis-related proteins from *Medicago truncatula* Gaertn.by two-dimensional electrophoresis and mass spectrometry. Electrophoresis 23: 122–137

Bever JD, Wang M (2005) Arbuscular mycorrhizal fungi - Hyphal fusion and multigenomic structure. Nature 433, E3-E4.

Bianciotto V, Bonfante P (1993) Evidence of DNA replication in an arbuscular mycorrhizal fungus in the absence of a host plant. Protoplasma 176:100–105.

Bianciotto V, Barbiero G, Bonfante P (1995) Analysis of the cell cycle in an arbuscular mycorrhizal fungus by flow cytometry and bromodeoxyuridine labelling. Protoplasma 188:161–169.

Bidochka MJ, Khachatourians GG (1990). Identification of *Beauveria bassiana* extracellular protease as a virulence factor in pathogenicity toward the migratory grasshopper, *Melanoplus sanguinipes*. J Invert Pathol 56:362–370.

Börstler B, Thiery O, Sykorova Z, Berner A, Redecker D (2010) Diversity of mitochondrial large subunit rDNA haplotypes of *Glomus intraradices* in two agricultural field experiments and two semi-natural grasslands. Mol Ecol 19:1497-1511

Brechenmacher L, Weidmann S, van Tuinen D, Chatagnier O, Gianinazzi S, Franken P, Gianinazzi-Pearson V (2004) Expression profiling of up-regulated plant and fungal genes in early and late stages of *Medicago truncatula-Glomus mosseae* interactions. Mycorrhiza 14:253-262

Breuninger M, Requena N (2004) Recognition events in AM symbiosis: analysis of fungal gene expression at the early appressorium stage. Fungal Gen Biol 41:794-804

Burt A, Carter DA, Koenig GL, White TJ, Taylor JW (1996) Molecular markers reveal cryptic sex in the human pathogen *Coccidioides immitis*. Proc Natl Acad Sci USA 93:770-773

Cappellazzo G, Lanfranco L, Fitz M, D. Wipf D, Bonfante P (2008). Characterization of an amino acid permease from the endomycorrhizal fungus *Glomus mosseae*. Plant Physiol 147:429-437

Carpentier SC, Panis B, Vertommen A, Swennen R, Sergeant K, Renaut J, Laukens K, Witters E, B. Samyn, Devreese B (2008) Proteome analysis of non-model organisms : a challenging but powerful approach. Mass Spectrom. Rev 27: 354-37

Casana MC, Bonfante P (1988) Intercellular hyphae and arbuscules of *Glomus fasciculatum* (Thaxter) Gerd & Trappe isolated after enzymatic digestion. Allionia 25:17-25

Chevalier BS, Stoddard BL (2001) Homing endonucleases: structural and functional insight into the catalysts of intron/intein mobility. Nucleic Acids Res 29:3757-3774.

Clapp JP, Fitter AH, Young JPW (1999) Ribosomal small subunit sequence variation within spores of an arbuscular mycorrhizal fungus, *Scutellospora* sp. Mol Ecol 8:915-921.

Croll D, Sanders IR (2009) Recombination in *Glomus intraradices*, a supposed ancient asexual arbuscular mycorrhizal fungus. BMC Evol Biol 9: 13.

Croll D, Giovannetti M, Kich AM, Sbrana C, Ehinger M, Lammers PJ, Sanders IR (2009) Nonself vegetative fusion and genetic exchange in the arbuscular mycorrhizal fungus *Glomus intraradices*. New Phytol 181:924-937

Dalgaard JZ, Klar AJ, Moser MJ, Holley WR, Chatterjee A, Mian IS (1997) Statistical modeling and analysis of the LAGLIDADG family of site-specific endonucleases and identification of an intein that encodes a site-specific endonuclease of the HNH family. Nucleic Acids Res 25:4626-4638

Delp G, Smith SE, Barker SJ (2000) Isolation by differential display of three partial cDNAs potentially coding for proteins from the VA mycorrhizal *Glomus intraradices*. Mycol Res 104:293-300

den Bakker HC, VanKuren NW, Morton JB, Pawlowska TE (2010) Clonality and recombination in the life history of an asexual arbuscular mycorrhizal fungus. Mol Biol Evol 27 :2474-2486.

Dumas-Gaudot E, Valot B, Bestel-Corre G, Recorbet G, St-Arnaud M, Dieu M, Raes M, Saravanan RS, Gianinazzi S (2004) Proteomics as a way to identify extra-radicular fungal proteins from *Glomus intraradices*-RiT-DNA carrot roots. FEMS Microbiol Ecol 48:401-411

Ferrol N, Barea JM, Azcón -Aguilar C (2000) The plasma membrane H^+-ATPase gene family in the arbuscular mycorrhizal fungus *Glomus mosseae*. Curr Genet 37:112–118

Ferrol N, Azcón-Aguilar C, Bago B, Franken P, Gollotte A, González-Guerrero M, Harrier LA, Lanfranco L, van Tuinen D, Gianinazzi-Pearson V (2004) Genomics of arbuscular mycorrhizal fungi. In: Arora DK, Khachatourians GG (eds) Applied Mycology & Biotechnology, vol 4, Fungal Genomics. Elsevier Science BV, St Louis, pp 379-403

Fontaneto D, Herniou EA, Boschetti C, Caprioli M, Melone G, Ricci C, Barraclough TG (2007) Independently evolving species in asexual bdelloid rotifers. Plos Biol 5: 914-921.

Gandolfi A, Bonilauri P, Rossi V, Menozzi P (2001) Intraindividual and intraspecies variability of ITS1 sequences in the ancient asexual *Darwinula stevensoni* (Crustacea : Ostracoda). Heredity 87:449-455.

Gemayel R, Vinces MD, Legendre M, Verstrepen KJ (2010) Variable tandem repeats accelerate evolution of coding and regulatory sequences. Ann Rev Genet 44:445-468

Gianinazzi S, Gollotte A, Binet M-N, van Tuinen D, Redecker D, Wipf D (2010) Agroecology: the key role of arbuscular mycorrhizas in ecosystem services. Mycorrhiza 20:519-530

Gianinazzi-Pearson V, Smith SE, Gianinazzi S, Smith FA (1991) Enzymatic studies on the metabolism of vesicular-arbuscular mycorrhizas. V. Is H^+-ATPase a component of ATP-hydrolysing enzyme activities in plant- fungus interfaces? New Phytol 117:61-74

Gianinazzi-Pearson V, Arnould C, Oufattole M, Arango M, Gianinazzi S (2000) Differential activation of H+ -ATPase genes by an arbuscular mycorrhizal fungus in root cells of transgenic tobacco. Planta 211:609-613

Gianinazzi-Pearson V, van Tuinen D, Dumas-Gaudot E, Dulieu H (2001) Exploring the genome of glomalean fungi. In: Hock B (ed) The Mycota IX. Springer-Verlag, Berlin, Heidelberg, pp 3-17

Gianinazzi-Pearson V, Azcón-Aguilar C, Bécard G, Bonfante P, Ferrol N, Franken P, Gollotte A, Harrier LA, Lanfranco L, van Tuinen D (2004) Structural and functional genomics of symbiotic arbuscular mycorrhizal fungi. In: Tkacz JS, Lange L (eds) Advances in Fungal Biotechnology for Industry, Agriculture and Medicine. Kluwer Academic/Plenum Publishers, New York, Boston, Dordrecht, London, Moscow, pp 405-424

Gianinazzi-Pearson V., Séjalon-Delmas N., Genre A., Jeandroz S., Bonfante P., 2007. Plants and arbuscular mycorrhizal fungi: cues and communication in the early steps of symbiotic interactions. Advances in Botanical Research 46:181-219.

Giovannetti M, Fortuna P, Citernesi AS, Morini S, Nuti MP (2001) The occurrence of anastomosis formation and nuclear exchange in intact arbuscular mycorrhizal networks. New Phytol 151:717-724

Giovannetti M, Sbrana C, Strani P, Agnolucci M, Rinaudo V, Avio L (2003) Genetic diversity of isolates of *Glomus mosseae* from different geographic areas detected by vegetative compatibility testing and biochemical and molecular analysis. Appl Environ Microbiol 69:616-624

Gladyshev EA, Arkhipova IR (2010) Genome structure of bdelloid rotifers: shaped by asexuality or desiccation? J Heredity 101:S85-S93

Gollotte A, L'Haridon F, Chatagnier O, Wettstein G, Arnould C., van Tuinen D, Gianinazzi-Pearson V (2006) Repetitive DNA sequences include retrotransposons in genomes of the Glomeromycota**.** Genetica 128:455-46

Gomez SK, Javot H, Deewatthanawong P, Torres-Jerez I, Tang Y, Blancaflor EB, Udvardi MK, Harrison MJ (2009) *Medicago truncatula* and *Glomus intraradices* gene expression in cortical cells harboring arbuscules in the arbuscular mycorrhizal symbiosis. BMC Plant Biol 9:10

González-Guerrero M, Azcón-Aguilar C, Mooney M, Valderas A, MacDiarmid CW, Eide DJ, Ferrol N (2005) Characterization of a *Glomus intraradices* gene encoding a putative Zn transporter of the cation diffusion facilitator family. Fungal Gen Biol 42:130-140

González-Guerrero M, Cano C, Azcón-Aguilar C, Ferrol N (2007) *GintMT1* encodes a functional metallothionein in *Glomus intraradices* that responds to oxidative stress. Mycorrhiza 17:327-335

González-Guerrero M, Benabdellah K, Valderas A, Azcón-Aguilar C, Ferrol N (2010a) *GintABC1* encodes a putative ABC transporter of the MRP subfamily induced by Cu, Cd and oxidative stress in *Glomus intraradices*. Mycorrhiza 20:137-146

González-Guerrero M, Oger E, Benabdellah K, Azcón-Aguilar C, Lanfranco L, Ferrol N (2010b) Characterization of a Cu,Zn superoxide dismutase gene in the arbuscular mycorrhizal fungus *Glomus intraradices*. Curr Genet 56:265-274

Govindarajulu M, Pfeffer PE, Jin HR, Abubaker J, Douds DD, Allen JW, Bucking H, Lammers PJ, Shachar-Hill Y (2005) Nitrogen transfer in the arbuscular mycorrhizal symbiosis. Nature 435:819-823

Harrier LA, Wright F, Hooker JE (1998) Isolation of the 3-phosphoglycerate kinase gene of the arbuscular mycorrhizal fungus *Glomus mosseae* (Nicol. & Gerd.) Gerdemann & Trappe. Current Genet 34:86-392

Harrison MJ (2005) Signaling in the arbuscular mycorrhizal symbiosis. Annu Rev Microbiol 59:19-42

Harrison MJ, van Buuren ML (1995) A phosphate transporter from the mycorrhizal fungus *Glomus versiforme*. Nature 378:626-629

Heckman DS, GeiserDM, Eidell BR, Stauffer RL, Kardos NL, Hedges SB (2001) Molecular evidence for the early colonization of land by fungi and plants. Science 293:1129-1133

Helber N, Requena N (2008) Expression of the fluorescence markers DsRed and GFP fused to a nuclear localization signal in the arbuscular mycorrhizal fungus *Glomus intraradices*. New Phytol 177**:**537 – 548

Heupel S, Roser B, Kuhn H, Lebrun MH, Villalba F, Requena N (2009) Erl1, a novel Era-like GTPase from *Magnaporthe oryzae*, is required for full root virulence and is conserved in the mutualistic symbiont *Glomus intraradices*. Mol Plant Microbe Interact 23:67–81

Hijri M, Sanders IR (2004) The arbuscular mycorrhizal fungus *Glomus intraradices* is haploid and has a small genome in the lower limit of eukaryotes. Fungal Gen Biol 41:253-261

Hijri M, Sanders IR (2005) Low gene copy number shows that arbuscular mycorrhizal fungi inherit genetically different nuclei. Nature 433:160-163

Hildebrandt U, Ouziad F, Marner FJ, Bothe H (2006) The bacterium *Paenibacillus validus* stimulates growth of the arbuscular mycorrhizal fungus *Glomus intraradices* up to the formation of fertile spores. FEMS Microbiol Lett 254:258-267

Hosny M, Gianinazzi-Pearson V, Dulieu H (1998a) Nuclear contents of eleven fungal species in Glomales. Genome 41:422-428

Hosny M, Païs de Barros, Gianinazzi-Pearson V, Dulieu H (1998b) Base composition of DNA from glomalean fungi: high amounts of methylated cytosine. Fungal Gen Biol 22:103-111

James TY, Kauff F, Schoch CL, Matheny PB, Hofstetter V, Cox C, Celio G, Gueidan C, Fraker E, Miadlikowska J et al. (2006) Reconstructing the early evolution of the fungi using a six gene phylogeny. Nature 443:818–822

Jany JL, Pawlowska TE (2010) Multinucleate spores contribute to evolutionary longevity of asexual Glomeromycota. Amer Nat 175:424-435.

Journet EP, van Tuinen D, Gouzy J, Crespeau H, Carreau V, Farmer MJ, Niebel A, Schiex T, Jaillon O, Chatagnier O, Godiard L, Micheli F, Kahn D, Gianinazzi-Pearson V, Gamas P (2002) Exploring root symbiotic programs in the model legume *Medicago truncatula* using EST analysis. Nucleic Acids Res 30:5579-5592

Judson OP, Normark BB (1996) Ancient asexual scandals. Trends Ecol Evol 11:A41-A46

Jun J, Abubaker J, Rehrer C, Pfeffer PE, Shachar-Hill Y, Lammers PJ (2002) Expression in an arbuscular mycorrhizal fungus of genes putatively involved in metabolism, transport, the cytoskeleton and the cell cycle. Plant Soil 244:141-148

Kloppholz S, Kuhn H, Requena N (2011) A secreted fungal effector of *Glomus intraradices* promotes symbiotic biotrophy. Current Biol 21 : 1204-1209.

Kuhn G, Hijri M, Sanders IR (2001) Evidence for the evolution of multiple genomes in arbuscular mycorrhizal fungi. Nature 414, 745-748

Kuznetsova E, Seddas-Dozolme PMA, Arnould C, Tollot M, van Tuinen D, Borisov A, Gianinazzi S, Gianinazzi-Pearson V (2010) Symbiosis–related pea genes modulate fungal and plant gene expression during the arbuscule stage of mycorrhiza with *Glomus intraradices*. Mycorrhiza 20:427-443

Lammers PJ, Jun J, Abubaker J, Arreola R, Gopalan A, Bago B, Hernandez-Sebastia C, Allen JW, Douds DD, Pfeffer PE, Shachar-Hill Y (2001) The glyoxylate cycle in an arbuscular mycorrhizal fungus. Carbon flux and gene expression. Plant Physiol 127:1287-1298

Lammers P, Tuskan GA, DiFazio SP, Podila GK, Martin F (2004) Mycorrhizal symbionts of *Populus* to be sequenced by the United States Department of Energy's Joint Genome Institut. Mycorrhiza 14 :63-64

Lanfranco L, Bolchi A, Cesale RE, Ottonello S, Bonfante P (2002) Differential expression of a metallothionein gene during the presymbiotic versus the symbiotic phase of an arbucular mycorrhizal fungus. Plant Physiol 130:58-67

Lanfranco L, Novero M, Bonfante P (2005) The mycorrhizal fungus *Gigaspora margarita* possesses a CuZn superoxide dismutase which is up-regulated during the symbiosis with legume hosts. Plant Physiol 137:1319-1330

Leake J, Johnson D, Donnelly D, Muckle G, Boddy L, Read D (2004) Networks of power and influence: the role of mycorrhizal mycelium in controlling plant communities and agroecosystem functioning. Can J Bot 82:1016–1045.

Lee J, Young JPW (2009) The mitochondrial genome sequence of the arbuscular mycorrhizal fungus *Glomus intraradices* isolate 494 and implications for the phylogenetic placement of *Glomus*. New Phytol 183:200-211

Li ZS, Szczypka M, Lu YP, Thiele DJ, Rea PA (1996) The yeast cadmium factor protein (YCF1) is a vacuolar glutathione S-conjugate pump. J Biol Chem 271:6509–6517

Lopez-Pedrosa A, Gonzalez-Guerrero M, Valderas A, Azcon-Aguilar C, Ferrol N (2006) *GintAMT1* encodes a functional high-affinity ammonium transporter that is expressed in the extraradical mycelium of *Glomus intraradices*. Fungal Genet Biol 43:102–110

Maldonado-Mendoza IE, Dewbre GR, Harrison MJ (2001) A phosphate transporter gene from the extraradical mycelium of an arbuscular mycorrhizal fungus *Glomus intraradices* is regulated in response to phosphate in the environment. Mol Plant-Microb Interact 14:1140–1148

Maldonado-Mendoza IE, Dewbre GR, Buuren MLv, Versaw WK, Harrison MJ (2002) Methods to estimate the proportion of plant and fungal RNA in an arbuscular mycorrhiza. Mycorrhiza 12:67-74

Martin F, Aerts A, Ahren D, Brun A, Danchin EGJ, Duchaussoy F, Gibon J, Kohler A, Liindquist E, Pereda V, et al. (2008a) The genome of *Laccaria bicolor* provides insights into mycorrhizal symbiosis. Nature 452:88-92

Martin F, Gianinazzi-Pearson V, Hijri M, Lammers PJ, Requena N, Sanders IR, Shachar-Hill Y, Shapiro H, Tuskan GA, Young PY (2008b) The long hard road to a completed *Glomus intraradices* genome. New Phytol 180:747-750

Martin F, Kohler A, Murat C, Balestrini R, Coutinho PM, Jaillon O, Montanini B, Morin E, Noel B, Percudani R, et al. (2010) Périgord Black Truffle genome uncovers evolutionary origins and mechanisms of symbiosis. Nature 464:1033-1038

Mitchell JI, Roberts PJ, Moss ST (1995) Sequence or structure? A short review on the application of nuclei acid sequence information to fungal taxonomy. Mycologist 9, 67-75

Morton JB (1993) Problems and solutions for the integration of glomalean taxonomy, systematics biology, and the study of endomycorrhizal phenomena. Mycorrhiza 2:97-109

Muller HJ (1932) Some Genetic Aspects of Sex. American Natur 66:118-138

O'Donnell K (1992) Ribosomal DNA internal transcribed spacers are highly divergent in the phytopathogenic ascomycete *Fusarium sambucinum (Gibberella pulicaris)*. Curr Gen 22:213-220.

Oeljeklaus S, Meyer HE. Warscheid B (2008) Advancements in plant proteomics using quantitative mass spectrometry. J Proteomics 72:545–554

Ouziad F, Hildebrandt U, Schmelzer E, Bothe H (2005) Differential gene expressions in arbuscular mycorrhizal-colonized tomato grown under heavy metal stress. J Plant Physiol 162:634-649

Pawlowska TE, Taylor JW (2004) Organization of genetic variation in individuals of arbuscular mycorrhizal fungi. Nature 427:733–737

Pettersson N, Filipsson C, Becit E, Brive L, Hohmann S (2005) Aquaporins in yeasts and filamentous fungi. Biol Cell 97:487-500

Pivato B, Mazurier S, Lemanceau P, Siblot S, Berta G, Mougel C, van Tuinen D (2007) *Medicago* species affect the community composition of arbuscular mycorrhizal fungi associated with roots. New Phytol 176:197-210

Purin S, Morton JB (2011) *In situ* analysis of anastomosis in representative genera of arbuscular mycorrhizal fungi. Mycorrhiza 21:505-514

Raab PA, Breenwald A, Redecker D (2005) Mitochondrial large ribosomal sub-unit sequences are homogenous within isolates of *Glomus* (arbuscular mycorrhizal fungi, Glomeromycota). Mycol Res 109:1315-1322

Recorbet G, Rogniaux H., Gianinazzi-Pearson V., Dumas-Gaudot E (2009). Fungal proteins in the extra-radical phase of mycorrhiza: a shotgun proteomic picture. New Phytol 181:248–260.

Recorbet G, Valot B, Robert F, Gianinazzi-Pearson V, Dumas-Gaudot E (2010) Identification of *in planta*-expressed arbuscular mycorrhizal fungal proteins upon comparison of the root proteomes of *Medicago truncatula* colonised with two *Glomus* species. Fungal Genet Biol 47:608-18

Reddy PV, Lam CK, Belanger FC (1996) Mutualistic fungal endophytes express a proteinase that is homologous to proteases suspected to be important in fungal pathogenicity. Plant Physiol 111:1209–1218.

Redecker D (2000) Specific PCR primers to identify arbuscular mycorrhizal fungi within colonized roots. Mycorrhiza 10:73-80

Requena N, Mann P, Hampp R, Franken P (2002) Early developmentally regulated genes in the arbuscular mycorrhizal fungus *Glomus mosseae*: Identification of *GmGIN*1, a novel gene with homology to the C-terminus of metazoan hedgehog proteins. Plant Soil 244:129-139

Requena N, Breuninger M, Franken P, Ocon A (2003) Symbiotic status, phosphate, and sucrose regulate the expression of two plasma membrane H^+-ATPase genes from the

mycorrhizal fungus *Glomus mosseae*. Plant Physiol 132:1540-1549

Rohrbough JG, Galgiani, JN, Wysocki VH (2007) The application of proteomic techniques to fungal protein identification and quantification. Ann NY Acad Sci 1111:133–146

Rosendahl S (2008) Communities, populations and individuals of arbuscular mycorrhizal fungi. New Phytol 178:253-266

Rosendahl S, Taylor JW (1997) Development of multiple genetic markers for studies of genetic variation in arbuscular mycorrhizal fungi using AFLP. Mol Ecol 6:821-829

Rosendahl S, McGee P, Morton JB (2009) Lack of global population genetic differentiation in the arbuscular mycorrhizal fungus *Glomus mosseae* suggests a recent range expansion which may have coincided with the spread of agriculture. Mol Ecol 18:4316-4329

Sanders IR, Alt M, Groppe K, Boller T, Wiemken A (1995) Identification of ribosomal DNA polymorphisms among and within spores of the Glomales: application to studies on the genetic diversity of arbuscular mycorrhizal fungal communities. New Phytol 130:419-427

Sanders IR, Croll D (2010) Arbuscular mycorrhiza: a challenge to understand the genetics of the fungal partner. Ann Rev Genet 44:271-292

Sawaki H, Saito M (2001) Expressed genes in the extraradical hyphae of an arbuscular mycorrhizal fungus, *Glomus intraradices*, in the symbiotic phase. FEMS Microbiol Lett 195:109-113

Schüssler A, Schwarzott D, Walker C (2001) A new fungal phylum, the Glomeromycota: phylogeny and evolution. Mycol Res 105:1413-1421

Schüssler A, Martin H, Cohen D, Fitz M, Wipf D (2006) Characterization of a carbohydrate transporter from symbiotic glomeromycotan fungi. Nature 444:933-936

Schüssler A, Martin H, Cohen D, Fitz M, Wipf D (2007) Arbuscular mycorrhiza - the first glomeromycotan sugar transporter is isolated and characterized. Plant Signal Behav 2:314-317

Schüssler A, Walker C (2010) The Glomeromycota: a species list with new families and new genera. Royal Botanic Garden Edinburgh, Kew; Botanische Staatssammlung, Munich; Oregon State University; www.amf-phylogeny.com

Seddas PMA, Arias CM, Arnould C, van Tuinen D, Godfroy O, Benhassou HA, Gouzy J, Morandi D, Dessaiint F, Gianinnazzi-Pearson V (2009) Symbiosis-related plant genes modulate molecular responses in an arbuscular mycorrhizal fungus during early root interactions. Mol Plant Microbe Interact 22:341-351

Simon L, Lalonde M, Bruns TD (1992) Specific amplification of the 18S fungal ribosomal genes from vesicular-arbuscular endomycorrhizal fungi colonizing roots. Appl Environ Microbiol 58:291-295.

Simon L, Bousquet J, Lévesque RC, Lalonde M (1993) Origin and diversification of endomycorrhizal fungi and coincidence with vascular land plants. Nature 363:67-69

Spanu PD, Abbott JC, Amselem J, Burgis TA, Soanes DM, Stüber K, van Themaat EVL, Brown JKM et al. (2010) Genome expansion and gene loss in powdery mildew fungi reveal tradeoffs in extreme parasitism. Science 330:1543-1546

Smith SE, Read DJ (2008) Mycorrhizal symbiosis. Academic Press, London, New York

Sreedhar L, Kobayashi DY, Bunting TE, Hillman BI, Belanger FC (1999) Fungal proteinase expression in the interaction of the plant pathogen *Magnaporthe poae* with its host. Gene 235:121–129

Stommel M, Mann P, Franken P (2001) Construction and analysis of an EST library using RNA from activated spores of the arbuscular mycorrhizal fungus *Gigaspora rosea*. Mycorrhiza 10:281-285

Stockinger H, Walker C, Schussler A (2009) '*Glomus intraradices* DAOM197198', a model fungus in arbuscular mycorrhiza research, is not *Glomus intraradices*. New Phytol 183:1176-1187

Sýkorová Z, Börstler B, Zvolenská S, Fehrer J, Gryndler M, Vosátka M, Redecker D (2011) Long-term tracing of *Rhizophagus irregularis* isolate BEG140 inoculated on *Phalaris arundinacea* in a coalmine spoil bank, using mitochondrial large subunit rDNA markers. Mycorrhiza DOI: 10.1007/s00572-011-0375-1.

