Functional characterization of non-JAZ TIFY proteins in *Arabidopsis thaliana*

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Contents

List of abbreviations

2CPB	2-CYS PEROXIREDOXIN B
ABA	Abscisic acid
ABP1	AUXIN BINDING PROTEIN1
AD	Activation domain
ADE2	Adenylosuccinate synthetase
ADH1	Alcohol dehydrogenase1
AFB	AUXIN F-BOX PROTEIN
AFP	ABI-FIVE BINDING PROTEIN
AGI	Arabidopsis Genome Identifier
Ala	Alanine
amiRNA	Artificial microRNA
Amp	Ampicillin
ANOVA	Analysis of Variance
ARF	AUXIN RESPONSE FACTOR
ASA1	ANTHRANILATE SYNTHASE α1
ASK1	ARABIDOPSIS SKP1 HOMOLOGUE 1
Asp	Aspartic acid
Aux/IAA	Auxin/Indole acetic acid protein
AuxRE	Auxin-responsive element
AXR1	AUXIN RESISTANT1
BD	Binding domain
BDL/IAA12	BODENLOS/IAA12
bHLH	basic helix-loop-helix
BR	Brassinosteroids
BU	Buffered salt solution
BY2	tobacco bright-yellow
CaMV	*Cauliflower Mosaic Virus*
CBP	CREB BINDING PROTEIN
CK	Cytokinin
COI1	CORONATINE INSENSITIVE1
Col-0	Arabidopsis thaliana Columbia-0 ecotype
COR	Coronatine
CP	Coat protein
CT	C-terminal
CTLH	C-terminal to LiSH domain
CUL1	CULLIN1
DAS	Days after stratification
DMC	Disperse meristematic cell
DMSO	Dimethyl sulfoxide

DO	Drop out supplements
E1	Ub-activating enzyme
E2	Ub-conjugating enzyme
E3	Ub-protein ligase enzyme
EAR	ERF–associated amphiphilic repression motif
EDTA	ethylenediaminetetraacetic acid
ER	Endoplasmic reticulum
ERF	ETHYLENE RESPONSE FACTOR
ET	Ethylene
fLUC	firefly Luciferase
GA	Gibberellic acid
Gal/Raf	Galactose/Raffinose
GAL4	Galactosidase 4
GFP	Green fluorescent protein
GH3	Gretchen Hagen3 acyl-adenylate/thioester-forming enzyme
Glu	Glutamic acid
Gm	Gentamycin
GS	G/streptavidin-binding peptide
GUS	β-Glucuronidase
HA	H*emagglutinin*
Ha	*Hyaloperonospora arabidopsidis*
HAT	Histone acetyl transferase
HDA	Histone deacetylase
His	Histidine
hpi	hours post inoculation
HRP	Horse radish peroxidase
IAA	Indole-acetic acid
IAR3	IAA-Ala resistant
IBR5	INDOLE-3-BUTYRIC ACID RESPONSE5
Ile	Isoleucine
ILL	ILR1-like
ILR1	IAA-LEUCINE RESISTANT1
InsP	Inositol phosphate
$InsP_3$	Inositol 1,4,5-trisphosphate
$InsP_5$	Inositol pentakisphosphate
$InsP_6$	Inositol hexakisphosphate
JA	Jasmonic acid / Jasmonate
jai3	*JASMONATE INSENSITIVE3/JAZ3*
JA-Ile	Jasmonate-isoleucine
JAR1	JASMONATE RESISTANT1
JAZ	JASMONATE-ZIM DOMAIN

JID	JAZ interaction domain
KEG	KEEP ON GOING
KIX	KID-binding domain
Km	Kanamycin
LacZ	β-galactosidase enzyme
Ler	Arabidopsis thaliana Lansdberg erecta ecotype
Leu	Leucine
LiAc	Lithium acetate
LisH	Lissencephaly homology domain
LRR	LEUCINE-RICH REPEAT domain
MAB2	MACCHI-BOU2
MED	Mediator
MeJA	Methyl jasmonate
Met	Methionine
MP	MONOPTEROS
MS	Mass spectrometry
MS	Murashige and Skoog medium
mTAP	C-terminal TAP tag
NINJA	NOVEL INTERACTOR OF JAZ
NLS	Nuclear localization signal
NO	nitric oxide
NPR1	NONEXPRESSOR OF PR GENES 1
NQR	NAD(P)H QUINONE OXIDOREDUCTASE
NRPB2	NUCLEAR RNA POLYMERASE B2
NT	N-terminal
OBE	OBERON
OD	Optical density
OE	Overexpression
PAP	Peroxidase antiperoxidase enzyme
PBS3	avrPphB susceptible3
PCR	Polymerase chain reaction
PEG	Polyethylene glycol
PI	Propidium iodide
PIN	PIN-FORMED
PP2A	PROTEIN PHOSPHATASE 2A
PPD	PEAPOD
Pst	*Pseudomonas syringae* pv. *tomato*
qRT-PCR	Quantitative real-time PCR
RBX1	RING BOX1
RNAi	RNA interference
rpm	revolutions per minute

RUB	RELATED TO UBIQUITIN
SA	Salicylic acid
SAUR	SMALL AUXIN UP RNAs
SCF	SKP1-Cullin-F-box E3 ubiquitin-ligase complex
SCL5	SCARECROW-like 5
SD	Synthetic defined media
SE	Standard Error
SHY2/IAA3	SHORT HYPOCOTYL 2/IAA3
SKP1	S PHASE KINASE-ASSOCIATED PROTEIN 1
SKP2A	S-Phase Kinase-Associated Protein 2A
SL	Strigolactones
SPY	SPINDLY
TAP	Tandem affinity purification
TE	Tris-EDTA buffer
TGMV	*Tomato golden mosaic virus*
THI1	THIAMINE1
TIR1	TRANSPORT INHIBITOR RESPONSE1
TPL	TOPLESS
TPR	TOPLESS-RELATED
TrAP	TMGV transcriptional activator protein
Tris	tris(hydroxymethyl)aminomethane
Trp	Tryptophan
TTA	TITANIA
UAS	Upstream activating sequence
Ub	Ubiquitin
UBP12	UBIQUITIN PROTEASE12
UPS	Ubiquitin-proteasome system
Ura	Uracil
WT	wild-type
WUS	WUSCHEL
X-gal	5-bromo-4-chloro-indolyl-β-D-galactopyranoside
Y2H	Yeast two-hybrid
Y3H	Yeast three-hybrid
YFP	Yellow fluorescent protein
YPD	Yeast peptone dextrose
YPDA	Yeast peptone dextrose adenine medium
ZIM	Zinc-finger protein expressed in Inflorescence Meristems
ZML	ZIM-like

Chapter 1

Introduction:
Jasmonate signalling: a copycat of auxin signalling?

Cover: Arabidopsis plant

Chapter 1

Introduction

Jasmonate signalling: a copycat of auxin signalling?

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ABSTRACT

Plant hormones regulate almost all aspects of plant growth and development. The last decades have provided breakthrough discoveries in phytohormone sensing and signal transduction, and highlighted the striking mechanistic similarities between the auxin and jasmonate (JA) signalling pathways.

In both cases, the activity of transcription factors regulating hormone-responsive gene expression is prevented by the formation of complexes that require the recruitment of the co-repressor TOPLESS via auxin and JA-specific repressors, i.e. the Aux/IAA and the JAZ and NINJA proteins, respectively. Perception of auxin and JA involves the formation of co-receptor complexes in which hormone-specific SKP1-Cullin-F-box protein E3-Ubiquitin ligases target the Aux/IAA and JAZ repressors for 26s proteasome-mediated degradation, that in turn releases the transcription factors and allow hormone-dependent gene expression.

In this chapter, we describe the similarities and differences in the auxin and JA signalling cascades, with respect to the protein families and the protein domains involved in the formation of the pathway-specific complexes.

INTRODUCTION: PLANT HORMONES AND COMMON ELEMENTS IN PLANT HORMONE SIGNALLING

Growth and development of an organism require correct perception, integration and transduction of signals coming from the outside and from within. In the case of plants, their sessile lifestyle requires tightly coordinated responses to variable environmental cues and (a)biotic stresses. Plant hormones are a group of structurally diverse small compounds whose perception and signalling orchestrate practically all cellular processes that steer plant growth, development and environmental adaptation, thereby ensuring an effective developmental plan and the rationalization in the use of resources for the fitness of the plant.

The study of plant hormones dates back to the early 20th century, when the five 'classical' hormones auxin, cytokinin, gibberelic acid (GA), abscisic acid (ABA) and ethylene (ET) were described. Nonetheless, also the last decades have been especially exciting in plant hormone research and several 'new' plant hormones and other signalling molecules have been described. These include the hormones jasmonic acid (JA), salicylic acid (SA), brassinosteroid (BR), strigolactones (SL), several signalling peptides and secondary messengers such as nitric oxide (NO) or polyamines (Kelly & Estelle, 2012; Santner & Estelle, 2009; Shan *et al.*, 2011).

Despite the wide variety of plant hormones, both in terms of their chemical structure and the processes they regulate, their signalling pathways share strikingly parallel or even common mechanisms. One example is the hormone-triggered degradation of repressor proteins through the ubiquitin-proteasome system (UPS), a molecular mechanism that is used by the vast majority of plant hormones (Kelly & Estelle, 2012; Santner & Estelle, 2010; Vierstra, 2009). The UPS consists in an Ubiquitin (Ub)-conjugating cascade, in which ubiquitin monomers are eventually transferred to a target protein. This cascade is based on a three-step enzymatic reaction that involves the action of the E1 Ub-activating, the E2 Ub-conjugating and E3 Ub-protein ligase enzymes. The E3 Ub-ligase renders specificity to the system and determines which protein(s) become ubiquitinated and targeted for 26s proteasome-mediated degradation (Santner & Estelle, 2010; Smalle & Vierstra, 2004).

A more exhaustive comparison of plant hormone signalling pathways points out the striking similarities between those of auxin and JA, in which the molecular machineries for hormone

perception and signal transduction are very alike and involve the same, homologous or very similar types of proteins (Santner & Estelle, 2009). Here, we compare the components of these two signalling pathways, which have widely been studied in the plant model species *Arabidopsis thaliana* (Arabidopsis), and focus on the protein complexes formed and the proteins or protein domains involved. We highlight the differences and similarities between the auxin and JA pathways, comment on the last findings and list some questions that remain to be addressed.

THE GENERAL MODEL FOR AUXIN AND JA SIGNALLING.

Auxin and JA perception and signal transduction are tightly regulated by the formation of protein complexes that involve different proteins, such as transcription factors (TFs), repressor proteins and members of the UPS.

Although a far more complex situation obviously takes place in the plant, a simplified vision of hormone signalling depicts the formation of different protein complexes based on the mere absence or presence of a single hormone.

In the absence of the hormone, the expression of hormone-responsive genes is prevented. The action of transcriptional activators such as the AUXIN RESPONSIVE FACTORS (ARF) or the basic helix-loop-helix (bHLH) MYC TFs, in auxin and JA signalling, respectively, is repressed, as they are bound by the Aux/IAA and JAZ repressor proteins, respectively (Fig. 1.1a-c) (Calderon-Villalobos *et al.*, 2010; Pauwels & Goossens, 2011). To exert their repressive function, both of the latter proteins recruit the co-repressor TOPLESS (TPL) through the ETHYLENE RESPONSE FACTOR (ERF)-associated amphiphilic repression (EAR) motif (Pauwels *et al.*, 2010; Szemenyei *et al.*, 2008). Since the Aux/IAA proteins contain an EAR motif themselves within their sequence, the interaction with TPL can be direct (Fig. 1.1a) (Szemenyei *et al.*, 2008). The same seems to occur for a subset of JAZ proteins, i.e. JAZ5 to JAZ8, that possess EAR motifs within their sequences (Fig. 1.1b) (Arabidopsis Interactome Mapping Consortium, 2011; Causier *et al.*, 2012; Kagale *et al.*, 2010; Kagale & Rozwadowski, 2010; Shyu *et al.*, 2012). The other Arabidopsis JAZ proteins, however, do not contain an EAR domain and recruit TPL through the adaptor protein NINJA that does contain a functional EAR motif (Fig. 1.1c) (Pauwels *et al*., 2010).

Perception of the hormone requires the formation of so-called co-receptor complexes. Closely related F-box proteins, namely TRANSPORT INHIBITOR RESPONSE1 (TIR1), AUXIN F-

BOX PROTEINS1-5 (AFB1-5) and CORONATINE INSENSITIVE1 (COI1) in auxin and JA signalling, respectively, provide the target specificity in SKP1-Cullin-F-box protein (SCF) E3 ubiquitin-ligase complexes. These SCF complexes play a dual function by serving as receptors for the bioactive hormone and recruiting their corresponding targets, the Aux/IAA and JAZ proteins. Upon interaction, a tripartite (SCF, hormone and target) co-receptor complex is formed, which furthermore requires the presence of inositol polyphosphates as cofactors (Mosblech *et al.*, 2011; Sheard *et al.*, 2010; Tan *et al.*, 2007). Subsequently, Aux/IAA and, presumably, also the JAZ proteins are ubiquitinated and directed for degradation via the 26s proteasome, thereby releasing the bound TFs, which in turn promotes hormone-dependent gene expression (Fig. 1.1d, e).

Table 1.1 provides an overview of the proteins involved. The similarities and differences between the components involved in these signalling pathways will be discussed in more detail in the following sections.

Figure 1.1. Schematic models for the protein complexes formed in auxin and JA signalling (next page).

a-c. In the absence of the hormone, the expression of hormone-responsive genes is repressed. **a.** In the absence of auxins, the Aux/IAA proteins repress the ARF TFs by interaction through their domains III/IV. To exert their repressive function the EAR motif-containing domain I in the Aux/IAA proteins recruits the co-repressor TPL. **b, c.** In the absence of JAs, the JAZ proteins repress JA-dependent TFs. Recruitment of the co-repressor TPL can occur either directly via an EAR motif present in the JAZ5-JAZ8 subset (**b**) or indirectly via the EAR motif-containing NINJA adaptor protein (**c**).

d, e. Formation of the co-receptor complexes in the presence of the hormone leads to ubiquitination of the Aux/IAA and JAZ repressors. The $SCF^{TIR1/AFB1-5}$ (**d**) or SCF^{COI1} (**e**) E3 ubiquitin-ligase complexes bind the bioactive form of the hormone and inositol polyphosphates and recruit the Aux/IAA (**d**) and JAZ (**e**) proteins to be ubiquitinated and targeted for 26S proteasome-mediated degradation.

The figure is adapted from Pauwels and Goossens, 2011 and Pauwels *et al.*, 2010. Abbreviations: ARF, AUXIN-RESPONSE FACTOR; ASK1, ARABIDOPSIS SKP1 HOMOLOGUE 1; Aux/IAA, AUXIN/INDOLE-ACETIC ACID; AuxRE, auxin-responsive element; bHLH, basic helix-loop-helix; COI1, CORONATINE INSENSITIVE1; CUL1, Cullin1; DBD, DNA-binding domain; EAR, ETHYLENE RESPONSE FACTOR (ERF)-associated amphiphilic repression; IAA, indole-acetic acid; $InsP_5$, inositol pentakisphosphate; $InsP_6$, inositol hexakisphosphate; JA-Ile, Jasmonate-isoleucine; JID, JAZ interaction domain; RBX1, RING BOX1; TIR1, TRANSPORT INHIBITOR1; Ub, ubiquitin; ZIM, Zinc-finger protein expressed in Inflorescence Meristems.

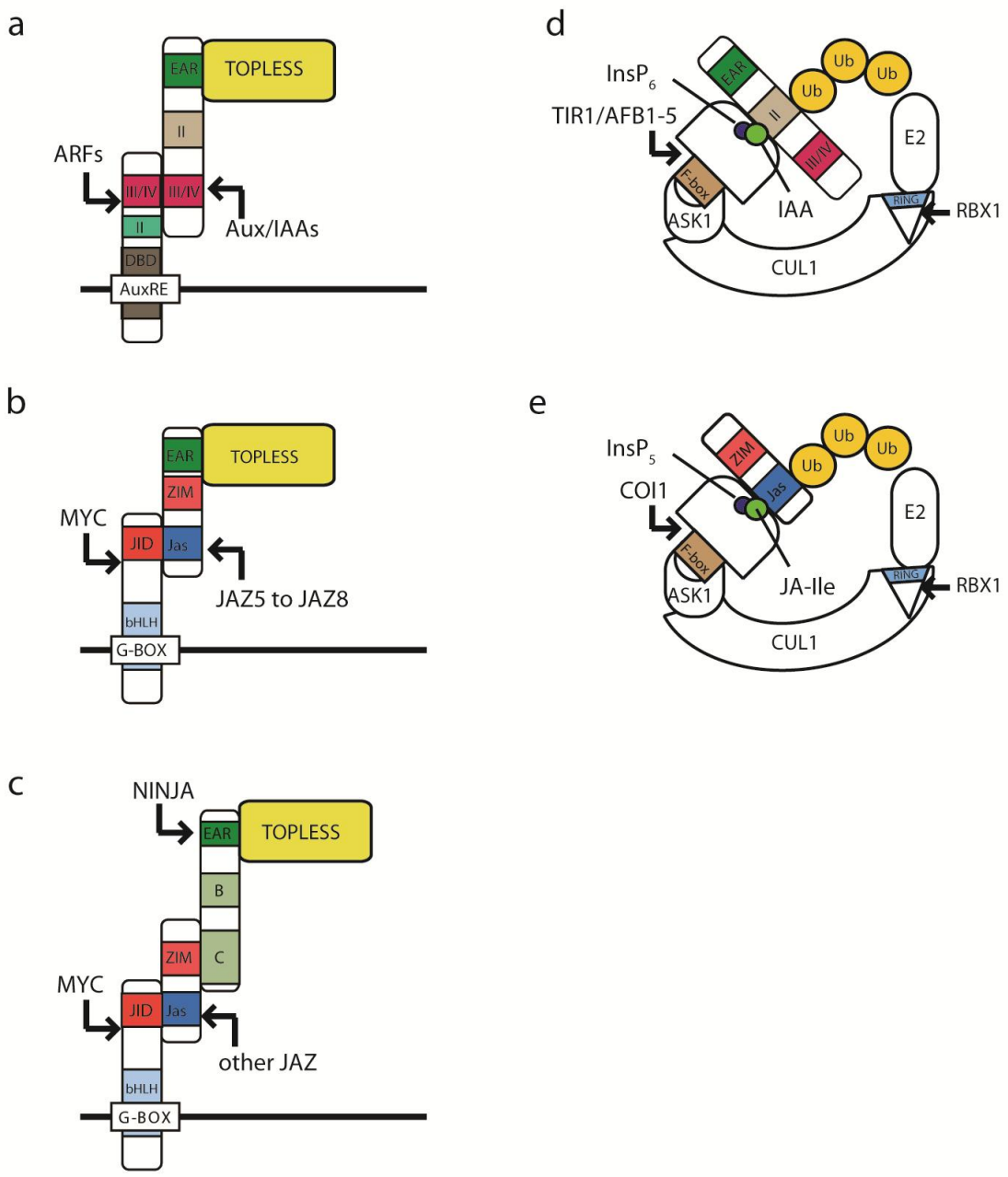

Table 1.1. Overview of the proteins involved in auxin and JA signalling.

Function	Auxin	JA	Same protein family?
Receptor(s) / F-box proteins	TIR1/AFB1-5	COI1	Yes
Hormone conjugating enzymes	GH3.2-6, 9 and 17	JAR1(GH3.11) GH3.3, 5, 6	Yes
Hormone deconjugating enzymes	ILR1, IAR3, ILL1-3,5, 6	IAR3	Yes
Target proteins	Aux/IAA1-29	JAZ1-12	No
Co-repressor protein	TPL/TPR	TPL	Yes
Adaptor protein	-	NINJA	No

CO-RECEPTOR ELEMENTS

Role of F-box proteins in SCF E3 Ubiquitin-ligase complexes

Both auxin and JA responses involve the UPS through the action of the multiprotein SCF complexes, which function as E3 ubiquitin-ligases and which constitute the largest E3 family in plants (Gagne *et al.*, 2002).

The SCF complexes are formed by four proteins (Santner & Estelle, 2010). CULLIN1 (CUL1) and RING BOX1 (RBX1) form the core of the complex, bind to S PHASE KINASE-ASSOCIATED PROTEIN 1 (SKP1) and recruit the E2 ubiquitin-conjugating enzyme (Fig 1.1d, e). SKP1, also known as ARABIDOPSIS SKP1 HOMOLOGUE 1 (ASK1) in plants, is essential for binding to the F-box protein. The latter provides the target specificity of the SCF complexes. Notably, there exist almost 700 different F-box proteins in Arabidopsis, classified as such based on the presence of a conserved N-terminal F-box domain (Vierstra, 2009). Proteins belonging to this family can be further classified according to the presence of other protein-protein interaction domains (Gagne *et al.*, 2002). The most abundant class consists of the F-box proteins containing LEUCINE-RICH REPEAT (LRR) domains, a 20 to 29 amino acid sequence that assembles to form a helical structure (Gagne *et al.*, 2002). TIR1/AFB1-5 and COI1, the F-box proteins that regulate auxin and JA signalling, respectively, both belong to the LRR class of F-box proteins.

TIR1 and AFBs are auxin receptors

TIR1 was first characterized based on the resistance of the *tir1* mutant to auxin and auxin transport inhibitors (Ruegger *et al.*, 1998). Due to its LRR-type F-box structure, TIR1 was postulated to mediate the degradation of negative regulators of auxin signalling, i.e. the Aux/IAA repressor proteins (see below).

Five TIR1-like proteins exist in Arabidopsis, namely AFB1-5. These proteins present high sequence similarity with TIR1: 70% for AFB1, 60% for AFB2-3, and around 50% for AFB4-5 (Dharmasiri *et al.*, 2005b; Parry *et al.*, 2009). All six TIR1 and AFB proteins possess the same structure, with an N-terminal F-box protein domain, several LRRs and conserved amino acids required for binding to auxin and the AUX/IAA proteins. AFB4 and AFB5 present an additional N-terminal region with a yet unknown function (Mockaitis & Estelle, 2008).

Unravelling the mechanisms for auxin perception and signal transduction signified a breakthrough in hormone signalling research. First, Dharmasiri *et al.* (2005a) demonstrated that TIR1 and AFB1-3 act as auxin receptors that mediate Aux/IAA degradation in the presence of the hormone. Soon after, these findings were complemented by those of Tan *et al*. (2007). The crystal structures of TIR1-ASK1 in the absence or presence of several auxin analogues and Aux/IAA peptides revealed that auxin acts as a 'molecular glue', promoting the interaction of TIR1 and Aux/IAA by direct binding to a hydrophobic pocket within the TIR1 structure. The co-receptor model proposed for auxin signalling is a unique receptor model that is not found in the animal kingdom, in which the hormone-binding and the active site are physically distant in the tridimensional structure of the receptor (Calderon-Villalobos *et al.*, 2010).

In the past decade, all six F-box proteins (TIR1 and AFB1-5) have been described as auxin receptors, and each of them might have specific functions in auxin signalling, based on their gene expression pattern, protein accumulation and the root development defects in the respective mutant lines (Dharmasiri *et al.*, 2005a; Greenham *et al.*, 2011; Parry *et al.*, 2009). Exceptionally, AFB4 seems to act as a negative regulator of auxin signalling, in contrast with the rest of the TIR1/AFB proteins (Greenham *et al.*, 2011).

COI1 is the JA receptor

Similarly to *TIR1*, the *COI1* locus was identified in a screen for JA-insensitive plants. In this case, the screen was performed with coronatine (COR), a phytotoxin produced by several strains of the pathogen *Pseudomonas syringae* that acts as a mimic of the JA-isoleucine (JA-Ile) conjugate, the bioactive form of the hormone (Feys *et al.*, 1994; Fonseca *et al.*, 2009b; Xie *et al.*, 1998). The *coi1-1* mutant is perturbed in all aspects of the JA response, which suggests it plays a central role in JA signal transduction.

Structurally, *COI1* encodes an F-box that is the closest related to TIR1 after the AFB proteins (Fig. 1.2). In contrast to the *TIR1* and *AFBs* that belong to the same gene family, *COI1* is a unique gene. COI1 is a member of the SCF^{COI1} complex, and was postulated to mediate the degradation of putative JA-signalling repressor proteins, later on characterized as the JAZ proteins (Chini *et al.*, 2007; Thines *et al.*, 2007; Xie *et al.*, 1998; Yan *et al.*, 2007). Likewise to

TIR1, COI1 acts as hormone receptor, hosting JA-Ile within a protein pocket, and recruiting the JAZ proteins for their subsequent ubiquitination (Sheard *et al.*, 2010).

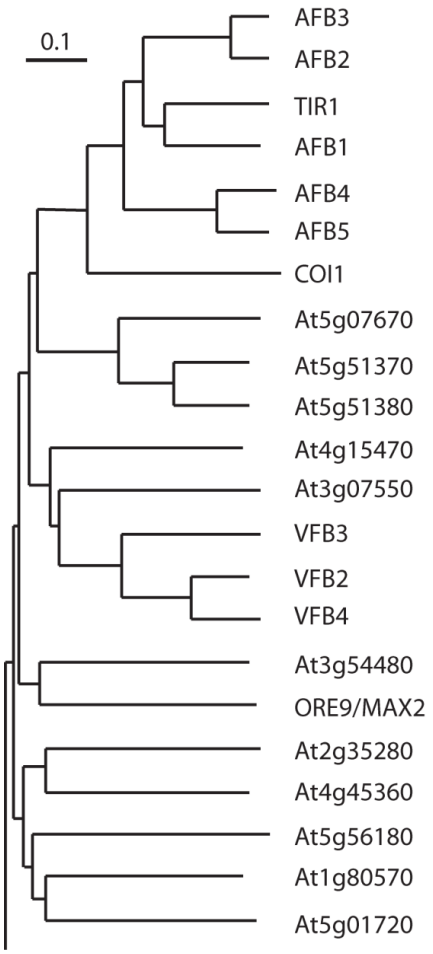

Figure 1.2. TIR1, AFBs and COI1 are closely related F-box proteins. Section a phylogenetic tree showing part of the C4 F-box subfamily. Bars represent the branch length equivalent to a 0.1 amino acid changes per residue. The bars represent the branch length equivalent to 0.1 amino acid changes per residue. Figure adapted from Gagne *et al*., 2002.

The hormone: IAA and JA-Ile

Auxin is one of the five 'classical' plant hormones and reports on its function in several processes, such as gravitropic responses, date back to more than a century ago. Despite its early discovery and extensive research, the biosynthetic route of auxin has not been completely unravelled. Both tryptophan (Trp)-dependent and -independent auxin synthesis pathways have been proposed (Mano & Keymoto, 2011; Zhao, 2010).

Jasmonates (JAs) have only been identified in the 1960s and had originally been described as secondary metabolites present in the essential oils of jasmine (*Jasminum* sp.) flowers. Two decades later, the first physiological effects of JAs in plants, such as inhibition of growth and promotion of senescence, were described and nowadays these oxylipin-type molecules are recognised as true plant hormones (Wasternack, 2007). The JA biosynthetic pathway is well defined. Starting from the precursor α-linoleic acid, several enzymatic steps lead to the formation of JA. The first steps take place in the chloroplasts, the later ones occur in the peroxisomes (Howe, 2001; Schaller & Stintzi, 2009; Wasternack, 2007).

Once produced, both IAA and JA can be conjugated to amino acids, a reaction performed by Gretchen Hagen3 (GH3) acyl-adenylate/thioester-forming enzymes, named after the GH3 protein first characterized in *Glycine max* as an early auxin responsive gene (Hagen & Guilfoyle, 1985; Hagen *et al.*, 1984). The Arabidopsis genome harbours 19 GH3 proteins classified in three groups (Staswick, 2009; Westfall *et al.*, 2010). Group I includes JASMONATE RESISTANT1 (JAR1, GH3.11), that conjugates JA to Ile, and GH3.10 which is involved in red light-specific hypocotyl elongation (Staswick & Tiryaki, 2004; Staswick *et al.*, 2002; Takase *et al.*, 2004; Westfall *et al.*, 2012). Group II members (GH3.2 to 6, 9 and 17) conjugate various amino acids to IAA and SA (Staswick *et al.*, 2005) and the GH3.3, GH3.5 and GH3.6 subset has very recently been shown to conjugate Asp, Met and Trp to JA as well (Gutierrez *et al.*, 2012). Within group III, formed by GH3.7, 8 and 12 to 19, only the GH3.12 member (avrPphB susceptible3 or PBS3) has been shown to use benzoates as substrates (Nobuta *et al.*, 2007; Okrent *et al.*, 2009; Westfall *et al.*, 2012).

The conjugation of a certain amino acid to IAA or JA can have different outcomes. In the case of auxin, where IAA is the bioactive form of the hormone, conjugation to form IAA-Glu or IAA-Asp results in IAA catabolism, whereas the conjugates IAA-Ala and IAA-Ile become inactive (Ludwig-Müller, 2011; Staswick, 2009). In the case of JA, in contrast, conjugation

leads to JA-Ile, whose (+)-7-*iso*-Jasmonoyl-L-isoleucine isomer has been pinpointed as the hitherto only known bioactive form of the hormone (Fonseca *et al.*, 2009b). Together with JA-Ile, other JA conjugates have very weak activity triggering COI1-JAZ interaction, whereas neither JA nor its methyl ester (MeJA) are bioactive, highlighting the necessity of JA conjugation for hormone activation (Fonseca *et al.*, 2009b; Gutierrez *et al.*, 2012; Thines *et al.*, 2007). To date, it remains unknown whether JA, MeJA or JA conjugates other than JA-Ile can act as active forms of the hormone through additional perception mechanisms.

Amino acid conjugation to plant hormones is reversed through the action of deconjugating enzymes. The IAA-LEUCINE RESISTANT1 (ILR1)-like proteins are a family of amidohydrolases that regulate free IAA and JA-Ile levels (Campanella *et al.*, 2003; LeClere *et al.*, 2002; Rampey *et al.*, 2004; Woldemariam *et al.*, 2012). In Arabidopsis, the family is formed by 7 proteins (ILR1, IAR3, and ILL1, ILL2, ILL3, ILL5 and ILL6) with distinct, but also overlapping, substrate specificities (Bartel & Fink, 1995; Davies *et al.*, 1999; LeClere *et al.*, 2002; Rampey *et al.*, 2004). Evolutionarily, IAR3 is the most conserved protein within the ILR1-like family and, remarkably, is able to deconjugate both JA-Ile and IAA-Ala *in vitro*, whereas the other family members act specifically on IAA (Campanella *et al.*, 2003; Woldemariam *et al.*, 2012). The functionality of the ILR1-like family in other plant species, e.g. *Triticum aestivum, Medicago truncatula, Populus trichocarpa, Brassica rapa* or *Nicotiana attenuata* has also been described, supporting the conservation of this mechanism in higher plants (Campanella *et al.*, 2004; Campanella *et al.*, 2010; Campanella *et al.*, 2008; Junghans *et al.*, 2006; Woldemariam *et al.*, 2012).

Hormone (de)conjugation from its amino acid functions as one of the mechanisms developed by plants to control hormone homeostasis. In the case of JA signalling, oxidation and carboxylation of JA-Ile by the CYP94B3 and the CYP94C1 cytochrome P450 enzymes, respectively, set an additional regulatory mechanism to control JA-Ile pools and therefore tailor JA-triggered responses in the plant (Heitz *et al.*, 2012; Kitaoka *et al.*, 2011; Koo *et al.*, 2011).

In auxin, catabolism also takes place through oxidative pathways that may require decarboxylation of either the side chain and the indole ring or the indole nucleus alone (Ruiz Rosquete *et al.*, 2011). The oxidative degradation of auxin seems to be important during development, although the mechanisms controlling this process are still poorly understood.

The targets: Aux/IAA and JAZ

Binding of the hormone to the F-box proteins promotes the recruitment of their targets, Aux/IAA and JAZ proteins. These proteins possess several protein domains that mediate interactions with different partners. In this section, we briefly describe these protein families and focus on the protein domains important for co-receptor formation.

The Aux/IAA proteins bind to the TIR1-IAA co-receptor complex

The Aux/IAA proteins are encoded by early auxin-induced genes and have first been described together with the *GH3* and *SMALL AUXIN UP RNAs* (*SAUR*) genes (Hagen & Guilfoyle, 2002). The Aux/IAA proteins constitute of four protein domains. Domain I is responsible for the repressor activity (see later) (Tiwari *et al.*, 2004). Domain II contains the degron important for Aux/IAA-auxin-$SCF^{TIR1/AFB}$ co-receptor formation. Domains III and IV share high homology with the homonymous domains present in auxin-responsive factors (ARFs, see below) and regulate the formation of homo- and heterodimers between Aux/IAA and ARFs (Calderon-Villalobos *et al.*, 2010).

The Arabidopsis genome encodes for 29 Aux/IAA proteins, most of them found to be auxin-inducible and functionally redundant, based on the study of their expression pattern and phenotypical analysis of single and double mutants (Mockaitis & Estelle, 2008; Overvoorde *et al.*, 2005). The study of some gain-of-function mutants showed that those caused by a point mutation within domain II stabilized the Aux/IAA protein, suggesting that this domain is responsible for protein degradation upon auxin perception. Accordingly, several studies have shown that auxin promotes the degradation of Aux/IAA proteins in a $SCF^{TIR1/AFB}$-dependent manner (Calderon-Villalobos *et al.*, 2010). The Aux/IAA proteins act as co-receptors of the hormone and, following auxin binding to the pocket of TIR1, are recruited to TIR1. Aux/IAA binding to TIR1 for co-receptor complex formation was mapped to a degron sequence within domain II of the Aux/IAA proteins, containing the hydrophobic consensus motif GWPPV (Tan *et al*., 2007). Additionally to this degron motif, it has recently been shown that other regions in the Aux/IAA proteins may contribute to determine auxin binding affinity in the different Aux/IAA-TIR1 pairs. The elegant work of Calderón-Villalobos *et al*. (2012) demonstrated the existence distinct hormone-binding affinities between a number of Aux/IAA-TIR1 pairs, which suggests a mechanism to fine-tune auxin signalling

throughout plant development, given the differential expression patterns of the Aux/IAA family members.

The JAZ proteins bind to the COI1-JA-Ile co-receptor complex

The discovery of the JAZ proteins was a major step forward in the JA research field. As in the case of auxin, it was postulated that COI1 might regulate JA signalling by targeting repressor proteins for degradation. Almost a decade after the first characterization of COI1 three independent research groups identified the JAZ proteins as targets of COI1 and repressors of MYC2 (Chini *et al.*, 2007; Thines *et al.*, 2007; Yan *et al.*, 2007).

The JAZ proteins belong to the plant-specific TIFY family, characterized by the presence of a core TIF[F/Y]XG motif within the Zinc-finger protein expressed in Inflorescence Meristems (ZIM) protein domain. This domain is conserved among the 18 TIFY family members in Arabidopsis, which are further classified in two groups based on the presence (class I) or absence (class II) of a C2C2-GATA domain (Fig. 1.3). The 12 existing JAZ proteins belong to Class II, together with the PEAPOD1 (PPD1), PPD2 and TIFY8 proteins, and contain other protein domains besides the ZIM (Bai *et al.*, 2011; Vanholme *et al.*, 2007).

Particularly, the JAZ proteins possess a characteristic C-terminal domain, the Jas domain, that is defined by the $SLX_2FX_2KRX_2RX_5PY$ conserved pattern and responsible for interaction with COI1 and several TFs regulating JA-dependent gene expression (Chung *et al.*, 2010; Melotto *et al.*, 2008; Pauwels *et al.*, 2010; Pauwels & Goossens, 2011; Yan *et al.*, 2007). These interactions are key for a proper JA-response and, consistently, disruption or loss of the Jas domain causes JA-insensitivity, as reported in mutants such as the *JASMONATE INSENSITIVE3/JAZ3* (*jai3*), or expressing JAZ splice variants missing the Jas domain (Chini *et al.*, 2007; Chung *et al.*, 2010; Chung & Howe, 2009; Melotto *et al.*, 2008; Thines *et al.*, 2007; Yan *et al.*, 2007). Analogous to the Aux/IAA proteins, the degron sequence within the Jas domain of some of the JAZ proteins has been identified, with e.g. ELPIARRA as the minimal JAZ1 amino acid sequence necessary for the formation of the co-receptor complex (Sheard *et al.*, 2010). Remarkably, this degron sequence is divergent between the JAZ proteins, and these differences may define the distinct affinities in the different JAZ-COI1 combinations (Sheard *et al.*, 2010). This is exemplified by JAZ8, whose degron motif is quite divergent from that of JAZ1 (PKASMK) and which presents only limited affinity for interaction with

COI1 (Shyu *et al.*, 2012). Nevertheless, a detailed study of the interaction between the different JAZ-COI1 pairs similar to that of the Aux/IAA-TIR1 pairs would be required to unravel whether additional regions besides the JAZ degron could also affect JAZ-COI1 binding affinities (Calderón-Villalobos *et al*., 2012).

The Jas domain is divergent or inexistent in the other class II TIFY proteins and, accordingly, no role for these proteins in JA signalling has been reported to date (Fig. 1.3) (Bai *et al.*, 2011; White, 2006).

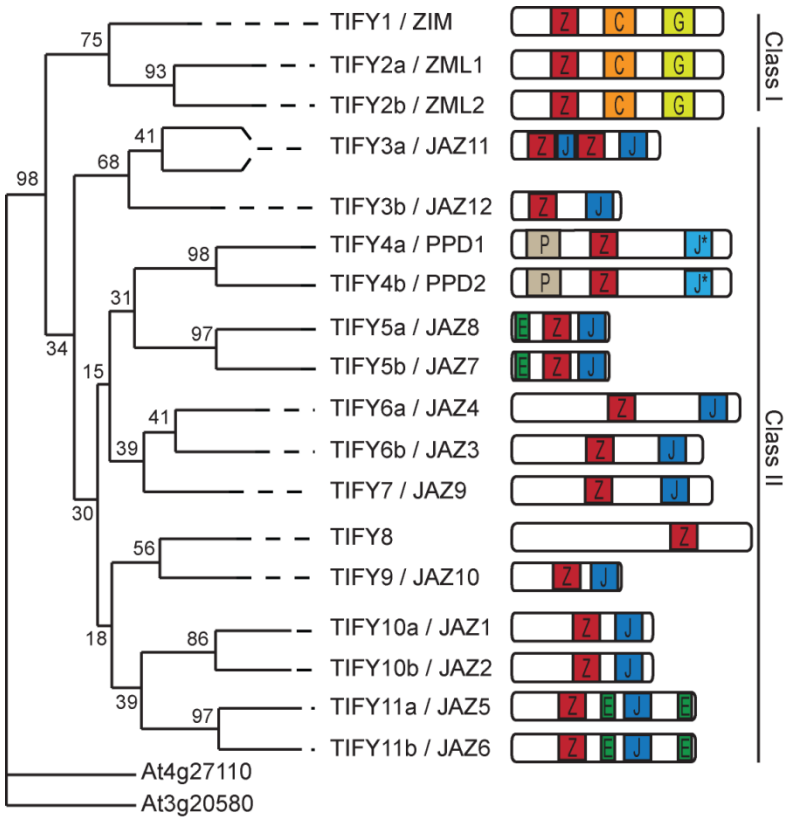

Figure 1.3. The TIFY protein family in Arabidopsis.
Phylogenetic tree of the Arabidopsis TIFY family members based on the ZIM domain (Z) protein sequence. AT4G27110 and AT3G20580 were chosen as the outgroup. AT4G27110 contains a TIFY motif but is not conserved in the domain outside this motif. Consequently, it is not considered to be a real TIFY protein. The second protein, AT3G20580, is its closest homologue within the parsed region. The numbers above the branches are bootstrap values from 100 replicates and assess the robustness of the tree. Additional protein domains are shown. C: CONSTANS, CO-like, and TOC1 (CCT) domain; G: C2C2-GATA Zn-finger; P: PEAPOD domain; J: Jas domain; J* Jas-like domain; E: EAR domain. Figure adapted from Vanholme *et al.* (2007).

Aux/IAA and JAZ ubiquitination and proteasomal degradation

Despite the generally assumed model in which Aux/IAA and JAZ proteins are ubiquitinated upon hormone perception and consequently targeted for proteasomal degradation, experimental data confirming this postulation are scarce. The instability of polyubiquitinated proteins, their rapid degradation and the need for extremely sensitive techniques might account for the few effective reports on protein ubiquitination regardless of the high number of putative target proteins undergoing this process. Two groups have reported Aux/IAA (Dos Santos Maraschin *et al.*, 2009) or JAZ polyubiquitination (Saracco *et al*., 2009) by means of two different approaches. Making use of a cell suspension-based protoplast system, Dos Santos Maraschin *et al.* (2009) showed SCF^{TIR1}-mediated polyubiquitination and proteasomal degradation of SHORT HYPOCOTYL2/IAA3 (SHY2/IAA3) and BODENLOS/IAA12 (BDL/IAA12), which occurs in an auxin and TIR1 dose-dependent manner. Polyubiquitination of JAZ6 was detected through a non-targeted approach that used a tandem affinity purification (TAP) assay coupled to mass spectrometry to identify ubiquitinated proteins in Arabidopsis (Saracco *et al.*, 2009). Ubiquitination of JAZ proteins by COI1 has not been experimentally proven to date.

Inositol phosphates act as cofactors for hormone perception

Inositol phosphates (InsP) were previously linked to plant responses to wounding. Interestingly, the formation of inositol polyphosphates such as inositol 1,4,5-trisphosphate ($InsP_3$) is known to be JA-mediated (Mosblech *et al.*, 2008). The characterization of the auxin co-receptor complex by Tan *et al*. (2007) revealed the presence of an inositol polyphosphate molecule acting as a TIR1 cofactor. Concretely, inositol hexakisphosphate ($InsP_6$) was found to bind to TIR1 through the LRR of the F-box protein that forms the binding pocket (Fig. 1.1d) (Tan *et al*., 2007). These reports, together with the high homology between TIR1 and COI1 suggested that, analogous to auxin signalling, InsPs might also play a role in JA signalling (Mosblech *et al*., 2008). Indeed, this postulation was confirmed with the identification of inositol pentakisphosphate ($InsP_5$) interacting with COI1 and JAZ1 adjacent to the hormone (Fig. 1.1e) (Sheard *et al*., 2010).

The residues in TIR1 and COI1 responsible for InsP binding are similar, although not identical, which might account for the selectivity of the corresponding InsP to the respective F-box protein. Functionally, binding of InsPs to the co-receptor complex has been shown to increase the efficiency of hormone perception (Mosblech *et al.*, 2011; Sheard *et al.*, 2010).

ACTIVATION AND REPRESSION ELEMENTS

Transcriptional activators

Hormone-dependent gene expression is regulated by TFs belonging to different protein families. The existence of several members within a particular TF family can be translated into rather opposite outcomes, acting either redundantly or specifically in the regulation of a given process.

Auxin-responsive gene expression

Auxin-responsive gene expression is regulated by AUXIN RESPONSE FACTORS (ARFs), a protein family composed of 23 members in Arabidopsis.

The ARFs consist of four protein domains. Domain I contains a B3-like DNA binding domain. Domain II is variable and formed by either Glu- or Pro-rich regions, associated with a function in transcriptional activation or repression, respectively (Chapman & Estelle, 2009; Guilfoyle & Hagen, 2007). The C-terminal region is composed by domains III and IV, that are analogous to those in the Aux/IAA proteins and serve for homo- and heterodimerization (Calderon-Villalobos *et al.*, 2010; Okushima *et al.*, 2005). ARFs control gene expression through binding to auxin-responsive elements (AuxRE) in the promoters of auxin-responsive genes (Fig. 1.1a).

The ARFs regulate several processes either by the redundant function of a set of ARFs or by the formation of concrete ARF-Aux/IAA pairs. One example of such specificity occurs during root embryonic formation, which is regulated by the interaction of the Aux/IAA protein BDL/IAA12 with the ARF5 protein, also known as MONOPTEROS (MP/ARF5) (Weijers *et al.*, 2005).

JA-responsive gene expression

The expression of JA-responsive genes is also regulated by TFs. The bHLH MYC2 protein is one of the most studied and has long been considered as the central regulator of JA-dependent responses (Dombrecht *et al.*, 2007; Lorenzo *et al.*, 2004). Nevertheless, the fact that the *myc2* mutant is not completely insensitive to JAs - e.g. it is still male fertile- pointed towards the existence of additional TFs involved in the regulation of JA-dependent gene expression (Feys *et al*., 1994; Fonseca *et al*., 2009a; Lorenzo *et al*., 2004). Indeed, besides MYC2, its close homologs MYC3 and MYC4, other bHLH, and R2R3 MYB TFs, also regulate diverse JA responses (Cheng *et al.*, 2011; Fernández-Calvo *et al.*, 2011; Niu *et al.*, 2011; Pauwels & Goossens, 2011; Qi *et al.*, 2011; Song *et al.*, 2011). The bHLH MYC TFs are partially redundant but also regulate independently different processes. For instance, MYC2 has a major role in JA-mediated root growth inhibition, while MYC3 and MYC4 seem to be more important for the defence responses in aerial tissues e.g. resistance to herbivores (Cheng *et al.*, 2011; Fernández-Calvo *et al.*, 2011). The MYB21 and MYB24 TFs function in JA-regulated anther development and filament elongation, whereas the MYB75 and the bHLH factors GL3 and EGL3 regulate anthocyanin accumulation and trichome initiation (Qi *et al*., 2011; Song *et al*., 2011).

Repression through EAR-domain proteins: Aux/IAA, JAZ, NINJA

The EAR motif was first described in a subset of the class II ERF and C2H2 protein families, as a conserved motif with the L/FDNL/F(x)P sequence, and capable to turn transcriptional activators into repressors when fused to them (Ohta *et al.*, 2001). This motif, later pinpointed to appear mainly as LxLxL or DLNxxP, is present in up to 219 proteins in Arabidopsis and is postulated to act as a repression motif in stress, development and hormone signalling pathways (Kagale *et al.*, 2010; Kagale & Rozwadowski, 2010; Kagale & Rozwadowski, 2011).

Several models for repression of gene expression by EAR motif-containing proteins have been proposed. The capacity for recruitment of co-repressor proteins and chromatin remodelling factors is one of the most favoured hypothesis for the role of EAR-containing proteins (Kagale & Rozwadowski, 2010; Kazan, 2006). In the case of auxin and JA signalling, the EAR motif present in the repressor proteins mediates the interaction with TPL. As such,

the EAR motif-containing proteins can be considered as a 'bridge' between transcriptional activators and repressors.

Aux/IAA proteins

The AUX/IAA proteins contain an LxLxL-type EAR repression motif within their domain I that is necessary and sufficient for TPL recruitment. Domain II has previously been described as necessary for the formation of the auxin co-receptor complex and domains III and IV for homo- and heterodimerization with other Aux/IAA or ARFs, respectively (Fig. 1.1a) (Calderon-Villalobos *et al.*, 2010; Mockaitis & Estelle, 2008).

JAZ proteins

A subset of the JAZ proteins, i.e. JAZ5 to JAZ8, has recently been shown to contain EAR domains suggesting that, analogously to the Aux/IAAs, they might directly interact with TPL (Fig. 1.1b) (Kagale *et al.*, 2010). So far, this has been experimentally verified for JAZ5 and JAZ8 (Arabidopsis Interactome Mapping Consortium, 2011; Shyu *et al.*, 2012).

The other JAZ proteins, however, do not have an EAR domain and apply a different strategy to recruit TPL. The ZIM domain, conserved in all the family members and known to mediate homo- and heterodimerization of the JAZ also provides a link to the TPL repressor protein (Bai *et al.*, 2011; Chini *et al.*, 2009; Chung & Howe, 2009; Vanholme *et al.*, 2007). ZIM is the domain necessary for recruitment of NINJA, an EAR motif-containing protein that serves as adaptor and bridges non-EAR JAZ proteins and TPL (Fig 1.1c).

Finally, the non-JAZ TIFY proteins PPD and TIFY8 do not have EAR motifs within their sequences, but contain the ZIM domain through which they are also able to interact with NINJA (Pauwels *et al*., 2010).

NINJA

The NINJA protein was first identified as an interactor of JAZ1 through TAP of Arabidopsis cell cultures overexpressing *JAZ1* (Pauwels *et al.*, 2010). NINJA is related to the ABI-FIVE BINDING PROTEINS (AFPs) that function in ABA signalling (Garcia *et al.*, 2008; Lopez-Molina *et al.*, 2003). In contrast to the latter proteins, *NINJA* expression is not regulated by ABA but rather by JAs (Pauwels *et al.*, 2010). NINJA possesses three protein domains, namely A, B

and C. The A domain of NINJA harbours an EAR motif, necessary and sufficient for the recruitment of the co-repressor TPL and TPL-related proteins, whereas the C domain of NINJA mediates the interaction with the ZIM domain of the JAZ and other class II TIFY proteins (Nagels Durand *et al.*, 2012; Pauwels *et al.*, 2010).

The co-repressor TPL

Co-repressors are transcriptional regulators that cannot bind DNA independently but are recruited by other TFs to regulate gene expression. First described as Groucho in *Drosophila melanogaster* and Tup1 in *Saccharomyces cerevisiae*, the group of Groucho/Tup1 co-repressors is present in animals, plants and fungi. Thirteen Groucho/Tup1 proteins are encoded in the Arabidopsis genome and are divided in two groups. LEUNIG and LEUNIG_HOMOLOG form one group and TPL and four TPL-related (TPR) proteins constitute the other (Lee & Golz, 2012; Liu & Karmarkar, 2008).

TPL was first described as an interactor of the homeodomain TF WUSCHEL (WUS), which regulates meristem homeostasis (Kieffer *et al.*, 2006; Laux *et al.*, 1996). During the past decade TPL has been linked to several processes, including meristem maintenance, auxin and JA signalling, defence responses and control of flowering time (Arabidopsis Interactome Mapping Consortium, 2011; Causier *et al.*, 2012; Gallavotti *et al.*, 2010; Long *et al.*, 2006; Long *et al.*, 2002; Pauwels *et al.*, 2010; Szemenyei *et al.*, 2008; Zhu *et al.*, 2010).

The TPL and TPR proteins possess an N-terminal lissencephaly homology (LisH) domain, a C-terminal to LiSH (CTLH) domain, a TOP domain, a Q-rich domain and several central and C-terminal WD-40 repeats (Krogan *et al*., 2012; Lee & Golz, 2012; Long *et al.*, 2006). The N-terminal region that comprises the LisH, the CTLH and the TOP domains is responsible for interaction with proteins such as WUS, BDL/IAA12, NINJA or APETALA2 (Krogan *et al*., 2012; Kieffer *et al.*, 2006; Pauwels *et al.*, 2010; Szemenyei *et al.*, 2008) which, in turn, provide an EAR domain to recruit TPL. Accordingly, the recently published TPL Interactome indicated the overrepresentation of EAR domains within the TFs interacting with TPL/TPR proteins. Furthermore, the TPL Interactome identified up to 20 out of the 29 Aux/IAA proteins, some of the JAZ proteins and several ARFs. The latter may account for the repressive function of some of these TFs (Causier *et al.*, 2012).

TPL- and TPR-mediated transcriptional repression is likely mediated by the recruitment of histone deacetylases (HDAs) such as HDA19 and/or demethylases like JUMONJI8. These proteins act on chromatin histones, preventing chromatin expansion and/or impairing the access of the transcriptional machinery, and eventually supressing gene expression (Long *et al.*, 2006; Macrae & Long, 2011).

CONSERVATION OF THE AUXIN AND JA SIGNALLING PATHWAYS IN THE PLANT KINGDOM

The auxin signalling pathway is conserved in Streptophyta, i.e. land plants and several orders of green algae. The major components of this mechanism are conserved in land plants. For instance, both the moss *Physcomitrella patens* and the fern *Selaginella moellendorffii* contain genes encoding for Aux/IAA, ARFs and TIR1 homologues within their genomes and hence, a signalling machinery comparable to that of flowering plants exists in these species (De Smet *et al.*, 2011; Finet & Jaillais, 2012; Paponov *et al.*, 2009). Remarkably, some of these orthologues are presented as truncated forms and/or present variations in the protein domains necessary for interaction. One example is the divergent domain I of some of the Aux/IAA proteins in *P. patens* and *S. moellendorffii*, with an LxLxPP motif instead of the LxLxL consensus EAR motif (Paponov *et al.*, 2009). The role of TPL in auxin signalling is also conserved, and TPL/TPR orthologues are found in *P. patens*. Interaction of the TPL and Aux/IAA orthologues suggests that the divergent LxLxPP motif can act as repression motif in mosses (Causier *et al.*, 2012).

In the case of Chlorophyta, a division of green algae, no orthologues for Aux/IAA, ARFs or TPL/TPR proteins are found in some the species whose genome is available (De Smet *et al*., 2011). Nevertheless, auxin signalling might still occur through a TIR1-independent mechanism since AUXIN BINDING PROTEN1 (ABP1, see discussion) and INDOLE-3-BUTYRIC ACID RESPONSE5 (IBR5) orthologues are found in both Chlorophyta and Streptophyta, and suggest that the more complex TIR1-dependent auxin signalling just emerged during the evolution from Chlorophyta towards green plants. For more details, we refer recent excellent reviews(De Smet *et al.,* 2011; Finet & Jaillais, 2012; Lau *et al.*, 2009 and Paponov *et al.,*(2009).

The evolutionary analysis of the core module components of JA signalling has not been as extensively covered as for auxin, although it has clearly defined their conserved presence in green plants (Streptophyta) but the absence of e.g. COI1 and JAZ (or other TIFY) proteins Chlorophyta (Bai *et al.*, 2011; Chico *et al.*, 2008; Vanholme *et al.*, 2007).

Remarkably, JAZ protein orthologues have been identified in several plant species ranging , amongst others, from the moss *P. patens* and the fern *S. moellendorffii* to tobacco, rice or poplar, and shown the conservation of the Jas domain, essential for JAZ regulation of JA signalling and interaction with COI1 (Bäckström *et al.*, 2007; Chung *et al.*, 2010; Rensing *et al.*, 2008; Shoji *et al.*, 2008; Ye *et al.*, 2009).

In the case of the GH3 enzymes, responsible for amino acid conjugation to the hormones, no obvious orthologues were found in Chlorophyta but they do exist in higher plants (De Smet *et al.*, 2011). Moreover, the number of GH3 enzymes encoded by the genomes of different organisms increases gradually with plant complexity, which suggests that the variety of GH3 enzymes is derived from a common ancestor that underwent genome duplication and specialization events (Ludwig-Müller, 2011; Ludwig-Müller *et al.*, 2009).

Taken together, the conservation patterns of the core module proteins in auxin and JA signalling suggest that these pathways appeared at least 450 million years ago, when plants conquered the land, and have further specified during plant evolution.

AUXIN AND JA SIGNALLING CROSSTALK

It is well known that plant hormones do not act independently but are tightly interconnected to fine-tune plant growth, development and interaction with the environment. Auxin and JA interact with practically all other hormones as reviewed elsewhere (Pieterse *et al.*, 2012; Robert-Seilaniantz *et al.*, 2011; Wasternack, 2007). In this section we will highlight some aspects of the crosstalk between auxin and JA (Fig. 1.4), a topic recently covered by Hoffmann *et al.* (2011).

One point of crosstalk between the two hormones occurs at the level of the hormone perception. Since both IAA and JA-Ile are perceived by SCF E3-ligases, disturbed functioning of these complexes leads to impaired responses to both hormones. This alteration can be caused by mutations in one of the SCF subunits, as in the case of mutations in CUL1 (Moon

et al., 2007; Quint *et al.*, 2006), but also on upstream regulators, as in mutants defective in AUXIN RESISTANT1 (*axr1*). The latter protein is responsible for CUL1 conjugation to RELATED TO UBIQUITIN (RUB) proteins, a process known as rubylation, and essential for proper CUL1 functioning (del Pozo *et al.*, 2002; Dharmasiri *et al.*, 2007).

Auxin-JA crosstalk has also been reported to occur through the interaction of auxin- and JA-related regulators of gene expression. For example, ARF6 and ARF8 regulate JA biosynthesis in flowers, auxin induces JAZ1 expression, and MYC2 mediates the repression of the *PLETHORA* genes, involved in auxin-induced regulation of root meristem maintenance (Chen *et al.*, 2011; Grunewald *et al.*, 2009; Nagpal *et al.*, 2005; Tabata *et al.*, 2010).

Another level of interaction is the modulation of each other's homeostasis and transport by auxin and JA. Auxin is known to induce JA biosynthesis and, similarly, JA mediates the expression of some of the auxin biosynthetic genes such as *ANTHRANILATE SYNTHASE α1* (*ASA1*) (Dombrecht *et al.*, 2007; Sun *et al.*, 2011; Sun *et al.*, 2009). Furthermore, high JA concentrations reduce the accumulation of the PIN-FORMED1 (PIN1) and PIN2 auxin transporters at the plasma membrane, impairing auxin transport and gravitropic responses (Sun *et al.*, 2011; Sun *et al.*, 2009).

Generally, auxin is considered to be the growth promoting hormone, whereas JA is known to repress plant growth. One example of the interaction between these hormones in time and space takes place during gravitropic responses. In rice coleoptiles, for instance, it has been proposed that concomitant to the auxin gradient formed due to gravistimulation, an opposite JA gradient is formed that contributes to the auxin-mediated acceleration of the gravitropic response (Gutjahr *et al.*, 2005; Hoffmann *et al.*, 2011).

Another nice example of the close interaction between the two pathways is found in adventitious root formation (Gutierrez *et al.*, 2012). In this process, the expression of a subset of GH3 proteins, i.e. *GH3.3*, *5* and *6*, is positively regulated by ARF6 and ARF8 and negatively by ARF17. The amido-acid-conjugating activity of these GH3 proteins also regulates the pools of bioactive (JA-Ile) and inactive (JA-Asp, JA-Met, JA-Trp) conjugates, to help controlling the formation of adventitious roots, which is negatively regulated by JA-Ile and the downstream JA-triggered responses.

Finally, the JA-Trp and IAA-Trp conjugates have been reported as auxin antagonists, highlighting the complexity and the tight regulation of auxin-mediated processes (Staswick, 2009).

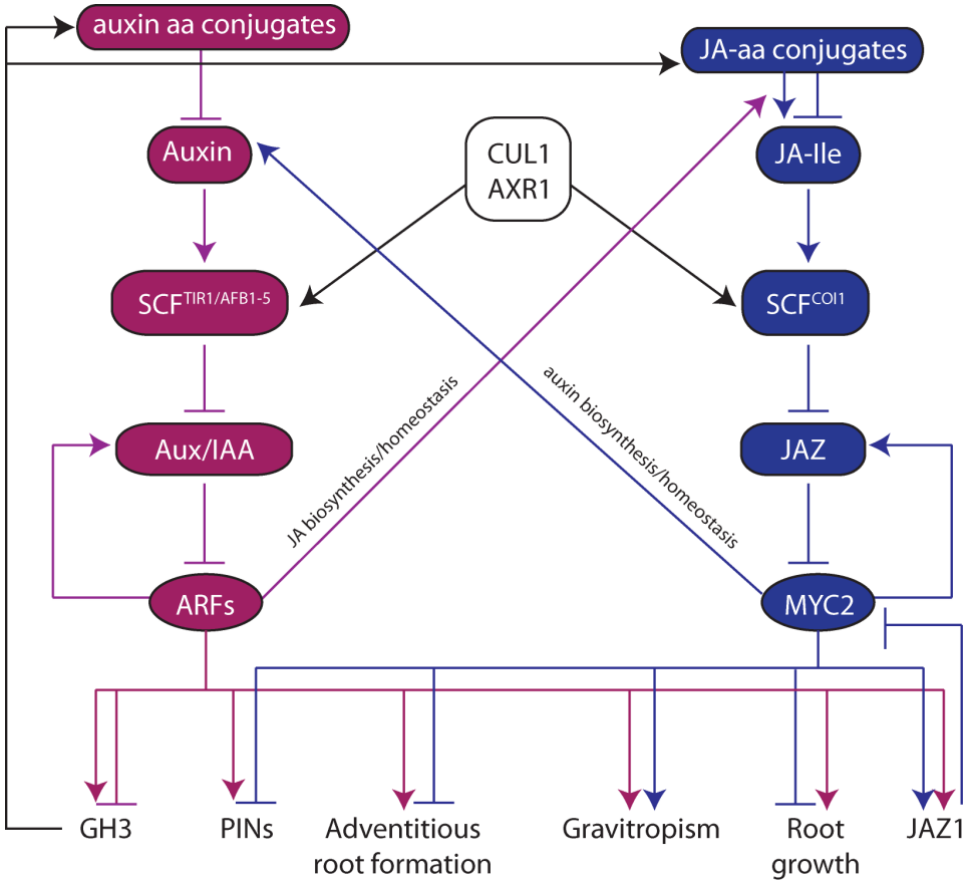

Figure 1.4. Auxin and JA crosstalk in Arabidopsis. Representative model for auxin and JA signalling pathways and their crosstalk during the regulation of several processes reported in literature. Blue and purple lines represent JA- and auxin-related processes, whereas black lines refer to processes affected in both hormone signalling pathways.

CONCLUSIONS AND PERSPECTIVES

The past two decades have been extremely fruitful for plant hormone research and have provided a detailed characterization of the core modules for auxin and JA signalling, thereby creating new paradigms in our understanding on how plant hormones are perceived and trigger the corresponding responses.

Nevertheless, plant hormone research has still much to offer, and the coming years will definitely bring more new insights and provide answers to the several questions that remain open. Some of the most intriguing ones are briefly touched in this outlook section.

It is not all about repression: the Mediator complex

In this chapter, we have focused on the main players involved in auxin and JA signalling. We have considered the Aux/IAA and JAZ proteins as the central players that serve as the bridge between transcriptional activators and co-repressor proteins to control hormone-dependent gene expression.

Whereas many reports tackled the repression mechanisms, less studied is the modulation of auxin- and JA-responsive gene expression upon release of the TFs. Two groups have recently demonstrated a positive role for the Mediator complex in the regulation of the activity of JA-regulated TFs such as MYC2 and ORA59 (Cevik *et al.*, 2012; Chen *et al.*, 2012). Mediator constitutes a multiprotein complex that bridges TFs to the RNA polymerase machinery and participates in the regulation of gene expression (Kidd *et al.*, 2011). Isolation of the Mediator complex retrieved 21 conserved proteins and six plant-specific ones (Bäckström *et al.*, 2007). From those subunits, MED25 is one of the most widely studied and known to regulate other processes besides JA-dependent gene expression, such as flowering, organ size and stress responses (Cerdán & Chory, 2003; Elfving *et al.*, 2011; Kidd *et al.*, 2010; Kidd *et al.*, 2009; Xu & Li, 2011). A direct involvement of the Mediator complex in the activity of any of the ARFs has not been described yet. However, it has been shown that mutations in the MED12 and MED13 subunits and in MACCHI-BOU2 (MAB2), a homolog of MED13, affect embryo patterning and cotyledon formation. The fact that *med12* and *med13* show phenotypes similar to those of *bdl* and *mp* mutants suggests that MED12 and MED13 can

promote auxin-dependent responses in embryo formation, but experimental data confirming such hypothesis is still lacking (Gillmor *et al.*, 2010; Ito *et al.*, 2011).

TIR1 nitrosylation

NO has been implicated in several physiological processes such as seed germination, plant defence, root development, stomatal closure, mitochondria and chloroplast functioning, and performs a role as secondary messenger in hormone signalling pathways (Lindermayr & Durner, 2009; Lindermayr *et al.*, 2010). In the case of SA signalling, S-nitrosylation of NONEXPRESSOR OF PR GENES 1 (NPR1), the plant immune co-activator, enhances nuclear localization and was shown to be important for the spatial and temporal activity of transcriptional regulators of the SA response (Lindermayr *et al.*, 2010).

A role of nitric oxide (NO) in auxin signalling has also been postulated recently. S-nitrosylation of TIR1 in two cysteine residues enhanced TIR1-Aux/IAA interaction, thus facilitating Aux/IAA degradation. Whether S-nitrosylation might modify TIR1 binding to the hormone remains currently unknown (Terrile *et al.*, 2011). Whether NO-nitrosylation may also affect COI1 functioning is an attractive parallel that arises given the similarities between these two F-box proteins. The possible involvement of these or other posttranslational modifications in auxin and JA perception and signalling will be strategic to study in the future.

Additional posttranslational modifications: JAZ phosphorylation

The previous data seem to indicate that the JA and auxin co-receptor complexes do not require phosphorylation of the target proteins (Sheard *et al.*, 2010; Tan *et al.*, 2007). However, a tobacco JAZ protein (NtPPS3) was identified as a kinase target (Katou *et al.*, 2005) and JAZ12 phosphopeptides have recently been identified in Arabidopsis (PhosPhAt 3.0; Durek *et al.* (2010)), suggesting a role for phosphorylation in JAZ regulation which, to date, has not been reported.

Hormone perception by several receptors

In the case of auxin, other receptors besides TIR1 and the AFB F-box proteins have been described and shown to rule a distinct set of auxin responses. The first, AUXIN BINDING PROTEIN1 (ABP1), is an ER-localized protein that is secreted to the extracellular space and regulates very rapid auxin-mediated responses in the plasma membrane such as cell expansion and clathrin-dependent endocytosis (Robert *et al.*, 2010; Sauer & Kleine-Vehn, 2011; Tromas *et al.*, 2010). The second, S-Phase Kinase-Associated Protein 2A (SKP2A) is an F-box protein involved in cell proliferation. SKP2A interacts with the cell cycle-related TFs E2FC and DPB and targets them and/or other hormone-binding proteins for degradation (Jurado *et al.*, 2010). Hence, the functioning of multiple hormone receptors is needed in the multi-level control of auxin-dependent responses. Given the similarities between the auxin and JA pathways, it is remarkable that no other JA receptors than COI1 have been identified so far. Similar to auxin, direct binding of JA-Ile or other forms of JAs to non-COI1 proteins might occur, as already reported in the case of mammalian cells. Indeed, MeJA can act as an anti-cancer drug that induces growth inhibition and cell death in cancer cells through various mechanisms (Cohen & Flescher, 2009). In a process known as bio-energetic mechanism, MeJA is able to directly bind mitochondrial hexokinase, the initial enzyme in the glycolytic pathway, thereby perturbing cellular biosynthetic processes and triggering cancer cell death (Cohen & Flescher, 2009; Goldin *et al.*, 2008).

