New Insights into Carbon Transport and Incorporation to Wood

Mir Amir Hossein Mahboubi
Faculty of Forest Sciences
Department of Forest Genetics and Plant Physiology
Umeå

Doctoral Thesis
Swedish University of Agricultural Sciences
Umeå 2014

Acta Universitatis agriculturae Sueciae

Cover: A leaf of *Populus tremula* × *tremuloides* connected to the stem through the petiole.

(photo: Amir Mahboubi)

ISSN 1652-6880
ISBN (print version) 978-91-576-8204-8
ISBN (electronic version) 978-91-576-8205-5

Print: Arkitektkopia, Umeå 2015

New Insights into Carbon Transport and Incorporation to Wood

Abstract

Wood formation in trees requires carbon import from the photosynthetic tissues. In several tree species the majority of this carbon is derived from sucrose transported in the phloem. This thesis describes experimental work on the mechanism of radial sucrose transport from phloem to developing wood and subsequent incorporation of carbon into wood polymers.

I investigated the role of active sucrose transport during secondary cell wall formation in hybrid aspen (*Populus tremula x tremuloides*). Reduction of a plasma membrane localised sucrose transporter (PttSUT3) decreased carbon allocation to secondary walls of wood fibers. The results show the importance of active sucrose transport for wood formation in a symplasmic phloem loading tree species, and identify PttSUT3 as a principal transporter for carbon delivery into secondary cell wall forming wood fibers.

To investigate the temporal dynamics of carbon transport and wood polymer biosynthesis, I labelled two-month-old hybrid aspen trees with $^{13}CO_2$ and tracked the flux of ^{13}C from leaves to developing wood. Analysis of the cell wall polymer labelling patterns using 2D-NMR revealed temporal differences in the labelling of carbohydrates and lignin subunits. Further analysis showed that ^{13}C incorporation into different wood polymers is influenced by the diurnal cycle.

Primary metabolism enzymes in the developing wood play an important role in carbon partitioning to wood cell wall polymers. In this part of the study, the activity of eight primary metabolism enzymes linking sucrose to cell wall precursor biosynthesis was determined in phloem, cambium and in different stages of wood development. Comparison of enzyme activity measurements with transcript and metabolite profiles across the developing wood suggested a central role for transcriptional regulation of carbon allocation to wood.

Combined, the results of the three projects provide new insights into the mechanism and regulation of carbon allocation to developing wood.

Keywords: Secondary cell wall, sucrose transport, $^{13}CO_2$, primary metabolism, developing wood

Author's address: Mir Amir Hossein Mahboubi, SLU, Department of Forest Genetics and Plant physiology, SLU, S-90183, Umeå, Sweden
E-mail: Miramirhossein.Mahboubi@ slu.se

If your tree of life gets the fruit of knowledge, you can bring the whole universe down.

Naser Khosrow

Contents

List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I **Amir Mahboubi**, Christine Ratke, Andras Gorzas, Manoj Kumar, Ewa J. Mellerowicz, and Totte Niittylä (2013). Aspen SUCROSE TRANSPORTER3 Allocates Carbon into Wood Fibers. *Plant Physiology* 163 (4), 1729–1740.

II **Amir Mahboubi***, Pernilla Linden*, Mattias Hedenström, Thomas Moritz and Totte Niittylä (2014). Carbon-13 tracking after $^{13}CO_2$ supply revealed diurnal patterns of wood biosynthesis in aspen. *Manuscript*.

III Melissa Roach, Stephanie Arrivault, **Amir Mahboubi**, Nicole Krohn, Ronan Sulpice, Mark Stitt and Totte Niittylä (2014). Spatial analysis of primary metabolism in developing wood of aspen suggested a central role for transcriptional regulation in carbon allocation to wood. *Manuscript.*

*Authors with equal contribution.

Abbreviations

3-PGA	3-phosphoglycerate
4CL	4-coumarate-CoA ligase
ADH	arogenate dehydratase
ADP	adenosine diphosphate
ATP	adenosine triphosphate
C4H	cinnamate 4-hydroxylase
CAD	cinnamyl alcohol dehydrogenase
CC	companion cell
CCRs	cinnamoyl-CoA reductases
CesA	cellulose synthase
CINV	cytosolic invertase
DNA	deoxyrebonucleic acid
dsRNA	double stranded RNA
EA	elemental analyzer
EA-IRMS	elemental analysis-isotope ratio mass spectrometry
F5H	ferulate 5-hydroxylase
FBP	fructose-1,6-bisphosphate
FBPases	fructose-1,6-bisphosphatase
FRK	fructokinase
GC	gas chromatography
GT	glycosyltransferase
GT43-B	*GLYCOSYLTRANSFERASE-43B*
HXK	hexokinase

LC	liquid chromatography
mRNA	messenger RNA
MS	mass spectrometry
NMR	nucleic magnetic resonance
PAL	phenylalanine ammonia-lyases
PCA	principle component analysis
PD	plasmodesmata
PGI	phosphoglucoisomerase
PGM	phosphoglucomutase
PMA4	PLASMA MEMBRANE ATPASE 4
Ptt	*Populus tremula x tremuloides*
PYPP-AT	phenylpyruvate aminotransferase
RBP	ribulose-1,5-bisphosphate
RISC	RNA-induced silencing complex
RNA	ribonucleic acid
RNAi	RNA interference
Rubisco	ribulose-1,5-bisphosphate carboxylase-oxygenase
SE-CCC	sieve element companion cell complex
SHDH	shikimate dehydrogenase
SPP	sucrose phosphate phosphatase
SPS	sucrose phosphate synthase
SUC2	SUCROSE TRANSPORTER 2
SUS	sucrose synthase
SUT	sucrose transporter
TALENs	transcription activator-like effector nucleases
T-DNA	transfer DNA
TFA	trifluoroacetic acid
TILLING	targeting induced local lesions in genomes
TP	triose phosphates
TPT	triose phosphate translocators
UDP	uridine diphosphate
UGPase	UDP-glucose pyrophosphorylase
USPases	UDP sugar pyrophosphorylase
UTP	uridine triphosphate

The contribution of Mir Amir Hossein Mahboubi to the papers included in this thesis was as follows:

I Planning, performance of the work, analysis and summary of the results, writing and preparation of the manuscript.

II Planning, performance of the work, analysis and summary of the results, writing and preparation of the manuscript.

III Sampling, Analysis and summary of the results, preparation and writing of the manuscript.

1 Introduction

1.1 Wood formation

Wood is used as a raw material for various industrial purposes such as, construction, furniture and pulp and paper industry. Over the past decade, woody biomass has become the center of research focus for production of biomass-based fuels due to the constraints concerning the use of fossil fuels. Wood formation also sequesters carbon from atmosphere and the carbon can be retained in wood for a relatively long time, therefore potentially reducing carbon emission to the atmosphere (Plomion et al., 2001). Wood also serves as structural support for trees that have evolved to grow tall in order to compete for light (Kenrick and Crane 1997).

Wood is formed from activity of the cambial meristem that comprises four main developmental steps: cell division, cell expansion, secondary cell wall deposition and maturation (cell death) (Fig. 1) (Mellerowicz et al., 2001). Three different cell types are formed during wood development in angiosperms: fibers, vessels and ray parenchyma. Fibers are long fusiform cells that confer mechanical support to the plant. Vessels are dead hollow tube-like cells that are responsible for water transport. Ray cells are bands of radially oriented living parenchymatous cells having a role in radial assimilate transport in wood (Plomion et al., 2001; Van bel 1990).

Wood formation process involves biosynthesis and deposition of secondary cell walls. Secondary cell walls in wood are composed of three major polymers; cellulose, hemicelluloses and lignin that interact with each other after being deposited to the cell wall matrix (Plomion et al., 2001). Biosynthesis of secondary cell wall polymers is a carbon demanding process that requires the import of assimilated carbon from photosynthetic tissues. Assimilates produced in source leaves are loaded into the phloem, transported throughout the vascular system and finally unloaded from phloem into developing wood and other sink tissues. The steps of carbon assimilation and transport from source

leaves to developing wood are of critical importance to provide carbon needed for secondary cell wall biosynthesis and wood development and will be discussed in the following sections.

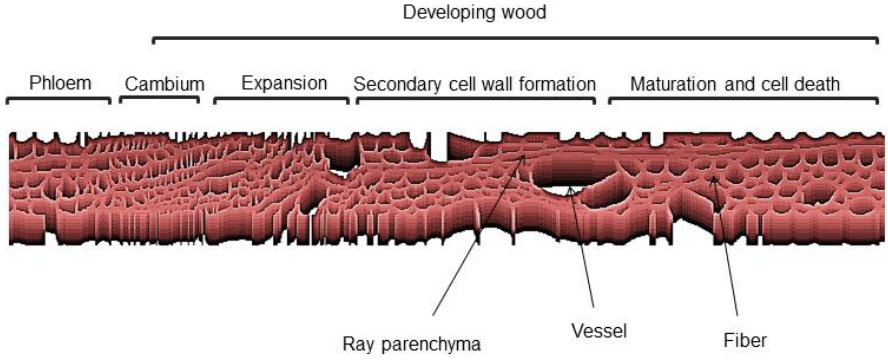


Figure 1. Overview of the developing wood displaying wood developmental stages (cambial division, cell expansion, secondary cell wall deposition and maturation and cell death) and wood cell types (fibers, vessel elements and ray parenchyma cells).

1.2 Carbon assimilation in source tissues

Carbon assimilation occurs in the chloroplast of photosynthetic cells, mainly leaf mesophyll cells, through the enzymatic reactions of the Calvin cycle (Calvin and Benson 1949) (Fig. 2). The first step of Calvin cycle is catalyzed by ribulose-1,5-bisphosphate carboxylase-oxygenase (Rubisco) that converts ribulose-1,5-bisphosphate (RBP) and CO_2 to 3-phosphoglycerate (3-PGA) (Miziorko and Lorimer 1983). 3-phosphoglycerate is then used as a substrate for triose phosphates (TP) production. Triose phosphates convert to fructose-1,6-bisphosphate (FBP) and the latter then converts to fructose-6-phophate in two successive reactions catalyzed by aldolases and fructose-1,6-bisphosphatases (FBPases) respectively.

Both aldolases and FBPases have been shown to be important for carbon assimilation and plant growth. Reduction of chloroplastic aldolase or FBPase activity in potato antisense lines impaired photosynthesis, leading to reduced sucrose and starch biosynthesis altogether resulting in a decrease in plant growth (Koßmann et al., 1994; Haake et al., 1998). Reduction of cytosolic FBPase activity in potato also limited photosynthesis and carbon assimilation in source leaves with no effect on plant and tuber growth. (Zrenner et al., 1996). These results indicate that the plastidial FBPase activity cannot be compensated by its cytosolic counterpart, highlighting the importance of the plastidial FBPase in provision of carbon needed for plant growth.

Phosphoglucoisomerases (PGIs) and phosphoglucomutases (PGMs) catalyze the interconversion of fructose-6-phosphate to glucose-6-phosphate and glucose-6-phosphate to glucose-1-phosphate respectively, giving rise to formation of the hexose phosphates pool. Reduced plastid PGI activity in *Clarkia xanthiana* and *Arabidopsis thaliana* mainly decreased starch synthesis, while reducing the activity of *Clarkia xanthiana* cytosolic PGI mainly reduced sucrose biosynthesis (Neuhaus et al., 1989; Yu et al., 2000). Reduction in plastid PGM activity in *Arabidopsis thaliana* and potato also impaired starch biosynthesis whereas reduced cytosolic PGM activity impaired sucrose biosynthesis (Caspar et al., 1985; Tauberger et al., 2000; Fernie et al., 2002). These results implied that PGI and PGM activities in plastids mainly contribute to starch biosynthesis whereas the cytosolic activity of these enzymes is part of sucrose biosynthesis pathway in cytosol and that sucrose biosynthesis mainly occurs in cytosol.

After carbon assimilation in the chloroplast, the assimilated carbon is then transported to cytosol to enter cytosolic carbon metabolism. Chloroplastic triose phosphate translocators (TPT) were shown to have a role in triose-phosphate transport across the chloroplast envelope to cytosol during the day as *Arabidopsis thaliana* and potato antisense lines having reduced TPT activity accumulated excessive starch and have reduced photosynthesis as well as sucrose biosynthesis (Riesmeier et al., 1993; Schneider et al., 2002). Maltose and glucose resulting from starch breakdown at night were also shown to be transported to cytosol by maltose and glucose exporters (Niittylä et al., 2004; Cho et al., 2011). Sugars translocated from chloroplast to cytosol build up the cytosolic pool of hexose phosphates that can enter the sucrose biosynthetic pathway.

Sucrose phosphate synthase (SPS) uses fructose-6-phosphate and UDP-Glucose to produce sucrose phosphate the latter converts to sucrose by sucrose phosphate phosphatase (SPP). UDP-glucose pyrophosphorylase (UGPase) is an enzyme in sucrose biosynthesis pathway that produces UDP-glucose from UTP and Glucose-1-phosphate (Kleczkowski et al., 2004). Tomato plants expressing maize SPS under Rubisco small subunit promoter, were reported to have reduced starch content and elevated sucrose levels (Worrell et al., 1991). Tobacco RNA interference (RNAi) lines with reduced SPP activity accumulated more starch and less sucrose (Chen et al., 2005). These results indicate the importance of both SPS and SPP in sucrose biosynthesis in source leaves. Sucrose produced in source cells is the main form of carbon source in majority of plant species that is loaded into phloem and transported throughout the whole plant to provide for the carbon demand of the heterotrophic sink tissues such as developing wood.

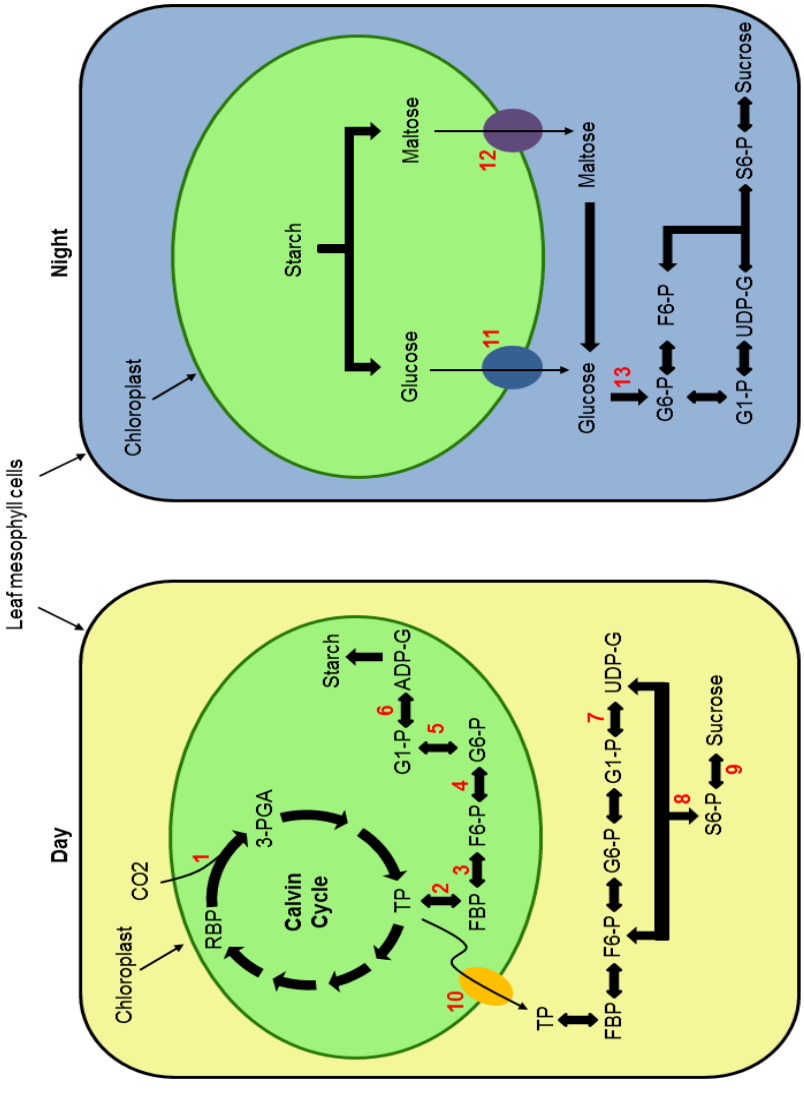


Figure 2. Overview of sucrose and starch biosynthesis in leaf mesophyll cells during day and night. During the day, photosynthesis is active and carbon is assimilated in chloroplast, part of which is stored as starch in chloroplast and the rest is exported to cytosol by TPTs to enter sucrose biosynthesis and other metabolic phathways. During the night, no photosynthesis is occurring and breakdown of starch is activated producing maltose and glucose in chloroplast that are exported to cytosol by maltose and glucose exporters to provide carbon for sucrose biosynthesis and primary metabolism. Enzymes and transporters included in this model are; Rubisco (1), aldolase (2), FBPase (3), PGI (4), PGM (5), AGPase (6), UGPase (7), SPS (8), SPP (9), TPT (10), glucose exporter (11), maltose exporter (12), Hexokinase (13).