Tamasloukht MB, Séjalon-Delmas N, Kluever A, Jauneau A, Roux C, Bécard G, Franken P (2003) Root factors induce mitochondrial-related gene expression and fungal respiration during development switch from asymbiosis to presymbiosis in the arbuscular mycorrhizal fungus *Gigaspora rosea*. Plant Physiol 131:1468-1478

Tamasloukht MB, Waschke A, Franken P (2007) Root exudates-stimulated RNA accumulation in the arbuscular mycorrhizal fungus *Gigaspora rosea*. Soil Biol Biochem 39:1824-1827

Thiéry O, Börstler B, Ineichen K, Redecker D (2010) Evolutionary dynamics of introns and homing endonuclease ORFs in a region of the large subunit of the mitochondrial rRNA in *Glomus* species (arbuscular mycorrhizal fungi, Glomeromycota). Mol Phylogen Evol 55:599-610

Tian C, Kasiborski B, Koul R, Lammers PJ, Bücking H, Shachar-Hill Y (2010) Regulation of the nitrogen transfer pathway in the arbuscular mycorrhizal symbiosis: gene characterization and the coordination of expression with nitrogen flux. Plant Physiol 153:1175-1187

Tisserant E, Kohler A, Dozolme-Seddas P, Balestrini R, Benabdellah K, Colard A, Croll D, Da Silva C, Gomez SK, Koul R, Ferrol N, Fiorilli V, Formey D, Franken Ph, Helber N, Hijri M, Lanfranco L, Lindquist E, Liu Y, Malbreil M, Morin E, Poulain J, Shapiro H, van Tuinen D, Waschke A, Azcón-Aguilar C, Bécard G, Bonfante P, Harrison MJ, Lammers P, Paszkowski U, Requena N, Rensing SA, Roux C, Sanders IR, Shachar-Hill Y, Tuskan G, Young JPW, Gianinazzi-Pearson V, Martin F The transcriptome of the arbuscular

mycorrhizal fungus *Glomus intraradices* reveals functional tradeoffs in an obligate symbiont. New Phytol (submitted)

Tollot M, Wong Sak Hoi J, van Tuinen D, Arnould C, Chatagnier O, Dumas B,Gianinazzi-Pearson V, Seddas PM (2009) An STE12 gene identified in the mycorrhizal fungus *Glomus intraradices* restores infectivity of a hemibiotrophic plant pathogen. New Phytol 181:693–707

Tommerup IC, Sivasithamparam K (1990) Zygospores and asexual spores of *Gigaspora decipiens*, an arbuscular mycorrhizal fungus. Mycol Res 94 :897-900

Tunlid A, Rosen SE, Rask L (1994) Purification and characterization of an extracellular serine protease from the nematode-trapping fungus *Arthrobotrys oligospora*. Microbiology 140, 1687–1695.

van Tuinen D, Jacquot E, Zhao B, Gollotte A, Gianinazzi-Pearson V (1998) Characterization of root colonization profiles by a microcosm community of arbuscular mycorrhizal fungi using 25S rDNA-targeted nested PCR. Mol Ecol 7:879-887

Waschke A, Sieh D, Tamasloukht M, Fischer K, Mann P, Franken P (2006) Identification of heavy metal-induced genes encoding glutathione S-transferases in the arbuscular mycorrhizal fungus *Glomus intraradices*. Mycorrhiza 17:1-10

Young JPW (2008) The genetic diversity of intraterrestrial aliens. New Phytol 178:465-468

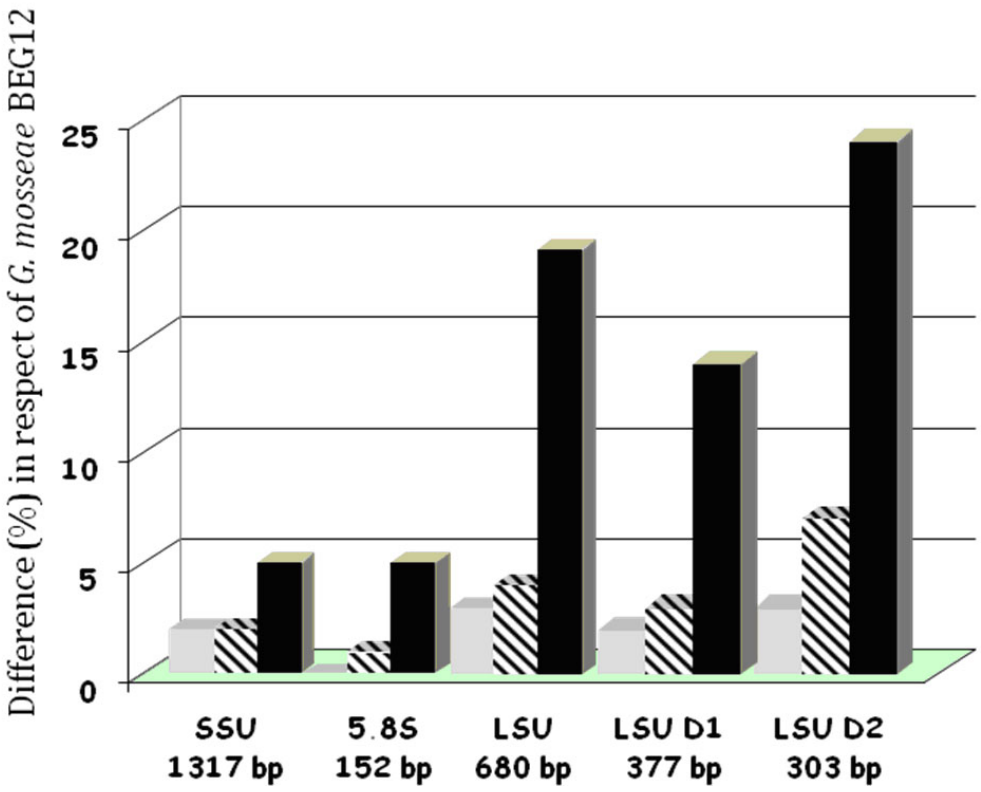
Figure Legends

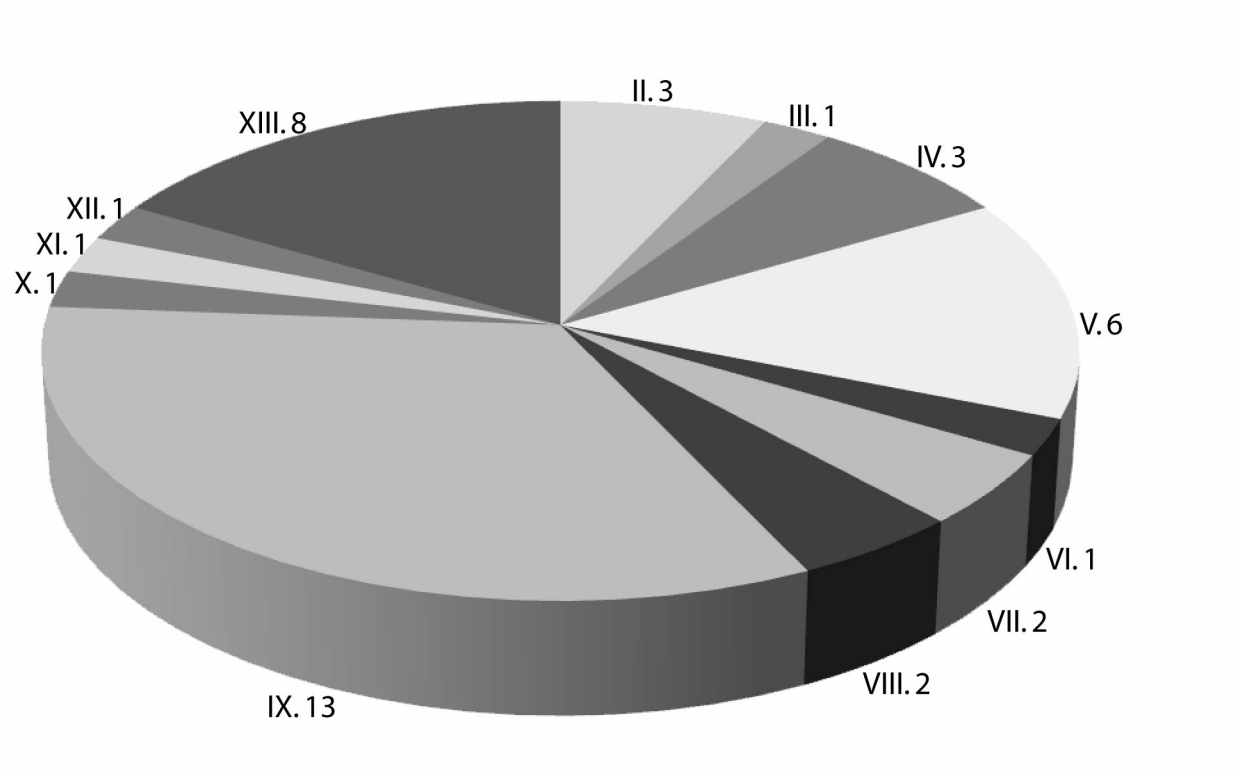
Figure 1. Differences between sequences of *Glomus mosseae* (BEG12), expressed as a percentage, and *G. mosseae* BEG69 (▫), *G. coronatum* BEG22 (▨) and *G. intraradices* AFTOL ID 48 (■), for the SSU, 5.8S, and 5' end of the LSU (including the variable D1 and D2 domains), and the variable D1 and D2 domains alone.

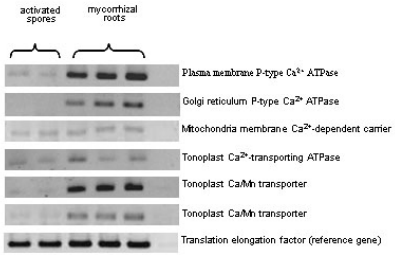
Figure 2. Functional categories (roman numbers) and numbers of putative fungal genes from a *M. truncatula*/*G. intraradices* cDNA library: II Cytoskeleton; III Membrane transport; IV Vesicular trafficking, secretion and protein sorting; V Primary metabolism; VI Secondary and hormone metabolism; VII Chromatin and DNA metabolism; VIII Gene expression and RNA metabolism; IX Protein synthesis and degradation; X Signal transduction and post-transcriptional modification; XI Miscellaneous; XII Defense and cell rescue; XIII No homology

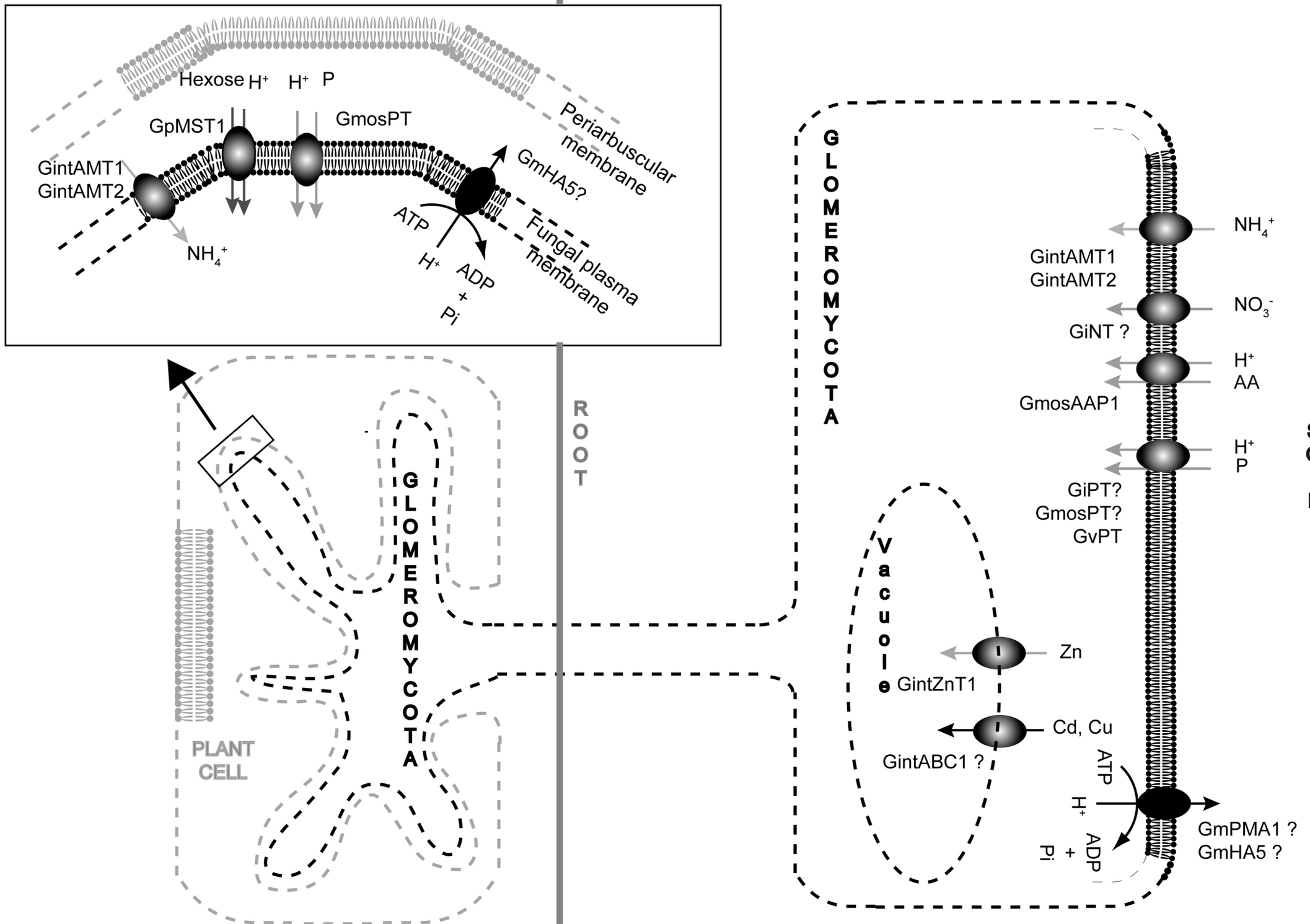
Figure 3. Expression profiling of *G. intraradices* genes encoding proteins putatively involved in calcium homeostasis; amplification by RT-PCR of cDNA from activated spores and mycorrhizal roots of *M. truncatula*.

Figure 4. Current knowledge of transporter genes from Glomeromycotan fungi and their putative role in the arbuscular mycorrhiza: GpMST1 (*Geosiphon pyriformis* monosaccharide transporter 1, Schüssler et al. 2006, 2007), GmPMA1 (*Glomus mosseae* plasma membrane ATPase 1, Requena et al. 2003), GmHA5 (*G. mosseae* H^+-ATPase, Ferrol et al. 2000), GintABC1 (*G. intraradi*ces ABC transporter 1, González-Guerrero et al. 2010), GintAMT1 (*G. intraradi*ces ammonium transporter 1, Lopez-Pedrosa et al. 2006), GintAMT2 (*G. intraradi*ces ammonium transporter 1, Pérez-Tienda et al., submitted), GiNT (*G. intraradi*ces nitrate transporter, Tian et al. 2010), GmosAAP1 (*G. mosseae* amino acid permease 1, Cappellazzo et al. 2008), GintZnT1 (*G. intraradi*ces zinc transporter 1, González-Guerrero et al. 2005), GiPT (*G. intraradi*ces phosphate transporter, Maldonado-Mendoza et al. 2001), GmosPT (*G. mosseae* phosphate transporter, Benedetto et al. 2005), GvPT (*G. versiforme* phosphate transporter, Harrison and van Buuren 1995) and GintAQP1 (*G. intraradi*ces aquaporin 1, Aroca et al. 2009). Question marks refer to transporters that have not been functionally characterized.


Difference (%) in respect of G. mosseae BEG12
25
20
15
10
5
0
SSU
1317 bp
5.8S
152 bp
LSU
680 bp
LSU D1
377 bp
LSU D2
303 bp


II. 3
III. 1
IV. 3
V. 6
VI. 1
VII. 2
VIII. 2
IX. 13
X. 1
XI. 1
XII. 1
XIII. 8


activated spores
mycorrhizal roots
Plasma membrane P-type Ca^{2+} ATPase
Golgi reticulum P-type Ca^{2+} ATPase
Mitochondria membrane Ca^{2+}-dependent carrier
Tonoplast Ca^{2+}-transporting ATPase
Tonoplast Ca/Mn transporter
Tonoplast Ca/Mn transporter
Translation elongation factor (reference gene)


Hexose
H+
H+
P
GpMST1
GmosPT
Periarbuscular membrane
GintAMT1
GintAMT2
GmHA5?
ATP
H+
ADP
+
Pi
Fungal plasma membrane
NH4+
ROOT
GLOMEROMYCOTA
PLANT CELL
GLOMEROMYCOTA
NH4+
GintAMT1
GintAMT2
NO3-
GiNT ?
H+
AA
GmosAAP1
H+
P
GiPT?
GmosPT?
GvPT
SOIL
Vacuole
Zn
GintZnT1
Cd, Cu
GintABC1 ?
ATP
H+
ADP
Pi
+
GmPMA1 ?
GmHA5 ?

Review

Cell PRESS

Sugar transporters in plants and in their interactions with fungi

Joan Doidy[1], Emily Grace[2], Christina Kühn[3], Françoise Simon-Plas[1], Leonardo Casieri[1] and Daniel Wipf[1]

[1] UMR INRA 1347, Agrosup, Université de Bourgogne, Agroécologie, Pôle Interactions Plantes Microorganismes ERL CNRS 6300, BP 86510, 21065 Dijon Cedex, France
[2] School of Environmental and Life Sciences, University of Newcastle, Callaghan, NSW 2308, Australia
[3] Institute of Biology, Department of Plant Physiology, Humboldt University, 10115 Berlin, Germany

Sucrose and monosaccharide transporters mediate long distance transport of sugar from source to sink organs and constitute key components for carbon partitioning at the whole plant level and in interactions with fungi. Even if numerous families of plant sugar transporters are defined; efflux capacities, subcellular localization and association to membrane rafts have only been recently reported. On the fungal side, the investigation of sugar transport mechanisms in mutualistic and pathogenic interactions is now emerging. Here, we review the essential role of sugar transporters for distribution of carbohydrates inside plant cells, as well as for plant–fungal interaction functioning. Altogether these data highlight the need for a better comprehension of the mechanisms underlying sugar exchanges between fungi and their host plants.

Sugar transport: from source leaves to fungal sinks

With the completion of the genome sequence of the dicot *Arabidopsis* (*Arabidopsis thaliana*) and the monocot rice (*Oryza sativa*), we learned that hexose and sucrose transport proteins belong to large multigenic families [1–4]. Main sugar transporters in plants comprising sucrose (SUTs) and monosaccharide (MSTs) transporters are members of the major facilitator superfamily and are predicted to share a common structure, with 12 transmembrane domains connected by hydrophilic loops and to function as H^+/sugar symporters. Sugar transport systems are necessary for coordination of carbon partitioning, plant development, cell to cell communication and environmental adaptation, thereby playing pivotal roles in optimal plant growth and crop yield. Taken together, sugars are not just energy rich metabolites providing fuel for cellular machinery, they also constitute key signalling molecules [5].

In plants, long distance transport from photosynthetic source leaves to sink organs comprises different crucial steps depending on species and organ types, some still under debate. Sucrose synthesized in the mesophyll is first loaded into the collection phloem, then long distance transport occurs in the transport phloem and finally sucrose is unloaded in the release phloem (Figure 1).

Alternatively, sugar can also be transferred to non-plant sinks. Indeed, plant colonization by heterotrophic organisms (mutualistic or pathogenic) represents increased sink strength. However, mechanisms of transport and transporters involved in carbon partitioning between organisms are still poorly understood. Uptake, exchanges and competition for sugar, at biotrophic interfaces, are controlled by membrane transporters and their regulation patterns are essential in determining the outcome of plant–fungal interactions and in adapting to changes in soil nutrient quantity and quality [6–8]. Recently, sugar transporters from pathogenic or mycorrhizal fungi have been identified. On the plant side an increasing number of studies are focusing on sugar transporters and their roles in plant–microorganism interactions such as the recent identification of the SWEET family [9].

The data highlight the need for a better comprehension of cellular and molecular mechanisms involved in sugar partitioning between fungi and their host plants. Here, we review the essential role of sugar transporters for the distribution of carbohydrates inside plant cells and between different plant organs, as well as for the functioning of plant–fungal interactions.

Plant sucrose transporters

All plants possess a small-sized family of sucrose transporters (SUT), also called SUC (sucrose carriers), involved in several crucial steps for long distance transport of sucrose from source to sink sites where sugars are used or stored (Figure 1). For detailed information on all SUT genes cited in this paper see Table S1 in the supplementary material online. The latest classification of SUTs describes five distinct clades: SUT1–SUT5 [10,11]. The SUT1 clade (dicot specific), the largest, comprises a single protein member responsible for sucrose phloem loading in so-called 'apoplastic loaders' species (③ in Figure 1) [12], as well as numerous paralogs thought to have a role in sucrose partitioning in sink organs [2]. The SUT3 clade (monocot specific) and SUT1 members, despite their evolutionary divergence appear to be functionally orthologous [13,14]. Newly separated from SUT3, the SUT5 clade is so far the least studied with a single protein characterized in rice [11,15]. Concerning the SUT2 clade, initially described as containing sugar sensors, some members have been reported to be functional transporters [16]. The majority of characterized SUTs exhibit sucrose affinities in the

Corresponding author: Wipf, D. (daniel.wipf@dijon.inra.fr).

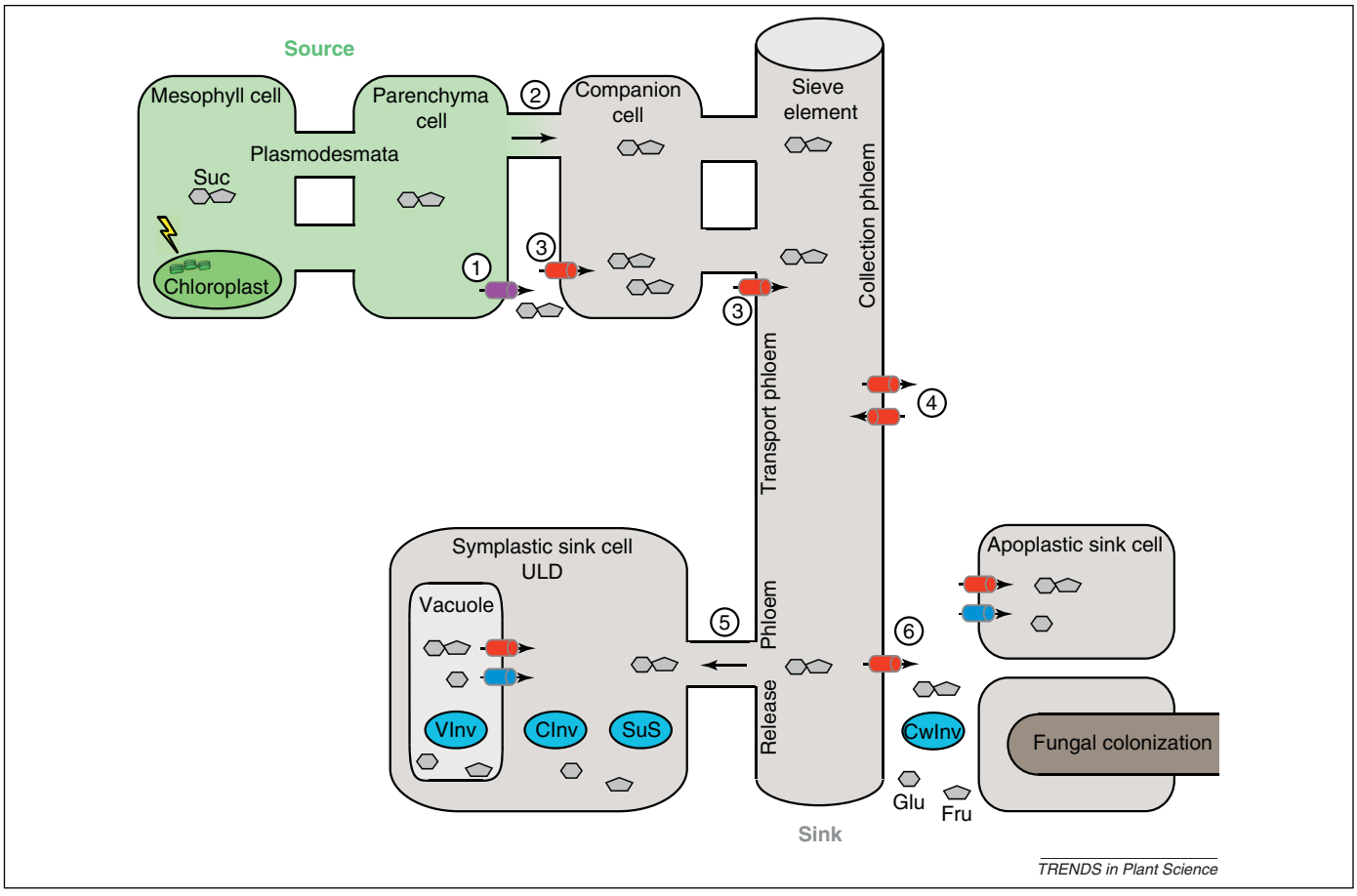


Figure 1. Long distance transport of sugars from source to sink organs in plants. In plants, sucrose (Suc) is synthesized in source leaves and constitutes the main carbohydrate form for long-distance transport; the first step consisting in sucrose export from mesophyll cells is still under debate; however, recent findings suggest that SWEET (in purple) are key components for sucrose export likely from parenchyma cells ① [23]. Following export, sucrose is either passively loaded in the companion cell sieve element complex via plasmodesmata in symplastic species ② or actively loaded by a single SUT1 (e.g. AtSUC2 in *Arabidopsis*) phloem loading protein in apoplastic species ③ [107] where trafficking of SUT1 is proposed between companion cell and enucleate sieve elements [10]. Once in the phloem sap, sucrose follows the flow of transport phloem where it can be unloaded and retrieved to supply flanking tissues ④. Upon release from the phloem, sucrose is either unloaded to symplastically connected sink organs, such as unloading domains (ULD) through plasmodesmata ⑤ or via SUTs (in red) to apoplastically connected sink cells ⑥ [21] and cleaved by sucrose-splitting enzymes (light blue) to yield glucose (Glu) and fructose (Fru) that are taken up by MSTs (in blue). Alternatively, sugar can also be transferred to fungal sinks and plant colonization by heterotrophic fungi increases sugar demand [85,86]. Sugar-splitting enzymes comprise cell wall-bound (CwInv), cytosolic (CInv) and vacuolar invertases (VInv) whereas sucrose synthase (SuS) catalyzes a reversible reaction and therefore is involved in both sucrose synthesis and catabolism.

millimolar range and also bind a large range of other naturally occurring or synthetic sugars. It is worthwhile to note, however, that biochemical properties of plant sugar transporters have been studied in heterologous systems rather than *in planta*.

Major advances concern the recent description of export capacities for sucrose transporters as well as the intracellular localization of SUT4 clade members. So far all SUTs have been characterized to mediate sucrose import, but recently the study of sucrose-induced currents of maize (*Zea mays*) ZmSUT1 in oocytes revealed an alternative transport mode [17,18]. Indeed, a rise in cytosolic sucrose concentration above 300 mM with variation of pH conditions, inverted the transport mode of ZmSUT1 resulting in sucrose efflux with a 100-fold lower affinity. *In planta*, the antisense inhibition of *StSUT1* in potato (*Solanum tuberosum*) led to lower tuber yield when phloem unloading towards tubers is apoplastic [19,20], indicating a major role for StSUT1 in efflux towards sink organs. In the release phloem, apoplastic sucrose concentration, membrane potential and proton motive force are in favour of sucrose export from the phloem by SUT1 [21]. Thereby, ZmSUT1 seems to mediate both sucrose influx for phloem loading (③ in Figure 1) and efflux for unloading towards apoplastic sinks (⑥ in Figure 1). Furthermore, leguminous transporters (PsSUF1, PsSUF4 and PvSUF1) were characterized as sucrose facilitators (SUF), supporting bidirectional diffusion of sucrose when expressed in baker's yeast (*Saccharomyces cerevisiae*) [22]. Leguminous SUFs mediate passive sucrose efflux and influx (Figure 2), but so far no other published work has reported the identification of additional SUF proteins. Recent findings suggest that besides SUTs, SWEETs which were initially characterized as glucose uniporters ([9], see below) are also involved in sucrose export *in planta* (Figure 2) [23]. AtSWEET11 and AtSWEET12 are key sucrose effluxers as evidenced by expression profiles, double mutant phenotypes and GFP fusions, consistent with a role in sucrose export from photosynthetic mesophyll cells (① in Figure 1).