Tight regulation of hormone homeostasis, hormone-mediated responses and hormonal crosstalk

To tailor hormone responses and fine-tune the plant developmental program, auxin and JA signalling are tightly controlled through various mechanisms. Amino acid conjugation through GH3 enzymes, alternative deconjugation via the ILR1-like proteins or hydroxydation and carboxylation by CYP450 enzymes can regulate hormone homeostasis (Campanella *et al.*, 2003; Gutierrez *et al.*, 2012; Heitz *et al.*, 2012; Kitaoka *et al.*, 2011; Koo *et al.*, 2011; Ludwig-Müller, 2011; Staswick, 2009; Woldemariam *et al.*, 2012). Hormone-mediated transcriptional reprogramming forms a negative feedback regulatory loop in which *Aux/IAA* and *JAZ* gene expression is promoted in a hormone-dependent manner by the transcriptional activators that are liberated from the very same repressors (Chini *et al.*,

2007; Hagen & Guilfoyle, 2002; Vanholme *et al.*, 2007). Besides the JAZ, many other genes involved in JA signalling are regulated through an amplification loop to modulate JA responses both positively and negatively. For instance, the primary JA response induces the boosting of JA signalling itself (Pauwels *et al.*, 2008). In addition, the complexity of hormone signalling is further increased through crosstalk between auxin and JA and with most other plant hormones (Pieterse *et al.* (2012); Robert-Seilaniantz *et al.* (2011). In this regard, the identification of crosstalk points that link different signalling pathways will help mapping the interaction amongst them and the relevance thereof for the fine-tuning of plant growth and development.

Identification of key players through different techniques

Most of the players described in this review have been found through screens based on phenotypical responses to exogenous hormone application. Nevertheless, supplementary screens to identify key hormonal signalling players are needed. Of special interest is the use of protein-protein interaction techniques such as TAP and high throughput yeast two-hybrid (Y2H) screens to find new leads for subsequent reverse genetics screens, given that hormone signalling is tightly regulated by the formation of specific protein complexes. One successful example of such an approach is the discovery of NINJA (Pauwels *et al.*,2010). Despite its key role in JA signalling, NINJA gain- or loss-of-function causes only slight, although significant, phenotypical differences in terms of root growth inhibition upon MeJA treatment, which quite probably would not have been perceived in screens based on root growth inhibition-based assays.

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Chapter 2
Scope and Objectives

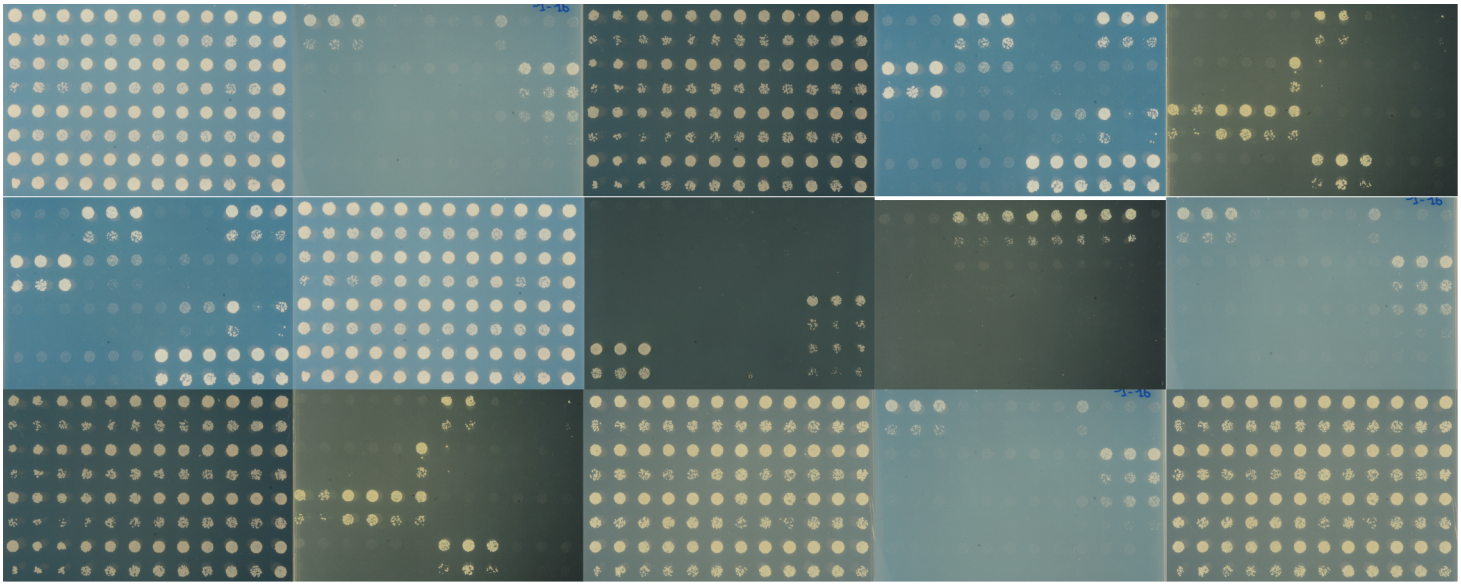
SCOPE AND OBJECTIVES

The discovery and functional characterization of the JASMONATE-ZIM DOMAIN (JAZ) proteins signified one of the major advances in the Jasmonate (JA) signalling research field. These proteins act as central regulators of JA signalling, functioning as repressors of JA-dependent gene expression by recruitment of the co-repressor TOPLESS (TPL) through NOVEL INTERACTOR OF JAZ (NINJA) (reviewed in Chapter 1).

The JAZ proteins belong to the plant-specific TIFY protein family, characterized by the 'TIFY' motif within the Zinc-finger protein expressed in Inflorescence Meristems (ZIM) protein domain, conserved in all family members (Bai et al., 2010; Vanholme et al., 2007). This family is divided in two classes, and class II of the model species *Arabidopsis thaliana* (Arabidopsis) comprises 15 proteins, including the 12 JAZ, two PEAPOD (PPD1 and PPD2) and one TIFY8 proteins.

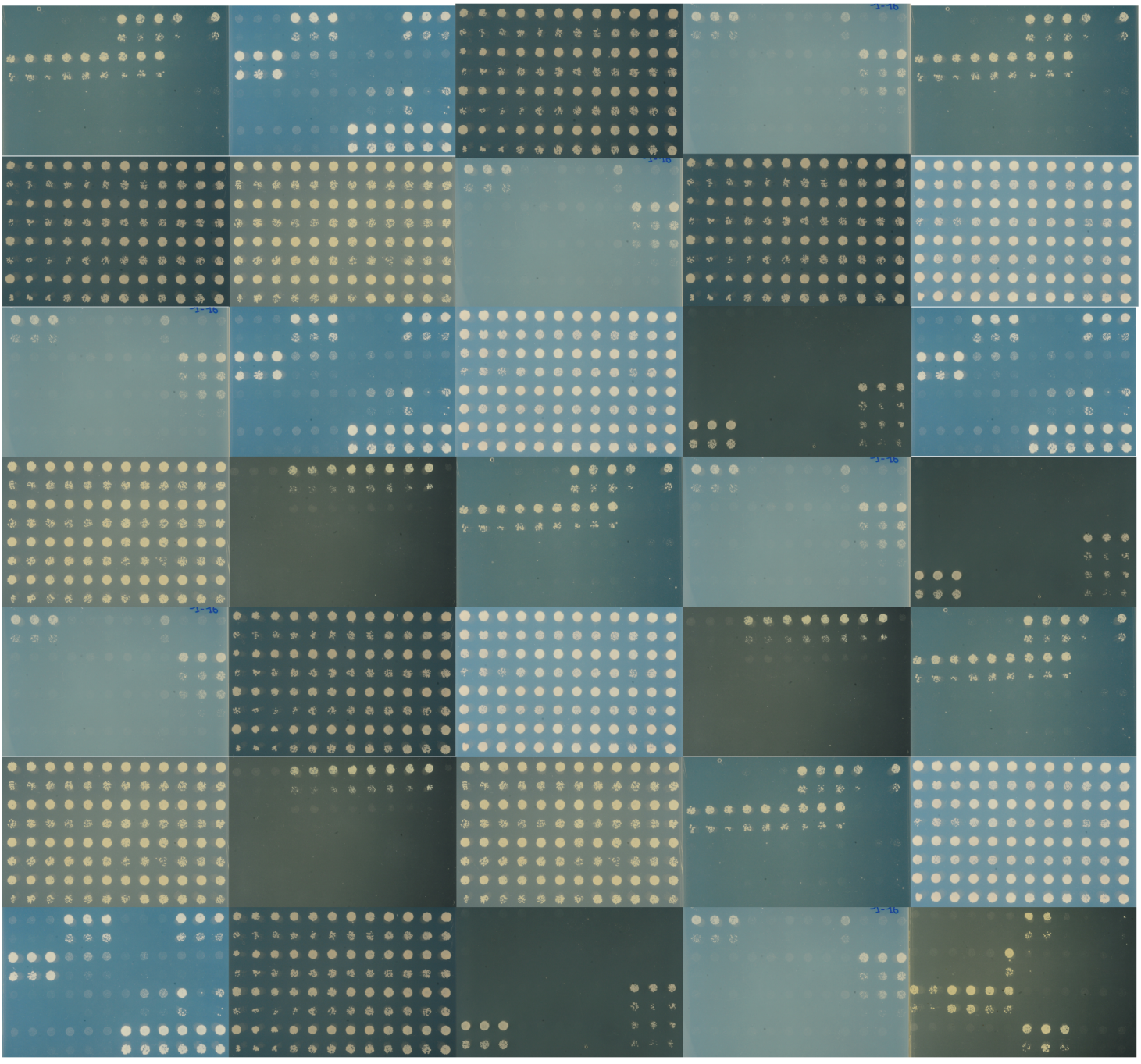
In contrast with the JAZ proteins, for which an in-depth functional characterization has been provided in the last years, few studies have focused on the non-JAZ TIFY proteins and, to date, just the characterization of PPD as growth regulators and putative transcription factors has been reported (White et al., 2006; Lacatus and Sunter, 2009). Therefore, detailed information about their role *in planta* is still scarce.

In this thesis, we initiated the characterization of the TIFY8 and PPD proteins. Given their similarity to the JAZ proteins, we decided to study their putative role in JA signalling by analysis of JA-responsiveness. Moreover, and since we previously demonstrated that both PPD and TIFY8 proteins are able to interact with NINJA (Pauwels et al., 2010), we also focused on the study of their interacting partners by means of Tandem affinity purification (TAP) and yeast two-hybrid (Y2H), with the aim to unravel the formation of specific protein complexes and, ultimately, the function these non-JAZ TIFY proteins.



Chapter 3

Yeast two-hybrid analysis of Jasmonate signalling proteins



Cover: Yeast colonies growing on control and selective media in Y2H assays

Chapter 3

Yeast Two-Hybrid Analysis of Jasmonate Signalling Proteins

Manuscript:

"Yeast two-hybrid analysis of Jasmonate signalling proteins"

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***Author contributions**: Optimization of the yeast two-hybrid protocol presented and writing of the manuscript.

ABSTRACT

Protein-protein interaction studies are crucial to unravel how jasmonate (JA) signals are transduced. Among the different techniques available, yeast two-hybrid (Y2H) is commonly used within the JA research community to identify proteins belonging to the core JA signalling module. The technique is based on the reconstitution of a transcriptional activator that drives the reporter gene expression upon protein-protein interactions. The method is sensitive, straightforward and can be adapted for different approaches. In this chapter, we provide a detailed protocol to perform targeted Y2H assays to test known proteins and/or protein domains for direct interaction in a pairwise manner and present the possibility to study ternary protein complexes through Y3H.

1. INTRODUCTION

Over the last decade, a core module of jasmonate (JA) signalling has been characterized in which hormone perception and consequent signal transduction are tightly regulated by protein complexes formed by different protein types. So far, the main players identified include transcription factors regulating JA-responsive genes (such as MYC2-like basic helix-loop-helix), the JASMONATE-ZIM DOMAIN (JAZ) repressor proteins, co-repressors, such as the Novel Interactor of JAZ (NINJA) and TOPLESS and the F-box protein CORONATINE INSENSITIVE1 (COI1) that forms part of the Skp1/Cullin/F-box $(SCF)^{COI1}$ ubiquitin E3 ligase complex (Pauwels *et al*., 2011). The protein complexes formed are determined by the absence or presence of bioactive JAs, demonstrating the importance of protein-protein interaction studies in this field.

Among the different protein-protein interaction techniques available (Brückner *et al*., 2009; Shoemaker *et al*., 2007; Berggård *et al*., 2007), yeast (*Saccharomyces cerevisiae*) two-hybrid (Y2H) is widely applied within the JA research community. For instance, by means of Y2H assays, the JAZ proteins were confirmed to be the COI1 targets (Thines *et al*., 2007) and to interact with many different transcription factors (for an overview, see Pauwels *et al*., 2011*,* Table 1). Moreover, Y2H can not only be used as an alternative but is often used to validate other protein-protein interaction techniques (Brückner *et al*., 2009; Causier, 2004; Cusick *et al*., 2005). For example, parallel tandem affinity purification (TAP) and Y2H screens had originally identified NINJA as a member of the JA signalling core module (Pauwels *et al*., 2010) and Y2H assays confirmed that both the JAZ-NINJA and the NINJA-TOPLESS interactions were direct. These findings extended the current model for JA signalling and demonstrated the power of combining multiple techniques in unravelling protein complexes.

The Y2H technique as described (Fields and Song, 1989) is based on the reconstitution of a functional transcriptional activator (such as the yeast galactosidase 4 [GAL4]) to promote the expression of (a) reporter gene(s). This transcriptional activator is split into its two functional protein domains: a DNA-binding domain and an activation domain. These domains are fused to a protein of interest, generating two “hybrid” proteins (bait and prey) that are transformed in a compatible yeast strain. In the case of bait-prey interaction, the transcriptional activator is reconstituted and the reporter gene(s) is (are) expressed (see

Figure 3.1) (Brückner *et al*., 2009; Causier, 2004; Cusick *et al*., 2005; Fields and Song, 1989; Causier and Davies, 2002).

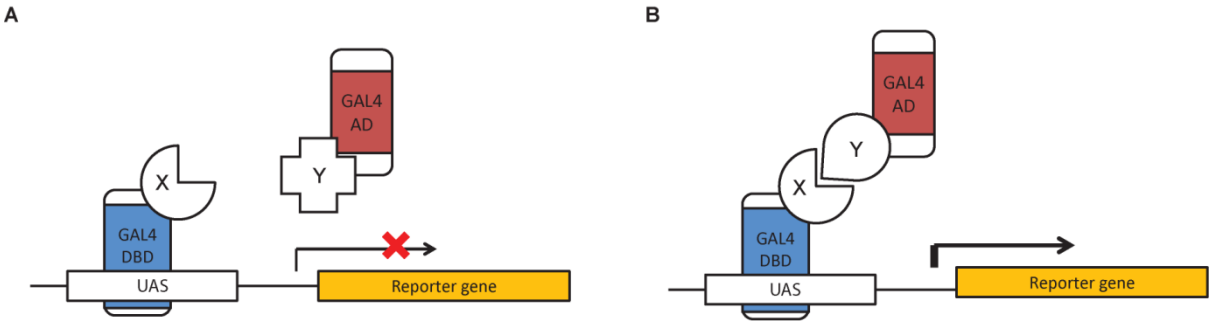


Figure. 3.1. Molecular basis of the Y2H technique.
A transcriptional activator (such as the yeast GAL4) is split into its DNA-binding domain (DBD) and activation domain (AD) that are fused to the proteins of interest (X and Y), generating two "hybrid" proteins, the DBD-X (bait) and AD-Y (prey). **A.** Lack of reporter gene expression, when the proteins do not interact. **B.** Reconstitution of the transcriptional activator upon bait and prey interaction, allowing that the reporter gene expression is driven by a promoter containing the upstream activating sequence (UAS).

The Y2H technique has many advantages: it is sensitive, easy to perform, relatively cheap and can be widely used thanks to its versatility, because it can be adapted to different approaches, such as cDNA-library screens to identify protein interactors. In the JA field, TOPLESS had been used as bait (Causier *et al*., 2012). In matrix-based Y2H assays, a large set of proteins is tested for interaction in a pair-wise manner. Such assays have been carried out with cloned ORFeomes in several model species and recently also in *Arabidopsis thaliana* (Arabidopsis Interactome Mapping Consortium, 2011). Finally, targeted Y2H assays are chosen when the objective is to confirm interactions between two proteins observed through other methods and/or map the domain(s) necessary and sufficient for the interaction.

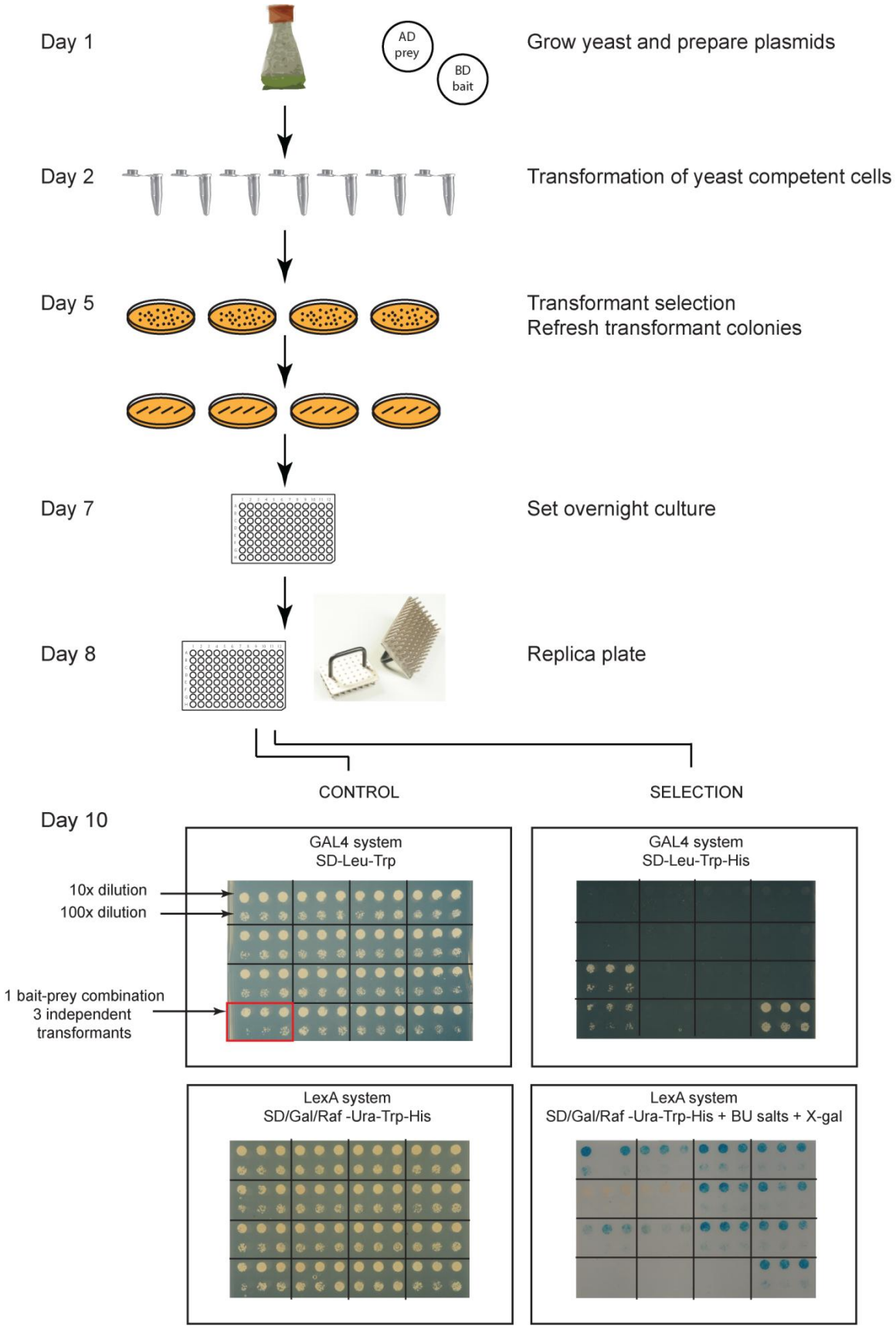
Despite the number of advantages, the Y2H technique also presents limitations (Causier, 2004). The most common are the presence of both false positive and negative interactions, derived from a series of factors. For instance, false positives can occur when using a transcription factor as bait, as its activation domain might lead to autoactivation and subsequent reporter gene expression. False negatives, however, can be caused by lack of protein expression, incorrect protein folding and/or the requirement of posttranslational

modifications in bait or prey. Considering these possibilities is of vital importance in the design of a Y2H assay and the interpretation of its outcome.

Together with Y2H, targeted yeast three-hybrid (Y3H) assays can also be performed to study the formation of ternary protein complexes by cotransformation with a third protein of interest acting as a bridge between bait and prey. Within the JA signalling field, it has recently been shown the formation of a ternary JAZ3-NINJA-TPL complex (Nagels Durand *et al*., 2012).

In this chapter, we focus on targeted Y2H and Y3H assays and we describe a comprehensive protocol that covers all the steps needed (see Figure 3.2). We also give an overview of the variations in the experimental setup regarding the use of different transcriptional activator systems (i.e., the GAL4 and LexA systems), yeast strains, vectors and reporter genes that are generally used in the JA signalling research field.

Figure. 3.2. Overview of the procedure for targeted Y2H assays (next page).
Day 1. Inoculation of a yeast culture overnight in 2× YPDA medium and preparation of bait and prey plasmid constructs.
Day 2. Preparation of competent yeast cells and cotransformation with bait and prey constructs, followed by plating on selective media and incubation for 2 days at 30ºC. For the GAL4 and LexA systems, SD-Leu-Trp and SD Gal/Raf-Ura-Trp-His are used, respectively.
Day 5. Picking up and transfer of independent transformants to a new selective plate, followed by incubation for 2 days at 30°C.
Day 7. Inoculation and incubation of three independent transformant cultures per each bait-prey combination overnight in liquid selective media in 96-well plates.
Day 8. Dilution of the overnight cultures to 1/10 and 1/100 in sterile water. The 16 bait-prey combinations and their corresponding dilutions are set up in one 96-well plate by means of a replica plater to drop yeast cultures on the desired control and selective media.
Day 10. After incubation for 2 days at 30°C, positive protein interactions are scored based on growth or staining on selective media.


Day 1
AD prey
BD bait
Grow yeast and prepare plasmids
Day 2
Transformation of yeast competent cells
Day 5
Transformant selection
Refresh transformant colonies
Day 7
Set overnight culture
Day 8
Replica plate
CONTROL
SELECTION
Day 10
GAL4 system
SD-Leu-Trp
GAL4 system
SD-Leu-Trp-His
10x dilution
100x dilution
1 bait-prey combination
3 independent
transformants
LexA system
SD/Gal/Raf -Ura-Trp-His
LexA system
SD/Gal/Raf -Ura-Trp-His + BU salts + X-gal

2. MATERIALS

2.1. Equipment

1. 250-ml Erlenmeyer flasks.
2. Incubator shaker set at 30^{o}C and 300-400 rpm.
3. Spectrophotometer set for reading optical densities (OD) at λ=600nm (OD_{600}).
4. Tabletop centrifuge (both for conical centrifugation and 1.5-mL microcentrifuge tubes).
5. Thermoblock.
6. Steel bacterial cell spreader or glass beads (3 mm).
7. Sterile Petri dishes: round (9 mm diameter) and square (120 × 120 mm).
8. Microporous tape sheets for covering 96-well plates.
9. Steel replica plater for 96-well plates (Sigma-Aldrich, St. Louis, MO, USA).
10. Both U-shaped and flat-bottom 96-well plates.
11. Vortex.
12. Sterile toothpicks.
13. Laminar flow.

2.2. Media, buffers and solutions

All solutions are prepared with purified water. There is no need to adjust the pH in the yeast growth media and, once sterile, they can be kept at room temperature.

2.2.1. Yeast Growth

1. 2× yeast peptone dextrose (YPD) adenine (YPDA) liquid medium: add 100 g of yeast YPD (Clontech, Mountain View, CA, USA) and 73 mg of adenine (Sigma-Aldrich, St. Louis, MO, USA) for 1 L of medium (see Note 1).
2. YPDA solid medium: add 50 g of YPD (Clontech), 73 mg of adenine (Sigma-Aldrich) and 20 g of agar for 1 L of medium. Autoclave and pour in sterile round Petri dishes.
3. Yeast synthetic defined (SD) media, both liquid and solid: yeast nitrogen base ammonium sulphate and a carbon source, either dextrose (Minimal SD Base) or galactose and raffinose (Minimal SD Base Gal/Raf) and Drop Out (DO) supplements (Clontech). For the GAL4 system, add 26.7 g/L of minimal SD Base (Clontech); for the LexA system, add 37 g/L of minimal SD Base Gal/Raf (Clontech). Amounts required

for the DO supplements (Clontech) depend on their composition. If preparing media to pour plates, add agar to 2% (20 g/L). Autoclave and pour in sterile round Petri dishes if used for transformant selection or in sterile square Petri dishes if used for replica plating.

2.2.2. Yeast transformation

All solutions are prepared with purified water.

1. 1 M lithium acetate (LiAc). Autoclave and keep at 4°C.
2. 50% (w/v) Polyethylene glycol (PEG) 3350: add the PEG 3350 to less water than the final volume, dissolve by heating to 60°C, add water to adjust to the correct volume and cool down on ice.
3. 10× tris(hydroxymethyl)aminomethane (Tris)-ethylenediaminetetraacetic acid (EDTA) (TE): 100 mM Tris-HCl, 10 mM EDTA (pH 8.0). Autoclave and keep at 4°C.
4. Carrier DNA: 10 mg/mL salmon sperm DNA.

2.2.3. 5-bromo-4-chloro-indolyl-β-D-galactopyranoside (X-gal) assay for LexA-based Y2H systems

1. X-gal liquid solution: Prepare a 20 mg/mL stock solution by dissolving the X-gal in dimethyl sulfoxide (DMSO). Store in the dark at -20°C.
2. 10× buffered (BU) salt solution: For 1 L, add 70 g of $Na_2HPO_4.7H_2O$ and 30 g of NaH_2PO4. Adjust pH to 7. Autoclave and store at room temperature.
3. X-gal plates: For 1 L, mix the SD Gal/Raf medium, DO supplements and 20 g/L agar in 900 mL water. Autoclave and cool down to 55°C. Add 100 mL of 10× stock BU salts for a final 1× BU salts concentration and add 4 mL of the 20 mg/mL X-gal stock, for a final 80 mg/L concentration. Mix and pour plates.

2.3. Selection of the Y2H system

The two predominantly Y2H systems that have been used are based on the GAL4 and LexA transcriptional activators, respectively (Brückner *et al.*, 2009; Causier, 2004). In the GAL4 system, reconstitution of the yeast transcriptional activator GAL4 allows reporter gene expression. The LexA system is based on the *Escherichia coli* repressor DNA-binding domain-providing protein LexA that is combined with the *E. coli* B42 activation domain. The

stringency of the latter system is based on the number of LexA operator elements present in the promoter of the reporter genes. Very sensitive reporters include up to eight LexA-binding sites, such as the p8opLacZ plasmid (see Note 2) (Estojak *et al*., 1995; Gyuris *et al*., 1993). In both systems bait and prey proteins are targeted to the nucleus either by the fusion with GAL4 or LexA DNA-binding domains or the nuclear localization signal (NLS) which is added to GAL4 and B42 activation domains in the corresponding vectors.

2.4. Yeast strains

Several yeast strains have been developed for Y2H (see Table 3.1 and Note 3). These strains contain auxotrophic markers, used to select plasmids or report protein interactions, and reporters, such as the gene encoding the enzyme β-galactosidase (*LacZ*), allowing interaction to be reported by yeast staining.

Table 3.1. Characteristics of the different yeast strains commonly used in Y2H assays

Strain	Markers	Other genotype characteristics	Reporter	Available marker	Reference
PJ69-4	*trp1-901* *leu2-3, 112* *ura3-52* *his3-200*	*gal4Δ* *gal80Δ* LYS2::GAL1-HIS3 GAL2-ADE2 met2::GAL7-LacZ	HIS3 ADE2 LacZ	TRP LEU URA	James *et al*., 1996
AH109	*trp1-901* *leu2-3, 112* *ura3-52* *his3-200*	*gal4Δ* *gal80Δ* LYS2::$GAL1_{UAS}$-$GAL1_{TATA}$-HIS3 $GAL2_{UAS}$-$GAL2_{TATA}$-ADE2 URA3::$MEL1_{UAS}$-$MEL1_{TATA}$-LacZ	HIS3 ADE2 LacZ	TRP LEU	Clontech
EGY48 (p8opLacZ)	*ura3* *his3* *trp1*	$LexA_{op(x6)}$-LEU2 p8opLacZ (Ura)	LEU LacZ	HIS TRP URA	Estojak *et al*., 1995

2.5. Vectors

Plasmid-assisted yeast complementation is used for transformant selection. Table 3.2 lists the properties of the most commonly used Y2H vectors (see Note 4). In the case of Y3H, a destination vector expressing the bridging protein fused to a nuclear localization signal and an epitope tag is desired. The use of the PJ69-4A strain together with the GAL4 system

allows the use of a vector with Ura as auxotrophic marker. This vector can be easily obtained with MultiSite gateway using pMG426 as a destination vector (Nagels Durand *et al*., 2012).

Table 3.2. Y2H vectors frequently used in JA signalling research in *Arabidopsis*

Vector	Fusion	Yeast Marker	*E. coli* Marker[a]	Promoter	Epitope	Replication Mechanism	Reference
pGADT7	GAL4AD	LEU2	Amp	ADH1	HA	2 µ	Clontech
pGBKT7	GAL4BD	TRP1	Km	ADH1	c-Myc	2 µ	Clontech
pGAD424	GAL4AD	LEU2	Amp	ADH1	--	2 µ	Clontech
pGBT9	GAL4BD	TRP1	Amp	ADH1	--	2 µ	Clontech
pDEST22	GAL4 AD	TRP1	Amp	ADH1	--	CEN	Invitrogen
pDEST32	GAL4DB	LEU2	Gm	ADH1	--	CEN	Invitrogen
pGILDA	LexA BD	HIS3	Amp	GAL1	--	CEN	Clontech
pB42AD (pJG4-5)	B42 AD	TRP1	Amp	GAL1	HA	2 µ	Clontech, Gyuris *et al.,* 1995

[a] Amp, ampicillin; Gm, gentamicin; Km, kanamycin

3. METHODS

In this section, we describe the methods to perform directed Y2H assays, designed to study the interaction between two known proteins and/or their protein domains. In this adapted yeast transformation (Gietz and Schiestl, 2007), yeast is cotransformed with bait and prey constructs as an alternative to yeast mating. The complete procedure takes approximately 10 to 11 days (see Fig. 3.2).

3.1. Cloning bait and prey

1. To obtain bait and prey constructs, clone the genes encoding the proteins of interest with attention to the reading frame and generate an entry clone by BP reaction with pDONR207 or pDONR223 (see Note 5) that confer gentamicin and spectinomycin resistance in *E. coli*, respectively, and are compatible with the Y2H Gateway® destination vectors (see Note 6).
2. Verify the entry clone by sequencing.

3. Generate an expression clone by LR reaction with the entry clone and the chosen destination vector(s) (Table 3.2, see Note 6).

3.2. Competent yeast cells (days 1 and 2)

1. Inoculate yeast (from plate or cryostock) in 5 mL of 2× YPDA.
2. Incubate overnight at 200 rpm and 30°C (see Note 7).
3. Preincubate 50 mL of 2× YPDA at 30°C overnight (see Note 8).
4. Determine the OD_{600} of the overnight culture (see Note 9).
5. Inoculate the preincubated 2× YPDA medium with the yeast culture grown overnight to yield an OD_{600}= 0.25.
6. Incubate for two cell divisions (approximately 4 h) until OD_{600} = 1.
7. Record the OD of the culture (OD_{600} = 1 is optimal).
8. When the optimal OD_{600} is reached, centrifuge at 800×*g* for 5 min in conical centrifugation tubes in a tabletop centrifuge.
9. Discard the supernatant.
10. Wash cells in 25 mL of precooled sterile water.
11. Centrifuge at 800×*g* for 5 min.
12. Discard the supernatant.
13. Resuspend the yeast cells in 1 mL of sterile water.
14. Transfer resuspension to a sterile 1.5-mL microcentrifuge tube.
15. Centrifuge at 21,000×*g* for 30 seconds.
16. Discard the supernatant.
17. Bring the yeast cell suspension back to OD_{600}= 1 by adding a volume of water equal to the OD_{600} recorded in step 6 (for instance, 0.9 mL if OD_{600} was 0.9), or use to prepare stocks of frozen yeast-competent cells (see Note 10).
18. Vortex vigorously to resuspend the cells.
19. Transfer by pipetting 100-µL aliquots to sterile 1.5-mL microcentrifuge tubes.
20. Centrifuge at 21,000×*g* for 30 seconds (see Note 11).
21. Remove supernatant.
22. Hold the cells on ice.

3.3. Yeast transformation (day 2)

1. For the preparation of DNA mixes, add 1 µg of both bait and prey constructs to a final volume of 34 µL of water in a 1.5-mL microcentrifuge tube (see Note 12).
2. In the meantime, boil just the needed aliquot of the carrier DNA for 10 minutes at 95°C.
3. For the transformation mix, add 240 µL of 50% (w/v) PEG , 36 µL of 1 M LiAc, 10 µL of 10 mg/mL carrier DNA, 34 µL of 30 ng/µL plasmid, 35 µL of sterile water and 5 µL of 10×TE (see Note 13).
4. Keep on ice.
5. Transfer the transformation mix to a microcentrifuge tube containing the yeast-competent cells.
6. Mix by pipetting up and down or vortexing shortly.
7. Incubate in a thermoblock set at 42^{o}C and shaking at 300-400 rpm for 40 minutes (see Note 14).
8. Centrifuge at 21,000×*g* for 30 seconds.
9. Remove the supernatant.
10. Resuspend the cells in 200 µL of sterile water.
11. Plate the cells on the appropriate selective SD medium either with a steel bacterial cell spreader or with sterile glass beads (see Note 15).
12. Incubate plates at 30^{o}C (see Note 16).

3.4. Isolation of yeast transformants (day 5)

1. Pick a number (five is usually sufficient) of transformant colonies with sterile toothpicks for each bait-prey combination.
2. Streak on a fresh selective plate.
3. Grow for 2 days at 30^{o}C.

3.5. Verification of the bait-prey interaction by replica plating (see Note 17)

3.5.1. Liquid culture overnight (day 7)

1. Take a U-bottom 96-well plate (with lid) and fill it with 200 µL of the appropriate selective liquid SD medium per well (see Note 18).
2. Select three transformants per bait-prey combination.

3. Inoculate the yeast to the corresponding well with sterile toothpicks (see Note 19).
4. Seal the plate with a microporous tape sheet.
5. Incubate overnight at 30°C and shaking at 200 rpm.

3.5.2. Preparation of 96-well plates for dilution series (*day 8*) (see Note 20)

1. Use a sterile flat-bottom, 96-well plate and fill it in with 180 µL of sterile water per well.
2. For each well from the overnight culture, transfer the culture by pipetting up and down and transfer 20 µL to another (corresponding) well in the fresh flat-bottom plate filled with water.
3. Mix by pipetting up and down to generate a well-mixed 10-fold dilution.
4. Repeat steps 1-3 to generate a 100-fold dilution (see Note 21).

3.5.3. Replica plate (day 8)

1. Sterilize the replica plater (RP) twice by flaming it using ethanol.
2. Let it cool down.
3. Insert the RP in the flat-bottom 96-well plate with the diluted cultures.
4. Lift the RP to the middle of the culture, keeping it perfectly horizontal.
5. Mix cells by rotating the RP horizontally by hand.
6. Lift the RP out the 96-well plate (see Notes 22 and 23).
7. Put the RP perfectly horizontal on the selective plate (see Note 22).
8. Lift the RP from the medium (see Notes 22 and 24).
9. Let the droplets dry in the laminar flow cabinet for 5 to 10 minutes.
10. Repeat steps 3 to 9 for replica plating of the transformants on the desired number of control and selective media (see Table 3.3).
11. Incubate the plates at 30^{o}C for 2 days (day 10).
12. Once colonies start appearing, score growth or staining (see Note 20).
13. Store plates at 4^{o}C.

Table 3.3. Drop out media for the different systems.

System	Control Media	Selective Media
GAL4 (Y2H)	SD Base-Leu-Trp	SD Base –Leu-Trp-His
GAL4 (Y3H)	SD Base-Leu-Trp-Ura	SD Base –Leu-Trp-Ura-His
LexA	SD Base Gal/Raf-Ura-Trp-His	SD Base Gal/Raf-Ura-Trp-His + 1× BU salts + X-gal

4. NOTES

1. YPD medium is a rich medium used to grow yeast without plasmid selection. Yeast with mutations in adenylosuccinate synthetase *(Ade2)* accumulate red pigment and are slowed down in growth. Adding extra adenine to the media is required (for instance PJ69-4 and AH109 strains) (Bergman, 2001; Saghbini *et al*., 2001).
2. Different results can be obtained depending on the chosen system, most prominently illustrated by the reported JAZ homodimerization and heterodimerization. The LexA system yielded 47 out of 132 possible interactions versus 14 for the GAL4 system. Although the LexA system might be more sensitive, four interactions observed with GAL4 were not detected with it (Chini *et al*., 2009; Chung and Howe, 2009). Moreover, a GAL4-based system with different setups can yield different interactions (Rajagopala *et al*., 2009).
3. For the GAL4 system, the most commonly used strains are PJ69-4 (James *et al*., 1996) and the derivative AH109 (Clontech), in which the endogenous *GAL4* and the inhibitory *GAL80* genes are knocked-out. For the LexA system, the strain EGY48 (p8opLacZ) is widely used (Estojak *et al*., 1995; Gyuris *et al*., 1993) and allows the use of the *GAL1*-inducible promoter, because it does not require the knocked out *GAL4*.
4. Some of the vectors carry epitope tags (i.e. Myc and hemagglutinin) that allow the verification of the fusion protein production by immunoblot analysis. The vectors pGAD424 and pGBT9 are very similar to pGADT7 and pGBKT7, but lack epitopes and T7. In the Gateway®-compatible pDEST22 and pDEST32 vectors (Invitrogen), the auxotrophic markers are swapped compared to the vectors above, so they cannot be combined.

5. We focus on the use of Gateway®-compatible vectors and we refer to the manufacturer's guidelines (Invitrogen) for detailed cloning protocols. The entry clones in the pDONR223 (pENTR223) can be obtained for most of the *Arabidopsis* genes from Arabidopsis Biological Resource Centre (http://www.abrc.osu.edu/).
6. When using the Multisite Gateway vectors for bridging protein expression, the ORF must be cloned without stop codon to allow C-terminal fusion. In a MultiSite Gateway reaction this ORF can then be combined with any desired yeast promoter and tag. Available tags include NLS-3xV5, NLS-3xc-myc, and NLS- 3xFLAG-6xHIS. These tags ensure nuclear localization and detection by immunoblot complementary to tags used in the Y2H vectors. For details about MultiSite Gateway cloning see Nagels Durand *et al*. (2012).
7. When the yeast culture is started from a fresh plate, resuspend the cells first in 1 mL of YPDA liquid medium in a microcentrifuge tube and vortex vigorously during 5 minutes to break yeast clumps. Transfer this 1-mL aliquot to set up the overnight culture.
8. This 50-mL volume is sufficient for 10 transformations and should be adapted according to the required transformation number.
9. To monitor the O_{D600} of the overnight culture, prepare 1/10 dilutions of the culture with the YPDA medium, because the overnight culture will have an $O_{D600} > 1$.
10. To prepare frozen yeast-competent cells, resuspend the cells in a volume corresponding to the recorded OD_{600} with a 5% (v/v) glycerol and 10% (v/v) DMSO. Transfer to microcentrifuge tubes and freeze the cells gradually and slowly to guarantee cell survival (for instance with a MrFrosty container; Nalgene, Rochester, NY, USA). Cells stored at -80°C can be used for up to one year (Gietz and Schiestl, 2007b).
11. If frozen yeast-competent cells are used for transformation, thaw the cell samples at 37°C. For each transformation, aliquot 100 µL in microcentrifuge tubes, spin down at 17,000×*g* for 2 min and proceed to step 20 of Section 3.3.3.
12. In the case of Y3H assays, mix 1 µg of each of the three constructs.
13. The volumes are given for one transformation reaction. Preparation of a master mix is recommended, including all components except the plasmid DNA, if multiple transformations are done simultaneously.

14. Incubation period and temperature might differ depending on the yeast strain used (Gietz and Schiestl, 2007). For the yeast strains used here, incubation at 42°C for 40 minutes is efficient.
15. For the GAL4 and LexA Y2H systems, use SD Base Leu-Trp and SD Base Gal/Raf-Ura-Trp-His, respectively. In the Y3H presented here, use SD Base Leu-Trp-Ura.
16. Transformants are visible after 3 to 4 days.
17. This method gives the greatest reproducibility and nicest pictures (see Fig. 3.2). Alternatively, independent transformant cultures can be streaked or dropped manually on the appropriate selective plates to assess bait-prey interactions.
18. One well needs to be filled per independent transformant to be tested.
19. Normalizing ODs at this point is not necessary.
20. By using the 96-well replica plate system, up to 48 different bait-prey combinations (in two dilutions each) can be tested in one plate. However, because it is highly recommended to test three biological replicates per bait-prey combination, we normally test up to 16 combinations, including negative controls (for instance, bait and prey with the corresponding empty vectors) to detect false positives derived from the autoactivation of either bait and prey constructs, and eventually also positive controls (known interactors). Sometimes, false negatives appear among the three replicates. Western blotting is then recommended to confirm that both bait and prey fusion proteins are expressed.
21. A 1,000-fold dilution is optional. For a typical experimental setup, see Fig. 3.2.
22. It is very important that steps 5 to 7 of Section 3.5.3. are performed in a fluent, swift manner, because hesitation will lead to unequal loading or deformed droplets. Also make sure the replica plater does not move horizontally when in contact with the plate medium.
23. In every pin, an equal amount (approximately 2 to 3 µL) of yeast culture will be loaded.
24. Droplets are formed and an equal volume per well is expected to be plated.

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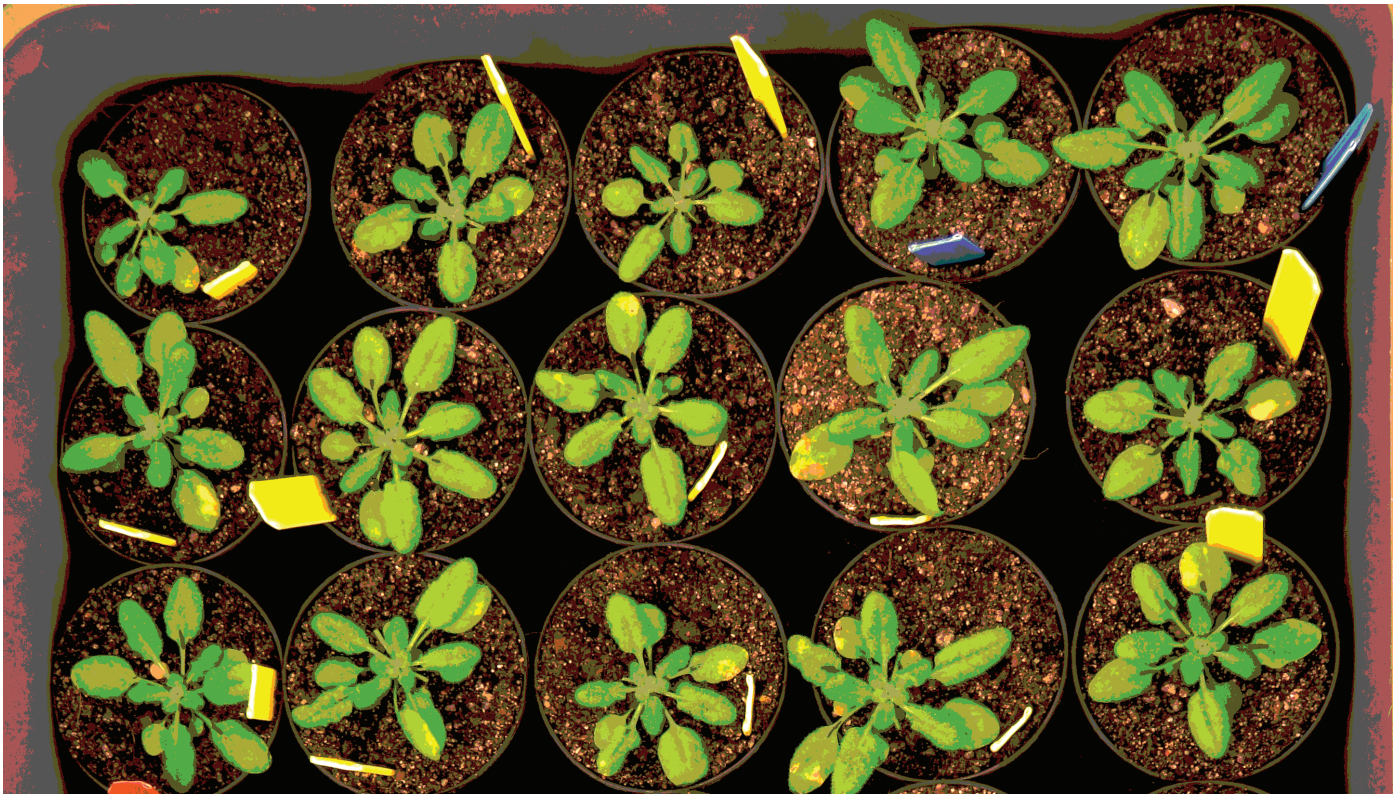

Chapter 4

TIFY8 is a transcriptional repressor modulating *JAZ* gene expression upon *Pseudomonas syringae* infection

Cover: 5 week-old Arabidopsis plants after infection with *Pseudomonas syringae*.

Chapter 4

TIFY8 is a transcriptional repressor modulating *JAZ* gene expression upon infection with *Pseudomonas syringae*

Manuscript:

"TIFY8 is a transcriptional repressor modulating *JAZ* gene expression upon infection by *Pseudomonas syringae*". In preparation for resubmission to the Plant Journal.

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ABSTRACT

Jasmonate (JA) signalling is mediated by the JASMONATE-ZIM DOMAIN (JAZ) repressor proteins, which are degraded upon JA perception to release downstream responses. The ZIM protein domain has been shown to be essential for JAZ homo- and heterodimerisation and binding to the co-repressor Novel Interactor of JAZ (NINJA). This domain is characteristic of the larger TIFY protein family within which the role of the atypical member TIFY8 is currently unknown. In this chapter we show that *TIFY8* expression is inversely correlated with the expression of several *JAZ* genes. Infection of *Arabidopsis thaliana* by *Pseudomonas syringae* pv. *tomato* DC3000 (*Pst* DC3000) resulted in repression of *TIFY8* expression and upregulation of *JAZ* gene expression. Moreover, the study of a *tify8* mutant producing truncated versions of the TIFY8 protein rendered enhanced sensitivity and hyperinduction of *JAZ* gene expression following infection with *Pst* DC3000. Tandem affinity purification of TIFY8 revealed *in vivo* interaction with NINJA, which is mediated through the ZIM domain of TIFY8. Correspondingly, we demonstrate that TIFY8 acts as a transcriptional repressor and forms a ternary complex with NINJA and the co-repressor TOPLESS.

INTRODUCTION

Jasmonates (JAs) are plant-specific hormones that regulate processes such as vegetative growth, cell cycle progression, trichome formation, senescence, male fertility and responses to both abiotic and biotic stresses. JAs are known to control the production of a myriad of species-specific secondary metabolites. Moreover, JA signals can be integrated with signals of other plant hormones such as auxins, abscisic acid, ethylene, gibberellins and salicylic acid (SA), which fine-tunes different responses, for example during plant defence (Glazebrook, 2005; Wasternack, 2007; Bari and Jones, 2009; Pauwels *et al.*, 2009; Lackman *et al.*, 2011; Robert-Seilaniantz *et al.*, 2011; Pieterse *et al.*, 2012). Conversely, pathogens have developed diverse mechanisms to suppress plant defences and successfully infect the plants (Grant and Jones, 2009; Pieterse *et al.*, 2012). One of the best studied cases is the hemibiotrophic bacterial pathogen *Pseudomonas syringae*. Several pathovars of this species produce the phytotoxin coronatine (COR), an important virulence factor and a structural analogue of (+)-7-*iso*-Jasmonoyl-L-isoleucine (JA-Ile), the bioactive form of JAs (Fonseca *et al.*, 2009b). Following *P. syringae* infection, COR mimics JA-Ile, and thereby induces the JA signalling pathway. In turn, the activation of the JA-responses partially inhibits the SA-dependent defence responses that are triggered after *P. syringae* infection, thereby allowing bacterial colonization and symptom development (Bender *et al.*, 1998; Brooks *et al.*, 2005; Kunkel and Chen, 2006; Melotto *et al.*, 2006; Pieterse *et al.*, 2012).

The discovery of the JAZ proteins signified a breakthrough in the study of JA perception and signalling (Chini *et al.*, 2007; Thines *et al.*, 2007; Yan *et al.*, 2007). JAZ proteins act as negative regulators of JA signalling: in the absence of JAs, they bind and repress multiple transcription factors controlling the expression of JA-responsive genes. The presence of JA-Ile targets JAZ proteins for proteasomal degradation, releasing the transcription factors to regulate JA-dependent gene expression (reviewed in Chapter 1, Pauwels and Goossens, 2011; Kazan and Manners, 2012).

Arabidopsis (*Arabidopsis thaliana*) comprises 12 JAZ proteins that belong to the plant-specific TIFY family, named after the core TIF[F/Y]XG motif within the Zinc-finger protein expressed in Inflorescence Meristem (ZIM) protein domain, conserved amongst all the family members (Vanholme *et al.*, 2007; Bai *et al.*, 2011). The TIFY family can be divided in two classes, according to the presence of a C2C2-GATA domain (Figure 4.1). The Arabidopsis

genome harbours three proteins that contain a C2C2-GATA and a divergent ZIM domain, ZIM (At4g24470), ZIM-LIKE1 (ZML1, At3g21175), and ZML2 (At1g51600), which are classified as group I TIFY proteins. All 12 JAZ proteins do not contain the C2C2-GATA domain and thus belong to Class II. Other members of class II are TIFY8 (At4g32570) and the PEAPOD (PPD) proteins PPD1 (At4g14713) and PPD2 (At4g14720) (Figure 4.1).

The different domains present in the JAZ proteins (Figure 4.1) provide the specificity for protein-protein interactions that determine the differential formation of complexes in the absence or presence of the hormone (Pauwels and Goossens, 2011). All 12 JAZ proteins possess a C-terminal Jas domain (Yan *et al.*, 2007), which mediates protein-protein interactions in a JA-Ile-dependent manner. On the one hand, the Jas domain is responsible for the interaction with several transcription factors, including bHLH and R2R3-MYB that regulate different JA-dependent responses (Pauwels and Goossens, 2011). On the other hand, the Jas domain also mediates the interaction of JAZ proteins with CORONATINE-INSENSITIVE1 (COI1) (Chini *et al.*, 2007; Thines *et al.*, 2007; Yan *et al.*, 2007). COI1 is the F-box subunit of SCF^{COI1}, an E3-ubiquitin ligase complex (Feys *et al.*, 1994; Xie *et al.*, 1998). The presence of JA-Ile or COR acts as a "molecular glue" between COI1 and the JAZ proteins (Fonseca *et al.*, 2009a; Yan *et al.*, 2009; Sheard *et al.*, 2010). This interaction targets the JAZ proteins for 26S-mediated proteasomal degradation.

The ZIM domain is known to mediate homo- and heterodimerisation between JAZ proteins (Chini *et al.*, 2009; Chung and Howe, 2009) and to exert the repressor function of the JAZ proteins, as it enables the recruitment of the co-repressor TOPLESS (TPL) through interaction with the NOVEL INTERACTOR OF JAZ (NINJA) protein (Pauwels *et al.*, 2010). NINJA possesses an ETHYLENE RESPONSE FACTOR (ERF)–associated amphiphilic repression (EAR) motif through which it can interact with TPL. A subset of the JAZ proteins, i.e. JAZ5 to JAZ8, has been found to contain EAR motifs as well (Kagale *et al.*, 2010) (Figure 4.1) and were recently reported to be capable of directly interacting with TPL without a need for NINJA (Arabidopsis Interactome Mapping Consortium, 2011; Causier *et al.*, 2012; Shyu *et al.*, 2012).

Compared to the JAZs, the PPD1 and PPD2 proteins contain an additional N-terminal PPD-domain and a divergent C-terminal Jas domain (Bai *et al.*, 2011). PPD proteins have been described to regulate leaf lamina size (White, 2006) and the PPD2 protein was reported as

an interactor of the coat protein promoter of the *Tomato golden mosaic virus*, suggesting DNA-binding activity (Lacatus and Sunter, 2009).

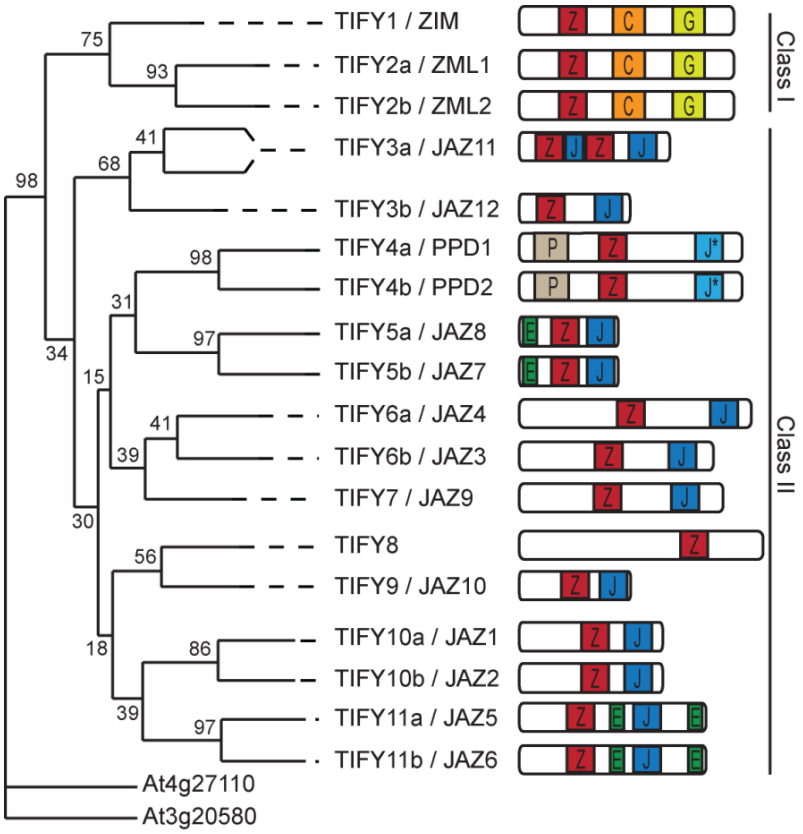


Figure 4.1. The TIFY protein family in Arabidopsis.
Phylogenetic tree of the Arabidopsis TIFY family members based on the ZIM domain (Z) protein sequence. AT4G27110 and AT3G20580 were chosen as the outgroup. AT4G27110 contains a TIFY motif but is not conserved in the domain outside this motif. Consequently, it is not considered to be a real TIFY protein. The second protein, AT3G20580, is its closest homologue within the parsed region. The numbers above the branches are bootstrap values from 100 replicates and assess the robustness of the tree. Additional protein domains are shown. C: CONSTANS, CO-like, and TOC1 (CCT) domain; G: C2C2-GATA Zn-finger; P: PEAPOD domain; J: Jas domain; J* Jas-like domain; E: EAR domain. Figure adapted from Vanholme *et al.* (2007).

The TIFY8 protein (encoded by *At4g32570*) is an atypical TIFY family member for which no other specific protein domains besides the ZIM domain have been described yet. TIFY8 has been reported to interact with the NINJA adaptor protein and a wide set of proteins in yeast two-hybrid assays (Pauwels *et al.*, 2010; Arabidopsis Interactome Mapping Consortium, 2011), and to be downregulated upon *Cabbage leaf curl virus* infection (Ascencio-Ibáñez *et al.*, 2008). To date, its function remains unknown.

In this chapter, we report the characterisation of TIFY8 as a transcriptional repressor that forms a ternary protein complex with TPL through interaction with NINJA. As such, TIFY8 can modulate gene expression, amongst which that of certain *JAZ* genes during infection with *P. syringae*.

RESULTS

TIFY8 is an atypical TIFY protein

A phylogenetic tree based on the ZIM domain sequence shows that the TIFY8 ZIM domain is closely related to that of the JAZ proteins, but TIFY8 lacks additional domains present in the TIFY family such as the Jas domain (Figure 4.1.) (Vanholme *et al.*, 2007). This atypical domain structure prompted us to study TIFY8 conservation in the plant kingdom. According to the PLAZA comparative genomics platform (http://bioinformatics.psb.ugent.be/plaza; Van Bel *et al.*, 2012) TIFY8 is present in the fern *Selaginella moellendorffii*, the moss *Physcomitrella patens* and in dicots, but appears to be lost in monocots. Within the dicots studied, TIFY8 orthologues are present as unique genes in several plant species, including Arabidopsis (Supplementary Figure S4.1).

Based on the conservation of the TIFY8 protein, we studied the alignment of TIFY8 orthologues predicted from Phytozome (http://www.phytozome.net/, Goodstein *et al.*, 2012). This alignment outlines the presence of a strongly conserved ZIM domain as well as additional conserved features of yet unknown function in the N-terminal region of these proteins (Supplementary Figure S4.2).

Unravelling TIFY8 interactors through TAP

To unravel the molecular function of TIFY8, Tandem Affinity Purification (TAP) of tagged TIFY8 expressed in Arabidopsis cell cultures (Van Leene *et al.*, 2008) was performed. Protein G/streptavidin-binding peptide (GS)-tagged TIFY8 (TIFY8-GS, hereafter called TIFY8-mTAP) was stably expressed under control of the *Cauliflower Mosaic Virus* (CaMV) 35S promoter and TIFY8 protein complexes were purified. MS analysis of co-purified proteins identified the TIFY protein PPD2, the co-repressor NINJA and a protein of yet unknown function encoded by *At4g32295* (later renamed to KIX1 protein (Chapter 5; Annex 2). These results were very similar to those obtained with the PPD2 TAP (Table 5.1).

Despite the effective identification of NINJA, PPD2 and KIX1 as TIFY8 interactors, we still performed a second TAP experiment. Two main reasons supported this approach. First, the use of a new, more sensitive, Orbitrap mass spectrometry for the identification of the isolated peptides after TAP, increasing the number of interactors retrieved (Eloy et al., 2012). Second, the possibility of performing treatments to the cell cultures prior to TAP, to identify treatment-dependent TIFY8 interacting partners. Accordingly, the new TAP experiment was performed using the same Arabidopsis cell culture as previously described (35s:TIFY8-mTAP), but in this case, besides mock-treated samples also JA-treated samples were used for TAP analysis (Annex 3). An overview of the TIFY8 interactors identified through TAP can be found in Table 4.1.

Table 4.1. Overview of prey proteins identified through TAP using TIFY8 as bait

AGI	Protein	**MALDI TOF/TOF mock**	**Orbitrap mock**	**Obitrap 50 µM JA**
TIFY proteins				
AT4G14720	PPD2	2	2	2
AT4G32570	TIFY8	**2**	2	2
Repressor proteins				
AT4G28910	NINJA	2	2	2
AT4G32295	KIX1	2	2	2
AT3G24150	KIX2		2	2
AT1G15750	TPL			2
Other proteins				
AT3G11540	SPY		2	2
AT1G51690	ATB alpha		2	2
AT5G48160	OBE2		2	2
AT3G63500	TTA2		2	2
AT3G08530/AT3G11130	Clathrin, heavy chain		1	2
AT4G23460/AT4G11380	Adaptin family protein		1	1
AT3G25800	PP2A-4			2
AT2G42500	PP2A-3			2
AT5G06600	UBP12			2

Proteins were identified using peptide-based homology analysis of MS data. Background proteins identified in control experiments were withdrawn. Number indicates the times the prey was identified in 2 experiments with each bait protein. Abbreviations: AGI, Arabidopsis Genome Identifier; PPD2, PEAPOD2; NINJA, NOVEL INTERACTOR OF JAZ; TPL, TOPLESS; SPY, SPINDLY; TTA2, TITANIA2; PP2A, PROTEIN PHOSPHATASE2A; UBP12, UBIQUITIN-SPECIFIC PROTEASE. Detailed MS data can be found in Annexes 2 and 3.

The list of interactors got notably extended by introduction of the Orbitrap technology. The two most remarkable new interactors are the only Arabidopsis KIX1 orthologue (hereafter

called KIX2 and encoded by *At3g24150*) and the repressor protein TPL. Remarkably, TPL is identified only after JA treatment, a fact that can be explained by an increase in the TPL protein pool available for interaction with TIFY8 following JA-mediated degradation of the JAZ proteins, also interacting with TPL. Notably, TAP with TIFY8 did not retrieve any of the JAZ proteins as potential interactors, in contrast to the PPD2 TAP (Chapter 5).

Our results reflect a wide diversity within the TIFY8 interactors. For instance, several protein phosphatases (PP2As) and an ubiquitin protease (UBP12) are reported. The latter has been shown to be an active ubiquitin protease that, together with its homologue UBP13, negatively regulates plant immunity (Ewan *et al*., 2011). Two PHD-finger proteins, OBERON2 (OBE2) and TITANIA (TTA2), are also found. These two proteins belong to a small protein family formed by 4 members (OBE1/2 and TTA1/2), which play a role in regulating MONOPTEROS-mediated gene expression during embryonic root meristem initiation (Saiga *et al.*, 2012). Also the N-acetylglucosaminyltransferase SPINDLY (SPY) was retrieved. SPY is known to function as a negative regulator of GA signalling and also mediates cytokinin responses in leaves and flowers (Qin *et al.*, 2011; Steiner *et al.*, 2012).
Within the framework of this doctoral thesis, we decided to focus on the interaction with NINJA and the KIX proteins, given our established interest in the first and the identification of the latters in both TIFY8 and PPD TAP experiments (Pauwels et al., 2010, Chapter 5).

TIFY8 interacts with NINJA and PPD but not the JAZs in Y2H assays

Since TAP is unable to discriminate between direct and indirect interactions within protein complexes, yeast two-hybrid (Y2H) assays were performed with special focus on the TAP hits retrieved in the first experiment (Table 4.1). Direct binding of TIFY8 to both NINJA and PPD2 was confirmed. Next, we tested truncations of TIFY8 and were able to map the ZIM domain of TIFY8 as necessary and sufficient for these interactions (Figure 4.b-c). Since the ZIM domain has been reported to be required for the formation of homo- and heterodimers within the TIFY family members (Chini *et al.*, 2009; Chung and Howe, 2009), we studied the potential interaction of TIFY8 with all class II TIFY proteins. Interaction was detected between all three non-JAZ class II TIFY proteins PPD1, PPD2 and TIFY8 itself, but not between TIFY8 and any of the JAZ proteins (Figure 4d). Interaction of all class II TIFY proteins

with NINJA was tested in parallel as a control and confirmed for most of the combinations, as previously reported (Pauwels *et al.*, 2010).

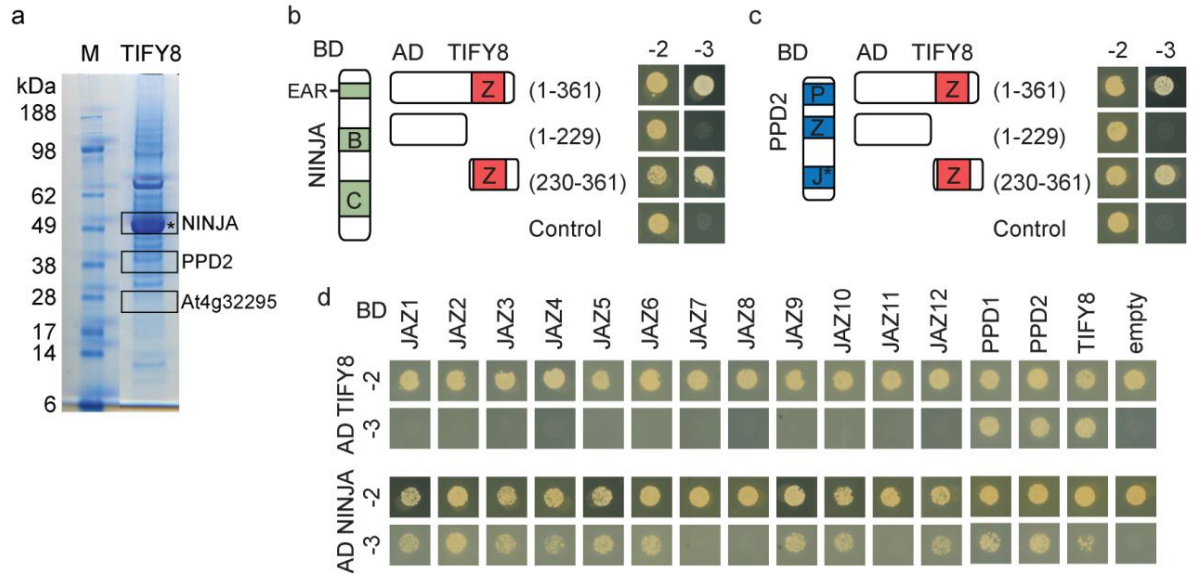


Figure 4.2. TIFY8 interacts with NINJA and PEAPOD, but not with the JAZ proteins.
a. Gel separation of proteins co-purified with TIFY8-TAP from Arabidopsis cell cultures. Boxes mark the approximated positions of proteins identified by MS after subtracting background proteins. Lane M shows molecular mass markers. The asterisk represents TIFY8, identified as bait.
b and c. TIFY8 interacts with NINJA (**b**) and PPD2 (**c**) through the ZIM domain of TIFY8 in Y2H assays. Drawings represent the proteins and their domains; numbers indicate terminal amino acid residues. Co-transformation of the PJ69-4A yeast strain with TIFY8 full-length and truncated versions, and NINJA and PPD2 in Gateway-compatible pGADT7 and pGBT9 vectors, respectively.
d. TIFY8 interacts with NINJA and PPD proteins, but not with the JAZ proteins in Y2H assays. Co-transformation of the PJ69-4A yeast strain with TIFY8 or NINJA and all TIFY family members in Gateway-compatible pGADT7 and pGBKT7 vectors, respectively. Transformed yeasts were spotted on control medium lacking Leu and Trp (-2) or selective medium additionally lacking His (-3). AD: activation domain; BD: DNA-binding domain. Controls for autoactivation are provided by transformation with the corresponding empty vector. Protein domains for TIFY8 and PPD2 are designated as in Figure 4.1.