1.3 Sucrose export and phloem loading

Higher plants can be divided into three categories based on how they load sucrose into phloem (Turgeon 1996) (Fig. 3). Members of the first group are apoplasmic loaders characterized by limited number of plasmodesmata between the sieve element companion cell complex (SE-CCC) and surrounding cells. Many plant species such as Arabidopsis, tobacco and rice belong to this group of phloem loaders (Sauer 2007). They first export sucrose from source cells out to the apoplasm by sucrose efflux carriers. The mechanism of sucrose export in apoplasmic phloem loaders was not understood until recently, when Chen et al (2012) demonstrated that Arabidopsis SWEET11 and SWEET12 function as sucrose efflux carriers and double mutants of the genes encoding these two transporters were defective in phloem loading (Chen et al., 2012). Sucrose-proton symporters localized to the plasma membrane of SE-CCC then mediate sucrose import from apoplasm into the SE-CCC. In *Arabidopsis thaliana,* SUCROSE TRANSPORTER 2 (SUC2) was shown to be localized at the plasma membrane of companion cells (CCs) and null mutants of *SUC2* (*suc2)* showed impaired phloem loading and severe plant growth defect (Stadler and Sauer 1996; Gottwald et al., 2000). Sucrose transport into SE-CCC in apoplasmic loaders occurs against the sucrose concentration gradient and requires energy. Energy for the active sucrose transport is provided by the activity of proton ATPase pumps that export protons out to the apoplasm creating an electrochemical gradient across the plasma membrane of SE-CCC. This proton gradient drives the activity of sucrose-proton symporters and facilitates sucrose import into the SE-CCC. In support of this transport model, suppressed expression of PLASMA MEMBRANE ATPASE 4 (PMA4) in *Nicotiana plumbaginifolia* resulted in reduced plant growth as well as decreased sucrose import into SE-CCC (Zhao et al., 2000).

Plants belonging to the second group of phloem loaders have plasmodesmal connectivity between the source cells and SE-CCC and load into phloem through symplasm. These plants have higher sucrose concentration in the SE-CCC than mesophyll cells that potentially forces sucrose to flow back to the mesophyll cells down the concentration gradient (Turgeon 1996). To circumvent this, plants of this group convert sucrose into raffinose (trisaccharide) and/or stachyose (tetrasaccharide) in phloem that are too large to flow back to the mesophyll cells (Turgeon 1996; Rennie and Turgeon 2009). This phenomenon is known as “polymer trapping” and cucurbits are the best example of this group of phloem loaders.

The third phloem loading strategy is very common amongst many trees that have higher solute content in mesophyll cells than in phloem and high plasmodesmal continuity between mesophyll and SE-CCC, hence are thought to load into phloem passively (Rennie and Turgeon 2009; Fu et al., 2011). Members of the plant family Salicaceae including *Populus spp.* belong to this group of phloem loaders (Russin and Evert 1985). In a recent study,

expression of yeast invertase (cleaving apoplasmic sucrose to glucose and fructose) under the 35S or the minor vein-specific galactinol synthase promoter was shown to impair phloem loading in the apoplasmic loader alfalfa (*Medicago sativa*), while no loading effect was observed in grey poplar (*Populus tremula* × *alba*), providing strong evidence indicating the symplasmic transport route to be sufficient for phloem loading in poplar. (Zhang et al., 2014).

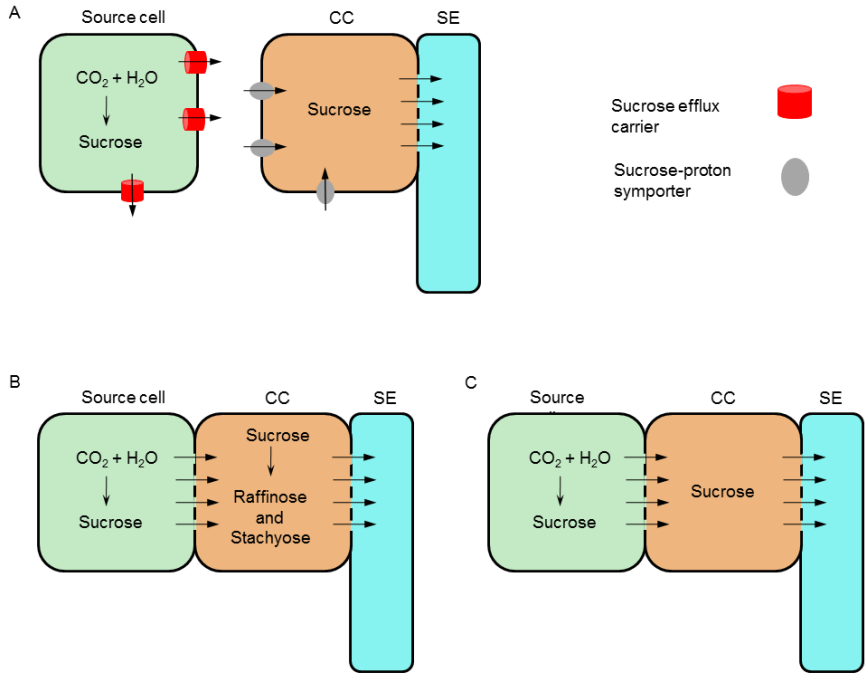


Figure 3. Schematic overview of phloem loading strategies in angiosperms. Loading strategies comprise apoplasmic loading (A), polymer trapping (B) and symplasmic loading (C)

1.4 Sucrose transport in the phloem

The driving force for solute transport (including sucrose transport) in the phloem, according to Munch's pressure flow model (1927), is the pressure difference between the source and sink ends of the sieve tube (pressure gradient). Munch's theory was challenged by Thompson (2006) who suggested that since plants lack a centralized regulatory system similar to the nervous system in animals, they cannot have any control over the turgor pressure along the transport phloem path. They put an idea forward that the phloem solute

exchange would be more efficient when pressure gradient is negligible. This gives a better control of unloading over the whole phloem path, as local reduction in turgor pressure at the site of unloading can be more easily balanced.

In trees, the increase in length of the phloem transport path (due to the height of the plant) should result in higher pressure difference between source and sink if Munch's theory was the driving force for phloem transport (Turgeon 2010). However, actual measurements showed lower phloem pressure in trees than what was reported for herbaceous species with pressure gradient being negligible in trees (Zimmermann 1957; Wright and Fisher 1980; Lee 1981). The imperfection of Munch's theory on phloem transport in trees is still an ongoing debate with many open questions regarding phloem transport mechanism/s in trees waiting to be addressed.

1.5 Radial sucrose transport into wood

Sucrose is ultimately transported from phloem to sink tissues through a process called phloem unloading. In trees, wood is an important sink that depending on the tree species accounts for 45 % to 80 % of aboveground biomass (Litton et al., 2007). Radial sucrose transport into wood is thought to comprise three steps, first unloading from the sieve tube into ray cells, second, transport through the ray cells and third, export from the ray cells into developing fibers and vessel elements (Van bel 1990) (Fig. 4). The transport from sieve tubes into ray cells in trees is thought to occur through the symplasm (Sauter 1980; Sauter and Kloth 1986). In conifers, high plasmodesmal connectivity was observed between sieve tube and a specific type of ray parenchyma cells known as Strasburger cells, suggesting the symplasm to be the main transport route from sieve elements into ray cells in conifers (Sauter 1980). In *Populus*, the rate of starch accumulation in wood ray cells during the growth season was about 8 to 80 times higher than the rate known for transmembrane transport suggesting that the radial translocation of sucrose to occur through the symplasm (Sauter and Kloth 1986). Transport through the ray cells also seems to be symplasmic based on electron microscopy images of plasmodesmata connecting the ray cells of *Populus canadensis* (Sauter and Kloth 1986). This model is also supported by tracer studies showing the movement of a symplasmic tracer through ray cells of *Acer pseudoplatanus* (Sokołowska and Zagórska-Marek 2012). Export from the ray cells into developing fiber cells and vessels is thought to occur through the contact pits at the interface between rays and vessels or fibers (Van bel 1990) and across the plasma membrane (Fig. 4) based on the evidence showing the efflux of sugars from ray parenchyma cells into the wood apoplasm in different tree species (Sauter et

al., 1973; Sauter 1980; Sauter 1988). This was based on the presence of sucrose in wood apoplasm that was suggested to be partly converted to hexoses since apoplasmic sap of *Populus* wood also contained high hexose contents (Sauter 1988). In case sucrose is exported from the ray cells to wood apoplasm, then one important question in wood formation is whether sucrose is actively imported from wood apoplasm into developing wood fibers and vessel elements, and which transporter/s are responsible for this import?

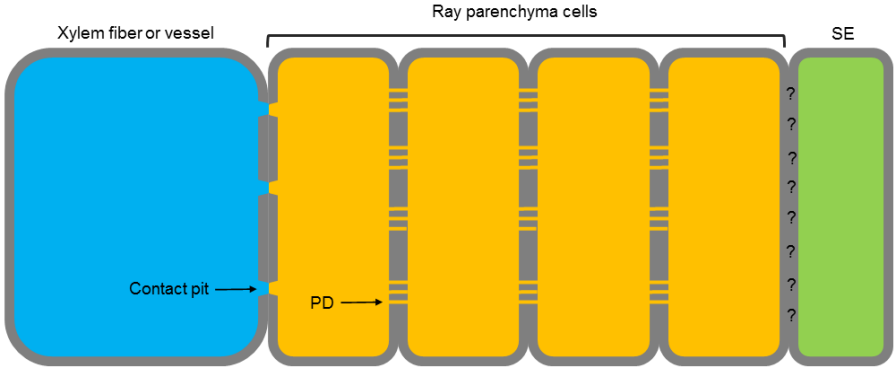


Figure 4. Schematic overview of phloem unloading and radial transport in angiosperm trees according to Van bel (1990). The unloading process first involves export from sieve elements (SE) into ray cells by an unknown mechanism. Second, symplasmic transport along the ray cells through plasmodesmata (PD) and third, export from ray cells to wood fibers or vessels that occurs through apoplasm.

1.6 Active sucrose transport into wood

The last step of sugar transport into wood is the import of sugars from wood apoplasm into developing fibers and vessel elements. Sucrose release from ray cells to wood apoplasm suggests that sucrose transporters (SUTs) may be involved in sucrose import into developing wood fibers and vessels.

SUTs are transmembrane proteins with 12 transmembrane domains and were shown to function as proton-sucrose symporters (Sauer 2007). The symport function of SUTs was shown by analyzing the stoichiometry of sucrose and proton transport across the plasma membrane of *Xenopus* oocyte expressing potato SUT1 (StSUT1) and Arabidopsis SUC2 (AtSUC2) (Boorer et al., 1996 and Zhou et al., 1997).

Phylogenetic analysis of sucrose transporters divides them into four groups (Sauer 2007). Group 1 contains monocot SUTs of which, ZmSUT1and

OsSUT1are the two well studied examples having a role in phloem loading in maize and long distance transport in rice respectively (Scofield et al., 2007; Slewinski et al., 2009). Members of group 2 SUTs belong to dicots among which AtSUC2 and NtSUT1 have been studied in detail and were shown to have a role in phloem loading in Arabidopsis and tobacco (Burkle et al., 1998; Gottwald et al., 2000). Group 3 SUTs differ from the other SUTs by having a longer central loop resulting in 15% to 20% larger proteins with low sucrose transport activity and similarity to yeast sugar sensors (Sauer 2007). Group 3 SUTs are expressed in different tissues such as SE and some sink tissues in several plant species and thought to have a role in sucrose retrieval along the phloem path, sucrose import into sink tissues and/or sugar sensing (Barker et al., 2000; Barth et al., 2003; Meyer et al., 2004). Group 4 SUTs are vacuolar transporters shown to be localized to the tonoplast (Endler et al., 2006).

In *Populus spp.*, there are five sucrose transporter genes of which *SUCROSE TRANSPORTER 4* (*SUT4*) and *SUCROSE TRANSPORTER 3* (*SUT3*) were shown to be more highly expressed in the stem with *SUT3* being specific to stem (Payyavula et al., 2011). SUT4 was shown to localize to the tonoplast and analysis of carbon allocation in *SUT4* RNAi lines with 50% to 90% lowered *SUT4* transcript level showed reduced sucrose export (based on steady state analysis of sucrose pool) from their leaves with no changes in plant growth and no report of changes in wood anatomy and/or cell wall composition (Payyavula et al., 2011). The role of other SUTs in carbon allocation to wood however remained to be investigated.

1.7 Sucrose cleavage pathways in developing wood

After being imported into developing wood fibers and vessels, sucrose is cleaved either by sucrose synthases (SUSs) or invertases. It has for long been a debate that which of the two alternatives is the predominant route of sucrose catabolism in plant cells. SUSs catalyze sucrose cleavage to fructose and UDP-glucose in a reversible manner with the stoichiometry of the reaction thought to be in the direction of sucrose cleavage in vivo (Delmer 1972; Geigenberger and Stitt 1993). Amor et al. (1995) identified a form of SUS that is associated to the surface of cotton seed (*Gossypium hirsutum*) fiber cells (seed trichomes) and consistently oriented to the path of cellulose or callose deposition. Consistently, reduction of SUS activity severely impaired initiation and elongation of cotton fiber cells (Ruan et al., 2003). Based on this finding, SUS was proposed to be associated with the cellulose biosynthesis machinery providing UDP-glucose specifically for cellulose biosynthesis.

Inhibition of SUS activity down to 4% of WT in hybrid aspen (*Populus tremula* × *tremuloides*) decreased carbon allocation into the cell walls and lowered the wood bulk density (Gerber et al., 2014). Although the SUSRNAi had no specific effect on cellulose biosynthesis it is possible to increase cellulose content in wood by increased expression of SUS as was shown in hybrid poplars expressing *Gossypium hirsutum SUS* under both 35S and p-coumarate-CoA ligase (4CL) promoters had slightly higher amount of cellulose in wood (Coleman 2009). These results establish a role for SUSs in sucrose metabolism in the direction of biosynthesis of cell wall polymers in the wood.

Invertases catalyze irreversible sucrose breakdown to glucose and fructose. In Arabidopsis CINV1 and CINV2 are the main cytosolic invertases in Arabidopsis root double mutants of which had a severe growth defect (Barratt et al., 2009). In the same study, SUS quadruple mutants of Arabidopsis lacking SUS activity in all tissues but phloem did not show any detectable phenotype suggested that SUS is not the main route of carbon entrance into the cellular metabolism. However, this was disputed later by a study demonstrating that the SUS activity in quadruple mutant is sufficient to provide carbon for cellular metabolism (Baroja-Fernandez et al., 2012). A very recent metabolic flux model of cell wall polysaccharide biosynthesis using Arabidopsis cell culture system supports the results obtained by Barrat et al. (1995) claiming the invertase pathway to be the main route of sucrose cleavage (Chen et al., 2013). In *Populus*, there are 8 acidic and 16 neutral/alkaline invertases (Bocock et al., 2008), but there has not been any functional characterization for any of them in trees.

Monosaccharides produced from sucrose cleavage then enter the carbon primary metabolism in developing wood cells that undergo continuous biosynthesis and deposition of the cell wall polymers (Fig. 5).