Members of the SUT4 clade from *Arabidopsis* AtSUT4, barley (*Hordeum vulgare*) HvSUT2 [24], *Lotus japonicus* LjSUT4 [25] and tobacco (*Nicotiana tabacum*) NtSUT4 [26], have been localized to the tonoplast by GFP fusion. All four SUTs are functional at the plasma membrane of

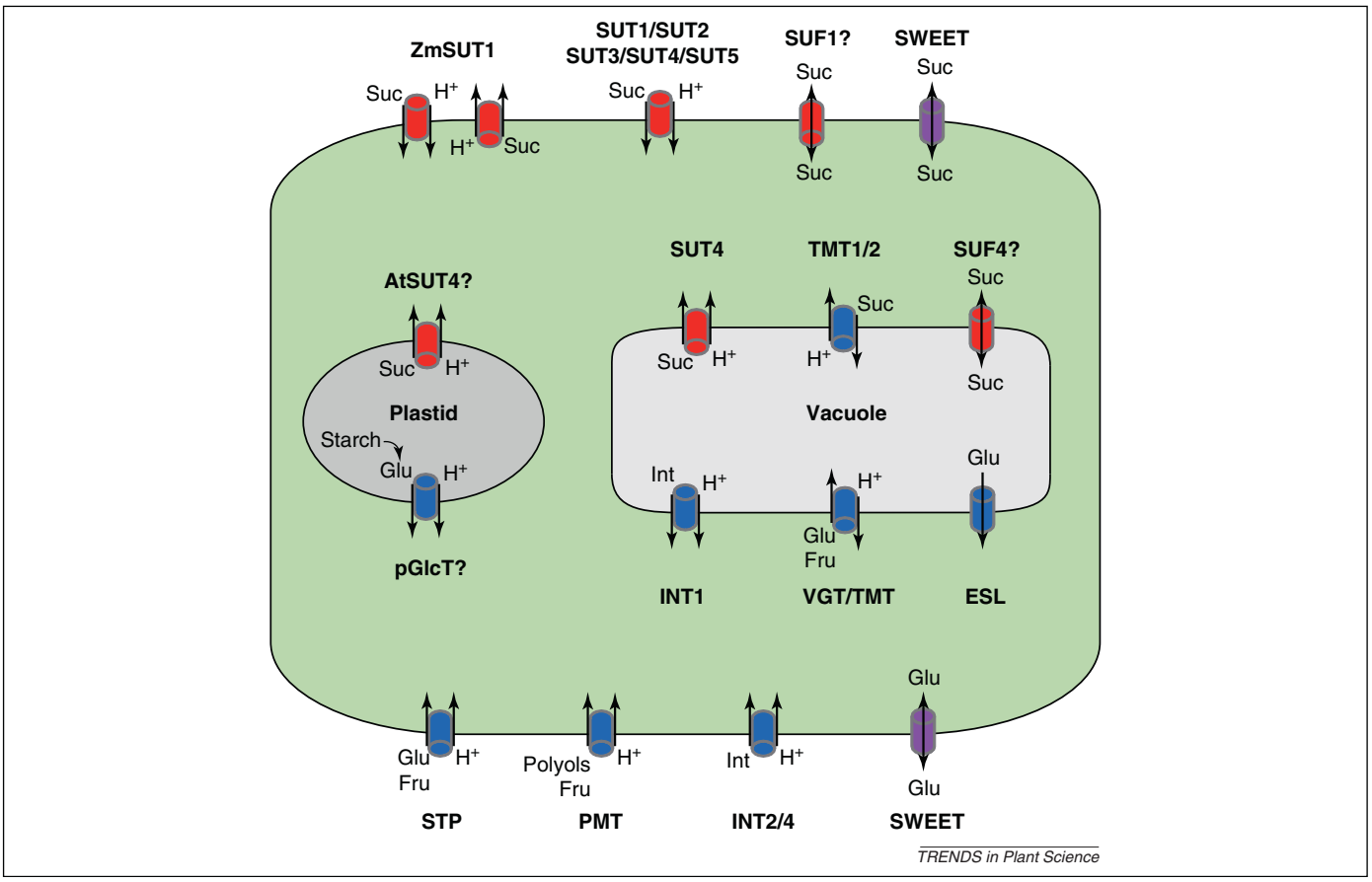


Figure 2. Model of intracellular distribution of plant sugar transporters. Three families of transporters are implicated in the distribution of sugars (sucrose in upper half and hexose in lower half) within the plant cell: SUTs (in red), MSTs (in blue) and SWEETs (in purple). At the plasma membrane, most transporters have been characterized as H^+/sugar importers, although recently ZmSUT1 was shown to also mediate active efflux of sucrose [17,18]. By contrast, SWEETs and SUFs function as energy-independent uniporters that mediate sugar influx and/or efflux [9,22]. SWEETs characterized to date localize to the plasma membrane, whereas localization of SUF1 and SUF4 has not been studied, and their proposed membrane localization in this figure is based upon phylogenetic grouping into particular clades. At the vacuolar membrane the MST subfamilies, VGT and TMT (vacuolar glucose transporter and tonoplast membrane transporter, respectively) function as sugar/H^+ antiporters loading sugars into the vacuole [32,56–58], while proteins of the MST subfamily ESLs (ERD six-like transporters) are likely to be involved in energy-independent sugar efflux from the vacuole [61,62]. Two families may also play a role in efflux of sugars from plastids, these being SUT4 and the MST subfamily, pGlcTs (plastid glucose transporters). Members of these families (AtSUT4 and SopGlcT) were isolated in chloroplast fractions [60,108] but their biochemical mechanisms of transport have not yet been shown. Finally, the MST subfamily transporters, INTs (inositol transporters) localize to both plasma and vacuolar membranes [109–111]; they transport myo-inositol, an important precursor in a number of metabolic pathways.

yeast cells or *Xenopus* oocytes [25,27,28], which was explained by mis-targeting of overexpressed proteins. Indeed, as revealed by patch-clamp analyses of AtSUC4-overexpressing vacuoles, AtSUC4 functions as a H^+/sucrose symporter at the tonoplast [29]. Very recently, SUT4 from poplar (*Populus tremula*, PtaSUT4) and rice (OsSUT2) were assumed to play a role in the orchestration of the intracellular sucrose partitioning, thereby affecting the sucrose efflux from source leaves and the utilization of sucrose in lateral and terminal sink organs [30,31].

In a recent publication the vacuolar sucrose import was described to be mainly performed by two members clustering in the MST family (TMT1 and TMT2, see below) potentially acting as H^+/sucrose antiporters (Figure 2), whereas AtSUC4 seems to play only a minor role in sucrose flux via the tonoplast under physiological conditions [32]. This is in agreement with the fact that overexpression of vacuolar *TMT1* in *Arabidopsis* altered sugar export rates and increased source capacity. *TMT1* overexpression affected assimilate allocation, seed biomass and development in *Arabidopsis* [33], whereas altered *AtSUC4* expression did not have any effect on growth and development. Very recently, vacuolar specific localization of various SUT4 proteins was reported [29]. However, a dual targeting to the vacuolar and plasma membrane already encountered for *Arabidopsis* aquaporins [34] can also be postulated for SUT4 proteins; this idea is reinforced by the localization of SUT4 members at the plasma membrane *in planta* (e.g. StSUT4) [27,35]. Another explanation for the vacuolar presence could be degradation of SUT4 protein in lytic vacuoles. Further investigations are needed to confirm whether dual targeting of SUT4 occurs in plants, considering that the localization and oligomerization of SUTs is dependent on transporter cycling and membrane dynamics [36,37].

Association to membrane raft and cycling of sugar transporters

The plant plasma membrane contains particular domains (rafts) that exhibit a specific molecular composition. Indeed, rafts are defined as 'small, heterogeneous, highly dynamic, sterol- and sphingolipid-enriched domains that

compartmentalize cellular processes' [38]. Proteomic analyses have indicated that a high proportion of proteins associated with detergent-resistant membranes, considered as the biochemical counterpart of rafts might be involved in signalling, protein activity, endocytosis, oligomerisation, degradation and transport pathways [39]. First evidence of the presence of SUTs in rafts came from the isolation of StSUT1 in detergent-resistant membrane fractions of the plant plasma membrane, mostly in its monomeric form, but also as homodimers [36,37]. After expression of GFP fusions in yeast, the plasma membrane-associated SlSUT1 from tomato (*Solanum lycopersicum*) and the hexose transporter (HUP1) of the green alga *Chlorella kessleri* are functional and assemble in heterogeneously concentrated spots resembling the patchy appearance observed previously for endogenous yeast symporters in raft-like microdomains [37,40,41]. By contrast, in yeast mutants, lacking the typical raft lipids (ergosterol and sphingolipids), both proteins distributed homogenously at the plasma membrane and the rate of glucose uptake for HUP1 was reduced to less than one-third of corresponding wild-type cells, indicating a functional impact of transporter association to membrane rafts. This association to raft-like domains seems to be essential to the endocytic cycling and polar distribution of transporters. Recently, internalization of solanaceous SUT1 proteins was investigated by means of Brefeldin A, an inhibitor of exocytosis that led to the formation of cytosolic vesicles containing StSUT1–GFP protein, suggesting a constant recycling of SUTs [41]. In addition, the use of cytoskeleton inhibitors revealed that internalization of SUTs via vesicles progresses along actin strands, but is independent of tubulin [41]. This confirms that the constant turnover of sugar transporters via recycling or degradation in lytic vacuoles is dependent on association with membrane microdomains and cytoskeleton in plant cells. There is no doubt that the recent advances in plant membrane raft biological significance will lead to a considerable development concerning the regulation of plant transporters, through their dynamic association to specific domains of the membrane, for plant nutrition and in nutrient exchanges during plant–fungal interactions.

Plant monosaccharide transporters

Two families of transporters are involved in the loading and unloading of plant monosaccharides across plasma membrane and between organelles within a cell. The plant MST gene family is large, comprising 53 MSTs in *Arabidopsis*, 58 in barrel medic (*Medicago truncatula*), 59 in grapevine (*Vitis vinifera*) and 65 in rice (Figure 3). For detailed information on all MST genes cited in this paper see Table S1 in the supplementary material online [3,4,42]. MSTs are distributed among seven subfamilies, but the current phylogenetic classification, especially clade nomenclature, remains ambiguous. Indeed, clades are either named according to substrate specificity [sugar transport protein (STP), polyol/monosaccharide transporter (PMT), inositol transporter (INT)], subcellular localization [vacuolar glucose transporter (VGT), tonoplast membrane transporter (TMT), plastidic glucose transporter (pGlcT)], mutant-recovering phenotype [suppressor of G Protein Beta1 (SGB1)] or even to a stress condition that induces gene expression [early-responsive to dehydration six-like (ESL)]. By contrast, the recently identified SWEET belonging to a distinct transporter family comprises only 17 transporters in *Arabidopsis*, 15 in *M. truncatula* and 21 in rice and presents a novel structural model including seven transmembrane domains [9]. With the identification of the SWEETs a more complete picture of plant sugar transport has begun to emerge (Figure 2).

In sink tissues hydrolysis of sucrose by invertase yields glucose and fructose which are taken up via STPs (Figures 1 and 2). All characterized STPs function as plasma membrane-localized H^+/hexose symporters, many of which show broad substrate specificity (with highest affinity for glucose) with the exceptions of AtSTP9 being a glucose-specific transporter [43] and AtSTP14, a galactose-specific transporter [44]. Although mechanisms controlling expression profiles are to be determined, STPs are clearly under tight regulatory control, responding to spatial, developmental and environmental cues [45–47], i.e. *AtSTP2*, *AtSTP6*, *AtSTP9* and *AtSTP11* are specifically expressed in pollen, but at different developmental stages [48].

In addition to STPs, members of the PMT subfamily may also be involved in hexose uptake across the plasma membrane. PMTs are broad-substrate spectrum proton symporters capable of transporting to varying degrees, hexoses and pentoses in addition to polyols. In plants for which polyols (mannitol or sorbitol) play a major role in sugar transport, PMTs are involved in phloem loading and unloading [49–51]. However, in species which do not transport polyols in their phloem, a physiological function is more difficult to assign. In *Arabidopsis*, *AtPMT1* and *AtPMT2* are expressed in developing sink tissues and show highest affinity for xylose and fructose, suggesting a role in cell-loading of fructose resulting from invertase activity [52]. Polyols being a major sugar in the metabolism of some fungi [53], PMTs have also been suggested to play a role in plant–fungal interactions [54].

Following uptake into the cell cytoplasm, sugars in excess to metabolic requirements are stored in the vacuole which functions in both transient and long-term storage. Vacuolar storage and remobilization involves membrane transport steps and plays a key role in maintenance of cellular metabolism, osmo-regulation and adaptation to environmental conditions [55]. As the names imply, VGTs and TMTs localize to the tonoplast (Figure 2). Transport assays in isolated yeast vacuoles expressing *AtVGT1* demonstrated an active uptake of glucose and fructose, indicating that AtVGT1 functions as a H^+/glucose antiporter [56]. Transport assays using vacuoles from *AtTMT* knockout mutants showed reduced glucose uptake activity, which was later shown to be complemented by overexpression of rice *OsTMT1* [57,58]. Mutant analysis indicates the key role of carbohydrate vacuolar storage and partitioning in controlling plant responses to developmental and environmental cues [32,33]. Loss of *AtVGT1* transcript by T-DNA insertion led to delayed flowering and reduced seed viability indicating an important role in plant development [56], whereas overexpression of *AtTMT1* increased glucose uptake into isolated vacuoles, stimulated source capacity

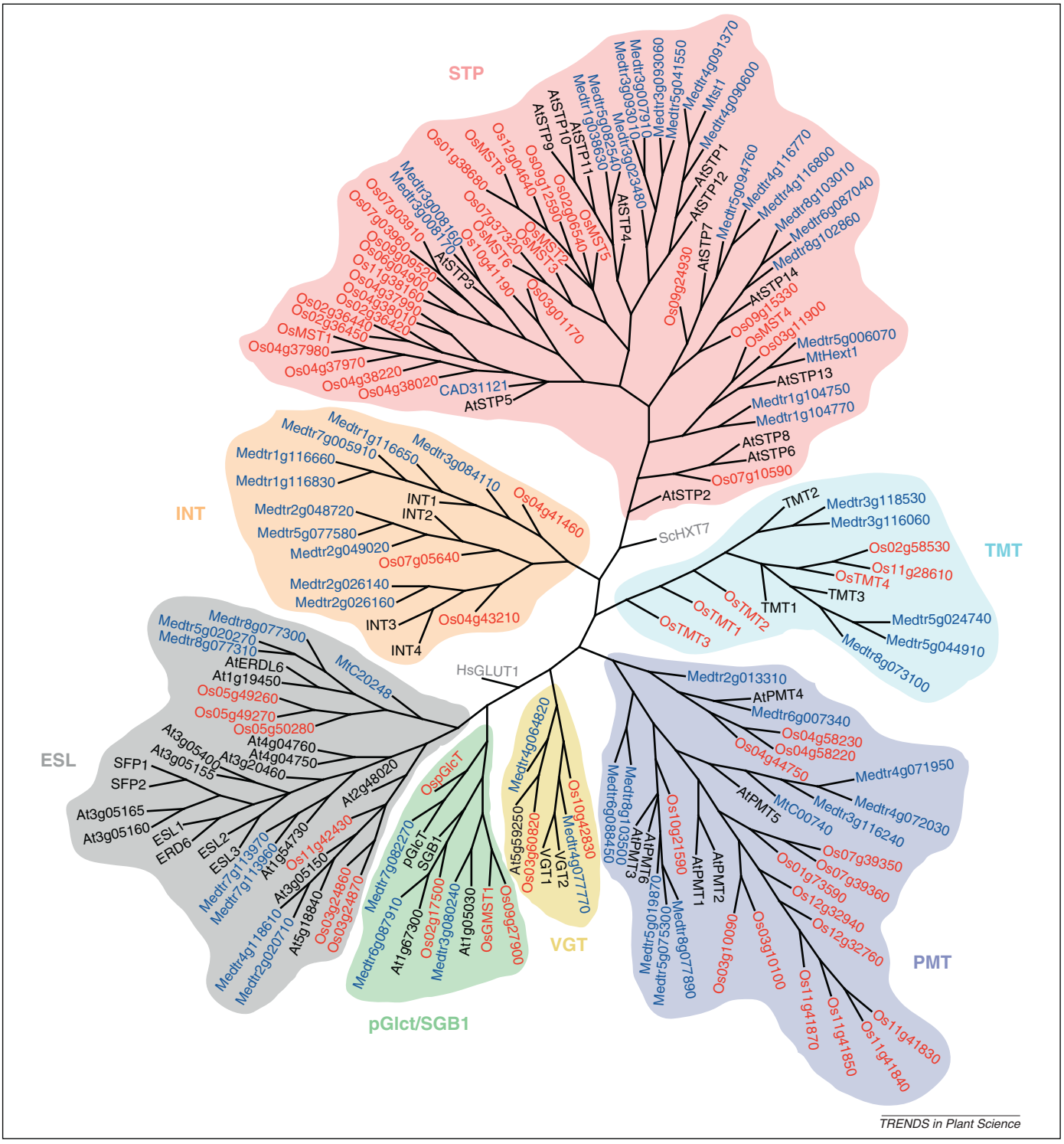


Figure 3. Phylogenetic tree of the MST family in *Arabidopsis*, *M. truncatula* and rice. Maximum parsimony consensus tree separates the MST family into seven subfamilies (highlighted in colours) [3] and comprises 183 plant accessions, 53 AtMSTs from *Arabidopsis* (black), 62 MtMSTs from *M. truncatula* (blue) and 68 OsMSTs from rice (red); the human and yeast transporters (grey) were used as outgroups. Characterized and published proteins are named according to their latest denomination; genomic locus or accession number was used for proteins which have not yet been assigned. For gene list and nomenclature see Table S1 in the supplementary material online. 53 AtMSTs were identified in the genome of *Arabidopsis* accession Columbia [3]; and 58 MtMSTs were identified in the genome of *M. truncatula*. Three additional accessions (CAD31121, MtC20248 and MtC00740) were identified from the A17 line but were not found on the current version of the genome (Mt3.5). An additional accession, Mtst1 (AAB06594) was identified from a different subpopulation (2828) of *M. truncatula* Jemalong [99]. 65 OsMSTs were identified in the genome of rice *japonica* subspecies cultivar Nipponbare [4]. An additional accession, OsMST1 (BAB19862) was identified from the cultivar Nipponbare but was not found on the current version of the genome. Two additional accessions, OsMST4 (AAQ24871) and OsMST6 (AAQ24872) were identified from the rice *indica* subspecies.
Outgroups: HsGLUT1 (NP_006507), ScHXT7 (NP_010629).

and led to increased seed biomass and accelerated early plant development [33]. Interestingly, electrophysiological characterization showed sucrose transport in vacuoles isolated from *Attmt1/tmt2* knockout mutants [32]. This discovery highlights the importance of mutant analysis in assigning physiological function, particularly with respect to apparently broad-substrate spectrum MST subfamilies such as STPs and PMTs.

The ability to remobilize carbohydrates is as important as their uptake and storage and a number of efflux transporters have been identified (Figure 2). At the chloroplast membrane, it has been proposed that pGlcTs function in the efflux of glucose derived from the amylolytic breakdown of transitory starch, and double mutant lines of *Atpglct* together with the maltose transporter *mex1* exhibited reduced photosynthetic activities as well as extreme chloroplast abnormalities [59,60]. At the vacuolar membrane, ESLs are implicated in carbohydrate remobilization. Functional and mutant analyses of *AtESL1* and *AtERDL6* suggest a role in energy-independent glucose efflux [61,62]. Expression profiling of ESLs in several plant species indicate a role in remobilization of stored sugars from the vacuole in response to environmental and perhaps also biotic stresses [61–65]. Finally, at the plasma membrane, SWEETs function as bidirectional sugar uniporters, facilitating energy-independent efflux or influx of sugars [9]. Although the identification of SWEETs is relatively recent, phenotypes of a number of *sweet* mutants have been previously characterized and SWEETs clearly play important roles in pollen development and senescence, as well as in nutrition of plant-interacting fungi and in resistance against pathogens [9]. For example the first identified SWEET, *MtN3* from *M. truncatula*, was found to be highly upregulated after rhizobial inoculation suggesting that SWEETs are also likely to play a role in biotrophic exchanges during development of plant symbioses [66].

Sugar transporters in plant–fungal interactions

Co-evolution of plants and fungi in various environments through several hundred million years made possible the emergence of close interactions among species belonging to these two kingdoms. These interactions can be divided into mutualistic (e.g. mycorrhiza) and pathogenic (from biotrophic to necrotrophic). Both types of interaction are dependent on the plant for supply of carbon in the form of photosynthates, but much remains to be understood on mechanisms of transport and partitioning of sugars toward the specialized membranes at plant–fungal interfaces. At the symbiotic and pathogenic interface, transport proteins specific to the uptake of sucrose and monosaccharides, have been identified (Figure 4). For detailed information on all fungal sugar transporter genes cited in this paper see Table S1 in the supplementary material online.

Sucrose partitioning in plant–fungal interactions

Initially, in 2001 only a single fungal SUT, SpSUT1 from fission yeast (*Schizosaccharomyces pombe*), had been characterized [67]. Nine years later, the first fungal SUT (UmSRT1) involved in plant–microorganism interactions was isolated from the maize pathogen *Ustilago maydis* [68]. The high affinity of UmSRT1 for sucrose and competition for sucrose with ZmSUT1 from the host plant, suggest direct uptake of sucrose by the pathogen as a strategy to avoid defence mechanisms induced by glucose utilization [68,69]. Indeed, plants have evolved mechanisms to sense extracellular changes in glucose concentrations

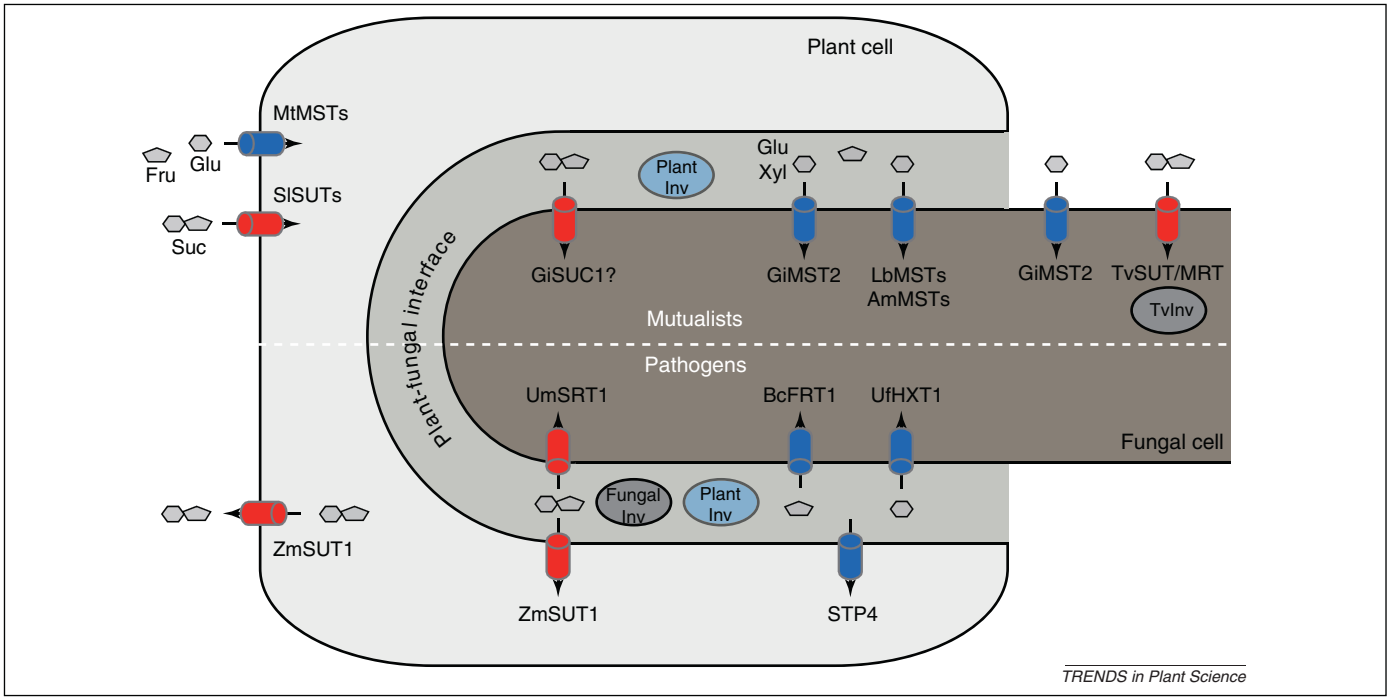


Figure 4. Sugar partitioning in plant–fungal interactions. Plant and fungal SUTs (in red) and MSTs (in blue) are involved in sugars partitioning in plant–fungal interactions. In some pathogenic interactions both plant and fungus strongly compete for the uptake of sucrose (ZmSUT1 and UmSRT1) at the interface [68,69]. Fungal invertases (fungal Inv) and monosaccharides uptake [fructose (*BcFRT1*); glucose (*UfHXT1*)] allow these fungi to efficiently use plant nutrients causing, eventually, host damage and/or death [91,92]. By contrast, mutualistic interactions (by BCF, AMF and EMF) are based on beneficial biotrophic exchanges and generally improve plant-nutritional status [8]. Tomato SlSUTs and *M. truncatula* MtMSTs (CAD31121; MtMst1) may support the carbon demand at the symbiotic interface [99,100], where also the activity of plant invertases (plant Inv) seems to play a role in regulating sugar fluxes to the fungus [80,81]. Mycorrhizal fungi present the machinery in order to efficiently, and preferentially, uptake glucose from the apoplastic interface (GiMST2; LbMSTs; AmMSTs) or through intercellular hyphae (GiMST2) [73,95,98]; only recently a putative SUT gene was identified in arbuscular mycorrhizal fungi (*GiSUC1*) [73] and its role is yet to be established. BCF and ericoid fungi are able to incorporate and hydrolyze sucrose (TvSUT; MRT; TvInv), which seems to loosen the plant-mediated control over the sugar exchange [71,72,79].

(e.g. produced from extracellular sucrose hydrolysis) and respond to these changes with the induction of defence responses [68,70].

Among the so-called biocontrol fungi (BCF) which reduce or prevent plant-pathogens occurrence and promote plant growth and stress resistance; two novel SUTs have been characterized from *Metarhizium robertsii* (MRT) and *Trichoderma virens* (TvSUT) and shown to be involved in sugar uptake from root exudates [71,72]. Although TvSUT and UmSRT1 are distantly related and involved in different interactions, both show a very high specificity for sucrose, whereas plant SUTs generally transport a broader substrate range. In arbuscular mycorrhizal (AM) symbiosis, sucrose is not generally considered to be taken up by the mycobiont, but recently a Glomeromycotan SUT (GiSUC1) was identified from *Glomus intraradices* EST contigs [73]. As this field opens up, a novel SUT family from fungi is emerging. First results suggest that SUTs are involved in the functionality of both symbiotic and pathogenic plant-fungal interactions.

Invertases, a group of functionally similar enzymes which hydrolyse sucrose, shed further light on the question of fungal utilization of sucrose. Recently, evolutionary reconstruction of the invertase gene family in numerous fungal phyla, including species with different nutritional strategies, revealed the presence of invertase-encoding genes in all sequenced pathogens, the majority of endophytes, half of the lichen-forming fungi and only a few ectomycorrhizal fungi (EMF). This highlights a negative correlation between invertase gene presence and degree of mutualism [74].

Generally, increased activities of cell-associated invertases have been observed in several pathogenic interactions. Indeed, increased invertase activity and plant monosaccharide importer expression (STP4 homologues) may result in limiting access of sugars to pathogens during plant infection [45,75,76]. So far it has not been possible to distinguish whether plant- or fungal-invertases contribute to this increase, but it seems unlikely that the nutritional demands of the pathogen can be solely supported by the host enzymatic machinery [77,78]. In BCF–plant interactions, an upregulation of plant photosynthesis is mediated by enhanced sink activity due to induction of fungal *TvInv* and *TvSUT* in colonized plant tissues [72,79]. The incorporation of an intracellular metabolic pathway that facilitates sucrose uptake and its cleavage may represent an evolutionary advantage over fungi missing this machinery, and could constitute a considerable divergence from pathogenic to mutualistic behaviour.

In the majority of AM fungal species, invertase activities have not been identified and it is assumed that plant-encoded enzymes catalyze sucrose breakdown. Expression and activity of all types of plant sucrose cleaving enzymes appear to be regulated in the presence of AMF [80–84]. Increases in photosynthetic and invertase activity coupled with higher SUT transcript levels, as well as accumulation of sucrose and monosaccharides in sink organs indicate a mycorrhizal-driven stronger sink and sugar allocation toward roots for fungal symbiont development [85,86]. Increasing sucrose export by overexpressing *SoSUT1* in potato increased AM fungal colonization, although only in high phosphate conditions [87]. In parallel, limiting hexose access by the downregulation of invertase activity in roots led to reduced mycorrhization levels. However, the inhibition of sucrose export from leaves by a knockdown of *StSUT1*, the gene coding for the phloem loading protein in potato [87], and the increase of hexose levels in tobacco roots through overexpression of yeast-derived invertases did not affect fungal colonization [88]. These contradictory findings suggest that carbon supply through sucrose breakdown is not alone the bottleneck for a functional interaction. This is in-line with the postulate that plants 'can detect, discriminate, and reward the best fungal partners with more carbohydrates; in turn, their fungal partners enforce cooperation by increasing nutrient transfer only to those roots providing more carbohydrates' [89].

Monosaccharide partitioning in plant–fungal interactions

Sucrose is hydrolyzed evenly, either by plant- or fungal-invertases, into glucose and fructose at the plant–fungal interface, but much evidence indicates glucose is preferentially taken up by the mycobiont [7,90].