TIFY8 recruits TOPLESS via NINJA

Interaction between TIFY8 and NINJA points towards a function of TIFY8 in transcriptional repression, which is further supported by the identification of TOPLESS through TAP (Table 4.1). Therefore, we first assessed the intracellular localisation of the TIFY8 protein. Confocal imaging of Arabidopsis plants expressing a TIFY8-GFP fusion protein showed that TIFY8 localises to the nucleus and hence can potentially play a role in the regulation of gene expression (Figure 4.3a, Supplementary Figure S4.3).

JAZ proteins function as transcriptional repressors by recruiting the repressor protein TPL through NINJA, forming a ternary repression complex (Pauwels *et al.*, 2010). Hence, we studied the capacity of TIFY8 to form such repressor protein complexes. Since TIFY8 lacks an EAR motif itself, it likely cannot directly interact with TPL. This is supported by Y2H assays in which we tested interactions with the N-terminal fragment of TPL (TPL-N), containing the LisH, CTLH and TOP domains which were shown to be essential for binding to the EAR motif and mediate other protein-protein interactions (Szemenyei *et al.*, 2008, Krogan et al., 2012). TIFY8 was unable to bind TPL-N in contrast to NINJA (Figure 4.3b). This corroborates previous reports showing direct interaction between TPL and NINJA (Pauwels *et al.*, 2010; Arabidopsis Interactome Mapping Consortium, 2011). Alternatively, we could demonstrate that NINJA can act as an adaptor protein between TIFY8 and TPL (Figure 4.3c). In conclusion, the formation of a ternary TIFY8/NINJA/TPL-N complex, suggests that, analogous to the JAZ proteins, TIFY8 might function as a transcriptional repressor.

Transient expression assays confirm TIFY8 activity as repressor

To assess repressor activity we performed transient expression assays in tobacco protoplasts. TIFY8 was fused to the GAL4 DNA binding domain (GAL4DBD) and co-expressed with a construct expressing the firefly luciferase (fLUC) reporter gene under the control of GAL4 binding elements. Targeting TIFY8 to the UAS promoter reduced basal expression strongly, comparable to the effect of JAZ1:GAL4DBD (Figure 4.3d). Taken together, our findings strongly demonstrate that TIFY8 acts as a repressor of gene expression.

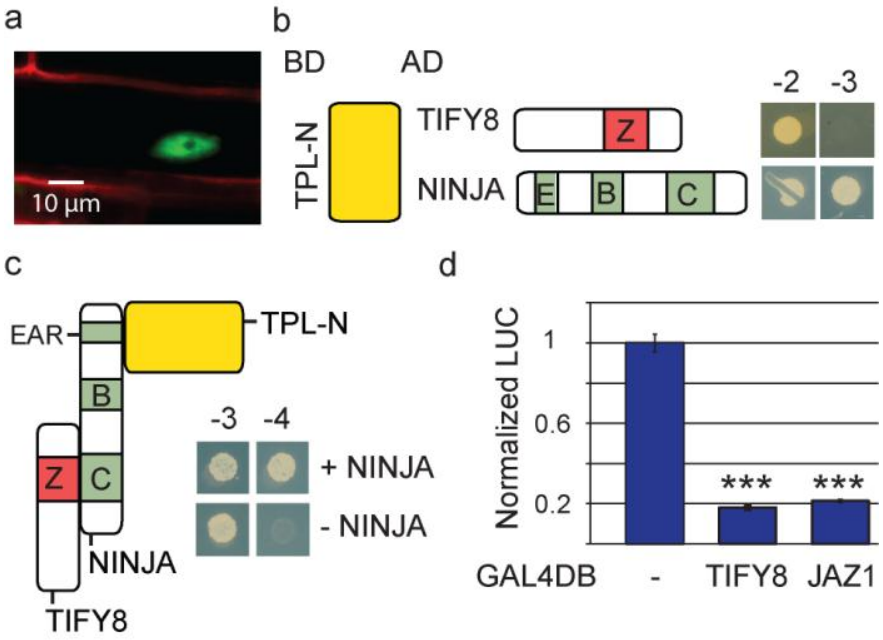


Figure 4.3. TIFY8 recruits TPL and can act as a transcriptional repressor.
a. TIFY8 localises to the nucleus. Confocal imaging of roots of a 2-week-old Arabidopsis seedling expressing the 35S:TIFY8-GFP transgene.
b. NINJA, but not TIFY8, interacts directly with TPL in Y2H assays. Co-transformation of the PJ69-4A yeast strain with TIFY8 or NINJA and the N-terminal fragment of TPL (TPL-N) in pGADT7 or pGBKT7 vectors, respectively. Transformed yeast were spotted on control medium lacking Leu and Trp (-2) or selective medium additionally lacking His (-3).
c. TIFY8 recruits TPL through interaction with NINJA in Y3H assays. Co-transformation of the PJ69-4A yeast strain with TIFY8 and TPL-N in Gateway-compatible pGADT7 and pGBKT7 vectors, respectively, together with NLS-3xFLAG-6xHis tagged NINJA in the pMG426 vector. Transformed yeast were spotted on control medium lacking Leu, Trp and Ura (-3) or selective medium additionally lacking His (-4). A negative control is provided by substitution of NINJA by the empty pMG426 vector. Protein domains for TIFY8 and NINJA are designated as in Figure 4.1 and Figure 4.2, respectively.
d. TIFY8 acts as a transcriptional repressor in transient expression assays. Transactivation activity in tobacco protoplasts transfected with a pUAS–fLUC reporter construct, effector constructs fused to GAL4DBD, and a 35S:rLUC normalization construct. Error bars represent ±SE of eight biological replicates. Asterisks represent significant differences ($p<0.001$, one-way ANOVA, Tukey HSD's Post Hoc test).

Generation of transgenic lines with altered TIFY8 expression

To help in the functional characterization of TIFY8, we generated several transgenic lines in which *TIFY8* expression was altered.

tify8-1

We selected a GABI-KAT T-DNA insertion line (Kleinboelting *et al.*, 2012) that holds a T-DNA in the first exon of TIFY8 (hereafter referred to as *tify8-1*). Sequencing analysis revealed that

the T-DNA was inserted right after the start codon of TIFY8 (Figure 4.4). Next, we investigated the generation of TIFY8 transcripts in the *tify8-1* line with multiple primer combinations covering the entire length of the gene. A reduction of 80% is seen in the TIFY8 transcript steady-state levels (Figure 4.4) when using primers specific for the first exon (primer combination 1 in Figure 4.4a). Unexpectedly, transcripts of downstream exons were present in levels that slightly exceeded those of wild-type Arabidopsis plants (Figure 4.4b). Likely, the latter can be accounted to the fact that the T-DNA contains a 35S promoter sequence before the right border of the T-DNA (Kleinboelting *et al.*, 2012) (Figure 4.4a) or to the existence of a regulatory region acting as an alternative promoter within the *TIFY8* sequence, which might lead to the generation of transcripts containing downstream exons. The first ATG present in frame in exon 2 would yield a truncated TIFY8 missing 110 N-terminal amino acids. Although we cannot conclude with certainty that *tify8-1* is a knock-out line, we assume it can be considered as a loss-of-function mutant expressing no or truncated versions of the TIFY8 protein.

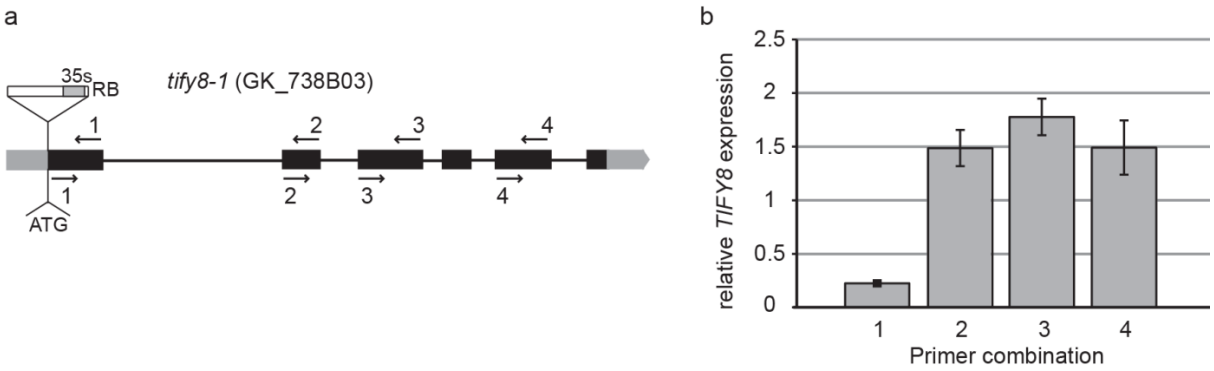


Figure 4.4. The tify8-1 T-DNA line generates truncated TIFY8 transcripts.
a. Schematic diagram of the *TIFY8* (At4g32570) locus. Black bars, black lines and grey bars represent exons, introns and the untranslated regions, respectively. The T-DNA in the *tify8-1* line (GK_738B03) is inserted immediately after the start codon of *TIFY8*, and the T-DNA contains the 35S promoter sequence next to the right border (RB). Arrows and numbers indicate different primer combinations covering different regions of *TIFY8*. Primer sequences can be found in Annex 1.
b. qRT-PCR analysis of TIFY8 transcripts in the *tify8-1* line. Transcript levels were studied in 5-week-old Arabidopsis *tify8-1* rosette leaves. Numbers represent the primer combination used, described in (a). *UBQ10* (*At4g05320*) was used as internal control and expression values (Y-axis) were normalised to those of the wildtype. Error bars represent ±SE of three biological replicates.

TIFY8 overexpression and silencing lines

Next to the loss-of-function *tify8-1*, we also generated lines overexpressing either the full length or truncated versions of the TIFY8 protein (Supplementary Figure S4.2) and TIFY8 was silenced by means of RNA interference (RNAi). For overexpression, the full length *TIFY8* was cloned and expressed under the control of the CaMV 35Spromoter and fused or not to a C-terminal GS TAP tag, hereafter referred to as TIFY8-mTAP or TIFY8 OE, respectively. The truncated versions of TIFY8 were designed based on the N-terminal conserved region (hereafter called TIFY8-NT) and the C-terminal region containing the conserved ZIM domain (hereafter referred to as TIFY8-CT), cloned and expressed under the CaMV 35S promoter without terminal tags. Finally, for the TIFY8-RNAi lines, a *TIFY8* fragment was cloned in a specific vector leading to hairpin RNA constructs in order to silence *TIFY8* expression (for details, see Experimental Procedures).

It is noteworthy that overexpression of the full length *TIFY8* led to smaller plants when grown *in vitro* and in early stages of plant growth (Figure 4.5a). The penetrance of the phenotype seemed to be directly related to the *TIFY8* expression levels, as revealed by qRT-PCR (Fig 4.5b). However, the TIFY8 OE lines grew normally when transferred to soil, and no significant differences in later development were observed.

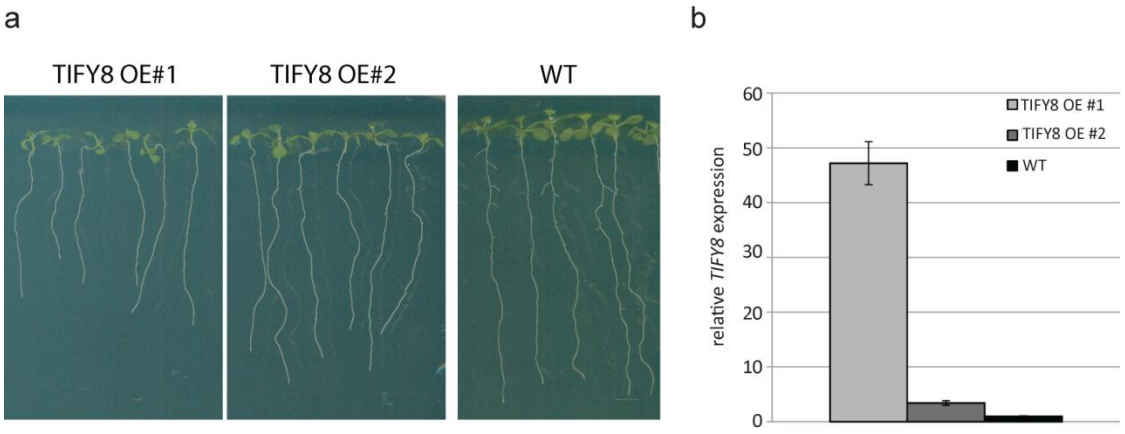


Figure 4.5. TIFY8 overexpression seedlings are smaller.
a. 7-day-old Arabidopsis seedlings grown vertically on plates MS solid media provided with sucrose.
b. qRT-PCR analysis of TIFY8 expression levels in the TIFY8 OE lines compared to wild type. Transcript levels were studied in two-week-old Arabidopsis TIFY8 OE and wildtype seedlings. *UBQ10* (*At4g05320*) was used as internal control and expression values were normalised to those of the wildtype. Error bars represent ±SE of three technical replicates.

In contrast, none of the plant lines overexpressing TIFY8-mTAP or the truncated TIFY8 fragments showed this phenotype, although high transgene overexpression levels were also detected. The *TIFY8*-RNAi lines did not show any remarkable phenotype either, and all developed normally (data not shown).

Altered expression of TIFY8 does not affect JA-responses

As a first approach to unravel whether TIFY8 could play a role in jasmonate signalling as the JAZ proteins do, the JA-responsiveness in transgenic lines with impaired *TIFY8* expression was studied. We focused on two of the most commonly used parameters to score JA-responsiveness in Arabidopsis: main root growth and anthocyanin accumulation, which are affected by JAs in a dose-dependent manner. On the one hand, JAs are known to decrease root growth by affecting both cell division and root meristem size (Pauwels *et al.*, 2009; Chen *et al.*, 2011). The JA-mediated inhibition of root growth has been used in numerous screens searching for key components of JA-signalling, with the characterization of the JA-insensitive mutant *coi1-1* as the most prominent example (Xie *et al.*, 1998). On the other hand, JA promotes the production of a myriad of species-specific secondary metabolites, including anthocyanins in Arabidopsis (Pauwels *et al.*, 2009; De Geyter *et al.*, 2012).

Here, we studied the JA-mediated responses of the *tify8-1* insertion line, the TIFY8-OE and the TIFY8-NT overexpression lines. JA-responsiveness of these transgenic lines together with wild-type (Col-0) was scored in terms of root growth and anthocyanin accumulation in seedlings grown on control MS media and on increasing MeJA concentrations. The hormone dose (2.5 and 10 µM MeJA) was chosen based on previous assays with wild-type and transgenic Arabidopsis lines as these concentrations, although relatively low, are optimal to report on different JA-responses between the lines tested (Master Thesis Astrid Nagels Durand). An overview of the outcome of these assays is found in Figure 4.6. In the case of the TIFY8-OE lines, however, the assay had to be slightly modified. The MeJA concentrations used before (2.5 and 10 µM) turned out to be too high and the small size of the plants, together with the MeJA-mediated growth inhibition, impeded an accurate measurement of the root length at the highest MeJA concentration (data not shown). Consequently, the two independent TIFY8-OE lines together with wild-type (Col-0) were tested on control MS media and 0.5 and 1 µM MeJA concentrations.

In the case of the *tify8-1* line, no significant differences were found except in plants grown on 2.5µM MeJA, but not on 10µM, and the T-DNA line behaved similarly to wild-type for both the inhibition of root growth and the accumulation of anthocyanins.

Despite their smaller size, the plants overexpressing the full-length TIFY8 overall responded normally to JA treatment. As mentioned before, the overexpressing lines are shorter than wild-type and significant differences between the lines were reported in control conditions. Strikingly, the interaction of the genotype and treatment turned out to be significant in terms of root length inhibition when the plants were grown in media supplied with 1µM MeJA, suggesting that TIFY8-ovexpression lines are hypersensitive to JAs. Nevertheless, this possibility was not further supported by the measurement of root growth inhibition when plants were grown on media supplied with 0.5µM MejA nor in the anthocyanin assays, where no significant differences between the lines were encountered.

Finally, in the case of plants overexpressing TIFY8-NT, the interaction between genotype and treatment was not significant for any of the conditions assayed for neither MeJA-mediated inhibition of root growth nor the anthocyanin accumulation assay.

Taken together, our results suggest that misregulation of *TIFY8* does not lead to altered JA-responsiveness.

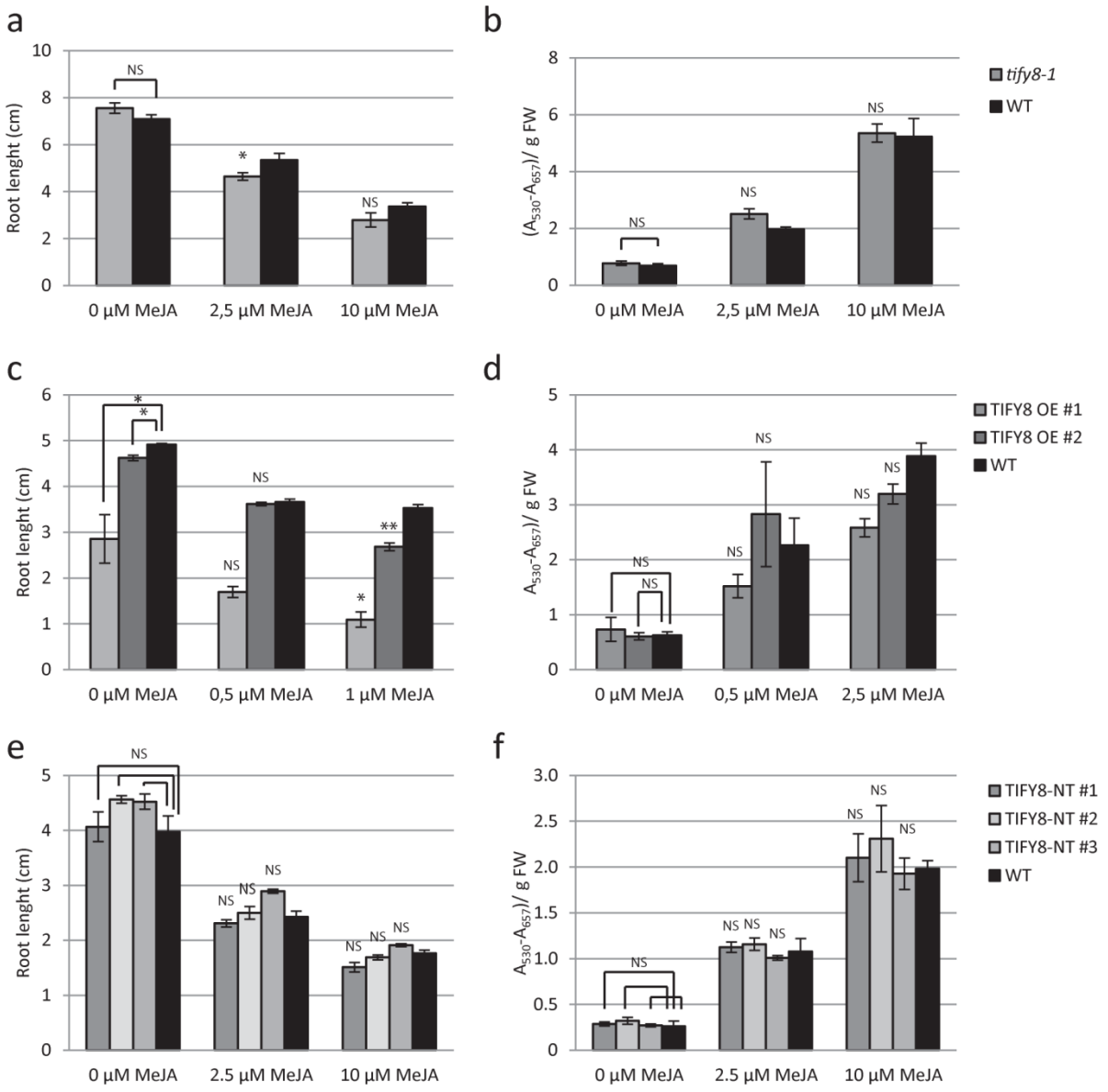


Figure 4.6. JA-mediated inhibition of root growth and anthocyanin accumulation in transgenic plants with altered TIFY8 expression. Three different TIFY8 transgenic constructs were tested: the *tify8-1* mutant line (**a**, **b**), two independent TIFY8-OE lines (**c**, **d**) and three independent truncated TIFY8 overexpression lines (**e**, **f**). Root growth inhibition was scored on 11 DAS (**a**, **c** and **e**); anthocyanins were extracted for the same samples used for root growth but harvested 14 DAS (**b**, **d**, and **f**). Four technical repeats per line and treatment, consisting on up to eight seedlings per repeat ($20 \leq n \leq 32$), were analysed. Bars represent average ± SE. Differences between the lines assayed in control conditions are shown (NS: no significant, *: $p<0.05$; t-test). Statistically significant differences for the interaction between genotype and treatment are shown (NS: no significant, $p>0.05$; *: $p<0.05$; **: $p<0.01$, one-way ANOVA).

TIFY8 protein is stable upon JA treatment

Alternative to the JAZ proteins, TIFY8 does not contain a Jas domain within its sequence. Consequently, TIFY8 is presumably not recruited by COI1 and might not be affected by JA treatment. To confirm this hypothesis, we tested the stability of the TIFY8 and the JAZ1 proteins by analysing protein accumulation in Arabidopsis seedlings overexpressing either the 35S:TIFY8-GS tag (TIFY8-mTAP) or the 35S:JAZ1-GS tag (JAZ1-mTAP) when treated for 1h with 50µM JA (Figure 4.7). Our results show that, in contrast to JAZ1, the TIFY8 protein is stable upon JA treatment.

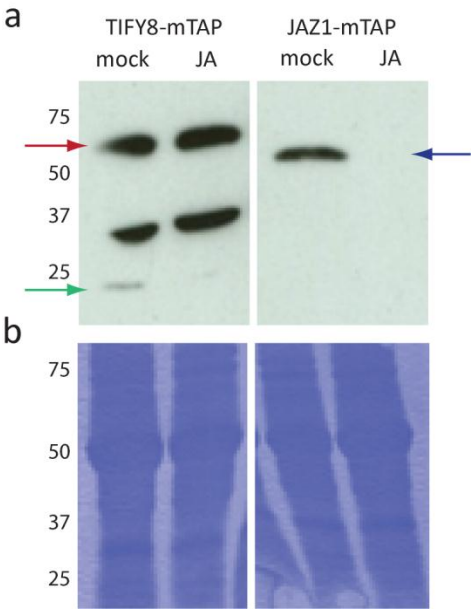


Figure 4.7. TIFY8 is stable upon JA treatment. Western Blot analysis of 10 day-old Arabidopsis seedlings overexpressing the TIFY8- or JAZ1-mTAP fusions after 1h treatment with either 50 µM JA or ethanol (mock). **a.** Western Blot using the Peroxidase Anti-Peroxidase (PAP) antibody. Red and blue arrows indicate the position of the TIFY8-mTAP and JAZ1-mTAP fusion proteins, respectively. The green arrow indicates the size of the mTAP-tag. **b.** Coomassie staining of the blot for verification of equal protein loading in gel.

TIFY8 expression is not JA-induced but is repressed by *Pst* DC3000 infection

Our previous results suggest that TIFY8 might not be directly involved in JA signalling, as altered *TIFY8* gene expression does not modify JA-responsiveness and JA treatment does not alter the stability of the TIFY8 protein (Figures 4.6 and 4.7, respectively). Furthermore, in contrast to the *JAZ* genes, *TIFY8* is not induced upon JA treatment (Vanholme *et al.*, 2007, Marcel Van Verk, personal communication). Therefore, we mined public microarray data and found that *TIFY8* gene expression is altered by different (a)biotic stresses. Amongst them, the hemibiotrophic pathogen *P. syringae* pv. *tomato* DC3000 (*Pst* DC3000) causes downregulation of *TIFY8* (http://www.genevestigator.com; Hruz *et al.* (2008)).

To validate these observations, bioassays with the virulent *Pst* DC3000 strain were performed in the lab of Prof. Dr. ir. Corné M.J. Pieterse, in which the transcriptional response of wild-type Arabidopsis was compared with that of the *tify8-1* line. Plant samples were harvested prior to and 24h after inoculation with *Pst* DC3000. *TIFY8* expression was analysed and found to be significantly downregulated following *Pst* DC3000 infection, in agreement with the public microarray data (Figure 4.8). Downregulation of *TIFY8* expression also occurred in the *tify8-1* line, suggesting that *TIFY8* regulation is not abolished by the T-DNA insertion and might occur post-transcriptionally.

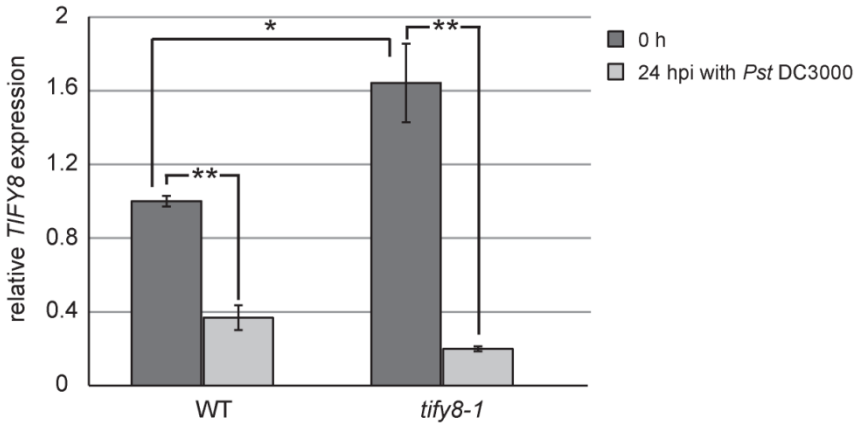


Figure 4.8. *TIFY8* is downregulated after infection with *Pst* DC3000. qRT-PCR analysis of *TIFY8* expression in wild-type and *tify8-1* plants after infection with *Pst* DC3000. Transcript levels were studied in 5-week-old Arabidopsis rosette leaves prior to (0 h) and 24 h post inoculation (24 hpi) with *Pst* DC3000.
Primer combination number 4 from Figure 4.4 was used to study *TIFY8* expression. *UBQ10* (*At4g05320*) was used as internal control and expression values were normalised to those of the wildtype prior to infection with *Pst* DC3000. Error bars represent ±SE of three biological replicates. Asterisks correspond to significant differences between wild-type and the *tify8-1* line prior to infection with *Pst* DC3000 (*: $p<0.05$; t-test) and within each of the lines prior to and after infection with *Pst* DC3000 (**: $p<0.01$; t-test).

tify8-1 plants are more sensitive to *Pst* DC3000 infection

Next to the analysis of *TIFY8* gene expression levels after infection with *Pst* DC3000, we performed bioassays to score the sensitivity of the *tify8-1* line. In these assays, *tify8-1* turned out to be slightly more susceptible to *Pst* DC3000 infection than wild-type. Three days post inoculation, bacterial growth was significantly increased, and a higher proportion of leaves showing disease symptoms in comparison to the wild-type was observed.

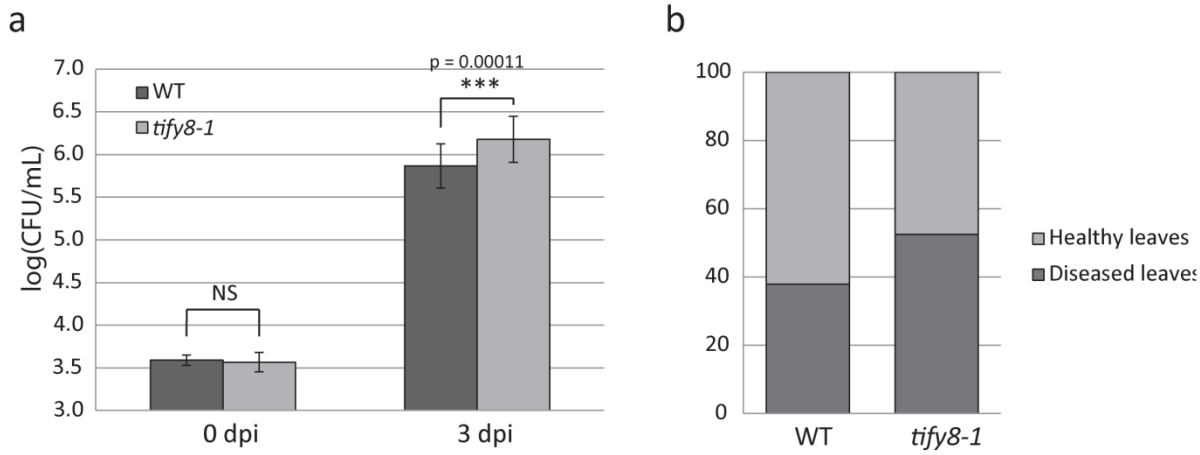


Figure 4.9. ***tify8-1* is more susceptible to *Pst* DC3000 infection. a.** Growth of *Pst* DC3000 in wild-type and *tify8-1* plants after inoculation. Bacteria were isolated from leaves 5 minutes and 3 days post inoculation (0 and 3 dpi, respectively). Data points represent the average of eight or sixteen samples for 0 and 3dpi, respectively. Error bars represent the standard deviation and asterisks represent significant differences (t-test, NS= no significant, ***p= 0.0001). **b.** Percentage of diseased and healthy leaves in wild-type and *tify8-1* plants 3dpi with *Pst* DC3000. Disease was annotated based on the presence of chlorosis.

JAZ gene induction upon *Pst* DC3000 infection is enhanced in *tify8-1*

Having verified the downregulation of *TIFY8* following *Pst* DC3000 infection and the slightly increased sensitivity of the *tify8-1* line, a pilot cDNA-AFLP transcript profiling experiment was performed to screen for genes that could be misregulated in the *tify8-1* line compared to wild-type. We identified 44 transcripts that were expressed differentially between the two lines. Within the selected gene set, *JAZ10* was upregulated following infection with *Pst* DC3000, but remarkably stronger in the *tify8-1* background than in the wild-type (Supplementary Figure S4.4). qRT-PCR analysis confirmed this observation (Figure 4.10). Our results are in agreement with those recently reported by Demianski *et al.* (2012), who showed that expression of several *JAZ* genes was induced after *Pst* DC3000 infection. This prompted us to test whether more *JAZ* genes might be hyperinduced in the *tify8-1* line. The

expression of all 12 *JAZ* genes was verified in qRT-PCR experiments which showed that, besides *JAZ10*, other *JAZ* genes were also hyperactivated in the *tify8-1* background upon infection by *Pst* DC3000 (Figure 4.11).

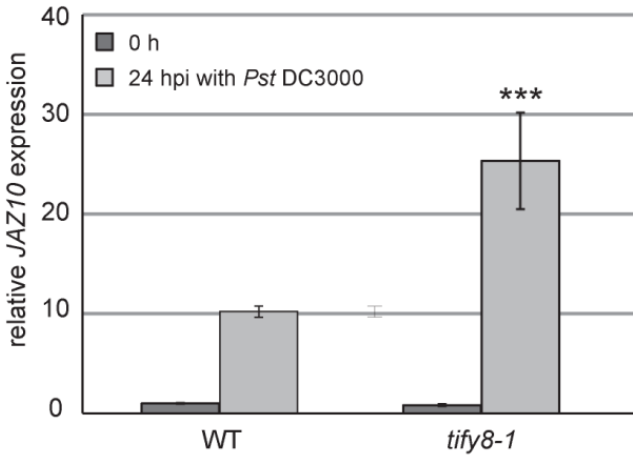
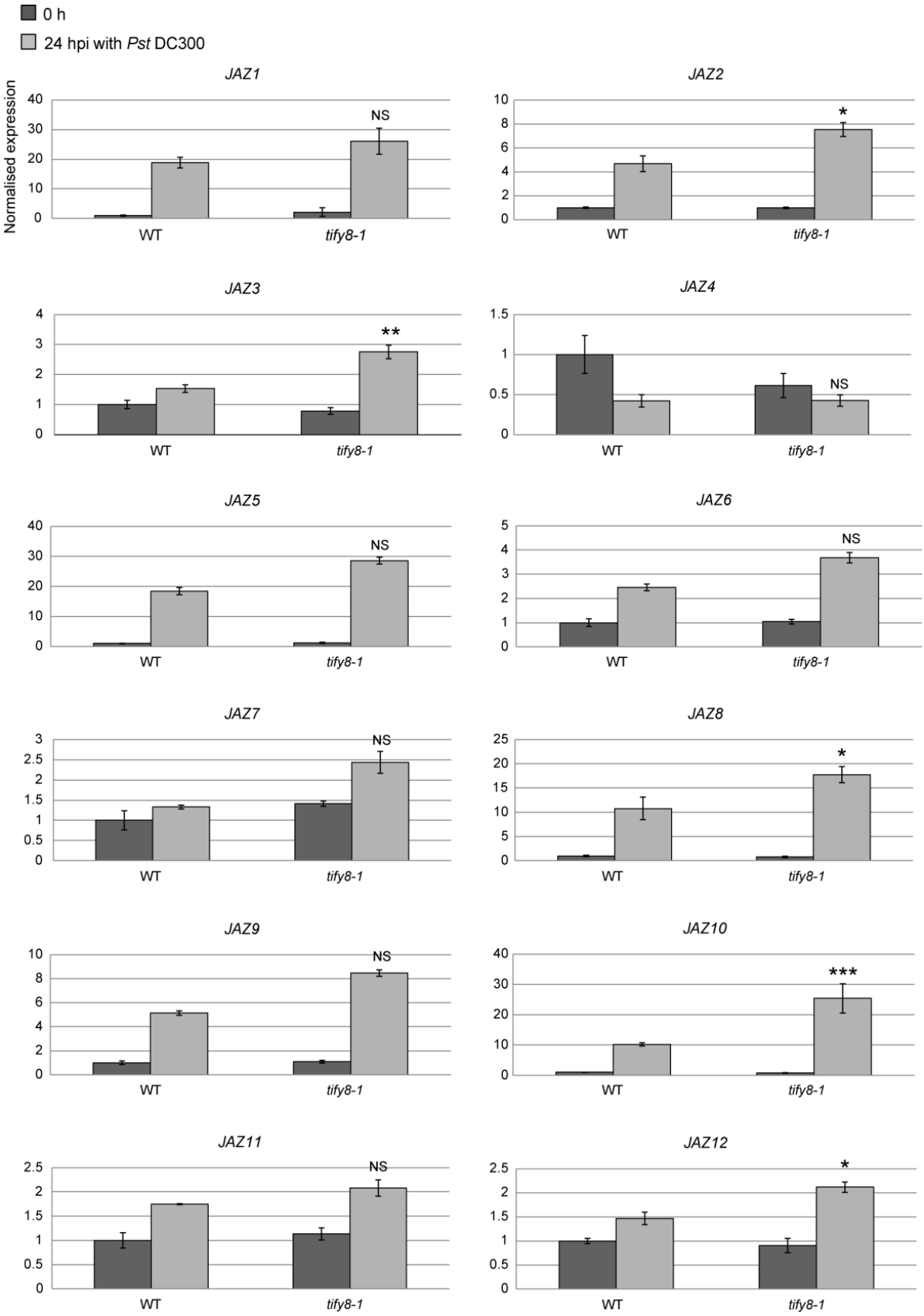


Figure 4.10. *JAZ10* is hyperinduced in the *tify8-1* line after infection by *Pst* DC3000. *JAZ10* expression in wild-type and *tify8-1* plants after infection with *Pst* DC3000 was studied. Transcript levels were studied in 5-week-old Arabidopsis rosette leaves prior to (0 h) and 24 h post inoculation (24 hpi) with *Pst* DC3000. *UBQ10* (*At4g05320*) was used as internal control and expression values were normalised to those of the wildtype prior to infection with *Pst* DC3000. Error bars represent ±SE of three biological replicates. Asterisks correspond to significant differences for the interaction between genotype and treatment (***: p<0.001; one-way ANOVA).

Figure 4.11. A subset of *JAZ* genes is hyperinduced following *Pst* DC3000 infection in the *tify8-1* line (see next page)

qRT-PCR analysis of *JAZ* expression in wild-type and *tify8-1* plants. Transcript levels were studied in 5-week-old Arabidopsis rosette leaves prior to (0 h) and 24 h post inoculation (hpi) with *Pst* DC3000. Primer sequences are provided in Annex 1. *UBQ10* (*At4g05320*) was used as internal control and expression values were normalised to those of the wild-type prior to infection. Error bars represent ±SE of three biological replicates. Asterisks represent significant differences for the interaction between genotype and treatment (NS: p>0.05, *: p<0.05, **: p<0.01, ***: p<0.001; one-way ANOVA).


0 h
24 hpi with Pst DC300
Normalised expression
JAZ1
JAZ2
JAZ3
JAZ4
JAZ5
JAZ6
JAZ7
JAZ8
JAZ9
JAZ10
JAZ11
JAZ12
NS
*
**
***
WT
tify8-1

TIFY8 repression by *Pst* DC3000 is COR-dependent but *JAZ* hyperinduction in the *tify8-1* line cannot be mimicked by exogenous COR

Induction of *JAZ* genes during infection with *Pst* DC3000 occurs in a COR-dependent manner (Demianski *et al.*, 2012). To verify whether the observed link between the regulation of *TIFY8* and *JAZ* gene expression was also COR-dependent, we analysed their expression in Arabidopsis seedlings exogenously treated with COR (Figure 4.12). *TIFY8* expression was downregulated after COR treatment, both in the wild-type and the *tify8-1* line, suggesting that the effect of *Pst* DC3000 infection on *TIFY8* expression is mediated by COR (Figure 4.12a). In agreement with the previous reports most of the *JAZ* genes, including *JAZ10*, were induced by COR (Figure 4.12b) (Demianski *et al.*, 2012). In contrast, no hyperinduction of any of the *JAZ* genes could be detected in the *tify8-1* line, suggesting that this effect is either not dependent on COR or involves multiple factors besides COR.

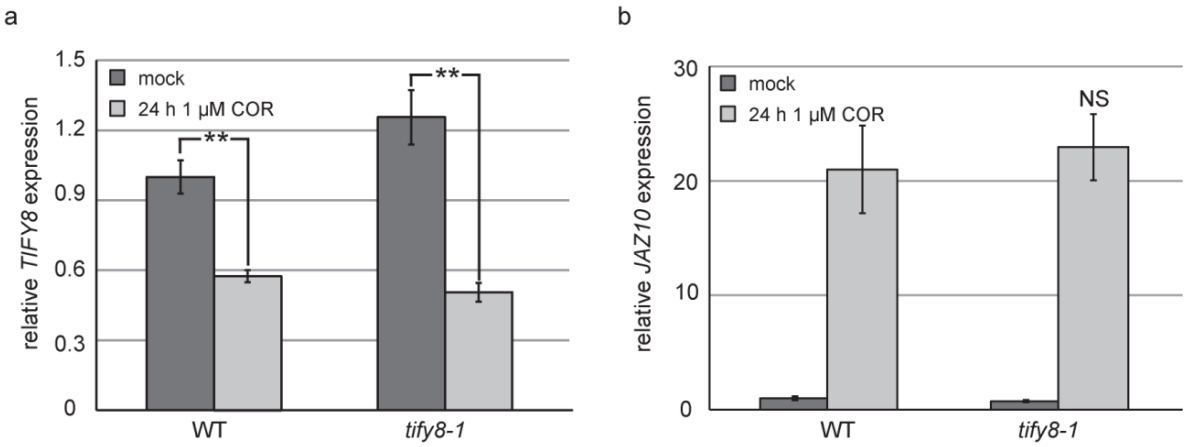


Figure 4.12. Exogenous COR treatment mediates *TIFY8* downregulation but not *JAZ* hyperinduction in the *tify8-1* line.
qRT-PCR analysis of *TIFY8* (a) and *JAZ10* (b) expression in wild-type and *tify8-1* plants upon exogenous COR treatment. Transcript levels were studied in 9-day-old Arabidopsis seedlings. Wild-type and *tify8-1* seeds were germinated on liquid MS media and, after 8 days, treated either with 1µM COR or DMSO (mock-treatment) for 24 h. Primer combination number 4 from Figure 4.4a was used to study *TIFY8* expression (a). *UBQ10* (*At4g05320*) was used as internal control and expression values were normalised to those of the mock-treated wildtype. Error bars represent ±SE of five biological replicates. Asterisks in (a) correspond to significant differences for the treatment (**: $p<0.01$; t-test); in (b) correspond to significant differences for the interaction between genotype and treatment (***: $p<0.001$, NS: $p>0.05$; one-way ANOVA).

JAZ10 **hyperinduction in the** ***tify8-1*** **line is mimicked by the overexpression of the C-terminal region of** ***TIFY8***

Within the bioassays performed in the lab of Prof. Dr. ir. Corné M. J. Pieterse, other transgenic lines with altered *TIFY8* expression besides the *tify8-1* mutant were tested, such as the previously presented TIFY8 overexpression (TIFY8-OE) and TIFY8 silenced lines (TIFY8 RNAi) (for *TIFY8* expression levels in these lines, see Supplementary Figure S4.5). Remarkably, *JAZ10* hyperinduction following bacterial infection was not detected in any of the latter lines (Figure 4.13a), suggesting that this effect is inherent to the special characteristics of the T-DNA insertion line. Indeed, the *tify8-1* line may produce no or truncated versions of the TIFY8 proteins which, presumably, may lack the first TIFY8 exon translated to the N-terminal region of TIFY8 but retain the C-terminal region that includes the ZIM domain (see Figure 4.4). According to these observations, the TIFY8-CT overexpression line could be considered as mimic of the *tify8-1* line. To verify this hypothesis, *JAZ10* induction in the TIFY8-CT overexpression line was analysed following *Pst* DC3000 infection. *JAZ10* expression in this line showed an induction level intermediate to that of the *tify8-1* and the wild-type lines (Figure 4.13b), which indicates that the overaccumulation of truncated TIFY8 proteins is correlated with the hyperinduction of *JAZ10*.

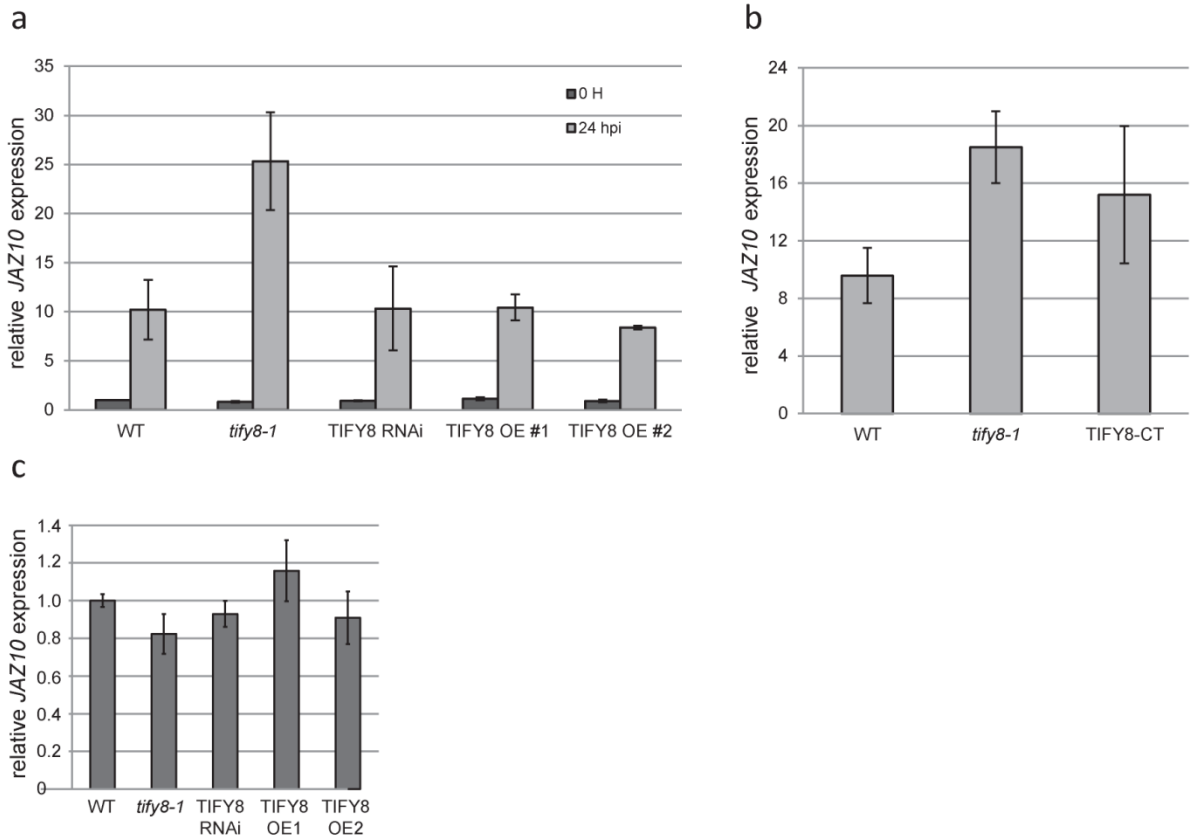


Figure 4.13. *JAZ10* is hyperinduced in the *tify8-1* and TIFY8-CT lines after infection by *Pst* DC3000. JAZ10 expression in wild-type and transgenic plants with altered TIFY8 expression after infection with Pst DC3000 was studied. Transcript levels were studied in 5-week-old Arabidopsis rosette leaves prior to (0 h) and 24 h post inoculation (24 hpi) with Pst DC3000. UBQ10 (At4g05320) was used as internal control and expression values were normalised to those of the wildtype prior to infection with Pst DC3000 (c) shows a zoomed image of JAZ10 expression prior to Pst infection in (a). Error bars represent ±SE of three biological replicates. No significant differences for the interaction between genotype and treatment were found in (a, b) ($p>0.05$, one-way ANOVA) nor by genotype in (c) ($p>0.05$, t-test).

A model for TIFY8: its conserved regions mediate the interaction with a transcription factor modulating *JAZ* gene expression upon *Pst* DC3000 infection

The data previously presented suggests that the different conserved regions/domains in TIFY8 mediate the interaction with transcription factor(s) eventually modulating *JAZ* gene expression upon infection with *Pst* DC3000. Here, we propose a model which can explain the different outcomes detected in the various plant lines analysed.

In wild-type, the full-length TIFY8 protein (or $TIFY8_{WT}$) is produced, which interacts with NINJA and recruits TPL. Moreover, $TIFY8_{WT}$ is able to interact with a transcription factor "X" different to MYC2 for the repression of *JAZ* gene expression (Figure 4.14a). This hypothesis is supported by the recently reported partial MYC2-independent *JAZ* gene induction upon *Pst* DC3000 infection (Demiansky et al., 2012) and the observation that TIFY8 does not interact with MYC2 in TAP nor in Y2H assays (Table 4.1 and data not shown, respectively).

In the *tify8-1* line, however, no $TIFY8_{WT}$ but truncated TIFY8 forms missing part of the N-terminal region are present (referred to as TIFY8*). Following infection with *Pst* DC3000, *JAZ* gene hyperinduction is detected in the *tify8-1* line, which could be explained by the protein-protein interaction capacities of TIFY8*. Taken together, our results pinpoint the N-terminal region of TIFY8, missing in TIFY8*, as the one mediating the interaction with the corresponding transcription factor "X". (Figure 4.14b).

Finally, in the TIFY8-CT line, truncated versions of the TIFY8 protein are overexpressed (referred to as TIFY8-CT). These TIFY8-CT forms are not effective interactors of the corresponding transcription factor "X" and thus *JAZ* hyperinduction as in the *tify8-1* line is expected. However, the TIFY8-CT line shows an intermediate phenotype for *JAZ* hyperinduction following *Pst* DC3000 infection (Figure 4.14c), a fact that can be explained by the presence of endogenous $TIFY8_{WT}$ forms, given that the TIFY8-CT transgenics were generated in the Col-0 ecotype background.

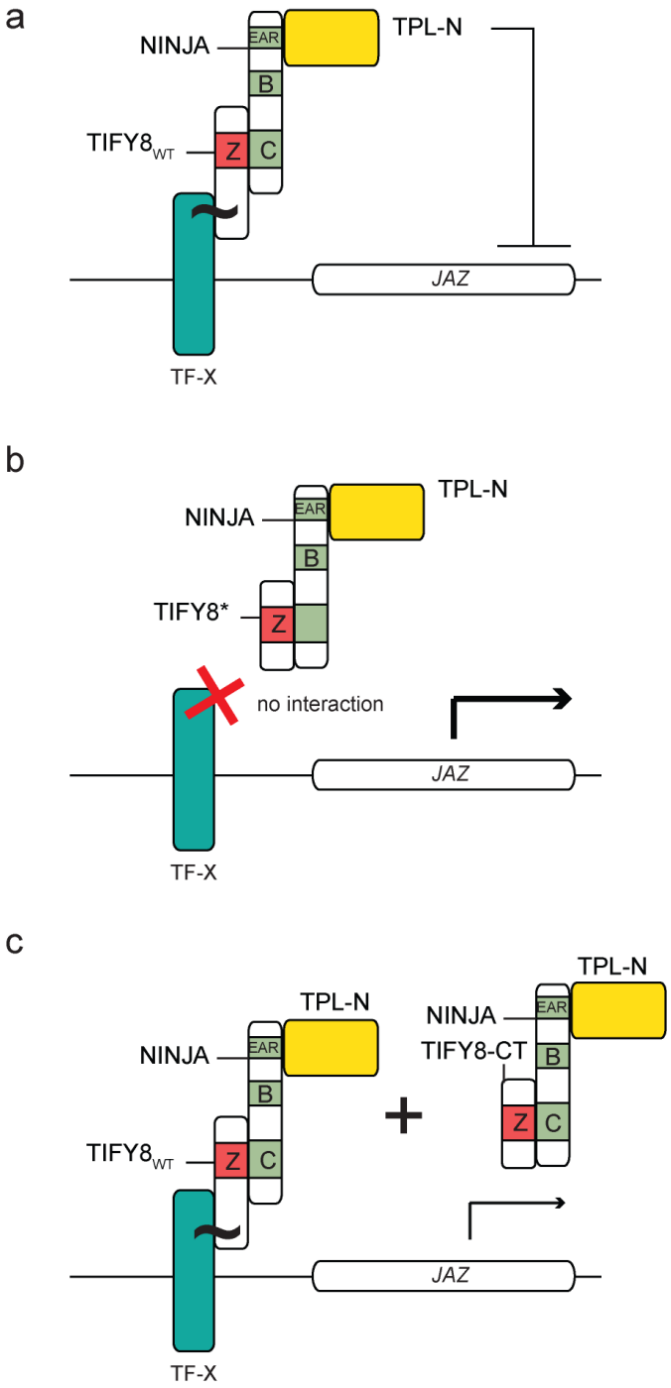


Figure 4.14. Model for TIFY8-mediated repression of gene expression upon *Pst* DC3000 infection. **a**. In wild-type plants, TIFY8 wild-type ($TIFY8_{WT}$) protein represses *JAZ* expression recruiting TPL by interaction with NINJA and binding yet unknown transcription factor 'X' (TF-X) through the conserved TIFY8 N-terminal region.
b. *tify8-1* produces truncated versions of the protein (TIFY8*) missing the N-terminal conserved region but retaining the ZIM domain. Accordingly, TIFY8* is able to interact with NINJA but not with TF-X and thus *JAZ* gene hyperinduction is detected. **c.** The TIFY8-CT overexpresses the C-terminal region of TIFY8, containing the ZIM domain. In this line, generated in the wild-type background, both $TIFY8_{WT}$ and TIFY8-CT proteins are present. Accordingly, a balance between repression and release of *JAZ* expression occurs, yielding an intermediate *JAZ* expression hyperinduction.

TIFY8 expression is inversely correlated to that of some JAZ genes

Our findings show that infection with *Pst* DC3000 triggers an opposite effect on *TIFY8* and *JAZ* gene expression (down- and upregulation, respectively). Notably, *TIFY8* shows an expression pattern opposite to that of *JAZ10* and other *JAZ* members, not only upon *Pst* DC3000 infection, but throughout plant development when analysing gene expression with publicly available microarray data (http://www.genevestigator.com; Hruz T *et al.*, 2008) or by means of the eFP Browser facility (http://bar.utoronto.ca/efp/cgi-bin/efpWeb.cgi; Winter *et al.*, 2007; Supplemental Figure S4.6). Taken together, these observations suggest that TIFY8 might negatively regulate *JAZ* gene expression.

Study of the _TIFY8_ promoter expression shows a non-overlapping pattern to that of _JAZ1_

To study TIFY8 expression pattern throughout the plant life span and compare it to that of the JAZ1 gene, we generated transgenic lines carrying the *TIFY8promoter::GUS:GFP* translational fusion. Nuclear localization was previously confirmed through confocal imaging of Arabidopsis seedlings overexpressing the TIFY8-GFP transgene (Figure 4.3). Here, we studied the overall expression pattern by means of GUS stains (Figure 4.15).

Sampling seedlings at several time points during early development showed that *TIFY8* is expressed both in shoots and roots. In shoots, the *TIFY8* promoter seems to be expressed in cotyledons and young true leaves, where its expression gradually diminishes towards the base of the leaf during its development (Figure 4.15a, b and j). Strong GUS induction was detected on the shoot apical meristem and emerging leaves (Figure 4.15e). In roots, we detected very strong *TIFY8* promoter expression in the root tip, which could be pinpointed to the quiescent centre and columella cells, (Figure 4.15g). Consistent with Genevestigator and eFP observations, we confirmed that the *TIFY8* promoter expression pattern is generally contrary to that of *JAZ1*. Two examples of this inversely correlation are the promoter expression levels detected in the root tip and cotyledons. In root tips, *TIFY8* promoter is strongly expressed whereas that of *JAZ1* seems to be inactive. Vice versa, the *TIFY8* promoter is not expressed in the cotyledon tip while that of *JAZ1* is clearly induced (Figures 4.15e-f and 4.15c-d, respectively). Additionally, *TIFY8* promoter expression was found in lateral roots, with high expression levels in the elongating region and the lateral root tip (Figure 4.15i). In later stages, no *TIFY8* promoter expression was detected in mature rosette

leaves (data not shown). In flowers, *TIFY8* promoter expression was restricted to younger flowers and no expression in older flowers nor in siliques was detected, whereas the *JAZ1* promoter was expressed in the stigma at later stages of flower development and both in the base and the tip of the siliques (Figure 4.15l-m).

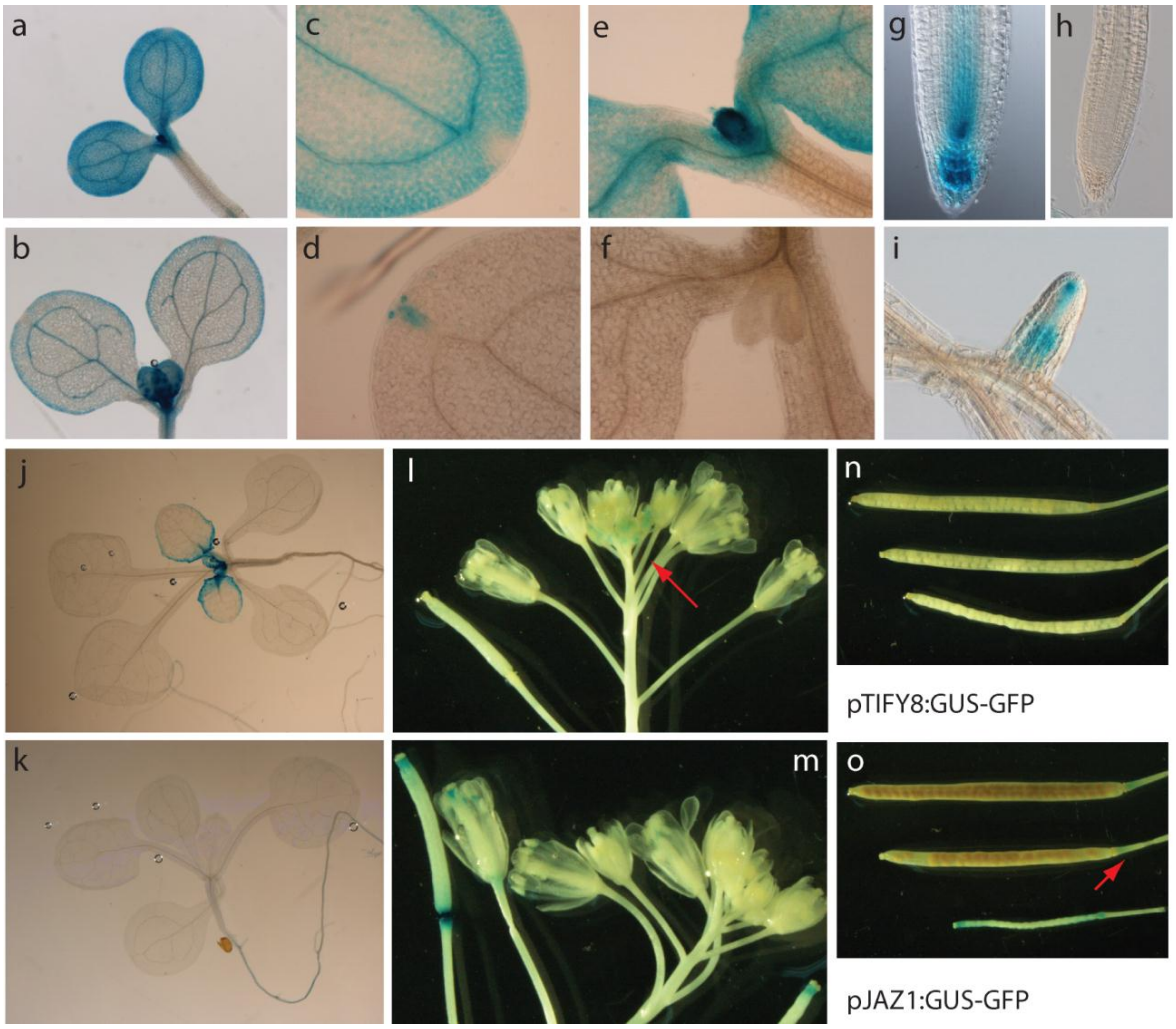


Figure 4.15. Overview or *TIFY8* and *JAZ1* promoter gene expression. GUS stains of Arabidopsis plants expressing either *TIFY8* or *JAZ1* promoter fusions to GUS and GFP. **a, b**. *TIFY8* promoter expression pattern in 5 and 7 day-old seedlings, respectively. **c, d.** *TIFY8* (**c**) and *JAZ1* (**d**) promoter expression in 5 day-old cotyledons. **e, f.** *TIFY8* (**e**) and *JAZ1* (**f**) promoter expression in the shot apical meristem and emerging leaves of 5 day-old seedlings. **g, h.** *TIFY8* (**g**) and *JAZ1* (**h**) promoter expression in the root tip of 5 day-old Arabidopsis seedlings. **i.** *TIFY8* promoter expression in lateral root of a 10 day-old seedling. **j, k.** TIFY8 (**j**) and JAZ1 (**k**) promoter expression in 14 day-old seedlings. **l-o.** *TIFY8* and *JAZ1* promoter expression in flowers (**l, m**) and siliques (**n, o**).

DISCUSSION

Within the plant-specific TIFY family, the JAZ proteins are the best studied members, as they are key regulators of JA signalling (Chapter 1). Functional characterisation of the other TIFY family members lags behind and in-depth studies on these proteins are scarce. In this chapter, we started characterising the TIFY8 protein.

TIFY8 expression is inversely correlated to that of several *JAZ* genes

To get insight in the biological processes TIFY8 is involved in, we consulted public microarray data, searching for conditions affecting *TIFY8* expression, given that altered *TIFY8* expression does not seem to affect JA-dependent responses and that the TIFY8 protein is stable upon JA treatment (Figures 4.6 and 4.7). Remarkably, *TIFY8* expression is not induced upon JA treatment, in contrast to the closely related *JAZ* genes (Vanholme *et al.*, 2007), but is regulated by several (a)biotic stress conditions, including repression upon infection with the virulent bacterium *Pst* DC3000. Intriguingly, we found that in the *tify8-1* mutant the expression of several *JAZ* genes was hyperinduced following *Pst* DC3000 infection (Figures 4.10 and 4.11).

Hence, an opposite effect of *Pst* DC3000 on *TIFY8* and *JAZ* gene expression (down- and upregulation, respectively) occurs. Notably, *TIFY8* shows an expression pattern opposite to that of *JAZ10* and other *JAZ* members, not only upon *Pst* DC3000 infection, but throughout plant development (Supplementary Figure S4.6, Figure 4.15). Taken together, these observations suggest that TIFY8 might negatively modulate *JAZ* gene expression.

The ZIM domain of TIFY8 mediates interaction with various proteins, but not with the JAZ

To investigate the molecular mechanisms behind this regulation, we undertook a biochemical approach by searching for TIFY8 interacting partners. TAP assays with Arabidopsis cell cultures overexpressing GS-tagged TIFY8 retrieved NINJA and PPD2 as interactors. Y2H confirmed the interaction with these proteins to be direct and, additionally, mapped the ZIM domain of TIFY8 as necessary and sufficient for all these interactions (Figure 4.2b, c). Although the ZIM domain, present in all TIFY family members, is known to be responsible for homo- and heterodimerisation (Chini *et al.*, 2009; Chung and Howe, 2009), Y2H indicated that TIFY8 did not interact with any of the JAZ proteins, but only with

itself and the PPD proteins (Figure 4.2d). Accordingly, no peptides corresponding to JAZ proteins were retrieved in TAP experiments using TIFY8 as bait. Conversely, TAP of NINJA, MYC2, MYC3, MYC4 or other JAZ proteins only identified JAZ proteins and no other TIFY proteins (Pauwels *et al.*, 2010; Fernández-Calvo *et al.*, 2011). Taken together this implies specificity of TIFY8 for the PPD proteins in terms of protein-protein interactions with other TIFY family members.

TIFY8 is a repressor of gene expression

The direct interaction of TIFY8 with NINJA points towards a role for TIFY8 as a repressor. Similarly to the JAZ proteins, TIFY8 recruits TPL through the adaptor protein NINJA. The formation of this hitherto unrevealed protein complex extends the functional scope for TPL, repressing gene expression also through TIFY8. These observations are in line with those of Causier *et al.* (2012), who describe TPL as a common repressor in several processes such as hormone signalling or stress responses.

To date, no DNA-binding activity for TIFY8 has been reported. Accordingly, using a protein-binding microarray for the identification of DNA-binding specificities of transcription factors (Godoy *et al.*, 2011) no evidence for a possible DNA-binding activity for TIFY8 could be obtained, which hints that TIFY8 represses gene expression through interaction with transcription factors.

Model for TIFY8-mediated regulation of gene expression upon *Pst* DC3000 infection

Here, we propose a model in which TIFY8-mediated *JAZ* gene expression upon infection with *Pst* DC3000 requires the formation of repressor complexes with NINJA and TPL, and interaction with transcription factors by means of the conserved N-terminal region within the TIFY8 protein (Figure 4.14).

Given that under control situations the *JAZ* expression levels in all the *TIFY8* transgenic lines assayed are similar to those in wild-type (Figures 4.10, 4.11, 4.12b and 4.13a), our results suggest that the plant recruits TIFY8 to the *JAZ* promoter upon *Pst* DC3000 infection to control the induction levels of *JAZ* genes and fine-tune the defence response. Meanwhile, *TIFY8* is downregulated in a COR-dependent manner (Figure 4.12a), a fact that could be

considered as a *Pst* DC3000-mediated mechanism to enhance pathogen virulence. Accordingly, the *tify8-1* line is more sensitive to *Pst* DC3000 than wild-type (Figure 4.9). It is known that *Pst* DC3000 exerts its virulence through the production of COR, able to mimic JA-Ile and trigger JA-dependent responses. Stimulation of JA signalling can result in a downregulation of the SA-mediated defences, necessary against *Pst* DC3000, thus promoting symptom appearance (Pieterse et al., 2012; Brooks, 2005). The recent study of Demiansky *et al*., (2012) shows JA-hypersensitivity of a *jaz10* knock-out mutant and JAZ10 RNAi lines, and their consequent hypersensitivity to *Pst* DC3000 infection. In our case, however, we report hyperinduction of the *JAZ* genes, and do not see hypersensitivity of the *tify8-1* line upon MeJA treatment (Figure 4.6). Identify the mechanisms that lead to an increased sensitivity to *Pst* DC3000 in the *tify8-1* line is one of the major questions that remain open and which are currently being addressed in our lab.

TIFY8 and JAZ regulate each other's gene expression?

In contrast with the *JAZ*s, *TIFY8* expression is drastically reduced after infection with *Pst* DC3000 (Figure 4.8), an opposite pattern also observed throughout plant development (Supplementary Figure S4.6, Figure 4.15). These observations, together with the fact that both JAZ and TIFY8 can form transcriptional repressor complexes, make tempting to speculate of JAZ and TIFY8 regulating each other's expression through a negative feedback loop. Whether *TIFY8* downregulation upon *Pst* DC3000 infection is mediated by JAZ and the mechanisms behind it would be interesting to test in the future. One possibility is that COR-dependent *TIFY8* downregulation (Figure 4.12a) is orchestrated as a consequence of COR-mediated JAZ degradation. Moreover, our results suggest *TIFY8* downregulation to be promoter-independent, since a reduction in the *TIFY8* expression levels is detected in all the lines tested following infection with *Pst* DC3000, regardless of the presence of the endogenous *TIFY8* promoter or ectopical expression using the 35S CaMV promoter (Supplementary Figure S4.5).