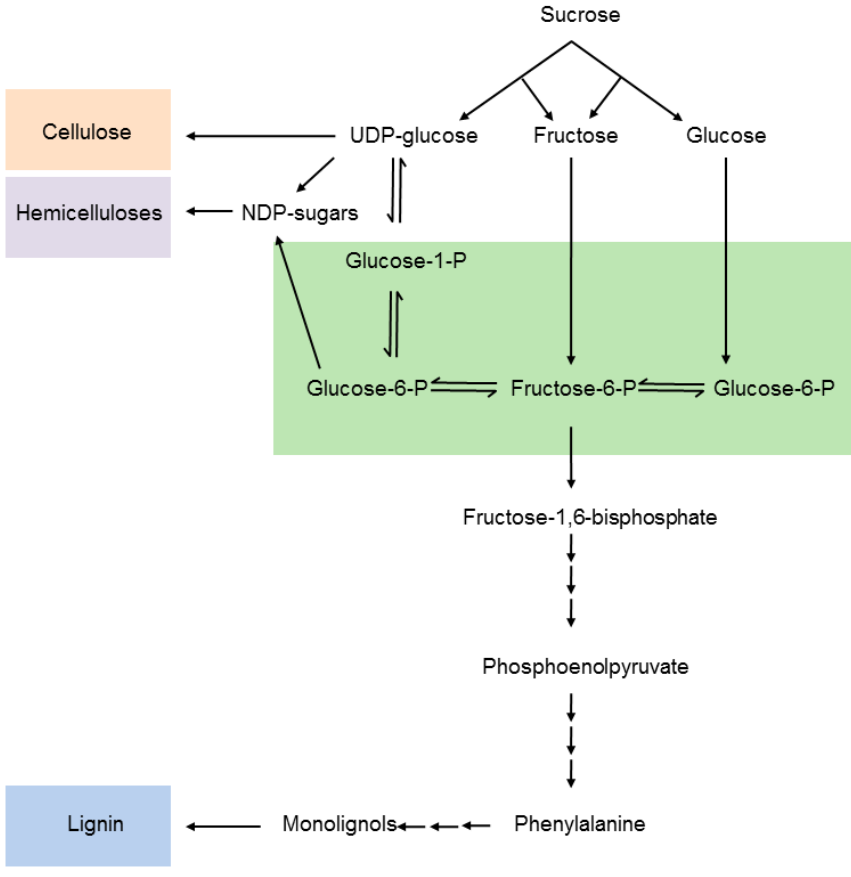


Figure 5. Overview of pathways leading to biosynthesis of the main wood cell wall polymers.

1.8 Carbon allocation to cell wall polymers in wood

1.8.1 Carbon allocation to Cellulose biosynthesis

Cellulose is the most abundant biopolymer in the world (Delmer and Haigler 2002). Cellulose polymer is a linear chain of β-(1-4)-linked glucose units that bind to each other by hydrogen bonds to form cellulose microfibrils (Tylor 2008). Cellulose is made by rosette-like protein assemblies that are composed of six hexameric protein complexes known as cellulose synthase (CesA) complexes. Each CesA complex is composed of six CesA protein subunits (Arioli et al., 1998; Tylor 2008). CesA enzymes are thought to use UDP-glucose as substrate for the biosynthesis of cellulose chains, therefore,

pathways that lead to UDP-glucose production can potentially have an impact on cellulose biosynthesis (Carpita and Delmer 1981).

SUSs and UGPases are the main enzymes that have been shown to produce UDP-glucose in plants. Inhibition of the SUS activity in hybrid aspen reduced carbon allocation to all the secondary cell wall polymers however its overexpression only increased cellulose deposition in hybrid poplar (Coleman et al., 2009; Gerber et al., 2014). Expression of *Acetobacter xylinum UGPase* in hybrid poplar (*Populus alba×grandidentata*) enhanced wood cellulose content but impaired plant growth drastically (Coleman et al., 2007). Fluxes into intermediary pools of UDP-glucose biosynthesis e.g. hexose phosphates pool can also have an effect on UDP-glucose pool size. Inhibition of fructokinase (FRK) activity attenuated hexose phosphates and as a result UDP-glucose pool sizes. FRK lines with reduced FRK activity also had reduced carbon allocation into cellulose in hybrid aspen (Roach et al., 2012). Moreover, PGIs and PGMs that function in inter-conversion of hexosephosphates can potentially affect the flux into UDP-glucose, however, their role in carbon allocation to cellulose in wood remains a question to be addressed.

1.8.2 Carbon allocation to biosynthesis of hemicelluloses

Hemicelluloses are complex polysaccharides that bind cellulose via hydrogen bonding (Lerouxel et al., 2006). Xyloglucans, mannans and xylans are the main hemicelluloses found in gymnosperm and angiosperm wood. Xyloglucans are made of a β-(1-4)-glucan backbone about 70% of which have (1-6)-xylose side-chains. Xylose side-chains can be further linked to galactose-galactose or galactose-fucose extending the side chain (Mellerowicz et al., 2001). Mannans consist of a β-(1-4)-mannose chain in which mannose can be occasionally substituted by glucose resulting in glucomannan (Lerouxel et al., 2006). Xylans, are β-(1-4)-xylose chains that contain glucose and arabinose side chains (Lerouxel et al., 2006). Xylan polymers are the most abundant hemicellulosic polysaccharides of secondary cell wall in angiosperm wood (Lerouxel et al., 2006).

Hemicelluloses are synthesized by glycosyltransferases (GTs) that are Golgi-membrane localized proteins. They use nucleotide sugars in Golgi lumen as substrate and add them to the reducing end of a sugar polymer backbone (Lerouxel et al., 2006). Nucleotide sugars are primarily made in cytosol and then transported into Golgi where they can be interconverted to other nucleotide sugars (Seifert 2004; Handford et l., 2006). UDP-Glucose dehydrogenase is an enzyme converting UDP-glucose to UDP-glucuronic acid in an irreversible manner and is the first committed step of carbon flux into other nucleotide sugars (Tenhaken and Thulke 1996). UDP- glucuronic acid is

then converted to UDP-xylose by UDP-xylose synthase, a step that can occur both in cytosol and in Golgi (Seifert 2004; Harper and Bar-Peled 2002). It is still not clear whether UDP-xylose made in cytosol is transported into Golgi or *de-novo* synthesis from other UDP-sugars in Golgi provides the precursor for xylan biosynthesis. UDP sugar pyrophosphorylases (USPases) can also produce different nucleotide sugar (Kleczkowski et al., 2011) and their role in biosynthesis of cell wall polysaccharides remains to be addressed.

1.8.3 Carbon allocation to lignin biosynthesis

Lignin is a polymer consisting of aromatic alcohols called monolignols that are added to the polymer via radical coupling (Boerjan et al., 2003). Lignin polymer is mainly composed of three monolignols, coniferyl alcohol (G lignin subunit), sinapyl alcohol (S lignin subunit) and *p*-coumaryl (H lignin subunit), however, that does not exclude the possibility for incorporation of other monolignols into lignin (Boerjan et al., 2003). Lignin composition can vary in different species, for instance monocots have higher H lignin contents than dicots and gymnosperms do not contain S lignin (Boerjan et al., 2003). Monolignols are synthesized through phenylpropanoid pathway and then exported to apoplasm where they are oxidized (dehydrogenated) to free radicals by laccases and proxidases that results in coupling of oxidized monolignols to lignin polymer (Vanholme et al., 2010).

Phenylalanine is the precursor of phenylpropanoid pathway and is synthesized in plastids through shikimate pathway that links carbohydrate metabolism to biosynthesis of aromatic compounds (Hermann 1999). Lignin biosynthesis was shown to be dependent upon shikimate pathway. One example is ozone induction of transcription of shikimate pathway genes that increased lignin content and altered lignin composition in European beech (*Fagus sylvatica*) (Betz et al., 2009). The last step of phenylalanine biosynthesis in plastids is the conversion of arogenate to phenylalanine that is catalyzed by arogenate dehydratase (ADH) enzyme (Corea et al., 2012). In Arabidopsis there are six ADHs that single, double, triple and quadruple T-DNA knockouts of them show lignin deficient phenotype ranging from close to wild type in single mutants down to about 68% reduction in quadruple mutant highlighting the role of this very early step in directing the flux of carbon towards lignin biosynthesis (Corea et al., 2012). An alternative cytosolic pathway of phenylalanine biosynthesis was recently reported in *Petunia hybrida* where a cytosolic phenylpyruvate aminotransferase (PYPP-AT) was shown to synthesize phenylalanine by using phenylpyruvate as phenyl ring donor and tyrosine as an amine group donor (Yoo et al., 2013). The role of this cytosolic phenylalanine biosynthesis pathway in carbon flux into lignin remains to be addressed.

Genetic manipulation of enzymes in phenylpropanoid pathway affected lignin content and/or composition. The first two steps of the phenylpropanoid pathway are catalyzed by phenylalanine ammonia-lyases (PAL) and cinnamate 4-hydroxylase (C4H). Inhibition of the activity for each of the two enzymes in tobacco reduced lignin content and altered lignin composition (Sewalt et al., 1997). Cinnamoyl-CoA reductases (CCRs) catalyzing conversion of cinnamoyl-CoA esters to cinnamaldehydes are the key enzymes towards biosynthesis of monolignols (Lacombe et al., 1997). Arabidopsis *irx4* mutant with collapsed xylem phenotype and reduction in lignin content is the result of a nucleotide change within *CCR1* highly conserved intron splicing site (Jones et al., 2001). Ferulate 5-hydroxylase (F5H) catalyzes a reaction that leads to formation of S lignin. Downregulation of F5H enzyme reduced S lignin in Arabidopsis, whereas overexpressing the enzyme under C4H promoter resulted in lignin composed of solely S lignin (Marita et al., 1999). Cinnamyl alcohol dehydrogenase (CAD) enzyme catalyzes the last step of lignin monomer biosynthesis that is the conversion of cinnamaldehydes to cinnamyl alcohol monomers (Halpin et al., 1994). In general, inhibition of CAD activity in several plant species resulted in dramatic changes in lignin composition favoring more incorporation of aldehyde monomers than alcohol monomers into the polymer (Halpin et al., 1994; Ralph et al., 1997; Lapierre et al., 1999).

1.9 Regulation of carbon partitioning between different cell wall polymers in wood

Considerable amounts of carbon (imported into wood as sucrose) are ultimately deposited into cellulose, hemicelluloses and lignin in secondary cell wall forming cells of developing wood. Carbon partitioning into these three polymers is highly genetically and environmentally regulated so that each polymer has a determined contribution to total woody biomass. To keep the equilibrium in carbon partitioning into different cell wall polymers, the metabolic pathways involved in their biosynthesis are possibly coordinated at both transcript and protein levels.

Regulation of UDP-glucose production can potentially affect the flux of carbon into cell wall polysaccharide biosynthesis. UGPases and SUSs are the main enzymes responsible for UDP-glucose biosynthesis. A *UGPase* gene was shown to be up-regulated by sucrose treatment in Arabidopsis (Ciereszko et al., 2001). SUSs were also shown to be sugar responsive at the transcriptional level in maize (Koch et al., 1992). These results suggest that sugars can potentially act as signal molecules to regulate transcription of the genes involved in provision of UDP-glucose for cell wall polysaccharide biosynthesis.

A number of MYB and NAC transcriptional factors were shown to regulate transcription of lignin biosynthesis enzymes (Zhong and Ye 2007). In Arabidopsis expression of AtMYB15 was shown to activate shikimate pathway genes and suggested to be the regulator of shikimate pathway (Chen et al., 2006). As discussed in the section 1.8 above the shikimate pathway has a pivotal role in diverting carbon from cell wall polysaccharide biosynthesis to lignin biosynthesis. In *Eucalyptus grandis* EgMYB2 was shown to bind *cis*-regulatory regions of *CCR* and *CAD* promoters and its expression in tobacco resulted in altered lignin content and composition (Goicoechea et al., 2005). AtMYB103 was reported to control lignin composition by positively regulating *F5H* expression resulting in formation of S type lignin (Öhman et al., 2013). Another MYB transcriptional factor (MYB75/PAP1) was demonstrated as a negative regulator of lignin biosynthesis (Bhargava et al., 2010). *MYB75* transcript was previously shown to be co-expressed with phenylpropanoid pathway enzymes in a day/night dependent manner (Harmer et al., 2000).

Diurnal cycle was reported in a few studies to have an influence on the expression of lignin biosynthesis genes. In Arabidopsis, lignin biosynthesis genes had diurnal expression pattern, mostly up-regulated prior to dawn (Harmer et al., 2000; Rogers et al., 2005). Shikimate pathway genes were shown to be positively regulated by dark in another study in Arabidopsis (Sharkhuu et al., 2014). In *Eucalyptus*, lignin biosynthesis genes were also shown to be diurnally regulated (Solomon et al., 2010).

Based on these results concerning diurnal regulation of cell wall biosynthesis (mainly lignin biosynthesis) I hypothesized that wood formation may be influenced by the diurnal cycle. This has remained as important unanswered question in wood biology.

2 Objectives

My aim in this study was to investigate carbon transport and allocations to cell walls in wood of hybrid aspen (*Populus tremula* × *tremuloides*).

In the first part of this study (paper I), I attempted to unravel the role of active transport in channeling the assimilated carbon into secondary cell wall forming cells in developing wood. The focus in this part of the study was on sucrose transporters (SUTs) since sucrose is the main form of transported carbon in hybrid aspen. RNAi mediated down-regulation of a *SUT* gene with a peak in expression during secondary cell wall formation in wood allowed characterizing the function of this *SUT* in carbon allocation into wood.

In the second part of this study (paper II), my aim was to track the carbon from the site of assimilation in leaves all the way down to wood cell wall polymers over a diurnal cycle through stable isotope labeling of wild type hybrid aspen trees. This allowed me to be able to monitor the cell wall deposition dynamics with a focus on the effect of day/night cycle on biosynthesis and deposition of cell wall polymers.

In the third part of this study (paper III), the activities of prominent enzymes of carbon primary metabolism were compared to the transcript level of their encoding genes and status of their related metabolites along the wood developmental stages. One reason of doing this was to find the developmental stages at which the enzymes show the highest activity. Comparison of the enzyme activity with transcript and metabolite levels also allows understanding the role of transcriptional regulation on activity of the enzymes involved in carbon primary metabolism in wood.

3 Materials and methods

3.1 Model organism

In this study, hybrid aspen (*Populus tremula* × *tremuloides*) was used as the model tree species. Species belonging to *Populus* genus are the most commonly used model tree species due to four main reasons.

First, the genome of *Populus tricocarpa* (black cottonwood) was sequenced in 2006 and since then serves as a publicly available resource giving the possibility to find specific genes and work with gene families of interest (Tuskan et al., 2006). *Populus trichocarpa* has a relatively compact genome of approximately 46000 genes accommodated across 19 chromosomes, rendering the organism ease of gene functional study and genetic manipulation.

The second reason behind the use of *Populus* as a model system is that it can be genetically transformed by *Agrobacterium* mediated genetic transformation approach (Nilsson et al., 1992). This gives the possibility of producing transgenic lines with altered expression of target gene/s in order to elucidate their role in different biological processes.

The third reason is the rapid growth rate of *Populus* trees that can produce large amount of biomass in a relatively short period of time (a few months). *Populus* can serve as a very useful model system in studies with focus on wood formation since substantial amounts of woody biomass is produced in these trees in just a few weeks providing sufficient amounts biomass for variety of analytical purposes.

The fourth reason that *Populus* trees are widely used as model organisms in tree biology is that in comparison to other tree species, *Populus* trees have closer phylogenetic relativity to Arabidopsis (Jonsson and Douglas 2007). This provides a better chance to transfer the knowledge obtained from Arabidopsis

studies to trees and can accelerate gene functional characterization studies in trees.