Among plant pathogens, the first conclusive proof of an MST role in nutrient uptake at the plant–fungal interface came from faba-bean rust (*Uromyces fabae*). A proton-dependent hexose transporter (UfHXT1), with high affinity for glucose was localized specifically to specialized infection structures (haustoria) [91]. Later, an MST (BcFRT1) from grey mould (*Botrytis cinerea*) was characterized as having high affinity for fructose [92], suggesting that pathogenic fungi have varied their transport mechanisms to take up a broader range of plant photosynthates.

Nutrient exchange during mutualistic interactions, and understanding of its role at the plant–fungal interface, has attracted much attention in past decades. However, in EMF, only three MSTs from *Amanita muscaria* (AmMST1 and AmMST2) and *Tuber borchii* (TbHXT1) have been characterized so far [93–95]. These transporters share high affinity for glucose, but different regulatory systems and spatial localization. Indeed, while *AmMST1 and AmMST2* were stimulated by extracellular monosaccharide concentration and suggested to be targeted at the plant–fungus interface, *Tbhxt1* expression was stimulated during carbohydrate starvation of fungal hyphae and is possibly responsible for sugars supply to the soil-growing mycelium. The preferential glucose uptake by these transporters is consistent with observations in other EMF [7]. Recently, understanding the mechanisms of sugar transport in EMF has benefited from the sequenced fungal genomes [96,97]. The *Laccaria bicolor* genome contains 15 putative MSTs (LbMSTs), and transport properties assessed through competition experiments showed glucose as the preferred monosaccharide substrate [98]. As observed for *A. muscaria* MSTs, the mycorrhizal developmental stage rather than apoplastic hexose concentration play a key role in MST regulation. Moreover, expression patterns confirmed a strong induction under carbon limitation.

Within the Glomeromycotan phylum (AM fungi) the first symbiosis-related MST was identified in *Geosiphon pyriformis* interacting with *Nostoc punctiforme*. This unique symbiotic model allowed the isolation of pure

fungal mRNA which then served to isolate GpMST1 [90]. Information obtained from this model, together with the available Glomeromycotan genomic data, recently led to the isolation of three MSTs from the model species *G. intraradices*, one of which GiMST2, is characterized as a high affinity H^+/glucose transporter expressed in arbuscules and intercellular mycelium [73]. Xylose binding by GiMST2 and GpMST1 suggest that cell wall monosaccharides may also be a carbon source for AMF [73,90]. Host-induced gene silencing (HIGS) of *GiMST2* resulted in impaired mycorrhizal formation, malformed arbuscules and reduced expression of the AM-specific plant phosphate transporter, *MtPT4*. This suggests GiMST2 is the primary transporter for sugar uptake by *G. intraradices* and has an indispensable role in functional symbiosis. Consistently, on the plant side, an STP member (Mtst1) shows higher expression levels in arbusculated cortical cells and in adjacent cells which are frequently in contact with the intercellular network [99]. By contrast, a second MtMST candidate (CAD31121) is downregulated in response to AM (see: http://mtgea.noble.org/v2/) and its protein product was detected in detergent-resistant membrane fractions of *M. truncatula* roots [100]. This suggests that raft-associated proteins also play a role in the regulation of trophic exchanges in mycorrhizal symbiosis.

Future milestones

Recent release of plant and fungal genomes as well as transcriptomic databases allowed the generation of an inventory of sugar transporter genes involved in plant–fungal interactions [96,97,101,102]. Nevertheless, the transportome puzzle is still far from complete and major pieces such as the system of cellular efflux at biotrophic interfaces are still missing, also the regulation of nutrient exchanges within and between organisms is still poorly understood. However, increasing application of molecular and post-genomic methodologies as well as technological improvements with higher spatial resolution (e.g. laser capture microdissection technology) are now available for the detection of nutrients, transcripts and proteins present at plant–fungal interfaces [103–106]. These recent technological achievements in model plants associated to fungal consortia will facilitate comprehensive identification of the key nutrient transporters involved in mutualistic and pathogenic interactions.

Acknowledgements

This work was supported by grants to JD, FSP, LC and DW from the Conseil Régional de Bourgogne and the Agence National de Recherche (Project TRANSMUT 'Impact of the biotrophic transportome on mutualistic plant-fungal interactions', ANR-10-BLAN-1604-01). The authors are grateful to Deborah Alongi (Castleton State College, USA) for sending genomic sequences of rice MSTs.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tplants.2012.03.009.

References

1 Lalonde, S. *et al.* (2004) Transport mechanisms for organic forms of carbon and nitrogen between source and sink. *Annu. Rev. Plant Biol.* 55, 341–372
2 Sauer, N. (2007) Molecular physiology of higher plant sucrose transporters. *FEBS Lett.* 581, 2309–2317
3 Buttner, M. (2007) The monosaccharide transporter(-like) gene family in *Arabidopsis*. *FEBS Lett.* 581, 2318–2324
4 Johnson, D.A. and Thomas, M.A. (2007) The monosaccharide transporter gene family in *Arabidopsis* and rice: A history of duplications, adaptive evolution, and functional divergence. *Mol. Biol. Evol.* 24, 2412–2423
5 Rolland, F. *et al.* (2006) Sugar sensing and signaling in plants: conserved and novel mechanisms. *Annu. Rev. Plant Biol.* 57, 675–709
6 Müller, T. *et al.* (2007) Nitrogen transport in the ectomycorrhiza association: the *Hebeloma cylindrosporum–Pinus pinaster* model. *Phytochemistry* 68, 41–51
7 Nehls, U. *et al.* (2010) Fungal carbohydrate support in the ectomycorrhizal symbiosis: a review. *Plant Biol.* 12, 292–301
8 Smith, S.E. and Read, D.J. (2008) *Mycorrhizal Symbiosis*, Academic Press
9 Chen, L.Q. *et al.* (2010) Sugar transporters for intercellular exchange and nutrition of pathogens. *Nature* 468, 527–532
10 Kuhn, C. and Grof, C. (2010) Sucrose transporters of higher plants. *Curr. Opin. Plant Biol.* 13, 1–11
11 Braun, D.M. and Slewinski, T.L. (2009) Genetic control of carbon partitioning in grasses: roles of sucrose transporters and Tie-dyed loci in phloem loading. *Plant Physiol.* 149, 71–81
12 Zhang, C. and Turgeon, R. (2009) Downregulating the sucrose transporter *VpSUT1* in *Verbascum phoeniceum* does not inhibit phloem loading. *Proc. Natl. Acad. Sci. U.S.A.* 106, 18849–18854
13 Ayre, B.G. (2011) Membrane-transport systems for sucrose in relation to whole-plant carbon partitioning. *Mol. Plant* 4, 377–394
14 Slewinski, T.L. *et al.* (2009) Sucrose transporter1 functions in phloem loading in maize leaves. *J. Exp. Bot.* 60, 881–892
15 Sun, Y. *et al.* (2010) Transport activity of rice sucrose transporters OsSUT1 and OsSUT5. *Plant Cell Physiol.* 51, 114–122
16 Barth, I. *et al.* (2003) PmSUC3: Characterization of a SUT2/SUC3-type sucrose transporter from *Plantago major*. *Plant Cell* 15, 1375–1385
17 Carpaneto, A. *et al.* (2005) Phloem-localized, proton-coupled sucrose carrier ZmSUT1 mediates sucrose efflux under the control of the sucrose gradient and the proton motive force. *J. Biol. Chem.* 280, 21437–21443
18 Carpaneto, A. *et al.* (2010) Sucrose- and H+-dependent charge movements associated with the gating of sucrose transporter ZmSUT1. *PLoS ONE* 5, e12605
19 Viola, R. *et al.* (2001) Tuberization in potato involves a switch from apoplastic to symplastic phloem unloading. *Plant Cell* 13, 385–398
20 Kühn, C. *et al.* (2003) The sucrose transporter StSUT1 localizes to sieve elements in potato tuber phloem and influences tuber physiology and development. *Plant Physiol.* 131, 102–113
21 Geiger, D. (2011) Plant sucrose transporters from a biophysical point of view. *Mol. Plant* 4, 395–406
22 Zhou, Y.C. *et al.* (2007) A suite of sucrose transporters expressed in coats of developing legume seeds includes novel pH-independent facilitators. *Plant J.* 49, 750–764
23 Chen, L-Q. *et al.* (2012) Sucrose efflux mediated by SWEET proteins as a key step for phloem transport. *Science* 335, 207–211
24 Endler, A. *et al.* (2006) Identification of a vacuolar sucrose transporter in barley and *Arabidopsis* mesophyll cells by a tonoplast proteomic approach. *Plant Physiol.* 141, 196–207
25 Reinders, A. *et al.* (2008) Functional analysis of LjSUT4, a vacuolar sucrose transporter from *Lotus japonicus*. *Plant Mol. Biol.* 68, 289–299
26 Okubo-Kurihara, E. *et al.* (2011) Sucrose transporter NtSUT4 from tobacco BY-2 involved in plant cell shape during miniprotoplast culture. *J. Plant Res.* 124, 395–403
27 Weise, A. *et al.* (2000) A new subfamily of sucrose transporters, SUT4, with low affinity/high capacity localized in enucleate sieve elements of plants. *Plant Cell* 12, 1345–1356
28 Weschke, W. *et al.* (2000) Sucrose transport into barley seeds: molecular characterization of two transporters and implications for seed development and starch accumulation. *Plant J.* 21, 455–467
29 Schneider, S. *et al.* (2011) Vacuoles release sucrose via tonoplast-localised SUC4-type transporters. *Plant Biol.* 14, 325–336
30 Payyavula, R.S. *et al.* (2011) The sucrose transporter family in *Populus*: the importance of a tonoplast PtaSUT4 to biomass and carbon partitioning. *Plant J.* 65, 757–770

31 Eom, J-S. *et al.* (2011) Impaired function of the tonoplast-localized sucrose transporter in rice (*Oryza sativa*), OsSUT2, limits the transport of vacuolar reserve sucrose and affects plant growth. *Plant Physiol.* 157, 109–119
32 Schulz, A. *et al.* (2011) Proton-driven sucrose symport and antiport are provided by the vacuolar transporters SUC4 and TMT1/2. *Plant J.* 68, 129–136
33 Wingenter, K. *et al.* (2010) Increased activity of the vacuolar monosaccharide transporter TMT1 alters cellular sugar partitioning, sugar signaling, and seed yield in *Arabidopsis*. *Plant Physiol.* 154, 665–677
34 Gattolin, S. *et al.* (2011) Mapping of tonoplast intrinsic proteins in maturing and germinating *Arabidopsis* seeds reveals dual localization of embryonic TIPs to the tonoplast and plasma membrane. *Mol. Plant* 4, 180–189
35 Chincinska, I.A. *et al.* (2008) Sucrose transporter StSUT4 from potato affects flowering, tuberization, and shade avoidance response. *Plant Physiol.* 146, 515–528
36 Krügel, U. *et al.* (2012) The potato sucrose transporter StSUT1 interacts with a DRM-associated protein disulfide isomerase. *Mol. Plant* 5, 43–62
37 Krügel, U. *et al.* (2008) Transport and sorting of the *Solanum tuberosum* sucrose transporter SUT1 is affected by posttranslational modification. *Plant Cell* 20, 2497–2513
38 Pike, L.J. (2006) Rafts defined: a report on the keystone symposium on lipid rafts and cell function. *J. Lipid Res.* 47, 1597–1598
39 Mongrand, S. *et al.* (2010) Membrane rafts in plant cells. *Trends Plant Sci.* 15, 656–663
40 Grossmann, G. *et al.* (2006) Lipid raft-based membrane compartmentation of a plant transport protein expressed in *Saccharomyces cerevisiae*. *Eukaryot. Cell* 5, 945–953
41 Liesche, J. *et al.* (2010) Recycling of *Solanum* sucrose transporters expressed in yeast, tobacco, and in mature phloem sieve elements. *Mol. Plant* 3, 1064–1074
42 Afoufa-Bastien, D. *et al.* (2010) The *Vitis vinifera* sugar transporter gene family: phylogenetic overview and macroarray expression profiling. *BMC Plant Biol.* 10, 245
43 Schneidereit, A. *et al.* (2003) Functional characterization and expression analyses of the glucose-specific AtSTP9 monosaccharide transporter in pollen of *Arabidopsis*. *Plant Physiol.* 133, 182–190
44 Poschet, G. *et al.* (2010) Identification and characterization of AtSTP14, a novel galactose transporter from *Arabidopsis*. *Plant Cell Physiol.* 51, 1571–1580
45 Truernit, E. *et al.* (1996) The sink-specific and stress-regulated *Arabidopsis STP4* gene: enhanced expression of a gene encoding a monosaccharide transporter by wounding, elicitors, and pathogen challenge. *Plant Cell* 8, 2169–2182
46 Norholm, M. *et al.* (2006) Expression of the *Arabidopsis* high-affinity hexose transporter STP13 correlates with programmed cell death. *FEBS Lett.* 580, 2381–2387
47 Hofmann, J. *et al.* (2009) Diversity and activity of sugar transporters in nematode-induced root syncytia. *J. Exp. Bot.* 60, 3085–3095
48 Büttner, M. (2010) The *Arabidopsis* sugar transporter (AtSTP) family: an update. *Plant Biol.* 12, 35–41
49 Noiraud, N. *et al.* (2001) Transport of polyols in higher plants. *Plant Physiol. Biochem.* 39, 717–728
50 Dusotoit-Coucaud, A. *et al.* (2010) Cloning and characterization of a new polyol transporter (HbPLT2) in *Hevea brasiliensis*. *Plant Cell Physiol.* 51, 1878–1888
51 Pommerrenig, B. *et al.* (2007) Differential regulation of sorbitol and sucrose loading into the phloem of Plantago major in response to salt stress. *Plant Physiol.* 144, 1029–1038
52 Klepek, Y. *et al.* (2010) *Arabidopsis thaliana* POLYOL/MONOSACCHARIDE TRANSPORTERS 1 and 2: fructose and xylitol/H+ symporters in pollen and young xylem cells. *J. Exp. Bot.* 61, 537–550
53 Solomon, P.S. *et al.* (2007) Decoding the mannitol enigma in filamentous fungi. *Trends Microbiol.* 15, 257–262
54 Juchaux-Cachau, M. *et al.* (2007) Characterization of AgMaT2, a plasma membrane mannitol transporter from celery, expressed in phloem cells, including phloem parenchyma cells. *Plant Physiol.* 145, 62–74
55 Neuhaus, H.E. (2007) Transport of primary metabolites across the plant vacuolar membrane. *FEBS Lett.* 581, 2223–2226
56 Aluri, S. and Buttner, M. (2007) Identification and functional expression of the *Arabidopsis thaliana* vacuolar glucose transporter 1 and its role in seed germination and flowering. *Proc. Natl. Acad. Sci. U.S.A.* 104, 2537–2542
57 Wormit, A. *et al.* (2006) Molecular identification and physiological characterization of a novel monosaccharide transporter from Arabidopsis involved in vacuolar sugar transport. *Plant Cell* 18, 3476–3490
58 Cho, J.I. *et al.* (2010) Expression analysis and functional characterization of the monosaccharide transporters, OsTMTs, involving vacuolar sugar transport in rice (Oryza sativa). *New Phytol.* 186, 657–668
59 Cho, M-H. *et al.* (2011) Role of the plastidic glucose translocator in the export of starch degradation products from the chloroplasts in *Arabidopsis thaliana*. *New Phytol.* 190, 101–112
60 Weber, A. *et al.* (2000) Identification, purification, and molecular cloning of a putative plastidic glucose translocator. *Plant Cell* 12, 787–801
61 Yamada, K. *et al.* (2010) Functional analysis of an *Arabidopsis thaliana* abiotic stress-inducible facilitated diffusion transporter for monosaccharides. *J. Biol. Chem.* 285, 1138–1146
62 Poschet, G. *et al.* (2011) A novel *Arabidopsis* vacuolar glucose exporter is involved in cellular sugar homeostasis and affects the composition of seed storage compounds. *Plant Physiol.* 157, 1664–1676
63 Quirino, B. *et al.* (2001) One of two tandem *Arabidopsis* genes homologous to monosaccharide transporters is senescence-associated. *Plant Mol. Biol.* 46, 447–457
64 Kiyosue, T. *et al.* (1998) *ERD6*, a cDNA clone for an early dehydration-induced gene of Arabidopsis, encodes a putative sugar transporter. *Biochim. Biophys. Acta* 1370, 187–191
65 Garcia-Rodriguez, S. *et al.* (2005) Expression of a tomato sugar transporter is increased in leaves of mycorrhizal or *Phytophthora parasitica*-infected plants. *Mycorrhiza* 15, 489–496
66 Gamas, P. *et al.* (1996) Use of a subtractive hybridization approach to identify new *Medicago truncatula* genes induced during root nodule development. *Mol. Plant Microbe Interact.* 9, 233–242
67 Reinders, A. and Ward, J.M. (2001) Functional characterization of the α-glucoside transporter Sut1p from *Schizosaccharomyces pombe*, the first fungal homologue of plant sucrose transporters. *Mol. Microbiol.* 39, 445–455
68 Wahl, R. *et al.* (2010) A novel high-affinity sucrose transporter is required for virulence of the plant pathogen *Ustilago maydis*. *PLoS Biol.* 8, e1000303
69 Wippel, K. *et al.* (2010) inverse pH regulation of plant and fungal sucrose transporters: a mechanism to regulate competition for sucrose at the host/pathogen interface? *PLoS ONE* 5, e12429
70 Ehness, R. *et al.* (1997) Glucose and stress independently regulate source and sink metabolism and defense mechanisms via signal transduction pathways involving protein phosphorylation. *Plant Cell* 9, 1825–1841
71 Fang, W. and St. Leger, R.J. (2010) Mrt, a gene unique to fungi, encodes an oligosaccharide transporter and facilitates rhizosphere competency in *Metarhizium robertsii*. *Plant Physiol.* 154, 1549–1557
72 Vargas, W.A. *et al.* (2011) Functional characterization of a plant-like sucrose transporter from the beneficial fungus *Trichoderma virens*. Regulation of the symbiotic association with plants by sucrose metabolism inside the fungal cells. *New Phytol.* 189, 777–789
73 Helber, N. *et al.* (2011) A versatile monosaccharide transporter that operates in the arbuscular mycorrhizal fungus *Glomus* sp is crucial for the symbiotic relationship with plants. *Plant Cell* 23, 3812–3823
74 Parrent, J. *et al.* (2009) Friend or foe? Evolutionary history of glycoside hydrolase family 32 genes encoding for sucrolytic activity in fungi and its implications for plant-fungal symbioses. *BMC Evol. Biol.* 9, DOI:10.1186/1471-2148-9-148
75 Fotopoulos, V. *et al.* (2003) The monosaccharide transporter gene, *AtSTP4*, and the cell-wall invertase, *Atβfruct1*, are induced in *Arabidopsis* during infection with the fungal biotroph *Erysiphe cichoracearum*. *Plant Physiol.* 132, 821–829
76 Sutton, P.N. *et al.* (2007) Powdery mildew infection of wheat leaves changes host solute transport and invertase activity. *Physiol. Plant.* 129, 787–795
77 Voegele, R.T. and Mendgen, K.W. (2011) Nutrient uptake in rust fungi: how sweet is parasitic life? *Euphytica* 179, 41–55

78 Guenther, J.C. *et al.* (2009) Triacylglyceride metabolism by *Fusarium graminearum* during colonization and sexual development on wheat. *Mol. Plant Microbe Interact.* 22, 1492–1503
79 Vargas, W.A. *et al.* (2009) Plant-derived sucrose is a key element in the symbiotic association between *Trichoderma virens* and maize plants. *Plant Physiol.* 151, 792–808
80 Schaarschmidt, S. *et al.* (2006) Arbuscular mycorrhiza induces gene expression of the apoplastic invertase LIN6 in tomato (*Lycopersicon esculentum*) roots. *J. Exp. Bot.* 57, 4015–4023
81 Blee, K.A. and Anderson, A.J. (2002) Transcripts for genes encoding soluble acid invertase and sucrose synthase accumulate in root tip and cortical cells containing mycorrhizal arbuscules. *Plant Mol. Biol.* 50, 197–211
82 Ravnskov, S. *et al.* (2003) Arbuscular mycorrhizal fungi differentially affect expression of genes coding for sucrose synthases in maize roots. *New Phytol.* 157, 539–545
83 Hohnjec, N. *et al.* (2003) The *Medicago truncatula* sucrose synthase gene *MtSucS1* is activated both in the infected region of root nodules and in the cortex of roots colonized by arbuscular mycorrhizal fungi. *Mol. Plant Microbe Interact.* 16, 903–915
84 Baier, M.C. *et al.* (2010) Knockdown of the symbiotic sucrose synthase *MtSucS1* affects arbuscule maturation and maintenance in mycorrhizal roots of *Medicago truncatula*. *Plant Physiol.* 152, 1000–1014
85 Wright, D.P. *et al.* (1998) Mycorrhizal sink strength influences whole plant carbon balance of *Trifolium repens* L. *Plant Cell Environ.* 21, 881–891
86 Boldt, K. *et al.* (2011) Photochemical processes, carbon assimilation and RNA accumulation of sucrose transporter genes in tomato arbuscular mycorrhiza. *J. Plant Physiol.* 168, 1256–1263
87 Gabriel-Neumann, E. *et al.* (2011) Constitutive overexpression of the sucrose transporter *SoSUT1* in potato plants increases arbuscular mycorrhiza fungal root colonization under high, but not under low, soil phosphorus availability. *J. Plant Physiol.* 168, 911–919
88 Schaarschmidt, S. *et al.* (2007) Regulation of arbuscular mycorrhization by carbon.The symbiotic interaction cannot be improved by increased carbon availability accomplished by root-specifically enhanced invertase activity. *Plant Physiol.* 143, 1827–1840
89 Kiers, E.T. *et al.* (2011) Reciprocal rewards stabilize cooperation in the mycorrhizal symbiosis. *Science* 333, 880–882
90 Schüßler, A. *et al.* (2006) Characterization of a carbohydrate transporter from symbiotic glomeromycotan fungi. *Nature* 444, 933–936
91 Voegele, R.T. *et al.* (2001) The role of haustoria in sugar supply during infection of broad bean by the rust fungus *Uromyces fabae*. *Proc. Natl. Acad. Sci. U.S.A.* 98, 8133–8138
92 Doehlemann, G. *et al.* (2005) Molecular and functional characterization of a fructose specific transporter from the gray mold fungus *Botrytis cinerea*. *Fungal Genet. Biol.* 42, 601–610
93 Wiese, J. *et al.* (2000) Functional characterization of the *Amanita muscaria* monosaccharide transporter, AmMst1. *Plant Biol.* 2, 278–282
94 Polidori, E. *et al.* (2007) Hexose uptake in the plant symbiotic ascomycete *Tuber borchii* Vittadini: biochemical features and expression pattern of the transporter TBHXT1. *Fungal Genet. Biol.* 44, 187–198
95 Nehls, U. (2008) Mastering ectomycorrhizal symbiosis: the impact of carbohydrates. *J. Exp. Bot.* 59, 1097–1108
96 Martin, F. *et al.* (2010) Perigord black truffle genome uncovers evolutionary origins and mechanisms of symbiosis. *Nature* 464, 1033–1038
97 Martin, F. *et al.* (2008) The genome of *Laccaria bicolor* provides insights into mycorrhizal symbiosis. *Nature* 452, 88–92
98 Fajardo Lopez, M. *et al.* (2008) The sugar porter gene family of *Laccaria bicolor*: function in ectomycorrhizal symbiosis and soil-growing hyphae. *New Phytol.* 180, 365–378
99 Harrison, M.J. (1996) A sugar transporter from *Medicago truncatula*: Altered expression pattern in roots during vesicular-arbuscular (VA) mycorrhizal associations. *Plant J.* 9, 491–503
100 Lefebvre, B. *et al.* (2007) Characterization of lipid rafts from *Medicago truncatula* root plasma membranes: a proteomic study reveals the presence of a raft-associated redox system. *Plant Physiol.* 144, 402–418
101 Tisserant, E. *et al.* (2011) The transcriptome of the arbuscular mycorrhizal fungus *Glomus intraradices* (DAOM 197198) reveals functional tradeoffs in an obligate symbiont. *New Phytol.* 193, 755–769
102 Young, N.D. *et al.* (2011) The *Medicago* genome provides insight into the evolution of rhizobial symbioses. *Nature* 480, 520–524
103 Balestrini, R. *et al.* (2007) Laser microdissection reveals that transcripts for five plant and one fungal phosphate transporter genes are contemporaneously present in arbusculated cells. *Mol. Plant Microbe Interact.* 20, 1055–1062
104 Gaude, N. *et al.* (2011) Arbuscule-containing and non-colonized cortical cells of mycorrhizal roots undergo a massive and specific reprogramming during arbuscular mycorrhizal development. *Plant J.* 69, 510–528
105 Lohaus, G. *et al.* (2001) Is the infiltration-centrifugation technique appropriate for the isolation of apoplastic fluid? A critical evaluation with different plant species. *Physiol. Plant* 111, 457–465
106 Hogekamp, C. *et al.* (2011) Laser-microdissection unravels cell-type specific transcription in arbuscular mycorrhizal roots, including CAAT-box TF gene expression correlating with fungal contact and spread. *Plant Physiol.* DOI:10.1104/pp.111.186635
107 Rennie, E.A. and Turgeon, R. (2009) A comprehensive picture of phloem loading strategies. *Proc. Natl. Acad. Sci. U.S.A.* 106, 14162–14167
108 Ferro, M. *et al.* (2003) Proteomics of the chloroplast envelope membranes from *Arabidopsis thaliana*. *Mol. Cell. Biol.* 2, 325–345
109 Schneider, S. *et al.* (2008) Functional and physiological characterization of *Arabidopsis* INOSITOL TRANSPORTER1, a novel tonoplast-localized transporter for myo-inositol. *Plant Cell* 20, 1073–1087
110 Schneider, S. *et al.* (2006) *Arabidopsis* INOSITOL TRANSPORTER4 mediates high-affinity H^+ symport of myoinositol across the plasma membrane. *Plant Physiol.* 141, 565–577
111 Schneider, S. *et al.* (2007) *Arabidopsis* INOSITOL TRANSPORTER2 mediates H^+ symport of different inositol epimers and derivatives across the plasma membrane. *Plant Physiol.* 145, 1395–1407

Supplementary Material

Sugar transporters in plants and in their interactions with fungi

Joan Doidy[1], Emily Grace[2], Christina Kühn[3], Françoise Simon-Plas[1], Leonardo Casieri[1] and Daniel Wipf[1]

[1]UMR INRA 1347, Agrosup, Université de Bourgogne, Agroécologie, Pôle Interactions Plantes Microorganismes ERL CNRS 6300, BP 86510, 21065 Dijon Cedex, France

[2]School of Environmental and Life Sciences, University of Newcastle, NSW 2308, Australia

[3]Institute of Biology, Department of Plant Physiology, Humboldt University, 10115 Berlin, Germany

Corresponding author: Wipf, D. (daniel.wipf@dijon.inra.fr)

Table S1. Detailed information of all sugar transporter genes mentioned in this article