TIFY8 transcription shows an unexpected complexity

It is noteworthy that the generation of truncated versions of the TIFY8 protein might affect the protein complexes formed for the regulation of *JAZ* gene expression (Figure 4.14). The special features of the *tify8-1* line, where transcripts of downstream exons are formed regardless the disruption of the TIFY8 transcription initiation site by the T-DNA insertion, suggest the existence of an internal promoter within the *TIFY8* sequence. Analysis of *TIFY8* in RNA-Seq data generated in wild-type in the lab of Prof. Dr. ir. Corné M. J. Pieterse verified the hypothesis that exon 1 is skipped. Moreover, several regulatory elements are found within the Intron 1 sequence of *TIFY8*, suggesting that this intron can function as an alternative *TIFY8* promoter (Marcel Van Verk, personal communication). Further study on this matter will shed some light in understanding the complex mechanisms behind *TIFY8* transcription, the formation of different splice variants and the functionality of the different protein forms presumably generated.

TIFY8 as a repressor in several processes?

Our results show that TIFY8 does not act as a JAZ protein despite the fact that they recruit common elements to form repressor protein complexes (i.e. NINJA and TPL). Alternatively, JAZ and TIFY8 proteins may play different roles *in planta.* JAZ proteins function in plant hormone signalling, with a key role in JA perception and signal transduction, as well as in cross-talk with other signalling pathways (Pauwels and Goossens, 2011; Kazan and Manners, 2012). TIFY8 seems to have a more general function according to the interactors retrieved in our TAP experiments, as well as in the recently reported Arabidopsis Interactome (Arabidopsis Interactome Mapping Consortium, 2011). The TAP analysis links TIFY8 to PPD2, reported to act as a transcription factor (Lacatus and Sunter, 2009) and to KIX1, which functions recruiting TPL in a NINJA-like manner (Chapter 5). The Arabidopsis Interactome indicates that TIFY8 interacts with several other proteins, some of them corresponding to transcription factors potentially involved in different pathways, such as INNER NO OUTER (INO, At1g23420), RESPONSE REGULATOR 14 (ARR14, At2g01760) or ABERRANT LATERAL ROOT FORMATION 14 (ALF4, At5g11030). Accordingly, TIFY8 might be recruited for the repression of gene expression in different processes and upon specific conditions. In the case of *Pst* DC3000 infection for instance, alteration of TIFY8 function affects downstream

gene regulation events, including *JAZ* gene expression. Further molecular and phenotypical characterisation of *TIFY8* gain- or loss-of-function lines in different growth conditions or challenged by different environmental changes or stresses, as well as exploration of the 'TIFY8 Interactome' will allow elucidating the myriad of processes TIFY8 is specifically involved in.

EXPERIMENTAL PROCEDURES

Phytozome alignment

TIFY8 orthologue sequences were retrieved by the Phytozome facility (http://www.phytozome.net/, Goodstein *et al.*, 2012) and aligned with the JalView software.

Tandem Affinity Purification (using MALDI TOF/TOF MS)

Entry clones containing the CaMV 35S promoter, the bait ORF and the GS-TAP tag (Bürckstümmer *et al.*, 2006) were recombined by MultiSite Gateway LR reaction with pKCTAP as destination vector (Van Leene *et al.*, 2008). Arabidopsis cell suspension cultures (PSB-D) were transformed without callus selection as described previously (Van Leene *et al.*, 2007). Tandem affinity purifications were performed as described (Van Leene *et al.*, 2007; Van Leene *et al.*, 2008) with the exception that the soluble protein fraction was obtained by centrifuging twice at 36,900 g for 20 min at 4°C.

Proteolysis and peptide isolation, acquisition of mass spectra by a 4800 MALDI TOF/TOF Proteomics Analyzer (AB SCIEX), and MS-based protein homology identification based on the TAIR genomic database (Swarbreck *et al.*, 2008) were performed as described in Van Leene *et al.* (2010). Experimental background proteins were subtracted based on approximately 40 TAP experiments on wild-type cultures and cultures expressing TAP-tagged mock GUS, RFP and GFP proteins (Van Leene et al., 2010).

Tandem Affinity Purification (using LC-MS/MS analysis)

Cloning of transgenes encoding tag fusions under control of the constitutive cauliflower tobacco mosaic virus 35S promoter and transformation of Arabidopsis cell suspension cultures were carried out as previously described (Van Leene et al., 2007). Tandem affinity purification of protein complexes was done using the GS tag (Van Leene et al., 2008) followed by a downscaled purification protocol based on the GS protocol as described in Van Leene et al. (2011). In short, cell extracts were made on 2.5 g cell culture and cleared by two subsequent centrifugation steps at 36,900 x *g* for 20 minutes. In the first purification step, a protein input of 25 mg was incubated with 25 µl of IgG-Sepharose 6 Fast Flow beads (GE Healthcare). For the second step, 25 µl of Streptavidin Sepharose High Performance (Amersham) was used. Final elution was done with 40 µl 1x NuPAGE sample buffer containing 20 mM Desthiobiotin for 5 minutes. Beads were separated from eluate in a 1-ml Mobicol column (MoBiTec, Göttingen, Germany).

Eluted proteins were separated in a short run of 7 minutes on a 4-12% gradient NuPAGE gel (Invitrogen) and visualized with colloidal Coomassie Brilliant Blue staining. The protein gel was washed for 2 hours in H2O, polypeptide disulfide bridges were reduced for 40 min in 25 mL of 6,66 mM DTT in 50 mM NH4HCO3 and sequentially the thiol groups were alkylated for 30 min in 25 mL 55 mM IAM in 50 mM NH4HCO3. After washing with H2O, a broad zone containing the proteins was cut from the protein gel, sliced into 24 gel plugs, and collected together in a single Eppendorf. Gel plugs were washed twice with H2O, dehydrated with 95% CH3CN (v/v), rehydrated with H2O and dehydrated again with 95% CH3CN (v/v). Dehydrated gel particles were rehydrated in 60 µL digest buffer containing 750 ng trypsin (MS Gold; Promega, Madison, WI), 50 mM NH4HCO3 and 10% CH3CN (v/v) for 30 min at 4° C. Proteins were digested at 37° C for 3.5 hours.

The obtained peptide mixtures were introduced into an LC-MS/MS system, the Ultimate 3000 RSLC nano (Dionex, Amsterdam, The Netherlands) in-line connected to an LTQ Orbitrap Velos (Thermo Fisher Scientific, Bremen, Germany). The sample mixture was loaded on a trapping column (made in-house, 100 µm internal diameter (I.D.) x 20 mm (length), 5 µm C18 Reprosil-HD beads, Dr. Maisch GmbH, Ammerbuch-Entringen, Germany). After back-flushing from the trapping column, the sample was loaded on a reverse-phase column (made in-house, 75 µm I.D. x 150 mm , 5 µm C18 Reprosil-HD beads, Dr. Maisch). Peptides were loaded with solvent A (0.1% trifluoroacetic acid, 2% acetonitrile), and separated with a linear gradient from 2% solvent A' (0.1% formic acid) to 50% solvent B' (0.1% formic acid and 80% acetonitrile) at a flow rate of 300 nl/min, followed by a wash step reaching 100% solvent B'.

The mass spectrometer was operated in data-dependent mode, automatically switching between MS and MS/MS acquisition for the ten most abundant peaks in a given MS spectrum. In the LTQ Orbitrap Velos, full scan MS spectra were acquired in the Orbitrap at a target value of 1E6 with a resolution of 60,000. The ten

most intense ions were then isolated for fragmentation in the linear ion trap, with a dynamic exclusion of 20 seconds. Peptides were fragmented after filling the ion trap at a target value of 1E4 ion counts.

From the MS/MS data in each LC run, Mascot Generic Files were created using the Mascot Distiller software (version 2.4.1.0, Matrix Science, www.matrixscience.com/Distiller.html). When generating these peak lists, grouping of spectra was allowed with a maximum intermediate retention time of 30 seconds and a maximum intermediate scan count of 5 was used where possible. Grouping was done with 0.005 Da precursor tolerance. A peak list was only generated when the MS/MS spectrum contained more than 10 peaks. There was no de-isotoping and the relative signal-to-noise limit was set to 2. These peak lists were then searched with the Mascot search engine (version 2.3, MatrixScience, www.matrixscience.com) using the Mascot Daemon interface (Matrix Science, www.matrixscience.com). Spectra were searched against the TAIR10 database containing 35386 sequence entries. Variable modifications were set to methionine oxidation and methylation of aspartic acid and glutamic acid. Fixed modifications were set to carbamidomethylation of cysteines. Mass tolerance on MS was set to 10 ppm (with Mascot's C13 option set to 1) and the MS/MS tolerance at 0.5 Da. The peptide charge was set to 1+, 2+ and 3+ and the instrument setting was set to ESI-TRAP. Trypsin was set as the protease used, allowing for 1 missed cleavage, and also cleavage was allowed when arginine or lysine is followed by proline. Only high confident peptides, ranked one and with scores above the threshold score, set at 99% confidence, were withheld. Only proteins with at least two matched high confident peptides were retained.

A list of non-specific background proteins was assembled by combining our previous background list (Van Leene et al, 2010) with background proteins from control GS purifications on mock, GFP-GS, and GUS-GS cell culture extracts identified with LTQ Orbitrap Velos. To obtain the final list of interactors, these background proteins were subtracted from the list of identified proteins.

Yeast two- and three-hybrid assays

Gateway-compatible entry clones with the open reading frames of the proteins of interest were used for LR II reaction (Invitrogen) with pGADT7, pGBKT7 or pGBT9 Gateway-compatible yeast two-hybrid vectors to generate bait and prey constructs. Next, the Saccharomyces cerevisiae PJ69-4A yeast strain was co-transformed with different bait-prey plasmid combinations, as described in Chapter 3. In the case of the Y2H assays, transformant yeast colonies were selected on SD media lacking Leu and Trp (-2). Three individual transformants were grown overnight in liquid (-2) media, and a tenfold dilution of these cultures was dropped on control (-2) and selective media additionally lacking His (-3). Correspondingly, empty pGADT7, pGBKT7 or pGBT9 Gateway-compatible vectors were used as negative controls.

In the case of the Y3H assays, the same procedure was followed, but the MultiSite pMG426 vector was used for expression of NINJA, driven by the GDP promoter and C-terminally fused to the SV40 NLS-3xFLAG-6xHis tag (http://gateway.psb.ugent.be). Co-transformation of the PJ69-4A yeast strain with all three constructs was performed and transformants were selected on SD media lacking Leu, Trp and Ura (-3). The same procedure as in the Y2H assays was followed, and diluted cultures were dropped on control (-3) and selective media additionally lacking His (-4). For both Y2H and Y3H assays, plates were allowed to grow for 2 days at 30°C, and interaction was scored in terms of growth on selective media.

Confocal microscopy

Plants expressing the *35S::TIFY8-GFP* fusion, 35S::GFP and 35S::NLS-GFP were germinated on solid MS plates (containing 10 g/L of sucrose and 8 g/L agar) placed vertically. On the day of imaging, seedlings were briefly incubated in propidium iodide (3 mg/L, Sigma) and subsequently washed and mounted in milliQ water. Fluorescence microscopy was performed with a Zeiss LSM 510 confocal microscope (Figure 4.3a) or an Olympus FV10 ASW confocal microscope (Supplementary Figure S4.3).

Transient expression assays in Arabidopsis protoplasts

Transient expression assays were performed as described previously (De Sutter *et al.*, 2005; Pauwels *et al.*, 2008). Protoplasts were prepared from a Bright Yellow-2 (BY-2) tobacco cell culture and co-transfected with a

reporter plasmid containing the firefly-Luciferase (fLUC) reporter gene driven by a promoter containing five GAL4-binding sites (Ohta *et al.*, 2000), a normalisation construct expressing Renilla luciferase (rLUC) under the control of the 35S promoter (De Sutter *et al.*, 2005) and effector constructs. GAL4DBD fusions were generated by combining pEN-L4-2-R1 (35S promoter), pEN-R2-GAL4DBD-L3 and an entry clone holding the ORF, combined by MultiSite Gateway LR reaction with pm43GW7 as destination vector. For each experiment, 2 µg of each plasmid were used. After transfection, protoplasts were incubated overnight in the dark, at room temperature and with gentle agitation. The next day, protoplasts were lysed, and fLUC and rLUC activities were determined with the Dual-Luciferase reporter assay system (Promega). Variations in transfection efficiency and technical error were corrected by normalisation of fLUC by rLUC activities. All transactivation assays were conducted in an automated experimental set-up. A one-way ANOVA and Tukey HSD's Post Hoc test were performed to confirm statistically significant differences between control and effector constructs ($p<0.05$).

Generation of plant lines

The single T-DNA knock-out line *tify8-1* (GK_738B03) was retrieved from GABI-KAT (www.gabi-kat.de; Kleinboelting *et al.*, 2012). Seeds were selected on MS media containing sulfadiazine (7,5 mg/mL) and genotyped as homozygous for the T-DNA insertion in the Col-0 ecotype background by PCR, using the LB primer for the GABI-KAT T-DNA and specific primers for this line (primer sequences listed in the Annex 1).

For generation of transgenic plants with 35S promoter-driven *TIFY8* overexpression fused C-terminally to GFP, to a TAP tag (TIFY8-mTAP) or without a fusion tag (TIFY8-OE), a destination clone containing the full-length *TIFY8* was retrieved from ABRC (DKLAT4G32570). From this construct, an entry clone without stop codon was generated by reverse BP reaction into the entry vector pDONR221. Next, the isolated plasmid was used for recombination with the pK7FWG2, the pKCTAP and the pFAST-G02 destination vectors, respectively (Karimi *et al.*, 2002; Van Leene *et al.*, 2008 and 2007; Shimada *et al.*, 2010) using the Gateway LR II kit (Invitrogen), yielding the corresponding expression clone. In the case of transgenic lines downregulating TIFY8, the CATMA4a34310 gene sequence-specific tag (GST; www.catma.org), a fragment targeting *TIFY8*, was amplified by and cloned into the pDONR221 entry vector by Gateway BP reaction. The obtained entry clone was used for recombination with the pK7GWIWG2D destination vector (Karimi et al., 2002) using the Gateway LR II kit (Invitrogen) and yielding an RNAi construct used for TIFY8 silencing. Finally, for generation of transgenic plants with 35S promoter-driven expression of *TIFY8* truncated forms, the corresponding TIFY8 fragments were first amplified from Arabidopsis cDNA by PCR and cloned into pDONR207 through Gateway BP reaction (primers are listed in Anex 1). The entry clones were then used for recombination with the pK7WG2D destination vector using the Gateway LR II kit (Invitrogen).

For gene promoter expression assays, a 1175bp fragment of the TIFY8 promoter was retrieved from the Arabidopsis Promoterome database (www.psb.ugent.be/SAP) and amplified by PCR to be cloned in the pDONRP4P1R entry vector. The entry clone was then used for LR reaction with the pmK7S*NFm14GW destination vector (Karimi et al., 2007), yielding the *TIFY8promoter*::GUS-GFP expression clone. Seeds expressing the *JAZ1promoter*::GUS-GFP fusion were obtained from Dr. Wim Grunewald.

Following cloning and sequence verification, the different expression clones were transformed into *Agrobacterium tumefaciens* C58C1 (pMP90) by electroporation. Transgenic Arabidopsis seeds were generated by floral dip (Clough and Bent, 1998), using Col-0 as the background ecotype. Transformants were selected on MS media supplied with the corresponding antibiotic and homozygous T3 plant lines were used in the assays.

TIFY8-OE gene expression analysis

TIFY8 gene expression was studied in Arabidopsis seedlings grown on solid MS media for 2 weeks. Seedlings were harvested in 1.5 mL eppendorf tubes provided with metal balls and frozen on liquid nitrogen. The frozen plant material was ground in a Retsch MM300 mixer. RNA extraction and cDNA synthesis for qRT-PCR analysis were performed as described below.

Root growth assay

Seeds were sterilized by the chlorine gas method and sown on MS media provided with 10 g/L sucrose, 8 g/L agar, pH 5,7 and the corresponding final MeJA concentration. The plates were kept during two days in the dark and at 4^{o}C for stratification in order to synchronize germination. Next, plates were placed vertically in a growth chamber with a 16h day/8h night regime and 21^{o}C, with the day of transfer was considered as day 0 after stratification (DAS). Plates were scanned on at 11 DAS at a 300 dpi resolution and root length was measured by means of the EzRhizo software (http://www.root-image-analysis.org/ez-rhizo). Samples were kept in the growth room for another three days for anthocyanin accumulation measurements.

Anthocyanin accumulation

On day 14 after stratification (DAS), samples from the root growth assay were harvested and weighted in pre-frozen 1.5 mL eppendorf tubes provided with metal balls. Samples were frozen on liquid nitrogen and ground in a Retsch MM300 mixer. For anthocyanin extraction, each sample was added 750 µL of extraction buffer (MeOH HCl 1%) and kept rotating in the dark for 10 minutes. Next, 500 µL of water and 200 µL of chloroform were added, mixing inverting the tubes after each step. Samples were centrifuged for 5 min at full speed and 200 µL of the supernatant were transferred to a 96-well plate. Anthocyanin accumulation was measured as A_{530}-A_{657} and referred to mg of fresh weight.

Protein degradation assays

For the study of TIFY8 stability, homozygous transgenic lines overexpressing either TIFY8 or JAZ1 fused to a GS C-terminal tag (TIFY8- or JAZ1-mTAP) were used. Seeds were grown on MS media complemented with 10g/L of sucrose, 8 g/L agar and pH 5,7. Samples were stratified for 2 days and transferred to a growth room with 16h light/8 h dark light regime and 21°C. After 10 days, the seedlings were transferred to liquid MS media complemented with 10g/L sucrose and pH 5,7 with 50 µM JA or ethanol (mock). Plant tissue was harvested after one hour, frozen in liquid Nitrogen and ground with a Retsch MM300 mixer. Total protein was extracted and 60 µg of protein were loaded for each sample in precasted 4-15% TGX gels (Bio-Rad). The gel run for 20 min at constant 300V and 200mA. Next, blotting was performed into Trans-blot Turbo transfer 0.2µm PVDF membranes (Bio-Rad). A 1/2500 dilution of the Anti-Peroxidase (PAP) antibody was used for incubation with the blot for one hour. Finally, 5-minute incubation the Western Bright ECL kit (Isogen) was performed to allow Chemiluminescent detection. Coomassie staining was performed confirm equal loading on gel.

Arabidopsis infection with *Pseudomonas syringae* pv. *tomato* DC3000

The virulent pathogen *Pseudomonas syringae* pv. *tomato* (*Pst*) DC3000 was incubated overnight at 28°C in liquid King's B (KB) medium as described previously (Pieterse *et al.*, 1998). Bacterial cells were collected by centrifugation (10 min, 2000x *g*) and resuspended in 10 mM $MgSO_4$ to a final density of $5x10^6$ colony-forming units per ml (CFU/ml). This suspension was used for pressure-infiltration of leaves of 5-week-old Arabidopsis plants as described (Pieterse *et al.*, 1998). Leaves were harvested prior to and 24 h after inoculation with *Pst* DC3000. Three biological replicates per genotype and time point were harvested and frozen in liquid nitrogen for subsequent RNA extraction to study gene expression.

***Pseudomonas syringae* bioassays**

Plants were infected as previously described but with a density of $5x10^5$ colony-forming units per ml (CFU/mL). Five minutes and three days after infection (0 and 3 dpi, respectively), leaf discs from infected leaves were collected. The leaf discs were washed first with 70% ethanol and then with water, thus removing bacteria from the surface of the leaf and allowing to count just *Pst* DC3000 growing within the plant tissue. Leaf discs were transferred to plastic tubes provided with metal balls and 500 µl of a 10 mM $MgSO_4$ solution. The samples were then ground with a Retsch MM300 mixer. 10-fold dilutions of the resulting mix were performed and plated on KB media. The plates were kept at room temperature for 2 days and the colonies formed counted.

Coronatine treatment of Arabidopsis seedlings

Arabidopsis seeds were sterilised by the chlorine gas method and divided in bulks of about 40 seeds, which were subsequently germinated in Erlenmeyers containing 50 mL of MS liquid media supplemented with 10 g/L of glucose and pH 5,7. After 8 days, samples were treated either with 1 µM COR (Sigma) or DMSO (mock treatment) for 24 h, after which were harvested as previously described. Seedlings were grown under a 16-h day/8-h night light regime, 21°C and orbital shaking at 130 rpm. Three biological repeats per line and time point were harvested to study gene expression.

RNA extraction and cDNA synthesis

Frozen plant material was ground and total RNA was extracted using the Qiagen RNeasy kit (Qiagen). An RNase-free DNase step was performed following manufacturer's instructions for preparation of RNA for quantitative real time (qRT)-PCR analysis. Next, 1 µg of total RNA was used for cDNA synthesis with the iScript kit (Bio-Rad).

Quantitative real time qRT-PCR experiments

qRT-PCR was performed on a LightCycler 480 system (Roche) using the Fast Start SYBR Green I PCR mix (Roche). The primer sequences are provided in Annex 1.

Samples were amplified as described: one pre-incubation step (95°C, 10 s) followed by 45 amplification cycles (incubation 95°C for 10 s, annealing at 65°C for 15 s, elongation at 72°C for 15 s). Primer efficiency cut off was set as 1.7. Reactions were performed in triplicate per cDNA sample and gene expression levels were quantified relative to the housekeeping gene *UBQ10* (*At4g05320*).

cDNA-AFLP

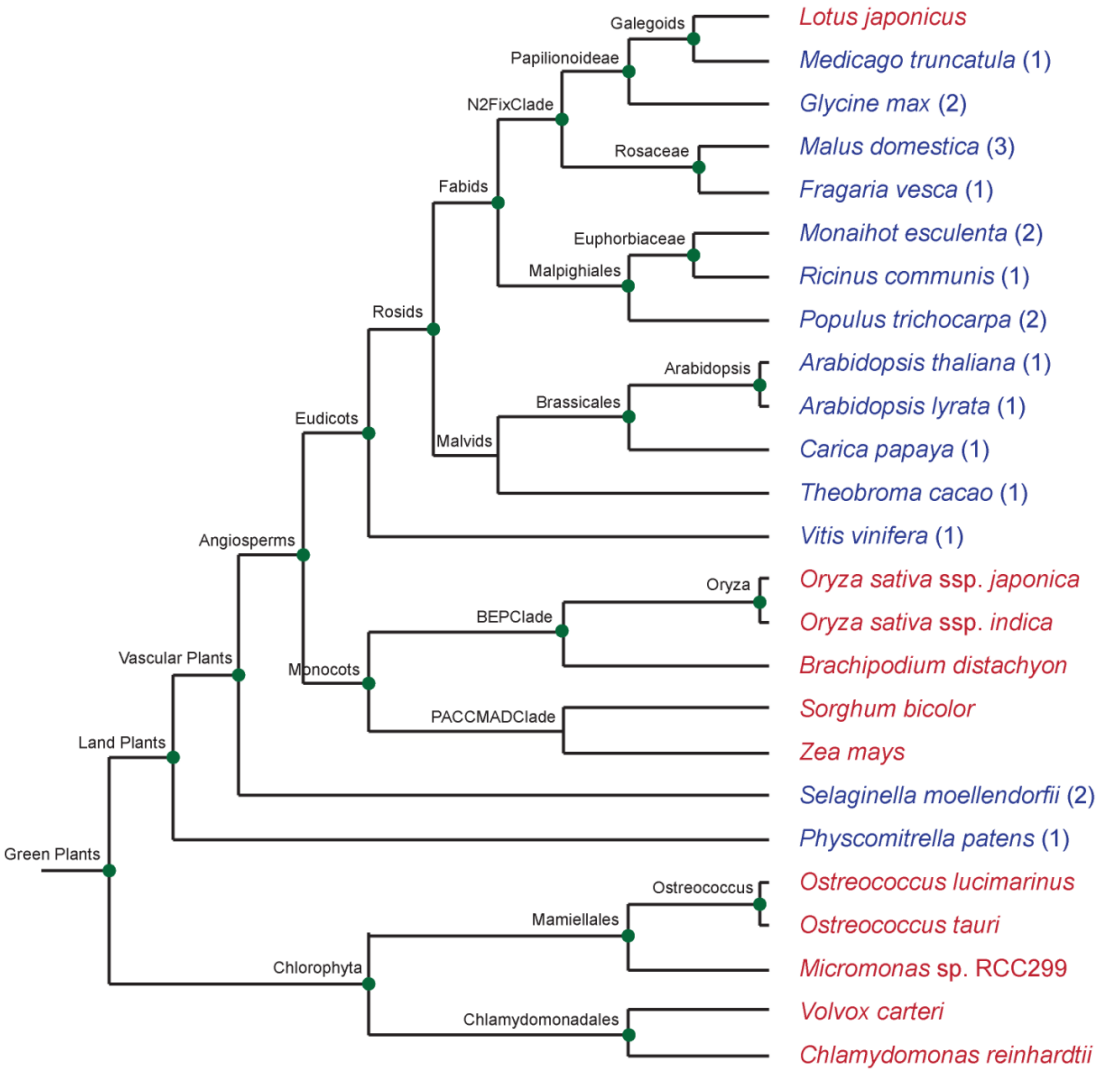
cDNA-AFLP transcript profiling was performed based on the method described by Vuylsteke *et al.* (2007). Selective amplification was performed with 12 out of the possible 128 primer combinations. AFPL-QuantarPro software (Keygene N.V.) was used for normalisation of gel band intensities. Next, clustering of the bands was performed with the Gene Cluster software (Eisen *et al.*, 1998) and its output was visualised through the Treeview software (Eisen Lab). Detection and analysis of differentially expressed tags was performed as described in Vuylsteke *et al.* (2007).

β-Glucuronidase (GUS) stains

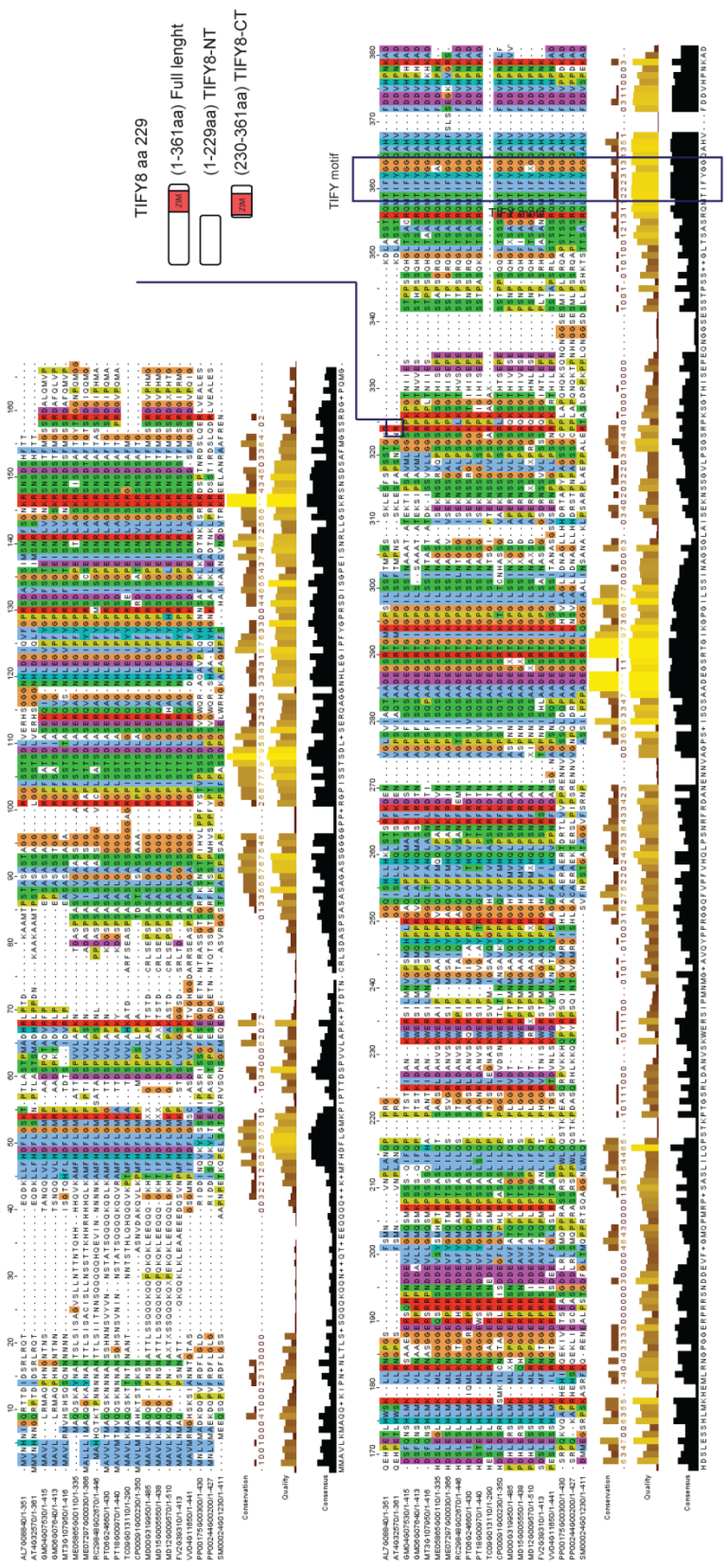
Samples were harvested on Falcon multiwell plates and kept in 90% acetone to clear the tissue. Next, the acetone was removed and replaced by the GUS staining solution containing 2mM X-Gluc solution in N,N-dimethylformamide (DMF), 0.1M $NaPO_4$ pH 7.0, 1mM $K_3Fe(CN)_6$ and 0.1% Triton X-100 diluted in distilled water. The samples were incubated at 37°C until blue coloration appeared. Next, the GUS staining solution was removed and the plant tissue was cleared with 70% ethanol at 4°C overnight.

Sample imaging was performed either in a light microscope (Leica BXL51) or a binocular (Leica MZ16).

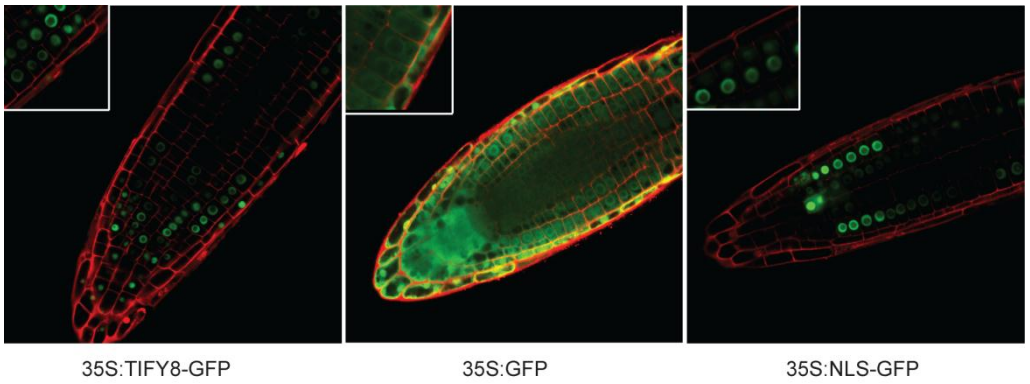
SUPPLEMENTARY INFORMATION



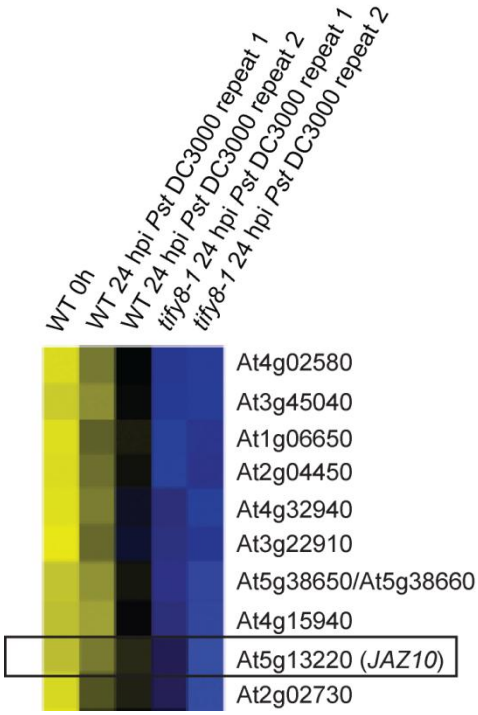
Supplementary Figure S4.1. AtTIFY8 orthologues.
Blue and red colours represent the existence or absence of putative AtTIFY8 orthologues in different species covered by the PLAZA comparative genomics resource. Numbers in brackets indicate the number of putative orthologues in each species (http://bioinformatics.psb.ugent.be/plaza). Orthologous gene families were inferred through sequence-based clustering with OrthoMCL (Van Bel *et al.*, 2012).



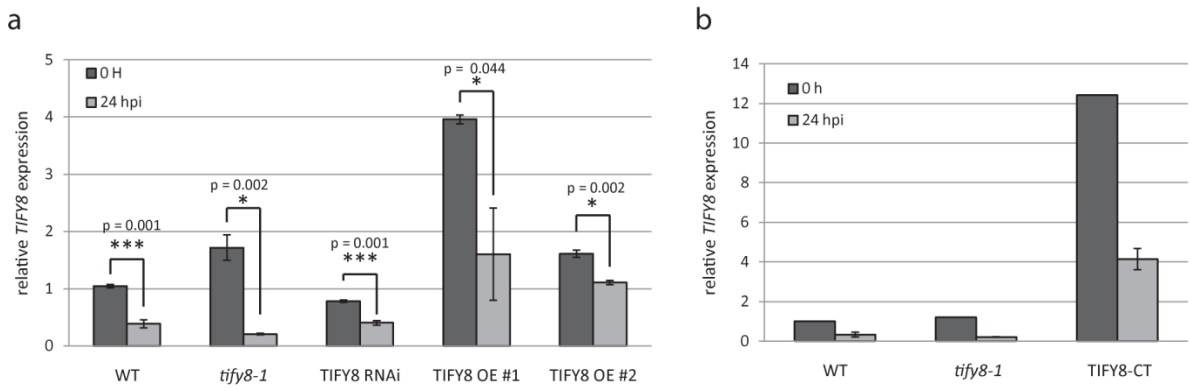
Supplementary Figure S4.2. TIFY8 is conserved among different plant species and presents additional protein domains of unknown function within its N-terminal region. Alignment of AtTIFY8 orthologues in several plant species retrieved from Phytozome. The TIFY8 aa 229 is also marked, as it delimits the N- and C-terminal truncated versions of TIFY8 generated in this study and schematically represented below. The TIFY motif within the ZIM domain is shown in a black box.



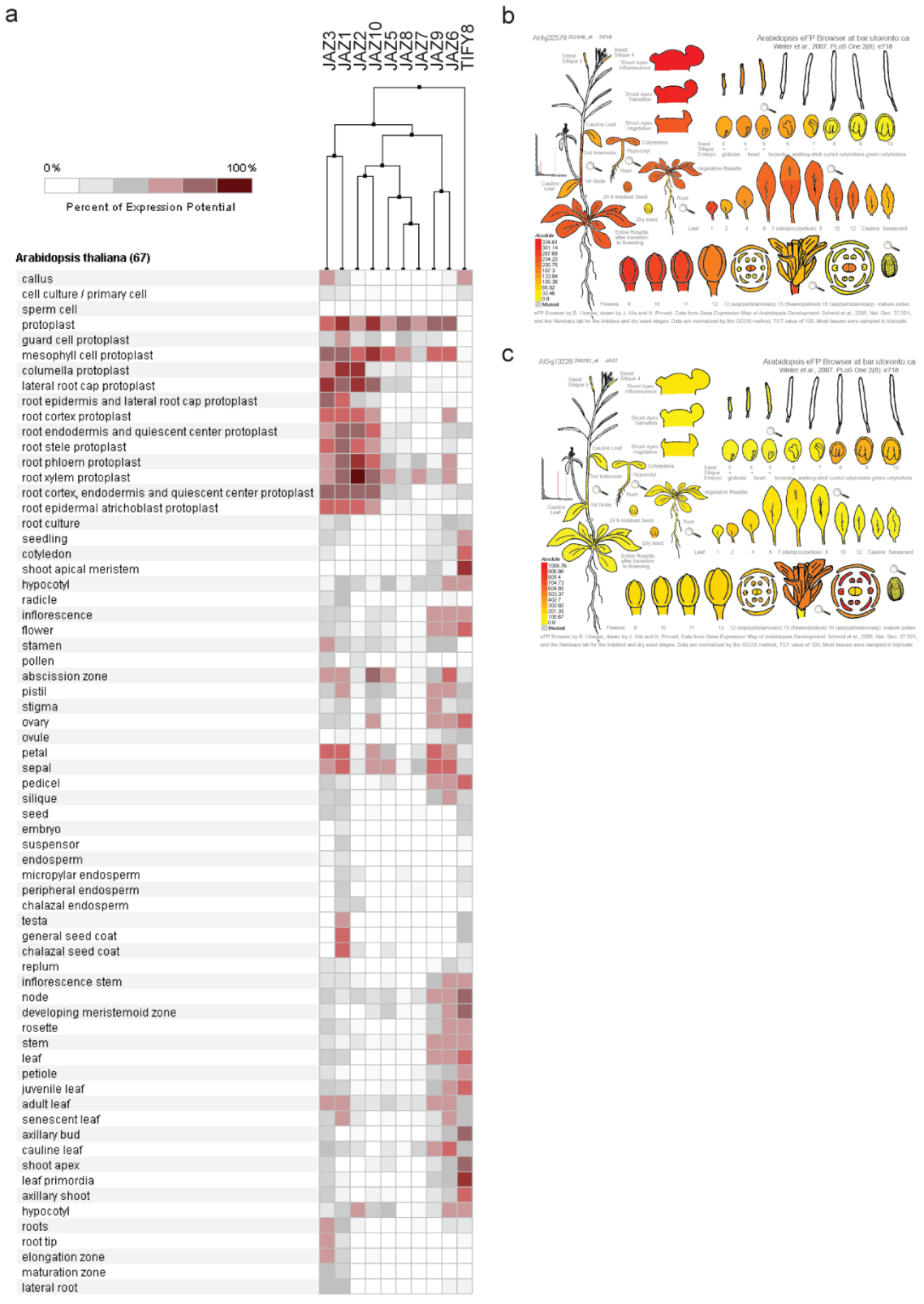
Supplementary Figure S4.3. TIFY8 localises to the nucleus.
Confocal root tip imaging of 4-day-old Arabidopsis seedlings overexpressing the TIFY8-GFP fusion protein, free GFP or nuclearly-localized GFP (NLS-GFP), respectively. Propidium iodide staining was performed prior to imaging to enhance the visualization of the cells.



Supplementary Figure S4.4. *JAZ10* is identified as hyperinduced in the *tify8-1* background during cDNA-AFLP transcript profiling upon infection by *Pst* DC3000.
Selected representative node extracted from the cDNA-AFLP cluster analysis of misregulated genes in the *tify8-1* line compared to wild-type prior to and 24 h after *Pst* DC3000 infection. Blue and yellow boxes correspond to induced or repressed gene expression relative to the average levels across all samples, respectively. Successfully sequenced gene tags within the node are shown.



Supplementary Figure S4.5. *TIFY8* expression levels in the different transgenic lines prior to and upon *Pst* DC3000 infection. *TIFY8* expression was studied in wild-type, *tify8-1* and different transgenic plants with altered *TIFY8* expression (0h or 24 hpi, respectively). Transcript levels were studied in 5-week-old Arabidopsis rosette leaves prior to or 24h after inoculation with *Pst* DC3000. Primer number 4 in figure 4.4 was used to analyse *TIFY8* and *UBQ10* (*At4g05320*) was used as internal control. Expression values were normalised to those of the wild-type. Error bars represent ±SE of three biological replicates. Statistical significance (t-test, *p<0.05, *** p<0.001) is shown in panel (a). No statistics were performed for panel (b) since just one biological repeat prior to infection with *Pst* DC3000 was analysed.



Supplementary Figure S4.6. The *TIFY8* expression pattern is opposite to that of *JAZ*.
a. Schematic representation of *TIFY8* and *JAZ* gene expression patterns in different plant tissues, based on the hierarchical clustering of publicly available microarray data (www.genevestigator.com, Hruz *et al*., 2008). *JAZ4* and *JAZ11* were not included since microarray data are not available. *JAZ12* was not studied as it is highly expressed in all tissues. **b, c.** Expression patterns of *TIFY8* (**b**) and *JAZ10* (**c**) extracted from the eFP Browser. (http://www.bar.utoronto.ca/efp; Winter *et al*., 2007).

Annex 1. List of primers used in this thesis.

Annex 2. List of the hits identified in TAP experiments using MALDI TOF/TOF MS.

Annex 3. Protein Identification details of the hits retrieved in TIFY8 TAP experiments treated with either 50 µM MeJA or DMSO (mock), obtained with the LTQ Orbitrap Velos (Thermo Fisher Scientific) and Mascot Distiller software (version 2.4, Matrix Science) combined with the Mascot search engine (version 2.3, Matrix Science) using the Mascot Daemon interface (Matrix Science) and database TAIR10. Proteins and peptides headers used in the table are listed below.
prot_score: protein score; **prot_mass**: protein mass; **prot_cover**: percentage of protein sequence covered by assigned peptide matches; **prot_len**: protein sequence length (AA); **prot_pi**: protein pI; **pep_query**: number assigned by Mascot to a specific MS2 spectrum; **pep_rank**: peptide rank number (1 for the top ranked peptide match); **pep_isbold**: peptide is in bold red. (Red and bold typefaces are used to highlight the most logical assignment of peptides to proteins. The first time a peptide match to a query appears in the report, it is shown in bold face. Whenever the top ranking peptide match appears, it is shown in red. Thus, a bold red match is the highest scoring match to a particular query listed under the highest scoring protein containing that match. This means that protein hits with many peptide matches that are both bold and red are the most likely assignments.); **pep_isunique**: peptide is unique for the protein family; **pep_exp_mz**: observed m/z value (precursor); **pep_exp_mr**: experimental relative molecular mass; **pep_exp_z**: observed peptide charge state; **pep_calc_mr**: calculated relative molecular mass; **pep_delta**: difference (error) between the experimental and calculated masses; **pep_start**: peptide start position in protein; **pep_end**: peptide end position in protein; **pep_miss**: number of missed enzyme cleavage sites; **pep_score**: peptide ions score; **pep_homol**: homology threshold score; **pep_ident**: identity threshold score; **pep_expect**: expectation value for the peptide match (The number of times we would expect to obtain an equal or higher score, purely by chance. The lower this value, the more significant the result); **pep_res_before**: amino acid before peptide sequence; **pep_seq**: peptide sequence; **pep_res_after**: amino acid after peptide sequence; **pep_var_mod**: any variable modifications found in the peptide; **ion_coverage**: ion coverage: y ions in red, b ions underlined

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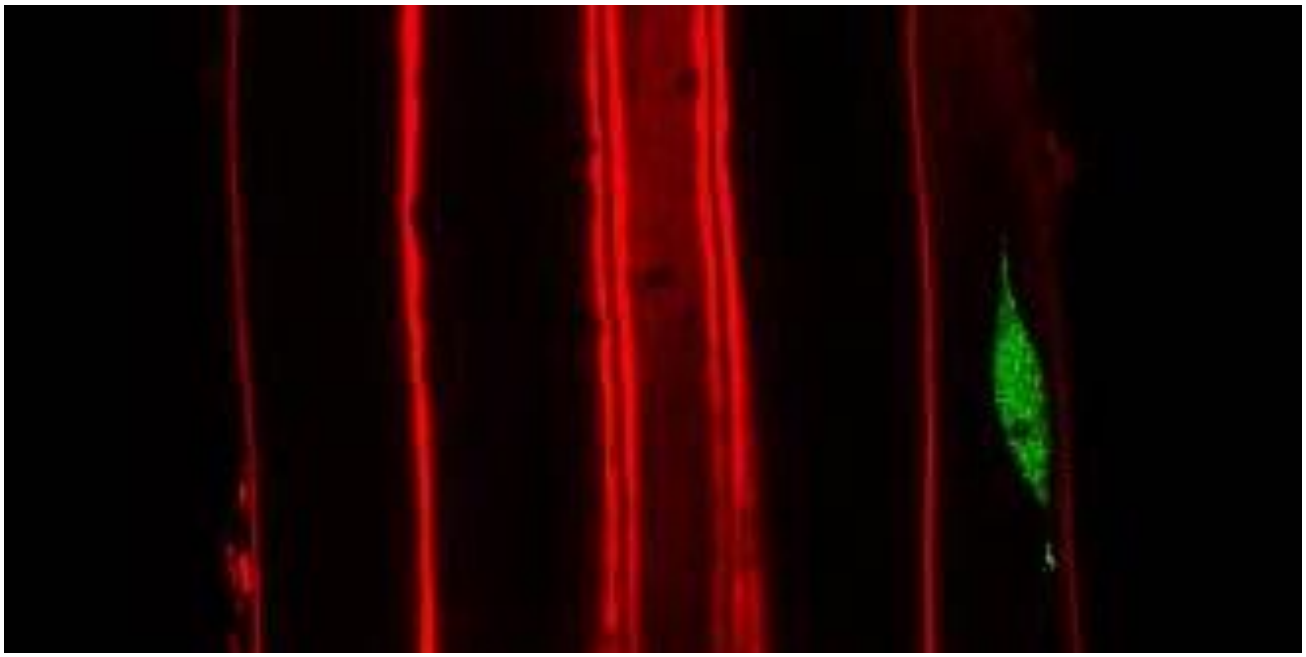
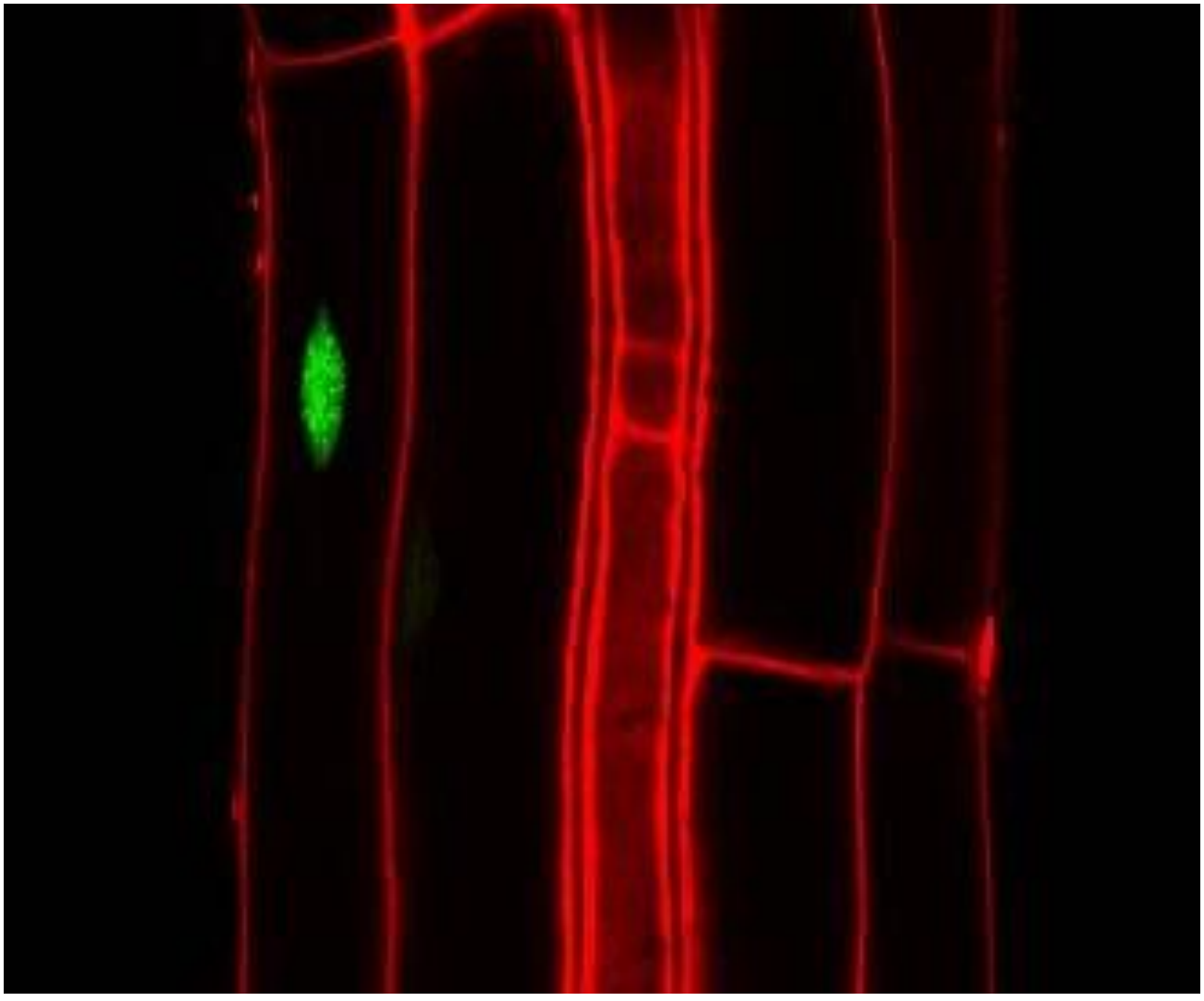


Chapter 5

The PPD proteins form a specific complex with the Arabidopsis KIX proteins



Cover: Confocal image of an Arabidopsis root overexpressing the KIX1-GFP fusion protein, which localises to the nucleus.

Chapter 5

The PPD proteins form a specific complex with the Arabidopsis KIX proteins

Manuscript in preparation:

"**Arabidopsis KIX-domain proteins are TOPLESS adaptors for PEAPOD2"**

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***Author contributions:** analysis of JA-responsiveness and JA-mediated protein degradation in Arabidopsis plants, gene cloning, yeast two- and three-hybrid assays, data interpretation and writing of the manuscript.

ABSTRACT

The JASMONATE-ZIM DOMAIN (JAZ) proteins are key regulators of jasmonate (JA) signalling, as they repress JA-dependent gene expression in the absence of the hormone through interaction with both JA-responsive transcription factors and NOVEL INTERACTOR OF JAZ (NINJA), an adaptor protein to the transcriptional co-repressor TOPLESS (TPL). The JAZ proteins belong to the plant-specific TIFY protein family, which, amongst others, also includes the two homologous PEAPOD (PPD) proteins. The PPD proteins have been characterized as regulators of leaf lamina size and as putative transcription factors. To date, no reports have focused on the possible role of these proteins in JA signalling, a tempting hypothesis given the protein domain similarities between the PPD and JAZ proteins and the fact that also PPDs interact with NINJA. In this chapter, we challenged that hypothesis and studied the protein complexes PPD2 is involved in. Our results show that PPDs do not interact with the core module JA signalling proteins MYC2 and COI1 but are in complexes with NINJA, JAZ and two previously uncharacterized proteins, here named KIX1 and KIX2. Both NINJA and KIXs are able to recruit TPL, forming repressive ternary complexes that, in turn, might regulate PPD-dependent gene expression.

INTRODUCTION

Within the plant-specific TIFY protein family, the JASMONATE-ZIM DOMAIN (JAZ) proteins are the most studied family members given their key function in the regulation of Jasmonate (JA) signalling. JAZ proteins act as repressors of JA signalling, as they bind to JA-responsive transcription factors and repress their activity by recruitment of the co-repressor TOPLESS (TPL) through NOVEL INTERACTOR OF JAZ (NINJA) (reviewed in Chapter 1).

In this chapter we focus on the non-JAZ TIFY proteins within the family, with special interest in the remaining class II members PEAPOD1 (PPD1) and PPD2 (Vanholme *et al*., 2007; Bai *et al*., 2010).

Two PPD proteins exist in Arabidopsis, encoded by *At4g14713* and *At4g14720* and named PPD1 and PPD2, respectively. The PPD proteins share 84% sequence similarity and, structurally, contain three different protein domains. PPDs show an N-terminal PPD-specific domain with yet unknown function, a central ZIM domain, conserved throughout the TIFY family, and a C-terminal Jas-like domain similar to that in the JAZ proteins (White *et al*., 2006; Vanholme *et al*., 2007; Katsir *et al*., 2008; Bai *et al*., 2010). The Jas domain in JAZ is responsible for interaction with JA signalling regulators such as the bHLH MYC transcription factors and the F-box protein COI1 (reviewed in Chapter 1).

To date, only few reports have addressed functional characterisation of the PPD proteins. Besides their interaction with NINJA, the PPD proteins have been described as regulators of leaf lamina size and as putative transcription factors (Pauwels *et al*., 2010; White, 2006; Lacatus and Sunter, 2009).

On the one hand, White (2006) showed that knock-out of both *PPD* genes in the Landsberg erecta (Ler) ecotype increased lamina growth and size, resulting in dome-shaped leaves. In the model he proposed, leaf development is regulated by two cell-cycle arrest fronts that define the transition from cell division to cell expansion and differentiation. The first front determines the arrest of cell proliferation and is PPD-independent. The second front regulates the proliferation of disperse meristematic cells (DMCs) and seems to be regulated in a *PPD* dose-dependent manner, given the opposite effect of *PPD* knock-out and overexpression lines, where DMC proliferation is prolonged or prematurely arrested, respectively.

On the other hand, PPD2 is able to bind the promoter of the *Tomato golden mosaic virus* (TGMV) coat protein (CP) and activates its expression upon association with the TMGV transcriptional activator protein (TrAP) (Lacatus and Sunter, 2009).

Beyond these reports, no follow-up studies on PPD function have been presented. Therefore, we investigated the putative function of PPD proteins in JA signalling, given their similarities with the JAZ proteins. Our results suggest that the PPD proteins do not affect JA-mediated responses whereas protein-protein interaction studies via tandem affinity purification (TAP) and yeast two-hybrid (Y2H) demonstrate the formation of PPD-specific protein complexes. We report the characterization of two hitherto unknown proteins named KIX based on the presence of a KIX domain within their structure. These KIX proteins also contain an ETHYLENE RESPONSE FACTOR (ERF)–associated amphiphilic repression (EAR) motif and, as NINJA, can act as adaptor proteins that link PPD to the TPL transcriptional co-repressor.

RESULTS

Altered PPD expression does not affect JA-mediated responses

The lack of studies reporting a putative function of the PPD proteins in JA signalling prompted us to investigate this possibility. In first instance, we studied the JA-responsiveness of transgenic lines with altered *PPD* expression.

Three different *PPD* transgenic constructs previously generated were chosen, as they would allow having a broad vision on a putative role of the PPD proteins on JA signalling. First, we made use of a transgenic line generated by the lab of Prof. Dr. Dirk Inzé. This line carries an artificial microRNA (amiRNA) targeting and downregulating the expression of both *PPD* genes. Next, we used several independent lines overexpressing *PPD2* C-terminally fused to GFP (35S:PPD2-GFP). Finally, given that plants expressing mutant or dominant negative forms of the *JAZ* genes missing the C-terminal Jas domain show insensitivity to JAs (Chini *et al.*, 2007; Thines *et al.*, 2007; Yan *et al.*, 2007; Melotto et al., 2008), we wondered whether the Jas-like domain in PPD would also regulate JA-responsiveness. Therefore, a line expressing a truncated version of PPD1 missing its C-terminal Jas-like domain (35S:PPD1ΔJas) was also used in these assays.

JA-responsiveness of these transgenic lines was scored in terms of root growth and anthocyanin accumulation in seedlings grown on control MS media and on various increasing (2.5 and 10 μM) MeJA concentrations.

The outcome of these assays, presented in Figure 5.1, shows that overall, the various transgenic lines with altered *PPD* expression tested do not show significantly altered JA-mediated responses under the conditions tested. Taken together, our results suggest that PPDs do not affect directly JA-responsiveness.

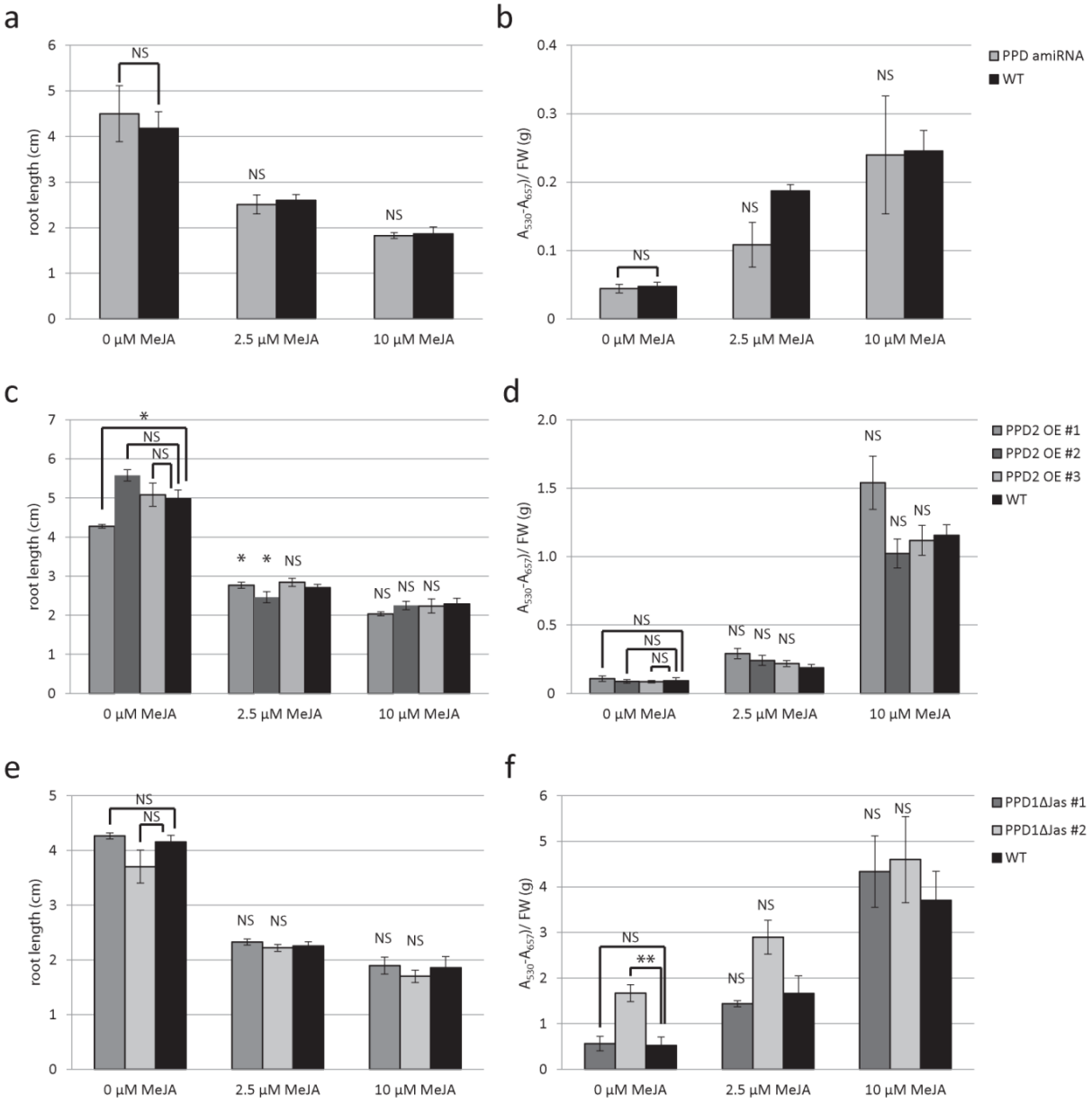

Figure 5.1. JA-mediated inhibition of root growth and anthocyanin accumulation in transgenic plants with altered PPD expression. Three different PPD transgenic constructs tested compared to wild-type: a silencing PPD amiRNA line (**a**, **b**), several independent PPD2 overexpression lines (**c**, **d**) and two independent truncated PPD1 overexpression lines (**e**, **f**). Root growth inhibition was scored on 11 DAS seedlings grown on MS media and increasing MeJA concentrations (**a**, **c** and **e**); anthocyanins in (**f**) were extracted for the same samples used for root growth but harvested 14 DAS, while in (**b**) and (**d**) were extracted from plants grown on liquid MS media supplemented with increasing MeJA concentrations. Four technical repeats per line and treatment, consisting on up to eight seedlings per repeat ($20 \leq n \leq 32$), were analysed. Bars represent average ± SE. Differences between the lines assayed in control conditions are shown (NS: no significant, *: $p<0.05$; t-test). Statistically significant differences for the interaction between genotype and treatment are shown (NS: no significant, $p>0.05$; **: $p<0.01$, one-way ANOVA).

PPD2 is not degraded upon JA treatment

The JAZ proteins are highly instable upon JA treatment. Hormone perception recruits these proteins to the SCF^{COI1} E3 ubiquitin-ligase complex and, following ubiquitination, the JAZ proteins are targeted for 26S proteasome-mediated degradation. The C-terminal Jas domain in the JAZ proteins mediates the interaction with COI1 (Chini *et al.*, 2007; Thines *et al.*, 2007; Yan *et al.*, 2007; Sheard *et al.*, 2010). Accordingly, *JAZ* mutants or splice variants (e.g. the JAZ10 splice variants) in which the Jas domain is disrupted render JA insensitivity (Yan *et al*., 2007; Chung and Howe, 2009).

The presence of a Jas-like C-terminal domain in the PPD proteins prompted us to study their stability upon JA treatment. The protein expression levels of transgenic plants overexpressing either PPD2 C-terminally fused to GFP, JAZ10 full length (JAZ10.1) or the stable JAZ10.3 splice variant C-terminally fused to YFP were studied in mock and JA-treated seedlings. This showed that PPD2 is not degraded, but rather modestly increased, upon JA treatment. The JAZ10.1 on the other hand, was degraded as expected while the JAZ10.3 spliced form was stable upon JA treatment.

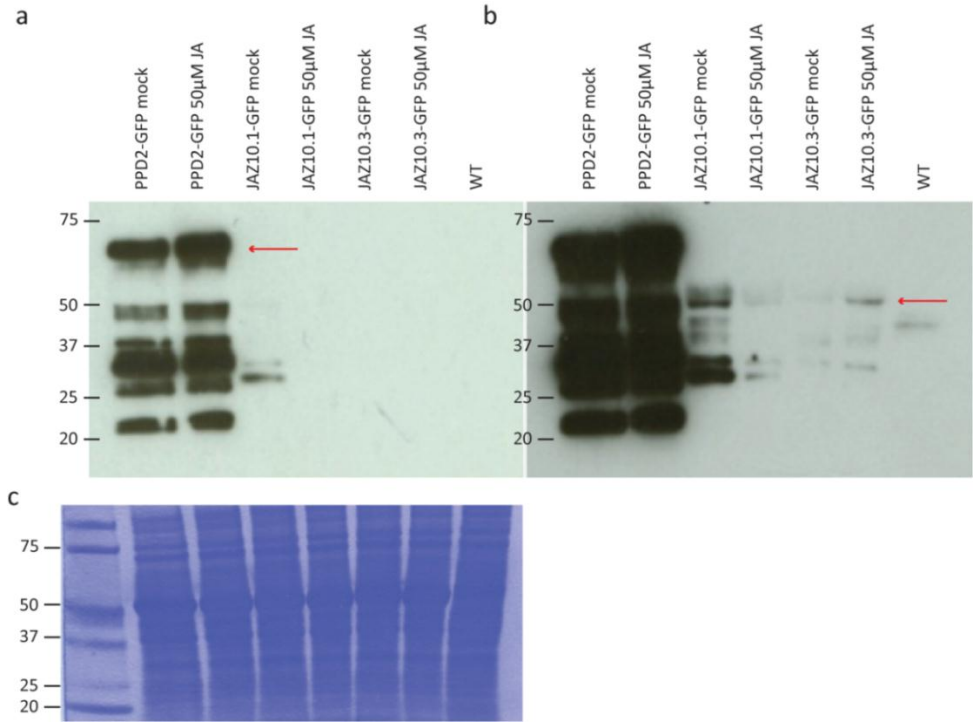

Figure 5.2. PPD proteins are not degraded upon JA treatment. Western blot analysis of 9 day-old Arabidopsis seedlings overexpressing the PPD2- JAZ10.1- or JAZ10.3-GFP fusions after 1h treatment with either 50 µM JA or ethanol (mock). **a.** Western Blot imaging following 30 seconds exposure. The red arrow indicates the size of the PPD2-GFP fusion protein. **b.** Western Blot imaging after 10 minutes exposure. Red arrow indicates the size of the JAZ10.1-GFP and JAZ10.3-GFP fusion proteins. **c.** Coomassie staining for verification of equal protein loading in gel.

TAP of PPD2 identifies other TIFY proteins, NINJA and Arabidopsis KIX proteins

Recently, tandem affinity purification (TAP) has successfully been applied in our group to isolate signal transduction protein complexes in Arabidopsis cell cultures overexpressing JA core module signalling proteins such as JAZ, NINJA or MYC (Pauwels *et al*., 2010, Fernández-Calvo *et al*., 2011). Here, we applied an updated and more sensitive protocol using Orbitrap mass spectrometry (Eloy *et al*., 2012; also Chapter 4) to unravel protein complexes holding the PPD2 protein (Table 5.1 and Annex 3).

PPD2 was found in complexes with the TIFY proteins JAZ3, JAZ10 and TIFY8, NINJA and two homologous proteins of yet unknown function, designated as KIX based on the presence of a KIX-like domain within their structure (see later). All these proteins, including PPD2, are predicted to localise to the nucleus, thus creating a potential network to which we focused our research. Most of the remaining interactors are predicted to localise in the cytoplasm or the chloroplasts. THI1 encodes a thiamine biosynthetic gene with a dual function in vitamin B1 formation and mitochondrial DNA damage tolerance and 2CBP is a 2-cysteine peroxiredoxin B involved in plant responses to oxidative stress, as well as NQR (Machado *et al*., 1997; Rey *et al*., 2007). The direct interaction of these proteins with PPD was not studied in detail within the scope of this thesis, but would be of great interest as these are also identified in TAP experiments using KIX2 as bait (see later).

The results of the TAP suggest that heterodimerization between PPD and JAZ proteins might be specific to some JAZ proteins. To study the JAZ10-PPD2 reciprocity for their *in vivo* interaction, we used Arabidopsis cell cultures overexpressing JAZ10 in a new TAP experiment. The outcome was similar to that obtained for other JAZ (Pauwels *et al*., 2010; Fernández-Calvo *et al*., 2011) and included the transcription factors MYC2, MYC3 and MYC4, other JAZ proteins (JAZ3 and JAZ12), and NINJA. Finally also PPD2 was detected, which was not the case with the previously reported JAZ-TAPs (JAZ1, JAZ3 and JAZ5; Pauwels et al., 2010, Fernandez-Calvo et al., 2011) (Table 5.1), pointing to some specificity of JAZ10 for PPD2.