3.2 Reverse genetics to study sucrose transport in *Populus*

Unlike forward genetics that starts with a phenotype first and finally leads to identification of the genetic components behind the phenotype, reverse genetics starts first with gene selection followed by studying the function of candidate gene/s in relevant biological processes (Alonso and Ecker 2006). In reverse genetics approach, genes are usually selected based on their expression level in the tissue of interest or during the cellular/physiological process of interest. Candidate gene can be also selected according to their annotations in publically available databases. The next step is then to generate mutant lines of the selected genes to study their function. The most commonly used methods for generating mutant lines include random methods such as T-DNA insertion as well as targeted methods such as, RNAi mediated gene silencing, targeting induced local lesions in genomes (TILLING) and transcription activator-like effector nucleases (TALENs) mediated gene silencing approaches (Alonso and Ecker 2006; Joung and Sander 2012).

In this study, reverse genetics was used to investigate the role of *Populus tremula* × *tremuloides* SUTs (*Ptt*SUTs) in carbon allocation into wood. The candidate *SUT* gene (*SUT3*) was selected based on its expression profile along the wood developmental zones. The selected gene was then targeted for down-regulation using RNAi mediated gene silencing approach. Down-regulation of *SUT3* was targeted to wood secondary cell wall forming cells using a wood promoter (*p-GLYCOSYLTRANSFERASE-43B*) (Ratke et al., 2014) and RNAi lines with reduced *SUT3* transcript levels were selected for further characterization.

3.3 RNAi mediated down-regulation of *PttSUT3*

RNAi mediated gene silencing is one of the common methods used for targeted down-regulation of gene/s of interest (Hannon 2002). Silencing is initiated by presence of a double-stranded RNA molecule (dsRNA) with homology to messenger RNA (mRNA) of the target gene that is cleaved by Dicer nucleases to about 22 nucleotide long dsRNA (Bernstein et al., 2001).The 22 nucleotide dsRNA then associates with the RNA-induced silencing complex (RISC) and guides the complex towards the target mRNA. Incorporation of dsRNA to RISC results in unwinding of dsRNA in an ATP dependent manner leading then to detection and degradation of target mRNA (Nykänen et al., 2001).

In this study, an RNAi vector was prepared using 140 bp *PttSUT3* fragment. The RNAi cassette was driven under *PttGLYCOSYLTRANSFERASE-43B* promoter (*pGT43-B*) targeting the *SUT3* mRNA specifically in the wood. Transcription of the RNAi cassette would result in a hairpin RNA structure that can be cleaved by Dicer enzyme producing 21-22 bp long dsRNA molecules that associates with RISC to mediate *SUT3* mRNA degradation (Fig. 6).

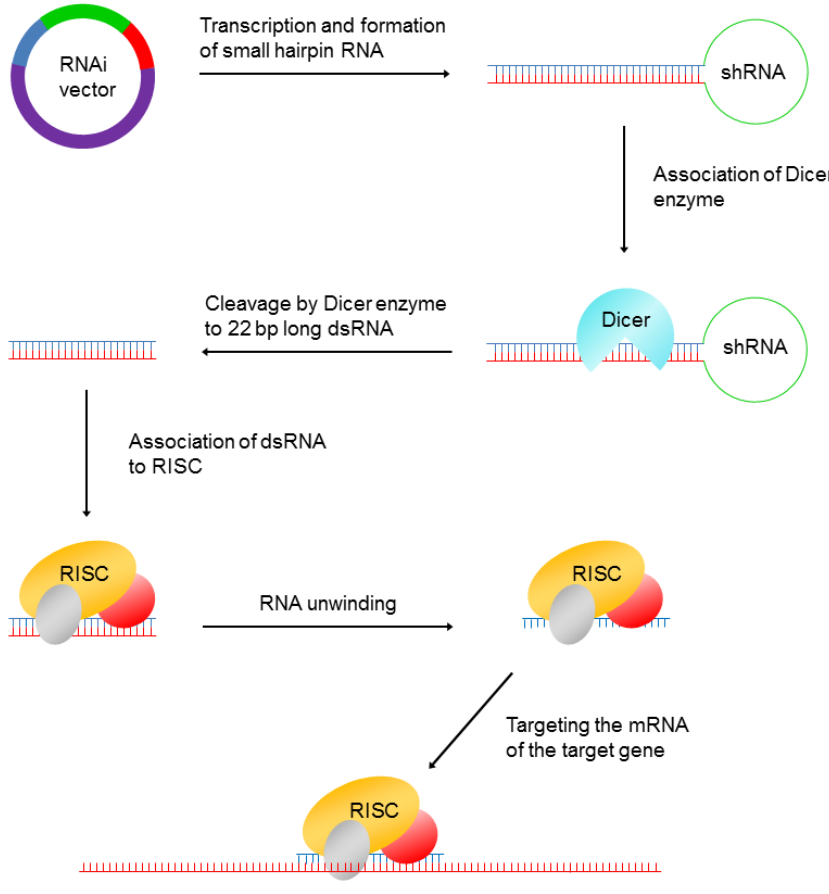


Figure 6. A model representing RNA interference gene silencing mechanism. Small hairpin RNA (shRNA) is transcribed and formed (using RNAi vector as template) and then is cleavege by Dicer to form the 22 bp dsRNA. Association of dsRNA to RISC results in dsRNA unwinding and subsequent targeting of the target mRNA to degradation.

3.4 Isotope labeling

Isotope labeling has been widely used in many studies leading to identification of metabolic pathways and characterization of metabolic fluxes in plants (Calvin and Benson 1949; Szecowka et al., 2013). Since measuring the size of metabolite pools alone cannot always provide a complete understanding of the whole metabolic pathway, labeling the metabolite pools provides additional information about dynamics of the metabolite pools as well as fluxes in and out of the metabolite pools deepening the understanding of metabolic pathways (Fell 2005, Ratcliffe and Shachar-Hill 2006).

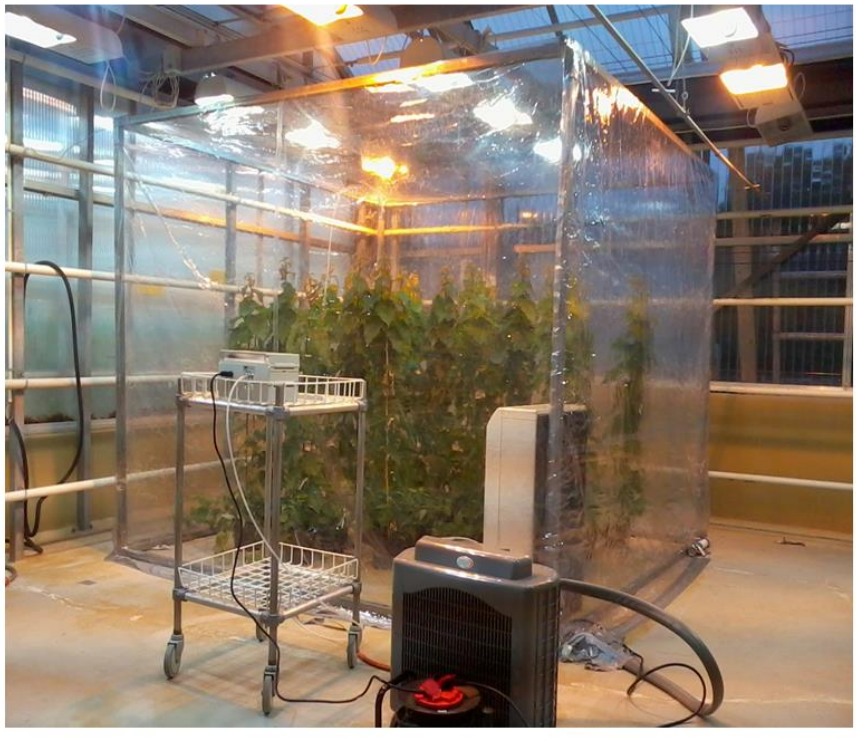
Use of isotope labeling is of a great potential when it comes to the analysis of cell wall polymers deposition. Cell wall polymers are stable pools of carbon polymers that are continuously added to the cell wall matrix; hence, carbon isotope labeling can serve as a robust tool to track cell wall formation. Carbon isotope labeling was used in a few studies for tracking the assimilated carbon into cell walls. In flax (*Linum usifafissimum*), $^{14}CO_2$ labelling has been used to study the dynamics of cell wall biosynthesis based on the proportion of radioactivity incorporation into different cell wall polymers over a time course (Gorshkova et al., 1997). Carbon isotope ($^{13}CO_2$) labeling was also used to study carbon allocation into earlywood and latewood in larch (*Larix gmelinii*) (Kagawa et al., 2006).

In this study, I investigated source to sink dynamics of carbon flow into the wood of hybrid aspen (*Populus tremula* × *tremuloides*). Two month old greenhouse grown trees were enclosed in a transparent chamber under controlled growth parameters and given a 4 hour $^{13}CO_2$ pulse (injected into the chamber by a 1 L syringe) at CO_2 level of 330-400 ppm (Fig. 7) followed by harvesting the leaf, stem phloem and developing wood at different time points after start of the pulse. In paper I, ^{13}C signal was only analyzed in the ethanol soluble fraction of developing xylem at 4 and 8 hours after start of the pulse to understand the changes in carbon flux in developing wood of *PttSUTRNAi* lines versus wild type. In paper II, ^{13}C was tracked into several intermediate metabolite pools (in leaf, phloem and developing wood) and cell wall polymers (in developing wood) at 4, 9, 14, 19 and 24 hours after start of the pulse.

3.5 Cell wall fractionation and analysis

Extractive and starch free developing wood powder was prepared as described by Foster et al., (2010) and used for cellulose, lignin and cell wall monosaccharide content measurements. Cellulose was fractionated using Updegraff method (Updegraff 1969) followed by Anthrone assay for

A



B

C

Figure 7. Labelling experiment set up. Two month old wild type (line T89) hyberid aspen trees were placed in a transparent chamber and labelled with $^{13}CO_2$ under controlled environment. The temprature and humidity were kept constat using an air conditioner unit (A). CO_2 level was monitored by a CO_2 detector (PP SYSTEMS) (B). CO_2 injection was performed using a 1 ml syringe (Hamilton) (C).

measurement of released glucose (Scott and Melvin 1953). Lignin fraction was prepared and the content was measured by Klason lignin method (Fengel and Wegner 1983). In paper I, cell wall monosaccharide fraction was prepared by 2 M HCL/methanol treatment followed by tri-sil reagent (1,1,3,3,3-hexamethyldisilazane + trimethylchlorosilane + pyridine, 3:1:9) derivatization using Sylon HTP kit (SUPELCO, http://www.sigmaaldrich.com) as described in (Sweeley et al., 1963). In paper II Cell wall monosaccharides were extracted by 2 M trifluoroacetic acid (TFA) hydrolysis of the cell wall material followed by alditol acetate derivatization, as described by Fox et al., (1989). Monosaccharide content was determined using gas chromatography mass spectrometry described in the next section. The flow chart of the fractionation procedure is illustrated in Figure 8.

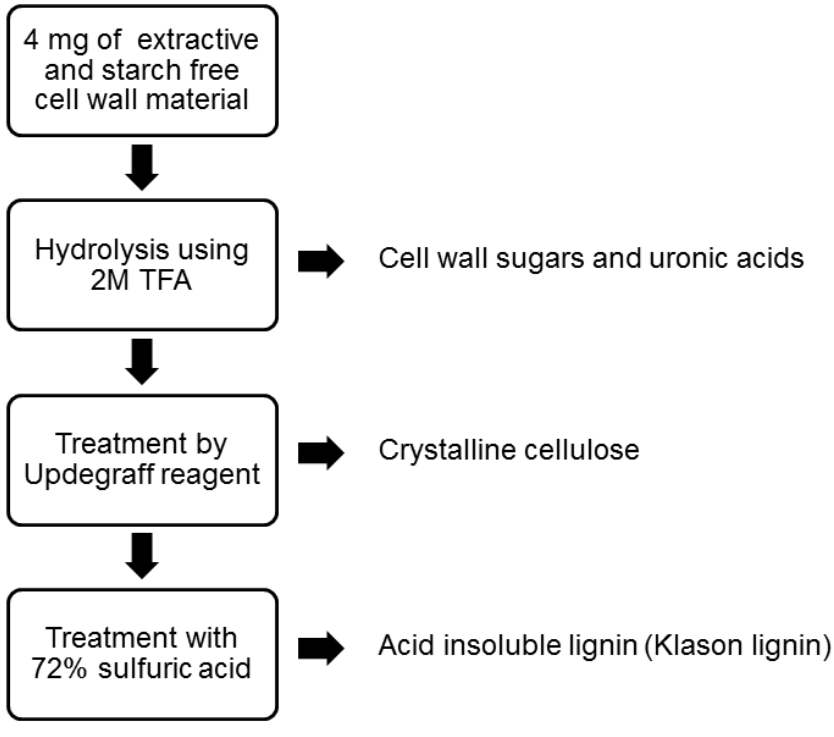


Figure 8. Overview of cell wall fractionation procedure .

3.6 Chemical analysis and isotope detection methods

3.6.1 Mass spectrometry

Mass spectrometry (MS) is a versatile analytical technique that is widely used in chemical identification and isotope detection studies. MS system includes an ionization chamber in which, compounds are ionized and directed to an electro-magnetic field, where ions are sorted based on their mass to charge ratio and finally detected by an ion detection system (Hoffmann 1996). MS system can be used in combination with an elemental analyzer (EA) in which samples are modified to CO_2 or N_2 that are then separated and directed to MS for carbon and/or nitrogen isotope analysis (Muccio and Jacksson 2009). This technique is referred to as elemental analysis-isotope ratio mass spectrometry (EA-IRMS). MS system can also be combined with gas chromatography (GC) or liquid chromatography (LC) columns to fractionate complex samples allowing separation and identification of higher number of compounds (Okazaki and Saito 2012).

In GC-MS, a carrier gas is used to carry metabolites along the GC capillary column. Therefore, metabolites should be vaporized in order to be transported through the column. Appropriate derivatization procedure is necessary to analyze many of the primary metabolites such as, sugars, amino acids and organic acids with polar functional groups by GC-MS. This facilitates the volatilization of these compounds which is critical to pass them through the gas phase of the capillary column (Okazaki and Saito 2012). In LC-MS though, there is no need for volatilization of compounds, therefore, is an ideal method for analysis of compounds with unstable structure and/or high vapor point (Okazaki and Saito 2012). In general, LC-MS method has some advantages over GC-MS such as, easier sample handling and shorter run time, however, in terms of maintenance, operation and availability of standard protocols and compound libraries GC-MS is more preferable (Ratcliffe and Sachar-hill 2006).

In this study, EA-IRMS was used to measure ^{13}C enrichment in developing wood soluble fraction (paper I) and in different developing wood cell wall fractions (paper II). GC-MS method was used to analyze the labeling pattern of sucrose, glucose, fructose and phenylalanine (paper II) as well as analysis of cell wall monosaccharides (paper I and paper II). LC-MS was used for sugar phosphates and UDP-glucose measurement (paper II).

3.6.2 Nucleic magnetic resonance

Nucleic magnetic resonance (NMR) method, exploits magnetic properties of certain atoms with net spin unequal to zero such as ^{1}H, ^{13}C, ^{15}N to resolve their molecular structure. In NMR, the atoms are placed in a magnetic field and

radiated with electromagnetic pulse of correct energy that excites the nuclei to higher energy state. The pulse should be then stopped to let the excited nuclei to relax back to the ground energy state losing energy that is detected by NMR (Edwards 2009). NMR is a powerful tool for characterization of chemical structure of substances. The method has a wide range of application in study areas such as, pharmaceutical research, proteomics and metabolomics (Yee et al., 2002; Ward et al., 2007; Holzgrabe 2011). In recent years there have been several studies characterizing cell wall polymers from several plant species using NMR that have broadened our knowledge of cell wall structure in further details (Hedenström et al., 2009; Dick-Perez et al., 2011; Mansfield et al., 2012).

In this study, we used 2D ^{13}C-^{1}H NMR method, providing the correlation between carbon atoms and their bound protons that have been used in many plant cell wall characterization studies (Ralph et al., 2004; Hedenström et al., 2009; Mansfield et al., 2012). Using this method enabled us to monitor the labeling pattern of cell wall residues in detail.