Plant sucrose transporters (SUTs)					
Accession number	**Gene name**	**Organism**	**Transport mode**	**Mutant phenotype**	**Refs**
SUT1 clade					
At1g22710	*AtSUC2*	*A. thaliana*	H+/sucrose symporter	Impaired phloem loading	[S1]
DQ221698	*PsSUF1*	*P. sativum*	Sucrose facilitator	NA	[S2]
DQ221700	*PvSUF1*	*P. vulgaris*	Sucrose facilitator	NA	[S2]
CAA57726	*SlSUT1*	*S. lycopersicum*	H+/sucrose symporter	Impaired phloem loading	[S3]
Q03411	*SoSUT1*	*S. oleracea*	H+/sucrose symporter	NA	[S4]
CAA48915	*StSUT1*	*S. tuberosum*	H+/sucrose symporter	Impaired phloem loading	[S5]
SUT3 clade					
BAA83501	*ZmSUT1*	*Z. mays*	H+/sucrose symporter	Impaired phloem loading	[S6]
SUT4 clade					
At1g09960	*AtSUT4*	*A. thaliana*	H+/sucrose symporter	NA	[S7]
Q9M423	*HvSUT2*	*H. vulgare*	H+/sucrose symporter	NA	[S8]
CAD61275	*LjSUT4*	*L. japonicus*	H+/sucrose symporter	NA	[S9]
BAI60050	*NtSUT4*	*N. tabacum*	NA	NA	[S10]
HQ540307	*OsSUT2*	*O. sativa japonica*	H+/sucrose symporter	Growth retardation	[S11]
DQ221697	*PsSUF4*	*P. sativum*	Sucrose facilitator	NA	[S2]
HM749900	*PtaSUT4*	*P. tremula x alba*	Sucrose transporter	Increased leaf-to-stem biomass ratios, elevated sucrose content in source leaves and stems, and altered phenylpropanoid metabolism	[S12]
AAG25923.2	*StSUT4*	*S. tuberosum*	H+/sucrose symporter	Early flowering, higher tuber yields and reduced sensitivity toward light enriched in far-red wavelength	[S13]

Plant monosaccharide transporters (MSTs)					
Accession number	**Gene name**	**Organism**	**Transport mode**	**Mutant phenotype**	**Refs**
STP clade					
At1g11260	*AtSTP1*	*A. thaliana*	H+/hexose symporter	Impaired extracellular response to sugars by the embryo and reduced sugar levels in seedlings	[S14, 15]
At1g07340	*AtSTP2*	*A. thaliana*	H+/hexose symporter	NA	[S16]
At5g61520	*AtSTP3*	*A. thaliana*	H+/hexose symporter	NA	[S17]
At3g19930	*AtSTP4*	*A. thaliana*	H+/hexose symporter	NA	[S18]
At1g34580	*AtSTP5*	*A. thaliana*	Not functional in yeast	NA	[S19]
At3g05960	*AtSTP6*	*A. thaliana*	H+/hexose symporter	No differences to WT	[S20]
At4g02050	*AtSTP7*	*A. thaliana*	Not functional in yeast	NA	[S19]
At5g26250	*AtSTP8*	*A. thaliana*	NA	NA	

At1g50310	*AtSTP9*	*A. thaliana*	H+/glucose symporter	NA	[S21]
At3g19940	*AtSTP10*	*A. thaliana*	NA	NA	
At5g23270	*AtSTP11*	*A. thaliana*	H+/hexose symporter	NA	[S22]
At4g21480	*AtSTP12*	*A. thaliana*	NA	NA	
At5g26340	*AtSTP13*	*A. thaliana*	H+/hexose symporter	NA	[S23]
At1g77210	*AtSTP14*	*A. thaliana*	H+/galactose symporter	No differences to WT	[S24]
Medtr1g104780	*MtHext1*	*M. truncatula A17*	NA	NA	
Medtr1g038630		*M. truncatula A17*	NA	NA	
Medtr1g104750		*M. truncatula A17*	NA	NA	
Medtr1g104770		*M. truncatula A17*	NA	NA	
Medtr3g007910		*M. truncatula A17*	NA	NA	
Medtr3g008160		*M. truncatula A17*	NA	NA	
Medtr3g008170		*M. truncatula A17*	NA	NA	
Medtr3g023480		*M. truncatula A17*	NA	NA	
Medtr3g093010		*M. truncatula A17*	NA	NA	
Medtr3g093060		*M. truncatula A17*	NA	NA	
Medtr4g090600		*M. truncatula A17*	NA	NA	
Medtr4g091370		*M. truncatula A17*	NA	NA	
Medtr4g116770		*M. truncatula A17*	NA	NA	
Medtr4g116800		*M. truncatula A17*	NA	NA	
Medtr5g006070		*M. truncatula A17*	NA	NA	
Medtr5g041550		*M. truncatula A17*	NA	NA	
Medtr5g082540		*M. truncatula A17*	NA	NA	
Medtr5g094760		*M. truncatula A17*	NA	NA	
Medtr6g087040		*M. truncatula A17*	NA	NA	
Medtr8g102860		*M. truncatula A17*	NA	NA	
Medtr8g103010		*M. truncatula A17*	NA	NA	
CAD31121		*M. truncatula A17*	NA	NA	[S25]
AAB06594	*Mtst1*	*M. truncatula 2828*	H+/hexose symporter	NA	[S26]
BAB19862	*OsMST1*	*O. sativa japonica*	Not functional in yeast	NA	[S27]
Os03g39710	*OsMST2*	*O. sativa japonica*	Hexose transporter	NA	[S27]
Os07g01560	*OsMST3*	*O. sativa japonica*	H+/hexose symporter	NA	[S27]
Os08g08070	*OsMST5*	*O. sativa japonica*	NA	NA	[S28]
Os01g38670	*OsMST8*	*O. sativa japonica*	NA	NA	[S28]
Os01g38680		*O. sativa japonica*	NA	NA	
Os02g06540		*O. sativa japonica*	NA	NA	
Os02g36420		*O. sativa japonica*	NA	NA	
Os02g36440		*O. sativa japonica*	NA	NA	
Os02g36450		*O. sativa japonica*	NA	NA	
Os03g01170		*O. sativa japonica*	NA	NA	
Os03g11900		*O. sativa japonica*	NA	NA	
Os04g37970		*O. sativa japonica*	NA	NA	
Os04g37980		*O. sativa japonica*	NA	NA	
Os04g37990		*O. sativa japonica*	NA	NA	
Os04g38010		*O. sativa japonica*	NA	NA	
Os04g38020		*O. sativa japonica*	NA	NA	
Os04g38220		*O. sativa japonica*	NA	NA	
Os06g04900		*O. sativa japonica*	NA	NA	
Os07g03910		*O. sativa japonica*	NA	NA	
Os07g03960		*O. sativa japonica*	NA	NA	
Os07g10590		*O. sativa japonica*	NA	NA	
Os07g37320		*O. sativa japonica*	NA	NA	
Os09g09520		*O. sativa japonica*	NA	NA	
Os09g12590		*O. sativa japonica*	NA	NA	
Os09g15330		*O. sativa japonica*	NA	NA	
Os09g24930		*O. sativa japonica*	NA	NA	
Os10g41190		*O. sativa japonica*	NA	NA	
Os11g38160		*O. sativa japonica*	NA	NA	
Os12g04640		*O. sativa japonica*	NA	NA	
AAQ24871	*OsMST4*	*O. sativa indica*	Hexose transporter	NA	[S28]
AAQ24872	*OsMST6*	*O. sativa indica*	Hexose transporter	NA	[S28]

TMTclade					
At1g20840	*TMT1*	*A. thaliana*	H+/ glucose and sucrose antiporter	Double tmt1-2: growth retardation, impaired seed quality	[S29-31]
At4g35300	*TMT2*	*A. thaliana*	H+/ glucose and sucrose antiporter	Double tmt1-2: growth retardation, impaired seed quality	[S29-31]
At3g51490	*TMT3*	*A. thaliana*	NA	NA	[S31]
Medtr8g073100		*M. truncatula A17*	NA	NA	
Medtr5g044910		*M. truncatula A17*	NA	NA	
Medtr5g024740		*M. truncatula A17*	NA	NA	
Medtr3g116060		*M. truncatula A17*	NA	NA	
Medtr3g118530		*M. truncatula A17*	NA	NA	
Os10g39440	*OsTMT1*	*O. sativa japonica*	Hexose transporter	NA	[S32]
Os02g13560	*OsTMT2*	*O. sativa japonica*	NA	NA	[S32]
Os03g03680	*OsTMT3*	*O. sativa japonica*	NA	NA	[S32]
Os11g40540	*OsTMT4*	*O. sativa japonica*	NA	NA	[S32]
Os02g58530		*O. sativa japonica*	NA	NA	
Os11g28610		*O. sativa japonica*	NA	NA	
PMT clade					
At2g16120	*AtPMT1*	*A. thaliana*	H+/hexose and polyol symporter	NA	[S33]
At2g16130	*AtPMT2*	*A. thaliana*	H+/hexose and polyol symporter	NA	[S33]
At2g18480	*AtPMT3*	*A. thaliana*	NA	NA	[S33]
At2g20780	*AtPMT4*	*A. thaliana*	NA	NA	[S33]
At3g18830	*AtPMT5*	*A. thaliana*	H+/hexose and polyol symporter	No differences to WT	[S34, 35]
At4g36670	*AtPMT6*	*A. thaliana*	NA	NA	[S33]
Medtr2g013310		*M. truncatula A17*	NA	NA	
Medtr3g116240		*M. truncatula A17*	NA	NA	
Medtr4g071950		*M. truncatula A17*	NA	NA	
Medtr4g072030		*M. truncatula A17*	NA	NA	
Medtr5g019870		*M. truncatula A17*	NA	NA	
Medtr5g075300		*M. truncatula A17*	NA	NA	
Medtr6g007340		*M. truncatula A17*	NA	NA	
Medtr6g088450		*M. truncatula A17*	NA	NA	
Medtr8g077890		*M. truncatula A17*	NA	NA	
Medtr8g103500		*M. truncatula A17*	NA	NA	
MtC00740		*M. truncatula A17*	NA	NA	
Os01g73590		*O. sativa japonica*	NA	NA	
Os03g10090		*O. sativa japonica*	NA	NA	
Os03g10100		*O. sativa japonica*	NA	NA	
Os04g44750		*O. sativa japonica*	NA	NA	
Os04g58220		*O. sativa japonica*	NA	NA	
Os04g58230		*O. sativa japonica*	NA	NA	
Os07g39350		*O. sativa japonica*	NA	NA	
Os07g39360		*O. sativa japonica*	NA	NA	
Os10g21590		*O. sativa japonica*	NA	NA	
Os11g41830		*O. sativa japonica*	NA	NA	
Os11g41840		*O. sativa japonica*	NA	NA	
Os11g41850		*O. sativa japonica*	NA	NA	
Os11g41870		*O. sativa japonica*	NA	NA	
Os12g32760		*O. sativa japonica*	NA	NA	
Os12g32940		*O. sativa japonica*	NA	NA	
VGT clade					
At3g03090	*VGT1*	*A. thaliana*	H+/hexose symporter	Delay in flowering, impaired seed germination	[S36]
At5g17010	*VGT2*	*A. thaliana*	NA	NA	[S36, 37]
At5g59250		*A. thaliana*	NA	NA	[S36, 37]
Medtr4g064820		*M. truncatula A17*	NA	NA	
Medtr4g077770		*M. truncatula A17*	NA	NA	

Os03g60820		*O. sativa japonica*	NA	NA	
Os10g42830		*O. sativa japonica*	NA	NA	
pGlct/SGB1 clade					
pGlct	*At5g16150*	*A. thaliana*	Hexose transporter	No differences to WT	[S38, 39]
SGB1	*At1g79820*	*A. thaliana*	Glucose transporter	NA	[S40]
At1g05030		*A. thaliana*	NA	NA	
At1g67300		*A. thaliana*	NA	NA	
Medtr3g080240		*M. truncatula A17*	NA	NA	
Medtr6g087910		*M. truncatula A17*	NA	NA	
Medtr7g082270		*M. truncatula A17*	NA	NA	
Os01g04190	*OspGlcT*	*O. sativa japonica*	NA	NA	[S41]
Os09g23110	*OsGMST1*	*O. sativa japonica*	NA	Hypersensitivity to salt stress	[S41]
Os02g17500		*O. sativa japonica*	NA	NA	
Os09g27900		*O. sativa japonica*	NA	NA	
ESL clade					
At1g08930	*ERD6*	*A. thaliana*	NA	NA	[S42]
At1g75220	*AtERDL6*	*A. thaliana*	NA	Impaired vacuolar sugar fluxes. Increased sensitivity against external glucose. Enhanced levels of seed sugars, proteins, and lipids	[S43]
At1g08920	*ESL1*	*A. thaliana*	Hexose facilitator	No differences to WT	[S44]
At1g08900	*ESL2*	*A. thaliana*	NA	NA	[S44]
At1g08890	*ESL3*	*A. thaliana*	NA	NA	[S44]
At5g27350	*SFP1*	*A. thaliana*	NA	No differences to WT	[S45]
At5g27360	*SFP2*	*A. thaliana*	NA	NA	[S45]
At1g19450		*A. thaliana*	NA	NA	
At1g54730		*A. thaliana*	NA	NA	
At2g48020		*A. thaliana*	NA	NA	
At3g05150		*A. thaliana*	NA	NA	
At3g05155		*A. thaliana*	NA	NA	
At3g05160		*A. thaliana*	NA	NA	
At3g05165		*A. thaliana*	NA	NA	
At3g05400		*A. thaliana*	NA	NA	
At3g20460		*A. thaliana*	NA	NA	
At4g04750		*A. thaliana*	NA	NA	
At4g04760		*A. thaliana*	NA	NA	
At5g18840		*A. thaliana*	NA	NA	
Medtr2g020710		*M. truncatula A17*	NA	NA	
Medtr4g118610		*M. truncatula A17*	NA	NA	
Medtr5g020270		*M. truncatula A17*	NA	NA	
Medtr7g113960		*M. truncatula A17*	NA	NA	
Medtr7g113970		*M. truncatula A17*	NA	NA	
Medtr8g077300		*M. truncatula A17*	NA	NA	
Medtr8g077310		*M. truncatula A17*	NA	NA	
MtC20248		*M. truncatula A17*	NA	NA	
Os03g24860		*O. sativa japonica*	NA	NA	
Os03g24870		*O. sativa japonica*	NA	NA	
Os05g49260		*O. sativa japonica*	NA	NA	
Os05g49270		*O. sativa japonica*	NA	NA	
Os05g50280		*O. sativa japonica*	NA	NA	
Os11g42430		*O. sativa japonica*	NA	NA	
INT clade					
At2g43330	*INT1*	*A. thaliana*	H+/ myoinositol symporter	Reduced root length	[S46]
At1g30220	*INT2*	*A. thaliana*	H+/ myoinositol symporter	No differences to WT	[S47]
At2g35740	*INT3*	*A. thaliana*	Pseudogene	NA	[S47]
At4g16480	*INT4*	*A. thaliana*	H+/ myoinositol symporter	No differences to WT	[S48]
Medtr1g116650		*M. truncatula A17*	NA	NA	
Medtr1g116660		*M. truncatula A17*	NA	NA	
Medtr1g116830		*M. truncatula A17*	NA	NA	

Medtr2g026140		*M. truncatula A17*	NA	NA	
Medtr2g026160		*M. truncatula A17*	NA	NA	
Medtr2g048720		*M. truncatula A17*	NA	NA	
Medtr2g049020		*M. truncatula A17*	NA	NA	
Medtr3g084110		*M. truncatula A17*	NA	NA	
Medtr5g077580		*M. truncatula A17*	NA	NA	
Medtr7g005910		*M. truncatula A17*	NA	NA	
Os04g41460		*O. sativa japonica*	NA	NA	
Os04g43210		*O. sativa japonica*	NA	NA	
Os07g05640		*O. sativa japonica*	NA	NA	
Fungal sugar transporters					
Accession number	**Gene name**	**Organism**	**Transport mode**	**Mutant phenotype**	**Refs**
Fungal sucrose transporters					
NP_594387.1	*SpSUT1*	*S. pombe*	H+/sucrose symporter	No differences to WT	[S49]
XP_758521.1	*UmSRT1*	*U. maydis*	H+/sucrose symporter	Loss of fungal virulence	[S50]
ACS83541.1	*MRT*	*M. robertsii*	Sucrose symporter	Reduced or no growth on sucrose and galactosides	[S51]
CBH19584.1	*TvSUT*	*T. virens*	H+/sucrose symporter	Reduced growth on sucrose	[S52]
HQ848966	*GiSUC1*	*G. intraradices*	NA	NA	[S53]
Fungal monosaccharide transporters					
AJ310209	*UfHXT1*	*U. fabae*	H+/hexose symporter	NA	[S54]
AAU87358	*BcFRT1*	*B. cinerea*	H+/fructose symporter	Delay in fructose-induced germination	[S55]
O13411	*AmMST1*	*A. muscaria*	Hexose transporter	NA	[S56]
AmMST2	*AmMST2*	*A. muscaria*	Hexose transporter	NA	[S57]
AY956320	*TbHXT1*	*T. borchii*	H+/hexose symporter	NA	[S58]
AM231332	*GpMST1*	*G. pyriformis*	H+/hexose symporter	NA	[S59]
HM143864	*GiMST2*	*G. intraradices*	H+/hexose symporter	Lower mycorrhization levels, abnormal arbuscule morphology	[S53]

Supplementary references

S1 Gottwald, J.R., et al. (2000) Genetic evidence for the in planta role of phloem-specific plasma membrane sucrose transporters. Proceedings of the National Academy of Sciences 97, 13979-13984

S2 Zhou, Y.C., et al. (2007) A suite of sucrose transporters expressed in coats of developing legume seeds includes novel pH-independent facilitators. Plant J. 49, 750-764

S3 Hackel, A., et al. (2006) Sucrose transporter LeSUT1 and LeSUT2 inhibition affects tomato fruit development in different ways. Plant J. 45, 180 - 192

S4 Riesmeier, J., et al. (1992) Isolation and characterization of a sucrose carrier cDNA from spinach by functional expression in yeast. EMBO J. 11, 4705 - 4713

S5 Riesmeier, J.W., et al. (1994) Evidence for an essential role of the sucrose transporter in phloem loading and assimilate partitioning. EMBO J. 13, 1-7

S6 Slewinski, T.L., et al. (2009) Sucrose transporter1 functions in phloem loading in maize leaves. J. Exp. Bot. 60, 881-892

S7 Weise, A., et al. (2000) A new subfamily of sucrose transporters, SUT4, with low affinity/high capacity localized in enucleate sieve elements of plants. Plant Cell 12, 1345-1356

S8 Weschke, W., et al. (2000) Sucrose transport into barley seeds: molecular characterization of two transporters and implications for seed development and starch accumulation. Plant J. 21, 455-467

S9 Reinders, A., et al. (2008) Functional analysis of LjSUT4, a vacuolar sucrose transporter from Lotus japonicus. Plant Mol. Biol. 68, 289-299

S10 Okubo-Kurihara, E., et al. (2011) Sucrose transporter NtSUT4 from tobacco BY-2 involved in plant cell shape during miniprotoplast culture. J. Plant Res. 124, 395-403

S11 Eom, J.-S., et al. (2011) Impaired function of the tonoplast-localized sucrose transporter in rice (Oryza sativa), OsSUT2, limits the transport of vacuolar reserve sucrose and affects plant growth. Plant Physiol. 157, 109-119

S12 Payyavula, R.S., et al. (2011) The sucrose transporter family in Populus: the importance of a tonoplast PtaSUT4 to biomass and carbon partitioning. Plant J. 65, 757-770

S13 Chincinska, I.A., et al. (2008) Sucrose transporter StSUT4 from potato affects flowering, tuberization, and shade avoidance response. Plant Physiol. 146, 515-528

S14 Sauer, N., et al. (1990) Primary structure, genomic organization and heterologous expression of a glucose transporter from Arabidopsis thaliana. EMBO J. 9, 3045-3050

S15 Sherson, S.M., et al. (2000) Monosaccharide/proton symporter AtSTP1 plays a major role in uptake and response of Arabidopsis seeds and seedlings to sugars. Plant J. 24, 849-857
S16 Truernit, E., et al. (1999) A male gametophyte-specific monosaccharide transporter in Arabidopsis. Plant J. 17, 191-201
S17 Buttner, M., et al. (2000) AtSTP3, a green leaf-specific, low affinity monosaccharide-H+ symporter of Arabidopsis thaliana. Plant Cell Environ. 23, 175-184
S18 Truernit, E., et al. (1996) The sink-specific and stress-regulated Arabidopsis STP4 gene: enhanced expression of a gene encoding a monosaccharide transporter by wounding, elicitors, and pathogen challenge. Plant Cell 8, 2169-2182
S19 Büttner, M. (2010) The Arabidopsis sugar transporter (AtSTP) family: an update. Plant biol. 12, 35-41
S20 Scholz-Starke, J., et al. (2003) AtSTP6, a new pollen-specific H+-monosaccharide symporter from Arabidopsis. Plant Physiol. 131, 70-77
S21 Schneidereit, A., et al. (2003) Functional characterization and expression analyses of the glucose-specific AtSTP9 monosaccharide transporter in pollen of Arabidopsis. Plant Physiol. 133, 182-190
S22 Schneidereit, A., et al. (2005) AtSTP11, a pollen tube-specific monosaccharide transporter in Arabidopsis. Planta 221, 48-55
S23 Norholm, M., et al. (2006) Expression of the Arabidopsis high-affinity hexose transporter STP13 correlates with programmed cell death. FEBS Lett. 580, 2381 - 2387
S24 Poschet, G., et al. (2010) Identification and characterization of AtSTP14, a novel galactose transporter from Arabidopsis. Plant Cell Physiol. 51, 1571-1580
S25 Lefebvre, B., et al. (2007) Characterization of lipid rafts from Medicago truncatula root plasma membranes: a proteomic study reveals the presence of a raft-associated redox system. Plant Physiol. 144, 402-418
S26 Harrison, M.J. (1996) A sugar transporter from Medicago truncatula: Altered expression pattern in roots during vesicular-arbuscular (VA) mycorrhizal associations. Plant J. 9, 491-503
S27 Toyofuku, K., et al. (2000) Characterization and Expression of Monosaccharide Transporters (OsMSTs) in Rice. Plant Cell Physiol. 41, 940-947
S28 Wang, Y., et al. (2008) Molecular cloning, functional characterization and expression analysis of a novel monosaccharide transporter gene OsMST6 from rice (Oryza sativa L.). Planta 228, 525-535
S29 Wingenter, K., et al. (2010) Increased activity of the vacuolar monosaccharide transporter TMT1 alters cellular sugar partitioning, sugar signaling, and seed yield in Arabidopsis. Plant Physiol. 154, 665-677
S30 Schulz, A., et al. (2011) Proton-driven sucrose symport and antiport are provided by the vacuolar transporters SUC4 and TMT1/2. Plant J. 68, 129-136
S31 Wormit, A., et al. (2006) Molecular identification and physiological characterization of a novel monosaccharide transporter from Arabidopsis involved in vacuolar sugar transport. Plant Cell 18, 3476-3490
S32 Cho, J.I., et al. (2010) Expression analysis and functional characterization of the monosaccharide transporters, OsTMTs, involving vacuolar sugar transport in rice (Oryza sativa). New Phytol. 186, 657-668
S33 Klepek, Y., et al. (2010) Arabidopsis thaliana POLYOL/MONOSACCHARIDE TRANSPORTERS 1 and 2: fructose and xylitol/H+ symporters in pollen and young xylem cells. J. Exp. Bot. 61, 537 - 550
S34 Klepek, Y., et al. (2005) Arabidopsis POLYOL TRANSPORTER5, a new member of the monosaccharide transporter-like superfamily, mediates H+-Symport of numerous substrates, including myo-inositol, glycerol, and ribose. Plant Cell 17, 204 - 218
S35 Reinders, A., et al. (2005) Analysis of transport activity of Arabidopsis sugar alcohol permease homolog AtPLT5. J. Biol. Chem. 280, 1594 - 1602
S36 Aluri, S. and Buttner, M. (2007) Identification and functional expression of the Arabidopsis thaliana vacuolar glucose transporter 1 and its role in seed germination and flowering. Proc. Natl. Acad. Sci. USA 104, 2537-2542
S37 Buttner, M. (2007) The monosaccharide transporter(-like) gene family in Arabidopsis. FEBS Lett. 581, 2318-2324
S38 Weber, A., et al. (2000) Identification, purification, and molecular cloning of a putative plastidic glucose translocator. Plant Cell 12, 787-801
S39 Cho, M.-H., et al. (2011) Role of the plastidic glucose translocator in the export of starch degradation products from the chloroplasts in Arabidopsis thaliana. New Phytol. 190, 101-112
S40 Wang, H.X., et al. (2006) A golgi-localized hexose transporter is involved in heterotrimeric G protein-mediated early development in Arabidopsis. Mol. Biol. Cell 17, 4257-4269
S41 Cao, H., et al. (2011) Reduced expression of a gene encoding a Golgi localized monosaccharide transporter (OsGMST1) confers hypersensitivity to salt in rice (Oryza sativa). J. Exp. Bot.
S42 Kiyosue, T., et al. (1998) ERD6, a cDNA clone for an early dehydration-induced gene of Arabidopsis, encodes a putative sugar transporter. Biochim. Biophys. Acta 1370, 187 - 191
S43 Poschet, G., et al. (2011) A novel Arabidopsis vacuolar glucose exporter is involved in cellular sugar homeostasis and affects the composition of seed storage compounds. Plant Physiol. 157, 1664-1676
S44 Yamada, K., et al. (2010) Functional analysis of an Arabidopsis thaliana abiotic stress-inducible facilitated diffusion transporter for monosaccharides J. Biol. Chem. 285, 1138-1146
S45 Quirino, B., et al. (2001) One of two tandem Arabidopsis genes homologous to monosaccharide transporters is senescence-associated. Plant Mol. Biol. 46, 447 - 457
S46 Schneider, S., et al. (2008) Functional and physiological characterization of Arabidopsis INOSITOL TRANSPORTER1, a novel tonoplast-localized transporter for myo-inositol. Plant Cell 20, 1073 - 1087
S47 Schneider, S., et al. (2007) Arabidopsis INOSITOL TRANSPORTER2 mediates H+ symport of different inositol epimers and derivatives across the plasma membrane. Plant Physiol. 145, 1395-1407
S48 Schneider, S., et al. (2006) Arabidopsis INOSITOL TRANSPORTER4 mediates high-affinity H+ symport of myoinositol across the plasma membrane. Plant Physiol. 141, 565-577
S49 Reinders, A. and Ward, J.M. (2001) Functional characterization of the α-glucoside transporter Sut1p from Schizosaccharomyces pombe, the first fungal homologue of plant sucrose transporters. Mol. Microbiol. 39, 445-455
S50 Wahl, R., et al. (2010) A novel high-affinity sucrose transporter is required for virulence of the plant pathogen Ustilago maydis. PLoS Biol. 8, e1000303
S51 Fang, W. and St. Leger, R.J. (2010) Mrt, a gene unique to fungi, encodes an oligosaccharide transporter and facilitates rhizosphere competency in Metarhizium robertsii. Plant Physiol. 154, 1549-1557
S52 Vargas, W.A., et al. (2011) Functional characterization of a plant-like sucrose transporter from the beneficial fungus Trichoderma virens. Regulation of the symbiotic association with plants by sucrose metabolism inside the fungal cells. New Phytol. 189, 777-789

S53 Helber, N., et al. (2011) A versatile monosaccharide transporter that operates in the arbuscular mycorrhizal fungus Glomus sp is Crucial for the symbiotic relationship with plants. Plant Cell 23, 3812-3823
S54 Voegele, R.T., et al. (2001) The role of haustoria in sugar supply during infection of broad bean by the rust fungus Uromyces fabae. Proc. Natl. Acad. Sci. USA 98, 8133-8138
S55 Doehlemann, G., et al. (2005) Molecular and functional characterization of a fructose specific transporter from the gray mold fungus Botrytis cinerea. Fungal Genet. Biol. 42, 601-610
S56 Wiese, J., et al. (2000) Functional characterization of the Amanita muscaria monosaccharide transporter, AmMst1. Plant biol. 2, 278-282
S57 Nehls, U. (2004) Carbohydrates and nitrogen: nutrients and signals in ectomycorrhizas. In Plant surface microbiology (Varma, A., et al., eds), pp. 373–392, Berlin: Springer Verlag
S58 Polidori, E., et al. (2007) Hexose uptake in the plant symbiotic ascomycete Tuber borchii Vittadini: biochemical features and expression pattern of the transporter TBHXT1. Fungal Genet. Biol. 44, 187-198
S59 Schüßler, A., et al. (2006) Characterization of a carbohydrate transporter from symbiotic glomeromycotan fungi. Nature 444, 933-936

Molecular Plant Advance Access published August 28, 2012

The *Medicago truncatula* Sucrose Transporter Family: Characterization and Implication of Key Members in Carbon Partitioning towards Arbuscular Mycorrhizal Fungi

Joan Doidy, Diederik van Tuinen, Olivier Lamotte, Marion Corneillat, Gérard Alcaraz and **Daniel Wipf**[1]

UMR INRA 1347, Agrosup, Université de Bourgogne, Agroécologie, Pôle Interactions Plantes Microorganismes ERL CNRS 6300, BP 86510, 21065 Dijon Cedex, France

ABSTRACT We identified *de novo* sucrose transporter (SUT) genes involved in long-distance transport of sucrose from photosynthetic source leaves towards sink organs in the model leguminous species *Medicago truncatula*. The identification and functional analysis of sugar transporters provide key information on mechanisms that underlie carbon partitioning in plant–microorganism interactions. In that way, full-length sequences of the *M. truncatula* SUT (MtSUT) family were retrieved and biochemical characterization of MtSUT members was performed by heterologous expression in yeast. The MtSUT family now comprises six genes which distribute among Dicotyledonous clades. MtSUT1-1 and MtSUT4-1 are key members in regard to their expression profiles in source leaves and sink roots and were characterized as functional H^+/sucrose transporters. Physiological and molecular responses to phosphorus supply and inoculation by the arbuscular mycorrhizal fungus (AMF) *Glomus intraradices* was studied by gene expression and sugar quantification analyses. Sucrose represents the main sugar transport form in *M. truncatula* and the expression profiles of *MtSUT1-1*, *MtSUT2*, and *MtSUT4-1* highlight a fine-tuning regulation for beneficial sugar fluxes towards the fungal symbiont. Taken together, these results suggest distinct functions for proteins from the SUT1, SUT2, and SUT4 clades in plant and in biotrophic interactions.