Table 5.1. Overview TAP purified proteins.

Prey		Bait		
AGI	Protein	**PPD2**	**JAZ10**	**KIX2**
PPD complex				
AT4G14720	PPD2	**2**	2	2
AT4G32570	TIFY8	2		
AT4G28910	NINJA	2	2	
AT4G32295	KIX1	2		
AT3G24150	KIX2	2		**2**
JAZ proteins				
AT3G17860	JAZ3	2	2	
AT5G13220	JAZ10	2	**2**	
AT5G20900	JAZ12		2	
MYC transcription factors				
AT1G32640	MYC2		2	
AT5G46760	MYC3		2	
AT4G17880	MYC4		2	
Other proteins				
AT5G54770	THI1	1		2
AT5G06290	2CPB	1		2
AT1G49670	NQR	2		
AT5G13530	KEG		2	
AT1G50600	SCL5			2
AT4G21710	NRPB2			2
AT5G06600	UBP12			1
AT5G52840	NADH-Ubiquinone reductase			1

Proteins were identified using peptide-based homology analysis of MS data. Background proteins identified in control experiments were withdrawn. Number indicates the times the prey was identified in 2 experiments with each bait protein. Abbreviations: AGI, Arabidopsis Genome Identifier; PPD2, PEAPOD2; NINJA, NOVEL INTERACTOR OF JAZ; JAZ, JASMONATE ZIM DOMAIN; THI1, THIAMINE1; 2CPB, 2-CYS PEROXIREDOXIN B; NQR, NAD(P)H: QUINONE OXIDOREDUCTASE; SCL5, SCARECROW-like 5; NRPB2, NUCLEAR RNA POLYMERASE B2; UBP12, UBIQUITIN PROTEASE12; KEG, KEEP ON GOING. Detailed MS data can be found in Annex 4.

PPD proteins contain a functional ZIM domain

The fact that PPD2 interacts with the TIFY proteins JAZ3, JAZ12 and TIFY8 in TAP suggests that heterodimerization within the TIFY family is not restricted to the JAZ proteins. To confirm this hypothesis, we tested all 12 JAZ proteins, PPD1, PPD2 and TIFY8 for interaction with the PPD proteins using yeast two-hybrid (Y2H) assays. These confirmed direct interaction of PPD with some of the JAZ proteins, including the TAP-retrieved JAZ3 and TIFY8 and provide evidence for homo- and heterodimerization between PPD1 and PPD2 (Figure 5.3a). Both dimerization of JAZ proteins and interaction with NINJA require the ZIM domain which is also present in PPD proteins (Bai *et al*., 2010; Vanholme *et al*., 2007; Pauwels *et al*.,

2010; Chini *et al*., 2009; Chung and Howe, 2009). Accordingly, we designed truncated versions of the PPD2 protein comprising different combinations of its N-terminal PPD, central ZIM and C-terminal Jas-like domains (Figure 4.1) and tested these fragments for interaction with NINJA and JAZ3 in Y2H. These results show that the ZIM domain is necessary and sufficient for interaction with NINJA and JAZ3, thus demonstrating that the PPD2 ZIM domain is a functional protein-protein interaction domain (Figure 5.3b-c). Finally, we could also confirm that PPD2 interacts with the domain C of NINJA, known to mediate the interaction with the JAZ proteins (Figure 5.3d, Pauwels *et al*., 2010).

TAP of PPD2 identifies the Arabdopsis KIX proteins

TAP with both the PPD2 and the TIFY8 proteins (Tables 5.1 and 4.1, respectively) retrieved two homolog proteins of yet unknown function encoded by *At4g32295* and *At3g24150* and which we named KIX1 and KIX2, respectively.

The search for orthologues of the KIX proteins in other species by means of Phytozome (http://www.phytozome.org/, Goodstein *et al*., 2012) and Plaza (Van Bel *et al*., 2011) pointed out the presence of the KIX subfamily in the fern *Selaginella moellendorffii*, but not in the moss *Physcomitrella patens* and the Chlamydomonadales, suggesting an evolutionary origin in the transition to vascular plants. They are present in all available eudicot genomes, but absence in the monocot lineage suggesting they were subject of gene loss.

The conservation of the KIX subfamily allowed us to map conserved regions and to identify the presence of additional protein domains. The alignment of KIX protein orthologs identified 5 conserved regions or motifs (Figure 5.4a). The N-terminus is highly conserved and consists of 2 distinct domains: the KIX domain (aa 1-69 in KIX1) and a domain fused to it and that which we denominated B (70-137). The C-terminus is less conserved and 3 distinct motifs are recognizable: a motif with a central proline (173-180), an ERF-associated amphiphilic repression (EAR) motif (212-220) and a putative nuclear localization signal (NLS, 228-231). Nuclear localization could indeed be confirmed in Arabidopsis seedlings expressing the KIX1-GFP fusion (Figure 5.4b). Fluorescence was seen only in the nucleus, corresponding with the observed nuclear localization of TIFY8, JAZ10 and PPD2 (Chapter 4, Chung and Howe, 2009).

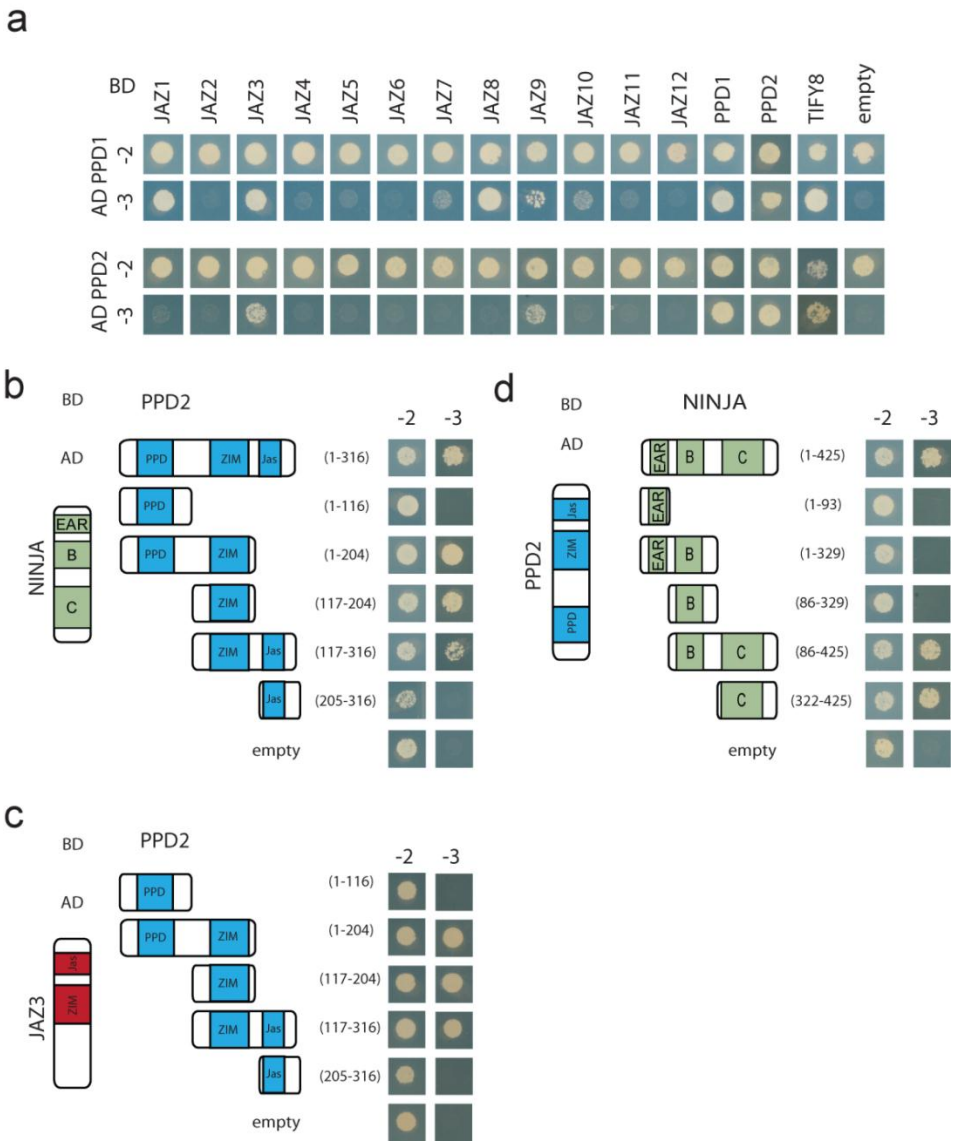

Figure 5.3. PPD proteins contain a functional ZIM domain.
a. PPD1 and PPD2 proteins form homo- and heterodimers with several class II TIFY proteins **b.** The ZIM domain of PPD2 is necessary and sufficient for interaction with NINJA. **c.** The ZIM domain of PPD2 is necessary and sufficient for interaction with JAZ3. **d.** The C domain of NINJA is necessary and sufficient for interaction with PPD2. For all Y2H assays, the *Saccharomyces cerevisiae* PJ69-4A yeast strain was co-transformed with bait and prey constructs. Transformed yeasts were spotted on control medium lacking Leu and Trp (-2) or selective medium additionally lacking His (-3). AD and BD: fusion of the protein of interest to the yeast GAL4 activation or binding domain in Gateway-compatible pGADT7 or pGBT9 vectors, respectively. Yeast co-transformation with the BD empty vector was used as control. Drawings represent the protein structure and domains; numbers indicate terminal amino acid residues.

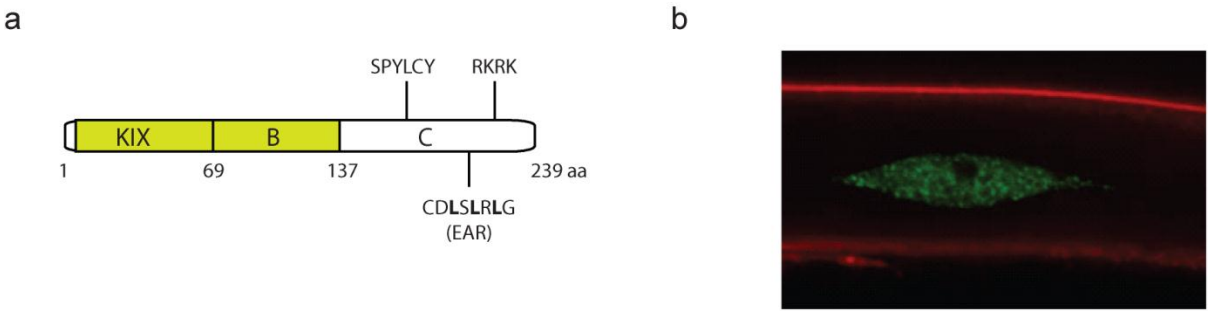

Figure 5.4. The KIX proteins present different protein domains and motifs and localize to the nucleus. a. Overview of the KIX1 protein structure. Numbers indicate terminal amino acid residues. **b.** Representative confocal microscopy image of an Arabidopsis root cell, expressing the KIX1-GFP fusion.

TAP of KIX proteins confirms interaction with PPD

To get more insight in these complexes, we performed a TAP with KIX2. This confirmed interaction *in vivo* with PPD2 but no TIFY8 peptides were obtained (Table 5.1). The absence of peptides belonging to any of the JAZ proteins suggests that the KIX proteins might form specific protein complexes with the non-JAZ class II TIFY proteins. Remarkably, TAP with KIX2 retrieved several other interactors, including some also identified as putative PPD2 interactors such as THI and 2CBP. Besides those interactions, TAP of KIX2 revealed interaction with NRPB2, the second biggest subunit of the DNA-dependent RNA Polymerase II (RNA Pol II; Larkin and Guilfoyle, 1993; Oxelman and Bremer, 2003), suggesting a link of the KIX proteins to RNA PolIII-mediated transcriptional activation, an attractive hypothesis that will be further studied (see discussion). Moreover, KIX2 was also found to interact with the uncharacterized transcription factor SCARECROW-like 5 which also contains an EAR domain (Kagale *et al*., 2010). Similarly, other EAR domain-containing transcription factors were found to interact with KIX2, as recently published by the Arabidopsis Interactome Mapping Consortium (2011).

The N-terminal PPD domain is necessary and sufficient for interaction with KIX

Since TAP cannot discriminate between indirect and direct interactions between bait and prey proteins, Y2H assays were performed and confirmed the direct interaction of the KIX and the PPD proteins (Figure 5.5a). Moreover, the use of truncated versions of PPD2 showed that the N-terminal PPD domain in PPD2 was necessary and sufficient for the interaction (Figure 5.5b).

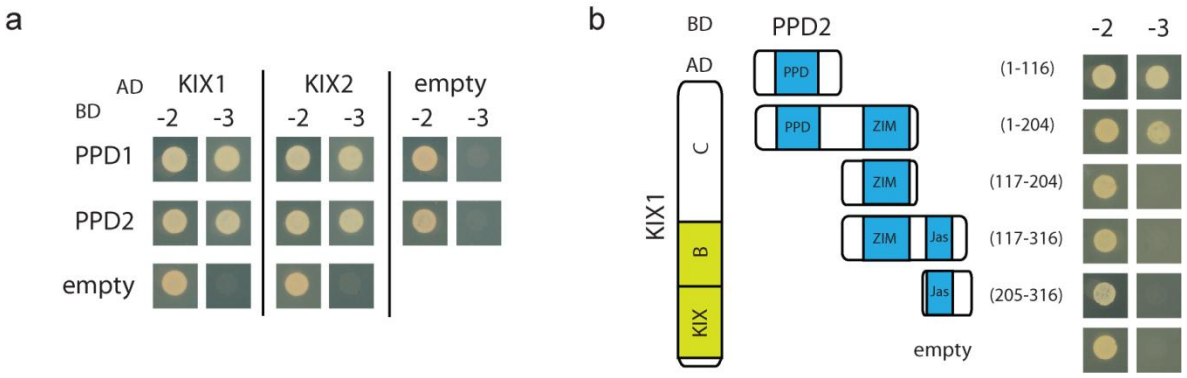

Figure 5.5. PPD proteins interact with KIX proteins through their N-terminal PPD domain. a. The KIX proteins interact with PPD proteins in Y2H assays. **b.** The N-terminal PPD domain in the PPD2 protein is necessary and sufficient for interaction with the KIX1 protein. The *Saccharomyces cerevisiae* PJ69-4A yeast strain was co-transformed with bait and prey constructs. Transformed yeasts were spotted on control medium lacking Leu and Trp (-2) or selective medium additionally lacking His (-3). AD and BD: fusion of the protein of interest to the yeast GAL4 activation or binding domain in Gateway-compatible pGADT7 or pGBKT7 vectors, respectively. Yeast co-transformation with the corresponding empty vector was used as control. Drawings represent the protein structure and domains; numbers indicate terminal amino acid residues.

KIX proteins are transcriptional repressors recruiting TPL via their EAR motifs

The EAR motif (Figure 5.4a) is known to mediate binding with the co-repressor TOPLESS (TPL, Szemenyei *et al*., 2008; Pauwels *et al*., 2010). Indeed, also the EAR motif in the KIX1 and KIX2 has been described and direct interaction with TPL in genome-wide Y2H screens has recently been reported (Kagale *et al*., 2010; Causier *et al*., 2011; Arabidopsis Interactome Mapping Consortium, 2011). To confirm the direct interaction between the KIX proteins and TPL, we performed targeted Y2H assays. KIX1 and KIX2 interacted directly with an N-terminal fragment of TPL, containing the three LiSH, CTLH and TOP domains important for protein-protein interaction (Szemenyei *et al*., 2008; Krogan *et al*., 2012). Moreover, we verified that this interaction is mediated by the EAR motif in KIX proteins, as mutations of the leucine residues of their EAR motifs abolished the interaction (Figure 5.6a).

The presence of this EAR motif in the KIX proteins prompted us to test whether they might have repressor activity. Therefore, the KIX proteins were fused to the GAL4 DNA binding domain (GAL4DBD) and co-expressed with a construct expressing the firefly luciferase (fLUC) reporter gene under the control of GAL4 binding elements in tobacco bright-yellow (BY2) protoplasts. Both KIXs were capable of strong repression and this was mediated by their EAR motif, similarly to NINJA (Figure 5.6b) (Pauwels *et al*., 2010). Finally, we tested if KIX and

NINJA were both capable of forming a molecular bridge between PPD2 and TPL. Due to the absence of an EAR motif within its sequence, PPD2 is unable to bind directly to TPL. However, co-expression with NINJA, KIX1 or KIX2 is sufficient for GAL4 reconstitution and subsequent yeast growth, providing evidence for the formation of PPD2/NINJA/TPL and PPD2/KIX/TPL ternary complexes (Figure 5.6c and d).

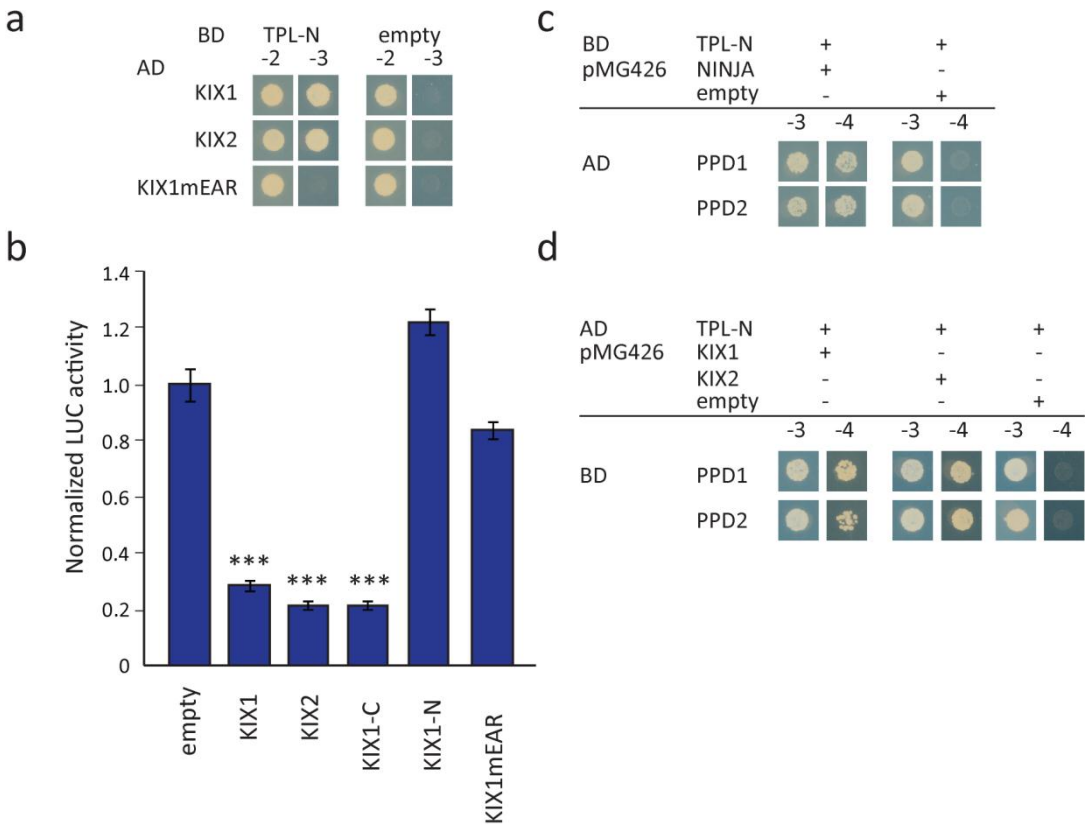

Figure 5.6. KIX proteins act as transcriptional repressors recruiting TPL. a. KIX proteins interact directly with TPL in Y2H assays. **b.** KIX proteins act as transcriptional repressors in transient expression assays. Transactivation activity in tobacco protoplasts transfected with a pUAS–fLUC reporter construct, effector constructs fused to GAL4DBD, and a 35S:rLUC normalization construct. Error bars represent ±SE of eight biological replicates. Asterisks represent significant differences (***, $p<0.001$, one-way ANOVA, Dunnett T3 Post Hoc test). **c.** NINJA act as adaptor proteins linking TPL to PPD. **d.** The KIX proteins can also act as adaptor proteins for PPD in order to recruit TPL.

DISCUSSION

TAP shows the formation of PPD-specific protein complexes

In this chapter, we focused on the putative role of the PPD proteins in JA signalling and the characterization of the protein complexes PPDs are involved in. The results obtained by means of TAP with either PPD2 or JAZ10 show that even though these proteins have common interactors such as NINJA and heterodimerization of PPD proteins with some of the JAZ proteins occur (Figure 5.3), there exist PPD- and JAZ-specific partners. Two examples are the differential identification of the yet unknown KIX proteins in TAP of PPD2 but not in that of JAZ10 and, vice versa, the identification of MYCs transcription factors interacting with JAZ10 but not with PPD2 (Table 5.1). The lack of interaction of PPD with MYCs or the JA-Ile receptor COI1, expected from an active Jas-like domain in PPD2, and the fact of PPD2 protein levels being stable following JA treatment (Figure 5.2), suggest that PPD proteins do not function as JAZ proteins but are involved in processes other than JA signalling.

The NINJA and KIX proteins link PPD towards repression

Within the list of interactors retrieved in TAP, we have analysed the direct interaction with NINJA and the KIX proteins and have proven that these occur through the central ZIM and the N-terminal PPD domain in PPD2, respectively (Figures 5.3 and 5.4; Pauwels *et al.*, 2010). Next, the presence of EAR motifs in both NINJA and the KIX proteins prompted us to study their direct interaction with PPD and the possibility for recruitment of the co-repressor TPL. Indeed, the ternary complexes PPD2/NINJA/TPL and PPD2/KIX1-2/TPL can be formed and might potentially repress gene expression (Figure 5.6c-d and Figure 5.8a-b).

The KIX proteins, dual function as repressors and activators?

Although we focused on the functionality of the EAR motif within the KIX proteins leading to recruitment of the co-repressor TPL, additional mechanisms for these proteins can be postulated.

The KIX proteins were named as such based on the presence of an N-terminal KIX domain. This protein domain has been described in non-plant species where it is present in co-

activator proteins such as the human histone acetyl transferases (HAT) CREB BINDING PROTEIN (CBP) or p300 or the Mediator subunit Med15 and mediate the interaction of these proteins with the activation domains of transcription factors (Chrivia *et al*., 1993, Chan and La Thangue, 2001; Yang *et al*., 2006; Thakur *et al*., 2009). In Arabidopsis, the KIX domain is present in proteins such HATs and the orthologue Mediator complex subunit MED15 (Pandey *et al*., 2002, Canet *et al*., 2012). Remarkably, the latter has recently been presented as essential in SA signalling since *Non-Recognition-Of-Bth4* (*nrb4*) mutants that express MED15 proteins with point mutations in the N-terminal KIX domain of Med15 show SA-insensitivity (Canet *et al*., 2012). These results suggest the KIX domain as important for connecting regulators of SA signalling to the Mediator complex, known to ultimately interact with the RNA PolII to regulate transcriptional activity (reviewed in Kidd et al., 2011).

The KIX proteins here presented are smaller proteins and, based on their protein structure, presumably do not function as HATs nor Mediator subunits. Nonetheless, the identification of the RNA Polymerase II subunit NRPB2 as an interactor of KIX2 in TAP suggests a direct KIX-mediated recruitment of the RNA PolII machinery, which, in turn, could promote the transcription of PPD target genes (see Figure 5.8). If that would be the case, KIX1 and KIX2 could perform a dual and antagonistic function, activating or repressing gene expression through interaction with the co-repressor TPL or the RNA Polymerase II, respectively. Confirmation of a direct interaction between KIX and NRPB2 would be the first step to prove the validity of this hypothesis, and different approaches are currently been launched in our lab.

PPD can act as a transcription factor and bind to DNA

Previously, the PPD2 protein has been reported to act as a transcription factor, as it is able to bind to the promoter of the coat protein of the *Tomato Golden Mosaic Virus* (Lacatus and Sunter, 2009). During this thesis, the DNA binding capacity of PPD2 has also been studied by some of our collaborators.

On the one hand, the group of Prof. Dr. Roberto Solano made use of a protein-binding microarray for the identification of DNA-binding specificities of transcription factors (Godoy *et al.*, 2011). This microarray covers all possible double-stranded 11-mers to identify the DNA-binding specificity of a candidate transcription factor. Study of the PPD2 binding

affinities retrieved the palindromic sequence GCCTnAGGC (Figure 5.7). A screen for promoters that presented these sequences throughout the Arabidopsis genome was performed but did not retrieve any candidate for which a straightforward link to PPD could be established (for a list of the hits retrieved, see Annex 5).

Figure 5.7. PPD2 predicted DNA-binding sequence. The palindromic sequence was retrieved from a protein binding microarray assay covering all possible double-stranded 11-mers (as in Godoy *et al.*, 2011).

On the other hand, the groups of Prof. Dr. Dirk Inzé and Prof. Dr. Geert De Jaeger have performed microarrays to identify genes misregulated by *PPD* silencing through amiRNA and chromatin-immunoprecipitation (ChIP) to retrieve gene promoter regions presumably bound by PPD2. Remarkably, the latter approach found a number of putative PPD2-binding sequences different to that identified by means of the protein-binding microarray. The differences in the methodology, the material used and the specific conditions of the experimental design might account for these non-overlapping outcomes.

A model for PPD regulation of gene expression

The identification of PPD interacting proteins by TAP suggest a dual model in which PPD can ultimately repress or activate target gene expression depending on the protein complexes formed. Recruitment of TPL by the formation of ternary PPD/NINJA/TPL or PPD/KIX/TPL complexes results in repression (Figure 5.8a-b). Alternatively, activation of gene expression could occur in case of the KIX proteins directly interacting with the RNA PolII (Figure 5.8c). Even though TAP was shown to be very effective in the identification of these complexes, it did not provide novel clues on the downstream processes that PPD proteins might regulate. Given the potential role of the PPD proteins as transcription factors, approaches such as the identification of the PPD DNA-binding domain and its putative targets should provide new insights. Accordingly, the first can be studied by means of yeast one-hybrid assays, for which we could use the PPD2 fragments generated here and test their interaction with the CP

promoter from TGMV. Next, of special interest is the identification of PPD-binding promoter sequences. The results from *PPD* amiRNA microarrays and ChIP of PPD-tagged cell cultures previously mentioned suggest PPD regulating its own expression and that of cell cycle regulators such as cyclin genes, which may account for the eventual PPD-mediated regulation of growth previously reported by White (2006). A detailed study of the outcome of these assays might help unravelling the actual role(s) of PPD proteins in plants.

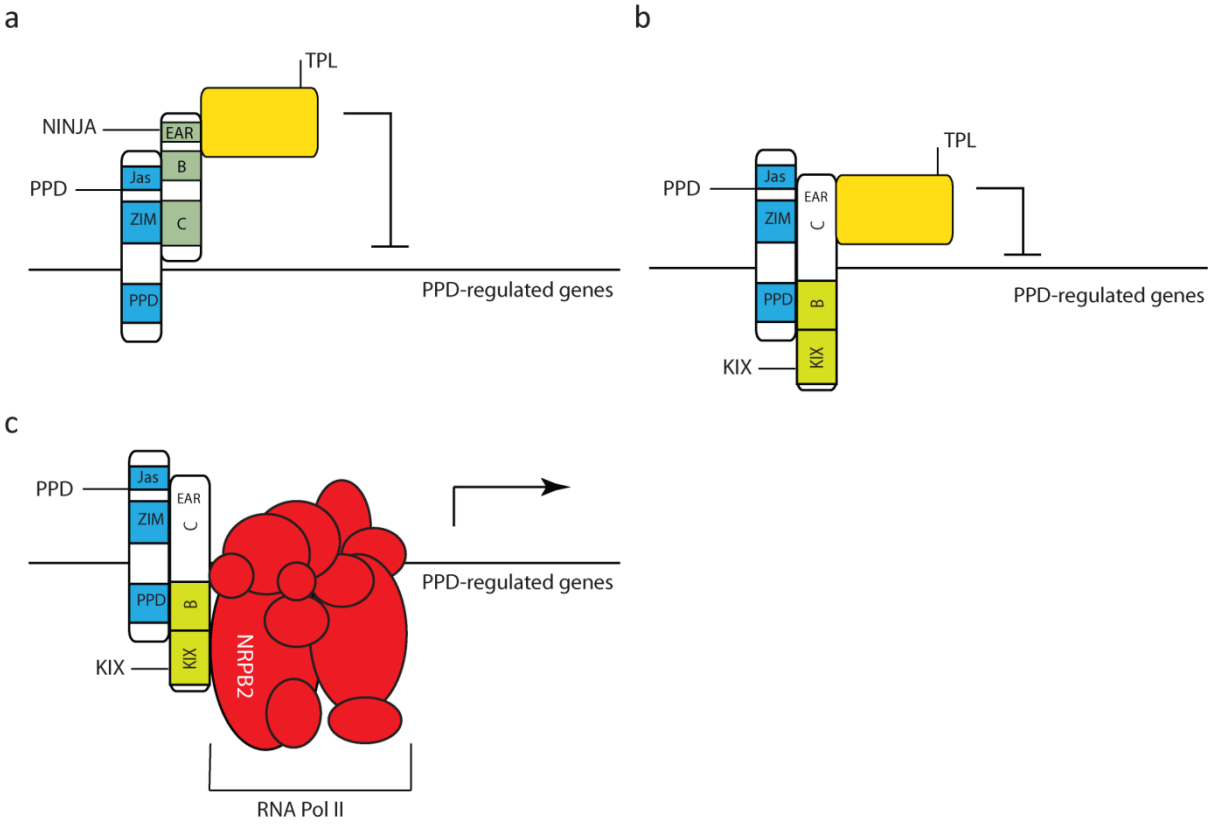

Figure 5.8. Model for PPD-mediated regulation of gene expression. a. PPD interacts with NINJA through their ZIM and C domains, respectively. The EAR motif in NINJA recruits the co-repressor TPL, inhibiting the transcription of PPD-regulated genes. **b.** PPD interacts with the KIX proteins through its N-terminal PPD domain and the N-terminal region of KIX. An EAR motif within domain C of KIX is able to recruit TPL similarly to NINJA. **c.** Alternatively to TPL, the KIX proteins can interact with the RNA PolII subunit NRPB2, thus promoting PPD target gene expression.

EXPERIMENTAL PROCEDURES

Generation of plant lines

The transgenic line carrying an artificial microRNA (amiRNA) targeting both PPD genes was obtained from the lab of Prof. Dr. Dirk Inzé. Those lines overexpressing PPD2 fused to GFP (PPD2-GFP) or a truncated version of PPD1 missing the C-terminal Jas-like domain (PPD1ΔJas) were generated in the lab of Prof. Dr. Alain Goossens. The transgenic lines overexpressing JAZ10.1 or the JAZ10.3 splice variant fused to YFP were obtained from the lab of Prof. Edward E. Farmer.

The ORFs of *PPD2* and the two *KIX* genes were cloned without stop codon into the pDONR207 vector, yielding an entry clone. For generation of transgenic plants with 35S promoter-driven expression of the gene of interest and C-terminal fusion to GFP, the entry clones obtained were recombined with the pK7FWG2 (Karimi *et al*., 2002) destination vector by LR II reaction (Invitrogen). The destination clone was sequence-verified and transformed by electroporation into *Agrobacterium tumefaciens* C58C1 (pMP90) cells. Transgenic Arabidopsis seeds were generated by floral dip (Clough and Bent, 1998), using Col-0 as the background ecotype. Transformants were selected on MS media supplied with 50 mg/mL of Kanamycin and homozygous T3 plant lines were used in the assays.

Root growth assay

Arabidopsis seeds were sterilized by the chlorine gas method and sown on MS media provided with 10 g/L sucrose, 8g/L agar, pH 5,7 and the corresponding final MeJA concentration (0, 2,5 or 10 µM). The plates were kept during two days in the dark at 4^{o}C for stratification in order to synchronize germination. Next, plates were placed vertically in a growth chamber with a 16h day/8h night regime and 21^{o}C and the day of transfer was considered as day 0 after stratification (DAS). Plates were scanned on at 11 DAS at a 300 dpi resolution and root length was measured by means of the EzRhizo software (http://www.root-image-analysis.org/ez-rhizo). Samples were kept in the growth room for another three days for anthocyanin accumulation measurements.

Anthocyanin accumulation

On day 14 after stratification (DAS), seedlings from the root growth assay or seedlings grown on liquid media were harvested and weighted in pre-frozen 1.5 mL eppendorf tubes provided with metal balls. Samples were frozen on liquid nitrogen and ground in a Retsch MM300. For anthocyanin extraction, each sample was added 750 µL of extraction buffer (MeOH HCl 1%) and kept rotating in the dark for 10 minutes. Next, 500 µL of water and 200 µL of chloroform were added, mixing inverting the tubes after each step. Samples were centrifuged for 5 min at full speed and 200 µL of the supernatant were transferred to a 96-well plate. Anthocyanin accumulation was measured as A_{530}-A_{657} and referred to mg of fresh weight.

Protein degradation assays

Transgenic Arabidopsis plant lines overexpressing PPD2, JAZ10.1 or JAZ10.3 under the control of the 35S promoter and C-terminally fused to GFP or YFP (in the case of PPD or JAZ10 splice variants, respectively) were used to study stability upon JA treatment. Seeds were grown on MS media plates containing 10g/L of sucrose, 8 g/L of agar and pH 5,7. Samples were stratified in the dark and 4°C for 2 days and transferred to a growth room with 16h light/8 h dark light regime and 21°C. After 8 days, the seedlings were transferred to liquid MS media with 10 g/L sucrose and pH 5,7, treated with 50 µM JA or ethanol (mock) and kept under orbital shaking. Plant tissue was harvested in 1.5 mL eppendorf tubes provided with metal balls after one hour treatment, frozen in liquid Nitrogen and ground with a Rechst mixer.

Total protein was extracted and 30 µg of protein were loaded for each sample in precasted 4-15% TGX gels (Bio-Rad). The gel run for 20 min at constant 300V and 200mA. Next, blotting was performed into Trans-blot Turbo transfer 0.2µm PVDF membranes (Bio-Rad). A 1/5000 dilution of the green fluorescent protein-horse raddish peroxidase (GFP-HRP) conjugated antibody (Milteny Biotech/MACS) was used for incubation with the blot for one hour. The Western Lightning ECL plus kit (Perkin Elmer) was used to allow Chemiluminescent detection, and SuperSignal West Femto Chemiluminescent Substrate (Thermo Scientific) was used to enhance signal visualization. Coomassie staining was performed confirm equal loading on gel.

Gene cloning

The ORFs of the genes of interest and the corresponding protein fragments were amplified from Arabidopsis cDNA using PCR using the Phusion® High-Fidelity DNA Polymerase (New England Biolabs), according to the manufacturer's protocol and using the primers listed on Annex 1. The PCR product was used for BP reaction (Invitrogen) with the Gateway-compatible pDONR207 vector, generating the corresponding entry clones. Next, LR II reactions (Invitrogen) with the corresponding destination vectors were performed, yielding expression clones to use in the several assays herein described. For mutagenesis of the EAR domain of the KIX proteins, the Gene Tailor Site-Directed Mutagenesis system (Invitrogen) was used following the manufacturer's protocol.

Tandem affinity purification (TAP) in Arabidopsis cell cultures using LC-MS/MS analysis

The bait ORFs were cloned for CaMV 35S promoter-driven expression and C-terminal fusion to the GS-TAP tag (Bürckstümmer *et al*., 2006) in entry clones that were subsequently recombined with the pKCTAP or pKNTAP destination vectors (Van Leene *et al*., 2008) by Multisite Gateway LR reaction. Arabidopsis cell suspension cultures (PSB-D) were transformed without callus selection as previously described (Van Leene *et al*., 2007).

Tandem affinity purification of protein complexes was done using the GS tag (Van Leene et al., 2008) followed by a downscaled purification protocol based on the GS protocol as described in Van Leene et al. (2011). In short, cell extracts were made on 2.5 g cell culture and cleared by two subsequent centrifugation steps at 36,900 x *g* for 20 minutes. In the first purification step, a protein input of 25 mg was incubated with 25 µl of IgG-Sepharose 6 Fast Flow beads (GE Healthcare). For the second step, 25 µl of Streptavidin Sepharose High Performance (Amersham) was used. Final elution was done with 40 µl 1x NuPAGE sample buffer containing 20 mM Desthiobiotin for 5 minutes. Beads were separated from eluate in a 1-ml Mobicol column (MoBiTec, Göttingen, Germany).

Eluted proteins were separated in a short run of 7 minutes on a 4-12% gradient NuPAGE gel (Invitrogen) and visualized with colloidal Coomassie Brilliant Blue staining. The protein gel was washed for 2 hours in H2O, polypeptide disulfide bridges were reduced for 40 min in 25 mL of 6,66 mM DTT in 50 mM NH4HCO3 and sequentially the thiol groups were alkylated for 30 min in 25 mL 55 mM IAM in 50 mM NH4HCO3. After washing with H2O, a broad zone containing the proteins was cut from the protein gel, sliced into 24 gel plugs, and collected together in a single Eppendorf. Gel plugs were washed twice with H2O, dehydrated with 95% CH3CN (v/v), rehydrated with H2O and dehydrated again with 95% CH3CN (v/v). Dehydrated gel particles were rehydrated in 60 µL digest buffer containing 750 ng trypsin (MS Gold; Promega, Madison, WI), 50 mM NH4HCO3 and 10% CH3CN (v/v) for 30 min at 4° C. Proteins were digested at 37° C for 3.5 hours.

The obtained peptide mixtures were introduced into an LC-MS/MS system, the Ultimate 3000 RSLC nano (Dionex, Amsterdam, The Netherlands) in-line connected to an LTQ Orbitrap Velos (Thermo Fisher Scientific, Bremen, Germany). The sample mixture was loaded on a trapping column (made in-house, 100 µm internal diameter (I.D.) x 20 mm (length), 5 µm C18 Reprosil-HD beads, Dr. Maisch GmbH, Ammerbuch-Entringen, Germany). After back-flushing from the trapping column, the sample was loaded on a reverse-phase column (made in-house, 75 µm I.D. x 150 mm , 5 µm C18 Reprosil-HD beads, Dr. Maisch). Peptides were loaded with solvent A (0.1% trifluoroacetic acid, 2% acetonitrile), and separated with a linear gradient from 2% solvent A' (0.1% formic acid) to 50% solvent B' (0.1% formic acid and 80% acetonitrile) at a flow rate of 300 nl/min, followed by a wash step reaching 100% solvent B'.

The mass spectrometer was operated in data-dependent mode, automatically switching between MS and MS/MS acquisition for the ten most abundant peaks in a given MS spectrum. In the LTQ Orbitrap Velos, full scan MS spectra were acquired in the Orbitrap at a target value of 1E6 with a resolution of 60,000. The ten most intense ions were then isolated for fragmentation in the linear ion trap, with a dynamic exclusion of 20 seconds. Peptides were fragmented after filling the ion trap at a target value of 1E4 ion counts.

From the MS/MS data in each LC run, Mascot Generic Files were created using the Mascot Distiller software (version 2.4.1.0, Matrix Science, www.matrixscience.com/Distiller.html). When generating these peak lists, grouping of spectra was allowed with a maximum intermediate retention time of 30 seconds and a maximum intermediate scan count of 5 was used where possible. Grouping was done with 0.005 Da precursor tolerance. A peak list was only generated when the MS/MS spectrum contained more than 10 peaks. There was no de-isotoping and the relative signal-to-noise limit was set to 2. These peak lists were then searched with the

Mascot search engine (version 2.3, MatrixScience, www.matrixscience.com) using the Mascot Daemon interface (Matrix Science, www.matrixscience.com). Spectra were searched against the TAIR10 database containing 35386 sequence entries. Variable modifications were set to methionine oxidation and methylation of aspartic acid and glutamic acid. Fixed modifications were set to carbamidomethylation of cysteines. Mass tolerance on MS was set to 10 ppm (with Mascot's C13 option set to 1) and the MS/MS tolerance at 0.5 Da. The peptide charge was set to 1+, 2+ and 3+ and the instrument setting was set to ESI-TRAP. Trypsin was set as the protease used, allowing for 1 missed cleavage, and also cleavage was allowed when arginine or lysine is followed by proline. Only high confident peptides, ranked one and with scores above the threshold score, set at 99% confidence, were withheld. Only proteins with at least two matched high confident peptides were retained.

A list of non-specific background proteins was assembled by combining our previous background list (Van Leene et al, 2010) with background proteins from control GS purifications on mock, GFP-GS, and GUS-GS cell culture extracts identified with LTQ Orbitrap Velos. To obtain the final list of interactors, these background proteins were subtracted from the list of identified proteins.

Yeast two- and three-hybrid assays

The ORFs of the proteins of interest were cloned without stop into Gateway-compatible entry clones as previously described. The entry vectors were then used for LR II reaction (Invitrogen) with Gateway-compatible versions of the pGADT7 and pGBKT7 (or pGBT9) yeast two-hybrid vectors, thus generating bait and prey constructs. Co-transformation of the *Saccharomyces cerevisiae* yeast PJ69-4A strain using different bait-prey plasmid combinations was performed as described in Chapter 3. Transformant yeast colonies in yeast two-hybrid (Y2H) assays were selected on SD media lacking Leu and Trp (-2). Several independent colonies for each bait-prey combination were grown overnight in liquid (-2) media. The next day, 10- and 100-fold dilutions of the cultures were dropped on control (-2) and selective (-3) media, the latter additionally lacking His. Co-transformation with the empty pGADT7 and pGBKT7 (or pGBT9) vectors was used as negative controls.

In yeast three-hybrid (Y3H) assays, the MultiSite pMG426 vector was used for expression of the third protein of interest (i.e. NINJA or KIXs), driven by the GDP promoter and C-terminally fused to the SV40 NLS-3xFLAG-6xHis tag (Nagels Durand *et al*., 2012; http://gateway.psb.ugent.be). The assay was performed as previously described for Y2H assays with modifications in the SD media used. For transformant selection and culturing in control media, SD media lacking Leu, Trp and Ura was used (-3), whereas selective media additionally lacked His (-4). Both in Y2H and Y3H assays, plates were allowed to grow for 2 days at 30°C, and interaction was scored in terms of growth on selective media.

Confocal imaging

Plants expressing the *35Spromoter::KIX1-GFP* fusions were germinated on solid MS plates (containing 10 g/L of sucrose and 8 g/L agar) placed vertically. On the day of imaging, seedlings were briefly incubated in 3mg/L propidium iodide (Sigma), subsequently washed and mounted in milliQ water. Fluorescence microscopy was performed with an Olympus FV10 ASW confocal microscope.

Transient Expression Assays in Arabidopsis protoplasts

Transient expression assays were performed as described previously (De Sutter *et al.*, 2005; Pauwels *et al.*, 2008). Protoplasts were prepared from a Bright Yellow-2 (BY-2) tobacco cell culture and co-transfected with a reporter plasmid containing the firefly-Luciferase (fLUC) reporter gene driven by a promoter containing five GAL4-binding sites (Ohta *et al.*, 2000), a normalisation construct expressing Renilla luciferase (rLUC) under the control of the 35S promoter (De Sutter *et al*., 2005) and effector constructs. GAL4DBD fusions were generated by combining pEN-L4-2-R1 (35S promoter), pEN-R2-GAL4DBD-L3 and an entry clone holding the ORF, combined by MultiSite Gateway LR reaction with pm43GW7 as destination vector. For each experiment, 2 µg of each plasmid were used. After transfection, protoplasts were incubated overnight in the dark, at room temperature and with gentle agitation. The next day, protoplasts were lysed, and fLUC and rLUC activities were determined with the Dual-Luciferase reporter assay system (Promega). Variations in transfection efficiency and technical error were corrected by normalisation of fLUC by rLUC activities. All transactivation

assays were conducted in an automated experimental set-up. A one-way ANOVA and Dunnett T3 Post Hoc test were performed to confirm statistically significant differences between control and effector constructs ($p<0.05$).

SUPPLEMENTARY INFORMATION

Annex 1. List of primers used in this thesis.

Annex 4. Protein Identification details obtained for PPD2, JAZ10 and KIX2 TAP assays, performed with the LTQ Orbitrap Velos (Thermo Fisher Scientific) and Mascot Distiller software (version 2.4, Matrix Science) combined with the Mascot search engine (version 2.3, Matrix Science) using the Mascot Daemon interface (Matrix Science) and database TAIR10. Proteins and peptides headers used in the table are listed below.
prot_score: protein score; **prot_mass**: protein mass; **prot_cover**: percentage of protein sequence covered by assigned peptide matches; **prot_len**: protein sequence length (AA); **prot_pi**: protein pI; **pep_query**: number assigned by Mascot to a specific MS2 spectrum; **pep_rank**: peptide rank number (1 for the top ranked peptide match); **pep_isbold**: peptide is in bold red. (Red and bold typefaces are used to highlight the most logical assignment of peptides to proteins. The first time a peptide match to a query appears in the report, it is shown in bold face. Whenever the top ranking peptide match appears, it is shown in red. Thus, a bold red match is the highest scoring match to a particular query listed under the highest scoring protein containing that match. This means that protein hits with many peptide matches that are both bold and red are the most likely assignments.); **pep_isunique**: peptide is unique for the protein family; **pep_exp_mz**: observed m/z value (precursor); **pep_exp_mr**: experimental relative molecular mass; **pep_exp_z**: observed peptide charge state; **pep_calc_mr**: calculated relative molecular mass; **pep_delta**: difference (error) between the experimental and calculated masses; **pep_start**: peptide start position in protein; **pep_end**: peptide end position in protein; **pep_miss**: number of missed enzyme cleavage sites; **pep_score**: peptide ions score; **pep_homol**: homology threshold score; **pep_ident**: identity threshold score; **pep_expect**: expectation value for the peptide match (The number of times we would expect to obtain an equal or higher score, purely by chance. The lower this value, the more significant the result); **pep_res_before**: amino acid before peptide sequence; **pep_seq**: peptide sequence; **pep_res_after**: amino acid after peptide sequence; **pep_var_mod**: any variable modifications found in the peptide; **ion_coverage**: ion coverage: y ions in red, b ions underlined

Annex 5. List of hits retrieved by the PPD-binding microarray with 0.95 confidence. AGI annotation and short description was obtained from the TAIR10 database.

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Chapter 6
Conclusions and Perspectives

Picture: Heading Monument Valley. Picture by Juan Carlos Navarro

CONCLUSIONS AND PERSPECTIVES

During the time frame of this thesis, we aimed to study and functionally characterize the non-JAZ TIFY proteins in the model species Arabidopsis. In the previous chapters, we reported our findings and proposed mechanistic models to integrate the data generated. This section provides an overview of the conclusions, discusses open questions and offers perspectives for further research.

Although belonging to the same family, neither TIFY8 nor PPD proteins are functionally redundant with the JAZ proteins. In contrast with them, the non-JAZ TIFY proteins are stable upon JA treatment, altered gene expression does not lead to differential JA-mediated responses and are present in distinct protein complexes.

Here, we made use of the TAP platform developed by the lab of Prof. Dr. Geert de Jaeger. The previous experiences with JAZ1, NINJA, JAZ3, JAZ5 and MYC transcription factors have shown this technique to be robust and reliable for the identification of protein complexes within the JA signalling core module (Fernández-Calvo et al., 2011; Pauwels et al., 2010). In this case, TAP of TIFY8, PPD2, JAZ10 and KIX2 allowed us to identify several partners, hinting the existence of interactive networks (Figure 6.1). Amongst the candidates, we focused in the interaction of NINJA with the non-JAZ TIFY proteins, which suggested the formation of repressor complexes ultimately recruiting TPL and which we confirmed by means of Y3H assays. Accordingly, the functionality of the NINJA-TPL module represents a general mechanism for repression of gene expression. The recruitment of the NINJA-TPL module to a certain class II TIFY protein might then regulate the corresponding downstream process, i.e. JA signalling in the case of the JAZs, leaf growth in the case of PPD.

Here, we reported the TIFY8 protein functioning as a transcriptional repressor. Remarkably, TIFY8 does not interact with the JAZ proteins but is able to regulate *JAZ* gene expression upon infection with the hemibiotrophic bacteria *Pst* DC3000, which suggests some specialization of TIFY8 for regulation of other TIFY members. Nonetheless, there exist several questions to be addressed to obtain a complete characterization of this atypical TIFY family member.

First, the conserved protein regions of TIFY8 seem to play a key role in the regulation of gene expression since they might mediate specific protein-protein interactions with NINJA

and with several transcription factors. Our results suggest the N-terminal region of TIFY8 to be important for TIFY8-mediated regulation of *JAZ* gene expression levels. Functionality of this protein region might explain its conservation amongst TIFY8 orthologues in other species, which are commonly present as a unique gene. To mimic the lack of this region, we made use of the TIFY8-CT line overexpressing, but additional experiments would be necessary to confirm our hypothesis. One possible approach, given that we lack a true *tify8* knock out, is a cross between the *tify8-1* and the TIFY8-CT line, were no transcripts expressing the N-terminal region of TIFY8 will be expressed. *JAZ10* hyperinduction upon infection with *Pst* DC3000 in this cross can then be analysed.

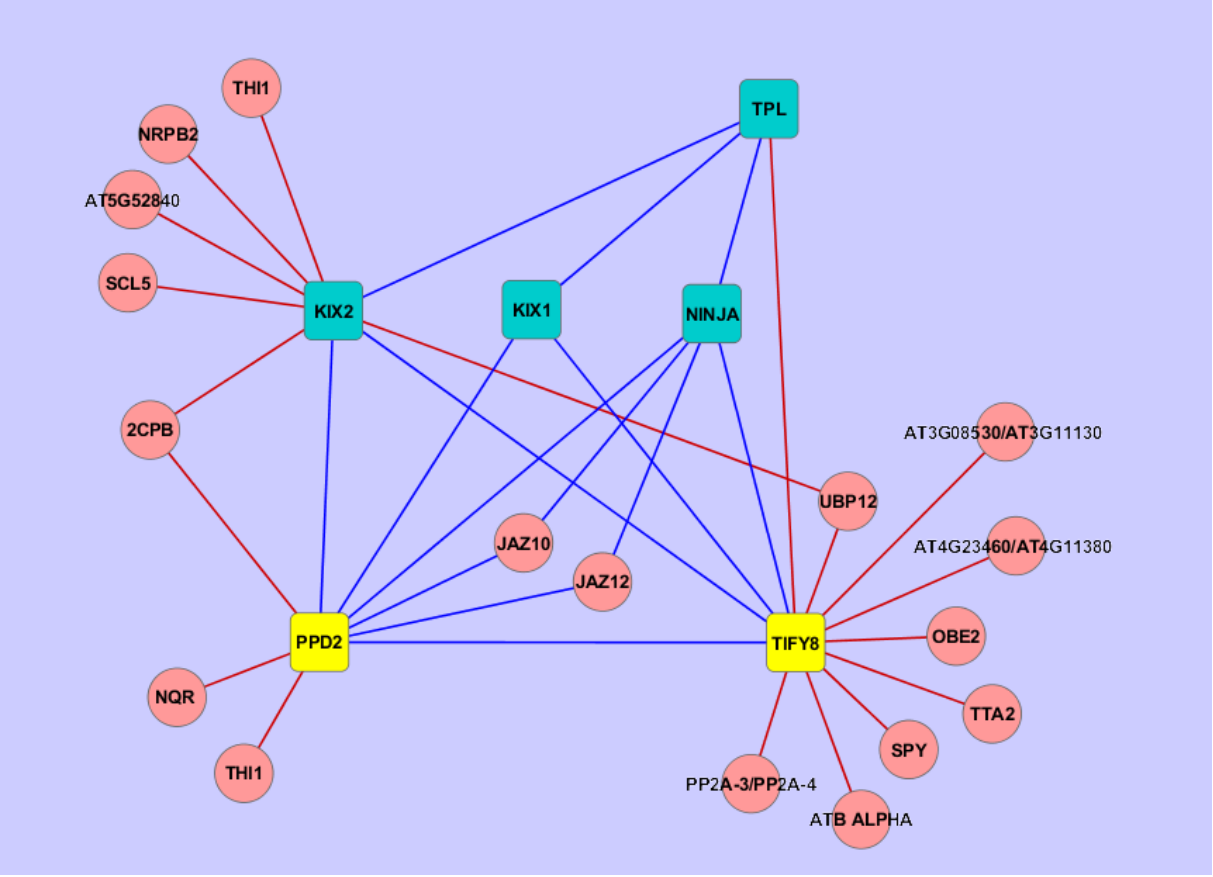

Figure 6.1. Overview of some of the protein-protein interactions reported in this thesis. The outcomes of the TIFY8, PPD2, KIX2 reported in this thesis and the NINJA TAP in Pauwels et al. (2010) were used for generating the network. Red lines represent the interactions reported solely by TAP, whereas blue lines indicate the interactions verified by means of TAP and Y2H. The non-JAZ TIFY proteins PPD2 and TIFY8 are shown in yellow and the KIX, NINJA and TPL, all linked to repression, are shown in blue.

Secondly, the finding of different *TIFY8* splice variants being generated adds an extra level of complexity in the regulation of *TIFY8* expression and its downstream effects. It will be key to determine which alternative transcripts are produced and attempt to predict the TIFY8 protein forms generated. This can be approached by means of RT-PCR-based experiments and a detailed analysis of RNA-Seq data publicly available or for example that generated in the lab of Prof. Dr. ir. Corné M. J. Pieterse.

Next, as just mentioned above, the *tify8-1* line cannot be considered a true knock-out line. The analysis of such a line is of crucial importance to understand the role of TIFY8, not just in the regulation of *JAZ* gene expression upon infection with *Pst* DC3000 but in other processes it might be involved in, given the long list of interactors retrieved in our TAP experiments and the recently published Arabidopsis Interactome. Nonetheless, the use of the various lines with altered *TIFY8* expression generated during this thesis could be used for microarray or phenotypical analysis to identify additional processes requiring TIFY8.

Regarding the PPD proteins, we reported their interaction with NINJA and two yet unknown proteins, here named KIX, for the recruitment of TPL. The identification of the KIX proteins opens an interesting line of research, given their special protein domain structure and the identification of the RNA polymerase II subunit 2 as an interactor of KIX2 in TAP experiments. These findings suggest a dual function for the KIX proteins, being able to interact with both transcriptional repressors and activators (i.e. TPL and RNA PolII, respectively). Different approaches for further research around the KIX proteins are foreseen, such as the confirmation of the interaction KIX-RNA Pol II subunit by means of Y2H and pull-down assays, or the generation and the phenotypical analysis of transgenic lines with altered *KIX* expression, such as overexpression, single knock-out or a *kix1kix2* double mutant.

The identification of other putative PPD2 interactors did not retrieve any candidate pointing towards a clear function of the PPD proteins. However, alternative approaches exploiting the potential capacity of PPD2 working as a transcription factor have been started in the lab of Prof. Dr. Geert de Jaeger and Prof. Dr. Dirk Inzé. Microarray analysis of a *PPD* loss of function line and ChIP to identify putative PPD targets points towards a role of PPD regulating cell cycle and plant growth, and are in line with the findings previously reported by White (2006), where *ppd* mutants lead to larger plant organs. The integration of the data

generated in these experiments, together with the identification of the PPD protein DNA-binding domain will be key in the future to determine the PPD targets and the downstream processes they regulate.

Finally, the reciprocity in the identification of TIFY8 and PPD proteins as interactors in both TAP and Y2H assays insinuates a cooperative function of the non-JAZ TIFY proteins and the existence of a complex regulatory network around these proteins. Unravelling the role of TIFY8 in PPD-regulated processes and vice-versa will be the next step for an in-depth functional characterization of non-JAZ TIFY proteins.

Chapter 7

Summary | Samenvatting

SUMMARY

Jasmonates (JAs) are phytohormones that regulate a myriad of processes such as plant growth, defence responses to necrotrophic pathogens and herbivores, and the production of a wide range of species-specific secondary metabolites. During the last years, a core module of JA signalling has been unravelled which show the striking similarities with the corresponding pathway of auxin. Both perception and signalling of these hormones involve the same, similar or homologous proteins which form specific protein complexes (**Chapter 1**).

The JAZ proteins are key components within the JA signalling pathway. JAZs orchestrate the repression of JA-dependent gene expression, which is released upon hormone perception and consequent JAZ ubiquitination and targeting for 26s proteasome-mediated degradation (**Chapter 1**). Despite the great knowledge about the JAZ proteins acquired in the last years, very few is known about non-JAZ proteins that belong to the same family, i.e. the plant-specific TIFY protein family. The PPD1, PPD2 and TIFY8 proteins have not been much the focus of study yet and an in-depth functional characterization of these proteins was still missing. This challenged us to undertake this line of research (**Chapter 2**).

One of the approaches chosen for the characterization of these proteins was the study of their protein-protein interactions by means of TAP and Y2H techniques. For the latter method, we described a detailed protocol that allows to effectively test a high number of putative interactions in a pair-wise manner (**Chapter 3**).

A detailed study of TIFY8 showed that JA did not affect TIFY8 protein stability and that JA-responsiveness was not changed in transgenic plants with altered *TIFY8* expression. This supports the hypothesis of TIFY8 being involved in other processes different than JA signalling. At the protein level, regardless the presence of the ZIM domain which is important for homo- and heterodimerization of the TIFY proteins, TIFY8 does not interact with any of the JAZ but with the PPD proteins. Accordingly, no JAZ peptides were identified in TAP with TIFY8, whereas the PPD and NINJA proteins were the most interesting interactors retrieved in these assays. Next, we confirmed that TIFY8 is able to recruit TPL through interaction with NINJA. The latter finding accounts for a function of TIFY8 as a transcriptional repressor. Finally, we showed that TIFY8 is able to regulate *JAZ* gene expression following infection with the hemibiotrophic bacteria *Pst* DC3000. The mutant

tify8, which produces truncated TIFY8 proteins, is hypersensitive to this pathogen and shows *JAZ* gene hyperinduction(**Chapter 4**).

Similarly to TIFY8, we performed TAP to identify PPD-interacting proteins. This retrieved, amongst others JAZ10, TIFY8, NINJA and two hitherto unknown proteins named KIX1 and KIX2. Even though interaction with some of the JAZ proteins occurred, PPD proteins do not act as JAZ proteins, since i) no other JA core module proteins - e.g. COI1, MYCs- are identified in TAP, ii) PPD proteins stay stable *in planta* upon JA treatment and iii) transgenic lines with altered *PPD* expression do not show differential JA-responsiveness. We focused our research on NINJA and the KIX proteins, all of them possessing EAR motifs. We confirmed the interaction of all three proteins with PPD and their ability to recruit TPL. We conclude that NINJA and the two KIX proteins generate repressing protein complexes which may regulate PPD-dependent gene expression. (**Chapter 5**).

SAMENVATTING

Jasmonaten (JAs) zijn plantenhormonen die een grote verscheidenheid aan processen reguleren zoals groei, verdediging tegen necrotrofe ziekteverwekkers of herbivoren en de productie van soortspecifieke secundaire metabolieten. De laatste jaren is de centrale signalisatiemodule van jasmonaten ontdekt en zijn de sterke gelijkenissen met die van auxine duidelijk geworden. De waarneming en signalisatie van auxine en JA maken gebruik van dezelfde, gelijkaardige of homologe eiwitten die specifieke eiwitcomplexen vormen (**Hoofdstuk 1**).

De JAZ eiwitten zijn sleutelcomponenten in de JA-signalisatie. De JAZ coördineren de repressie van JA-afhankelijke genexpressie. Deze wordt opgeheven na waarneming van het hormoon waarna de JAZ geübiquitineerd worden en gemerkt voor afbraak door het 26S proteasoom (**Hoofdstuk 1**).

Desondanks de grote hoeveelheid kennis die de laatste jaren is opgedaan rond de JAZ eiwitten is er weinig geweten over de niet-JAZ eiwitten die tot dezelfde familie behoren, i.e. de plant-specifieke TIFY eiwitfamilie. De eiwitten PPD1, PPD2 en TIFY8 waren nog niet veel het onderwerp van onderzoek en een diepgaande functionele analyse van deze eiwitten ontbrak nog. Dit motiveerde ons om deze onderzoekslijn te starten (**Hoofdstuk 2**).

Eén van de gekozen methoden om deze eiwitten te bestuderen is de identificatie van interagerende eiwitten door middel van TAP en Y2H. Voor de laatste methode beschrijven we een gedetailleerd protocol dat toelaat om efficiënt een groot aantal mogelijke interacties in een paarsgewijze manier te testen **(Hoofdstuk 3).**

Een gedetailleerde studie van TIFY8 toonde aan dat JA niet de TIFY8 eiwitstabiliteit beïnvloedt en dat de gevoeligheid voor JA onveranderd blijft in transgene planten met gewijzigde *TIFY8*-expressie. Deze bevindingen bevestigen de hypothese dat TIFY8 betrokken is in andere processen dan JA- signalisatie. Op het eiwitniveau, desondanks de aanwezigheid van een ZIM-domain belangrijk voor homo- en heterodimerisatie van de TIFY-eiwitten, interageert TIFY8 niet met de JAZ, maar wel met de PPD eiwitten. Overeenkomstig werden geen JAZ peptiden gevonden in de TAP van TIFY8, maar werden wel PPD en NINJA als de meest interessante interactoren geïdentificeerd. Vervolgens bevestigden we dat TIFY8 in staat is om TPL te recruteren door zijn interactie met NINJA. Deze laatste vinding bevestigt

de functie van TIFY8 als een transcriptionele repressor. Finaal toonden we aan dat TIFY8 *JAZ* genexpressie kan reguleren na infectie met de hemibiotrofe bacterie *Pst* DC3000. De mutant *tify8,* die afgekorte TIFY8 eiwitten produceert, vertoont hypergevoeligheid voor deze ziekteverwekker en toont hyperinductie van *JAZ*-genexpressie (**Hoofdstuk 4**).

Gelijkaardig aan TIFY8 voerden we een TAP uit om PPD-bindende eiwitten te identificeren. Deze analyse resulteerde onder andere in de ontdekking van JAZ10, TIFY8, NINJA en twee tot dan toe onbekende eiwitten KIX1 en KIX2 als interactoren. Desondanks er interactie van PPD met sommige JAZ werd gevonden, concludeerden we dat PPD eiwitten niet functioneren als JAZ doordat i) geen andere eiwitten uit de module (eg. COI1, MYC) werden geïdentificeerd, ii) PPD eiwitten stabiel blijven *in planta* na behandeling met JA en iii) transgene planten met gewijzigde *PPD*-expressie geen veranderde gevoeligheid voor JA vertonen. We spitsten ons onderzoek vervolgens toe op NINJA en de twee KIX die allen een EAR-repressiemotief bezitten. We bevestigden de interactie van alle drie met de PPD en hun eigenschap om TPL te recruteren. We besluiten dat NINJA en KIX repressieve eiwitcomplexen vormen die PPD-afhankelijke genexpressie kunnen reguleren (**Hoofdstuk 5**).

[redacted]

Curriculum vitae

[redacted]

Curriculum Vitae

PERSONAL DETAILS
Amparo Cuéllar Pérez
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EDUCATION

March 2009- January 2012: PhD student in Sciences (Biotechnology) at Ghent University.

2002-2008: Agricultural Engineer. Master in Biotechnology and Plant Breeding. School of Agricultural Engineering (ETSIA). Polytechnic University of Valencia (UPV; Spain).

RESEARCH EXPERIENCE

March 2009- January 2012: Predoctoral fellow at the department of Plant Biotechnology and Genetics of Ghent University (UGent) and the department of Plant Systems Biology of the Flanders Institute of Biotechnology (VIB). Promoter: Prof. Dr. Alain Goossens.
Title: "*Functional characterization of non-JAZ TIFY proteins in Arabidopsis thaliana".*

September 2011 and February 2012: Research stay in the Plant-Microbe Interactions group of Prof. Dr. ir. Corné M. J. Pieterse at the Institute of Environmental Biology. Utrecht University, Utrecht, The Netherlands.

Sept 2007-June 2008: Master thesis project. Hosted at UGent-VIB within the Erasmus Exchange program. Promoter: Dr. Pierre Hilson.
Title: *"Characterization of the GOLVEN peptides controlling auxin homeostasis"*.
Awarded by the School of Agricultural Engineering (ETSIA). Polytechnic University of Valencia (UPV, Spain).

Oct 2006- July 2007: Internship-collaboration grant at Institute for Plant Molecular and Cell Biology, Polytechnic University of Valencia, Spain (IBMCP, CSIC-UPV). Promoter: Dr. Pablo Tornero.

June - August 2005: Internship in Seminis. Vegetable Research Station placed in Miranda (Cartagena, Spain).

PUBLICATIONS

Cuéllar Pérez A and Goossens A. Jasmonate signaling: a copycat of auxin signaling?. Submitted to Plant, Cell and Environment.

Cuéllar Pérez A, Pauwels L, de Clercq R, Goossens A.(2012). Yeast two-hybrid analysis of Jasmonate signaling proteins . Methods in Molecular Biology. Springer, New York (In press).

Whitford R, Fernandez A, Tejos R, Cuéllar Pérez A, Kleine-Vehn J, Vanneste S, Drozdzecki A, Leitner J, Abas L, Aerts M, Hoogewijs K, Baster P, De Groodt R, Lin YC, Storme V, Van de Peer Y, Beeckman T, Madder A, Devreese B, Luschnig C, Friml J, Hilson P. GOLVEN secretory peptides regulate auxin carrier turnover during plant gravitropic responses. Dev Cell. 2012; 22(3):678-85.

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Pauwels L, Fernández-Barbero G, Geerinck J, Tilleman S, Grunewald W, Cuéllar Pérez A, Chico JM, Bossche RV, Sewell J, Gil E, García-Casado G, Witters E, Inzé D, Long JA, De Jaeger G, Solano R, Goossens A. NINJA connects the co-repressor TOPLESS to jasmonate signalling. *Nature. 2010; 464(7289):788-91.*

OTHER SCIENTIFIC COMMUNICATIONS

Pauwels L, Cuéllar Pérez A, Nagels Durand A, Colling J, Vanden Bossche R, de Clercq R, Van Wees SCM, De Jaeger G, Pieterse CMJ, Goossens, A. TIFY8 is a transcriptional repressor regulating *JAZ* gene expression upon infection by *Pseudomonas syringae*. Poster at the 23rd International Conference on Arabidopsis Research (ICAR). Viena (Austria), July 2012.