4 Results and discussion

4.1 Aspen SUCROSE TRANSPORTER 3 allocates carbon to wood fibers (paper I)

To study the role of active sucrose transport in provision of carbon for wood formation and development, I first looked at the transcript level of all *PttSUT*s along the wood developmental stages. Among all *PttSUT*s, *PttSUT3* was discovered to have highest transcript level in the cambial and secondary cell wall forming zones of developing wood (Fig. 10A; paper I, Fig. 1). Since both cambial cell division and secondary cell wall formation are highly metabolically active processes, and have a high demand for sucrose (carbon) import, a role was hypothesized for *PttSUT3* to mediate sucrose transport into the cells in these two developmental stages. PttSUT3 protein belongs to group 2 sucrose transporters according to Payyavula et al., (2011). Members of this group of SUTs have high affinity to sucrose compared to SUTs from other groups (Lalonde 2004). The most well characterized member of this group of SUTs is AtSUC2 that is responsible for phloem loading in Arabidopsis (Fig. 9) (Gottwald et al., 2000). PttSUT3 was shown to complement yeast sucrose transport deficient mutant (SUS7/ura3) (Payyavula et al., 2011). Transient expression of YFP:PttSUT3 in tobacco leaf localized the YFP signal to the plasma membrane (paper I, Fig.2).

In the next step, I decided to down-regulate *SUT3* transcription using RNAi method to study its role during secondary cell wall formation in wood. To avoid pleiotropic effects of *SUT3* transcript reduction in other parts of the plant, I used *GT43-B* promoter active during secondary cell wall formation in the wood (Ratke et al., 2014) to confine RNAi targeting of *SUT3* transcript to this region. Three lines with approximately 50% to 60% reduction in *SUT3* transcript level were selected for further characterization (Fig. 10B; paper I, Fig. 3). All *SUT3RNAi* lines showed a slight reduction in stem height and diameter as well as clear reduction in leaf size (paper I, Table I and Fig. 4).

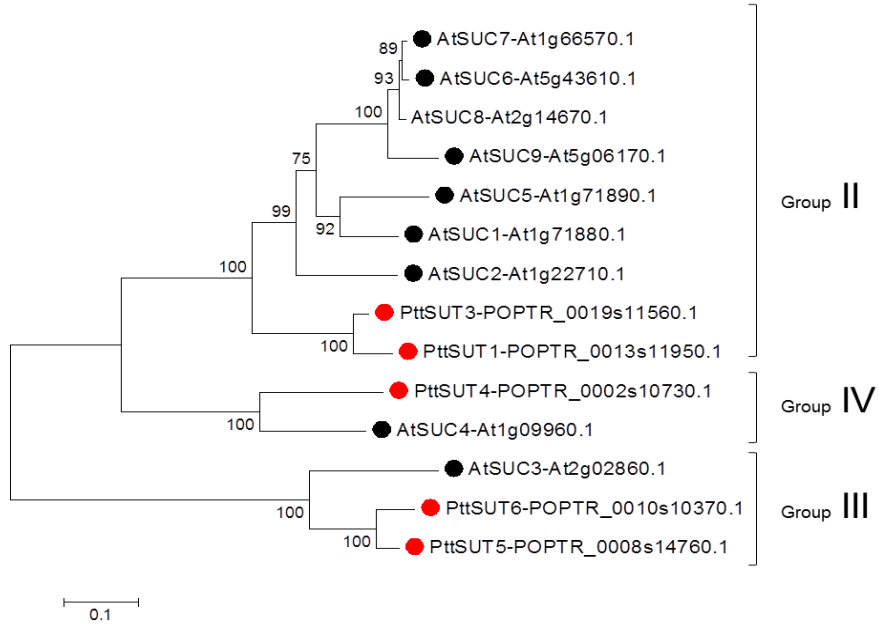


Figure 9. Neighbourhood-joining phylogenetic tree of *Arabidopsis* and *Populus* sucrose transporters. PttSUT3 as well as PttSUT1 are the closest homologs of group II AtSUTs.

Interestingly, mature wood cells in *SUT3RNAi* lines had thinner cell wall in comparison to the wild type trees with clear reduction in wood fiber cell wall area (Fig. 11A; paper I, Fig. 5). Consistent with this, wood bulk density was also reduced in *SUT3RNAi* lines (paper I, Table II). These results indicate that SUT3 has an important role in carbon allocation into wood fibers.

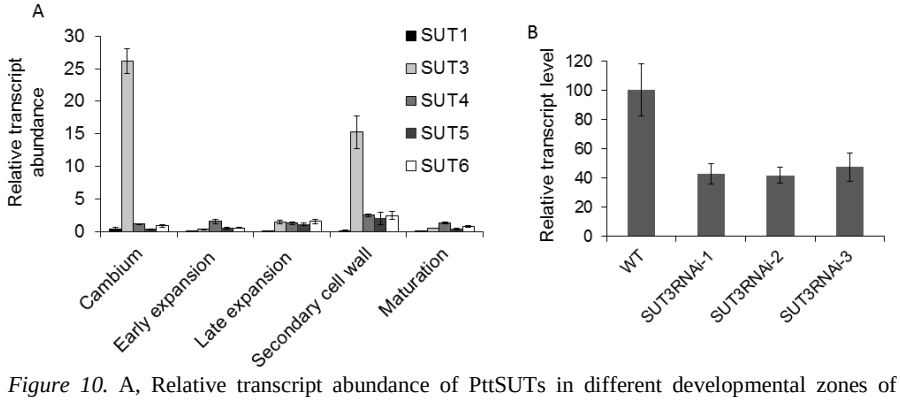


Figure 10. A, Relative transcript abundance of PttSUTs in different developmental zones of developing wood. Error bars represent standard error of mean (SEM) of three biological replicates. B, *PttSUT3* transcript level in developing wood of the WT and *SUT3RNAi* lines. Error bars represent SEM of four biological replicates.

Analysis of cell wall composition of mature wood in *SUT3RNAi* lines revealed that they have reduced carbohydrate to lignin ratio in comparison to the wild type (Fig. 11B; paper I, Fig. 7) suggesting that PttSUT3 function specifically favors carbon allocation to cell wall carbohydrates. In line with this, transcriptional analysis of the genes expressed at different wood developmental stages showed that cell wall carbohydrate genes peak at secondary cell wall formation zone similar to *PttSUT3*, whereas monolignol biosynthesis genes are also expressed later during maturation where *PttSUT3* is not highly expressed (Hertzberg et al., 2001).

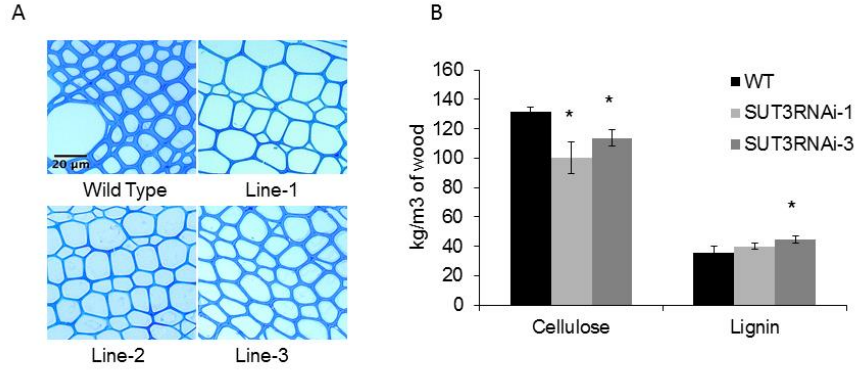


Figure 11. A, Light microscopy images of representative stem sections of WT and *SUT3RNAi* lines. B, Estimation of cellulose and lignin content per volume of wood. Error bars represent SEM of four biological replicates. Asterisks indicate *P* value comparison with WT. $*P < 0.05$ (Student's *t*-test).

Measurement of soluble sugar pools showed that sucrose level was not affected but sucrose to hexose ratio was increased in the developing wood of *SUT3RNAi* lines (Fig 12A; paper I, Fig. 8). Also, $^{13}CO_2$ labeling of *SUT3RNAi* lines resulted in higher ^{13}C accumulation in ethanol soluble fraction of developing wood in comparison to the wild type (Fig 12B; paper I, Fig. 9). Unchanged sucrose levels as well as higher ^{13}C accumulation in wood soluble fraction of RNAi lines imply that there is no impairment in the first step of sucrose transport into the developing wood that is sucrose transport from phloem into ray cells (Van bel 1990). Transport along the ray cells was also shown to be through the symplasm (Sauter and Kloth 1986) ruling out the possible role of PttSUT3 in ray to ray transport. Presence of a membrane transport step from ray cells out to the wood apoplasm in trees (Sauter et al., 1973; Sauter 1980; Sauter 1988) can suggest that *PttSUT3* mediates sucrose transport from wood apoplasm into the developing fibers and vessel elements. However, this still does not exclude the possibility for SUT3 to have other roles such as, sucrose retrieval from apoplasm into ray cells and sieve tube.

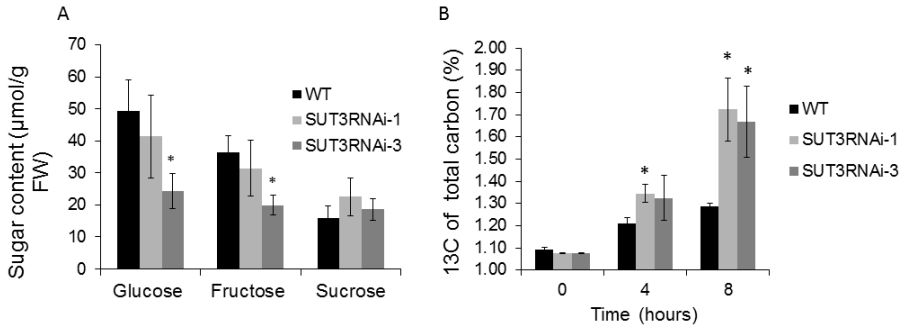


Figure 12. A, Analysis of sucrose, glucose and fructose content in the developing wood of WT and *SUT3RNAi* lines. Error bars represent SEM of four biological replicates. B, Accumulation of ^{13}C in the ethanol soluble fraction of developing wood in two-month-old trees labeled with $^{13}CO_2$. $^{13}CO_2$ was supplied for 4 hours and samples were harvested at 0, 4 and 8 hours after the start of the labeling. Error bars represent SEM of three biological replicates. Asterisks indicate *P* value comparison with WT. $*P < 0.05$ (Student's *t*-test).

Results from this study showed that an active sugar transporter (PttSUT3) is involved in carbon allocation to secondary cell walls of developing wood fibers.

4.2 Carbon-13 tracking after $^{13}CO_2$ supply revealed diurnal patterns of wood biosynthesis in aspen (paper II)

Based on evidence suggesting diurnal regulation of several transcripts encoding for enzymes involved in cell wall polymer biosynthesis in plants, one central question is whether carbon that has been transported to the developing wood is uniformly allocated into different cell wall polymers at a constant rate over the diurnal cycle. To address this question I labeled two month old hybrid aspen ((*Populus tremula* × *tremuloides*) trees with $^{13}CO_2$ for 4 hours followed by harvesting the trees after 0, 4, 9, 14, 19 and 24 hours (T0 to T24) from start of the labeling with the dark period occurring between T9 and T14. Then I tracked the ^{13}C incorporation into some of the intermediate metabolites in the pathway (paper II, Fig. 1) as well as the actual cell wall polymers.

For metabolite analysis, the labeling of sucrose pool was monitored in source leaves, phloem and developing wood as well as monitoring glucose, fructose, UDP glucose, hexose phosphates and phenylalanine labeling in developing wood. Sucrose pools in source leaves and phloem were similarly labeled peaking at T4 with approximately 30% of the total pool labeled decreasing to approximately 9% and 13% at T24 (Fig. 13A; paper II, Fig. 2). Sucrose pool of developing wood however was labeled with a delay peaking at T9 with approximately 25% of the total pool labeled decreasing down to nearly 10% of the pool at T24 (Fig. 13A; paper II, Fig. 3A). This delay in developing wood

sucrose labeling can be explained by the multi-step phloem to developing wood transport involving both symplasmic and apoplasmic steps slowing down the phloem to developing wood transport of sucrose (Van bel 1990). In the developing wood, glucose and fructose pools were labeled very slowly so that less than 7% of the total pool was labeled after T24 (Fig. 13B; paper II, Fig. 3 D and E). In contrast, hexose phosphates and UDP-glucose mimic sucrose labeling pattern in the developing wood (Fig. 13B; paper II, Fig. 3 B and C). These data suggest the presence of large pools of glucose and fructose that are hardly labeled but very small cytosolic pools that are rapidly phosphorylated to generate the hexose phosphates pool. In line with this, vacuolar glucose and fructose pools were shown to contribute to 65% and 88% of the total in Arabidopsis leaves respectively and similar to our results little ^{13}C was accumulated into glucose or fructose pools after $^{13}CO_2$ labelling (Szecowka et al., 2013).

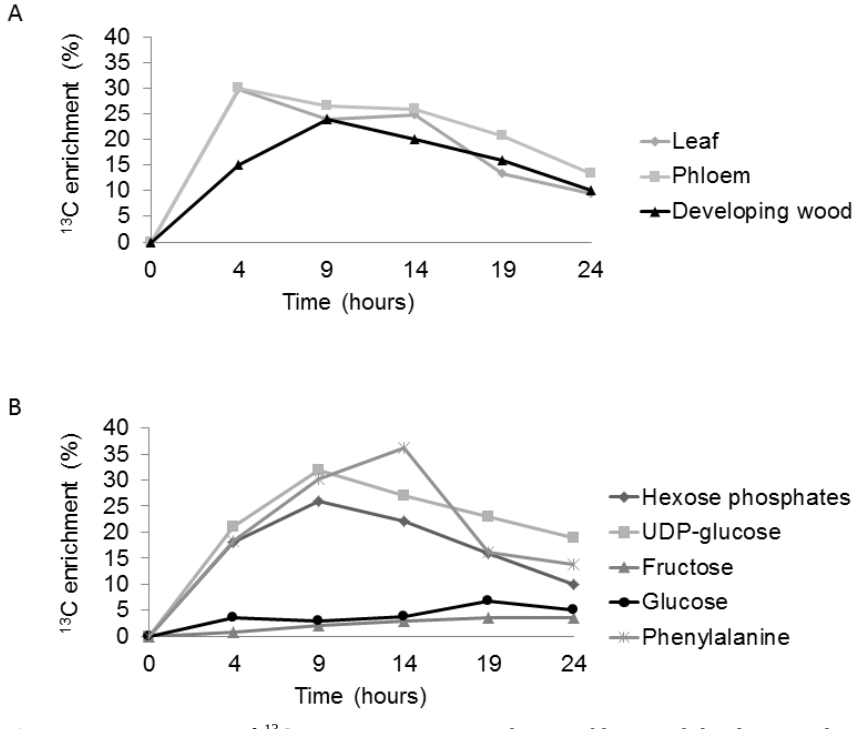


Figure 13. Incorporation of ^{13}C into sucrose in source leaves, phloem and developing xylem (A) and into hexose-phosphates, UDP-glucose, glucose, fructose and phenylalanine in developing wood (B). Dark period was from 9 to 14 hours from the start of the experiment.

Phenylalanine pool was also labelled differently than sucrose pool peaking at T14 with approximately 36% of the total pool labelled (Fig. 13B).

Phenylalanine biosynthesis was previously shown to be critical for lignin biosynthesis (Corea et al., 2012), therefore, the difference between UDP-glucose and phenylalanine labelling patterns can potentially cause differential carbon incorporation to lignin and cell wall polysaccharides.