Key words: sugar transport; sucrose transporter; SUT; sugar partitioning; *Medicago truncatula*; *Glomus intraradices*.

INTRODUCTION

In plants, sucrose (fructose $\beta 2 \leftrightarrow 1 \alpha$ glucose) constitutes the main form of carbohydrate for long-distance transport. This disaccharide is synthesized in the photosynthetic source leaves and is then loaded into the phloem sap, where the disaccharide follows its route through the transport phloem. Finally, sucrose is unloaded to supply sink tissues. Thereby, sucrose transporters (SUTs also called SUCs; for review, see Lalonde et al., 2004; Sauer, 2007; Kühn and Grof, 2010; Slewinski and Braun, 2010; Ayre, 2011; Geiger, 2011; Doidy et al., 2012) are key components of carbon partitioning from source to sink organs. SUTs are members of the major facilitator superfamily (MFS) and present a common structure with 12 transmembrane domains that are assumed to form a single pore for sucrose and most SUTs have been characterized as H^+/sucrose co-transporters. Although plant genome sequencing projects and library analysis led to the identification of the sucrose transporter family in numerous reference plant species (Aoki et al., 2003; Sauer, 2007; Afoufa-Bastien et al., 2010; Payyavula et al., 2011), the SUT family of the model legume *Medicago truncatula* had not yet been identified.

Sugars can also be transferred to non-plant sinks and root colonization by beneficial symbionts constitutes an additional sugar demand for the host plant (Doidy et al., 2012). Here, we focused on the model symbiotic interaction between the model legume *M. truncatula* and the arbuscular mycorrhizal fungus (AMF) *Glomus intraradices*. Arbuscular mycorrhiza (AM) is a mutualistic interaction based on biotrophic exchanges between the plant and the fungal partners (Smith and Read, 2008). The fungus supplies the autotrophic host

[1] To whom correspondence should be addressed. E-mail daniel.wipf@dijon.infra.fr, tel. +33 (0)3 80 69 34 52, fax +33 (0)3 80 69 37 53.

doi:10.1093/mp/sss079

Received 30 December 2011; accepted 14 July 2012

with water and nutrients, mainly phosphate, and thereby enhances plant growth. In return, the plant provides sugar photosynthates to the heterotrophic symbiont. Therefore, root colonization by AMF increases the global sink strength. Indeed, heterotrophic fungal growth, spore formation, and respiration as well as increased metabolism in several plant tissues augment the sugar demand and it has been estimated that between 4% and 20% of total photoassimilates are redirected towards colonized parts (Douds et al., 2000; Graham, 2000). This increased sink strength is balanced by greater CO_2 assimilation and higher photosynthetic rate in source leaves (Wright et al., 1998; Boldt et al., 2011).

In leaves, sucrose can be loaded actively by SUTs to the companion cell sieve element complex via the apoplast (for review of phloem loading strategies, see Rennie and Turgeon, 2009; Eom et al., 2012). Indeed, in apoplastic loading species, a single protein member of the SUT1 clade is responsible for the loading of sucrose into the phloem (Zhang and Turgeon, 2009). The down-regulation (Riesmeier et al., 1994; Kühn et al., 1996; Bürkle et al., 1998; Schulz et al., 1998; Hackel et al., 2006) or total gene disruption (Gottwald et al., 2000) of the phloem loading protein causes sugar accumulation and chlorotic lesions in source leaves as well as reduced growth of sink organs and thereby an overall stunted plant growth. Up to now, a single *SUT1* knockdown mutant has been investigated in mycorrhizal conditions; the *StSUT1* mutation in potato with impaired sucrose export from source leaves, had no effect on fungal colonization rates (Gabriel-Neumann et al., 2011). These results have to be tempered by the observation of increased sugar transport towards colonized parts to feed AMF (Wright et al., 1998; Bago et al., 2000; Douds et al., 2000; Graham, 2000), likely by means of SUTs, which present differential transcript accumulation in AM conditions (Ge et al., 2008; Boldt et al., 2011; Gaude et al., 2011). In that way, potato lines overexpressing an orthologous SUT1 gene had higher colonization rates but only when cultivated under high phosphate supply (Gabriel-Neumann et al., 2011).

At the cellular level, NMR spectrometry experiments revealed that the fungal intraradical mycelium can take up hexoses, mainly glucose and to a smaller extent fructose, but not sucrose (Solaiman and Saito, 1997; Pfeffer et al., 1999; Bago et al., 2003). Consistently with this, tomato roots colonized by *G. mosseae* showed a higher accumulation of sucrose and fructose (Boldt et al., 2011). Indeed, before the transfer of hexoses at the plant–fungal interface, sucrose is cleaved by either plant enzymes, sucrose synthase, and/or invertases. In *M. truncatula*, a study showed promoter activity of the sucrose synthase gene (*MtSucS1*) around internal hyphae and arbuscules which represent the major fungal structures for sugar transfer (Hohnjec et al., 2003; Helber et al., 2011). Furthermore, expression and activity of all types of sucrose cleaving enzymes appeared to be up-regulated in the presence of AMF (Wright et al., 1998; Ravnskov et al., 2003; Garcia-Rodriguez et al., 2007; Tejeda-Sartorius et al., 2008).

Although sucrose cleaving enzymes are being studied, the complete sucrose transporter family of the model leguminous species *M. truncatula* is still not identified and the mechanisms controlling nutrient exchanges in AM symbiosis remain to be deciphered. In this work, we report a comprehensive study of sucrose transporters in *M. truncatula* (MtSUTs) and their implication in carbon partitioning towards the fungal symbiont during the AM interaction with *G. intraradices*.

RESULTS

The *Medicago truncatula* SUT Family

In silico screening led to the identification of two genomic loci (*Medtr4g131920.1* and *Medtr5g067470.1*), one full-length contig, and three partial ESTs corresponding to putative MtSUTs (Supplemental Table 1). Furthermore, two additional accessions were identified in the version 3.5 of the *M. truncatula* genome (Young et al., 2011); however, both open reading frames of these tandem loci are interrupted by a stop codon and therefore were considered as pseudogenes encoding for truncated proteins (Supplemental Table 1). After rapid amplification of cDNA ends (RACE) to amplify full-length coding sequences, we cloned the MtSUT family that now comprises six members. The six MtSUTs share an amino acid similarity ranging from 35% to 77% and common SUT features with 12 predicted transmembrane domains (Supplemental Figure 1).

A SUT phylogenetic tree based on the amino acid sequence alignment of 88 plant SUTs including the newly found MtSUTs was constructed (Figure 1). The six MtSUTs are distributed in all Dicotyledonous SUT clades; we thus named the different MtSUTs according to their phylogenetic position. Three MtSUTs belong to the SUT1 clade (MtSUT1-1, MtSUT1-2, and MtSUT1-3). MtSUT2 is linked to the SUT2 clade, being the most distant protein due to the longer N-terminal region and the extended central loop between transmembrane domains 6 and 7 (Supplemental Figure 1), a common trait of the type IIA members of the SUT2 clade. MtSUT4-1 and MtSUT4-2 belong to the SUT4 clade. So far, duplicated genes in the SUT4

Table 1. Leguminous SUT Properties.

Gene name	Km for sucrose	Transport properties	Reference
MtSUT1-1	1.7 mM (pH 5)	Active symporter	
GmSUT1	5.6 mM (pH4)	Active symporter	Aldape et al., 2003
PsSUT1	1.5 mM (pH 5.5)	Active symporter	Zhou et al., 2007
VfSUT1	ND	ND	Weber et al., 1997
MtSUT4-1	13.7 mM (pH 5)	Active symporter	
LjSUT4	12.9 mM (pH 5)	Active symporter	Reinders et al., 2008
PsSUF4	37.8 mM (pH 5.5)	Facilitator	Zhou et al., 2007

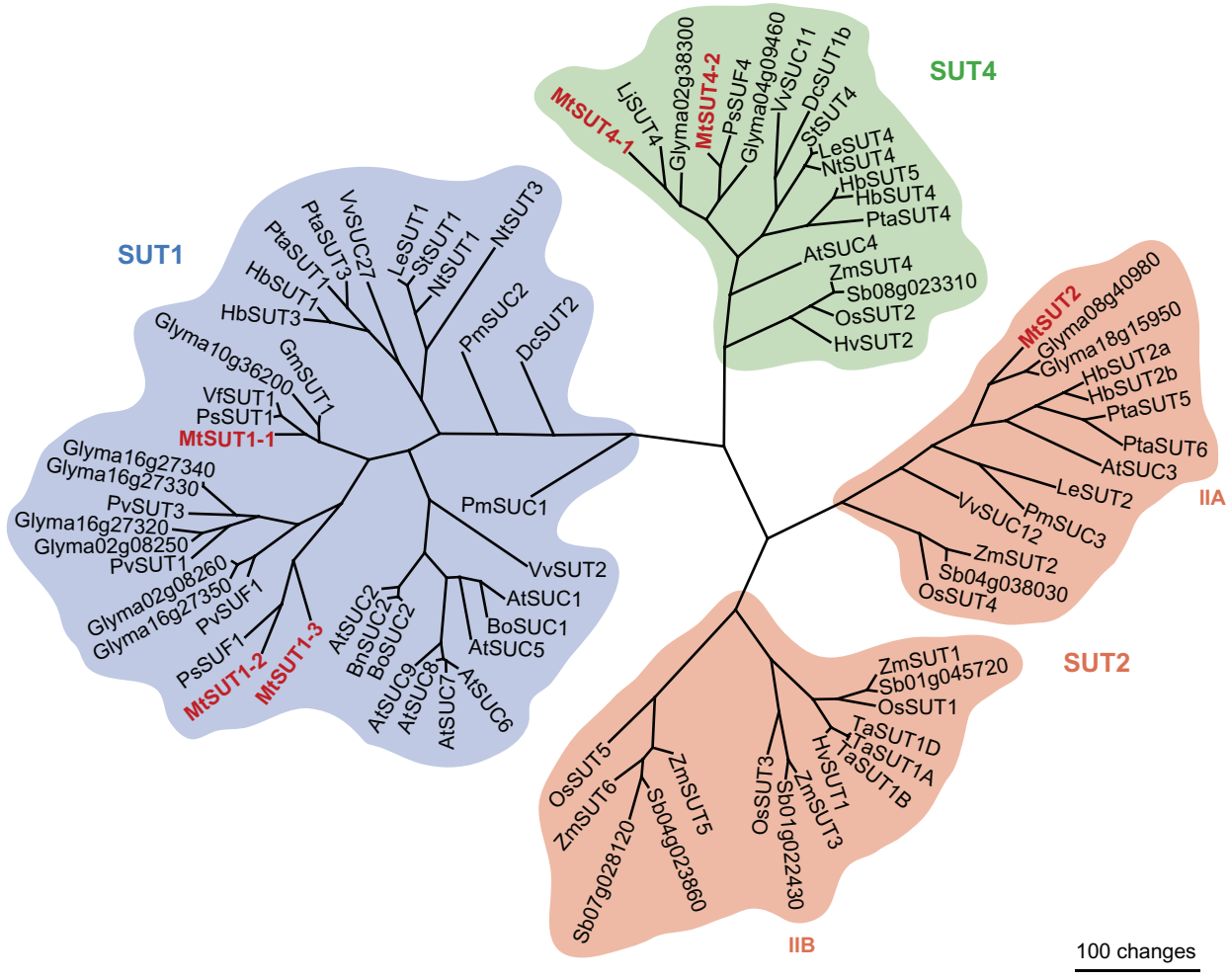


Figure 1. Phylogenetic Tree of the Plant SUT Family. SUT proteins can be divided into the SUT1, SUT2, and SUT4 clades highlighted in colors. Within the SUT2 clade, type IIA and Monocotylodenous-specific type IIB subclades were separated. The following 88 SUT sequences are included: *Arabidopsis thaliana*, nine AtSUTs: AtSUC1 (At1g71880), AtSUC2 (At1g22710), AtSUC3 (At2g02860), AtSUC4 (At1g09960), AtSUC5 (At1g71890), AtSUC6 (At5g43610), AtSUC7 (At1g66570), AtSUC8 (At2g14670), AtSUC9 (At5g06170). *Brassica napus*: BnSUC2 (ACB47398). *Brassica oleracea*: BoSUC1 (AAL58071), BoSUC2 (AAL58072). *Daucus carota*: DcSUT2 (O65803), DcSUT1b (O65929). *Glycine max*, twelve GmSUTs: GmSUT1 (CAD91334), Glyma10g36200, Glyma02g08250, Glyma02g08260, Glyma02g38300, Glyma04g09460, Glyma08g40980, Glyma16g27320, Glyma16g27330, Glyma16g27340, Glyma16g27350, Glyma18g15950. *Hevea brasiliensis*, six HbSUTs: HbSUT1 (ABJ51933), HbSUT2a (ABJ51934), HbSUT2b (ABJ51932), HbSUT3 (ABK60190), HbSUT4 (ABK60191), HbSUT5 (EF067333). *Hordeum vulgare*: HvSUT1 (Q9M422), HvSUT2 (Q9M423). *Lotus japonicus*: LjSUT4 (CAD61275). *M. truncatula*, six MtSUTs: MtSUT1-1 (JN255789), MtSUT1-2 (JN255790), MtSUT1-3 (JN255791), MtSUT2 (JN255792), MtSUT4-1 (JN255793), MtSUT4-2 (JN255794). *Nicotiana tabacum*: NtSUT1 (Q40583), NtSUT3 (Q9XFM1), NtSUT4 (BAI60050). *Oriza sativa*, five OsSUTs: OsSUT1 (AAF90181), OsSUT2 (BAC67163), OsSUT3 (BAB68368), OsSUT4 (BAC67164), OsSUT5 (BAC67165). *Phaseolus vulgaris*: PvSUT1 (ABB30164), PvSUF1 (DQ221700), PvSUT3 (ABB30166). *Pisum sativum*: PsSUT1 (AAD41024), PsSUF1 (DQ221698), PsSUF4 (DQ221697). *Plantago major*: PmSUC1 (CAI59556), PmSUC2 (CAA53390), PmSUC3 (CAD58887). *Populus tremula x alba*, five PtaSUTs: PtaSUT1 (HM749898), PtaSUT3 (HM749899), PtaSUT4 (HM749900), PtaSUT5 (HM749901), PtaSUT6 (HM749902). *Solanum lycopersicum*: SlSUT1 (CAA57726), SlSUT2 (AAG12987), SlSUT4 (AAG09270). *Solanum tuberosum*: StSUT1 (CAA48915), StSUT4 (AAG25923.2). *Sorghum bicolor*, six SbSUTs: Sb01g022430, Sb01g045720, Sb04g023860, Sb04g038030, Sb07g028120, Sb08g023310. *Triticum aestivum*: TaSUT1A (AAM13408), TaSUT1B (AAM13409), TaSUT1D (AAM13410). *Vicia faba*: VfSUT1 (CAB07811). *Vitis vinifera*: VvSUC11 (AF021808), VvSUC12 (AF021809), VvSUC27 (AF021810), VvSUCy (ADP37124). *Zea mays*, six ZmSUTs: ZmSUT1 (BAA83501), ZmSUT2 (AAS91375), ZmSUT3 (ACF86653), ZmSUT4 (AAT51689), ZmSUT5 (ACF85284), ZmSUT6 (ACF85673).

clade were only reported in rubber tree (*Hevea brasiliensis*) and soybean (*Glycine max*).

Promoter regions of the two genomic loci show regulatory *cis*-elements conserved with previously described sugar transporters from *Arabidopsis*, rice, and grape (Supplemental Table 2; Afoufa-Bastien et al., 2010; Ibraheem et al., 2010). In addition, the intron splicing pattern is well conserved between the SUT1 and SUT4 clade members of *M. truncatula*, with *MtSUT1-2*, *MtSUT1-3*, and *MtSUT4-1* presenting a very well conserved motif for exons 3 and 4 (Supplemental Figure 2).

These features suggests a common origin and evolution of *MtSUTs* through gene duplication (Johnson and Thomas, 2007).

Functional Characterization of MtSUTs

The full open reading frames of the six *MtSUTs* were cloned into the yeast expression vector pDRf1–GW and transformed in the yeast mutant strain SUSY7/ura3- suitable for sucrose uptake characterization (Figure 2). A clear complementation was obtained for the yeast strains expressing *MtSUT1-1* and *MtSUT4-1* when grown on minimal medium with 2% sucrose as the sole carbon source. Also, yeast expressing *MtSUT1-2* and to a smaller extent *MtSUT1-3* showed improved growth when compared to the control transformed with the empty vector. *MtSUT2* and *MtSUT4-2* did not restore the growth of the mutant strain. The weak growth of strains expressing *MtSUT1-2* and *MtSUT1-3* was not increased by the addition of 4% and 10% sucrose (Supplemental Figure 3).

The biochemical characterization of the strongly complementing members, MtSUT1-1 and MtSUT4-1, was performed by yeast uptake experimentation using radiolabeled [^{14}C] sucrose. Both transporters present kinetics similar to the previously characterized plant SUTs. MtSUT1-1 shows an apparent Km for sucrose of 1.7 mM whereas MtSUT4-1 has a Km of 13.7 mM at pH 5 (Figure 3A and 3B, and Table 1). Transport dependency on membrane potential revealed that both transporters are H^+/sucrose co-transporters, since their transport properties were curbed upon addition of protonophores and H^+-ATPase inhibitors (Figure 3C). Thereby, we report the first characterization of two *M. truncatula* SUTs, MtSUT1-1 and MtSUT4-1, as functional H^+/sucrose importers.

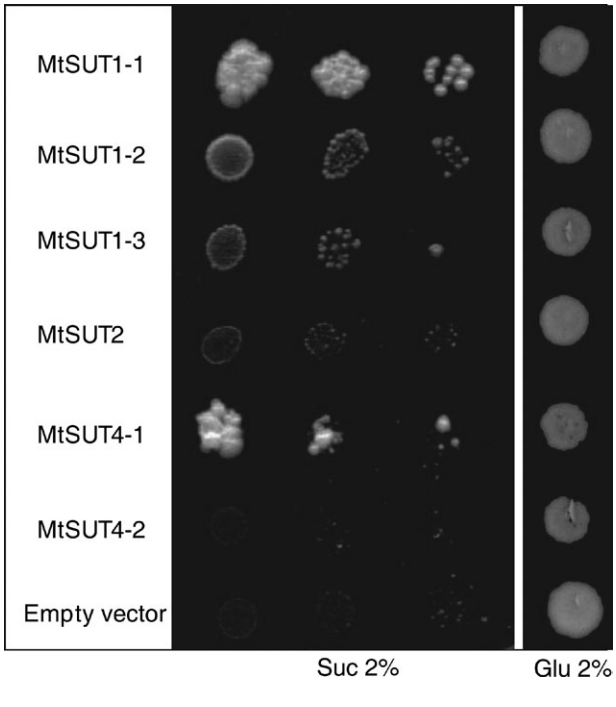


Figure 2. Complementation of the Sucrose Uptake Deficiency of the Yeast Strain SUSY7/ura3-. Serial dilutions of yeast cells were grown on minimum medium without uracil with 2% sucrose (Suc) or glucose (Glu, control) as sole carbon source.

Substrate competition assays showed that, in addition to sucrose, only the disaccharide maltose is bound by MtSUT1-1 and MtSUT4-1 (Figure 3D). However, trehalose did not compete with sucrose uptake and therefore seems not to be a possible substrate for both transporters. The SUT4 clade protein LjSUT4 and the SUT1 clade protein AtSUC9 have also been characterized as sucrose and maltose, but not trehalose transporters by heterologous expression in *Xenopus laevis* oocytes (Sivitz et al., 2007; Reinders et al., 2008). These findings show that SUT specificity is tightly modulated among disaccharides.

Expression Analysis of *MtSUTs*

Transcript accumulation of each member of the MtSUT family was analyzed by quantitative reverse transcription PCR (q-RT-PCR) in plants fertilized with a high-phosphate nutrient solution. While *MtSUT1-3*, *MtSUT2*, *MtSUT4-1*, and *MtSUT4-2* are expressed at a similar level in leaves and roots, *MtSUT1-1* shows a 20-fold higher transcript accumulation in leaves compared to roots (Figure 4). This information suggests a major role for MtSUT1-1 in sucrose fluxes from photosynthetic source leaves to the phloem. Furthermore, SUTs were detected in both sink and source parts. Indeed, transcripts of the six MtSUT members were present in leaves and root tissues with the exception of *MtSUT1-2* only present in leaves (Figure 4).

Analysis of *M. truncatula* Plant Development and *MtSUT* Expression under Differential Phosphate Conditions and upon AM Symbiosis

Plants were cultivated for 4 weeks in low-phosphate (LP) conditions in the absence of fungus for control conditions or upon fungal inoculation of the AMF *G. intraradices* for AM conditions (AM). In addition, a second group of control plants was cultivated in high-phosphate (HP) conditions to balance the provision of phosphate by AMF. After 28 d, no significant differences ($P \leq 0.05$) of the measured growth parameters (Table 2) could be observed between the different experimental conditions. However, the aerial parts of LP plants showed a reduced growth ($P \leq 0.1$) compared to that supplied with HP solution or inoculated by AMF.

The sugar content of source leaves and root compartments was quantified (Figure 5). Sucrose represents the main sugar in leaves and roots of *M. truncatula*. Stachyose and raffinose were respectively detected at substantial levels in leaves and root compartments. Trehalose, already known to be a major form of sugar storage for AMF (Bago et al., 2003), was found exclusively in mycorrhized roots. A decrease of total soluble sugars was observed in source leaves in response to AMF inoculation (Figure 5A); however, only a slight increase in sucrose could be observed in AM-colonized roots compared to the HP-treated plants (Figure 5B).

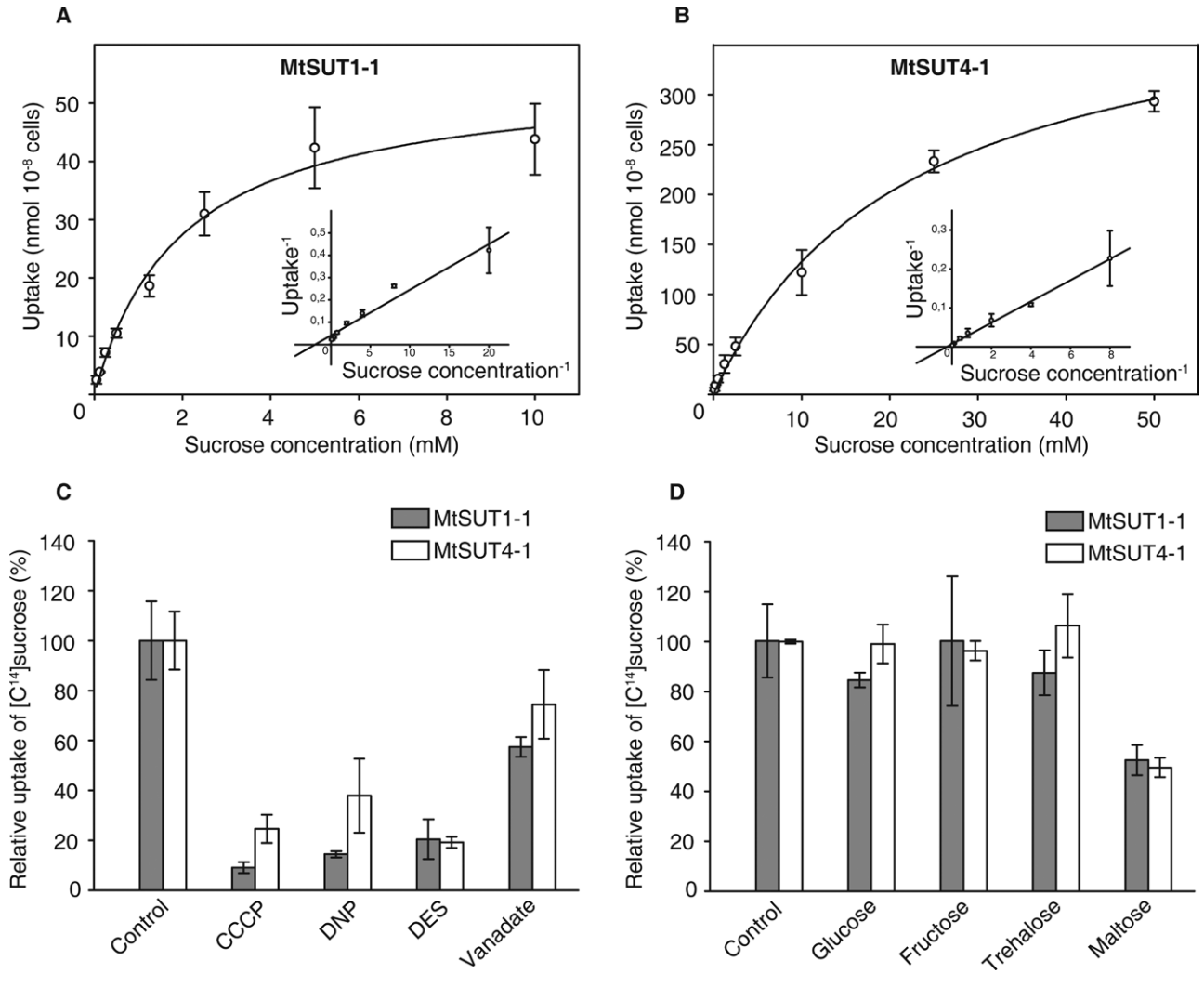


Figure 3. Biochemical Characterization of MtSUT1-1 and MtSUT4-1.

(A, B) Uptake kinetics of [^{14}C]sucrose by the yeast strain SUSY7/ura3- expressing *MtSUT1-1* and *MtSUT4-1* at the indicated sucrose concentration at pH 5. Michaelis-Menten and Lineweaver-Burk representation of the data are illustrated.

(C) Influence of plasma membrane energization on the uptake rate of [^{14}C]sucrose in the yeast mutant expressing *MtSUT1-1* or *MtSUT4-1*.

(D) Substrate specificity of MtSUT1-1 and MtSUT4-1. (C, D) Data are expressed as percentage of the sucrose control.