Cuéllar Pérez A, Pauwels L, Nagels Durand A, Geerinck J, Vanden Bossche R, de Clercq R, Goossens A. Characterization of novel proteins involved in jasmonate signalling. Oral presentation and poster at the Belgian Plant Biotechnology Association Symposium. Lovain-la-Neuve (Belgium), November 2011.

Cuéllar Pérez A, Pauwels L, Nagels Durand A, Geerinck J, Vanden Bossche R, de Clercq R, Goossens A. Characterization of novel proteins involved in jasmonate signalling. Poster presented at the 6th SummerSchool on Environmental Signalling. Utrecht (The Netherlands), August 2011.

Pauwels L, Cuéllar Pérez A, Geerinck J, Nagels Durand A, Stone S, Callis J, De Jaeger G and Goossens A. Tandem affinity purification of JAZ proteins reveals new interactors involved in (de-)ubiquitination in *Arabidopsis thaliana*. Poster presented at The Ubiquitin Family, Cold Spring Harbor (USA). May, 2011.

Pauwels L, Fernández Barbero G, Geerinck J, Tilleman S, Grunewald W, Cuellar Pérez A, Van De Slijcke E, Vanden Bossche R, Chico JM, Witters E, Inzé D, Long J, De Jaeger G, Solano R and Goossens A. NINJA connects JAZ proteins to the co-repressor TOPLESS. Poster presented on the Keystone Symposia Conference: Plant Receptors and Signaling in Plant Development and Biotic Interactions, Tahoe City (California, USA), March 2010.

Pauwels L, Geerinck J, Tilleman S, Grunewald W, Cuellar Pérez A, Van De Slijcke E, Vanden Bossche R, Witters E, Inzé D, De Jaeger G and Goossens A. (2009) Tandem affinity purifcation of JAZ protein complexes. Poster presented at Regulatory Oxylipins, Lausanne (Switzerland), June, 2009.

ATTENDED MEETINGS

- Partnership meetings within the SmartCell FP7 EU Project.
- Belgian Plant Biotechnology Association symposium. November 2011, Louvain-la-Neuve (Belgium).
- Utrecht 6th SummerSchoool on Environmental Signalling. August 2011, Utrecht (The Netherlands).
- Advances in Genomics symposium. January 2010, Ghent (Belgium).

SUPERVISION OF UNDERGRADUATED STUDENTS

2010-2011: Ajay Kakad (2nd year Master of Biochemistry and Biotechnology).
Title: *"Are the PPD proteins functional JAZ proteins?"*

2010-2011: Jonas Goossens (1st year Master in Biochemistry and Biotechnology).
Title: "*Protein ubiquitination in jasmonate signal transduction".*

2009-2010: Astrid Nagels Durand (2nd year Master in Biochemistry and Biotechnology).
Title: *"Phenotypic screening of plants with modulated expression of candidate jasmonate signalling proteins"*.

COURSES and WORKSHOPS

English Writing Skills. UGent, February-April 2012.

Workshop on Statistical thinking and Reasoning in Experimental Research. VIB Research Training Course (VRTC), November 2011.

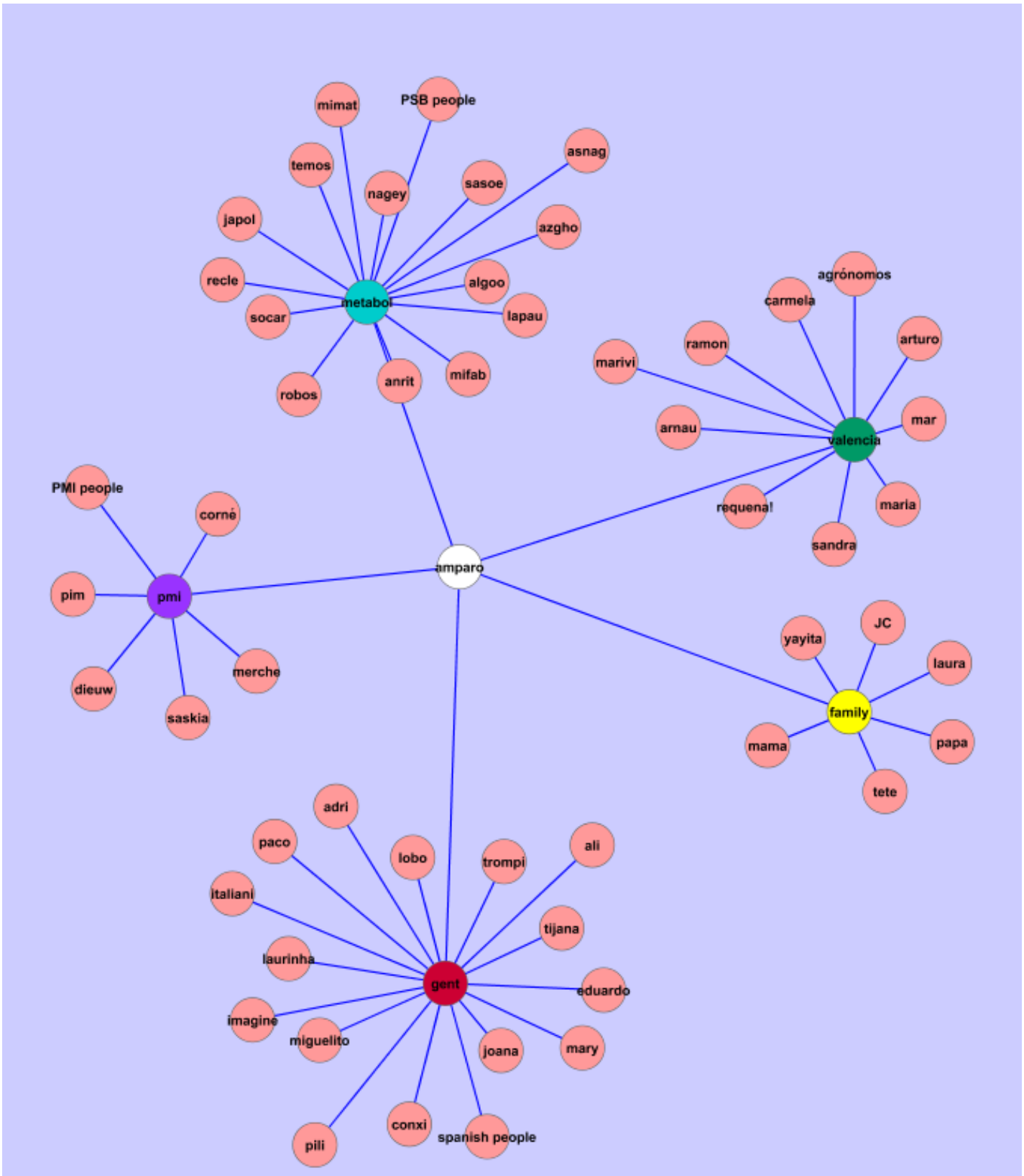
Workshops on Transferable Skills: Contracts, Intellectual Property and Project Management. Institute for Continuing Education in Science of Ghent University (ICES), March-April 2011.

Science Ethics I. VIB Research Training Course (VRTC), March 2011.

Bioinformatics courses: "Basic bioinformatics, concepts, databases and tools", Genevestigator and Vector NTi workshops. VIB Bioinformatics Training Services (VIB-BITS). 2009-2011

Effective Science communication. Ghent University, February 2010.

Acknowledgements



After all the struggle to get this thesis ready, I didn't expect this section to be the most complicated to write, but it definitely is. It is difficult to get words together to express my gratitude to everyone that has helped me during this important phase in my life.

Alain, I really want to thank you for giving me the chance to come back to Gent and start this PhD. Also, thanks for your guidance and support during these years and for the nice atmosphere you bring to the group. It will be almost impossible to find a better working place than the Metabol lab. I really enjoyed working here. The chats, the jokes, the laughs, the smoking breaks (not for me anymore!), the coffee breaks, the drinks, the (swampy) team building activities and, of course, the scientific discussions! Seriously guys, you are not colleagues but friends, and I am gonna miss you so much. Laurens, it's been a pleasure to work with you side by side. I've learned a lot from you and I really appreciate your help during my PhD. Robin (a.k.a. piticli), thanks for being there; Rebecca for being always so nice, Andrés and Astrid for bringing 'el toque latino' to the lab. Tess and Jacob, for their example of devoted scientists and their help, especially in the last months. Michele, grazie tra altro per le lezioni di italiano and Michiel, I really enjoyed your humour! And I could continue: Janine, Azra, Jonas, Sophie, Sandra, Alex, Philip, Jan (x2), Ajay, Lander, Timo... It's just great to work with such nice people!

Besides the Metabol, I am very grateful to the entire PSB. The great environment and all the people I have encountered through these years. Thank you so much! Hartelijk bedankt ook aan de mensen bij de PMI in Utrecht, for making my stay there a great experience, and especially Corné and Saskia for being so friendly and for their help.

I also have to thank my family. Papá, mamá, Laura, Juan Carlos, yaya, tete, gracias por vuestra confianza en mí y vuestro apoyo incondicional. And my friends back in Spain, for their friendship and for making me feel close to Valencia even though it's been a while that I am far away.

Finally I also want to share my gratitude to all the friends in made Gent. The PhD experience, especially abroad, is not just a professional but also an important personal learning process. And it was my pleasure to learn with you. Gracias a la gran 'familia' española emigrada en Gante: Ali, Lobo, Miguel, Rubén, Víctor… I might need several pages to mention you all and the great moments we've shared. Anche grazie al settore italiano, especially to Mary and Tijana (yes Tj, I got you within the Italian pack!). And of course... a Conchi, gracias por tu paciencia y por tu apoyo, por ser la mejor amiga que alguien puede pedir. Y a Adri, por creer en mí, por todo lo compartido hasta ahora y lo que aún nos queda por compartir.

I could keep on writing pages and pages… but I've always preferred to do this personally and hope I get the chance to do so. Thus, making a long story short… I just want to tell, to all of you:

¡Gracias! - Thank you! - Dank U! - Grazie! - Gràcies! – Merci! - Danke!

Annexes

Annex 1. List of primers used in this thesis

Gene	Use	Sequence	USE	comment
ACTIN	FwP	GTTGCACCACCTGAAAGGAAG	genotyping	
ACTIN	RvP	CAATGGGACTAAAACGCAAAA	genotyping	
ADH1-terminator	RvP	CCGGTAGAGGTGTGGTCAAT	cloning	To check pGADT7, pGBKT7 and pGBT9 Gateway-compatible vectors
GAL4AD	FwP	GCGTATAACGCGTTTGGAAT	cloning	To check pGADT7 Gateway-compatible vectors (968bp + insert)
GAL4DBD	FwP	TGCCGTCACAGATAGATTGG	cloning	To check pGBT9 and pGBKT7 Gateway-compatible vectors (619bp +insert)
GK LB	LB	CCCATTTGGACGTGAATGTAGACAC	genotyping	left border GABK-KAT T-DNA
GK-738B03	LP	AAGAGGTCAAGCAACCACATG	genotyping	left primer specific for GK-738B03 (*tify8-1*)
GK-738B03	RP	AATGCCATGATTTTACAATCG	genotyping	right primer specific for GK-738B03 (*tify8-1*)
JAZ1	FwP	GAGCAAAGGCACCGCTAATA	qRT-PCR	
JAZ1	RvP	TGCGATAGTAGCGATGTTGC	qRT-PCR	
JAZ10	FwP	ACGCTCCTAAGCCTAAGTTCC	qRT-PCR	
JAZ10	RvP	TCGAAATCGCACCTTGAATA	qRT-PCR	
JAZ10	FwP	AAAAAGCAGGCTCG**ATG**TCGAAAGCTACCATAGA	cloning	Partial AttB1 sequence attached
JAZ10	RvP	AGAAAGCTGGGTTTAGGCCGATGTCGGATAGT	cloning	Partial AttB2 sequence attached
JAZ11	FwP	GATCGATTCACGAAGCTAGGTC	qRT-PCR	
JAZ11	RvP	TCCCACCAAAGATAATAGTAAGTTGA	qRT-PCR	
JAZ12	FwP	CATCTAATGTGGCATCACCAG	qRT-PCR	
JAZ12	RvP	TGCCTCCTTGCAATAGGTAGA	qRT-PCR	
JAZ2	FwP	GCTTCACTTCATCGGTTCCT	qRT-PCR	
JAZ2	RvP	TTGGTATGGTGCCTTTGATG	qRT-PCR	
JAZ3	FwP	GGCTCCAACAGTGGCATT	qRT-PCR	
JAZ3	RvP	ATGGGGATACGCTCGTGAC	qRT-PCR	
JAZ4	FwP	CGGGCTGGGATCAAAGTTA	qRT-PCR	
JAZ4	RvP	GAATGACCAATCCATCATACCTC	qRT-PCR	
JAZ5	FwP	AAAGATGTTGCTGACCTCAGTG	qRT-PCR	
JAZ5	RvP	CCCTCCGAAGAATATGGTCA	qRT-PCR	
JAZ6	FwP	AACGAGTTCCGGGAACAATG	qRT-PCR	
JAZ6	RvP	ACCTGATGTTGCTGCCCAG	qRT-PCR	
JAZ7	FwP	ATGCGACTTGGAACTTCGCCTT	qRT-PCR	
JAZ7	RvP	AGAGCTGCTTGATTCGTCCAACG	qRT-PCR	
JAZ8	FwP	CGATCGCAAGCAGAGAAATG	qRT-PCR	
JAZ8	RvP	GATCCGACCCGTTTGAGGAT	qRT-PCR	
JAZ9	FwP	TGCTGTCGAAGAACGAGGGT	qRT-PCR	
JAZ9	RvP	CTTCCCCCATTCTCTAGCTGC	qRT-PCR	
KIX1 B	FwP	GGGGACAAGTTTGTACAAAAAAGCAGGCTCC**ATG**AATTCTGAGGCTGAG	cloning	AttB1 site attached. ATG added
KIX1 C	FwP	GGGGACAAGTTTGTACAAAAAAGCAGGCTCC**ATG**CAAGTATTCATGAAACC	cloning	AttB1 site attached. ATG added
KIX1 FL	RvP	GGGGACCACTTTGTACAAGAAAGCTGGGTC**TCM**GTTGTTATTGTTGCTGC	cloning	AttB2 site attached. Ambiguous for cloning +- stop
KIX1 KIX	RvP	GGGGACCACTTTGTACAAGAAAGCTGGGTC**TCM**GGCTTTGGAATACATG	cloning	
KIX1 KIX+B	RvP	GGGGACCACTTTGTACAAGAAAGCTGGGTC**TCM**GGACAACATGTTGTCCAAG	cloning	AttB2 site attached. Ambiguous for cloning +- stop
KIX1 mEAR	FwP	TTGGTGGGTGTGAATGTGAT**GCA**TCT**GCG**CGC**GCA**GGTCCTCTTG	cloning	Mutation of Leu to Ala
KIX1 mEAR	RvP	ATCACATTCACACCCACCAAAGGTAATGCC	cloning	
KIX1/KIX2 FL	FwP	GGGGACAAGTTTGTACAAAAAAGCAGGCTCC**ATG**CCGAGGCCAGGGCCAAG	cloning	AttB1 site attached
KIX2 B	RvP	GGGGACAAGTTTGTACAAAAAAGCAGGCTCC**ATG**AATTCCGAGGAAGAGTATAC	cloning	AttB1 site attached ATG added
KIX2 C	FwP	GGGGACAAGTTTGTACAAAAAAGCAGGCTCC**ATG**AATGAGCCGTCTTACC	cloning	AttB2 site attached. Ambiguous for cloning +- stop
KIX2 FL	RvP	GGGGACCACTTTGTACAAGAAAGCTGGGTC**TCM**AAGGAAGTCTCCACAC	cloning	AttB2 site attached. Ambiguous for cloning +- stop
KIX2 KIX	RvP	GGGGACCACTTTGTACAAGAAAGCTGGGTC**TCM**GGCTTTAGAGTACATGATTTC	cloning	AttB2 site attached. Ambiguous for cloning +- stop
KIX2 KIX+B	RvP	GGGGACCACTTTGTACAAGAAAGCTGGGTC**TCM**AGTCGACGCAGAAACCGGTTC	cloning	AttB2 site attached. Ambiguous for cloning +- stop
KIX2 mEAR	FwP	CAACTGAGAGGGTCTGTGAT**GCA**TCC**GCG**AGG**GCC**GGTATATCTTC	cloning	Mutation of Leu to Ala
KIX2 mEAR	RvP	CAGAAGAAGCAACTGAGAGGGTCTGTGAT	cloning	
NINJA B	FwP	CAACTGCGACAGATGAAGGA	cloning	no AttB site attached, requires a 2nd PCR to include them
NINJA B	RvP	CATTTTGCGGATGAACTGTG	cloning	no AttB site attached, requires a 2nd PCR to include them

Gene	Use	Sequence	USE	comment
NINJA C	FwP	GCAACCTCAATACCGCTTTC	cloning	no AttB site attached, requires a 2nd PCR to include them
NINJA C	RvP	CCGGTATGTGACACCTGAGA	cloning	no AttB site attached, requires a 2nd PCR to include them
NINJA EAR	FwP	GCTTGGGTCTTTCTTGTGGA	cloning	no AttB site attached, requires a 2nd PCR to include them
NINJA EAR	RvP	GCTGTTGACCCGAATCACTT	cloning	no AttB site attached, requires a 2nd PCR to include them
pDONR201/207	FwP	TCGCGTTAACGCTAGCATGGATCTC	cloning	to amplify GOI, colony PCR and sequencing (250bp + insert)
pDONR201/207	RvP	GTAACATCAGAGATTTTGAGACAC	cloning	to amplify GOI, colony PCR and sequencing (250bp + insert)
PPD2 END	RvP	GGGGACCACTTTGTACAAGAAAGCTGGGTC**TCM**ATTATCTTCGCTGTTTAGATC	cloning	AttB2 site attached. Ambiguous for cloning +- stop
PPD2 Jas*	FwP	GGGGACAAGTTTGTACAAAAAAGCAGGCTCC**ATG**CCCATGAGTAAAGAGAAGATG	cloning	AttB1 site attached. ATG added
PPD2 PPD	RvP	GGGGACCACTTTGTACAAGAAAGCTGGGTC**TCM**CCTTCTATGGCACGCACCATC	cloning	AttB2 site attached. Ambiguous for cloning +- stop
PPD2 START	FwP	GGGGACAAGTTTGTACAAAAAAGCAGGCTCC**ATG**GATGTAGGAGTTACTAC	cloning	AttB1 site attached
PPD2 ZIM	FwP	GGGGACAAGTTTGTACAAAAAAGCAGGCTCC**ATG**GATTCTCCAAGATCAGC	cloning	AttB1 site attached. ATG added
PPD2 ZIM	RvP	GGGACCACTTTGTACAAGAAAGCTGGGTC**TCM**TTTCGAAATCATTCTACTAG	cloning	AttB2 site attached. Ambiguous for cloning +- stop
TIFY8 #1	FwP	CCGACAGACAGAACAAGATAAGC	qRT-PCR	
TIFY8 #1	RvP	AAGCAGAAGCCGTGGAAGG	qRT-PCR	
TIFY8 #2	FwP	GGGAATCATCTTGATGGGATAC	qRT-PCR	
TIFY8 #2	RvP	CCTGAAAACCGATTGCTCAT	qRT-PCR	
TIFY8 #3	FwP	CCATCAGTTATTGCTCAGACAG	qRT-PCR	
TIFY8 #3	RvP	GCTTGAGTTTGGCATTGTAAAAG	qRT-PCR	
TIFY8 #4	FwP	AGGCGGATGTGATAATGG	qRT-PCR	
TIFY8 #4	RvP	CTGGAGGTAGGTTGATGAC	qRT-PCR	
TIFY8 C-term	FwP	GGGGACAAGTTTGTACAAAAAAGCAGGCTTA**ATG**AAAGACTTGGCGTCG	cloning	AttB1 site attached. ATG added
TIFY8 C-term	RvP	GGGGACCACTTTGTACAAGAAAGCTGGGTA**TCA**TGTGGCTTCTTTTTC	cloning	AttB2 site attached. Ambiguous for cloning + stop
TIFY8 N-term	FwP	GGGGACAAGTTTGTACAAAAAAGCAGGCTTA**ATG**ATGGTGAACCACAAC	cloning	AttB1 site attached
TIFY8 N-term	RvP	GGGGACCACTTTGTACAAGAAAGCTGGGTATCACCGATTTCCGGTATTTGAAGGG	cloning	AttB2 site attached. Ambiguous for cloning +- stop
TIFY8 promoter	FwP	GGGGACAACTTTGTATAGAAAAGTTGTCCTGAAGAGCCTCGATTTT	cloning	
TIFY8 promoter	RvP	GGGGACTGCTTTTTTGTACAAACTTGGAAACCAGTGTGTCAGTGTGG	cloning	
TIFY8 RNAi	FwP	GGGGACAAGTTTGTACAAAAAAGCAGGCTCCCGGATGTGATAATGGCCTTG	cloning	CATMA4a34310, GST for TIFY8 silencing
TIFY8 RNAi	RvP	GGGGACCACTTTGTACAAGAAAGCTGGGTTGGTTGTGTAAAGATCCGGTG	cloning	CATMA4a34310, GST for TIFY8 silencing
TPL-N	FwP	GGGGACAAGTTTGTACAAAAAAGCAGGCTCC**ATG**TCTTCTCTTAGTAGAGAG	cloning	AttB1 site attached
TPL-N	RvP	GGGGACCACTTTGTACAAGAAAGCTGGGTC**TCM**ATTTTTACAAAGCTGGTGTTG	cloning	AttB2 site attached. Ambiguous for cloning +- stop
TPL-N	FwP	GGGGACAAGTTTGTACAAAAAAGCAGGCTCC**ATG**TCTTCTCTTAGTAGAGAG	cloning	AttB1 site attached
TPL-N	RvP	GGGGACCACTTTGTACAAGAAAGCTGGGTC**TCM**ATTTTTACAAAGCTGGTGTTG	cloning	AttB2 site attached. Ambiguous for cloning +- stop
UBQ10	FwP	CTGCGACTCAGGGAATCTTCTAA	qRT-PCR	
UBQ10	RvP	TTGTGCCATTGAATTGAACCC	qRT-PCR	

Annex 2. List of hits identifed by TAP of TIFY8 using MALDI-TOF/TOF mass spectrometry

Prey					PMF data					MSMS data											
Prey Locus	Prey Name	# Found/ # exp	Database version	Protein Score	Expect	RMS error (ppm)	Sequence coverage %	Unique Peptides	Total Ion Score	Peptide Number	Start	End	Observed	Mr(Exp)	Mr(Calc)	Delta (Da)	Miss	Ions Score	Expect	Peptide	Variable Modification
AT4G14720	PPD2	2/2	TAIR10	217	7,10E-18	8	31	9	162	*1*	*76*	*86*	1217,6917	1216,684	1216,6928	-0,0084	0	25	5,90E-02	ILVSQPPNPPR	
										2	18	29	1417,7452	1416,738	1416,746	-0,0081	0	61	2,40E-05	LLTEEDISQLTR	
										3	60	74	1533,7501	1532,743	1532,7471	-0,0042	0	48	3,80E-04	ALYEPGDDSGAGILR	
										4	179	200	2403,1855	2402,178	2402,1689	0,0094	0	28	3,00E-02	SIMHFAANPIDLPENGIFASSR	Oxidation (M)
AT4G28910	NINJA	2/2	TAIR10	73	1,70E-03	4	13	3	60	1	276	292	1618,8217	1617,814	1617,8111	0,0033	0	60	2,20E-05	DGSGGIVALSQSPFAGR	
AT4G32295	unknown protein	2/2	TAIR10	/					36	1	26	34	1088,6483	1087,641	1087,639	0,0021	0	36	2,40E-03	GLLIQEIFR	

Annex 3. List of peptides identified in TAP of TIFY8 with Orbitrap mass spectrometry in mock and JA-treated samples

Exp	Bait	Treatment	AT number	Name	prot_ score	prot_ mass	prot_ cover	prot_ len	pep_ exp_mz	pep_ exp_mr	pep_ exp_z	pep_ calc_mr	pep_ delta	pep_ start	pep_ end	pep_ miss	pep_ score	pep_ homol	pep_ ident	pep_ expect	pep_res_ before	pep_ seq	pep_res_ after	pep_ var_mod
1	TIFY8	mock	AT4G32570	TIFY8	2919	38766	66,5	361	513,7704	1025,5263	2	1025,5254	0,001	335	343	0	50,85		36	0,00033	R	EHQGSIISR	G	
1	TIFY8	mock	AT4G32570	TIFY8	2919	38766	66,5	361	584,767	1167,5194	2	1167,519	0,0004	103	113	0	83,22		34	1,40E-07	R	SDVSGSIMSNR	F	Oxidation (M)
1	TIFY8	mock	AT4G32570	TIFY8	2919	38766	66,5	361	588,8238	1175,633	2	1175,6339	-0,0009	26	35	0	69,2		38	7,80E-06	K	LLFHDFLGSK	N	
1	TIFY8	mock	AT4G32570	TIFY8	2919	38766	66,5	361	593,774	1185,5334	2	1185,5336	-0,0002	293	303	0	83,82		34	1,10E-07	K	LGQMYEGGSSK	E	Methyl (DE); Oxidation (M)
1	TIFY8	mock	AT4G32570	TIFY8	2919	38766	66,5	361	624,2901	1246,5656	2	1246,5652	0,0004	304	313	0	42,38		34	0,0016	K	ETPQMPPEFR	A	Oxidation (M)
1	TIFY8	mock	AT4G32570	TIFY8	2919	38766	66,5	361	646,8143	1291,614	2	1291,6157	-0,0017	218	229	0	56,85		37	0,00011	K	LESFAPSNTGNR	K	
1	TIFY8	mock	AT4G32570	TIFY8	2919	38766	66,5	361	700,8343	1399,654	2	1399,6514	0,0027	36	48	0	75		35	1,30E-06	K	NPTLASTSMADHR	L	
1	TIFY8	mock	AT4G32570	TIFY8	2919	38766	66,5	361	761,8832	1521,7518	2	1521,7497	0,0021	203	217	0	72,07	32	37	3,80E-06	K	GPGILSSFTMPNSSK	L	
1	TIFY8	mock	AT4G32570	TIFY8	2919	38766	66,5	361	881,4422	1760,8699	2	1760,8707	-0,0008	86	102	0	123,75	35	38	2,70E-11	R	HSGGGNHLDGIQLFGPR	S	
1	TIFY8	mock	AT4G32570	TIFY8	2919	38766	66,5	361	958,4538	1914,893	2	1914,8919	0,0011	180	198	0	150,62		36	4,00E-14	K	DENVGPSVIAQTAADEGSR	T	
1	TIFY8	mock	AT4G32570	TIFY8	2919	38766	66,5	361	998,9855	1995,9564	2	1995,9585	-0,0021	143	161	0	90,16	25	37	5,60E-08	R	NGPGSFSMNVNPLANQPPR	G	
1	TIFY8	mock	AT4G32570	TIFY8	2919	38766	66,5	361	711,3525	2131,0357	3	2131,0368	-0,0011	258	279	0	60,24	28	37	5,80E-05	K	ADVIMALAGSSGGSWSTGLSHK	P	
1	TIFY8	mock	AT4G32570	TIFY8	2919	38766	66,5	361	1096,035	2190,0554	2	2190,0553	0,0002	178	198	1	200,38		37	5,20E-19	R	FKDENVGPSVIAQTAADEGSR	T	
1	TIFY8	mock	AT4G32570	TIFY8	2919	38766	66,5	361	1152,5483	2303,082	2	2303,0794	0,0027	238	257	0	120,53	28	37	4,60E-11	K	QMTIFYGGQAHVFDDVHPNK	A	
1	TIFY8	mock	AT4G32570	TIFY8	2919	38766	66,5	361	1184,5324	2367,0502	2	2367,0516	-0,0014	120	139	0	85,68	29	34	7,50E-08	R	SNSDSHFTTQEHPETLHWSK	L	
1	TIFY8	mock	AT4G32570	TIFY8	2919	38766	66,5	361	1187,0963	2372,178	2	2372,1794	-0,0014	258	281	1	113,02	32	37	2,90E-10	K	ADVIMALAGSSGGSWSTGLSHKPK	S	Oxidation (M)
1	TIFY8	mock	AT4G32570	TIFY8	2919	38766	66,5	361	1306,1293	2610,244	2	2610,2443	-0,0002	58	85	0	176,77	34	37	1,20E-16	K	AAMTPSTASASSAGGLGGLSSTSDLVER	H	Methyl (DE); Oxidation (M)
1	TIFY8	mock	AT4G14720	PPD2	447	35056	38,7	315	515,3009	1028,5873	2	1028,5866	0,0007	87	95	0	65,25		35	1,10E-05	R	VTTTLIEPR	N	
1	TIFY8	mock	AT4G14720	PPD2	447	35056	38,7	315	607,8587	1213,7029	2	1213,703	-0,0001	49	59	0	67,5		35	6,10E-06	K	SQAIQQVLSLK	A	
1	TIFY8	mock	AT4G14720	PPD2	447	35056	38,7	315	608,8148	1215,6151	2	1215,6136	0,0015	166	176	0	42,29		37	0,0032	K	VNVYDGVPPEK	A	
1	TIFY8	mock	AT4G14720	PPD2	447	35056	38,7	315	470,8856	1409,635	3	1409,6358	-0,0007	104	115	0	50,21		35	0,00035	R	IPLQEDDGACHR	R	
1	TIFY8	mock	AT4G14720	PPD2	447	35056	38,7	315	709,3809	1416,7473	2	1416,746	0,0013	18	29	0	93,46		37	2,80E-08	K	LLTEEDISQLTR	E	
1	TIFY8	mock	AT4G14720	PPD2	447	35056	38,7	315	758,849	1515,6834	2	1515,6842	-0,0008	121	135	0	100,53		34	2,40E-09	R	SAEFSGSSGQFVADK	D	
1	TIFY8	mock	AT4G14720	PPD2	447	35056	38,7	315	767,3803	1532,7461	2	1532,7471	-0,0009	60	74	0	72,26		38	3,80E-06	K	ALYEPGDDSGAGILR	K	
1	TIFY8	mock	AT4G14720	PPD2	447	35056	38,7	315	797,8976	1593,7806	2	1593,7821	-0,0014	258	272	0	92,36	29	38	3,90E-08	K	APGVASSSLEMFLNR	Q	Oxidation (M)
1	TIFY8	mock	AT4G14720	PPD2	447	35056	38,7	315	801,7299	2402,1678	3	2402,1689	-0,001	179	200	0	66,91	30	37	1,20E-05	R	SIMHFAANPIDLPENGIFASSR	M	Oxidation (M)
1	TIFY8	mock	AT3G11540	SPY	447	102790	19,9	914	434,2381	866,4616	2	866,461	0,0006	76	83	0	38,94		36	0,0053	K	NVEAHIGK	G	
1	TIFY8	mock	AT3G11540	SPY	447	102790	19,9	914	482,2976	962,5806	2	962,5801	0,0005	515	523	0	46,22		28	0,00019	K	VVVYSAVVK	A	
1	TIFY8	mock	AT3G11540	SPY	447	102790	19,9	914	517,2436	1032,4727	2	1032,4724	0,0004	2	10	0	50,68		34	0,00026	M	VGLEDDTER	E	
1	TIFY8	mock	AT3G11540	SPY	447	102790	19,9	914	569,2932	1136,5719	2	1136,5713	0,0005	127	136	0	72,02	34	37	3,80E-06	R	LVEAAESYQK	A	
1	TIFY8	mock	AT3G11540	SPY	447	102790	19,9	914	579,8102	1157,6059	2	1157,604	0,0019	164	174	0	63,52		36	2,10E-05	K	LAGNTQEGIQK	Y	
1	TIFY8	mock	AT3G11540	SPY	447	102790	19,9	914	611,3071	1220,5997	2	1220,5997	0	26	38	0	54,15		38	0,00029	R	SSSSSAGVLSPSR	K	
1	TIFY8	mock	AT3G11540	SPY	447	102790	19,9	914	681,3582	1360,7019	2	1360,702	-0,0001	258	270	0	67,86	37	38	1,20E-05	K	NNMAIALTDLGTK	V	
1	TIFY8	mock	AT3G11540	SPY	447	102790	19,9	914	721,8628	1441,711	2	1441,7089	0,0021	273	285	0	94,37	36	37	2,00E-08	K	LEGDVTQGVAYYK	K	
1	TIFY8	mock	AT3G11540	SPY	447	102790	19,9	914	739,9096	1477,8046	2	1477,8028	0,0018	702	714	0	90,22		34	2,90E-08	R	FLTTLEQLGLESK	R	
1	TIFY8	mock	AT3G11540	SPY	447	102790	19,9	914	750,3514	1498,6882	2	1498,6875	0,0008	93	105	0	66,38		35	8,30E-06	K	GNLAFDCFSEAIR	L	
1	TIFY8	mock	AT3G11540	SPY	447	102790	19,9	914	624,6761	1871,0064	3	1871,0074	-0,001	146	163	0	42,83	31	35	0,0021	K	PAAECLAIVLTDLGTSLK	L	
1	TIFY8	mock	AT3G11540	SPY	447	102790	19,9	914	737,3896	2209,147	3	2209,1531	-0,0061	389	408	0	66,9		36	8,70E-06	K	AILANPTYAEAFNNLGVLYR	D	
1	TIFY8	mock	AT3G11540	SPY	447	102790	19,9	914	849,1072	2544,2999	3	2544,3013	-0,0014	584	606	0	65,1	29	36	1,50E-05	R	PAPVQVTWIGYPNTTGLPTVDYR	I	
1	TIFY8	mock	AT3G11540	SPY	447	102790	19,9	914	941,4934	2821,4585	3	2821,4605	-0,002	137	163	1	59,72	33	35	3,90E-05	K	ALMADASYKPAAECLAIVLTDLGTSLK	L	
1	TIFY8	mock	AT4G28910	NINJA	374	45081	40,9	425	368,6975	735,3804	2	735,3803	0,0001	48	53	0	41,89		36	0,0032	K	VIDDFK	N	
1	TIFY8	mock	AT4G28910	NINJA	374	45081	40,9	425	448,7457	895,4769	2	895,4763	0,0006	372	379	0	39,01		34	0,0034	R	TISGVTYR	Y	
1	TIFY8	mock	AT4G28910	NINJA	374	45081	40,9	425	763,3198	1524,6251	2	1524,6263	-0,0012	199	212	0	64,89		29	2,80E-06	R	SNHGGSGTEEFTMR	N	Oxidation (M)
1	TIFY8	mock	AT4G28910	NINJA	374	45081	40,9	425	809,9126	1617,8107	2	1617,8111	-0,0004	276	292	0	130,26		38	6,00E-12	K	DGSGGIVALSQSPFAGR	V	
1	TIFY8	mock	AT4G28910	NINJA	374	45081	40,9	425	954,9411	1907,8677	2	1907,865	0,0027	73	89	0	69,9		35	3,60E-06	R	SDSGQQPPQNFFNDLSK	A	
1	TIFY8	mock	AT4G28910	NINJA	374	45081	40,9	425	1062,5552	2123,0958	2	2123,0971	-0,0013	179	197	1	84,4	29	37	1,90E-07	K	EVVRPPTDTNIVDNLTGQR	R	
1	TIFY8	mock	AT4G28910	NINJA	374	45081	40,9	425	771,3187	2310,9342	3	2310,9369	-0,0027	404	425	0	82,47		27	3,40E-08	R	HASEEYVSPESSMGMTAASAHT	-	2 Oxidation (M)
1	TIFY8	mock	AT4G28910	NINJA	374	45081	40,9	425	830,1124	3316,4204	4	3316,42	0,0005	145	178	0	39,12		29	0,0011	K	ASHVSTATDEGSTAENEDVAESEVGGGSSSNHAK	E	
1	TIFY8	mock	AT4G28910	NINJA	374	45081	40,9	425	978,9866	3911,9173	4	3911,9186	-0,0012	239	275	0	60,89	30	35	2,70E-05	K	ESGQHAAATSLLQPNANAGNLPIMFGYSPVQLPMLDK	D	2 Oxidation (M)
1	TIFY8	mock	AT1G51690	ATB ALPHA	316	57526	22,8	513	381,7291	761,4436	2	761,4436	0	62	67	0	39,12		34	0,0038	R	VVLFER	T	
1	TIFY8	mock	AT1G51690	ATB ALPHA	316	57526	22,8	513	593,7958	1185,5771	2	1185,5778	-0,0008	414	424	0	74,55		35	1,30E-06	R	AATGSYSNLFR	V	
1	TIFY8	mock	AT1G51690	ATB ALPHA	316	57526	22,8	513	617,8054	1233,5963	2	1233,5877	0,0086	79	88	0	41,2		37	0,0046	R	ELEEADYPLR	H	
1	TIFY8	mock	AT1G51690	ATB ALPHA	316	57526	22,8	513	778,9141	1555,8136	2	1555,8134	0,0002	336	349	0	127,47		36	8,80E-12	K	SFFTEIIASVSDIK	F	
1	TIFY8	mock	AT1G51690	ATB ALPHA	316	57526	22,8	513	526,6265	1576,8577	3	1576,8573	0,0004	210	224	0	50,92		35	0,00031	R	LPVVVTSHESSPVAR	C	
1	TIFY8	mock	AT1G51690	ATB ALPHA	316	57526	22,8	513	585,9331	1754,7775	3	1754,7788	-0,0013	96	109	0	52,06		33	0,00014	K	TEFQSHDPEFDYLK	S	
1	TIFY8	mock	AT1G51690	ATB ALPHA	316	57526	22,8	513	896,9581	1791,9016	2	1791,9003	0,0013	425	442	0	89,09	35	38	8,40E-08	R	VFGVAPGSTETATLEASR	N	
1	TIFY8	mock	AT1G51690	ATB ALPHA	316	57526	22,8	513	1025,1501	3072,4285	3	3072,4272	0,0013	30	58	0	73,14		35	1,80E-06	R	SAGEEVQEVDIISAIEFDNSGNHLATGDR	G	
1	TIFY8	mock	AT4G32295	KIX1	270	27551	38,2	238	398,2761	794,5375	2	794,5378	-0,0003	54	60	0	45,86		20	2,60E-05	K	LPVVVLR	A	
1	TIFY8	mock	AT4G32295	KIX1	270	27551	38,2	238	485,7315	969,4484	2	969,4477	0,0008	61	68	0	57,03		34	5,40E-05	R	AEEIMYSK	A	
1	TIFY8	mock	AT4G32295	KIX1	270	27551	38,2	238	504,2984	1006,5822	2	1006,5811	0,0011	219	228	0	41,14		32	0,0013	R	LGPLGPPTQK	R	
1	TIFY8	mock	AT4G32295	KIX1	270	27551	38,2	238	544,8267	1087,6389	2	1087,639	-0,0001	26	34	0	60,06		33	2,50E-05	R	GLLIQEIFR	I	
1	TIFY8	mock	AT4G32295	KIX1	270	27551	38,2	238	565,8108	1129,6071	2	1129,6091	-0,002	85	94	0	54,2	37	38	0,00027	R	TNDAINTIIR	L	
1	TIFY8	mock	AT4G32295	KIX1	270	27551	38,2	238	665,3324	1328,6503	2	1328,6507	-0,0004	35	45	0	57,6		36	7,80E-05	R	IVCEIHSQSTR	K	
1	TIFY8	mock	AT4G32295	KIX1	270	27551	38,2	238	715,3588	1428,7031	2	1428,7031	0	160	172	0	88,27	35	37	7,50E-08	K	NLAVAQENCPVSK	Y	
1	TIFY8	mock	AT4G32295	KIX1	270	27551	38,2	238	823,3917	2467,1533	3	2466,1519	1,0014	196	218	0	85,41		36	1,30E-07	R	PASLIDATNGITFGGCECDLSLR	L	
1	TIFY8	mock	AT5G48160	OBE2	238	66126	7,8	574	545,8088	1089,603	2	1089,603	0	149	158	0	92,86		36	2,50E-08	R	TDLSSVTLVR	A	

1	TIFY8	mock	AT5G48160	OBE2	238	66126	7,8	574	579,3296	1156,6446	2	1156,6452	-0,0006	393	402	0	94,99		36	1,30E-08	R	IAEVVQETLR	K	
1	TIFY8	mock	AT5G48160	OBE2	238	66126	7,8	574	609,8162	1217,6178	2	1217,618	-0,0002	111	120	0	62,25		38	4,40E-05	R	LPDEFLDELK	N	
1	TIFY8	mock	AT5G48160	OBE2	238	66126	7,8	574	885,9532	1769,8919	2	1769,8909	0,001	359	373	0	96,21	35	37	1,30E-08	K	LILMFFQEIESDSAK	S	
1	TIFY8	mock	AT3G24150	KIX22	105	38771	7,3	343	384,273	766,5315	2	766,5317	-0,0002	54	60	0	45,5		20	2,80E-05	K	LPVVVLK	A	
1	TIFY8	mock	AT3G24150	KIX22	105	38771	7,3	343	485,7315	969,4484	2	969,4477	0,0008	61	68	0	57,03		34	5,40E-05	K	AEEIMYSK	A	
1	TIFY8	mock	AT3G24150	KIX22	105	38771	7,3	343	565,3107	1128,6069	2	1128,6139	-0,007	85	94	0	52,08		36	0,0003	R	VNDAIDTIIR	R	
1	TIFY8	mock	AT3G63500	TTA2	84	101204	4,5	887	498,2691	994,5237	2	994,5236	0,0001	633	640	0	40,63		36	0,0043	K	EVFLNFAR	E	
1	TIFY8	mock	AT3G63500	TTA2	84	101204	4,5	887	1128,2988	3381,8746	3	3381,8759	-0,0013	218	249	0	74,38		26	1,70E-07	K	LLLEPLDLSLSLPDVLLPIGGQDTNQLGSPVR	S	
2	TIFY8	mock	AT4G32570	TIFY8	3477	38766	74,2	361	513,7699	1025,5253	2	1025,5254	-0,0001	335	343	0	52,49		36	0,00025	R	EHQGSIISR	G	
2	TIFY8	mock	AT4G32570	TIFY8	3477	38766	74,2	361	576,7694	1151,5242	2	1151,5241	0,0001	103	113	0	91,03		36	3,20E-08	R	SDVSGSIMSNR	F	
2	TIFY8	mock	AT4G32570	TIFY8	3477	38766	74,2	361	588,8237	1175,6328	2	1175,6339	-0,0011	26	35	0	59,25		38	7,70E-05	K	LLFHDFLGSK	N	
2	TIFY8	mock	AT4G32570	TIFY8	3477	38766	74,2	361	593,7737	1185,5328	2	1185,5336	-0,0008	293	303	0	70,19		33	2,30E-06	K	LGQMYEGGSSK	E	Methyl (DE); Oxidation (M)
2	TIFY8	mock	AT4G32570	TIFY8	3477	38766	74,2	361	631,2977	1260,5808	2	1260,5809	-0,0001	304	313	0	48,55		35	0,00047	K	ETPQMPPEFR	A	Methyl (DE); Oxidation (M)
2	TIFY8	mock	AT4G32570	TIFY8	3477	38766	74,2	361	646,814	1291,6134	2	1291,6157	-0,0023	218	229	0	54,72		37	0,00017	K	LESFAPSNTGNR	K	
2	TIFY8	mock	AT4G32570	TIFY8	3477	38766	74,2	361	455,542	1363,6042	3	1363,6051	-0,0009	316	327	0	66,05		34	6,80E-06	R	PSHQPTSSACHR	I	
2	TIFY8	mock	AT4G32570	TIFY8	3477	38766	74,2	361	700,8331	1399,6517	2	1399,6514	0,0003	36	48	0	73,38		35	1,60E-06	K	NPTLASTSMADHR	L	
2	TIFY8	mock	AT4G32570	TIFY8	3477	38766	74,2	361	769,8786	1537,7427	2	1537,7446	-0,0019	203	217	0	66,07	29	37	1,50E-05	K	GPGILSSFTMPNSSK	L	Oxidation (M)
2	TIFY8	mock	AT4G32570	TIFY8	3477	38766	74,2	361	845,9525	1689,8905	2	1689,8911	-0,0006	162	177	0	74,78	34	37	1,80E-06	R	GGGQISHLLHQLSTSR	F	
2	TIFY8	mock	AT4G32570	TIFY8	3477	38766	74,2	361	881,4429	1760,8711	2	1760,8707	0,0005	86	102	0	125,11	33	38	2,10E-11	R	HSGGGNHLDGIQLFGPR	S	
2	TIFY8	mock	AT4G32570	TIFY8	3477	38766	74,2	361	965,4608	1928,9071	2	1928,9076	-0,0004	180	198	0	133,66		36	2,00E-12	K	DENVGPSVIAQTAADEGSR	T	Methyl (DE)
2	TIFY8	mock	AT4G32570	TIFY8	3477	38766	74,2	361	998,9859	1995,9572	2	1995,9585	-0,0012	143	161	0	93,09	27	37	3,00E-08	R	NGPGSFSMNVNPLANQPPR	G	
2	TIFY8	mock	AT4G32570	TIFY8	3477	38766	74,2	361	1074,5223	2147,03	2	2147,0317	-0,0017	258	279	0	114,79	31	37	2,00E-10	K	ADVIMALAGSSGGSWSTGLSHK	P	Oxidation (M)
2	TIFY8	mock	AT4G32570	TIFY8	3477	38766	74,2	361	1096,0345	2190,0544	2	2190,0553	-0,0008	178	198	1	193,17		37	2,80E-18	R	FKDENVGPSVIAQTAADEGSR	T	
2	TIFY8	mock	AT4G32570	TIFY8	3477	38766	74,2	361	774,0306	2319,07	3	2319,0743	-0,0042	238	257	0	37,55	25	36	0,0077	K	QMTIFYGGQAHVFDDVHPNK	A	Oxidation (M)
2	TIFY8	mock	AT4G32570	TIFY8	3477	38766	74,2	361	1184,5348	2367,055	2	2367,0516	0,0034	120	139	0	87,54		34	5,00E-08	R	SNSDSHFTTQEHPETLHWSK	L	
2	TIFY8	mock	AT4G32570	TIFY8	3477	38766	74,2	361	1187,0955	2372,1764	2	2372,1794	-0,003	258	281	1	114,3	31	37	2,20E-10	K	ADVIMALAGSSGGSWSTGLSHKPK	S	Oxidation (M)
2	TIFY8	mock	AT4G32570	TIFY8	3477	38766	74,2	361	1291,1227	2580,2308	2	2580,2337	-0,0029	58	85	0	168,71	32	37	7,60E-16	K	AAMTPSTASASSAGGLGGLSSTSDLVER	H	
2	TIFY8	mock	AT3G11540	SPY	511	102790	16,7	914	569,293	1136,5715	2	1136,5713	0,0002	127	136	0	72,04	34	37	3,80E-06	R	LVEAAESYQK	A	
2	TIFY8	mock	AT3G11540	SPY	511	102790	16,7	914	579,8102	1157,6059	2	1157,604	0,0019	164	174	0	67,52		36	8,50E-06	K	LAGNTQEGIQK	Y	
2	TIFY8	mock	AT3G11540	SPY	511	102790	16,7	914	594,827	1187,6394	2	1187,6398	-0,0004	877	888	0	80,27		38	6,30E-07	R	VSVTGEATPSLK	A	
2	TIFY8	mock	AT3G11540	SPY	511	102790	16,7	914	611,3068	1220,5991	2	1220,5997	-0,0006	26	38	0	53,24		38	0,00033	R	SSSSSAGVLSPSR	K	
2	TIFY8	mock	AT3G11540	SPY	511	102790	16,7	914	681,358	1360,7014	2	1360,702	-0,0006	258	270	0	76,09		38	1,80E-06	K	NNMAIALTDLGTK	V	
2	TIFY8	mock	AT3G11540	SPY	511	102790	16,7	914	721,8627	1441,7108	2	1441,7089	0,0019	273	285	0	81,7	36	37	3,70E-07	K	LEGDVTQGVAYYK	K	
2	TIFY8	mock	AT3G11540	SPY	511	102790	16,7	914	739,9098	1477,8051	2	1477,8028	0,0023	702	714	0	103,91		34	1,20E-09	R	FLTTLEQLGLESK	R	
2	TIFY8	mock	AT3G11540	SPY	511	102790	16,7	914	750,351	1498,6873	2	1498,6875	-0,0001	93	105	0	73		35	1,90E-06	K	GNLAFDCFSEAIR	L	
2	TIFY8	mock	AT3G11540	SPY	511	102790	16,7	914	792,9025	1583,7905	2	1583,7905	0	59	72	0	113,72		37	2,20E-10	K	FADALALYEAMLEK	D	
2	TIFY8	mock	AT3G11540	SPY	511	102790	16,7	914	624,6756	1871,0049	3	1871,0074	-0,0024	146	163	0	78,12		36	6,50E-07	K	PAAECLAIVLTDLGTSLK	L	
2	TIFY8	mock	AT3G11540	SPY	511	102790	16,7	914	849,1074	2544,3005	3	2544,3013	-0,0008	584	606	0	65,94	28	36	1,30E-05	R	PAPVQVTWIGYPNTTGLPTVDYR	I	
2	TIFY8	mock	AT4G14720	PPD2	412	35056	41,3	315	464,2969	926,5793	2	926,58	-0,0007	10	17	1	29,42		29	0,0091	K	SILEKPLK	L	
2	TIFY8	mock	AT4G14720	PPD2	412	35056	41,3	315	515,3008	1028,5871	2	1028,5866	0,0005	87	95	0	74,02		35	1,50E-06	R	VTTTLIEPR	N	
2	TIFY8	mock	AT4G14720	PPD2	412	35056	41,3	315	607,8586	1213,7027	2	1213,703	-0,0004	49	59	0	84,17		35	1,30E-07	K	SQAIQQVLSLK	A	
2	TIFY8	mock	AT4G14720	PPD2	412	35056	41,3	315	608,814	1215,6134	2	1215,6136	-0,0002	166	176	0	42,38	31	37	0,0031	K	VNVYDGVPPEK	A	
2	TIFY8	mock	AT4G14720	PPD2	412	35056	41,3	315	470,8856	1409,6349	3	1409,6358	-0,0008	104	115	0	68,2		35	5,50E-06	R	IPLQEDDGACHR	R	
2	TIFY8	mock	AT4G14720	PPD2	412	35056	41,3	315	709,3808	1416,747	2	1416,746	0,001	18	29	0	96,38		37	1,40E-08	K	LLTEEDISQLTR	E	
2	TIFY8	mock	AT4G14720	PPD2	412	35056	41,3	315	758,8493	1515,684	2	1515,6842	-0,0001	121	135	0	87,19		34	5,40E-08	R	SAEFSGSSGQFVADK	D	
2	TIFY8	mock	AT4G14720	PPD2	412	35056	41,3	315	767,3803	1532,7461	2	1532,7471	-0,001	60	74	0	70,3		38	6,00E-06	K	ALYEPGDDSGAGILR	K	
2	TIFY8	mock	AT4G14720	PPD2	412	35056	41,3	315	797,8986	1593,7827	2	1593,7821	0,0006	258	272	0	79,86	31	38	7,20E-07	K	APGVASSSLEMFLNR	Q	Oxidation (M)
2	TIFY8	mock	AT4G14720	PPD2	412	35056	41,3	315	796,3978	2386,1717	3	2386,1739	-0,0022	179	200	0	61,13	24	37	4,50E-05	R	SIMHFAANPIDLPENGIFASSR	M	
2	TIFY8	mock	AT4G28910	NINJA	369	45081	26,6	425	448,745	895,4755	2	895,4763	-0,0008	372	379	0	47,85		35	0,00056	R	TISGVTYR	Y	
2	TIFY8	mock	AT4G28910	NINJA	369	45081	26,6	425	763,32	1524,6255	2	1524,6263	-0,0008	199	212	0	65,28		29	2,60E-06	R	SNHGGSGTEEFTMR	N	Oxidation (M)
2	TIFY8	mock	AT4G28910	NINJA	369	45081	26,6	425	809,9124	1617,8102	2	1617,8111	-0,0009	276	292	0	113,72	37	38	2,70E-10	K	DGSGGIVALSQSPFAGR	V	
2	TIFY8	mock	AT4G28910	NINJA	369	45081	26,6	425	554,9491	1661,8254	3	1661,8274	-0,002	356	371	0	53,21	24	38	0,00033	R	PNLPWVSTTGSGPHGR	T	
2	TIFY8	mock	AT4G28910	NINJA	369	45081	26,6	425	954,9391	1907,8637	2	1907,865	-0,0012	73	89	0	76,44		35	7,40E-07	R	SDSGQQPPQNFFNDLSK	A	
2	TIFY8	mock	AT4G28910	NINJA	369	45081	26,6	425	1062,5553	2123,096	2	2123,0971	-0,0011	179	197	1	88,22	32	37	7,90E-08	K	EVVRPPTDTNIVDNLTGQR	R	
2	TIFY8	mock	AT4G28910	NINJA	369	45081	26,6	425	771,3192	2310,9358	3	2310,9369	-0,0011	404	425	0	70		27	6,10E-07	R	HASEEYVSPESSMGMTAASAHT	-	2 Oxidation (M)
2	TIFY8	mock	AT1G51690	ATB ALPHA	332	57526	18,9	513	381,7284	761,4423	2	761,4436	-0,0013	62	67	0	36,19		35	0,0083	R	VVLFER	T	
2	TIFY8	mock	AT1G51690	ATB ALPHA	332	57526	18,9	513	593,7959	1185,5773	2	1185,5778	-0,0006	414	424	0	78,99		35	4,90E-07	R	AATGSYSNLFR	V	
2	TIFY8	mock	AT1G51690	ATB ALPHA	332	57526	18,9	513	778,9141	1555,8137	2	1555,8134	0,0003	336	349	0	126,23		36	1,20E-11	K	SFFTEIIASVSDIK	F	
2	TIFY8	mock	AT1G51690	ATB ALPHA	332	57526	18,9	513	526,6266	1576,8581	3	1576,8573	0,0007	210	224	0	43,81		35	0,0016	R	LPVVVTSHESSPVAR	C	
2	TIFY8	mock	AT1G51690	ATB ALPHA	332	57526	18,9	513	585,9334	1754,7783	3	1754,7788	-0,0004	96	109	0	37,42		33	0,0042	K	TEFQSHDPEFDYLK	S	
2	TIFY8	mock	AT1G51690	ATB ALPHA	332	57526	18,9	513	896,9579	1791,9013	2	1791,9003	0,001	425	442	0	101,27	36	38	5,10E-09	R	VFGVAPGSTETATLEASR	N	
2	TIFY8	mock	AT1G51690	ATB ALPHA	332	57526	18,9	513	963,4402	1924,8658	2	1924,865	0,0008	469	487	0	118,26		35	4,80E-11	R	GSESPGVDGNTNALDYTTK	L	
2	TIFY8	mock	AT4G32295	KIX1	265	27551	53,8	238	398,2761	794,5376	2	794,5378	-0,0002	54	60	0	49,65		20	1,10E-05	K	LPVVVLR	A	
2	TIFY8	mock	AT4G32295	KIX1	265	27551	53,8	238	485,7312	969,4479	2	969,4477	0,0002	61	68	0	45,54		34	0,00078	R	AEEIMYSK	A	
2	TIFY8	mock	AT4G32295	KIX1	265	27551	53,8	238	504,297	1006,5795	2	1006,5811	-0,0016	219	228	0	38,67		33	0,0033	R	LGPLGPPTQK	R	
2	TIFY8	mock	AT4G32295	KIX1	265	27551	53,8	238	544,8267	1087,6389	2	1087,639	0	26	34	0	58,09		33	3,90E-05	R	GLLIQEIFR	I	
2	TIFY8	mock	AT4G32295	KIX1	265	27551	53,8	238	565,8111	1129,6077	2	1129,6091	-0,0014	85	94	0	47,7		38	0,0011	R	TNDAINTIIR	L	
2	TIFY8	mock	AT4G32295	KIX1	265	27551	53,8	238	665,3323	1328,6499	2	1328,6507	-0,0007	35	45	0	60,98		36	3,60E-05	R	IVCEIHSQSTR	K	
2	TIFY8	mock	AT4G32295	KIX1	265	27551	53,8	238	713,8272	1425,6398	2	1425,6387	0,0011	173	183	0	51,39		35	0,00023	K	YSAYPLCYSFR	P	

2	TIFY8	mock	AT4G32295	KIX1	265	27551	53,8	238	715,359	1428,7034	2	1428,7031	0,0003	160	172	0	88,03	33	37	8,00E-08	K	NLAVAQENCPVSK	Y	
2	TIFY8	mock	AT4G32295	KIX1	265	27551	53,8	238	823,0559	2466,146	3	2466,1519	-0,006	196	218	0	45,15		36	0,0014	R	PASLIDATNGITFGGCECDLSLR	L	
2	TIFY8	mock	AT4G32295	KIX1	265	27551	53,8	238	986,8042	2957,3907	3	2957,3899	0,0008	95	120	0	68,69	27	36	5,70E-06	R	LDETTETGEFLQPCIEAALHLGCTPR	R	
2	TIFY8	mock	AT5G48160	OBE2	158	66126	7	574	545,8083	1089,6021	2	1089,603	-0,0009	149	158	0	89,71		37	5,60E-08	R	TDLSSVTLVR	A	
2	TIFY8	mock	AT5G48160	OBE2	158	66126	7	574	579,3292	1156,6438	2	1156,6452	-0,0013	393	402	0	76,55		36	9,10E-07	R	IAEVVQETLR	K	
2	TIFY8	mock	AT5G48160	OBE2	158	66126	7	574	596,2643	1190,5141	2	1190,5172	-0,0031	383	392	0	34,55		32	0,0067	R	LMAPQDACNR	I	Oxidation (M)
2	TIFY8	mock	AT5G48160	OBE2	158	66126	7	574	609,8163	1217,618	2	1217,618	0,0001	111	120	0	62,5	35	38	4,10E-05	R	LPDEFLDELK	N	
2	TIFY8	mock	AT4G23460	Adaptin family proteir	123	99776	3,6	893	690,3441	1378,6736	2	1378,6802	-0,0067	120	130	0	50,43	29	38	0,00058	K	ITEYLCDPLQK	C	
2	TIFY8	mock	AT4G23460	Adaptin family proteir	123	99776	3,6	893	776,4283	2326,2631	3	2326,2606	0,0025	284	304	0	70,08		33	2,10E-06	K	MAPPLVTLLSAEPEIQYVALR	N	Oxidation (M)
2	TIFY8	mock	AT3G08530	Clathrin, heavy chain	117	194402	2,1	1703	508,2744	1014,5342	2	1014,5346	-0,0004	998	1007	0	53,63		35	0,00017	K	SPEQVSAAVK	A	
2	TIFY8	mock	AT3G08530	Clathrin, heavy chain	117	194402	2,1	1703	683,8759	1365,7371	2	1365,7365	0,0007	368	380	0	43,9		35	0,0013	R	GNLPGAENLVVQR	F	
2	TIFY8	mock	AT3G08530	Clathrin, heavy chain	117	194402	2,1	1703	718,375	1434,7354	2	1434,7354	0	1132	1144	0	87,02	32	38	1,50E-07	R	EGLVSDAIESFIR	A	
2	TIFY8	mock	AT3G63500	TTA2	113	101204	3,5	887	498,2691	994,5237	2	994,5236	0,0001	633	640	0	39,34		36	0,0058	K	EVFLNFAR	E	
2	TIFY8	mock	AT3G63500	TTA2	113	101204	3,5	887	593,7776	1185,5406	2	1185,5414	-0,0008	319	327	0	52,14		34	0,00016	K	YNENTVYQR	L	
2	TIFY8	mock	AT3G63500	TTA2	113	101204	3,5	887	787,4068	1572,7991	2	1572,8008	-0,0017	469	482	0	93,24		37	2,80E-08	K	NVQLGAFQDALQNR	T	
2	TIFY8	mock	AT3G24150	KIX22	88	38771	7,3	343	384,2729	766,5312	2	766,5317	-0,0005	54	60	0	33,05		20	0,0005	K	LPVVVLK	A	
2	TIFY8	mock	AT3G24150	KIX22	88	38771	7,3	343	485,7312	969,4479	2	969,4477	0,0002	61	68	0	45,54		34	0,00078	K	AEEIMYSK	A	
2	TIFY8	mock	AT3G24150	KIX22	88	38771	7,3	343	565,314	1128,6134	2	1128,6139	-0,0005	85	94	0	65,23		36	1,40E-05	R	VNDAIDTIIR	R	
3	TIFY8	JA	AT4G32570	TIFY8	3858	38766	66,5	361	513,7696	1025,5247	2	1025,5254	-0,0007	335	343	0	60,03		36	4,30E-05	R	EHQGSIISR	G	
3	TIFY8	JA	AT4G32570	TIFY8	3858	38766	66,5	361	576,7694	1151,5243	2	1151,5241	0,0002	103	113	0	85,47		36	1,10E-07	R	SDVSGSIMSNR	F	
3	TIFY8	JA	AT4G32570	TIFY8	3858	38766	66,5	361	585,7763	1169,5381	2	1169,5387	-0,0006	293	303	0	81,69		34	2,00E-07	K	LGQMYEGGSSK	E	Methyl (DE)
3	TIFY8	JA	AT4G32570	TIFY8	3858	38766	66,5	361	588,8239	1175,6333	2	1175,6339	-0,0006	26	35	0	64,54		38	2,30E-05	K	LLFHDFLGSK	N	
3	TIFY8	JA	AT4G32570	TIFY8	3858	38766	66,5	361	631,298	1260,5815	2	1260,5809	0,0006	304	313	0	45,81		35	0,00088	K	ETPQMPPEFR	A	Methyl (DE); Oxidation (M)
3	TIFY8	JA	AT4G32570	TIFY8	3858	38766	66,5	361	646,8142	1291,6137	2	1291,6157	-0,0019	218	229	0	61,37		37	3,80E-05	K	LESFAPSNTGNR	K	
3	TIFY8	JA	AT4G32570	TIFY8	3858	38766	66,5	361	700,833	1399,6515	2	1399,6514	0,0001	36	48	0	76,03		35	8,80E-07	K	NPTLASTSMADHR	L	
3	TIFY8	JA	AT4G32570	TIFY8	3858	38766	66,5	361	761,882	1521,7494	2	1521,7497	-0,0004	203	217	0	68,58	28	38	9,00E-06	K	GPGILSSFTMPNSSK	L	
3	TIFY8	JA	AT4G32570	TIFY8	3858	38766	66,5	361	881,4414	1760,8683	2	1760,8707	-0,0024	86	102	0	139,98		38	6,60E-13	R	HSGGGNHLDGIQLFGPR	S	
3	TIFY8	JA	AT4G32570	TIFY8	3858	38766	66,5	361	958,4531	1914,8916	2	1914,8919	-0,0003	180	198	0	143,04		36	2,20E-13	K	DENVGPSVIAQTAADEGSR	T	
3	TIFY8	JA	AT4G32570	TIFY8	3858	38766	66,5	361	1006,9826	2011,9506	2	2011,9534	-0,0028	143	161	0	115,33	30	37	1,60E-10	R	NGPGSFSMNVNPLANQPPR	G	Oxidation (M)
3	TIFY8	JA	AT4G32570	TIFY8	3858	38766	66,5	361	1074,5229	2147,0312	2	2147,0317	-0,0005	258	279	0	102,3	27	37	3,50E-09	K	ADVIMALAGSSGGSWSTGLSHK	P	Oxidation (M)
3	TIFY8	JA	AT4G32570	TIFY8	3858	38766	66,5	361	1096,0346	2190,0546	2	2190,0553	-0,0006	178	198	1	200,42		37	5,20E-19	R	FKDENVGPSVIAQTAADEGSR	T	
3	TIFY8	JA	AT4G32570	TIFY8	3858	38766	66,5	361	1160,5447	2319,0748	2	2319,0743	0,0006	238	257	0	82,64	26	36	2,40E-07	K	QMTIFYGGQAHVFDDVHPNK	A	Oxidation (M)
3	TIFY8	JA	AT4G32570	TIFY8	3858	38766	66,5	361	1184,5326	2367,0506	2	2367,0516	-0,001	120	139	0	90,96	28	34	2,20E-08	R	SNSDSHFTTQEHPETLHWSK	L	
3	TIFY8	JA	AT4G32570	TIFY8	3858	38766	66,5	361	791,7327	2372,1762	3	2372,1794	-0,0032	258	281	1	63,59		37	2,50E-05	K	ADVIMALAGSSGGSWSTGLSHKPK	S	Oxidation (M)
3	TIFY8	JA	AT4G32570	TIFY8	3858	38766	66,5	361	1291,1239	2580,2332	2	2580,2337	-0,0005	58	85	0	183,41	35	37	2,50E-17	K	AAMTPSTASASSAGGLGGLSSTSDLVER	H	
3	TIFY8	JA	AT3G11540	SPY	940	102790	28,7	914	434,2377	866,4608	2	866,461	-0,0002	76	83	0	44,43		36	0,0015	K	NVEAHIGK	G	
3	TIFY8	JA	AT3G11540	SPY	940	102790	28,7	914	436,2612	870,5079	2	870,5076	0,0003	670	676	0	34,32		33	0,0085	K	VLQVWAR	I	
3	TIFY8	JA	AT3G11540	SPY	940	102790	28,7	914	447,2818	892,5491	2	892,5494	-0,0004	780	788	0	47,83		27	9,90E-05	K	VGLGHLVAK	N	
3	TIFY8	JA	AT3G11540	SPY	940	102790	28,7	914	482,2977	962,5808	2	962,5801	0,0008	515	523	0	66,84		28	1,60E-06	K	VVVYSAVVK	A	
3	TIFY8	JA	AT3G11540	SPY	940	102790	28,7	914	517,2435	1032,4725	2	1032,4724	0,0001	2	10	0	50,14		34	0,00029	M	VGLEDDTER	E	
3	TIFY8	JA	AT3G11540	SPY	940	102790	28,7	914	531,2739	1060,5332	2	1060,5335	-0,0003	84	92	0	47,76		38	0,0012	K	GICLQTQNK	G	
3	TIFY8	JA	AT3G11540	SPY	940	102790	28,7	914	569,2935	1136,5725	2	1136,5713	0,0012	127	136	0	72,88		37	3,10E-06	R	LVEAAESYQK	A	
3	TIFY8	JA	AT3G11540	SPY	940	102790	28,7	914	579,8098	1157,605	2	1157,604	0,001	164	174	0	66,95	34	36	1,00E-05	K	LAGNTQEGIQK	Y	
3	TIFY8	JA	AT3G11540	SPY	940	102790	28,7	914	611,3068	1220,5991	2	1220,5997	-0,0006	26	38	0	78,3		38	1,00E-06	R	SSSSSAGVLSPSR	K	
3	TIFY8	JA	AT3G11540	SPY	940	102790	28,7	914	636,8194	1271,6242	2	1271,6245	-0,0003	607	618	0	57,86		36	7,10E-05	R	ITDSLADPPDTK	Q	
3	TIFY8	JA	AT3G11540	SPY	940	102790	28,7	914	681,3578	1360,7011	2	1360,702	-0,0009	258	270	0	99,52		38	8,20E-09	K	NNMAIALTDLGTK	V	
3	TIFY8	JA	AT3G11540	SPY	940	102790	28,7	914	721,8625	1441,7105	2	1441,7089	0,0016	273	285	0	104,26		37	2,00E-09	K	LEGDVTQGVAYYK	K	
3	TIFY8	JA	AT3G11540	SPY	940	102790	28,7	914	739,91	1477,8055	2	1477,8028	0,0027	702	714	0	95,66		34	8,10E-09	R	FLTTLEQLGLESK	R	
3	TIFY8	JA	AT3G11540	SPY	940	102790	28,7	914	750,351	1498,6875	2	1498,6875	0,0001	93	105	0	67,56		35	6,40E-06	K	GNLAFDCFSEAIR	L	
3	TIFY8	JA	AT3G11540	SPY	940	102790	28,7	914	792,9028	1583,7911	2	1583,7905	0,0007	59	72	0	128,31		37	7,50E-12	K	FADALALYEAMLEK	D	
3	TIFY8	JA	AT3G11540	SPY	940	102790	28,7	914	804,9	1607,7854	2	1607,7865	-0,001	438	451	0	74,96	29	38	2,10E-06	R	LLAMNYINEGLDDK	L	
3	TIFY8	JA	AT3G11540	SPY	940	102790	28,7	914	832,9335	1663,8524	2	1663,8529	-0,0006	40	54	0	80,94		38	5,40E-07	K	VTQGNDTLSYANILR	A	
3	TIFY8	JA	AT3G11540	SPY	940	102790	28,7	914	624,6764	1871,0073	3	1871,0074	-0,0001	146	163	0	85,08		36	1,30E-07	K	PAAECLAIVLTDLGTSLK	L	
3	TIFY8	JA	AT3G11540	SPY	940	102790	28,7	914	1105,5819	2209,1492	2	2209,1531	-0,0039	389	408	0	129,84	34	36	4,30E-12	K	AILANPTYAEAFNNLGVLYR	D	
3	TIFY8	JA	AT3G11540	SPY	940	102790	28,7	914	849,1069	2544,299	3	2544,3013	-0,0023	584	606	0	70,22	30	36	4,70E-06	R	PAPVQVTWIGYPNTTGLPTVDYR	I	
3	TIFY8	JA	AT3G11540	SPY	940	102790	28,7	914	941,4938	2821,4597	3	2821,4605	-0,0008	137	163	1	54,48	31	35	0,00013	K	ALMADASYKPAAECLAIVLTDLGTSLK	L	
3	TIFY8	JA	AT4G28910	NINJA	608	45081	46,1	425	755,3227	1508,6309	2	1508,6314	-0,0005	199	212	0	83,18		30	5,50E-08	R	SNHGGSGTEEFTMR	N	
3	TIFY8	JA	AT4G28910	NINJA	608	45081	46,1	425	809,9127	1617,8109	2	1617,8111	-0,0002	276	292	0	135,87	37	38	1,60E-12	K	DGSGGIVALSQSPFAGR	V	
3	TIFY8	JA	AT4G28910	NINJA	608	45081	46,1	425	554,9495	1661,8267	3	1661,8274	-0,0007	356	371	0	76,13	26	38	1,60E-06	R	PNLPWVSTTGSGPHGR	T	
3	TIFY8	JA	AT4G28910	NINJA	608	45081	46,1	425	954,9392	1907,8638	2	1907,865	-0,0012	73	89	0	93,38		35	1,50E-08	R	SDSGQQPPQNFFNDLSK	A	
3	TIFY8	JA	AT4G28910	NINJA	608	45081	46,1	425	1062,5563	2123,098	2	2123,0971	0,0009	179	197	1	88,02		36	7,80E-08	K	EVVRPPTDTNIVDNLTGQR	R	
3	TIFY8	JA	AT4G28910	NINJA	608	45081	46,1	425	739,6935	2216,0587	3	2216,0597	-0,001	90	109	1	68,58	30	37	7,50E-06	K	APTTEAEASTKPLWVEDESR	K	
3	TIFY8	JA	AT4G28910	NINJA	608	45081	46,1	425	771,3189	2310,9348	3	2310,9369	-0,0021	404	425	0	88,17		27	9,10E-09	R	HASEEYVSPESSMGMTAASAHT	-	2 Oxidation (M)
3	TIFY8	JA	AT4G28910	NINJA	608	45081	46,1	425	830,1113	3316,4163	4	3316,42	-0,0037	145	178	0	35,71	25	29	0,0024	K	ASHVSTATDEGSTAENEDVAESEVGGGSSSNHAK	E	
3	TIFY8	JA	AT4G28910	NINJA	608	45081	46,1	425	978,9863	3911,9162	4	3911,9186	-0,0024	239	275	0	61,33	24	35	2,40E-05	K	ESGQHAAATSLLQPNANAGNLPIMFGYSPVQLPMLDK	D	2 Oxidation (M)
3	TIFY8	JA	AT4G14720	PPD2	601	35056	48,6	315	464,2973	926,5801	2	926,58	0	10	17	1	46,18		28	0,00019	K	SILEKPLK	L	
3	TIFY8	JA	AT4G14720	PPD2	601	35056	48,6	315	515,3005	1028,5864	2	1028,5866	-0,0002	87	95	0	59,42		36	4,80E-05	R	VTTTLIEPR	N	
3	TIFY8	JA	AT4G14720	PPD2	601	35056	48,6	315	607,8585	1213,7024	2	1213,703	-0,0006	49	59	0	70,13		35	3,40E-06	K	SQAIQQVLSLK	A	
3	TIFY8	JA	AT4G14720	PPD2	601	35056	48,6	315	608,8147	1215,6147	2	1215,6136	0,0012	166	176	0	53,03		37	0,00027	K	VNVYDGVPPEK	A	