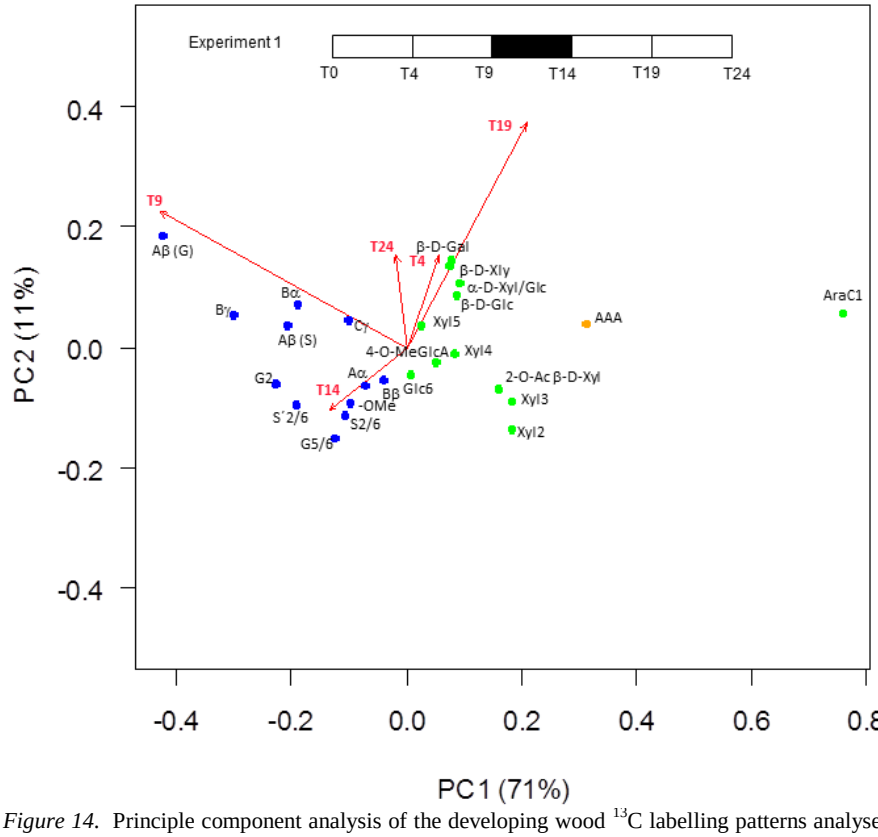


Figure 14. Principle component analysis of the developing wood ^{13}C labelling patterns analysed with 2D NMR for experiment 1 with dark period between 9 and 14 hours. PCA plot shows the distribution of the 2D NMR ^{13}C peak integrals for cell wall polysaccharides (green dots), lignin (blue dots) and aromatic amino acids (yellow dot). Red arrows (loadings) show the contribution of the time points to the observed variation between the NMR peaks. Data derived from the analysis of mean of four biological replicates. Aromatic amino acids (AAA), G-lignin (G5/6, G2), S-lignin (S'2/6, S2/6), Lignin linkage types (Bα, Aβ (S), Aβ (G), Aα, Cγ, Bγ, Bβ), lignin methoxy groups (-OMe), α-L-arabinose (α-L-Ara), β-D-galactose (β-D-Gal), Glucose (β -D-Glc, Glc6, α-D-Xyl/Glc?), Xylose (β -D-Xyl, 2-O-Ac β -D-Xyl, Xyl2, Xyl3, Xyl4, Xyl5, α-D-Xyl/Glc?), Glucuronic acid (4-0-MeGlcA).

To analyse the ^{13}C incorporation into different cell wall polymers, cell wall material from the developing wood was fractionated into cellulose, cell wall monosaccharides (mainly consisting of hemicelluloses) and lignin fractions. ^{13}C incorporation into each fraction was then measured by isotope analysis method. Results of these measurements showed that cellulosic fraction accumulates ^{13}C with the highest rate in comparison to the other two fractions

(paper II, Fig. 4B). Lignin fraction was labeled with a slightly lower rate than cellulose while cell wall monosaccharides fraction showed significantly lower rate of ^{13}C incorporation than cellulose and lignin fractions (paper II, Fig. 4B).

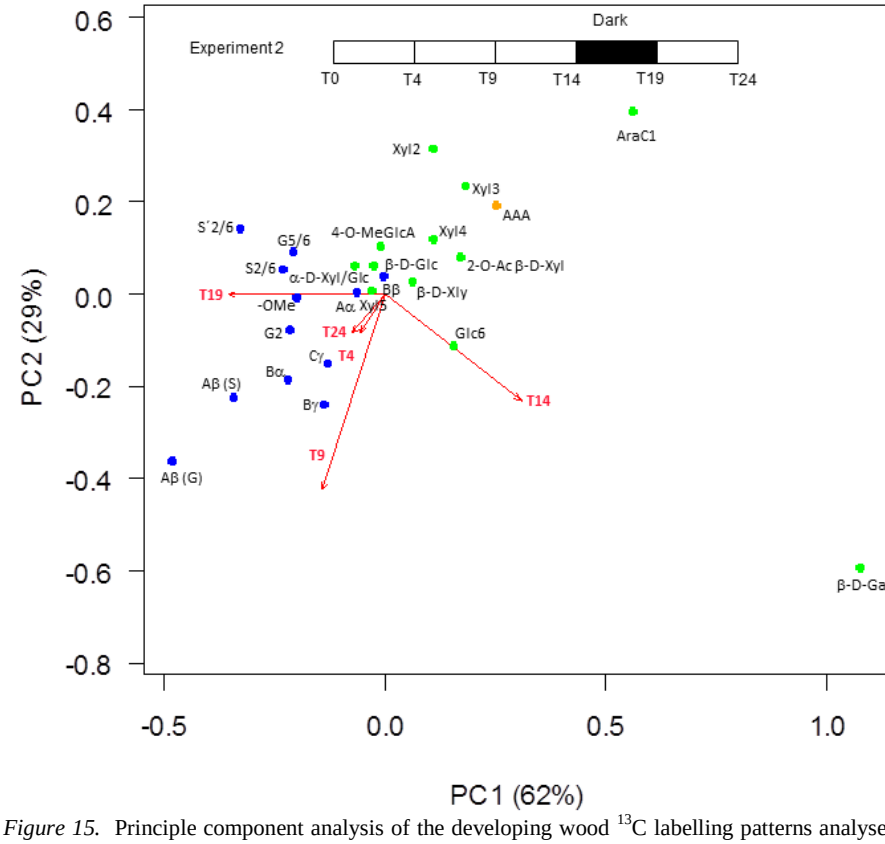


Figure 15. Principle component analysis of the developing wood ^{13}C labelling patterns analysed with 2D NMR for experiment 2 with dark period between 14 and 19 hours. PCA plot shows the distribution of the 2D NMR ^{13}C peak integrals for cell wall polysaccharides (green dots), lignin (blue dots) and aromatic amino acids (yellow dot). Red arrows (loadings) show the contribution of the time points to the observed variation between the NMR peaks. Data derived from the analysis of mean of four biological replicates. Aromatic amino acids (AAA), G-lignin (G5/6, G2), S-lignin (S'2/6, S2/6), Lignin linkage types (Bα, Aβ (S), Aβ (G), Aα, Cγ, Bγ, Bβ), lignin methoxy groups (-OMe), α-L-arabinose (α-L-Ara), β-D-galactose (β-D-Gal), Glucose (β -D-Glc, Glc6, α-D-Xyl/Glc?), Xylose (β -D-Xyl, 2-O-Ac β -D-Xyl, Xyl2, Xyl3, Xyl4, Xyl5, α-D-Xyl/Glc?), Glucuronic acid (4-0-MeGlcA).

Analysis of ^{13}C incorporation into different cell wall fractions only gives an understanding about how the polymers are labelled but not about their monomeric constituents especially in the case of lignin and hemicelluloses that are made of different monomers. To understand the cell wall deposition dynamics in further detail, ^{13}C labelling of different cell wall residues was

analysed using 2D-NMR. Peaks from 2D-NMR spectra of *Populus* wood cell wall have been well studied and assigned to different carbon atoms in each cell wall polymer (paper II, Fig. 5) (Ralph et al., 2004; Hedenström et al., 2009; Mansfield et al., 2013). A dataset was then generated using the NMR peaks from all the time points throughout the labeling experiment. Principle component analysis (PCA) was performed using the NMR dataset to find the correlation between the time points and the NMR peaks. PCA results showed a clear separation of lignin peaks from cell wall polysaccharide peaks with lignin peaks correlating positively with T9 and T14 and negatively with T19 (Fig. 14; paper II, Fig. 6). This was consistent with the different labelling patterns observed between UDP-glucose and phenylalanine. Peaks from S and G lignin were observed to correlate tightly with T14 which was the end of the dark period made us hypothesize that the dark period has an effect on the labelling pattern of cell wall polymers. To test this hypothesis, I performed another experiment under similar experimental conditions as the first experiment with only one difference that the trees were labelled 5 hours earlier during the day so that the dark period lies between T14 and T19 in the second experiment. Interestingly, PCA results from the second experiment showed S and G peaks correlating with T19 which is the end of the dark period in the new experiment (Fig 15; paper II, Fig. 7). This is while, the peaks from hemicelluloses, especially xylose peaks, correlated negatively with the end of the dark period in both experiments. NMR analysis in this study only reflects relative changes in the labelling pattern of cell wall polymers, therefore, it is not still clear whether the effect of the dark period favours lignin deposition or it inhibits cell wall polysaccharide deposition. But since lignin biosynthesis genes were previously reported to be up-regulated during the dark period (Harmer et al., 2000; Sharkhuu et al., 2014), therefore, it can be hypothesized that it is the lignin biosynthesis that is enhanced during the dark. According to these results, carbon incorporated differently into lignin and cell wall polysaccharides in the developing wood of hybrid aspen. Also, carbon incorporation into cell wall polymers was shown to be under diurnal influence.

4.3 Spatial analysis of primary metabolism in developing wood of aspen suggested a central role for transcriptional regulation in carbon allocation to wood. (paper III)

Wood developmental stages comprise cambial cell division, cell expansion, secondary cell wall deposition and maturation (cell death). Wood formation process is dependent upon sucrose transport and metabolism to provide the carbon for cellular metabolism and biosynthesis of secondary cell wall polymers. Expression of sucrose metabolism genes were shown to vary across

the wood developmental gradient (Hertzberg et al., 2001), which in turn can cause changes in cellular metabolism in different developmental stages. These expression data have been used for selection of candidate genes for reverse genetics and gene functional analysis in wood formation. However, whether the transcript data reflect status of protein and metabolite levels in the context of primary metabolism has remained to be addressed. To address this, we have measured the enzyme activity for 8 primary metabolism enzymes across the wood development (Table I, paper III, Fig. 1) followed by comparing them to their transcript abundance and corresponding metabolite levels.

Table I. List of carbon metabolism enzymes selected for enzyme activity measurement across wood developmental zones.

Enzyme	Reaction
Sucrose phosphate synthase (SPS)	UDP-glucose + D-Fructose 6-phosphate <=> UDP + Sucrose 6-phosphate
Sucrose synthase (SUS)	UDP-glucose + D-Fructose <=> UDP + Sucrose
UDP-glucose pyrophosphorylase (UGPase)	UTP + D-Glucose 1-phosphate <=> Diphosphate + UDP-glucose
Hexokinase (HXK)	ATP + D-Hexose <=> ADP + D-Hexose 6-phosphate
Fructokinase (FRK)	ATP + D-Fructose <=> ADP + D-Hexose 6-phosphate
Phosphoglucomutase (PGM)	D-Glucose 1-phosphate <=> D-Glucose 6-phosphate
Phosphoglucoisomerase (PGI)	D-Glucose 6-phosphate <=> D-Fructose 6-phosphate
Shikimate dehydrogenase (SHDH)	Shikimate + $NADP^+$ <=> 3-Dehydroshikimate + NADPH + H^+

For SPS, the enzyme activity was constant across the wood to the start of the maturation zone where the activity started to decline (paper III, Fig. 2A). Consistently, SPS transcripts also showed a constant expression over the wood developmental stages (paper III, Fig. 3A). SUS activity showed two peaks, one in the phloem and the other one during the secondary cell wall formation (paper III, Fig. 2B) correlating with SUS transcript expression along the developing wood (paper III, Fig. 3B). UGPase activity was high all the way from phloem to the expansion zone, increased remarkably during the secondary cell wall formation with a decrease at the onset of maturation (paper III, Fig 2C) correlating well with *UGPase* transcripts in the developing wood (paper III, Fig. 3C). HXK and FRK showed a very similar activity pattern peaking in cambium and early expansion zones, then decreasing during late expansion followed by a marked increase during the secondary cell wall formation and finally declining in the beginning of maturation (paper III, Fig. 2 D and E). For *FRK* one transcript was found to match the enzyme activity across the wood

development with no correlating HXK transcript (paper III, Fig. 3 D and E). PGM activity showed a peak in phloem and cambium, decreased during the expansion, an increase during the secondary cell wall formation and finally declining in the beginning of maturation (paper III, Fig. 2F) with two transcripts having similar patterns of transcript abundance (paper III, Fig. 3F). The PGI activity was high in phloem and cambium, and then stayed relatively similar across the developing wood followed by a decline in maturation zone (paper III, Fig. 2G) with one transcript having nearly similar abundance pattern (paper III, Fig. 3G). SHDH activity is a link between primary metabolism and phenylalanine biosynthesis the latter is the precursor for monolignol biosynthesis. SHDH activity had a small peak in the cambial region and then increased steadily towards the end of the secondary cell wall forming zone followed by a decrease in the beginning of maturation (paper III, Fig. 2H). One *SHDH* transcript was found with a tight correlation with the enzyme activity (paper III, Fig. 3H).

To find out whether the enzyme activities measured correlate with corresponding metabolite pools across the wood development, key metabolites of sugar primary metabolism (Sucrose, glucose, fructose, glucose-6-P, fructose-6-P, glucose-1-P and UDP-glucose) were analyzed. Sucrose level is high in the phloem, then drops down in cambium and remains constant during the expansion and secondary cell wall formation with only a little peak during the secondary cell wall formation and decreases towards the maturation zone (paper III, Fig. 4A). Both glucose and fructose levels increased from phloem to the middle of secondary cell wall forming zone and then started to decline in the maturation zone (paper III, Fig. 4 B and C). Hexose phosphates and UDP-glucose levels showed two peaks, one in cambium and the other one in secondary cell wall forming zone ((paper III, Fig. 5).

Based on these results, for all carbon metabolism enzymes investigated in this study, at least one transcript was found to correlate with the enzyme activity pattern. In most cases, activity of the enzymes and the level of their correlating transcripts peaked during secondary cell wall formation where, high carbon amounts incorporate into cell wall polymers. Supporting these results, reducing the activity of FRK and SUS through transcriptional modification in hybrid aspen impaired carbon allocation to cell wall polymers (Roach et al., 2012; Greber et al., 2014).

Activity patterns for carbon metabolism enzymes were also consistent with the level of their related metabolites (soluble sugars, sugar phosphates and UDP-glucose) along the wood developmental stages. This tight correlation between transcript, protein and metabolite levels for carbon primary metabolism

enzymes implies that transcriptional regulation plays an important role in carbon metabolism.

5 Conclusion and future perspectives

In most of the angiosperm trees, carbon is loaded into phloem symplasmically and in the form of sucrose. Sucrose is then transported through the phloem to sink tissues where it is needed as a carbon source for sink metabolism. Wood is an important sink in trees undergoing continuous biosynthesis and deposition of secondary cell walls during active growth. Sucrose transport into wood is therefore of a great importance in providing carbon for wood biosynthesis. In paper I, A sucrose transporter (SUT3) was shown to have a role in carbon supply for cell wall biosynthesis in wood. Now the question is where and how SUT3 plays its role. In the future it will be important to establish where SUT3 protein is localized in the developing wood and also investigate the role of other transporters, such as sucrose exporters and monosaccharide transporters, in carbon allocation to wood. Another important issue to be addressed is the description of the symplasmic path of assimilates transport in wood. This can clarify which steps are symplasmic and which have to be through apoplasm. Use of symplasmic tracers in wood can be a useful approach to resolve this issue.

In paper II, carbon-13 tracking of wood formation revealed differences between lignin and cell wall carbohydrate deposition patterns. Also, ^{13}C incorporation into cell wall polymers was shown to be influenced by diurnal cycle. These findings open a new window towards a new aspect of wood biology that is, the molecular basis for diurnal regulation of wood formation. To follow up, omics approach can be useful in understanding the diurnal regulation of wood cell wall biosynthesis machinery at transcriptome, proteome and metabolome levels along the wood development. Combining $^{13}CO_2$ labeling with omics approach followed by obtaining transcript, protein (or enzyme activity) and metabolite labeling data for all the enzymes of cell wall biosynthesis pathways and from primary cell wall, secondary cell wall and

maturation zones along the wood development would allow temporal and spatial understanding of the wood formation process.