MtSUT transcript accumulation of plants cultivated under differential phosphate supply and upon inoculation of *G. intraradices* was analyzed by q-RT-PCR. As *MtSUT1-2*, *MtSUT1-3*, and *MtSUT4-2* transcript levels were close to detection thresholds in all tested conditions (Figure 4), *MtSUT1-1*, *MtSUT2*, and *MtSUT4-1* were the candidates retained for further expression analyses. The expression pattern of the analyzed *MtSUTs* shows a fine-tuning regulation in regard to plant nutritional status and AM inoculation. Indeed, transcript accumulation of all *MtSUTs* decreased significantly (Figure 6) in plants fertilized with 10-times phosphate (HP). In contrast, all *MtSUTs* showed a higher transcript accumulation in leaves of AM plants (Figure 6A) when compared to plants treated with the same amount of phosphate (LP) or with HP but in the absence of AMF. Moreover, when compared to the plants treated with HP, to mimic phosphate allocation of AMF, all analyzed *MtSUTs* showed a higher transcript accumulation in source leaves and sink roots of AM plants (Figure 6). These findings indicate a higher sucrose export from source leaves and higher sucrose transfer to the roots when the sink strength is enhanced by the colonization of AMF. The strongly complemented candidate *MtSUT1-1* (Figure 2), which presents a high affinity for sucrose (Figure 3A), also showed the highest transcript accumulation in roots and leaves of AM plants (Figure 6). In contrast to the phosphate supply towards the host plant mediated by *MtPT4* which shows a mycorrhizal specific activation of its gene expression, long-distance transport of sugar from source leaves towards colonized roots mediated by MtSUTs present a fine-tuning regulation of their expression profiles (Figure 6).

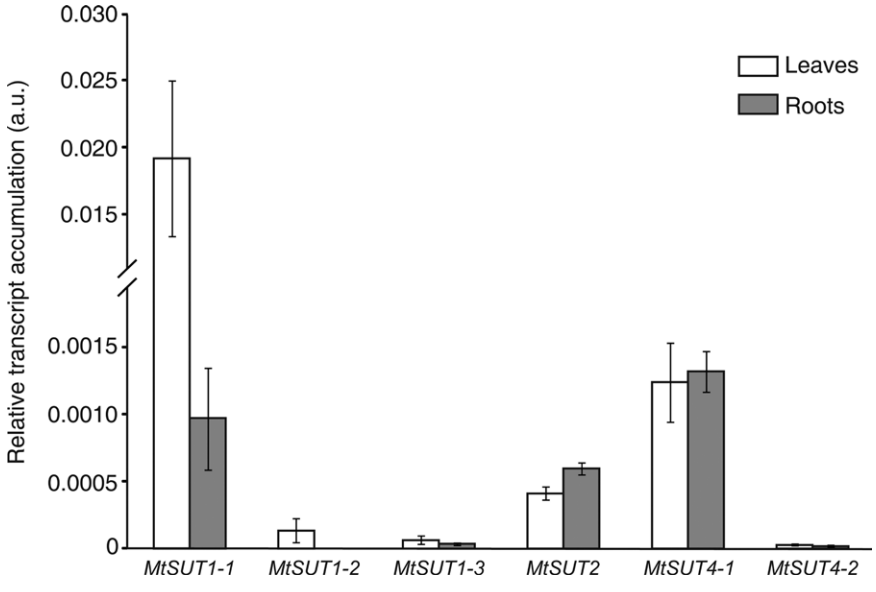


Figure 4. Analysis of Transcript Level of *MtSUT* Genes. Quantitative reverse transcription polymerase chain reaction (q-RT-PCR) analysis of *MtSUT1-1*, *MtSUT1-2*, *MtSUT1-3*, *MtSUT2*, *MtSUT4-1*, and *MtSUT4-2* in leaves (white bar) and roots (tinted bar) of plants treated with high-phosphate 1.3 mM conditions (HP). Data are expressed in arbitrary units (a.u.).

Table 2. Growth Parameters at Harvest.

	Fresh weight (mg)	Dry weight (mg)	Leaf area (cm²)
Leaves			
HP	492.8 ± 24.6[b]	191.6 ± 7.6[b]	9.1 ± 1.5[a]
LP	450.8 ± 53.0[a]	166.2 ± 25.6[a]	8.0 ± 0.9[a]
AM	496.6 ± 12.7[b]	191.2 ± 8.0[b]	9.3 ± 1.6[a]
Roots			
HP	966.0 ± 154.5[a]	177.0 ± 39.2[a]	
LP	1075.2 ± 208.0[a]	193.8 ± 24.5[a]	
AM	984.6 ± 48.2[a]	176.4 ± 18.2[a]	

Means ± standard deviations are shown. Significant differences are indicated with a letter ($P < 0.1$; $n = 5$).

DISCUSSION

This study reports for the first time the identification of the MtSUT family that comprises six members that were annotated according to the latest and consistent classification (Lalonde and Frommer, 2012; Reinders et al., 2012). As Braun and Slewinski (2009) proposed for the nomenclature of Monocotyledonous SUTs, we suggest in future work that all SUTs be named according to this annotation version to avoid gene confusion and synonymy. In that way, we renamed MtSut1 (Gaude et al., 2011) as MtSUT4-1 upon phylogenetic grouping to this particular clade.

MtSUT1-1 and MtSUT4-1, Key Members of the MtSUT Family

We performed the functional characterization of MtSUTs in the yeast heterologous system. The key members, MtSUT1-1 and MtSUT4-1, which strongly complement the deficient yeast strain, are functional transporters and present sucrose uptake kinetics similar to previously characterized plant SUTs (Sauer, 2007). MtSUT1-1 presents a Km for sucrose of 1.7 mM consistent with the saturable high-affinity/low-capacity (HALC) system, while MtSUT4-1 with a Km of 13.7 mM may represent the low-affinity/high-capacity (LAHC) component as already reported for SUT4 clade proteins (Weise et al., 2000). These transport kinetics are consistent with previously characterized leguminous SUTs (Table 1) relatively to their phylogenetic position (Figure 1).

In parallel, MtSUT1-2, MtSUT1-3, and MtSUT4-2 clustered with the recently characterized SUFs. In contrast to related leguminous facilitators which present a complementation of the yeast mutant when grown on minimal medium containing 4% sucrose (Zhou et al., 2007), the weak growth of strains expressing *MtSUT1-2* and *MtSUT1-3* was not increased when grown on 4% sucrose and MtSUT4-2 showed no transport capacity (Supplemental Figure 3).

Interestingly, the gene coding for the H^+/sucrose co-transporters MtSUT1-1 and MtSUT4-1 were also shown to have the highest expression profile among MtSUTs. MtSUT1-1 showed at least a 20-fold transcript accumulation in source leaves of all culture conditions. In contrast, *MtSUT2* allowed no sucrose transport in yeast but was substantially expressed in root and leave tissues (Figures 2 and 4). SUT2 clade members are in general not able to complement mutant yeast strain (Barker et al., 2000; Hackel et al., 2006) and were first described as sugar sensors (Lalonde et al., 1999). However, AtSUC3 (Meyer et al., 2000) and PmSUC3 (Barth et al., 2003) are characterized as low-affinity sucrose importers and GFP fusion experiments showed a SUT2 protein localization

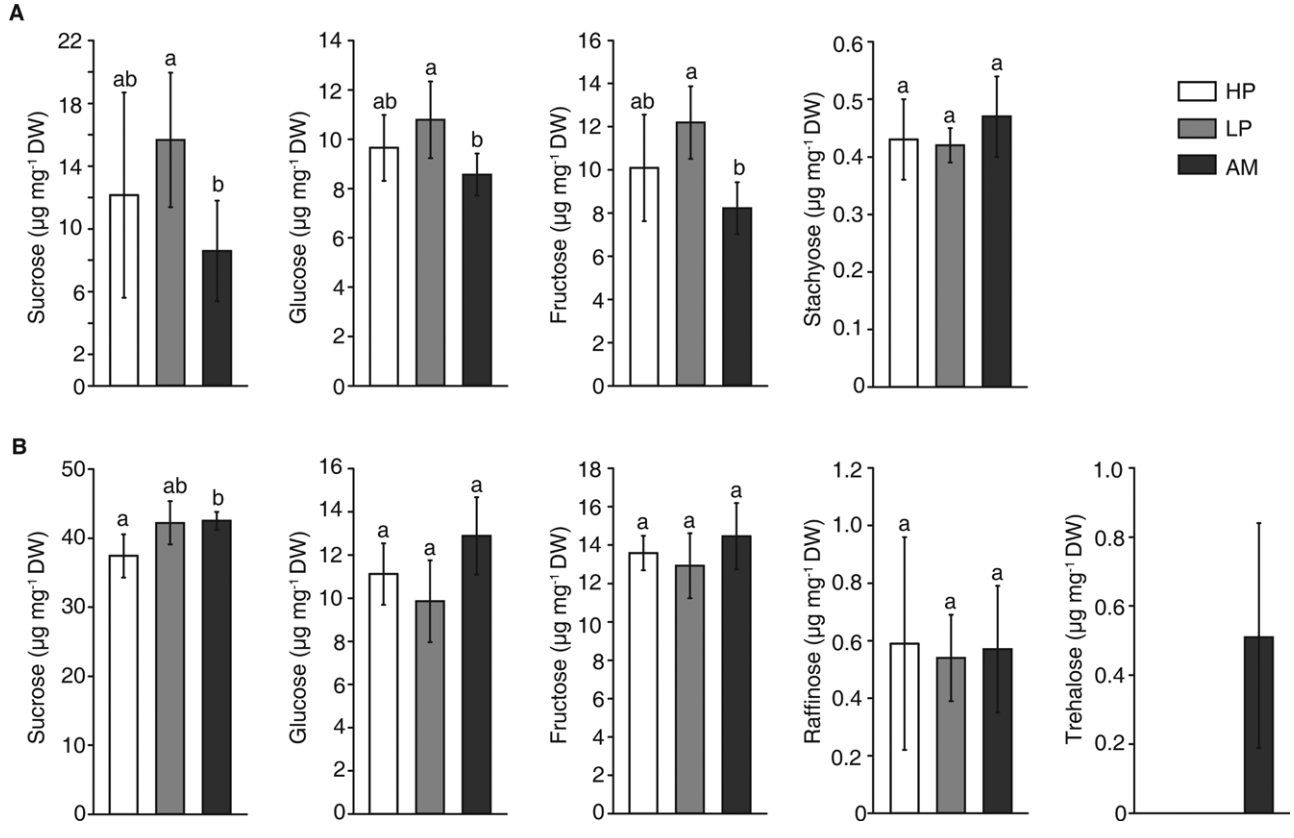


Figure 5. Sugar Content of *M. truncatula* Leaves and Roots. Quantification of soluble sugars in plants treated with either high-phosphate (HP) 1.3 mM conditions (white bars) or with low-phosphate (LP) 0.13 mM conditions (light tinted bar) or treated with low phosphate and inoculated (AM) with *G. intraradices* (dark tinted bar) in leaves **(A)** and roots **(B)**. Mycorrhization rates of AM plants were estimated to F% 73.3 ± 14.5; M% 51.1 ± 14.4; m% 69.0 ± 10.4; a% 92.5 ± 1.3; A% 47.4 ± 13.9. Independent statistical analyses were performed for each sugar, with letters indicating a statistically significant difference (Student's *t*-test, $P < 0.05$ upon accomplishing a Fischer test).

notably to sieve elements of source leaves and in sink root tips. *AtSUC3* expression is induced by physical wounding (Meyer et al., 2004) and, here, *MtSUT2* shows fine expression tuning according to the plant phosphate status and under AM inoculation. The exact role of SUT2 clade proteins is still not deciphered.

Altogether, the high-affinity active transport system of MtSUT1-1, its expression profile in leaves, and its phylogenetic position in the SUT1 clade close to the principal phloem loading orthologs of potato (StSUT1, Riesmeier et al., 1994), tobacco (NtSUT1, Bürkle et al., 1998), and *Arabidopsis* (AtSUC2, Gottwald et al., 2000) strongly suggest a potential role for MtSUT1-1 as the protein responsible for phloem loading in *M. truncatula*. In addition, MtSUT4-1 also shows conserved biochemical kinetics with its leguminous orthologs (Table 1). MtSUT4-1 and LjSUT4 are both H^+/sucrose co-transporters and cluster to a separated branch encompassing MtSUT4-2 and the facilitator protein PsSUF4 (Figure 1). Numerous proteins of the SUT4 clade localize at the tonoplast in several species (AtSUC4 and HvSUT2, Endler et al., 2006; OsSUT2, Eom et al., 2011; NtSUT4, Okubo-Kurihara et al., 2011; PtaSUT4, Payyavula et al., 2011). MtSUT1-1 and MtSUT4-1 GFP fusions did not allow us to confirm the localization of these proteins in tobacco cells (data not shown). Nevertheless, the hypothesis of a tonoplast localization for MtSUT4-1 is reinforced by the presence of a dileucine-based motif in the N-terminal region (Supplemental Figure 1; Larisch et al., 2012), recently shown to be necessary and sufficient for tonoplast targeting of numerous plant transporters (Komarova et al., 2012). This motif is also present in LjSUT4, which is targeted at the vacuolar membrane (Reinders et al., 2008, 2012).

Fine-Tuning of Sugar Partitioning from Leaves towards AM Roots

The quantification of sugar content in *M. truncatula* plants highlighted the importance of sucrose as the predominant carbohydrate. AM plants showed lower levels of sucrose, glucose, and fructose in source leaves, consistently with the higher sugar export. In all tested conditions, only small variations of sugar content were measured in sink roots. These results are in relation to the study of Schliemann et al. (2008)

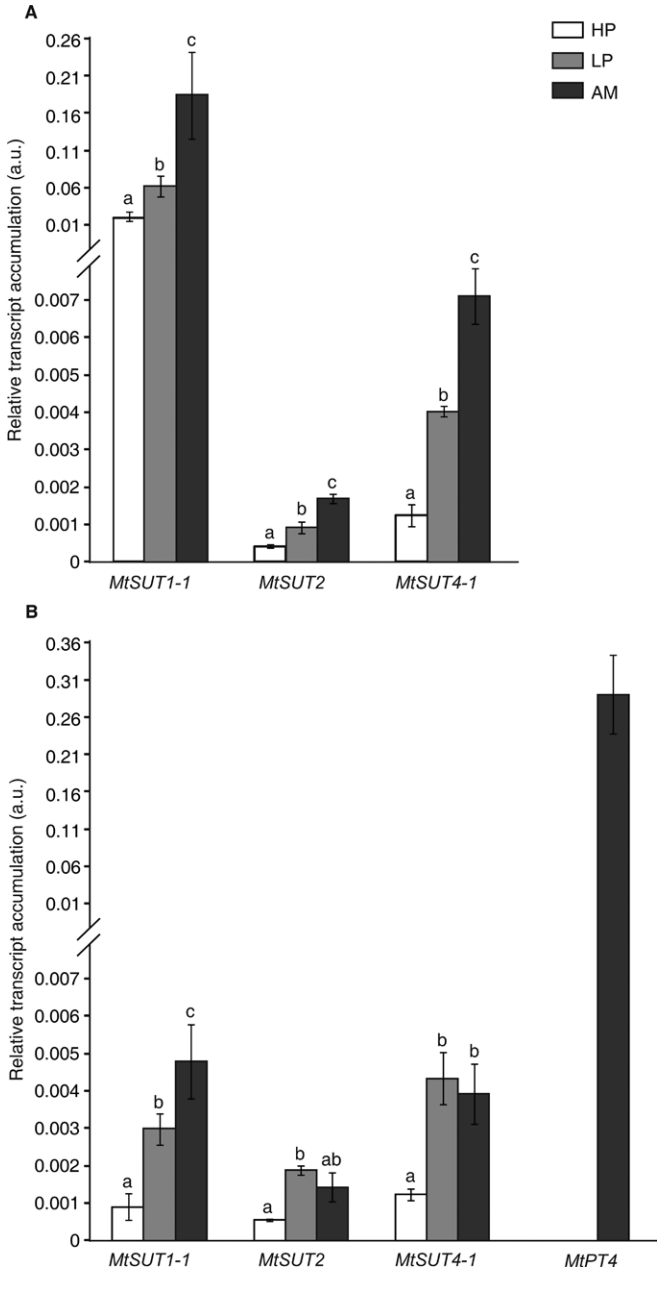


Figure 6. Effect of Phosphate Nutrition and AMF on Transcript Level of MtSUT Genes.

(A) Quantitative reverse transcription polymerase chain reaction (q-RT-PCR) analysis of *MtSUT1-1*, *MtSUT2*, and *MtSUT4-1* in leaves.

(B) q-RT-PCR analysis of *MtSUT1-1*, *MtSUT2*, *MtSUT4-1*, and *MtPT4* (phosphate transporter) in roots. Plants were treated with either high-phosphate (HP) 1.3 mM conditions (white bars) or with low-phosphate (LP) 0.13 mM conditions (light tinted bar) or treated with low phosphate and inoculated (AM) with *G. intraradices* (dark tinted bar). Mycorrhization rates of AM plants were estimated to F% 78.89±7.07; M% 44.41±7.25; m% 56.88±13,49; a% 95.5±4.43; A% 46.03±2.26. Data are expressed in arbitrary units (a.u.). Independent statistical analyses were performed for each gene, with letters indicating a statistically significant difference (Student's *t*-test, $P<0.05$ upon accomplishing a Fischer test).

showing similar accumulation of sucrose, glucose, and fructose in *M. truncatula* roots colonized by *G. intraradices* and in control plants fertilized with differential amounts of phosphate. In addition, we were able to detect the fungus-derived metabolite trehalose synthesized from plant hexoses in AM roots (Bago et al., 2003; Schliemann et al., 2008). In tomato, contradictory results subsist. Boldt et al. (2011) showed an increase in root sucrose and fructose consistently with a preferential glucose uptake by the AMF, while similar levels or even a decrease in all main sugars (sucrose, glucose, and fructose) is also reported in AM roots (Garcia-Rodriguez et al., 2007; Tejeda-Sartorius et al., 2008). However, such differences observed in studies could be explained by different model plants and AMF species combinations as well as different culture conditions.

In this study, we focused on *MtSUT* expression upon differential phosphate concentration and inoculation of *G. intraradices*. *MtSUT* expression was tightly regulated according to the plant nutritional status. Indeed, in HP plants, all *MtSUTs* transcripts in roots and leaves accumulated at lower levels. In tomato, *SlSUT* expression was not regulated when fertilized with 10-fold higher phosphate concentration (Ge et al., 2008). Moreover, the expression profile in AM conditions and the addition of 10-fold phosphate in HP conditions showed that *MtSUT* expression pattern is not related to the phosphorus supply by the fungus, but seems rather related to the increase sink strength upon AM colonization. Indeed, in our study, AM colonization led to an overall up-regulation of all *MtSUTs* in source leaves; in roots, only *MtSUT2* did not present a higher transcript accumulation upon inoculation with *G. intraradices* (Figure 6). In tomato, Boldt et al. (2011) also reported that all *SlSUT* genes are up-regulated in source leaves of tomato plants, while, in roots, only *SlSUT1* and *SlSUT4* accumulated at higher levels in roots colonized by *G. mosseae* compared to non-mycorrhizal control. Thereby, we confirm a comparable expression profile for *M. truncatula* orthologous genes in agreement with the observation from Boldt et al. (2011) indicating a conserved regulation pattern for respective solanaceous and leguminous SUTs in response to colonization by at least two different glomeromycotan fungi. Nevertheless, using different *Glomus* species, Ge et al. (2008) observed a differential regulation of genes coding for tomato sugar transporters. These discrepancies could be explained by fine-tuning at the transcriptional level of sugar transporters, including SUTs, and sucrose cleaving enzymes by phytohormones, jasmonic acid being part of the signal cascade regulating the strength of the mycorrhizal-driven sink (Hause et al., 2002; Tejeda-Sartorius et al., 2008). Actually, the expression of *MtSUT1-1*, the putative phloem loader, is up-regulated in cell suspension treated with exogenous jasmonate (see http://mtgea.noble.org/v2/).

Proteins necessary for phloem loading are also suggested to play a role in unloading mechanisms (Geiger, 2011). Indeed, the antisense inhibition of *StSUT1* in potato led to lower tuber yield when phloem unloading towards the sink tuber is apoplastic (Viola et al., 2001; Kühn et al., 2003) and ZmSUT1 is likely to mediate both import and export for sucrose loading and unloading (Carpaneto et al., 2005; Slewinski et al., 2009; Geiger, 2011). In our study, *MtSUT1-1* showed a higher transcript accumulation in AM roots; this information could indicate a further role for SUT1 proteins in phloem unloading towards AM-colonized sink tissues. Moreover, a higher transcript accumulation was observed for *MtSUT4-1* in leaves upon AM inoculation (Figure 6A) and, recently, proteins from the SUT4 clade were also shown to play a role in sucrose export from source leaves (Eom et al., 2011, 2012). *MtSUT4-1* is also expressed in roots and promoter–GUS fusion combined with staining of AM fungal structures showed that this gene is activated in cortical cells adjacent to arbusculated cells (Gaude et al., 2011). The vacuolar transporter LjSUT4 is suggested to play a role in sucrose release towards nodules in plant–rhizobial symbioses (Reinders et al., 2008). SUT4 transporters may be responsible for carbon reallocation of vacuolar sucrose storage towards colonized parts during plant symbioses.

Impaired sink sucrose utilization simulated with *MtSucS1* synthase antisense lines resulted in an overall down-regulation of several carbon-related genes and plants were affected during AM colonization, more particularly in the establishment and maintenance of arbuscules (Baier et al., 2010). However, increasing available hexoses in roots with plants transformed to overexpress apoplastic, cytosolic, and vacuolar-located invertases did not show an increase in fungal growth, colonization rate, or fungal sugar content, whereas plant with decreased invertase activity showed diminished mycorrhization rates (Schaarschmidt et al., 2007). Moreover, monosaccharide as well as sucrose transporters show differential regulation of their gene expression in response to AM inoculation (Figure 6; Harrison, 1996; Garcia-Rodriguez et al., 2005; Wright et al., 2005; Ge et al., 2008; Boldt et al., 2011; Gabriel-Neumann et al., 2011; Gaude et al., 2011; Hogekamp et al., 2011). In contrast to phosphate transfer generated by the phosphate transporter MtPT4, which represents a clear 'switch' marker of trophic exchanges in AM symbiosis (Figure 6B; Harrison et al., 2002; Javot et al., 2007), carbon partitioning towards AM-colonized roots involves fine modulation of plant sucrose cleaving enzymes and sugar transport proteins.

In conclusion, the present study shows for the first time the identification of the SUT family of the model legume *M. truncatula*. Biochemical characterization and transcript accumulation analyses revealed that MtSUT1-1 and MtSUT4-1 are key H^+/sucrose symporters. Expression study and sugar quantification under differential nutritional conditions and upon mycorrhizal inoculation suggest distinct functions for proteins from different SUT clades in source leaves and AM-colonized sink organs. The properties of MtSUTs give new insight into the conservation of transport kinetics among leguminous SUTs as well as conservation of expression patterns for respective orthologs in response to AM symbiosis.

METHODS

Cultivation and Inoculation of Plant Material

Seeds of *M. truncatula* J5 were surface-sterilized and germinated on 0.7% sterile agar for 3 d in darkness at 25°C. Then, plantlets were transferred in 75 ml of a sterile mix (2:1) of Terragreen (OilDri-US special, Mettman) and Epoisses soil (non-mycorrhized plants) or a in mix (2:1) of Terragreen and Epoisses soil base of *G. intraradices* BEG141 inoculum (syn. *Rhizophagus* sp., D. Redecker, personal communication). Plants were grown under controlled conditions (420 $\mu E\, m^{-2}\, s^{-1}$ for 16 h of light; 24 and 19°C day and night temperature, 70% humidity), watered daily with distilled water, and supplemented twice a week with 5 ml modified Long Ashton solution (double quantity of nitrate KNO_3 and 1/10 quantity of phosphate NaH_2PO_4 = 0.13 mM phosphate) for LP control plants and mycorrhized plants and with 5 ml modified Long Ashton solution (double quantity of nitrate and 1.3 mM phosphate) for HP control plants (Hewitt, 1966). Plants were harvested at 28 d post inoculation (dpi); mature leaves and root materials were rinsed with distilled watered and dried with paper. Materials for molecular analyses and sugar content quantification were frozen in liquid N_2 upon harvest and stored at –80°C.

Growth Parameter Measurements

Before harvest, digital pictures of the plants were taken and the total leaf surface was estimated using Visilog 6.8 (Noesis). At harvest, five plants of each treatment were separated into shoots and roots, and fresh weight was directly monitored. Dry weight of shoots and roots was measured after drying the material at 55°C for 3 d.

Root staining was performed according to Vierheilig et al. (1998) and mycorrhizal rates were estimated as described by Trouvelot et al. (1986) and calculated with the Mycocalc software (http://www2.dijon.inra.fr/mychintec/Mycocalc-prg/download.html).

Sequence Identification and Cloning

Putative MtSUT sequences were screened from the *M. truncatula* genome version 3.5 (www.medicagohapmap.org/index.php; Young et al., 2011) and from the DFCI *Medicago* Gene Index (http://compbio.dfci.harvard.edu/cgi-bin/tgi/gimain.pl?gudb=medicago) with a blast of previously published plant SUT sequences. For partial sequences, full-length cDNA was obtained with the 5′ and 3′ GeneRacer™ Kit (Invitrogen)

according to the manufacturers' instructions using EST-specific primers (Supplemental Table 3) and mRNA (250 ng) from *M. truncatula* roots and leaves purified by the means of the Dynabeads® mRNA Purification Kit (Invitrogen). PCR products were cloned into the pCR®2.1-TOPO® (Invitrogen) and sequenced. Specific primers (Supplemental Table 3) were designed for full-length cDNA amplification and were purified using the Qiaquick® purification kit (Qiagen). Subsequent cloning steps into the entry pDONR™221 (Invitrogen) and destination yeast expression pDRf1–GW vectors (Loqué et al., 2007) were carried out using the Gateway® Technology with Clonase™ II (Invitrogen).

Functional Analysis of Sucrose Transporters

Respective MtSUTs cloned in the pDRf1–GW vector and empty vector control pDRf1 (Loqué et al., 2007) were transformed according to Gietz and Schiestl (2007) into *Saccharomyces cerevisiae* strain SUSY7/ura3- (Riesmeier et al., 1992; Barker et al., 2000) and plated on minimal selective glucose (2%) medium without uracil (SDura$^-$). Correct incorporation of the plasmid was checked by sequencing after yeast colony PCR.

Drop test complementation assays were performed on minimal selective sucrose (2%) medium without uracil by pipetting a 3-µl sterile water drop containing respectively 10^4, 10^3, and 10^2 yeast cells per drop. A 3-µl drop containing 10^3 yeast cells per drop was deposited on SDura$^-$ as a loading control. Plates were incubated at 30°C, 20 d on sucrose medium, 5 d for the glucose control.

For uptake assays, single yeast colonies were grown in liquid SDura$^-$ to logarithmic phase. Cells were harvested at an OD_{600} of 0.5, washed twice in sterile water, and re-suspended in 50 mM, pH 5, NaPi buffer to a final OD_{600} of 5. Prior to the uptake measurements, cells were energized with 10 mM glucose and incubated for 5 min at 30°C. To start the reaction, 100 µl of this cell suspension was added to 100 µl of NaPi buffer containing 7.5 kBq of [^{14}C] sucrose, and unlabelled sucrose to the concentrations used in the experiments. 50 µl aliquots were removed over 3 min and transferred in 4 ml of ice-cold NaPi buffer. Cells were vacuum-collected onto GF/C microfiber filters (Whatman) and washed twice with 5 ml of NaPi buffer. Inhibitor assays were performed with the addition of inhibitors 30 s prior the incubation time. Inhibition of 500 µM [^{14}C]sucrose uptake (control) by the addition of 50 µM respective inhibitors: protonophores carbonyl cyanide *m*-chlorophenylhydrazone (CCCP), 2,4-dinitrophenol (DNP), and the plasma membrane H^+-ATPase inhibitors diethylstilbestrol (DES) and vanadate were tested. For sugar specificity, inhibition of 500 µM [^{14}C] sucrose uptake (control) by a 10-fold molar excess of competing sugars: glucose, fructose, trehalose, or maltose was used. For all uptake experiments, [^{14}C]sucrose uptake was radio-assayed by liquid scintillation counting and three biological replicates were performed.