3	TIFY8	JA	AT4G14720	PPD2	601	35056	48,6	315	705,8245	1409,6345	2	1409,6358	-0,0013	104	115	0	83,73		35	1,60E-07	R	IPLQEDDGACHR	R	
3	TIFY8	JA	AT4G14720	PPD2	601	35056	48,6	315	709,3809	1416,7472	2	1416,746	0,0012	18	29	0	110,5		38	5,70E-10	K	LLTEEDISQLTR	E	
3	TIFY8	JA	AT4G14720	PPD2	601	35056	48,6	315	758,8491	1515,6836	2	1515,6842	-0,0005	121	135	0	105,12		34	8,60E-10	R	SAEFSGSSGQFVADK	D	
3	TIFY8	JA	AT4G14720	PPD2	601	35056	48,6	315	767,3807	1532,7468	2	1532,7471	-0,0003	60	74	0	75,27		38	1,90E-06	K	ALYEPGDDSGAGILR	K	
3	TIFY8	JA	AT4G14720	PPD2	601	35056	48,6	315	797,8982	1593,7819	2	1593,7821	-0,0002	258	272	0	84,04	29	38	2,70E-07	K	APGVASSSLEMFLNR	Q	Oxidation (M)
3	TIFY8	JA	AT4G14720	PPD2	601	35056	48,6	315	796,3978	2386,1716	3	2386,1739	-0,0023	179	200	0	75,15	26	37	1,80E-06	R	SIMHFAANPIDLPENGIFASSR	M	
3	TIFY8	JA	AT4G14720	PPD2	601	35056	48,6	315	847,363	2539,0672	3	2539,0703	-0,0032	276	298	0	70,45		29	8,80E-07	R	MNAAYSQNLSGTGHCESPENQTK	S	Oxidation (M)
3	TIFY8	JA	AT1G51690	ATB ALPHA	438	57526	30	513	381,7289	761,4433	2	761,4436	-0,0003	62	67	0	37,8		35	0,0057	R	VVLFER	T	
3	TIFY8	JA	AT1G51690	ATB ALPHA	438	57526	30	513	485,2442	968,4738	2	968,4716	0,0022	22	29	0	54,64		35	0,00013	R	FSQVFGER	S	
3	TIFY8	JA	AT1G51690	ATB ALPHA	438	57526	30	513	593,7959	1185,5772	2	1185,5778	-0,0006	414	424	0	69,68		35	4,10E-06	R	AATGSYSNLFR	V	
3	TIFY8	JA	AT1G51690	ATB ALPHA	438	57526	30	513	597,7475	1193,4804	2	1193,4805	-0,0001	153	162	0	64,07		28	2,80E-06	K	ICDMNSDPSR	T	
3	TIFY8	JA	AT1G51690	ATB ALPHA	438	57526	30	513	617,8022	1233,5899	2	1233,5877	0,0022	79	88	0	57,49		37	0,0001	R	ELEEADYPLR	H	
3	TIFY8	JA	AT1G51690	ATB ALPHA	438	57526	30	513	778,9141	1555,8136	2	1555,8134	0,0002	336	349	0	127,99		36	7,80E-12	K	SFFTEIIASVSDIK	F	
3	TIFY8	JA	AT1G51690	ATB ALPHA	438	57526	30	513	526,6266	1576,8581	3	1576,8573	0,0008	210	224	0	56,7		35	8,10E-05	R	LPVVVTSHESSPVAR	C	
3	TIFY8	JA	AT1G51690	ATB ALPHA	438	57526	30	513	585,9334	1754,7783	3	1754,7788	-0,0005	96	109	0	37,75		33	0,0039	K	TEFQSHDPEFDYLK	S	
3	TIFY8	JA	AT1G51690	ATB ALPHA	438	57526	30	513	896,9586	1791,9027	2	1791,9003	0,0024	425	442	0	101,14	34	38	5,80E-09	R	VFGVAPGSTETATLEASR	N	
3	TIFY8	JA	AT1G51690	ATB ALPHA	438	57526	30	513	963,44	1924,8655	2	1924,865	0,0005	469	487	0	125,23		34	9,50E-12	R	GSESPGVDGNTNALDYTTK	L	
3	TIFY8	JA	AT1G51690	ATB ALPHA	438	57526	30	513	1025,1495	3072,4267	3	3072,4272	-0,0005	30	58	0	42,85	29	35	0,0019	R	SAGEEVQEVDIISAIEFDNSGNHLATGDR	G	
3	TIFY8	JA	AT4G32295	KIX1	417	27551	48,7	238	398,2761	794,5377	2	794,5378	-0,0001	54	60	0	42,58		20	5,50E-05	K	LPVVVLR	A	
3	TIFY8	JA	AT4G32295	KIX1	417	27551	48,7	238	485,7314	969,4482	2	969,4477	0,0006	61	68	0	57,7		34	4,70E-05	R	AEEIMYSK	A	
3	TIFY8	JA	AT4G32295	KIX1	417	27551	48,7	238	504,2973	1006,58	2	1006,5811	-0,0011	219	228	0	41,42		32	0,0013	R	LGPLGPPTQK	R	
3	TIFY8	JA	AT4G32295	KIX1	417	27551	48,7	238	544,8267	1087,6388	2	1087,639	-0,0002	26	34	0	57,59		33	4,30E-05	R	GLLIQEIFR	I	
3	TIFY8	JA	AT4G32295	KIX1	417	27551	48,7	238	565,8111	1129,6077	2	1129,6091	-0,0014	85	94	0	55,72		38	0,00018	R	TNDAINTIIR	L	
3	TIFY8	JA	AT4G32295	KIX1	417	27551	48,7	238	644,7638	1287,513	2	1287,5111	0,002	69	79	0	69,28		27	6,30E-07	K	ANSEAEYMDMK	T	
3	TIFY8	JA	AT4G32295	KIX1	417	27551	48,7	238	665,332	1328,6494	2	1328,6507	-0,0013	35	45	0	84,76		36	1,40E-07	R	IVCEIHSQSTR	K	
3	TIFY8	JA	AT4G32295	KIX1	417	27551	48,7	238	713,8261	1425,6376	2	1425,6387	-0,0011	173	183	0	68,59		34	4,30E-06	K	YSAYPLCYSFR	P	
3	TIFY8	JA	AT4G32295	KIX1	417	27551	48,7	238	715,358	1428,7015	2	1428,7031	-0,0016	160	172	0	90,47	36	37	4,60E-08	K	NLAVAQENCPVSK	Y	
3	TIFY8	JA	AT4G32295	KIX1	417	27551	48,7	238	986,8039	2957,3897	3	2957,3899	-0,0002	95	120	0	97,42	31	36	7,50E-09	R	LDETTETGEFLQPCIEAALHLGCTPR	R	
3	TIFY8	JA	AT1G15750	TOPLESS	218	125075	5	1131	536,811	1071,6074	2	1071,6077	-0,0002	150	158	0	62,03		37	3,20E-05	K	LIEANPLFR	D	
3	TIFY8	JA	AT1G15750	TOPLESS	218	125075	5	1131	653,3087	1304,6028	2	1304,6031	-0,0003	748	758	0	42,67		36	0,0022	K	LTEVSEPSQCR	S	
3	TIFY8	JA	AT1G15750	TOPLESS	218	125075	5	1131	868,4796	1734,9446	2	1734,9444	0,0002	7	20	0	112,72		34	1,60E-10	R	ELVFLILQFLDEEK	F	
3	TIFY8	JA	AT1G15750	TOPLESS	218	125075	5	1131	660,0081	1977,0024	3	1977,0027	-0,0004	690	711	0	94,02	34	38	2,50E-08	K	PAINSIAAAAAAAATSAGHADR	S	
3	TIFY8	JA	AT5G06600	UBP12	193	131152	8	1116	365,7469	729,4792	2	729,4789	0,0003	86	91	0	37,8		31	0,0022	R	ILIFPK	G	
3	TIFY8	JA	AT5G06600	UBP12	193	131152	8	1116	537,2535	1072,4924	2	1072,4924	0,0001	980	988	0	38,63		33	0,0029	R	AEEIPEEEK	N	
3	TIFY8	JA	AT5G06600	UBP12	193	131152	8	1116	620,3146	1238,6147	2	1238,6143	0,0004	253	263	0	69,14	33	37	6,60E-06	K	LQYNDTSVATK	E	
3	TIFY8	JA	AT5G06600	UBP12	193	131152	8	1116	638,298	1274,5813	2	1274,5819	-0,0006	184	193	0	45,2		34	0,00089	K	VLDYWSYDSK	K	
3	TIFY8	JA	AT5G06600	UBP12	193	131152	8	1116	651,8487	1301,6828	2	1301,6827	0,0001	925	936	0	73,25		37	3,00E-06	K	QSTVGDVINELK	T	
3	TIFY8	JA	AT5G06600	UBP12	193	131152	8	1116	731,8406	1461,6666	2	1461,6671	-0,0004	848	859	0	63,12		35	1,80E-05	R	LTSHNCYSQQPK	P	
3	TIFY8	JA	AT5G06600	UBP12	193	131152	8	1116	1120,8842	3359,6308	3	3359,631	-0,0002	1005	1033	0	62,63	26	36	2,40E-05	K	ETGQNQQVQNFGEPPFLVIHEGETLEEIK	N	
3	TIFY8	JA	AT3G08530	Clathrin, heavy chain	182	194402	4,9	1703	508,274	1014,5334	2	1014,5346	-0,0012	998	1007	0	58,79		35	5,20E-05	K	SPEQVSAAVK	A	
3	TIFY8	JA	AT3G08530	Clathrin, heavy chain	182	194402	4,9	1703	718,3751	1434,7357	2	1434,7354	0,0002	1132	1144	0	90,15	34	38	7,00E-08	R	EGLVSDAIESFIR	A	
3	TIFY8	JA	AT3G08530	Clathrin, heavy chain	182	194402	4,9	1703	814,4491	1626,8837	2	1626,8828	0,0008	983	997	0	44,85	31	35	0,0011	R	QLIDQVVSTALPESK	S	
3	TIFY8	JA	AT3G08530	Clathrin, heavy chain	182	194402	4,9	1703	865,7497	2594,2273	3	2594,2289	-0,0016	896	917	0	63	28	37	2,70E-05	K	IIIDSNNNPEHFLTTNPYYDSK	V	
3	TIFY8	JA	AT3G08530	Clathrin, heavy chain	182	194402	4,9	1703	887,8183	2660,433	3	2660,4326	0,0003	414	437	0	50,54	29	32	0,00015	K	FQSVPVQAGQTPPLLQYFGTLLTR	G	
3	TIFY8	JA	AT5G48160	OBE2	168	66126	5,2	574	545,8088	1089,6031	2	1089,603	0,0001	149	158	0	93,02		36	2,50E-08	R	TDLSSVTLVR	A	
3	TIFY8	JA	AT5G48160	OBE2	168	66126	5,2	574	579,3294	1156,6442	2	1156,6452	-0,001	393	402	0	88,92		36	5,20E-08	R	IAEVVQETLR	K	
3	TIFY8	JA	AT5G48160	OBE2	168	66126	5,2	574	609,8158	1217,6171	2	1217,618	-0,0009	111	120	0	58,37	36	38	0,00011	R	LPDEFLDELK	N	
3	TIFY8	JA	AT2G42500	PP2A-3/PP2A-4	163	36437	20,1	313	476,2377	950,4609	2	950,461	-0,0001	141	148	0	66,78		37	1,10E-05	K	YGNANVWK	I	
3	TIFY8	JA	AT2G42500	PP2A-3/PP2A-4	163	36437	20,1	313	478,7609	955,5073	2	955,5087	-0,0014	26	33	0	48		34	0,0005	K	PLSEQQVR	A	
3	TIFY8	JA	AT2G42500	PP2A-3/PP2A-4	163	36437	20,1	313	728,8697	1455,7249	2	1455,7259	-0,0009	287	298	0	54,64	31	37	0,00018	R	NHTFIQFEPAPR	R	
3	TIFY8	JA	AT2G42500	PP2A-3/PP2A-4	163	36437	20,1	313	896,4209	1790,8273	2	1790,8298	-0,0025	126	139	0	89,46		36	5,10E-08	R	QITQVYGFYDECLR	K	
3	TIFY8	JA	AT2G42500	PP2A-3/PP2A-4	163	36437	20,1	313	832,3703	2494,0891	3	2494,0893	-0,0003	190	210	0	40,46	32	32	0,0018	R	VQEVPHEGPMCDLLWSDPDDR	C	
3	TIFY8	JA	AT4G23460	Adaptin family proteir	142	99776	4,7	893	609,8394	1217,6642	2	1217,6656	-0,0013	350	359	0	54,34		36	0,00016	R	NIDQVLLEFK	E	
3	TIFY8	JA	AT4G23460	Adaptin family proteir	142	99776	4,7	893	690,3469	1378,6792	2	1378,6802	-0,001	120	130	0	54,48	33	38	0,00024	K	ITEYLCDPLQK	C	
3	TIFY8	JA	AT4G23460	Adaptin family proteir	142	99776	4,7	893	776,428	2326,262	3	2326,2606	0,0014	284	304	0	76,18		33	5,40E-07	K	MAPPLVTLLSAEPEIQYVALR	N	Oxidation (M)
3	TIFY8	JA	AT3G63500	TTA2	133	101204	7,4	887	498,2694	994,5242	2	994,5236	0,0006	633	640	0	41,06		36	0,0039	K	EVFLNFAR	E	
3	TIFY8	JA	AT3G63500	TTA2	133	101204	7,4	887	593,7774	1185,5403	2	1185,5414	-0,0012	319	327	0	67,69		34	4,40E-06	K	YNENTVYQR	L	
3	TIFY8	JA	AT3G63500	TTA2	133	101204	7,4	887	652,6584	1954,9535	3	1954,9537	-0,0003	302	318	0	43,62	26	37	0,0027	R	PIFQGIDWQALSHNDSK	Y	
3	TIFY8	JA	AT3G63500	TTA2	133	101204	7,4	887	1128,2993	3381,8761	3	3381,8759	0,0002	218	249	0	72,37		26	2,70E-07	K	LLLEPLDLSLSLPDVLLPIGGQDTNQLGSPVR	S	
3	TIFY8	JA	AT3G24150	KIX22	122	38771	7,3	343	384,2731	766,5316	2	766,5317	0	54	60	0	45,16		20	3,00E-05	K	LPVVVLK	A	
3	TIFY8	JA	AT3G24150	KIX22	122	38771	7,3	343	485,7314	969,4482	2	969,4477	0,0006	61	68	0	57,7		34	4,70E-05	K	AEEIMYSK	A	
3	TIFY8	JA	AT3G24150	KIX22	122	38771	7,3	343	565,3146	1128,6145	2	1128,6139	0,0007	85	94	0	74,62		36	1,60E-06	R	VNDAIDTIIR	R	
4	TIFY8	JA	AT4G32570	TIFY8	4262	38766	74,2	361	513,7702	1025,5258	2	1025,5254	0,0005	335	343	0	51,06		36	0,00033	R	EHQGSIISR	G	
4	TIFY8	JA	AT4G32570	TIFY8	4262	38766	74,2	361	576,769	1151,5234	2	1151,5241	-0,0007	103	113	0	93,26		36	1,90E-08	R	SDVSGSIMSNR	F	
4	TIFY8	JA	AT4G32570	TIFY8	4262	38766	74,2	361	588,8242	1175,6339	2	1175,6339	0	26	35	0	61,31	34	37	4,20E-05	K	LLFHDFLGSK	N	
4	TIFY8	JA	AT4G32570	TIFY8	4262	38766	74,2	361	593,7736	1185,5327	2	1185,5336	-0,0009	293	303	0	81,52		33	1,70E-07	K	LGQMYEGGSSK	E	Methyl (DE); Oxidation (M)
4	TIFY8	JA	AT4G32570	TIFY8	4262	38766	74,2	361	631,2979	1260,5812	2	1260,5809	0,0004	304	313	0	41,06		35	0,0026	K	ETPQMPPEFR	A	Methyl (DE); Oxidation (M)
4	TIFY8	JA	AT4G32570	TIFY8	4262	38766	74,2	361	646,8149	1291,6153	2	1291,6157	-0,0004	218	229	0	63,13		37	2,70E-05	K	LESFAPSNTGNR	K	
4	TIFY8	JA	AT4G32570	TIFY8	4262	38766	74,2	361	455,5421	1363,6044	3	1363,6051	-0,0008	316	327	0	62,24		34	1,60E-05	R	PSHQPTSSACHR	I	

4	TIFY8	JA	AT4G32570	TIFY8	4262	38766	74,2	361	700,8329	1399,6512	2	1399,6514	-0,0002	36	48	0	79,45		35	3,80E-07	K	NPTLASTSMADHR	L	
4	TIFY8	JA	AT4G32570	TIFY8	4262	38766	74,2	361	769,8787	1537,7428	2	1537,7446	-0,0018	203	217	0	77,22	25	37	1,20E-06	K	GPGILSSFTMPNSSK	L	Oxidation (M)
4	TIFY8	JA	AT4G32570	TIFY8	4262	38766	74,2	361	564,3039	1689,8898	3	1689,8911	-0,0013	162	177	0	55,27	29	37	0,00017	R	GGGQISHLLHQLSTSR	F	
4	TIFY8	JA	AT4G32570	TIFY8	4262	38766	74,2	361	881,4424	1760,8701	2	1760,8707	-0,0005	86	102	0	123,41	36	38	2,90E-11	R	HSGGGNHLDGIQLFGPR	S	
4	TIFY8	JA	AT4G32570	TIFY8	4262	38766	74,2	361	965,4607	1928,9068	2	1928,9076	-0,0008	180	198	0	140,91		36	3,70E-13	K	DENVGPSVIAQTAADEGSR	T	Methyl (DE)
4	TIFY8	JA	AT4G32570	TIFY8	4262	38766	74,2	361	998,986	1995,9575	2	1995,9585	-0,001	143	161	0	112,59		37	3,30E-10	R	NGPGSFSMNVNPLANQPPR	G	
4	TIFY8	JA	AT4G32570	TIFY8	4262	38766	74,2	361	1074,5224	2147,0302	2	2147,0317	-0,0015	258	279	0	108,93	29	37	7,70E-10	K	ADVIMALAGSSGGSWSTGLSHK	P	Oxidation (M)
4	TIFY8	JA	AT4G32570	TIFY8	4262	38766	74,2	361	1096,0344	2190,0542	2	2190,0553	-0,001	178	198	1	183,56		37	2,50E-17	R	FKDENVGPSVIAQTAADEGSR	T	
4	TIFY8	JA	AT4G32570	TIFY8	4262	38766	74,2	361	768,6993	2303,0761	3	2303,0794	-0,0033	238	257	0	40,71	33	36	0,0042	K	QMTIFYGGQAHVFDDVHPNK	A	
4	TIFY8	JA	AT4G32570	TIFY8	4262	38766	74,2	361	1184,5348	2367,055	2	2367,0516	0,0034	120	139	0	94,01	28	34	1,10E-08	R	SNSDSHFTTQEHPETLHWSK	L	
4	TIFY8	JA	AT4G32570	TIFY8	4262	38766	74,2	361	1187,0957	2372,1768	2	2372,1794	-0,0026	258	281	1	124,07	31	37	2,30E-11	K	ADVIMALAGSSGGSWSTGLSHKPK	S	Oxidation (M)
4	TIFY8	JA	AT4G32570	TIFY8	4262	38766	74,2	361	1306,1292	2610,2438	2	2610,2443	-0,0004	58	85	0	189,5	33	37	6,40E-18	K	AAMTPSTASASSAGGLGGLSSTSDLVER	H	Methyl (DE); Oxidation (M)
4	TIFY8	JA	AT3G11540	SPY	834	102790	28,6	914	393,7051	785,3955	2	785,3959	-0,0004	175	180	0	35,4		33	0,0068	K	YYEALK	I	
4	TIFY8	JA	AT3G11540	SPY	834	102790	28,6	914	434,2377	866,4608	2	866,461	-0,0002	76	83	0	41,77		36	0,0028	K	NVEAHIGK	G	
4	TIFY8	JA	AT3G11540	SPY	834	102790	28,6	914	482,2974	962,5803	2	962,5801	0,0002	515	523	0	37,99		28	0,0013	K	VVVYSAVVK	A	
4	TIFY8	JA	AT3G11540	SPY	834	102790	28,6	914	517,2433	1032,4721	2	1032,4724	-0,0002	2	10	0	43,54		34	0,0013	M	VGLEDDTER	E	
4	TIFY8	JA	AT3G11540	SPY	834	102790	28,6	914	531,273	1060,5313	2	1060,5335	-0,0022	84	92	0	52,35	37	38	0,00046	K	GICLQTQNK	G	
4	TIFY8	JA	AT3G11540	SPY	834	102790	28,6	914	569,2932	1136,5718	2	1136,5713	0,0005	127	136	0	80,82	36	37	5,00E-07	R	LVEAAESYQK	A	
4	TIFY8	JA	AT3G11540	SPY	834	102790	28,6	914	579,8105	1157,6065	2	1157,604	0,0024	164	174	0	59,95		36	4,80E-05	K	LAGNTQEGIQK	Y	
4	TIFY8	JA	AT3G11540	SPY	834	102790	28,6	914	594,8282	1187,6418	2	1187,6398	0,0021	877	888	0	78,66		38	8,80E-07	R	VSVTGEATPSLK	A	
4	TIFY8	JA	AT3G11540	SPY	834	102790	28,6	914	611,3067	1220,5988	2	1220,5997	-0,0009	26	38	0	60,25		38	6,70E-05	R	SSSSSAGVLSPSR	K	
4	TIFY8	JA	AT3G11540	SPY	834	102790	28,6	914	636,8195	1271,6244	2	1271,6245	-0,0001	607	618	0	57,42		36	7,90E-05	R	ITDSLADPPDTK	Q	
4	TIFY8	JA	AT3G11540	SPY	834	102790	28,6	914	681,3576	1360,7006	2	1360,702	-0,0015	258	270	0	95,32		38	2,20E-08	K	NNMAIALTDLGTK	V	
4	TIFY8	JA	AT3G11540	SPY	834	102790	28,6	914	721,8631	1441,7117	2	1441,7089	0,0028	273	285	0	95,44	36	37	1,60E-08	K	LEGDVTQGVAYYK	K	
4	TIFY8	JA	AT3G11540	SPY	834	102790	28,6	914	739,9095	1477,8044	2	1477,8028	0,0017	702	714	0	99,14		34	3,70E-09	R	FLTTLEQLGLESK	R	
4	TIFY8	JA	AT3G11540	SPY	834	102790	28,6	914	750,3511	1498,6876	2	1498,6875	0,0002	93	105	0	62,87		35	1,90E-05	K	GNLAFDCFSEAIR	L	
4	TIFY8	JA	AT3G11540	SPY	834	102790	28,6	914	792,9028	1583,7911	2	1583,7905	0,0006	59	72	0	113,95		37	2,10E-10	K	FADALALYEAMLEK	D	
4	TIFY8	JA	AT3G11540	SPY	834	102790	28,6	914	812,8985	1623,7825	2	1623,7814	0,0011	438	451	0	76,56	28	37	1,20E-06	R	LLAMNYINEGLDDK	L	Oxidation (M)
4	TIFY8	JA	AT3G11540	SPY	834	102790	28,6	914	624,6762	1871,0067	3	1871,0074	-0,0007	146	163	0	98,59		36	5,70E-09	K	PAAECLAIVLTDLGTSLK	L	
4	TIFY8	JA	AT3G11540	SPY	834	102790	28,6	914	737,3907	2209,1502	3	2209,1531	-0,0029	389	408	0	62,22		36	2,40E-05	K	AILANPTYAEAFNNLGVLYR	D	
4	TIFY8	JA	AT3G11540	SPY	834	102790	28,6	914	1148,5627	2295,1108	2	2295,1118	-0,0009	789	809	0	120,81	32	37	5,10E-11	K	NEDEYVQLSVDLASDVTALSK	L	
4	TIFY8	JA	AT3G11540	SPY	834	102790	28,6	914	849,4417	2545,3034	3	2544,3013	1,0021	584	606	0	59,1	29	36	6,10E-05	R	PAPVQVTWIGYPNTTGLPTVDYR	I	
4	TIFY8	JA	AT4G28910	NINJA	646	45081	45,2	425	448,7454	895,4763	2	895,4763	0	372	379	0	37,96		34	0,0043	R	TISGVTYR	Y	
4	TIFY8	JA	AT4G28910	NINJA	646	45081	45,2	425	560,2798	1118,545	2	1118,5455	-0,0005	90	100	0	47,56	32	37	0,00099	K	APTTEAEASTK	P	Methyl (DE)
4	TIFY8	JA	AT4G28910	NINJA	646	45081	45,2	425	592,7537	1183,4929	2	1183,4928	0,0001	132	141	0	46,7		31	0,0003	K	DSSHVDMHEK	K	
4	TIFY8	JA	AT4G28910	NINJA	646	45081	45,2	425	763,3199	1524,6252	2	1524,6263	-0,0012	199	212	0	69,75		29	9,10E-07	R	SNHGGSGTEEFTMR	N	Oxidation (M)
4	TIFY8	JA	AT4G28910	NINJA	646	45081	45,2	425	795,8478	1589,6809	2	1589,6805	0,0005	306	320	0	80,95		31	1,10E-07	K	QPVAEEGSSEDASER	P	
4	TIFY8	JA	AT4G28910	NINJA	646	45081	45,2	425	809,9124	1617,8103	2	1617,8111	-0,0008	276	292	0	100,14		37	6,10E-09	K	DGSGGIVALSQSPFAGR	V	
4	TIFY8	JA	AT4G28910	NINJA	646	45081	45,2	425	554,9493	1661,826	3	1661,8274	-0,0015	356	371	0	56,83	24	38	0,00014	R	PNLPWVSTTGSGPHGR	T	
4	TIFY8	JA	AT4G28910	NINJA	646	45081	45,2	425	954,9403	1907,866	2	1907,865	0,001	73	89	0	97,03		35	6,90E-09	R	SDSGQQPPQNFFNDLSK	A	
4	TIFY8	JA	AT4G28910	NINJA	646	45081	45,2	425	1062,5556	2123,0966	2	2123,0971	-0,0005	179	197	1	88,79	30	37	6,90E-08	K	EVVRPPTDTNIVDNLTGQR	R	
4	TIFY8	JA	AT4G28910	NINJA	646	45081	45,2	425	739,6934	2216,0582	3	2216,0597	-0,0015	90	109	1	67,9	30	37	8,90E-06	K	APTTEAEASTKPLWVEDESR	K	
4	TIFY8	JA	AT4G28910	NINJA	646	45081	45,2	425	771,3193	2310,936	3	2310,9369	-0,0008	404	425	0	101,08		27	4,80E-10	R	HASEEYVSPESSMGMTAASAHT	-	2 Oxidation (M)
4	TIFY8	JA	AT4G28910	NINJA	646	45081	45,2	425	1106,4808	3316,4206	3	3316,42	0,0006	145	178	0	131,16		29	7,00E-13	K	ASHVSTATDEGSTAENEDVAESEVGGGSSSNHAK	E	
4	TIFY8	JA	AT4G14720	PPD2	400	35056	37,8	315	464,2973	926,58	2	926,58	0	10	17	1	37,47		28	0,0014	K	SILEKPLK	L	
4	TIFY8	JA	AT4G14720	PPD2	400	35056	37,8	315	515,3011	1028,5875	2	1028,5866	0,0009	87	95	0	59,63		36	5,00E-05	R	VTTTLIEPR	N	
4	TIFY8	JA	AT4G14720	PPD2	400	35056	37,8	315	607,859	1213,7034	2	1213,703	0,0004	49	59	0	76,03		35	8,70E-07	K	SQAIQQVLSLK	A	
4	TIFY8	JA	AT4G14720	PPD2	400	35056	37,8	315	608,8149	1215,6153	2	1215,6136	0,0017	166	176	0	45,31		37	0,0016	K	VNVYDGVPPEK	A	
4	TIFY8	JA	AT4G14720	PPD2	400	35056	37,8	315	653,8395	1305,6644	2	1305,6638	0,0005	211	221	0	53,05	35	38	0,00036	K	MVELPQYGLEK	A	
4	TIFY8	JA	AT4G14720	PPD2	400	35056	37,8	315	470,8861	1409,6364	3	1409,6358	0,0007	104	115	0	72,55		35	2,10E-06	R	IPLQEDDGACHR	R	
4	TIFY8	JA	AT4G14720	PPD2	400	35056	37,8	315	709,3808	1416,7471	2	1416,746	0,0011	18	29	0	93,58		37	2,70E-08	K	LLTEEDISQLTR	E	
4	TIFY8	JA	AT4G14720	PPD2	400	35056	37,8	315	758,8489	1515,6833	2	1515,6842	-0,0008	121	135	0	100,59		34	2,40E-09	R	SAEFSGSSGQFVADK	D	
4	TIFY8	JA	AT4G14720	PPD2	400	35056	37,8	315	767,3805	1532,7464	2	1532,7471	-0,0006	60	74	0	72,16		38	4,00E-06	K	ALYEPGDDSGAGILR	K	
4	TIFY8	JA	AT4G14720	PPD2	400	35056	37,8	315	797,8986	1593,7825	2	1593,7821	0,0005	258	272	0	85,27	27	38	2,10E-07	K	APGVASSSLEMFLNR	Q	Oxidation (M)
4	TIFY8	JA	AT4G32295	KIX1	373	27551	58,4	238	398,2763	794,5381	2	794,5378	0,0003	54	60	0	42,74		20	5,30E-05	K	LPVVVLR	A	
4	TIFY8	JA	AT4G32295	KIX1	373	27551	58,4	238	485,7312	969,4478	2	969,4477	0,0001	61	68	0	48,38		34	0,00041	R	AEEIMYSK	A	
4	TIFY8	JA	AT4G32295	KIX1	373	27551	58,4	238	504,2978	1006,5811	2	1006,5811	0	219	228	0	39,05		32	0,0023	R	LGPLGPPTQK	R	
4	TIFY8	JA	AT4G32295	KIX1	373	27551	58,4	238	544,8267	1087,6389	2	1087,639	0	26	34	0	52,52		33	0,00014	R	GLLIQEIFR	I	
4	TIFY8	JA	AT4G32295	KIX1	373	27551	58,4	238	565,8109	1129,6073	2	1129,6091	-0,0018	85	94	0	53,58	36	38	0,00031	R	TNDAINTIIR	L	
4	TIFY8	JA	AT4G32295	KIX1	373	27551	58,4	238	652,7605	1303,5064	2	1303,506	0,0004	69	79	0	75,04		22	5,50E-08	K	ANSEAEYMDMK	T	Oxidation (M)
4	TIFY8	JA	AT4G32295	KIX1	373	27551	58,4	238	665,3321	1328,6496	2	1328,6507	-0,001	35	45	0	73,51		36	2,00E-06	R	IVCEIHSQSTR	K	
4	TIFY8	JA	AT4G32295	KIX1	373	27551	58,4	238	713,8264	1425,6382	2	1425,6387	-0,0005	173	183	0	56,26		34	7,40E-05	K	YSAYPLCYSFR	P	
4	TIFY8	JA	AT4G32295	KIX1	373	27551	58,4	238	715,3583	1428,702	2	1428,7031	-0,001	160	172	0	95,48	34	37	1,40E-08	K	NLAVAQENCPVSK	Y	
4	TIFY8	JA	AT4G32295	KIX1	373	27551	58,4	238	823,3952	2467,1638	3	2466,1519	1,0119	196	218	0	51,64	28	37	0,00036	R	PASLIDATNGITFGGCECDLSLR	L	
4	TIFY8	JA	AT4G32295	KIX1	373	27551	58,4	238	986,8039	2957,3898	3	2957,3899	-0,0001	95	120	0	84,53	31	36	1,50E-07	R	LDETTETGEFLQPCIEAALHLGCTPR	R	
4	TIFY8	JA	AT1G51690	ATB ALPHA	359	57526	21,1	513	593,7962	1185,5779	2	1185,5778	0	414	424	0	71,19		36	3,10E-06	R	AATGSYSNLFR	V	
4	TIFY8	JA	AT1G51690	ATB ALPHA	359	57526	21,1	513	617,8032	1233,5919	2	1233,5877	0,0042	79	88	0	45,72	36	38	0,0018	R	ELEEADYPLR	H	
4	TIFY8	JA	AT1G51690	ATB ALPHA	359	57526	21,1	513	778,9144	1555,8143	2	1555,8134	0,0009	336	349	0	130,37		36	4,60E-12	K	SFFTEIIASVSDIK	F	
4	TIFY8	JA	AT1G51690	ATB ALPHA	359	57526	21,1	513	526,6266	1576,8579	3	1576,8573	0,0006	210	224	0	53,8		35	0,00016	R	LPVVVTSHESSPVAR	C	

4	TIFY8	JA	AT1G51690	ATB ALPHA	359	57526	21,1	513	585,9335	1754,7788	3	1754,7788	0	96	109	0	49,87		33	0,00024	K	TEFQSHDPEFDYLK	S	
4	TIFY8	JA	AT1G51690	ATB ALPHA	359	57526	21,1	513	896,9583	1791,902	2	1791,9003	0,0017	425	442	0	98,59	34	38	1,00E-08	R	VFGVAPGSTETATLEASR	N	
4	TIFY8	JA	AT1G51690	ATB ALPHA	359	57526	21,1	513	963,4406	1924,8667	2	1924,865	0,0017	469	487	0	118,73		35	4,30E-11	R	GSESPGVDGNTNALDYTTK	L	
4	TIFY8	JA	AT1G15750	TOPLESS	242	125075	5,6	1131	379,2447	756,4748	2	756,4745	0,0003	93	99	0	47,41		32	0,0003	K	AVDILVK	D	
4	TIFY8	JA	AT1G15750	TOPLESS	242	125075	5,6	1131	536,8109	1071,6073	2	1071,6077	-0,0003	150	158	0	68,78		37	6,70E-06	K	LIEANPLFR	D	
4	TIFY8	JA	AT1G15750	TOPLESS	242	125075	5,6	1131	868,4796	1734,9447	2	1734,9444	0,0003	7	20	0	112,63		34	1,60E-10	R	ELVFLILQFLDEEK	F	
4	TIFY8	JA	AT1G15750	TOPLESS	242	125075	5,6	1131	660,0082	1977,0027	3	1977,0027	-0,0001	690	711	0	112,37	35	38	3,80E-10	K	PAINSIAAAAAAAATSAGHADR	S	
4	TIFY8	JA	AT5G06600	UBP12	228	131152	9,3	1116	365,7469	729,4793	2	729,4789	0,0004	86	91	0	39,07		31	0,0017	R	ILIFPK	G	
4	TIFY8	JA	AT5G06600	UBP12	228	131152	9,3	1116	422,7244	843,4342	2	843,4338	0,0004	838	845	0	40,54		34	0,0025	K	LGLDDPSK	L	
4	TIFY8	JA	AT5G06600	UBP12	228	131152	9,3	1116	565,7707	1129,5269	2	1129,5265	0,0004	137	145	0	36,19		34	0,0064	K	ETQHQFNAR	E	
4	TIFY8	JA	AT5G06600	UBP12	228	131152	9,3	1116	586,8248	1171,6351	2	1171,635	0,0002	617	626	0	46,24	35	37	0,0013	K	EFGVPVQLQR	F	
4	TIFY8	JA	AT5G06600	UBP12	228	131152	9,3	1116	620,3143	1238,6141	2	1238,6143	-0,0002	253	263	0	75,2	31	37	1,60E-06	K	LQYNDTSVATK	E	
4	TIFY8	JA	AT5G06600	UBP12	228	131152	9,3	1116	638,2984	1274,5823	2	1274,5819	0,0004	184	193	0	60,8		34	2,40E-05	K	VLDYWSYDSK	K	
4	TIFY8	JA	AT5G06600	UBP12	228	131152	9,3	1116	651,8486	1301,6826	2	1301,6827	-0,0001	925	936	0	69,81	36	38	6,80E-06	K	QSTVGDVINELK	T	
4	TIFY8	JA	AT5G06600	UBP12	228	131152	9,3	1116	731,8411	1461,6677	2	1461,6671	0,0007	848	859	0	58,14		35	5,60E-05	R	LTSHNCYSQQPK	P	
4	TIFY8	JA	AT5G06600	UBP12	228	131152	9,3	1116	973,785	2918,3332	3	2918,3358	-0,0027	484	509	0	64,98	26	35	1,00E-05	R	ALEEQYGGEEELPQTNPGFNNNPPFK	F	
4	TIFY8	JA	AT5G48160	OBE2	198	66126	6,8	574	516,7897	1031,5648	2	1031,5651	-0,0004	291	299	0	64,9		38	2,10E-05	R	TSELLGWVK	D	
4	TIFY8	JA	AT5G48160	OBE2	198	66126	6,8	574	545,8079	1089,6012	2	1089,603	-0,0018	149	158	0	98,06	35	37	7,90E-09	R	TDLSSVTLVR	A	
4	TIFY8	JA	AT5G48160	OBE2	198	66126	6,8	574	579,3294	1156,6442	2	1156,6452	-0,001	393	402	0	84,56		36	1,40E-07	R	IAEVVQETLR	K	
4	TIFY8	JA	AT5G48160	OBE2	198	66126	6,8	574	609,8164	1217,6183	2	1217,618	0,0004	111	120	0	58,66	35	38	0,0001	R	LPDEFLDELK	N	
4	TIFY8	JA	AT3G63500	TTA2	185	101204	5,6	887	498,2692	994,5237	2	994,5236	0,0001	633	640	0	40,72		36	0,0042	K	EVFLNFAR	E	
4	TIFY8	JA	AT3G63500	TTA2	185	101204	5,6	887	561,2956	1120,5766	2	1120,5764	0,0002	781	789	0	51,28		37	0,00047	R	FEELESIVR	M	
4	TIFY8	JA	AT3G63500	TTA2	185	101204	5,6	887	591,7635	1181,5124	2	1181,5135	-0,0011	792	801	0	49,47		33	0,00028	K	QAEAEMFQGR	A	Oxidation (M)
4	TIFY8	JA	AT3G63500	TTA2	185	101204	5,6	887	593,7779	1185,5412	2	1185,5414	-0,0002	319	327	0	75,47		34	8,40E-07	K	YNENTVYQR	L	
4	TIFY8	JA	AT3G63500	TTA2	185	101204	5,6	887	787,407	1572,7994	2	1572,8008	-0,0015	469	482	0	109,27		37	7,00E-10	K	NVQLGAFQDALQNR	T	
4	TIFY8	JA	AT4G23460	Adaptin family protein	142	99776	4,7	893	577,7935	1153,5724	2	1153,5727	-0,0004	96	105	0	38,84		36	0,0064	K	DSQDPNPLIR	A	
4	TIFY8	JA	AT4G23460	Adaptin family protein	142	99776	4,7	893	690,3458	1378,6771	2	1378,6802	-0,0031	120	130	0	59,11	32	37	7,60E-05	K	ITEYLCDPLQK	C	
4	TIFY8	JA	AT4G23460	Adaptin family protein	142	99776	4,7	893	776,4272	2326,2597	3	2326,2606	-0,0009	284	304	0	82,83	33	33	1,20E-07	K	MAPPLVTLLSAEPEIQYVALR	N	Oxidation (M)
4	TIFY8	JA	AT3G08530	Clathrin, heavy chain	117	194402	2,8	1703	508,2745	1014,5345	2	1014,5346	-0,0001	998	1007	0	46,13		35	0,00095	K	SPEQVSAAVK	A	
4	TIFY8	JA	AT3G08530	Clathrin, heavy chain	117	194402	2,8	1703	643,3188	1284,6231	2	1284,6237	-0,0006	1219	1229	0	58,53		35	5,40E-05	R	LYDEALYEAAK	I	
4	TIFY8	JA	AT3G08530	Clathrin, heavy chain	117	194402	2,8	1703	683,8752	1365,7358	2	1365,7365	-0,0007	368	380	0	36,49		35	0,0078	R	GNLPGAENLVVQR	F	
4	TIFY8	JA	AT3G08530	Clathrin, heavy chain	117	194402	2,8	1703	718,3752	1434,7358	2	1434,7354	0,0003	1132	1144	0	78,83	32	38	9,60E-07	R	EGLVSDAIESFIR	A	
4	TIFY8	JA	AT3G24150	KIX22	115	38771	7,3	343	384,2731	766,5316	2	766,5317	-0,0001	54	60	0	45,3		20	3,00E-05	K	LPVVVLK	A	
4	TIFY8	JA	AT3G24150	KIX22	115	38771	7,3	343	485,7312	969,4478	2	969,4477	0,0001	61	68	0	48,38		34	0,00041	K	AEEIMYSK	A	
4	TIFY8	JA	AT3G24150	KIX22	115	38771	7,3	343	565,3144	1128,6143	2	1128,6139	0,0005	85	94	0	70,93		36	3,70E-06	R	VNDAIDTIIR	R	
4	TIFY8	JA	AT2G42500	PP2A-3/PP2A-4	71	36437	5,1	313	465,2433	928,4721	2	928,4726	-0,0005	299	306	1	46,98		34	0,0006	R	RGEPDVTR	R	
4	TIFY8	JA	AT2G42500	PP2A-3/PP2A-4	71	36437	5,1	313	478,7607	955,5068	2	955,5087	-0,0019	26	33	0	57		34	5,90E-05	K	PLSEQQVR	A	

Annex 4. List of peptides identified in TAP of PPD2, JAZ10 and KIX2 with Orbitrap mass spectrometry

Exp	Bait	Treatment	AT number	Name	prot_ score	prot_ mass	prot_ cover	prot_ len	pep_ exp_mz	pep_ exp_mr	pep_ exp_z	pep_ calc_mr	pep_ delta	pep_ start	pep_ end	pep_ miss	pep_ score	pep_ homol	pep_ ident	pep_ expect	pep_res_ before	pep_ seq	pep_res_ after	pep_ var_mod
1	PPD2	mock	AT4G14720	PPD2	4078	35056	68,6	315	365,7324	729,4501	2	729,4497	0,0004	237	242	1	45,9		34	0,00075	R	KVSLQR	Y	
1	PPD2	mock	AT4G14720	PPD2	4078	35056	68,6	315	464,2972	926,5798	2	926,58	-2E-04	10	17	1	37,77		28	0,0013	K	SILEKPLK	L	
1	PPD2	mock	AT4G14720	PPD2	4078	35056	68,6	315	474,7136	947,4127	2	947,4131	-3E-04	96	103	0	49,35		32	0,0002	R	NELEACGR	I	
1	PPD2	mock	AT4G14720	PPD2	4078	35056	68,6	315	515,3008	1028,587	2	1028,587	0,0005	87	95	0	70,89		35	3,10E-06	R	VTTTLIEPR	N	
1	PPD2	mock	AT4G14720	PPD2	4078	35056	68,6	315	607,859	1213,704	2	1213,703	0,0004	49	59	0	94,12		35	1,30E-08	K	SQAIQQVLSLK	A	
1	PPD2	mock	AT4G14720	PPD2	4078	35056	68,6	315	608,8135	1215,612	2	1215,614	-0,001	166	176	0	86,03		36	1,20E-07	K	VNVYDGVPPEK	A	
1	PPD2	mock	AT4G14720	PPD2	4078	35056	68,6	315	406,5714	1216,692	3	1216,693	-5E-04	76	86	0	42,31		35	0,0019	K	ILVSQPPNPPR	V	
1	PPD2	mock	AT4G14720	PPD2	4078	35056	68,6	315	653,8394	1305,664	2	1305,664	0,0004	211	221	0	81,04		38	5,70E-07	K	MVELPQYGLEK	A	
1	PPD2	mock	AT4G14720	PPD2	4078	35056	68,6	315	673,4005	1344,787	2	1344,788	-0,001	75	86	1	67,48		30	2,20E-06	R	KILVSQPPNPPR	V	
1	PPD2	mock	AT4G14720	PPD2	4078	35056	68,6	315	705,8245	1409,634	2	1409,636	-0,001	104	115	0	91,25		35	2,80E-08	R	IPLQEDDGACHR	R	
1	PPD2	mock	AT4G14720	PPD2	4078	35056	68,6	315	709,3792	1416,744	2	1416,746	-0,002	18	29	0	106,5		37	1,30E-09	K	LLTEEDISQLTR	E	
1	PPD2	mock	AT4G14720	PPD2	4078	35056	68,6	315	758,8486	1515,683	2	1515,684	-0,002	121	135	0	104,9		34	8,90E-10	R	SAEFSGSSGQFVADK	D	
1	PPD2	mock	AT4G14720	PPD2	4078	35056	68,6	315	767,3806	1532,747	2	1532,747	-3E-04	60	74	0	99,7		38	7,00E-09	K	ALYEPGDDSGAGILR	K	
1	PPD2	mock	AT4G14720	PPD2	4078	35056	68,6	315	797,4127	1592,811	2	1592,812	-0,001	209	221	1	66,27	33	38	1,60E-05	K	EKMVELPQYGLEK	A	Methyl (DE); Oxidation (M)
1	PPD2	mock	AT4G14720	PPD2	4078	35056	68,6	315	804,906	1607,798	2	1607,798	-2E-04	258	272	0	99,37	29	38	7,50E-09	K	APGVASSSLEMFLNR	Q	Methyl (DE); Oxidation (M)
1	PPD2	mock	AT4G14720	PPD2	4078	35056	68,6	315	853,9475	1705,88	2	1705,882	-0,002	257	272	1	122,9	31	38	3,30E-11	K	KAPGVASSSLEMFLNR	Q	
1	PPD2	mock	AT4G14720	PPD2	4078	35056	68,6	315	999,4628	1996,911	2	1996,913	-0,002	121	139	1	119,7		36	4,30E-11	R	SAEFSGSSGQFVADKDSHK	T	Methyl (DE)
1	PPD2	mock	AT4G14720	PPD2	4078	35056	68,6	315	1000,493	1998,971	2	1998,972	-0,001	147	165	0	149,7	34	37	6,00E-14	R	SPAETNAVVGQMTIFYSGK	V	
1	PPD2	mock	AT4G14720	PPD2	4078	35056	68,6	315	1194,094	2386,173	2	2386,174	-0,001	179	200	0	123,9	32	37	2,40E-11	R	SIMHFAANPIDLPENGIFASSR	M	
1	PPD2	mock	AT4G14720	PPD2	4078	35056	68,6	315	1277,55	2553,085	2	2553,086	-8E-04	276	298	0	89,63		30	1,40E-08	R	MNAAYSQNLSGTGHCESPENQTK	S	Methyl (DE); Oxidation (M)
1	PPD2	mock	AT4G28910	NINJA	1612	45081	72	425	425,7244	849,4342	2	849,4344	-2E-04	380	386	0	44,44		38	0,0024	R	YNANQIK	I	
1	PPD2	mock	AT4G28910	NINJA	1612	45081	72	425	448,7453	895,476	2	895,4763	-3E-04	372	379	0	39,62		34	0,0029	R	TISGVTYR	Y	
1	PPD2	mock	AT4G28910	NINJA	1612	45081	72	425	560,2794	1118,544	2	1118,546	-0,001	90	100	0	59,89		37	6,00E-05	K	APTTEAEASTK	P	Methyl (DE)
1	PPD2	mock	AT4G28910	NINJA	1612	45081	72	425	565,7773	1129,54	2	1129,54	-3E-04	101	109	0	48,26		34	0,00046	K	PLWVEDESR	K	
1	PPD2	mock	AT4G28910	NINJA	1612	45081	72	425	572,2422	1142,47	2	1142,47	-4E-04	118	127	0	42,47		28	0,00044	K	FGFPGMNDDK	K	Oxidation (M)
1	PPD2	mock	AT4G28910	NINJA	1612	45081	72	425	592,7536	1183,493	2	1183,493	-1E-04	132	141	0	41,15		31	0,0011	K	DSSHVDMHEK	K	
1	PPD2	mock	AT4G28910	NINJA	1612	45081	72	425	729,3198	1456,625	2	1456,625	-2E-04	130	141	1	54,17		32	6,40E-05	K	EKDSSHVDMHEK	K	Oxidation (M)
1	PPD2	mock	AT4G28910	NINJA	1612	45081	72	425	755,3223	1508,63	2	1508,631	-0,002	199	212	0	86,24		30	2,60E-08	R	SNHGGSGTEEFTMR	N	
1	PPD2	mock	AT4G28910	NINJA	1612	45081	72	425	802,8551	1603,696	2	1603,696	-5E-04	306	320	0	84,77		32	5,90E-08	K	QPVAEEGSSEDASER	P	Methyl (DE)
1	PPD2	mock	AT4G28910	NINJA	1612	45081	72	425	806,3405	1610,666	2	1610,667	-5E-04	24	38	0	91,17		29	7,50E-09	K	GNNNNNAGSSSENYR	A	Methyl (DE)
1	PPD2	mock	AT4G28910	NINJA	1612	45081	72	425	809,9124	1617,81	2	1617,811	-9E-04	276	292	0	107,4	35	37	1,10E-09	K	DGSGGIVALSQSPFAGR	V	
1	PPD2	mock	AT4G28910	NINJA	1612	45081	72	425	831,9204	1661,826	2	1661,827	-0,001	356	371	0	70,1	31	38	6,50E-06	R	PNLPWVSTTGSGPHGR	T	
1	PPD2	mock	AT4G28910	NINJA	1612	45081	72	425	954,939	1907,863	2	1907,865	-0,002	73	89	0	93,38		35	1,50E-08	R	SDSGQQPPQNFFNDLSK	A	
1	PPD2	mock	AT4G28910	NINJA	1612	45081	72	425	1016,445	2030,874	2	2030,876	-0,002	387	403	0	62,15	28	32	1,10E-05	K	IVCACHGSHMSPEEFVR	H	Oxidation (M)
1	PPD2	mock	AT4G28910	NINJA	1612	45081	72	425	1062,556	2123,097	2	2123,097	-5E-04	179	197	1	89,64	27	37	5,60E-08	K	EVVRPPTDTNIVDNLTGQR	R	
1	PPD2	mock	AT4G28910	NINJA	1612	45081	72	425	1116,044	2230,074	2	2230,075	-0,001	90	109	1	127,1		37	1,10E-11	K	APTTEAEASTKPLWVEDESR	K	Methyl (DE)
1	PPD2	mock	AT4G28910	NINJA	1612	45081	72	425	771,3184	2310,933	3	2310,937	-0,004	404	425	0	107,7		27	9,80E-11	R	HASEEYVSPESSMGMTAASAHT	-	2 Oxidation (M)
1	PPD2	mock	AT4G28910	NINJA	1612	45081	72	425	977,7985	2930,374	3	2930,376	-0,002	321	348	1	51,49	35	36	0,00032	R	PTGDNSNLNTAFSFDFSAIKPGMAADVK	F	Oxidation (M)
1	PPD2	mock	AT4G28910	NINJA	1612	45081	72	425	990,8161	2969,427	3	2969,43	-0,004	213	238	0	55,34	30	36	0,00014	R	NMSYTVPFTVHPQNVVTSMPYSLPTK	E	2 Oxidation (M)
1	PPD2	mock	AT4G28910	NINJA	1612	45081	72	425	1106,479	3316,416	3	3316,42	-0,004	145	178	0	134,4		29	3,30E-13	K	ASHVSTATDEGSTAENEDVAESEVGGGSSSNHAK	E	
1	PPD2	mock	AT4G32570	TIFY8	218	38766	25,5	361	513,7696	1025,525	2	1025,525	-6E-04	335	343	0	44,79		36	0,0014	R	EHQGSIISR	G	
1	PPD2	mock	AT4G32570	TIFY8	218	38766	25,5	361	584,7666	1167,519	2	1167,519	-4E-04	103	113	0	78,89		34	3,70E-07	R	SDVSGSIMSNR	F	Oxidation (M)
1	PPD2	mock	AT4G32570	TIFY8	218	38766	25,5	361	586,7661	1171,518	2	1171,518	-2E-04	293	303	0	63,87		33	8,90E-06	K	LGQMYEGGSSK	E	Oxidation (M)
1	PPD2	mock	AT4G32570	TIFY8	218	38766	25,5	361	646,8146	1291,615	2	1291,616	-9E-04	218	229	0	51,08	36	37	0,00045	K	LESFAPSNTGNR	K	
1	PPD2	mock	AT4G32570	TIFY8	218	38766	25,5	361	708,8299	1415,645	2	1415,646	-0,001	36	48	0	55,83		34	8,10E-05	K	NPTLASTSMADHR	L	Oxidation (M)
1	PPD2	mock	AT4G32570	TIFY8	218	38766	25,5	361	769,8788	1537,743	2	1537,745	-0,002	203	217	0	64,24	28	37	2,30E-05	K	GPGILSSFTMPNSSK	L	Oxidation (M)
1	PPD2	mock	AT4G32570	TIFY8	218	38766	25,5	361	731,0253	2190,054	3	2190,055	-0,001	178	198	1	61,68	35	37	3,90E-05	R	FKDENVGPSVIAQTAADEGSR	T	
1	PPD2	mock	AT1G49670	NQR | ARP protein (REF)	207	68414	10,7	629	431,7609	861,5072	2	861,5072	-1E-04	173	180	0	51,4		34	0,0002	K	AGVVLFTR	S	
1	PPD2	mock	AT1G49670	NQR | ARP protein (REF)	207	68414	10,7	629	475,253	948,4914	2	948,4916	-2E-04	77	85	0	37,82	35	37	0,0092	R	GDLLAAFDK	H	
1	PPD2	mock	AT1G49670	NQR | ARP protein (REF)	207	68414	10,7	629	495,7632	989,5117	2	989,5142	-0,002	43	51	0	59,14		39	9,70E-05	K	GQETTSLVR	E	
1	PPD2	mock	AT1G49670	NQR | ARP protein (REF)	207	68414	10,7	629	561,7875	1121,56	2	1121,56	-1E-04	228	237	0	56,77		37	0,00012	K	GAFELITDEK	K	
1	PPD2	mock	AT1G49670	NQR | ARP protein (REF)	207	68414	10,7	629	616,8387	1231,663	2	1231,664	-6E-04	192	201	0	52,47		37	0,00031	R	INVLCPEFIK	T	
1	PPD2	mock	AT1G49670	NQR | ARP protein (REF)	207	68414	10,7	629	1038,554	2075,093	2	2075,094	-0,001	348	368	0	129,4	34	37	6,00E-12	K	LPFDAGFEGVGLIAAVGESVK	N	
1	PPD2	mock	AT5G13220	JAZ10	173	21800	20,8	197	674,872	1347,729	2	1347,729	0,0009	4	15	0	83,45		36	1,90E-07	K	ATIELDFLGLEK	K	
1	PPD2	mock	AT5G13220	JAZ10	173	21800	20,8	197	726,3513	1450,688	2	1450,69	-0,002	53	67	0	93,32	32	37	2,40E-08	K	SLLASTGNNSDSSAK	S	
1	PPD2	mock	AT5G13220	JAZ10	173	21800	20,8	197	771,9172	1541,82	2	1541,82	-3E-04	158	171	0	65,27		36	1,30E-05	K	LFGQNLEGDLPIAR	R	
1	PPD2	mock	AT4G32295	KIX1	157	27551	19,3	238	398,2761	794,5377	2	794,5378	-1E-04	54	60	0	45,69		20	2,70E-05	K	LPVVVLR	A	
1	PPD2	mock	AT4G32295	KIX1	157	27551	19,3	238	493,7284	985,4423	2	985,4426	-3E-04	61	68	0	40,68		33	0,0018	R	AEEIMYSK	A	Oxidation (M)
1	PPD2	mock	AT4G32295	KIX1	157	27551	19,3	238	544,8266	1087,639	2	1087,639	-3E-04	26	34	0	47,11		33	0,00049	R	GLLIQEIFR	I	
1	PPD2	mock	AT4G32295	KIX1	157	27551	19,3	238	660,7574	1319,5	2	1319,501	-7E-04	69	79	0	74,9		20	3,20E-08	K	ANSEAEYMDMK	T	2 Oxidation (M)
1	PPD2	mock	AT4G32295	KIX1	157	27551	19,3	238	665,3314	1328,648	2	1328,651	-0,003	35	45	0	62,22		36	2,60E-05	R	IVCEIHSQSTR	K	
1	PPD2	mock	AT5G06290	2-cysteine peroxiredoxin B	128	29932	17,2	273	743,4248	1484,835	2	1484,835	-1E-04	189	202	0	49,12		34	0,00035	K	SFGVLIPDQGIALR	G	
1	PPD2	mock	AT5G06290	2-cysteine peroxiredoxin B	128	29932	17,2	273	928,4412	1854,868	2	1854,868	0,0002	86	101	0	95,74		36	1,30E-08	K	APDFEAEAVFDQEFIK	V	
1	PPD2	mock	AT3G24150	KIX2	105	38771	10,5	343	384,2731	766,5316	2	766,5317	-1E-04	54	60	0	21,37		20	0,0073	K	LPVVVLK	A	
1	PPD2	mock	AT3G24150	KIX2	105	38771	10,5	343	493,7284	985,4423	2	985,4426	-3E-04	61	68	0	40,68		33	0,0018	K	AEEIMYSK	A	Oxidation (M)