Results from Paper III suggest that carbon metabolism enzymes in the developing wood are partly controlled at the transcriptional level. Therefore, discovering the basis for transcriptional regulation of carbon metabolism genes involved in cell wall precursor biosynthesis would deepen our understanding of carbon partitioning to wood cell wall polymer. Transcriptional and environmental factors described in section 1.9 can be suitable candidates to be investigated for their role in carbon partitioning to different cell wall polymers in wood.

References

Alonso, J. M., & Ecker, J. R. (2006). Moving forward in reverse: genetic technologies to enable genome-wide phenomic screens in Arabidopsis. *Nature Reviews Genetics*, *7*(7), 524-536.

Amor, Y., Haigler, C. H., Johnson, S., Wainscott, M., & Delmer, D. P. (1995). A membrane-associated form of sucrose synthase and its potential role in synthesis of cellulose and callose in plants. *Proceedings of the National Academy of Sciences*, *92*(20), 9353-9357.

Arioli, T., Peng, L., Betzner, A. S., Burn, J., Wittke, W., Herth, W., ... & Williamson, R. E. (1998). Molecular analysis of cellulose biosynthesis in Arabidopsis. *Science*, *279*(5351), 717-720.

Barker, L., Kühn, C., Weise, A., Schulz, A., Gebhardt, C., Hirner, B., ... & Frommer, W. B. (2000). SUT2, a putative sucrose sensor in sieve elements. *The Plant Cell Online*, *12*(7), 1153-1164.

Baroja-Fernández, E., Muñoz, F. J., Li, J., Bahaji, A., Almagro, G., Montero, M., ... & Pozueta-Romero, J. (2012). Sucrose synthase activity in the sus1/sus2/sus3/sus4 Arabidopsis mutant is sufficient to support normal cellulose and starch production. *Proceedings of the National Academy of Sciences*, *109*(1), 321-326.

Barratt, D. P., Derbyshire, P., Findlay, K., Pike, M., Wellner, N., Lunn, J., ... & Smith, A. M. (2009). Normal growth of Arabidopsis requires cytosolic invertase but not sucrose synthase. *Proceedings of the National Academy of Sciences*,*106*(31), 13124-13129.

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

Bernstein, E., Caudy, A. A., Hammond, S. M., & Hannon, G. J. (2001). Role for a bidentate ribonuclease in the initiation step of RNA interference. *Nature*,*409*(6818), 363-366.

Betz, G. A., Knappe, C., Lapierre, C., Olbrich, M., Welzl, G., Langebartels, C., ... & Ernst, D. (2009). Ozone affects shikimate pathway transcripts and monomeric lignin composition in European beech (Fagus sylvatica L.). *European Journal of Forest Research*, *128*(2), 109-116.

Bhargava, A., Mansfield, S. D., Hall, H. C., Douglas, C. J., & Ellis, B. E. (2010). MYB75 functions in regulation of secondary cell wall formation in the Arabidopsis inflorescence stem. *Plant Physiology*, *154*(3), 1428-1438.

Bocock, P. N., Morse, A. M., Dervinis, C., & Davis, J. M. (2008). Evolution and diversity of invertase genes in Populus trichocarpa. *Planta*, *227*(3), 565-576.

Boorer, K. J., Loo, D. D., Frommer, W. B., & Wright, E. M. (1996). Transport mechanism of the cloned potato H+/sucrose cotransporter StSUT1. *Journal of Biological Chemistry*, *271*(41), 25139-25144.

Boerjan, W., Ralph, J., & Baucher, M. (2003). Lignin biosynthesis. *Annual review of plant biology*, *54*(1), 519-546.

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

Calvin, M., & Benson, A. A. (1949). The path of carbon in photosynthesis IV: the identity and sequence of the intermediates in sucrose synthesis. *Science*,*109*(2824), 140-142.

Carpita, N. C., & Delmer, D. P. (1981). Concentration and metabolic turnover of UDP-glucose in developing cotton fibers. *Journal of Biological Chemistry*,*256*(1), 308-315.

Caspar, T., Huber, S. C., & Somerville, C. (1985). Alterations in growth, photosynthesis, and respiration in a starchless mutant of Arabidopsis thaliana (L.) deficient in chloroplast phosphoglucomutase activity. *Plant Physiology*,*79*(1), 11-17.

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

Chen, S., Hajirezaei, M., Peisker, M., Tschiersch, H., Sonnewald, U., & Börnke, F. (2005). Decreased sucrose-6-phosphate phosphatase level in transgenic tobacco inhibits photosynthesis, alters carbohydrate partitioning, and reduces growth. *Planta*, *221*(4), 479-492.

Chen, X., Alonso, A. P., & Shachar-Hill, Y. (2013). Dynamic metabolic flux analysis of plant cell wall synthesis. *Metabolic engineering*, *18*, 78-85.

Chen, Y., Zhang, X., Wu, W., Chen, Z., Gu, H., & Qu, L. J. (2006). Overexpression of the Wounding-Responsive Gene AtMYB15 Activates the Shikimate Pathway in Arabidopsis. *Journal of Integrative Plant Biology*, *48*(9), 1084-1095.

Cho, M. H., Lim, H., Shin, D. H., Jeon, J. S., Bhoo, S. H., Park, Y. I., & Hahn, T. R. (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.

Ciereszko, I., Johansson, H., & Kleczkowski, L. (2001). Sucrose and light regulation of a cold-inducible UDP-glucose pyrophosphorylase gene via a hexokinase-independent and abscisic acid-insensitive pathway in Arabidopsis. *Biochem. J*, *354*, 67-72.

Coleman, H. D., Canam, T., Kang, K. Y., Ellis, D. D., & Mansfield, S. D. (2007). Over-expression of UDP-glucose pyrophosphorylase in hybrid poplar

affects carbon allocation. *Journal of experimental botany*, *58*(15-16), 4257-4268.

Coleman, H. D., Yan, J., & Mansfield, S. D. (2009). Sucrose synthase affects carbon partitioning to increase cellulose production and altered cell wall ultrastructure. *Proceedings of the National Academy of Sciences*, *106*(31), 13118-13123.

Corea, O. R., Ki, C., Cardenas, C. L., Kim, S. J., Brewer, S. E., Patten, A. M., ... & Lewis, N. G. (2012). Arogenate dehydratase isoenzymes profoundly and differentially modulate carbon flux into lignins. *Journal of Biological Chemistry*,*287*(14), 11446-11459.

Delmer, D. P. (1972). The purification and properties of sucrose synthetase from etiolated Phaseolus aureus seedlings. *Journal of Biological Chemistry*,*247*(12), 3822-3828.

Delmer, D. P., & Haigler, C. H. (2002). The regulation of metabolic flux to cellulose, a major sink for carbon in plants. *Metabolic engineering*, *4*(1), 22-28.

Dick-Pérez, M., Zhang, Y., Hayes, J., Salazar, A., Zabotina, O. A., & Hong, M. (2011). Structure and interactions of plant cell-wall polysaccharides by two-and three-dimensional magic-angle-spinning solid-state NMR. *Biochemistry*, *50*(6), 989-1000.

Edwards, J. C. (2009). Principles of NMR. *Process NMR Associates LLC, 87A Sand Pit Rd, Danbury CT*, *6810*.

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

Fell, D. A. (2005). Enzymes, metabolites and fluxes. *Journal of experimental botany*, *56*(410), 267-272.

Fengel, D., & Wegener, G. (Eds.). (1983). *Wood: chemistry, ultrastructure, reactions*. Walter de Gruyter.

Fernie, A. R., Tauberger, E., Lytovchenko, A., Roessner, U., Willmitzer, L., & Trethewey, R. N. (2002). Antisense repression of cytosolic phosphoglucomutase in potato (Solanum tuberosum) results in severe growth retardation, reduction in tuber number and altered carbon metabolism. *Planta*,*214*(4), 510-520.

Foster, C. E., Martin, T. M., & Pauly, M. (2010). Comprehensive compositional analysis of plant cell walls (lignocellulosic biomass) part II: carbohydrates.*Journal of visualized experiments: JoVE*, (37).

Fox, A., Morgan, S. L., & Gilbart, J. (1989). Preparation of alditol acetates and their analysis by gas chromatography (GC) and mass spectrometry (MS). *Analysis of Carbohydrates by GLC and MS*, 87-117.

Fu, Q., Cheng, L., Guo, Y., & Turgeon, R. (2011). Phloem loading strategies and water relations in trees and herbaceous plants. *Plant Physiology*, *157*(3), 1518-1527.

Geigenberger, P., & Stitt, M. (1993). Sucrose synthase catalyses a readily reversible reaction in vivo in developing potato tubers and other plant tissues. *Planta*, *189*(3), 329-339.

Gerber, L., Zhang, B., Roach, M., Rende, U., Gorzsás, A., Kumar, M., ... & Sundberg, B. (2014). Deficient sucrose synthase activity in developing wood does not specifically affect cellulose biosynthesis, but causes an overall decrease in cell wall polymers. *New Phytologist*, *203*(4), 1220-1230.

Goicoechea, M., Lacombe, E., Legay, S., Mihaljevic, S., Rech, P., Jauneau, A., ... & Grima-Pettenati, J. (2005). EgMYB2, a new transcriptional activator from Eucalyptus xylem, regulates secondary cell wall formation and lignin biosynthesis. *The Plant Journal*, *43*(4), 553-567.

Gorshkova, T. A., Chemikosova, S. B., Lozovaya, V. V., & Carpita, N. C. (1997). Turnover of galactans and other cell wall polysaccharides during development of flax plants. *Plant physiology*, *114*(2), 723-729.

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

Haake, V., Zrenner, R., Sonnewald, U., & Stitt, M. (1998). A moderate decrease of plastid aldolase activity inhibits photosynthesis, alters the levels of sugars and starch, and inhibits growth of potato plants. *The Plant Journal*, *14*(2), 147-157.

Halpin, C., Knight, M. E., Foxon, G. A., Campbell, M. M., Boudet, A. M., Boon, J. J., ... & Schuch, W. (1994). Manipulation of lignin quality by downregulation of cinnamyl alcohol dehydrogenase. *The Plant Journal*, *6*(3), 339-350.

Handford, M., Rodriguez-Furlán, C., & Orellana, A. (2006). Nucleotide-sugar transporters: structure, function and roles in vivo. *Brazilian journal of medical and biological research*, *39*(9), 1149-1158.

Hannon, G. J. (2002). RNA interference. *Nature*, *418*(6894), 244-251.

Harmer, S. L., Hogenesch, J. B., Straume, M., Chang, H. S., Han, B., Zhu, T., ... & Kay, S. A. (2000). Orchestrated transcription of key pathways in Arabidopsis by the circadian clock. *Science*, *290*(5499), 2110-2113.

Harper, A. D., & Bar-Peled, M. (2002). Biosynthesis of UDP-xylose. Cloning and characterization of a novel Arabidopsis gene family, UXS, encoding soluble and putative membrane-bound UDP-glucuronic acid decarboxylase isoforms. *Plant physiology*, *130*(4), 2188-2198.

Hedenström, M., Wiklund-Lindström, S., Öman, T., Lu, F., Gerber, L., Schatz, P., ... & Ralph, J. (2009). Identification of lignin and polysaccharide modifications in Populus wood by chemometric analysis of 2D NMR spectra from dissolved cell walls. *Molecular plant*, *2*(5), 933-942.

Herrmann, K. M., & Weaver, L. M. (1999). The shikimate pathway. *Annual review of plant biology*, *50*(1), 473-503.

Hertzberg, M., Aspeborg, H., Schrader, J., Andersson, A., Erlandsson, R., Blomqvist, K., ... & Sandberg, G. (2001). A transcriptional roadmap to wood formation. *Proceedings of the National Academy of Sciences*, *98*(25), 14732-14737.

Hoffmann, E. (1996). *Mass spectrometry*. John Wiley & Sons, Inc..

Holzgrabe, U. (2011). *NMR spectroscopy in pharmaceutical analysis*. I. Wawer, & B. Diehl (Eds.). Elsevier.

Jansson, S., & Douglas, C. J. (2007). Populus: a model system for plant biology. *Annu. Rev. Plant Biol.*, *58*, 435-458

Jones, L., Ennos, A. R., & Turner, S. R. (2001). Cloning and characterization of irregular xylem4 (irx4): a severely lignin-deficient mutant of Arabidopsis. *The Plant Journal*, *26*(2), 205-216.

Joung, J. K., & Sander, J. D. (2012). TALENs: a widely applicable technology for targeted genome editing. *Nature reviews Molecular cell biology*, *14*(1), 49-55.

Kagawa, A., Sugimoto, A., & Maximov, T. C. (2006). $^{13}CO_2$ pulse-labelling of photoassimilates reveals carbon allocation within and between tree rings. *Plant, Cell & Environment*, *29*(8), 1571-1584.

Kenrick, P., & Crane, P. R. (1997). The origin and early evolution of plants on land. *Nature*, *389*(6646), 33-39.

Kleczkowski, L. A., Geisler, M., Ciereszko, I., & Johansson, H. (2004). UDP-glucose pyrophosphorylase. An old protein with new tricks. *Plant Physiology*,*134*(3), 912-918.

Kleczkowski, L. A., Decker, D., & Wilczynska, M. (2011). UDP-sugar pyrophosphorylase: a new old mechanism for sugar activation. *Plant physiology*, *156*(1), 3-10.

Koch, K. E., Nolte, K. D., Duke, E. R., McCarty, D. R., & Avigne, W. T. (1992). Sugar levels modulate differential expression of maize sucrose synthase genes. *The Plant Cell Online*, *4*(1), 59-69.

Koßmann, J., Sonnewald, U., & Willmitzer, L. (1994). Reduction of the chloroplastic fructose-1, 6-bisphosphatase in transgenic potato plants impairs photosynthesis and plant growth. *The Plant Journal*, *6*(5), 637-650.

Lacombe, E., Hawkins, S., Doorsselaere, J., Piquemal, J., Goffner, D., Poeydomenge, O., ... & Grima-Pettenati, J. (1997). Cinnamoyl CoA reductase, the first committed enzyme of the lignin branch biosynthetic pathway: cloning, expression and phylogenetic relationships. *The Plant Journal*, *11*(3), 429-441.

Lalonde, S., Wipf, D., & Frommer, W. B. (2004). Transport mechanisms for organic forms of carbon and nitrogen between source and sink. *Annu. Rev. Plant Biol.*, *55*, 341-372.

Lapierre, C., Pollet, B., Petit-Conil, M., Toval, G., Romero, J., Pilate, G., ... & Jouanin, L. (1999). Structural alterations of lignins in transgenic poplars with depressed cinnamyl alcohol dehydrogenase or caffeic acidO-methyltransferase activity have an opposite impact on the efficiency of industrial kraft pulping. *Plant Physiology*, *119*(1), 153-164.

Lee, D. R. (1981). Synchronous pressure-potential changes in the phloem of Fraxinus americana L. *Planta*, *151*(4), 304-308.

Lerouxel, O., Cavalier, D. M., Liepman, A. H., & Keegstra, K. (2006). Biosynthesis of plant cell wall polysaccharides—a complex process. *Current opinion in plant biology*, *9*(6), 621-630.

Litton, C. M., Raich, J. W., & Ryan, M. G. (2007). Carbon allocation in forest ecosystems. *Global Change Biology*, *13*(10), 2089-2109.

Mansfield, S. D., Kim, H., Lu, F., & Ralph, J. (2012). Whole plant cell wall characterization using solution-state 2D NMR. *Nature protocols*, *7*(9), 1579-1589.

Marita, J. M., Ralph, J., Hatfield, R. D., & Chapple, C. (1999). NMR characterization of lignins in Arabidopsis altered in the activity of ferulate 5-hydroxylase. *Proceedings of the National Academy of Sciences*, *96*(22), 12328-12332.

Mellerowicz, E. J., Baucher, M., Sundberg, B., & Boerjan, W. (2001). Unravelling cell wall formation in the woody dicot stem. In *Plant Cell Walls* (pp. 239-274). Springer Netherlands.