Gene Expression Analysis

Total RNA was extracted from roots and mature leaves harvested at 28 dpi using the SV Total RNA Isolation System (Promega) and eluted in RNAse-free water. RNA quality was checked by gel electrophoresis and quantity measurements were performed with a NanoDrop (Thermo Scientific). cDNA was produced with oligo(dT)$_{15}$ and SuperScript®III Reverse Transcriptase (Invitrogen) using 1 µg of RNA. Thereafter, cDNA template was diluted 40 times.

Quantitative real-time PCR reactions were performed in a finale volume of 15 µl using Absolute SYBR green ROX Mix (Thermo Scientific), 70 nM of gene-specific primers (Supplemental Table 3), and 2 µl of cDNA template in an ABI Prism 7900HT Sequence Detection System (Applied Biosystems). Reaction conditions were set as 95°C for 15 min and 40 cycles of 95°C for 15 s, 60°C for 30 s, and 72°C for 30 s. Three biological and three technical repetitions were performed. To check the absence of nonspecific amplicons, dissociation curves were generated at the end of the PCR cycles by heating the PCR products from 70 to 95°C. Primer pair efficiency (90%–110%) was estimated for all primer pairs by serial dilutions of the cDNA. RNA samples were checked to exclude genomic DNA contamination and no-template controls were used for each primer pair.

All data were analyzed using the SDS 2.3 software (Applied Biosystems) with a threshold of 0.25 to obtain cycle threshold values. Values were normalized (ΔCt) to the constitutively expressed translation initiation factor (*MtTef1*α, Baier et al., 2010) and expression coefficients were calculated ($2^{-\Delta Ct}$).

Analysis of Soluble Sugars

Samples were ground in liquid nitrogen followed by 1 d of lyophilization. The lyophilized powder (60 mg) was treated for 30 min by liquid extraction with 1 ml 80% (v/v) ethanolic solution in an ultrasonic bath at 80°C. After centrifugation (10 min at 15 000 *g*), 10 µl of supernatant were injected on an ICS 3000 Dionex instrument (Dionex) and sugar content was assessed by high-performance anion exchange-pulsed amperometric detection (HPAE-PAD) using a guard and analytical column CarboPac PA100 (Dionex) according to Dionex instructions (Dionex application note 122 [2004]). Five biological repetitions were performed.

Phylogenetic Analyses

Alignment of amino acid sequences of 88 plant SUTs was performed with Mafft version 6 (Katoh and Toh, 2008) and maximum parsimony analyses were performed using PAUP 4.0b10 (Swofford, 1998). Heuristic tree searches were executed using 1000 random sequence additions and the tree bisection–reconnection branch-swapping algorithm with random sequence analysis. The complete alignment was based on 730 sites; 513 were phylogenetically informative. Unrooted tree was displayed on Dendroscope2.7.4 (Huson et al., 2007).

Accession Numbers

Accession numbers of the sequences reported in this paper have been submitted to GenBank (www.ncbi.nlm.nih.gov/): MtSUT1-1 (JN255789), MtSUT1-2 (JN255790), MtSUT1-3 (JN255791), MtSUT2 (JN255792), MtSUT4-1 (JN255793), MtSUT4-2 (JN255794), Pseudogene1 (JN255795), and Pseudogene2 (JN255796).

SUPPLEMENTARY DATA

Supplementary Data are available at *Molecular Plant Online.*

FUNDING

This work was supported by the Conseil Régional de Bourgogne (Joan Doidy thesis grant FABER 2008_9201AAO036S00220 and PARI 20100112095254682–1) and the Agence National de la Recherche (project TRANSMUT, ANR-10-BLAN-1604–01).

ACKNOWLEDGMENTS

The authors are grateful to A. Colombet and V. Monfort (IBG, Dijon, France) for inoculum provision and to A. Schüβler and A. Brachman (LMU, Munich, Germany) for helpful discussions, as well as T. Adam, C. Arnould, L. Casieri, N. Leborgne-Castel, and H.-N. Truong (IPM, Dijon, France) for technical assistance. No conflict of interest declared.

REFERENCES

Afoufa-Bastien, D., et al. (2010). The *Vitis vinifera* sugar transporter gene family: phylogenetic overview and macroarray expression profiling. BMC Plant Biol. **10**, 245.

Aldape, M.J., Elmer, A.M., Chao, W.S., and Grimes, H.D. (2003). Identification and characterization of a sucrose transporter isolated from the developing cotyledons of soybean. Archives Biochem. Biophys. **409**, 243–250.

Aoki, N., Hirose, T., Scofield, G.N., Whitfeld, P.R., and Furbank, R.T. (2003). The sucrose transporter gene family in rice. Plant Cell Physiol. **44**, 223–232.

Ayre, B.G. (2011). Membrane-transport systems for sucrose in relation to whole-plant carbon partitioning. Mol. Plant. **4**, 377–394.

Bago, B., et al. (2003). Carbon export from arbuscular mycorrhizal roots involves the translocation of carbohydrate as well as lipid. Plant Physiol. **131**, 1496–1507.

Bago, B., Pfeffer, P.E., and Shachar-Hill, Y. (2000). Carbon metabolism and transport in arbuscular mycorrhizas. Plant Physiol. **124**, 949–957.

Baier, M.C., Keck, M., Gödde, V., Niehaus, K., Küster, H., and Hohnjec, N. (2010). Knockdown of the symbiotic sucrose synthase *MtSucS1* affects arbuscule maturation and maintenance in mycorrhizal roots of *Medicago truncatula*. Plant Physiol. **152**, 1000–1014.

Barker, L., et al. (2000). SUT2, a putative sucrose sensor in sieve elements. Plant Cell. **12**, 1153–1164.

Barth, I., Meyer, S., and Sauer, N. (2003). PmSUC3: characterization of a SUT2/SUC3-type sucrose transporter from *Plantago major*. Plant Cell. **15**, 1375–1385.

Boldt, K., et al. (2011). Photochemical processes, carbon assimilation and RNA accumulation of sucrose transporter genes in tomato arbuscular mycorrhiza. J. Plant Physiol. **168**, 1256–1263.

Braun, D.M., and Slewinski, T.L. (2009). Genetic control of carbon partitioning in grasses: roles of sucrose transporters and tie-dyed loci in phloem loading. Plant Physiol. **149**, 71–81.

Bürkle, L., Hibberd, J.M., Quick, W.P., Kühn, C., Hirner, B., and Frommer, W.B. (1998). The H+-sucrose cotransporter NtSUT1 is essential for sugar export from tobacco leaves. Plant Physiol. **118**, 59–68.

Carpaneto, A., Geiger, D., Bamberg, E., Sauer, N., Fromm, J., and Hedrich, R. (2005). Phloem-localized, proton-coupled sucrose carrier ZmSUT1 mediates sucrose efflux under the control of the sucrose gradient and the proton motive force. J. Biolog. Chem. **280**, 21437–21443.

Doidy, J., Grace, E., Kühn, C., Simon-Plas, F., Casieri, L., and Wipf, D. (2012). Sugar transporters in plants and in their interactions with fungi. Trends Plant Sci. **17**, 413–422.

Douds, D.D., Pfeffer, P.E., and Shachar-Hill, Y. (2000). Carbon partitioning, cost and metabolism of arbuscular mycorrhizae. In Arbuscular Mycorrhizas: Physiology and Function, **Kapulnick, Y., and Douds, D.D.**, eds (London: Kluwer Academic Press).

Endler, A., et al. (2006). Identification of a vacuolar sucrose transporter in barley and *Arabidopsis* mesophyll cells by a tonoplast proteomic approach. Plant Physiol. **141**, 196–207.

Eom, J.-S., Choi, S.-B., Ward, J., and Jeon, J.-S. (2012). The mechanism of phloem loading in rice (*Oryza sativa*). Molecules and Cells. **33**, 431–438.

Eom, J.-S., et al. (2011). Impaired function of the tonoplast-localized sucrose transporter in rice (*Oryza sativa*), OsSUT2, limits the transport of vacuolar reserve sucrose and affects plant growth. Plant Physiol. **157**, 109–119.

Gabriel-Neumann, E., Neumann, G., Leggewie, G., and George, E. (2011). Constitutive overexpression of the sucrose transporter *SoSUT1* in potato plants increases arbuscular mycorrhiza fungal root colonization under high, but not under low, soil phosphorus availability. J. Plant Physiol. **168**, 911–919.

Garcia-Rodriguez, S., Azcón-Aguilar, C., and Ferrol, N. (2007). Transcriptional regulation of host enzymes involved in the cleavage of sucrose during arbuscular mycorrhizal symbiosis. Physiologia Plantarum. **129**, 737–746.

Garcia-Rodriguez, S., Pozo, M.J., Azcón-Aguilar, C., and Ferrol, N. (2005). Expression of a tomato sugar transporter is increased in leaves of mycorrhizal or *Phytophthora parasitica*-infected plants. Mycorrhiza. **15**, 489–496.

Gaude, N., Bortfeld, S., Duensing, N., Lohse, M., and Krajinski, F. (2011). Arbuscule-containing and non-colonized cortical cells of mycorrhizal roots undergo a massive and specific reprogramming during arbuscular mycorrhizal development. Plant J. **69**, 510–528.

Ge, L., Sun, S.B., Chen, A.Q., Kapulnik, Y., and Xu, G.H. (2008). Tomato sugar transporter genes associated with mycorrhiza and phosphate. Plant Growth Regulation. **55**, 115–123.

Geiger, D. (2011). Plant sucrose transporters from a biophysical point of view. Mol. Plant. **4**, 395–406.

Gietz, R.D., and Schiestl, R.H. (2007). High-efficiency yeast transformation using the LiAc/SS carrier DNA/PEG method. Nature Protocols. **2**, 31–34.

Gottwald, J.R., Krysan, P.J., Young, J.C., Evert, R.F., and Sussman, M.R. (2000). Genetic evidence for the in planta role of phloem-specific plasma membrane sucrose transporters. Proc. Natl Acad. Sci. U S A. **97**, 13979–13984.

Graham, J.H. (2000). Assessing costs of arbuscular mycorrhizal symbiosis agroecosystems fungi. In Current Advances in Mycorrhizae Research, **Podila, G.K., and Douds, D.D.**, eds (St Paul: APS Press), pp. 127–140.

Hackel, A., Schauer, N., Carrari, F., Fernie, A., Grimm, B., and Kühn, C. (2006). Sucrose transporter LeSUT1 and LeSUT2 inhibition affects tomato fruit development in different ways. Plant J. **45**, 180–192.

Harrison, M.J. (1996). A sugar transporter from *Medicago truncatula*: altered expression pattern in roots during vesicular-arbuscular (VA) mycorrhizal associations. Plant J. **9**, 491–503.

Harrison, M.J., Dewbre, G.R., and Liu, J. (2002). A phosphate transporter from *Medicago truncatula* involved in the acquisition of phosphate released by arbuscular mycorrhizal fungi. Plant Cell. **14**, 2413–2429.

Hause, B., Maier, W., Miersch, O., Kramell, R., and Strack, D. (2002). Induction of jasmonate biosynthesis in arbuscular mycorrhizal barley roots. Plant Physiol. **130**, 1213–1220.

Helber, N., Wippel, K., Sauer, N., Schaarschmidt, S., Hause, B., and Requena, N. (2011). A versatile monosaccharide transporter that operates in the arbuscular mycorrhizal fungus *Glomus sp* is crucial for the symbiotic relationship with plants. Plant Cell. **23**, 3812–3823.

Hewitt, E. (1966). Sand and water culture methods used in the study of plant nutrition. Technical Communications, No. 22. Commonwealth Bureau of Horticulture and Plantation Crops. East Malling, England, pp. 479–534.

Hogekamp, C., Arndt, D., Pereira, P., Becker, J.D., Hohnjec, N., and Küster, H. (2011). Laser-microdissection unravels cell-type specific transcription in arbuscular mycorrhizal roots, including CAAT-box TF gene expression correlating with fungal contact and spread. Plant Physiol. published online October 26, 2011, doi: org/2010.1104/pp.2111.186635.

Hohnjec, N., Perlick, A.M., Pühler, A., and Küster, H. (2003). The *Medicago truncatula* sucrose synthase gene *MtSucS1* is activated both in the infected region of root nodules and in the cortex of roots colonized by arbuscular mycorrhizal fungi. Mol. Plant–Microbe Interact. **16**, 903–915.

Huson, D., Richter, D., Rausch, C., Dezulian, T., Franz, M., and Rupp, R. (2007). Dendroscope: an interactive viewer for large phylogenetic trees. BMC Bioinformat. **8**, 460.

Ibraheem, O., Botha, C.E.J., and Bradley, G. (2010). *In silico* analysis of *cis*-acting regulatory elements in 5′ regulatory regions of sucrose transporter gene families in rice (*Oryza sativa* Japonica) and *Arabidopsis thaliana*. Computational Biol. Chem. **34**, 268–283.

Javot, H., Penmetsa, R.V., Terzaghi, N., Cook, D.R., and Harrison, M.J. (2007). A *Medicago truncatula* phosphate transporter indispensable for the arbuscular mycorrhizal symbiosis. Proc. Natl Acad. Sci. U S A. **104**, 1720–1725.

Johnson, D.A., and Thomas, M.A. (2007). The monosaccharide transporter gene family in *Arabidopsis* and rice: a history of duplications, adaptive evolution, and functional divergence. Mol. Biol. Evolut. **24**, 2412–2423.

Katoh, K., and Toh, H. (2008). Recent developments in the MAFFT multiple sequence alignment program. Briefings in Bioinformatics. **9**, 286–298.

Komarova, N.Y., Meier, S., Meier, A., Grotemeyer, M.S., and Rentsch, D. (2012). Determinants for *Arabidopsis* peptide transporter targeting to the tonoplast or plasma membrane. Traffic. doi: 10.1111/j.1600–0854.2012.01370.x.

Kühn, C., and Grof, C. (2010). Sucrose transporters of higher plants. Curr. Opin. Plant Biol. **13**, 1–11.

Kühn, C., et al. (2003). The sucrose transporter StSUT1 localizes to sieve elements in potato tuber phloem and influences tuber physiology and development. Plant Physiol. **131**, 102–113.

Kühn, C., Quick, W.P., Schulz, A., Riesmeier, J.W., Sonnewald, U., and Frommer, W.B. (1996). Companion cell-specific inhibition of the potato sucrose transporter SUT1. Plant Cell Environ. **19**, 1115–1123.

Lalonde, S., and Frommer, W.B. (2012). SUT sucrose and MST monosaccharide transporter inventory of the *Selaginella* genome. Frontiers in Plant Science. **3**.

Lalonde, S., et al. (1999). The dual function of sugar carriers: transport and sugar sensing. Plant Cell. **11**, 707–726.

Lalonde, S., Wipf, D., and Frommer, W.B. (2004). Transport mechanisms for organic forms of carbon and nitrogen between source and sink. Ann. Rev. Plant Biol. **55**, 341–372.

Larisch, N., Schulze, C., Galione, A., and Dietrich, P. (2012). An N-terminal dileucine motif directs two-pore channels to the tonoplast of plant cells. Traffic. **13**, 1012–1022.

Loqué, D., Lalonde, S., Looger, L.L., von Wirén, N., and Frommer, W.B. (2007). A cytosolic *trans*-activation domain essential for ammonium uptake. Nature. **446**, 195–198.

Meyer, S., et al. (2000). AtSUC3, a gene encoding a new *Arabidopsis* sucrose transporter, is expressed in cells adjacent to the vascular tissue and in a carpel cell layer. Plant J. **24**, 869–882.

Meyer, S., Lauterbach, C., Niedermeier, M., Barth, I., Sjolund, R., and Sauer, N. (2004). Wounding enhances expression of AtSUC3, a sucrose transporter from *Arabidopsis* sieve elements and sink tissues. Plant Physiol. **134**, 684–693.

Okubo-Kurihara, E., Higaki, T., Kurihara, Y., Kutsuna, N., Yamaguchi, J., and Hasezawa, S. (2011). Sucrose transporter NtSUT4 from tobacco BY-2 involved in plant cell shape during miniprotoplast culture. J. Plant Res. **124**, 395–403.

Payyavula, R.S., Tay, K.H.C., Tsai, C.-J., and Harding, S.A. (2011). The sucrose transporter family in *Populus*: the importance of a tonoplast PtaSUT4 to biomass and carbon partitioning. Plant J. **65**, 757–770.

Pfeffer, P.E., Douds, D.D., Becard, G., and Shachar-Hill, Y. (1999). Carbon uptake and the metabolism and transport of lipids in an arbuscular mycorrhiza. Plant Physiol. **120**, 587–598.

Ravnskov, S., Wu, Y., and Graham, J.H. (2003). Arbuscular mycorrhizal fungi differentially affect expression of genes coding for sucrose synthases in maize roots. New Phytol. **157**, 539–545.

Reinders, A., Sivitz, A., Starker, C., Gantt, J., and Ward, J. (2008). Functional analysis of LjSUT4, a vacuolar sucrose transporter from *Lotus japonicus*. Plant Mol. Biol. **68**, 289–299.

Reinders, A., Sivitz, A.B., and Ward, J.M. (2012). Evolution of plant sucrose uptake transporters (SUTs). Frontiers in Plant Sci. **3**.

Rennie, E.A., and Turgeon, R. (2009). A comprehensive picture of phloem loading strategies. Proc. Natl Acad. Sci. U S A. **106**, 14162–14167.

Riesmeier, J.W., Willmitzer, L., and Frommer, W.B. (1992). Isolation and characterization of a sucrose carrier cDNA from spinach by functional expression in yeast. EMBO J. **11**, 4705–4713.

Riesmeier, J.W., Willmitzer, L., and Frommer, W.B. (1994). Evidence for an essential role of the sucrose transporter in phloem loading and assimilate partitioning. EMBO J. **13**, 1–7.

Sauer, N. (2007). Molecular physiology of higher plant sucrose transporters. FEBS Lett. **581**, 2309–2317.

Schaarschmidt, S., González, M.-C., Roitsch, T., Strack, D., Sonnewald, U., and Hause, B. (2007). Regulation of arbuscular mycorrhization by carbon: the symbiotic interaction cannot be improved by increased carbon availability accomplished by root-specifically enhanced invertase activity. Plant Physiol. **143**, 1827–1840.

Schliemann, W., Ammer, C., and Strack, D. (2008). Metabolite profiling of mycorrhizal roots of *Medicago truncatula*. Phytochemistry. **69**, 112–146.

Schulz, A., Kühn, C., Riesmeier, J.W., and Frommer, W.B. (1998). Ultrastructural effects in potato leaves due to antisense-inhibition of the sucrose transporter indicate an apoplasmic mode of phloem loading. Planta. **206**, 533–543.

Sivitz, A.B., et al. (2007). *Arabidopsis* Sucrose Transporter AtSUC9: high-affinity transport activity, intragenic control of expression, and early flowering mutant phenotype. Plant Physiol. **143**, 188–198.

Slewinski, T.L., and Braun, D.M. (2010). Current perspectives on the regulation of whole-plant carbohydrate partitioning. Plant Sci. **178**, 341–349.

Slewinski, T.L., Meeley, R., and Braun, D.M. (2009). Sucrose transporter1 functions in phloem loading in maize leaves. J. Exper. Bot. **60**, 881–892.

Smith, S.E., and Read, D.J. (2008). Mycorrhizal Symbiosis (Amsterdam and Boston: Academic Press).

Solaiman, M.D.Z., and Saito, M. (1997). Use of sugars by intraradical hyphae of arbuscular mycorrhizal fungi revealed by radiorespirometry. New Phytol. **136**, 533–538.

Swofford, D.L. (1998). PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4. Sinauer Associates, Sunderland, Massachusetts.

Tejeda-Sartorius, M., Martínez de la Vega, O., and Délano-Frier, J.P. (2008). Jasmonic acid influences mycorrhizal colonization in tomato plants by modifying the expression of genes involved in carbohydrate partitioning. Physiologia Plantarum. **133**, 339–353.

Trouvelot, A., Kough, J.L., and Gianinazzi-Pearson, V. (1986). Mesure du taux de mycorhization VA d'un système radiculaire: recherche de méthodes d'estimation ayant une signification fonctionnelle. Physiological and Genetical Aspects of Mycorrhizae, 217–221.

Vierheilig, H., Coughlan, A.P., Wyss, U., and Piché, Y. (1998). Ink and vinegar, a simple staining technique for arbuscular-mycorrhizal fungi. Applied Environement Microbiology. **64**, 5004–5007.

Viola, R., et al. (2001). Tuberization in potato involves a switch from apoplastic to symplastic phloem unloading. Plant Cell. **13**, 385–398.

Weber, H., Borisjuk, L., Heim, U., Sauer, N., and Wobus, U. (1997). A role for sugar transporters during seed development: molecular characterization of a hexose and a sucrose carrier in fava bean seeds. Plant Cell. **9**, 895–908.

Weise, A., et al. (2000). A new subfamily of sucrose transporters, SUT4, with low affinity/high capacity localized in enucleate sieve elements of plants. Plant Cell. **12**, 1345–1356.

Wright, D.P., Read, D.J., and Scholes, J.D. (1998). Mycorrhizal sink strength influences whole plant carbon balance of *Trifolium repens* L. Plant Cell Environ. **21**, 881–891.

Wright, D.P., Scholes, J.D., Read, D.J., and Rolfe, S.A. (2005). European and African maize cultivars differ in their physiological and molecular responses to mycorrhizal infection. New Phytol. **167**, 881–896.

Young, N.D., et al. (2011). The *Medicago* genome provides insight into the evolution of rhizobial symbioses. Nature. **480**, 520–524.

Zhang, C., and Turgeon, R. (2009). Downregulating the sucrose transporter *VpSUT1* in *Verbascum phoeniceum* does not inhibit phloem loading. Proc. Natl Acad. Sci. U S A. **106**, 18849–18854.

Zhou, Y.C., Qu, H.X., Dibley, K.E., Offler, C.E., and Patrick, J.W. (2007). A suite of sucrose transporters expressed in coats of developing legume seeds includes novel pH-independent facilitators. Plant J. **49**, 750–764.

DOIDY Joan

Date of birth: 18th May 1983

Address: **19 Boulevard Voltaire, 21000 Dijon, France**

Telephone: **0679858309**
E-mail address: **joan.doidy@gmail.com**
Nationality: French

EDUCATION

● Nov 2008 – May 2012 : Phd Student at the **Institut National de Recherche Agronomique (INRA) UMR** CNRS (5184)/INRA (1088)/Université de Bourgogne, BP 86510, 21065 Dijon Cedex in cotutelle with the **Ludwig Maximilians Universität, Münich, Gernamy**.
Title : **The *Medicago truncatula* sucrose transporter family: sugar transport from plant source leaves towards the arbuscular mycorrhizal fungus.**
Supervisors **Pr. Daniel Wipf (UB), Pr. Arthur Schüßler (LMU).**

● 2006 – 2007: **Master 2, Msc Plants, Probiotics and Biotechnologies** at the **University of Burgundy**.

● 2005 – 2006: **Master 1, Msc Plant Sciences and Technologies** at the **University of Burgundy**.

● 2001 – 2005: **Bachelor, Bsc. in Biology Geology** (DEUG BG and Licence BG) at the **University of Burgundy**.

● 1998 – 2001: **A' levels in science** majored in biology (baccalauréat S SVT) at Hippolyte Fontaine High school. 21000 Dijon, France.

WORK EXPERIENCE

● Sep 2007 - May 2008: **Marie Curie Fellow** at the **National Institute of Agricultural Botany**, **Cambridge, United Kingdom**.
Project title: **The re-invention of sainfoin (*Onobrychis viciifolia*): an example of a novel resource for sustainable agriculture.**

● Feb – Aug 2007: Master 2 project at **Central Science Laboratory**, **York**, **United Kingdom**.
Project title: **SINEs transposon based molecular markers for quantifying gene flows in oilseed rape (*Brassica napus*) varieties**.

● Feb – April 2006: Training period at the **Jules Guyot Wine Universitary Institute,** Dijon, France.
Project title: **Phosphate solubilizing organisms**.

OTHERS

● November 2007: **Project Management** workshop at the **Technische Universitat of Vienna.** Courses by **Roland Gareis Consulting**: Project Management, European Project Network, Research Proposal Application, Proposal Evaluation, Project Funding, Microsoft MS project.
→ International certification obtained: **Level D of Project Management: "Junior Project Manager".**

● English knowledge certification: - **IELTS** score: 7.5/9
- **TOEIC** score: 885/990

PUBLICATIONS AND PRESENTATIONS

Publication in Peer reviewed journals

1. Couturier J., Doidy J., Wipf D., Blaudez D. & Chalot M. (2010) Glutamine, arginine and the amino acid transporter Pt-CAT11 play important roles during senescence in poplar. Annals of Botany 105(7): 1159-1169.
2. Gianinazzi-Pearson V., van Tuinen D., Wipf D., Dumas-Gaudot E., Recorbet G., Liu Y., Doidy J., Redecker D., Ferrol N. (2012). Exploring the Genome of Glomeromycotan Fungi. Mycota IX
3. Doidy J., Grace E., Kühn C., Simon-Plas F., Casieri L. and Wipf D. (2012). Sugar transporters in plants and in their interactions with fungi. Trends in Plant Science 17 (7): 413-422.
4. Doidy J., van Tuinen D., Corneillat M., Alcaraz G., Wipf D. (submitted) The *Medicago truncatula* sucrose transporter family. Characterization and implication of key members in carbon partitioning towards arbuscular mycorrhizal fungi. Molecular plant doi:10.1093/mp/sss079

Oral communications

1. Doidy J., Schüssler A., van Tuinen D. & Wipf D.* (2009) Importance of fungal transporters in successful plant-fungus collaboration. 6th International Conference on Mycorrhizae, Belo Horizonte (Brazil) 9-14 August 2009.
2. Doidy J.*, van Tuinen D., Schüssler A. & Wipf D. (2010) Sugar transport in abuscular mycorrhiza. First PME-IGZ joint meeting on mycorrhizas, Dijon (France) 18 May 2010.
3. Doidy J.*, van Tuinen D., Schüssler A. & Wipf D. (2010) Transport de sucres au sein de la mycorhize à arbuscules. JFM2, 2nd Journées Francophones Mycorhizes, Brussels (Belgium) 15-17 September 2010.
4. Doidy J.*, van Tuinen D., Schüssler A. & Wipf D. (2011) The *Medicago truncatula* sucrose transporter family: From plant leaves to fungal symbiont. 3ème Journées des doctorants INRA SPE (Santé Plante Environnement), Dijon (France) 8-10 June 2011.

Poster presentation

1. Hayot C*., Doidy J*., Smith L. (2008) Pre-breeding and development of sainfoin. SHE2008, the first Symposium on Horticulture in Europe, Vienna (Austria) 17-20 February 2008.
2. Doidy J.*, van Tuinen D. Schüssler A., & Wipf D. (2009) Sugar transport in arbuscular mycorrhiza: Transfer of knowledge between two glomeromycotan models. XVéme Forum des Jeunes Chercheurs ED E2S Dijon (France) 25,26 June 2009.
3. Doidy J.*, D. van Tuinen, A. Schüssler & Wipf D. (2009) Transport de sucres au sein de la mycorhize à arbuscules : Transfert de connaissances entre deux modèles symbiotiques. 1ère Journée des doctorants INRA SPE (Santé Plante Environnement), Rennes (France) 1,2 September 2009.
4. Doidy J.*, Mercy L.*, & Wipf D*. (2009) Programmes cellulaires des interactions durables entre symbiotes mycorhiziens, et exploitations biotechnologiques. Exposition des champignons du sol à l'assiette, Dijon (France) 10,11 October 2009.
5. Doidy J.*, van Tuinen D. Schüssler A., & Wipf D. (2010) Sugar transport in abuscular mycorrhiza IMC9, 9th International mycorrhiza congress, Edinburgh, (UK) 1-6 August 2010.

* Presenting authors