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

Miziorko, H. M., & Lorimer, G. H. (1983). Ribulose-1, 5-bisphosphate carboxylase oxygenase. *Annual review of biochemistry*, *52*(1), 507-535.

Muccio, Z., & Jackson, G. P. (2009). Isotope ratio mass spectrometry. *Analyst*, *134*(2), 213-222.

Münch E. (1927) Versuche über den Saftkreislauf. *Ber Dtsch Bot Ges 45*: 340–356

Neuhaus, H. E., Kruckeberg, A. L., Feil, R., & Stitt, M. (1989). Reduced-activity mutants of phosphoglucose isomerase in the cytosol and chloroplast of Clarkia xantiana. *Planta*, *178*(1), 110-122.

Niittylä, T., Messerli, G., Trevisan, M., Chen, J., Smith, A. M., & Zeeman, S. C. (2004). A previously unknown maltose transporter essential for starch degradation in leaves. *Science*, *303*(5654), 87-89.

Nilsson, O., Aldén, T., Sitbon, F., Little, C. A., Chalupa, V., Sandberg, G., & Olsson, O. (1992). Spatial pattern of cauliflower mosaic virus 35S promoter-luciferase expression in transgenic hybrid aspen trees monitored by enzymatic assay and non-destructive imaging. *Transgenic research*, *1*(5), 209-220.

Nykänen, A., Haley, B., & Zamore, P. D. (2001). ATP requirements and small interfering RNA structure in the RNA interference pathway. *Cell*, *107*(3), 309-321.

Öhman, D., Demedts, B., Kumar, M., Gerber, L., Gorzsás, A., Goeminne, G., ... & Sundberg, B. (2013). MYB103 is required for FERULATE-5-HYDROXYLASE expression and syringyl lignin biosynthesis in Arabidopsis stems. *The Plant Journal*, *73*(1), 63-76.

Okazaki, Y., & Saito, K. (2012). Recent advances of metabolomics in plant biotechnology. *Plant biotechnology reports*, *6*(1), 1-15.

Payyavula, R. S., Tay, K. H., Tsai, C. J., & Harding, S. A. (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.

Plomion, C., Leprovost, G., & Stokes, A. (2001). Wood formation in trees. *Plant Physiology*, *127*, 1513–1523.

Ralph, J., MacKay, J. J., Hatfield, R. D., O'Malley, D. M., Whetten, R. W., & Sederoff, R. R. (1997). Abnormal lignin in a loblolly pine mutant. *Science*,*277*(5323), 235-239.

Ralph, S. A., Ralph, J., Landucci, L. L., & Landucci, L. L. (2004). NMR database of lignin and cell wall model compounds. *US Forest Prod. Lab., Madison, WI (http://ars. usda. gov/Services/docs. htm*.

Ratcliffe, R. G., & Shachar-Hill, Y. (2006). Measuring multiple fluxes through plant metabolic networks. *The Plant Journal*, *45*(4), 490-511.

Ratke, C., Pawar, P. M. A., Balasubramanian, V. K., Naumann, M., Duncranz, M. L., Derba-Maceluch, M., ... & Mellerowicz, E. J. (2014). Populus GT43 family members group into distinct sets required for primary and secondary wall xylan biosynthesis and include useful promoters for wood modification. *Plant biotechnology journal*.

Rennie, E. A., & Turgeon, R. (2009). A comprehensive picture of phloem loading strategies. *Proceedings of the National Academy of Sciences*, *106*(33), 14162-14167.

Riesmeier, J. W., Flügge, U. I., Schulz, B., Heineke, D., Heldt, H. W., Willmitzer, L., & Frommer, W. B. (1993). Antisense repression of the chloroplast triose phosphate translocator affects carbon partitioning in transgenic potato plants. *Proceedings of the National Academy of Sciences*,*90*(13), 6160-6164.

Roach, M., Gerber, L., Sandquist, D., Gorzsás, A., Hedenström, M., Kumar, M., ... & Niittylä, T. (2012). Fructokinase is required for carbon partitioning to cellulose in aspen wood. *The Plant Journal*, *70*(6), 967-977.

Rogers, L. A., Dubos, C., Cullis, I. F., Surman, C., Poole, M., Willment, J., ... & Campbell, M. M. (2005). Light, the circadian clock, and sugar perception in the control of lignin biosynthesis. *Journal of Experimental Botany*, *56*(416), 1651-1663.

Ruan, Y. L., Llewellyn, D. J., & Furbank, R. T. (2003). Suppression of sucrose synthase gene expression represses cotton fiber cell initiation, elongation, and seed development. *The Plant Cell Online*, *15*(4), 952-964.

Russin, W. A., & Evert, R. F. (1985). Studies on the leaf of Populus deltoides (Salicaceae): ultrastructure, plasmodesmatal frequency, and solute concentrations. *American journal of botany*, 1232-1247.

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

Sauter, J. J., Iten, W., & Zimmermann, M. H. (1973). Studies on the release of sugar into the vessels of sugar maple (Acer saccharum). *Canadian Journal of Botany*, *51*(1), 1-8.

Sauter, J. J. (1980). The Strasburger Cells—Equivalents of Compagnion Cells. *Berichte der Deutschen Botanischen Gesellschaft*, *93*(1), 29-42.

Sauter, J. J. (1980). Seasonal Variation of Sucrose Content in the Xylem Sap of< i> Salix</i>. *Zeitschrift für Pflanzenphysiologie*, *98*(5), 377-391.

Sauter, J. J., & Kloth, S. (1986). Plasmodesmatal frequency and radial translocation rates in ray cells of poplar (Populus x canadensis Moench 'robusta'). *Planta*, *168*(3), 377-380.

Sauter, J. J. (1988). Seasonal changes in the efflux of sugars from parenchyma cells into the apoplast in poplar stems (Populus× canademis "robusta"). *Trees*, *2*(4), 242-249.

Schleucher, J., Vanderveer, P. J., & Sharkey, T. D. (1998). Export of carbon from chloroplasts at night. *Plant Physiology*, *118*(4), 1439-1445.

Schneider, A., Häusler, R. E., Kolukisaoglu, Ü., Kunze, R., Van Der Graaff, E., Schwacke, R., ... & Flügge, U. I. (2002). An Arabidopsis thaliana knock-out mutant of the chloroplast triose phosphate/phosphate translocator is severely compromised only when starch synthesis, but not starch mobilisation is abolished. *The Plant Journal*, *32*(5), 685-699.

Scofield, G. N., Hirose, T., Aoki, N., & Furbank, R. T. (2007). Involvement of the sucrose transporter, OsSUT1, in the long-distance pathway for assimilate transport in rice. *Journal of Experimental Botany*, *58*(12), 3155-3169.

Scott Jr, T. A., & Melvin, E. H. (1953). Determination of dextran with anthrone.*Analytical Chemistry*, *25*(11), 1656-1661.

Seifert, G. J. (2004). Nucleotide sugar interconversions and cell wall biosynthesis: how to bring the inside to the outside. *Current opinion in plant biology*, *7*(3), 277-284.

Sewalt, V. J., Ni, W., Blount, J. W., Jung, H. G., Masoud, S. A., Howles, P. A., ... & Dixon, R. A. (1997). Reduced lignin content and altered lignin composition in transgenic tobacco down-regulated in expression of L-phenylalanine ammonia-lyase or cinnamate 4-hydroxylase. *Plant Physiology*,*115*(1), 41-50.

Sharkhuu, A., Narasimhan, M. L., Merzaban, J. S., Bressan, R. A., Weller, S., & Gehring, C. (2014). A red and far-red light receptor mutation confers resistance to the herbicide glyphosate. *The Plant Journal*.

Slewinski, T. L., Meeley, R., & Braun, D. M. (2009). Sucrose transporter1 functions in phloem loading in maize leaves. *Journal of Experimental Botany*,*60*(3), 881-892.

Sokołowska, K., & Zagórska-Marek, B. (2012). Symplasmic, long-distance transport in xylem and cambial regions in branches of Acer pseudoplatanus (Aceraceae) and Populus tremula × P. tremuloides (Salicaceae). *American journal of botany*, *99*(11), 1745-1755.

Solomon, O. L., Berger, D. K., & Myburg, A. A. (2010). Diurnal and circadian patterns of gene expression in the developing xylem of< i> Eucalyptus</i> trees. *South African Journal of Botany*, *76*(3), 425-439.

Stadler, R., & Sauer, N. (1996). The Arabidopsis thaliana AtSUC2 gene is specifically expressed in companion cells. *Botanica Acta*, *109*(4), 299-306.

Sweeley, C. C., Bentley, R., Makita, M., & Wells, W. W. (1963). Gas-liquid chromatography of trimethylsilyl derivatives of sugars and related substances. *Journal of the American Chemical Society*, *85*(16), 2497-2507.

Szecowka, M., Heise, R., Tohge, T., Nunes-Nesi, A., Vosloh, D., Huege, J., ... & Arrivault, S. (2013). Metabolic fluxes in an illuminated Arabidopsis rosette. *The Plant Cell Online*, *25*(2), 694-714.

Tauberger, E., Fernie, A. R., Emmermann, M., Renz, A., Kossmann, J., Willmitzer, L., & Trethewey, R. N. (2000). Antisense inhibition of plastidial phosphoglucomutase provides compelling evidence that potato tuber amyloplasts import carbon from the cytosol in the form of glucose-6-phosphate. *The Plant Journal*, *23*(1), 43-53.

Taylor, N. G. (2008). Cellulose biosynthesis and deposition in higher plants. *New Phytologist*, *178*(2), 239-252.

Tenhaken, R., & Thulke, O. (1996). Cloning of an enzyme that synthesizes a key nucleotide-sugar precursor of hemicellulose biosynthesis from soybean: UDP-glucose dehydrogenase. *Plant Physiology*, *112*(3), 1127-1134.

Thompson, M. V. (2006). Phloem: the long and the short of it. *Trends in plant science*, *11*(1), 26-32.

Turgeon, R. (1996). Phloem loading and plasmodesmata. *Trends in Plant Science*, *1*(12), 418-423.

Turgeon, R. (2010). The puzzle of phloem pressure. *Plant physiology*, *154*(2), 578-581.

Tuskan, G. A., Difazio, S., Jansson, S., Bohlmann, J., Grigoriev, I., Hellsten, U., ... & Henrissat, B. (2006). The genome of black cottonwood, Populus trichocarpa (Torr. & Gray). *Science*, *313*(5793), 1596-1604.

Updegraff, D. M. (1969). Semimicro determination of cellulose inbiological materials. *Analytical biochemistry*, *32*(3), 420-424.

Van Bel, A. J. E. (1990). Xylem-phloem exchange via the rays: The undervalued route of transport. *Journal of Experimental Botany*, *41*, 631–644.

Vanholme, R., Demedts, B., Morreel, K., Ralph, J., & Boerjan, W. (2010). Lignin biosynthesis and structure. *Plant Physiology*, *153*(3), 895-905.

Ward, J. L., Baker, J. M., & Beale, M. H. (2007). Recent applications of NMR spectroscopy in plant metabolomics. *FEBS Journal*, *274*(5), 1126-1131.

Weise, S. E., Weber, A. P., & Sharkey, T. D. (2004). Maltose is the major form of carbon exported from the chloroplast at night. *Planta*, *218*(3), 474-482.

Worrell, A. C., Bruneau, J. M., Summerfelt, K., Boersig, M., & Voelker, T. A. (1991). Expression of a maize sucrose phosphate synthase in tomato alters leaf carbohydrate partitioning. *The Plant Cell Online*, *3*(10), 1121-1130.

Wright, J. P., & Fisher, D. B. (1980). Direct measurement of sieve tube turgor pressure using severed aphid stylets. *Plant physiology*, *65*(6), 1133-1135.

Yee, A., Chang, X., Pineda-Lucena, A., Wu, B., Semesi, A., Le, B., ... & Arrowsmith, C. H. (2002). An NMR approach to structural proteomics. *Proceedings of the National Academy of Sciences*, *99*(4), 1825-1830.

Yoo, H., Widhalm, J. R., Qian, Y., Maeda, H., Cooper, B. R., Jannasch, A. S., ... & Dudareva, N. (2013). An alternative pathway contributes to phenylalanine biosynthesis in plants via a cytosolic tyrosine: phenylpyruvate aminotransferase. *Nature communications*, *4*.

Yu, T. S., Lue, W. L., Wang, S. M., & Chen, J. (2000). Mutation of Arabidopsis plastid phosphoglucose isomerase affects leaf starch synthesis and floral initiation. *Plant Physiology*, *123*(1), 319-326.

Zimmermann, M. H. (1957). Translocation of Organic Substances in Trees. II. On the Translocation Mechanism in the Phloem of White Ash (Fraxinus Americana L.). *Plant physiology*, *32*(5), 399.

Zhang, C., Han, L., Slewinski, T. L., Sun, J., Zhang, J., Wang, Z. Y., & Turgeon, R. (2014). Symplastic Phloem Loading in Poplar. *Plant physiology*,*166*(1), 306-313.

Zhao, R., Dielen, V., Kinet, J. M., & Boutry, M. (2000). Cosuppression of a plasma membrane H+-ATPase isoform impairs sucrose translocation, stomatal

opening, plant growth, and male fertility. *The Plant Cell Online*, *12*(4), 535-546.

Zhong, R., & Ye, Z. H. (2007). Regulation of cell wall biosynthesis. *Current opinion in plant biology*, *10*(6), 564-572.

Zhou, J. J., Theodoulou, F., Sauer, N., Sanders, D., & Miller, A. J. (1997). A kinetic model with ordered cytoplasmic dissociation for SUC1, an Arabidopsis H+/sucrose cotransporter expressed in Xenopus oocytes. *The Journal of membrane biology*, *159*(2), 113-125.

Zrenner, R., Krause, K. P., Apel, P., & Sonnewald, U. (1996). Reduction of the cytosolic fructose-1, 6-bisphosphatase in transgenic potato plants limits photosynthetic sucrose biosynthesis with no impact on plant growth and tuber yield. *The Plant Journal*, *9*(5), 671-681.

Acknowledgement

First of all I want to express my deepest gratitude to my supervisor Dr. Totte Niittylä for all his advises, his trust and patience on me, for always being available for my questions and for giving me this opportunity to carry out my research in his lab.

I also want to show my appreciation to Totte's lab members, Weiya, Melissa Titti, Umut, Wei and Louise, Gaia and Tina for all the helpful support and all I learned from them.

Thanks to professor Björn Sundberg and all the members of cell wall group for providing me a very friendly atmosphere and for helping me whenever I asked them for help.

Great thanks to Hannale and all Bioimprove members and graduate students for providing the very wonderful educational and research training environment during my PhD.

Thanks to Mattias and Andras for their support and time spent on NMR and FTIR analysis as well as very helpful discussions.

Pernilla, your contribution to ^{13}C manuscript was of a great value. Thanks for everything.

Thank to Peter, Junko and Lorenz in the cell wall analysis lab for their helpful advices and technical support on wood chemical analysis.

Christine and Veronica, I really appreciated your help and support for wood promoter study and plant transformation respectively.

Thanks to Benjamin for kind donation of wood viability staining image.

Thanks to Kjell and Lenor for their great support on microscopy.

More thanks to Kjell for preparation of wood developmental sections.

Ewa, thanks to all the help and support I have received from you since I started at UPSC.

Stephanie, Thanks for your kind help with the confocal for SUT3 localization.

Thanks to Judith and Manoj for their kind technical help with RNA preparation and qPCR analysis as well as their friendly advices.

Thanks to my reference committee members Vaughan and Leszek for their great critical advices and all the time they spent for me.

Thanks to Åsa Bengtsson and all the staff at isotope analysis facility of SLU campus in Umeå.

Thanks to all the staff at Wallenberg greenhouse specially Britt-Marie for all their help and support.

Thanks to administrative staff specially Inga-Lis at UPSC for their kind support whenever I needed any help.

Special thanks to Maria Israelsson for always being nice to provide information about the guidelines of graduate studies.

Thanks to Thomas Moritz for always being there for all the official issues and signatures needed.

One special thanks to all UPSC for shaping me a wonderful environment during all these years.

I thank my parents for their heartwarming presence.

And Finally Shokoufeh for all her love and warmth in my life and her parents for all their kind supports.