1	PPD2	mock	AT3G24150	KIX2	105	38771	10,5	343	565,3144	1128,614	2	1128,614	0,0003	85	94	0	71,71		36	3,10E-06	R	VNDAIDTIIR	R	
1	PPD2	mock	AT3G24150	KIX2	105	38771	10,5	343	587,2875	1172,56	2	1172,561	-4E-04	35	45	0	63,98		35	1,40E-05	R	LAMEAHSAATR	K	Oxidation (M)
1	PPD2	mock	AT3G17860	JAZ3	99	38064	8,5	352	418,7294	835,4443	2	835,444	0,0003	4	11	0	41,29		36	0,0036	R	DFLGLGSK	N	
1	PPD2	mock	AT3G17860	JAZ3	99	38064	8,5	352	532,2801	1062,546	2	1062,546	-2E-04	89	98	0	56,32	33	38	0,00015	K	APYSSVQGVR	M	
1	PPD2	mock	AT3G17860	JAZ3	99	38064	8,5	352	596,327	1190,639	2	1190,641	-0,001	88	98	1	56,19	37	37	0,00014	R	KAPYSSVQGVR	M	
1	PPD2	mock	AT3G17860	JAZ3	99	38064	8,5	352	634,8129	1267,611	2	1267,612	-6E-04	320	330	0	49,04	32	37	0,00067	R	VTSVSPYCLDK	K	
1	PPD2	mock	AT5G54770	THI1, TZ, THI4	95	36755	7,4	349	701,9006	1401,787	2	1401,787	0	181	193	0	58,05		35	5,00E-05	K	LFNAVAAEDLIVK	G	
1	PPD2	mock	AT5G54770	THI1, TZ, THI4	95	36755	7,4	349	717,8484	1433,682	2	1433,682	0,0002	259	271	0	72,27		36	2,80E-06	K	ALDMNTAEDAIVR	L	Oxidation (M)
2	PPD2	mock	AT4G14720	PPD2	4295	35056	71,1	315	365,7325	729,4504	2	729,4497	0,0007	237	242	1	45,77		34	0,00077	R	KVSLQR	Y	
2	PPD2	mock	AT4G14720	PPD2	4295	35056	71,1	315	464,2972	926,5798	2	926,58	-2E-04	10	17	1	40,01		28	0,00078	K	SILEKPLK	L	
2	PPD2	mock	AT4G14720	PPD2	4295	35056	71,1	315	469,2457	936,4768	2	936,4772	-4E-04	201	208	1	37,28	33	37	0,0097	R	MISKPMSK	E	Oxidation (M)
2	PPD2	mock	AT4G14720	PPD2	4295	35056	71,1	315	474,7139	947,4133	2	947,4131	0,0003	96	103	0	51,8		32	0,00011	R	NELEACGR	I	
2	PPD2	mock	AT4G14720	PPD2	4295	35056	71,1	315	522,3082	1042,602	2	1042,602	-5E-04	87	95	0	73,51		35	1,70E-06	R	VTTTLIEPR	N	Methyl (DE)
2	PPD2	mock	AT4G14720	PPD2	4295	35056	71,1	315	607,8589	1213,703	2	1213,703	0,0003	49	59	0	90,27		35	3,30E-08	K	SQAIQQVLSLK	A	
2	PPD2	mock	AT4G14720	PPD2	4295	35056	71,1	315	608,8133	1215,612	2	1215,614	-0,002	166	176	0	79,68		37	5,60E-07	K	VNVYDGVPPEK	A	
2	PPD2	mock	AT4G14720	PPD2	4295	35056	71,1	315	406,5721	1216,695	3	1216,693	0,0017	76	86	0	39,05		34	0,0036	K	ILVSQPPNPPR	V	
2	PPD2	mock	AT4G14720	PPD2	4295	35056	71,1	315	653,8395	1305,665	2	1305,664	0,0006	211	221	0	85,38		38	2,10E-07	K	MVELPQYGLEK	A	
2	PPD2	mock	AT4G14720	PPD2	4295	35056	71,1	315	673,4004	1344,786	2	1344,788	-0,001	75	86	1	74,75		30	4,10E-07	R	KILVSQPPNPPR	V	
2	PPD2	mock	AT4G14720	PPD2	4295	35056	71,1	315	705,8243	1409,634	2	1409,636	-0,002	104	115	0	91,31		35	2,70E-08	R	IPLQEDDGACHR	R	
2	PPD2	mock	AT4G14720	PPD2	4295	35056	71,1	315	709,3802	1416,746	2	1416,746	-2E-04	18	29	0	96,43		37	1,40E-08	K	LLTEEDISQLTR	E	
2	PPD2	mock	AT4G14720	PPD2	4295	35056	71,1	315	758,8492	1515,684	2	1515,684	-3E-04	121	135	0	103,1		34	1,40E-09	R	SAEFSGSSGQFVADK	D	
2	PPD2	mock	AT4G14720	PPD2	4295	35056	71,1	315	767,3807	1532,747	2	1532,747	-2E-04	60	74	0	100,2		38	6,30E-09	K	ALYEPGDDSGAGILR	K	
2	PPD2	mock	AT4G14720	PPD2	4295	35056	71,1	315	790,4049	1578,795	2	1578,796	-0,001	209	221	1	66,48		38	1,50E-05	K	EKMVELPQYGLEK	A	Oxidation (M)
2	PPD2	mock	AT4G14720	PPD2	4295	35056	71,1	315	804,906	1607,797	2	1607,798	-3E-04	258	272	0	105,4	30	38	1,90E-09	K	APGVASSSLEMFLNR	Q	Methyl (DE); Oxidation (M)
2	PPD2	mock	AT4G14720	PPD2	4295	35056	71,1	315	831,428	1660,841	2	1660,842	-7E-04	60	75	1	77,1	32	38	1,30E-06	K	ALYEPGDDSGAGILRK	I	
2	PPD2	mock	AT4G14720	PPD2	4295	35056	71,1	315	853,9475	1705,881	2	1705,882	-0,002	257	272	1	127,1		38	1,20E-11	K	KAPGVASSSLEMFLNR	Q	
2	PPD2	mock	AT4G14720	PPD2	4295	35056	71,1	315	999,4619	1996,909	2	1996,913	-0,004	121	139	1	127		36	8,10E-12	R	SAEFSGSSGQFVADKDSHK	T	Methyl (DE)
2	PPD2	mock	AT4G14720	PPD2	4295	35056	71,1	315	1000,493	1998,972	2	1998,972	-4E-04	147	165	0	155,3	35	37	1,70E-14	R	SPAETNAVVGQMTIFYSGK	V	
2	PPD2	mock	AT4G14720	PPD2	4295	35056	71,1	315	1194,094	2386,173	2	2386,174	-0,001	179	200	0	131,9	32	37	3,80E-12	R	SIMHFAANPIDLPENGIFASSR	M	
2	PPD2	mock	AT4G14720	PPD2	4295	35056	71,1	315	1270,542	2539,069	2	2539,07	-0,001	276	298	0	108,4		30	1,50E-10	R	MNAAYSQNLSGTGHCESPENQTK	S	Oxidation (M)
2	PPD2	mock	AT4G28910	NINJA	1972	45081	65,6	425	425,7232	849,4318	2	849,4344	-0,003	380	386	0	54,19		37	0,00023	R	YNANQIK	I	
2	PPD2	mock	AT4G28910	NINJA	1972	45081	65,6	425	448,7455	895,4763	2	895,4763	0	372	379	0	51,97		34	0,00017	R	TISGVTYR	Y	
2	PPD2	mock	AT4G28910	NINJA	1972	45081	65,6	425	550,2854	1098,556	2	1098,557	-7E-04	54	62	0	41,96		35	0,0025	K	NFLHPTSQR	P	
2	PPD2	mock	AT4G28910	NINJA	1972	45081	65,6	425	560,2797	1118,545	2	1118,546	-7E-04	90	100	0	61,58		37	3,90E-05	K	APTTEAEASTK	P	Methyl (DE)
2	PPD2	mock	AT4G28910	NINJA	1972	45081	65,6	425	565,7774	1129,54	2	1129,54	-2E-04	101	109	0	44,56		34	0,0011	K	PLWVEDESR	K	
2	PPD2	mock	AT4G28910	NINJA	1972	45081	65,6	425	572,2422	1142,47	2	1142,47	-4E-04	118	127	0	46,97		28	0,00016	K	FGFPGMNDDK	K	Oxidation (M)
2	PPD2	mock	AT4G28910	NINJA	1972	45081	65,6	425	592,7534	1183,492	2	1183,493	-5E-04	132	141	0	47,98		30	0,00019	K	DSSHVDMHEK	K	
2	PPD2	mock	AT4G28910	NINJA	1972	45081	65,6	425	628,2924	1254,57	2	1254,57	-1E-04	118	128	1	38,24		35	0,0054	K	FGFPGMNDDKK	K	
2	PPD2	mock	AT4G28910	NINJA	1972	45081	65,6	425	721,3222	1440,63	2	1440,63	-5E-04	130	141	1	64,9		33	7,50E-06	K	EKDSSHVDMHEK	K	
2	PPD2	mock	AT4G28910	NINJA	1972	45081	65,6	425	755,3219	1508,629	2	1508,631	-0,002	199	212	0	103,1		30	5,50E-10	R	SNHGGSGTEEFTMR	N	
2	PPD2	mock	AT4G28910	NINJA	1972	45081	65,6	425	802,8552	1603,696	2	1603,696	-4E-04	306	320	0	71,64		32	1,20E-06	K	QPVAEEGSSEDASER	P	Methyl (DE)
2	PPD2	mock	AT4G28910	NINJA	1972	45081	65,6	425	806,3404	1610,666	2	1610,667	-6E-04	24	38	0	82,47		29	5,40E-08	K	GNNNNNAGSSSENYR	A	Methyl (DE)
2	PPD2	mock	AT4G28910	NINJA	1972	45081	65,6	425	809,9124	1617,81	2	1617,811	-8E-04	276	292	0	101,1	33	37	4,80E-09	K	DGSGGIVALSQSPFAGR	V	
2	PPD2	mock	AT4G28910	NINJA	1972	45081	65,6	425	554,9495	1661,827	3	1661,827	-9E-04	356	371	0	82,46	27	38	3,80E-07	R	PNLPWVSTTGSGPHGR	T	
2	PPD2	mock	AT4G28910	NINJA	1972	45081	65,6	425	954,9391	1907,864	2	1907,865	-0,001	73	89	0	99,47		35	3,70E-09	K	SDSGQQPPQNFFNDLSK	A	
2	PPD2	mock	AT4G28910	NINJA	1972	45081	65,6	425	1016,443	2030,872	2	2030,876	-0,004	387	403	0	67,44		32	2,90E-06	K	IVCACHGSHMSPEEFVR	H	Oxidation (M)
2	PPD2	mock	AT4G28910	NINJA	1972	45081	65,6	425	1062,556	2123,097	2	2123,097	-3E-04	179	197	1	99,27	32	37	6,00E-09	K	EVVRPPTDTNIVDNLTGQR	R	
2	PPD2	mock	AT4G28910	NINJA	1972	45081	65,6	425	1109,036	2216,058	2	2216,06	-0,002	90	109	1	145,5	37	37	1,50E-13	K	APTTEAEASTKPLWVEDESR	K	
2	PPD2	mock	AT4G28910	NINJA	1972	45081	65,6	425	771,3186	2310,934	3	2310,937	-0,003	404	425	0	114,1		27	2,20E-11	R	HASEEYVSPESSMGMTAASAHT	-	2 Oxidation (M)
2	PPD2	mock	AT4G28910	NINJA	1972	45081	65,6	425	985,4855	2953,435	3	2953,435	-9E-04	213	238	0	55,95	30	37	0,00013	R	NMSYTVPFTVHPQNVVTSMPYSLPTK	E	Oxidation (M)
2	PPD2	mock	AT4G28910	NINJA	1972	45081	65,6	425	1111,152	3330,434	3	3330,436	-0,002	145	178	0	163,7		30	4,60E-16	K	ASHVSTATDEGSTAENEDVAESEVGGGSSSNHAK	E	Methyl (DE)
2	PPD2	mock	AT4G32570	TIFY8	136	38766	12,5	361	513,7696	1025,525	2	1025,525	-8E-04	335	343	0	39,2		36	0,0052	R	EHQGSIISR	G	
2	PPD2	mock	AT4G32570	TIFY8	136	38766	12,5	361	584,7666	1167,519	2	1167,519	-3E-04	103	113	0	89,9		34	3,00E-08	R	SDVSGSIMSNR	F	Oxidation (M)
2	PPD2	mock	AT4G32570	TIFY8	136	38766	12,5	361	646,8142	1291,614	2	1291,616	-0,002	218	229	0	52,09		37	0,00032	K	LESFAPSNTGNR	K	
2	PPD2	mock	AT4G32570	TIFY8	136	38766	12,5	361	708,8299	1415,645	2	1415,646	-0,001	36	48	0	60,59		34	2,70E-05	K	NPTLASTSMADHR	L	Oxidation (M)
2	PPD2	mock	AT5G13220	JAZ10	126	21800	20,8	197	674,8715	1347,729	2	1347,729	-1E-04	4	15	0	75,01		36	1,30E-06	K	ATIELDFLGLEK	K	
2	PPD2	mock	AT5G13220	JAZ10	126	21800	20,8	197	726,3517	1450,689	2	1450,69	-0,001	53	67	0	73,5	31	37	2,40E-06	K	SLLASTGNNSDSSAK	S	
2	PPD2	mock	AT5G13220	JAZ10	126	21800	20,8	197	771,9176	1541,821	2	1541,82	0,0005	158	171	0	44,67	34	36	0,0015	K	LFGQNLEGDLPIAR	R	
2	PPD2	mock	AT3G24150	KIX2	107	38771	8,5	343	493,7285	985,4424	2	985,4426	-2E-04	61	68	0	33,23		33	0,0099	K	AEEIMYSK	A	Oxidation (M)
2	PPD2	mock	AT3G24150	KIX2	107	38771	8,5	343	565,3143	1128,614	2	1128,614	0,0001	85	94	0	76,03		36	1,20E-06	R	VNDAIDTIIR	R	
2	PPD2	mock	AT3G24150	KIX2	107	38771	8,5	343	587,2884	1172,562	2	1172,561	0,0015	35	45	0	66,87		35	7,00E-06	R	LAMEAHSAATR	K	Oxidation (M)
2	PPD2	mock	AT3G17860	JAZ3	106	38064	8,5	352	418,7297	835,4448	2	835,444	0,0009	4	11	0	38,7		36	0,0065	R	DFLGLGSK	N	
2	PPD2	mock	AT3G17860	JAZ3	106	38064	8,5	352	532,2801	1062,546	2	1062,546	-1E-04	89	98	0	49,17	35	38	0,00079	K	APYSSVQGVR	M	
2	PPD2	mock	AT3G17860	JAZ3	106	38064	8,5	352	596,3271	1190,64	2	1190,641	-0,001	88	98	1	69,5		37	6,40E-06	R	KAPYSSVQGVR	M	
2	PPD2	mock	AT3G17860	JAZ3	106	38064	8,5	352	634,8128	1267,611	2	1267,612	-8E-04	320	330	0	53,9	32	37	0,00021	R	VTSVSPYCLDK	K	
2	PPD2	mock	AT4G32295	KIX1	93	27551	14,7	238	398,2762	794,5378	2	794,5378	0	54	60	0	39,53		20	0,00011	K	LPVVVLR	A	
2	PPD2	mock	AT4G32295	KIX1	93	27551	14,7	238	493,7285	985,4424	2	985,4426	-2E-04	61	68	0	33,23		33	0,0099	R	AEEIMYSK	A	Oxidation (M)
2	PPD2	mock	AT4G32295	KIX1	93	27551	14,7	238	544,8266	1087,639	2	1087,639	-3E-04	26	34	0	47,48		33	0,00045	R	GLLIQEIFR	I	

2	PPD2	mock	AT4G32295	KIX1	93	27551	14,7	238	665,3319	1328,649	2	1328,651	-0,001	35	45	0	64,02		36	1,80E-05	R	IVCEIHSQSTR	K	
2	PPD2	mock	AT1G49670	NQR | ARP protein (REF)	86	68414	7,3	629	431,7609	861,5072	2	861,5072	0	173	180	0	52,76		34	0,00014	K	AGVVLFTR	S	
2	PPD2	mock	AT1G49670	NQR | ARP protein (REF)	86	68414	7,3	629	475,2529	948,4913	2	948,4916	-4E-04	77	85	0	39,1	36	37	0,0071	R	GDLLAAFDK	H	
2	PPD2	mock	AT1G49670	NQR | ARP protein (REF)	86	68414	7,3	629	495,7627	989,5109	2	989,5142	-0,003	43	51	0	47,39		38	0,0014	K	GQETTSLVR	E	
2	PPD2	mock	AT1G49670	NQR | ARP protein (REF)	86	68414	7,3	629	561,7873	1121,56	2	1121,56	-3E-04	228	237	0	51,58		37	0,00039	K	GAFELITDEK	K	
2	PPD2	mock	AT1G49670	NQR | ARP protein (REF)	86	68414	7,3	629	616,8389	1231,663	2	1231,664	-2E-04	192	201	0	40,7		37	0,0048	R	INVLCPEFIK	T	
1	JAZ10	mock	AT4G28910	NINJA	1406	45081	55,5	425	394,702	787,3895	2	787,3898	-3E-04	341	348	0	49,81		34	0,00032	K	PGMAADVK	F	
1	JAZ10	mock	AT4G28910	NINJA	1406	45081	55,5	425	448,7453	895,476	2	895,4763	-3E-04	372	379	0	39,23		34	0,0032	R	TISGVTYR	Y	
1	JAZ10	mock	AT4G28910	NINJA	1406	45081	55,5	425	550,2855	1098,557	2	1098,557	-5E-04	54	62	0	39,66		35	0,0043	K	NFLHPTSQR	P	
1	JAZ10	mock	AT4G28910	NINJA	1406	45081	55,5	425	560,2795	1118,545	2	1118,546	-0,001	90	100	0	63,11		37	2,80E-05	K	APTTEAEASTK	P	Methyl (DE)
1	JAZ10	mock	AT4G28910	NINJA	1406	45081	55,5	425	564,2449	1126,475	2	1126,475	-1E-04	118	127	0	52,37		31	7,90E-05	K	FGFPGMNDDK	K	
1	JAZ10	mock	AT4G28910	NINJA	1406	45081	55,5	425	565,7777	1129,541	2	1129,54	0,0004	101	109	0	43,75		35	0,0015	K	PLWVEDESR	K	
1	JAZ10	mock	AT4G28910	NINJA	1406	45081	55,5	425	592,7535	1183,492	2	1183,493	-4E-04	132	141	0	45,14		30	0,00037	K	DSSHVDMHEK	K	
1	JAZ10	mock	AT4G28910	NINJA	1406	45081	55,5	425	721,3222	1440,63	2	1440,63	-5E-04	130	141	1	82,15		33	1,40E-07	K	EKDSSHVDMHEK	K	
1	JAZ10	mock	AT4G28910	NINJA	1406	45081	55,5	425	755,3223	1508,63	2	1508,631	-0,001	199	212	0	100,3		30	1,00E-09	R	SNHGGSGTEEFTMR	N	
1	JAZ10	mock	AT4G28910	NINJA	1406	45081	55,5	425	802,8537	1603,693	2	1603,696	-0,003	306	320	0	88,74		32	2,30E-08	K	QPVAEEGSSEDASER	P	Methyl (DE)
1	JAZ10	mock	AT4G28910	NINJA	1406	45081	55,5	425	806,3402	1610,666	2	1610,667	-0,001	24	38	0	77,16		29	1,80E-07	K	GNNNNNAGSSSENYR	A	Methyl (DE)
1	JAZ10	mock	AT4G28910	NINJA	1406	45081	55,5	425	809,9125	1617,81	2	1617,811	-6E-04	276	292	0	121,1		38	4,90E-11	K	DGSGGIVALSQSPFAGR	V	
1	JAZ10	mock	AT4G28910	NINJA	1406	45081	55,5	425	831,9183	1661,822	2	1661,827	-0,005	356	371	0	45,76	22	38	0,0018	R	PNLPWVSTTGSGPHGR	T	
1	JAZ10	mock	AT4G28910	NINJA	1406	45081	55,5	425	954,9392	1907,864	2	1907,865	-0,001	73	89	0	95,41		35	9,40E-09	R	SDSGQQPPQNFFNDLSK	A	
1	JAZ10	mock	AT4G28910	NINJA	1406	45081	55,5	425	1062,556	2123,097	2	2123,097	-3E-04	179	197	1	96,15	30	37	1,20E-08	K	EVVRPPTDTNIVDNLTGQR	R	
1	JAZ10	mock	AT4G28910	NINJA	1406	45081	55,5	425	1156,475	2310,935	2	2310,937	-0,002	404	425	0	111,1	27	27	4,70E-11	R	HASEEYVSPESSMGMTAASAHT	-	2 Oxidation (M)
1	JAZ10	mock	AT4G28910	NINJA	1406	45081	55,5	425	1111,152	3330,433	3	3330,436	-0,003	145	178	0	162,4		30	6,00E-16	K	ASHVSTATDEGSTAENEDVAESEVGGGSSSNHAK	E	Methyl (DE)
1	JAZ10	mock	AT5G13220	JAZ10	1229	21800	50,3	197	416,2323	830,45	2	830,4498	0,0002	38	45	0	47,64		39	0,0014	R	DIQGAISK	I	
1	JAZ10	mock	AT5G13220	JAZ10	1229	21800	50,3	197	643,3184	1284,622	2	1284,624	-0,002	186	197	0	66,56		36	9,40E-06	R	LVSTSPYYPTSA	-	
1	JAZ10	mock	AT5G13220	JAZ10	1229	21800	50,3	197	674,8715	1347,728	2	1347,729	-2E-04	4	15	0	95,45		36	1,20E-08	K	ATIELDFLGLEK	K	
1	JAZ10	mock	AT5G13220	JAZ10	1229	21800	50,3	197	726,3504	1450,686	2	1450,69	-0,004	53	67	0	107,5		37	9,30E-10	K	SLLASTGNNSDSSAK	S	
1	JAZ10	mock	AT5G13220	JAZ10	1229	21800	50,3	197	778,9251	1555,836	2	1555,836	-2E-04	158	171	0	107,8		36	7,00E-10	K	LFGQNLEGDLPIAR	R	Methyl (DE)
1	JAZ10	mock	AT5G13220	JAZ10	1229	21800	50,3	197	929,4938	1856,973	2	1856,974	-0,001	77	93	0	95,36		36	1,40E-08	R	EDQPQIPISPVHASLAR	S	
1	JAZ10	mock	AT5G13220	JAZ10	1229	21800	50,3	197	1076,046	2150,077	2	2150,078	-8E-04	137	155	1	89,04	34	37	7,20E-08	K	KDESSMETDLSVILPTTLR	P	Oxidation (M)
1	JAZ10	mock	AT5G13220	JAZ10	1229	21800	50,3	197	1124,572	2247,129	2	2247,13	-0,002	138	157	1	96,33	32	37	1,40E-08	K	DESSMETDLSVILPTTLRPK	L	Oxidation (M)
1	JAZ10	mock	AT5G13530	KEG	914	180725	17	1625	376,1903	750,3661	2	750,366	0,0001	249	255	0	57,09		36	8,00E-05	R	YGADVAR	G	
1	JAZ10	mock	AT5G13530	KEG	914	180725	17	1625	405,7633	809,5121	2	809,5123	-2E-04	392	399	0	40,65		23	0,00022	R	IVGVGIPR	E	
1	JAZ10	mock	AT5G13530	KEG	914	180725	17	1625	436,2661	870,5176	2	870,5175	0,0002	916	923	0	49,02		33	0,00028	R	VLANEVVK	L	
1	JAZ10	mock	AT5G13530	KEG	914	180725	17	1625	459,7023	917,3901	2	917,3913	-0,001	979	986	0	47,71		29	0,00014	K	ADPAEMER	V	
1	JAZ10	mock	AT5G13530	KEG	914	180725	17	1625	465,2379	928,4613	2	928,4614	-1E-04	819	827	0	52,35		34	0,00016	R	SPDAAVDVR	N	
1	JAZ10	mock	AT5G13530	KEG	914	180725	17	1625	480,7611	959,5077	2	959,5076	0,0001	836	843	0	44,69		38	0,0025	R	DFLEALPR	E	
1	JAZ10	mock	AT5G13530	KEG	914	180725	17	1625	493,3056	984,5966	2	984,5968	-2E-04	241	248	0	72,19		33	1,40E-06	R	LTLEQILR	Y	
1	JAZ10	mock	AT5G13530	KEG	914	180725	17	1625	499,8029	997,5913	2	997,592	-7E-04	1568	1576	1	53,6		30	5,00E-05	K	IKDGLVTPR	W	
1	JAZ10	mock	AT5G13530	KEG	914	180725	17	1625	526,7774	1051,54	2	1051,541	-8E-04	1588	1598	0	62,61		36	2,70E-05	K	GHVVGVDANGK	L	
1	JAZ10	mock	AT5G13530	KEG	914	180725	17	1625	531,7951	1061,576	2	1061,576	0	373	381	0	47,94		36	0,00077	R	EEIFQAVVK	A	
1	JAZ10	mock	AT5G13530	KEG	914	180725	17	1625	545,2957	1088,577	2	1088,577	0,0002	1308	1316	0	56,41	33	38	0,00015	K	VGQFVHFQK	G	
1	JAZ10	mock	AT5G13530	KEG	914	180725	17	1625	572,2847	1142,555	2	1142,557	-0,002	436	447	0	57,19		35	7,00E-05	R	SPSASPDNGIAK	I	
1	JAZ10	mock	AT5G13530	KEG	914	180725	17	1625	580,7984	1159,582	2	1159,581	0,0014	205	214	0	53,49		38	0,00029	R	NVCTFHGVVK	M	
1	JAZ10	mock	AT5G13530	KEG	914	180725	17	1625	610,3193	1218,624	2	1218,625	-5E-04	475	485	0	61,28		38	5,90E-05	R	VVLEGDFEGVR	N	
1	JAZ10	mock	AT5G13530	KEG	914	180725	17	1625	610,8477	1219,681	2	1219,681	-5E-04	892	902	0	51,02		34	0,00022	K	SVGFVQTILEK	E	
1	JAZ10	mock	AT5G13530	KEG	914	180725	17	1625	638,273	1274,532	2	1274,532	-4E-04	7	16	0	52,29		29	5,00E-05	K	VPCCSVCHTR	Y	
1	JAZ10	mock	AT5G13530	KEG	914	180725	17	1625	649,8474	1297,68	2	1297,681	-9E-04	448	458	0	77,8		35	6,20E-07	K	ICEVNIVQAPR	A	
1	JAZ10	mock	AT5G13530	KEG	914	180725	17	1625	663,7958	1325,577	2	1325,578	-7E-04	215	225	0	74,55		33	8,40E-07	K	MDGSLCLLMDR	C	Oxidation (M)
1	JAZ10	mock	AT5G13530	KEG	914	180725	17	1625	684,3671	1366,72	2	1366,721	-8E-04	1334	1346	0	74,1		36	1,90E-06	R	GIITTVHADGEVR	V	
1	JAZ10	mock	AT5G13530	KEG	914	180725	17	1625	740,3579	1478,701	2	1478,704	-0,002	1212	1225	0	93,89		37	2,20E-08	R	IDMDGTLSAQVTGR	Q	Oxidation (M)
1	JAZ10	mock	AT5G13530	KEG	914	180725	17	1625	764,8914	1527,768	2	1527,769	-9E-04	23	35	0	64,73	35	37	1,90E-05	R	VPLLLQCGHGFCK	D	
1	JAZ10	mock	AT5G13530	KEG	914	180725	17	1625	810,3562	1618,698	2	1618,697	0,0011	41	54	0	82,69		32	8,60E-08	K	MFSTSSDTTLTCPR	C	Oxidation (M)
1	JAZ10	mock	AT5G13530	KEG	914	180725	17	1625	834,4064	1666,798	2	1666,799	-2E-04	625	639	0	69,75	36	37	6,10E-06	K	YTDCAIVILENGGSR	S	
1	JAZ10	mock	AT5G13530	KEG	914	180725	17	1625	905,453	1808,891	2	1808,892	-4E-04	459	474	0	109,5	36	38	7,50E-10	R	ATNIGVFQDNPNNLHR	V	
1	JAZ10	mock	AT5G13530	KEG	914	180725	17	1625	1160,574	2319,134	2	2319,134	-6E-04	598	619	0	146,9	33	37	1,30E-13	R	ELLVAGADPNAVDDEGETVLHR	A	
1	JAZ10	mock	AT5G46760	MYC3	728	65294	26,4	592	369,6968	737,3791	2	737,3782	0,001	539	544	0	36,53		35	0,0071	R	FMEALK	E	
1	JAZ10	mock	AT5G46760	MYC3	728	65294	26,4	592	475,7335	949,4525	2	949,4539	-0,001	316	323	0	57,93		38	0,0001	R	QSSCLVEK	D	
1	JAZ10	mock	AT5G46760	MYC3	728	65294	26,4	592	517,785	1033,556	2	1033,556	-2E-04	192	201	0	59,67		37	5,90E-05	R	AGQGQIYGLK	T	
1	JAZ10	mock	AT5G46760	MYC3	728	65294	26,4	592	546,3082	1090,602	2	1090,602	-3E-04	324	333	0	72,75		35	2,10E-06	K	DLTFQGGLLK	S	
1	JAZ10	mock	AT5G46760	MYC3	728	65294	26,4	592	555,8055	1109,596	2	1109,597	-4E-04	388	397	0	51,99		35	0,00021	K	EAIVVEPPEK	K	
1	JAZ10	mock	AT5G46760	MYC3	728	65294	26,4	592	645,3071	1288,6	2	1288,601	-0,001	305	315	0	82,2		35	2,10E-07	K	NDISSVENQNR	Q	Methyl (DE)
1	JAZ10	mock	AT5G46760	MYC3	728	65294	26,4	592	661,8203	1321,626	2	1321,626	-2E-04	411	421	0	67,78		37	9,10E-06	R	EEPLNHVEAER	Q	
1	JAZ10	mock	AT5G46760	MYC3	728	65294	26,4	592	726,3397	1450,665	2	1450,666	-0,001	570	581	0	64		35	1,50E-05	K	MGSQFFNHDQLK	V	
1	JAZ10	mock	AT5G46760	MYC3	728	65294	26,4	592	773,3911	1544,768	2	1544,768	-5E-04	464	476	1	84,97		37	1,90E-07	K	LQQAESDKEEIQK	K	
1	JAZ10	mock	AT5G46760	MYC3	728	65294	26,4	592	780,335	1558,655	2	1558,657	-0,002	334	347	0	66,77		30	2,40E-06	K	SNETLSFCGNESSK	K	
1	JAZ10	mock	AT5G46760	MYC3	728	65294	26,4	592	864,8962	1727,778	2	1727,779	-7E-04	355	370	0	121		35	2,60E-11	K	GSNNDEGMLSFSTVVR	S	Oxidation (M)
1	JAZ10	mock	AT5G46760	MYC3	728	65294	26,4	592	872,9022	1743,79	2	1743,791	-0,001	371	387	0	97,81		35	5,50E-09	R	SAANDSDHSDLEASVVK	E	

1	JAZ10	mock	AT5G46760	MYC3	728	65294	26,4	592	978,9383	1955,862	2	1955,863	-9E-04	500	517	0	103,6		34	1,10E-09	K	SSNQDSTASSIEMEIDVK	I	Oxidation (M)
1	JAZ10	mock	AT1G32640	MYC2	593	68136	24,2	623	430,2295	858,4444	2	858,4447	-2E-04	513	519	0	38,19		36	0,0065	K	NQLEEVK	L	
1	JAZ10	mock	AT1G32640	MYC2	593	68136	24,2	623	443,2143	884,414	2	884,4141	0	341	347	0	48,37		33	0,00033	K	FNNTFSR	E	
1	JAZ10	mock	AT1G32640	MYC2	593	68136	24,2	623	452,7401	903,4656	2	903,4661	-6E-04	240	247	0	50,02		39	0,00082	R	QSSDLINK	V	
1	JAZ10	mock	AT1G32640	MYC2	593	68136	24,2	623	525,7815	1049,548	2	1049,551	-0,002	608	615	0	50,62		38	0,00056	R	IYTQEQLR	A	
1	JAZ10	mock	AT1G32640	MYC2	593	68136	24,2	623	633,3035	1264,593	2	1264,594	-0,001	363	374	0	81,84		37	3,40E-07	R	SGEILNFGDEGK	R	
1	JAZ10	mock	AT1G32640	MYC2	593	68136	24,2	623	661,8203	1321,626	2	1321,626	-2E-04	448	458	0	67,78		37	9,10E-06	R	EEPLNHVEAER	Q	
1	JAZ10	mock	AT1G32640	MYC2	593	68136	24,2	623	706,8663	1411,718	2	1411,72	-0,001	348	360	0	68,89		36	6,10E-06	R	ELNFSTSSSTLVK	P	
1	JAZ10	mock	AT1G32640	MYC2	593	68136	24,2	623	822,8882	1643,762	2	1643,764	-0,002	413	428	0	104,7		36	1,30E-09	K	TAGESDHSDLEASVVK	E	
1	JAZ10	mock	AT1G32640	MYC2	593	68136	24,2	623	833,4439	1664,873	2	1664,873	-1E-04	348	362	1	102,7		36	2,60E-09	R	ELNFSTSSSTLVKPR	S	
1	JAZ10	mock	AT1G32640	MYC2	593	68136	24,2	623	834,883	1667,751	2	1667,743	0,0086	121	135	0	61,94		35	2,30E-05	R	SSSPPFSTPADQEYR	K	
1	JAZ10	mock	AT1G32640	MYC2	593	68136	24,2	623	1015,439	2028,862	2	2028,866	-0,004	376	394	0	132,6		30	6,50E-13	R	SSGNPDPSSYSGQTQFENK	R	
1	JAZ10	mock	AT1G32640	MYC2	593	68136	24,2	623	1184,568	3550,681	3	3550,681	-5E-04	308	340	0	116,3		35	9,20E-11	K	SIQFENGSSSTITENPNLDPTPSPVHSQTQNPK	F	
1	JAZ10	mock	AT4G17880	MYC4	453	65005	12,9	589	369,6968	737,3791	2	737,3782	0,001	536	541	0	36,53		35	0,0071	K	FMEALK	E	
1	JAZ10	mock	AT4G17880	MYC4	453	65005	12,9	589	515,72	1029,425	2	1029,426	-5E-04	330	337	0	39,07		28	0,00092	K	SCEMVNFK	N	Oxidation (M)
1	JAZ10	mock	AT4G17880	MYC4	453	65005	12,9	589	648,7998	1295,585	2	1295,585	-4E-04	122	133	0	91,82		35	2,20E-08	K	SNPASAAEQEHR	K	
1	JAZ10	mock	AT4G17880	MYC4	453	65005	12,9	589	661,8203	1321,626	2	1321,626	-2E-04	412	422	0	67,78		37	9,10E-06	R	EEPLNHVEAER	Q	
1	JAZ10	mock	AT4G17880	MYC4	453	65005	12,9	589	708,3319	1414,649	2	1414,651	-0,002	314	326	0	71,15		35	2,60E-06	K	LCNGSSVENPNPK	V	
1	JAZ10	mock	AT4G17880	MYC4	453	65005	12,9	589	728,8458	1455,677	2	1455,682	-0,005	567	578	0	75,67		36	1,20E-06	K	MGNQFFTQDQLK	V	
1	JAZ10	mock	AT4G17880	MYC4	453	65005	12,9	589	767,8345	1533,654	2	1533,654	0,0001	338	351	0	78,91		31	1,70E-07	K	NGIENGQEEDSSNK	K	Methyl (DE)
1	JAZ10	mock	AT4G14720	PPD2	381	35056	41,3	315	464,2968	926,579	2	926,58	-0,001	10	17	1	29,77		29	0,0084	K	SILEKPLK	L	
1	JAZ10	mock	AT4G14720	PPD2	381	35056	41,3	315	515,3008	1028,587	2	1028,587	0,0005	87	95	0	59,09		35	4,70E-05	R	VTTTLIEPR	N	
1	JAZ10	mock	AT4G14720	PPD2	381	35056	41,3	315	607,8587	1213,703	2	1213,703	-3E-04	49	59	0	69,93		35	3,50E-06	K	SQAIQQVLSLK	A	
1	JAZ10	mock	AT4G14720	PPD2	381	35056	41,3	315	608,8136	1215,613	2	1215,614	-8E-04	166	176	0	49,42		37	0,00061	K	VNVYDGVPPEK	A	
1	JAZ10	mock	AT4G14720	PPD2	381	35056	41,3	315	609,3533	1216,692	2	1216,693	-8E-04	76	86	0	40,13		34	0,003	K	ILVSQPPNPPR	V	
1	JAZ10	mock	AT4G14720	PPD2	381	35056	41,3	315	661,8365	1321,658	2	1321,659	-4E-04	211	221	0	52,67		38	0,00039	K	MVELPQYGLEK	A	Oxidation (M)
1	JAZ10	mock	AT4G14720	PPD2	381	35056	41,3	315	705,8245	1409,635	2	1409,636	-0,001	104	115	0	56,52		35	8,20E-05	R	IPLQEDDGACHR	R	
1	JAZ10	mock	AT4G14720	PPD2	381	35056	41,3	315	709,3802	1416,746	2	1416,746	-1E-04	18	29	0	105,9		37	1,60E-09	K	LLTEEDISQLTR	E	
1	JAZ10	mock	AT4G14720	PPD2	381	35056	41,3	315	758,849	1515,684	2	1515,684	-7E-04	121	135	0	107,4		34	5,00E-10	R	SAEFSGSSGQFVADK	D	
1	JAZ10	mock	AT4G14720	PPD2	381	35056	41,3	315	767,3808	1532,747	2	1532,747	0	60	74	0	57,69		38	0,00012	K	ALYEPGDDSGAGILR	K	
1	JAZ10	mock	AT4G14720	PPD2	381	35056	41,3	315	797,8982	1593,782	2	1593,782	-3E-04	258	272	0	75,15	25	38	2,10E-06	K	APGVASSSLEMFLNR	Q	Oxidation (M)
1	JAZ10	mock	AT5G20900	JAZ12	315	20035	36,4	187	379,2319	756,4492	2	756,4494	-1E-04	77	82	0	41,88		33	0,0013	K	VQEILR	I	
1	JAZ10	mock	AT5G20900	JAZ12	315	20035	36,4	187	534,7531	1067,492	2	1067,492	-7E-04	161	169	0	38,75		35	0,0049	K	NPYPTSDFK	K	
1	JAZ10	mock	AT5G20900	JAZ12	315	20035	36,4	187	765,4008	1528,787	2	1528,785	0,0026	92	106	0	83,35	36	37	2,60E-07	K	NSTSISPVSSPALNR	A	
1	JAZ10	mock	AT5G20900	JAZ12	315	20035	36,4	187	1204,036	2406,058	2	2406,061	-0,002	10	36	0	157,3		34	5,10E-15	R	ASVEGGCGVADGDGGAAEIGGTGSVEK	S	
1	JAZ10	mock	AT3G17860	JAZ3	101	38064	3,1	352	532,2803	1062,546	2	1062,546	0,0002	89	98	0	62,5	34	38	3,70E-05	K	APYSSVQGVR	M	
1	JAZ10	mock	AT3G17860	JAZ3	101	38064	3,1	352	596,327	1190,64	2	1190,641	-0,001	88	98	1	73,78		37	2,40E-06	R	KAPYSSVQGVR	M	
2	JAZ10	mock	AT4G28910	NINJA	1569	45081	61,2	425	394,702	787,3895	2	787,3898	-3E-04	341	348	0	47,21		34	0,00058	K	PGMAADVK	F	
2	JAZ10	mock	AT4G28910	NINJA	1569	45081	61,2	425	425,7244	849,4342	2	849,4344	-3E-04	380	386	0	43,38		38	0,0031	R	YNANQIK	I	
2	JAZ10	mock	AT4G28910	NINJA	1569	45081	61,2	425	448,7454	895,4762	2	895,4763	-1E-04	372	379	0	55,4		34	7,70E-05	R	TISGVTYR	Y	
2	JAZ10	mock	AT4G28910	NINJA	1569	45081	61,2	425	550,2855	1098,556	2	1098,557	-7E-04	54	62	0	36,2		35	0,0095	K	NFLHPTSQR	P	
2	JAZ10	mock	AT4G28910	NINJA	1569	45081	61,2	425	560,2796	1118,545	2	1118,546	-8E-04	90	100	0	70,17		37	5,40E-06	K	APTTEAEASTK	P	Methyl (DE)
2	JAZ10	mock	AT4G28910	NINJA	1569	45081	61,2	425	564,2448	1126,475	2	1126,475	-3E-04	118	127	0	49,35		31	0,00016	K	FGFPGMNDDK	K	
2	JAZ10	mock	AT4G28910	NINJA	1569	45081	61,2	425	565,7771	1129,54	2	1129,54	-7E-04	101	109	0	35,38		34	0,0089	K	PLWVEDESR	K	
2	JAZ10	mock	AT4G28910	NINJA	1569	45081	61,2	425	721,3224	1440,63	2	1440,63	-1E-04	130	141	1	78,85		33	3,00E-07	K	EKDSSHVDMHEK	K	
2	JAZ10	mock	AT4G28910	NINJA	1569	45081	61,2	425	755,3227	1508,631	2	1508,631	-6E-04	199	212	0	93,75		30	4,80E-09	R	SNHGGSGTEEFTMR	N	
2	JAZ10	mock	AT4G28910	NINJA	1569	45081	61,2	425	795,8473	1589,68	2	1589,681	-5E-04	306	320	0	79,15		31	1,70E-07	K	QPVAEEGSSEDASER	P	
2	JAZ10	mock	AT4G28910	NINJA	1569	45081	61,2	425	806,3407	1610,667	2	1610,667	-1E-04	24	38	0	93,12		29	4,80E-09	K	GNNNNNAGSSSENYR	A	Methyl (DE)
2	JAZ10	mock	AT4G28910	NINJA	1569	45081	61,2	425	809,9127	1617,811	2	1617,811	-3E-04	276	292	0	129	37	38	8,00E-12	K	DGSGGIVALSQSPFAGR	V	
2	JAZ10	mock	AT4G28910	NINJA	1569	45081	61,2	425	831,9204	1661,826	2	1661,827	-0,001	356	371	0	80,71	27	38	5,70E-07	R	PNLPWVSTTGSGPHGR	T	
2	JAZ10	mock	AT4G28910	NINJA	1569	45081	61,2	425	954,9396	1907,865	2	1907,865	-3E-04	73	89	0	106,7		35	7,40E-10	R	SDSGQQPPQNFFNDLSK	A	
2	JAZ10	mock	AT4G28910	NINJA	1569	45081	61,2	425	677,966	2030,876	3	2030,876	0	387	403	0	36,3	32	32	0,0043	K	IVCACHGSHMSPEEFVR	H	Oxidation (M)
2	JAZ10	mock	AT4G28910	NINJA	1569	45081	61,2	425	1062,556	2123,097	2	2123,097	0,0001	179	197	1	87,05	33	37	1,00E-07	K	EVVRPPTDTNIVDNLTGQR	R	
2	JAZ10	mock	AT4G28910	NINJA	1569	45081	61,2	425	1140,482	2278,95	2	2278,947	0,003	404	425	0	113,7		29	3,70E-11	R	HASEEYVSPESSMGMTAASAHT	-	
2	JAZ10	mock	AT4G28910	NINJA	1569	45081	61,2	425	1106,48	3316,417	3	3316,42	-0,003	145	178	0	143,6		29	4,00E-14	K	ASHVSTATDEGSTAENEDVAESEVGGGSSSNHAK	E	
2	JAZ10	mock	AT5G13530	KEG	1153	180725	20,8	1625	376,1902	750,3658	2	750,366	-2E-04	249	255	0	52,62		35	0,00022	R	YGADVAR	G	
2	JAZ10	mock	AT5G13530	KEG	1153	180725	20,8	1625	386,258	770,5015	2	770,5014	0,0001	1432	1438	1	36,74		33	0,0043	K	KLVVGQK	T	
2	JAZ10	mock	AT5G13530	KEG	1153	180725	20,8	1625	405,7634	809,5122	2	809,5123	-2E-04	392	399	0	48,87		23	3,20E-05	R	IVGVGIPR	E	
2	JAZ10	mock	AT5G13530	KEG	1153	180725	20,8	1625	436,2661	870,5176	2	870,5175	0,0001	916	923	0	49,09		33	0,00027	R	VLANEVVK	L	
2	JAZ10	mock	AT5G13530	KEG	1153	180725	20,8	1625	459,7022	917,3899	2	917,3913	-0,001	979	986	0	42,69		28	0,00042	K	ADPAEMER	V	
2	JAZ10	mock	AT5G13530	KEG	1153	180725	20,8	1625	465,2379	928,4612	2	928,4614	-2E-04	819	827	0	54,35		34	9,90E-05	R	SPDAAVDVR	N	
2	JAZ10	mock	AT5G13530	KEG	1153	180725	20,8	1625	480,761	959,5075	2	959,5076	-1E-04	836	843	0	48,16		38	0,0011	R	DFLEALPR	E	
2	JAZ10	mock	AT5G13530	KEG	1153	180725	20,8	1625	493,3056	984,5967	2	984,5968	0	241	248	0	69,67		33	2,40E-06	R	LTLEQILR	Y	
2	JAZ10	mock	AT5G13530	KEG	1153	180725	20,8	1625	499,8029	997,5912	2	997,592	-8E-04	1568	1576	1	65,55		30	3,20E-06	K	IKDGLVTPR	W	
2	JAZ10	mock	AT5G13530	KEG	1153	180725	20,8	1625	526,7777	1051,541	2	1051,541	-2E-04	1588	1598	0	65,73		36	1,10E-05	K	GHVVGVDANGK	L	
2	JAZ10	mock	AT5G13530	KEG	1153	180725	20,8	1625	531,7951	1061,576	2	1061,576	0	373	381	0	45,86		36	0,0012	R	EEIFQAVVK	A	
2	JAZ10	mock	AT5G13530	KEG	1153	180725	20,8	1625	533,7613	1065,508	2	1065,509	-0,001	302	310	1	52,9		37	0,00027	K	TRPEFDSSK	V	
2	JAZ10	mock	AT5G13530	KEG	1153	180725	20,8	1625	545,2957	1088,577	2	1088,577	0,0001	1308	1316	0	54,37	33	38	0,00024	K	VGQFVHFQK	G	

2	JAZ10	mock	AT5G13530	KEG	1153	180725	20,8	1625	572,2852	1142,556	2	1142,557	-0,001	436	447	0	73,78		35	1,40E-06	R	SPSASPDNGIAK	I	
2	JAZ10	mock	AT5G13530	KEG	1153	180725	20,8	1625	580,798	1159,581	2	1159,581	0,0005	205	214	0	47,73	36	38	0,0012	R	NVCTFHGVVK	M	
2	JAZ10	mock	AT5G13530	KEG	1153	180725	20,8	1625	610,3195	1218,624	2	1218,625	-1E-04	475	485	0	59,76		39	8,40E-05	R	VVLEGDFEGVR	N	
2	JAZ10	mock	AT5G13530	KEG	1153	180725	20,8	1625	610,8477	1219,681	2	1219,681	-5E-04	892	902	0	53,16		34	0,00013	K	SVGFVQTILEK	E	
2	JAZ10	mock	AT5G13530	KEG	1153	180725	20,8	1625	638,2728	1274,531	2	1274,532	-8E-04	7	16	0	58,39		29	1,20E-05	K	VPCCSVCHTR	Y	
2	JAZ10	mock	AT5G13530	KEG	1153	180725	20,8	1625	649,8475	1297,68	2	1297,681	-8E-04	448	458	0	79,25		35	4,50E-07	K	ICEVNIVQAPR	A	
2	JAZ10	mock	AT5G13530	KEG	1153	180725	20,8	1625	663,7959	1325,577	2	1325,578	-5E-04	215	225	0	60,05		33	2,30E-05	K	MDGSLCLLMDR	C	Oxidation (M)
2	JAZ10	mock	AT5G13530	KEG	1153	180725	20,8	1625	684,367	1366,72	2	1366,721	-0,001	1334	1346	0	69,49		36	5,50E-06	R	GIITTVHADGEVR	V	
2	JAZ10	mock	AT5G13530	KEG	1153	180725	20,8	1625	719,3651	1436,716	2	1436,716	0	404	415	1	60,85	28	38	5,40E-05	K	MIGECLQFKPSK	R	
2	JAZ10	mock	AT5G13530	KEG	1153	180725	20,8	1625	740,3583	1478,702	2	1478,704	-0,002	1212	1225	0	116,6		37	1,20E-10	R	IDMDGTLSAQVTGR	Q	Oxidation (M)
2	JAZ10	mock	AT5G13530	KEG	1153	180725	20,8	1625	742,358	1482,702	2	1482,703	-0,001	1163	1175	1	63,42	33	37	2,40E-05	R	SKPFSCSVTDVEK	V	
2	JAZ10	mock	AT5G13530	KEG	1153	180725	20,8	1625	764,8914	1527,768	2	1527,769	-8E-04	23	35	0	67,96	34	37	8,80E-06	R	VPLLLQCGHGFCK	D	
2	JAZ10	mock	AT5G13530	KEG	1153	180725	20,8	1625	810,3554	1618,696	2	1618,697	-6E-04	41	54	0	85,63		31	4,20E-08	K	MFSTSSDTTLTCPR	C	Oxidation (M)
2	JAZ10	mock	AT5G13530	KEG	1153	180725	20,8	1625	863,949	1725,883	2	1725,883	0,0002	728	743	0	68,87		37	6,90E-06	R	TALHTAAMANNVELVR	V	Oxidation (M)
2	JAZ10	mock	AT5G13530	KEG	1153	180725	20,8	1625	905,4528	1808,891	2	1808,892	-8E-04	459	474	0	106,1	32	38	1,70E-09	R	ATNIGVFQDNPNNLHR	V	
2	JAZ10	mock	AT5G13530	KEG	1153	180725	20,8	1625	1027,506	2052,998	2	2053,001	-0,003	502	520	0	129		37	7,80E-12	R	SLLEAQNADGQSALHLACR	R	
2	JAZ10	mock	AT5G13530	KEG	1153	180725	20,8	1625	1160,574	2319,133	2	2319,134	-0,001	598	619	0	160,8		38	5,30E-15	R	ELLVAGADPNAVDDEGETVLHR	A	
2	JAZ10	mock	AT5G13220	JAZ10	1053	21800	53,3	197	414,2479	826,4812	2	826,48	0,0012	46	52	0	36,26		31	0,0035	K	IDPEIIK	S	
2	JAZ10	mock	AT5G13220	JAZ10	1053	21800	53,3	197	416,2323	830,45	2	830,4498	0,0002	38	45	0	47,6		39	0,0015	R	DIQGAISK	I	
2	JAZ10	mock	AT5G13220	JAZ10	1053	21800	53,3	197	643,3182	1284,622	2	1284,624	-0,002	186	197	0	67,78		35	6,60E-06	R	LVSTSPYYPTSA	-	
2	JAZ10	mock	AT5G13220	JAZ10	1053	21800	53,3	197	674,8713	1347,728	2	1347,729	-5E-04	4	15	0	91,05		37	4,40E-08	K	ATIELDFLGLEK	K	
2	JAZ10	mock	AT5G13220	JAZ10	1053	21800	53,3	197	726,3516	1450,689	2	1450,69	-0,001	53	67	0	101,3	37	37	4,10E-09	K	SLLASTGNNSDSSAK	S	
2	JAZ10	mock	AT5G13220	JAZ10	1053	21800	53,3	197	771,916	1541,818	2	1541,82	-0,003	158	171	0	109,1		36	5,70E-10	K	LFGQNLEGDLPIAR	R	
2	JAZ10	mock	AT5G13220	JAZ10	1053	21800	53,3	197	929,4935	1856,973	2	1856,974	-0,002	77	93	0	98,28		36	7,40E-09	R	EDQPQIPISPVHASLAR	S	
2	JAZ10	mock	AT5G13220	JAZ10	1053	21800	53,3	197	1124,573	2247,13	2	2247,13	0	138	157	1	100,1	30	37	5,70E-09	K	DESSMETDLSVILPTTLRPK	L	Oxidation (M)
2	JAZ10	mock	AT5G46760	MYC3	763	65294	26,4	592	369,6966	737,3786	2	737,3782	0,0005	539	544	0	36,43		34	0,007	R	FMEALK	E	
2	JAZ10	mock	AT5G46760	MYC3	763	65294	26,4	592	475,7337	949,4528	2	949,4539	-0,001	316	323	0	59,71		38	6,90E-05	R	QSSCLVEK	D	
2	JAZ10	mock	AT5G46760	MYC3	763	65294	26,4	592	517,785	1033,556	2	1033,556	-2E-04	192	201	0	55,34		37	0,00016	R	AGQGQIYGLK	T	
2	JAZ10	mock	AT5G46760	MYC3	763	65294	26,4	592	546,3082	1090,602	2	1090,602	-5E-04	324	333	0	70,69		35	3,40E-06	K	DLTFQGGLLK	S	
2	JAZ10	mock	AT5G46760	MYC3	763	65294	26,4	592	555,8055	1109,596	2	1109,597	-4E-04	388	397	0	57,41		35	6,00E-05	K	EAIVVEPPEK	K	
2	JAZ10	mock	AT5G46760	MYC3	763	65294	26,4	592	645,3075	1288,6	2	1288,601	-4E-04	305	315	0	87,33		35	6,70E-08	K	NDISSVENQNR	Q	Methyl (DE)
2	JAZ10	mock	AT5G46760	MYC3	763	65294	26,4	592	661,8201	1321,626	2	1321,626	-5E-04	411	421	0	64,71		37	1,90E-05	R	EEPLNHVEAER	Q	
2	JAZ10	mock	AT5G46760	MYC3	763	65294	26,4	592	734,3384	1466,662	2	1466,661	0,0011	570	581	0	63,46		35	1,70E-05	K	MGSQFFNHDQLK	V	Oxidation (M)
2	JAZ10	mock	AT5G46760	MYC3	763	65294	26,4	592	773,3909	1544,767	2	1544,768	-9E-04	464	476	1	89,82		37	6,20E-08	K	LQQAESDKEEIQK	K	
2	JAZ10	mock	AT5G46760	MYC3	763	65294	26,4	592	780,3351	1558,656	2	1558,657	-0,001	334	347	0	78,49		30	1,60E-07	K	SNETLSFCGNESSK	K	
2	JAZ10	mock	AT5G46760	MYC3	763	65294	26,4	592	864,8963	1727,778	2	1727,779	-5E-04	355	370	0	121,4		35	2,30E-11	K	GSNNDEGMLSFSTVVR	S	Oxidation (M)
2	JAZ10	mock	AT5G46760	MYC3	763	65294	26,4	592	872,9021	1743,79	2	1743,791	-0,002	371	387	0	108,5		35	4,70E-10	R	SAANDSDHSDLEASVVK	E	
2	JAZ10	mock	AT5G46760	MYC3	763	65294	26,4	592	978,9384	1955,862	2	1955,863	-8E-04	500	517	0	117,1		34	4,90E-11	K	SSNQDSTASSIEMEIDVK	I	Oxidation (M)
2	JAZ10	mock	AT1G32640	MYC2	501	68136	18,9	623	430,2295	858,4445	2	858,4447	-1E-04	513	519	0	40,88		36	0,0035	K	NQLEEVK	L	
2	JAZ10	mock	AT1G32640	MYC2	501	68136	18,9	623	443,2142	884,4138	2	884,4141	-3E-04	341	347	0	50,3		33	0,00021	K	FNNTFSR	E	
2	JAZ10	mock	AT1G32640	MYC2	501	68136	18,9	623	452,7403	903,466	2	903,4661	-2E-04	240	247	0	57,77		39	0,00014	R	QSSDLINK	V	
2	JAZ10	mock	AT1G32640	MYC2	501	68136	18,9	623	468,2287	934,4428	2	934,443	-2E-04	398	405	0	37,53		36	0,0087	R	SMVLNEDK	V	
2	JAZ10	mock	AT1G32640	MYC2	501	68136	18,9	623	633,3034	1264,592	2	1264,594	-0,001	363	374	0	55,77		36	0,00013	R	SGEILNFGDEGK	R	
2	JAZ10	mock	AT1G32640	MYC2	501	68136	18,9	623	661,8201	1321,626	2	1321,626	-5E-04	448	458	0	64,71		37	1,90E-05	R	EEPLNHVEAER	Q	
2	JAZ10	mock	AT1G32640	MYC2	501	68136	18,9	623	706,8666	1411,719	2	1411,72	-9E-04	348	360	0	52,06		36	0,00031	R	ELNFSTSSSTLVK	P	
2	JAZ10	mock	AT1G32640	MYC2	501	68136	18,9	623	822,8883	1643,762	2	1643,764	-0,002	413	428	0	123,1		35	1,90E-11	K	TAGESDHSDLEASVVK	E	
2	JAZ10	mock	AT1G32640	MYC2	501	68136	18,9	623	833,445	1664,875	2	1664,873	0,0021	348	362	1	103		36	2,50E-09	R	ELNFSTSSSTLVKPR	S	
2	JAZ10	mock	AT1G32640	MYC2	501	68136	18,9	623	834,8789	1667,743	2	1667,743	0,0004	121	135	0	91,23		34	2,30E-08	R	SSSPPFSTPADQEYR	K	
2	JAZ10	mock	AT1G32640	MYC2	501	68136	18,9	623	1015,439	2028,862	2	2028,866	-0,004	376	394	0	132,7		30	6,40E-13	R	SSGNPDPSSYSGQTQFENK	R	
2	JAZ10	mock	AT4G14720	PPD2	482	35056	44,1	315	464,2971	926,5796	2	926,58	-4E-04	10	17	1	35,59		28	0,0022	K	SILEKPLK	L	
2	JAZ10	mock	AT4G14720	PPD2	482	35056	44,1	315	474,7135	947,4124	2	947,4131	-6E-04	96	103	0	34,39		32	0,0063	R	NELEACGR	I	
2	JAZ10	mock	AT4G14720	PPD2	482	35056	44,1	315	515,3009	1028,587	2	1028,587	0,0007	87	95	0	69,66		35	4,00E-06	R	VTTTLIEPR	N	
2	JAZ10	mock	AT4G14720	PPD2	482	35056	44,1	315	607,8587	1213,703	2	1213,703	-2E-04	49	59	0	84,38		35	1,30E-07	K	SQAIQQVLSLK	A	
2	JAZ10	mock	AT4G14720	PPD2	482	35056	44,1	315	608,8138	1215,613	2	1215,614	-5E-04	166	176	0	48,13		37	0,00083	K	VNVYDGVPPEK	A	
2	JAZ10	mock	AT4G14720	PPD2	482	35056	44,1	315	661,8364	1321,658	2	1321,659	-6E-04	211	221	0	69,52		38	8,00E-06	K	MVELPQYGLEK	A	Oxidation (M)
2	JAZ10	mock	AT4G14720	PPD2	482	35056	44,1	315	709,3802	1416,746	2	1416,746	-2E-04	18	29	0	105,5		37	1,80E-09	K	LLTEEDISQLTR	E	
2	JAZ10	mock	AT4G14720	PPD2	482	35056	44,1	315	758,8487	1515,683	2	1515,684	-0,001	121	135	0	98,94		34	3,50E-09	R	SAEFSGSSGQFVADK	D	
2	JAZ10	mock	AT4G14720	PPD2	482	35056	44,1	315	767,3804	1532,746	2	1532,747	-8E-04	60	74	0	60,46		38	5,70E-05	K	ALYEPGDDSGAGILR	K	
2	JAZ10	mock	AT4G14720	PPD2	482	35056	44,1	315	797,8983	1593,782	2	1593,782	0	258	272	0	78,77		38	9,10E-07	K	APGVASSSLEMFLNR	Q	Oxidation (M)
2	JAZ10	mock	AT4G14720	PPD2	482	35056	44,1	315	861,9455	1721,877	2	1721,877	-6E-04	257	272	1	113,1	29	37	3,00E-10	K	KAPGVASSSLEMFLNR	Q	Oxidation (M)
2	JAZ10	mock	AT4G14720	PPD2	482	35056	44,1	315	847,3634	2539,068	3	2539,07	-0,002	276	298	0	30,8	24	30	0,0085	R	MNAAYSQNLSGTGHCESPENQTK	S	Oxidation (M)
2	JAZ10	mock	AT4G17880	MYC4	481	65005	12,9	589	369,6966	737,3786	2	737,3782	0,0005	536	541	0	36,43		34	0,007	K	FMEALK	E	
2	JAZ10	mock	AT4G17880	MYC4	481	65005	12,9	589	515,7202	1029,426	2	1029,426	0	330	337	0	39,01		28	0,00084	K	SCEMVNFK	N	Oxidation (M)
2	JAZ10	mock	AT4G17880	MYC4	481	65005	12,9	589	648,7998	1295,585	2	1295,585	-4E-04	122	133	0	87,69		35	5,80E-08	K	SNPASAAEQEHR	K	
2	JAZ10	mock	AT4G17880	MYC4	481	65005	12,9	589	661,8201	1321,626	2	1321,626	-5E-04	412	422	0	64,71		37	1,90E-05	R	EEPLNHVEAER	Q	
2	JAZ10	mock	AT4G17880	MYC4	481	65005	12,9	589	708,3323	1414,65	2	1414,651	-9E-04	314	326	0	84,89		35	1,10E-07	K	LCNGSSVENPNPK	V	
2	JAZ10	mock	AT4G17880	MYC4	481	65005	12,9	589	728,8474	1455,68	2	1455,682	-0,002	567	578	0	81,39		36	3,30E-07	K	MGNQFFTQDQLK	V	
2	JAZ10	mock	AT4G17880	MYC4	481	65005	12,9	589	767,8342	1533,654	2	1533,654	-5E-04	338	351	0	88,53		31	1,80E-08	K	NGIENGQEEDSSNK	K	Methyl (DE)

2	JAZ10	mock	AT5G20900	JAZ12	237	20035	34,8	187	765,4001	1528,786	2	1528,785	0,0011	92	106	0	75,48		37	1,70E-06	K	NSTSISPVSSPALNR	A	
2	JAZ10	mock	AT5G20900	JAZ12	237	20035	34,8	187	902,9516	1803,889	2	1803,889	-5E-04	171	187	1	56,33	36	38	0,00015	K	TDVPTGNVSIKEEFPTA	-	
2	JAZ10	mock	AT5G20900	JAZ12	237	20035	34,8	187	1204,037	2406,059	2	2406,061	-0,002	10	36	0	140,3		34	2,50E-13	R	ASVEGGCGVADGDGGAAEIGGTGSVEK	S	
2	JAZ10	mock	AT3G17860	JAZ3	103	38064	3,1	352	532,2797	1062,545	2	1062,546	-0,001	89	98	0	72,55		38	3,90E-06	K	APYSSVQGVR	M	
2	JAZ10	mock	AT3G17860	JAZ3	103	38064	3,1	352	596,327	1190,64	2	1190,641	-0,001	88	98	1	67,58		37	9,90E-06	R	KAPYSSVQGVR	M	
1	KIX2	mock	AT3G24150	KIX2	5069	38771	76,7	343	367,1957	732,3768	2	732,3766	0,0002	289	295	0	45,19		38	0,0021	R	IDVGSSR	A	
1	KIX2	mock	AT3G24150	KIX2	5069	38771	76,7	343	384,2731	766,5317	2	766,5317	0	54	60	0	56,84		20	2,10E-06	K	LPVVVLK	A	
1	KIX2	mock	AT3G24150	KIX2	5069	38771	76,7	343	431,7267	861,4388	2	861,4378	0,001	272	278	0	47,22		38	0,0015	R	VCDLSLR	L	
1	KIX2	mock	AT3G24150	KIX2	5069	38771	76,7	343	464,2307	926,4468	2	926,4471	-2E-04	14	20	1	39,43		35	0,0037	K	RAWHSDR	H	
1	KIX2	mock	AT3G24150	KIX2	5069	38771	76,7	343	485,731	969,4474	2	969,4477	-3E-04	61	68	0	57,16		34	5,40E-05	K	AEEIMYSK	A	
1	KIX2	mock	AT3G24150	KIX2	5069	38771	76,7	343	530,7849	1059,555	2	1059,556	-7E-04	279	288	0	51,43	32	39	0,0006	R	LGISSEPSTR	I	Methyl (DE)
1	KIX2	mock	AT3G24150	KIX2	5069	38771	76,7	343	565,3143	1128,614	2	1128,614	0,0002	85	94	0	85,84		36	1,20E-07	R	VNDAIDTIIR	R	
1	KIX2	mock	AT3G24150	KIX2	5069	38771	76,7	343	579,2897	1156,565	2	1156,566	-0,001	35	45	0	90,76		35	2,90E-08	R	LAMEAHSAATR	K	
1	KIX2	mock	AT3G24150	KIX2	5069	38771	76,7	343	740,361	1478,708	2	1478,708	0	301	312	0	85,11		37	1,70E-07	R	NQEELCLFAEVK	K	
1	KIX2	mock	AT3G24150	KIX2	5069	38771	76,7	343	774,4652	1546,916	2	1546,916	0,0001	249	263	0	90,85		25	3,00E-09	R	VPEAPIIIGMPIGIK	P	
1	KIX2	mock	AT3G24150	KIX2	5069	38771	76,7	343	882,8948	1763,775	2	1763,776	-8E-04	192	205	0	44,14		33	0,00083	R	GYPFLHESMQMHQK	P	2 Oxidation (M)
1	KIX2	mock	AT3G24150	KIX2	5069	38771	76,7	343	895,3738	1788,733	2	1788,734	-0,001	317	331	0	103,1		28	3,30E-10	R	FEWFSNSEGQNSDSR	V	
1	KIX2	mock	AT3G24150	KIX2	5069	38771	76,7	343	973,3981	1944,782	2	1944,78	0,0021	69	84	0	129,1		26	5,60E-13	K	ANSEEEYTDADTMWNR	V	Methyl (DE)
1	KIX2	mock	AT3G24150	KIX2	5069	38771	76,7	343	991,5131	1981,012	2	1981,012	0	167	185	0	146,3	32	37	1,30E-13	K	PSQTVQAAVPVDVLDNSNK	R	
1	KIX2	mock	AT3G24150	KIX2	5069	38771	76,7	343	1069,563	2137,111	2	2137,113	-0,002	167	186	1	95,78		36	1,20E-08	K	PSQTVQAAVPVDVLDNSNKR	V	
1	KIX2	mock	AT3G24150	KIX2	5069	38771	76,7	343	1087,959	2173,904	2	2173,905	-9E-04	314	331	1	91,54		28	5,50E-09	K	NDRFEWFSNSEGQNSDSR	V	
1	KIX2	mock	AT3G24150	KIX2	5069	38771	76,7	343	1129,527	2257,039	2	2257,04	-9E-04	139	157	0	138,3		35	5,90E-13	K	IQEPVSASTNEPSYHHEYR	Q	Methyl (DE)
1	KIX2	mock	AT3G24150	KIX2	5069	38771	76,7	343	1224,164	2446,313	2	2446,314	-9E-04	249	271	1	102		34	1,70E-09	R	VPEAPIIIGMPIGIKPSEEATER	V	
1	KIX2	mock	AT3G24150	KIX2	5069	38771	76,7	343	928,1297	2781,367	3	2781,368	-5E-04	96	121	0	51,49	33	37	0,0004	R	DESTETGPLLPPCVEAALNLGCIAVR	A	
1	KIX2	mock	AT3G24150	KIX2	5069	38771	76,7	343	980,1628	2937,467	3	2937,469	-0,002	95	121	1	95,75		36	1,30E-08	R	RDESTETGPLLPPCVEAALNLGCIAVR	A	
1	KIX2	mock	AT3G24150	KIX2	5069	38771	76,7	343	1484,752	2967,489	2	2967,49	-3E-04	158	185	1	103,2		36	2,10E-09	R	QQAQQSSTKPSQTVQAAVPVDVLDNSNK	R	
1	KIX2	mock	AT3G24150	KIX2	5069	38771	76,7	343	1003,974	4011,865	4	4011,87	-0,004	211	248	0	105,1	31	34	9,30E-10	R	QGTGPASAPAPAPVNLGSVYPLYYEGNNQTQQADMSFR	V	Oxidation (M)
1	KIX2	mock	AT5G06290	2-cysteine peroxiredoxin B	151	29932	21,6	273	620,8296	1239,645	2	1239,646	-0,001	74	85	0	58,84		35	5,20E-05	K	AQADDLPLVGNK	A	
1	KIX2	mock	AT5G06290	2-cysteine peroxiredoxin B	151	29932	21,6	273	743,4251	1484,836	2	1484,835	0,0006	189	202	0	63,28		34	1,40E-05	K	SFGVLIPDQGIALR	G	
1	KIX2	mock	AT5G06290	2-cysteine peroxiredoxin B	151	29932	21,6	273	928,4413	1854,868	2	1854,868	0,0005	86	101	0	97,09		36	9,20E-09	K	APDFEAEAVFDQEFIK	V	
1	KIX2	mock	AT4G21710	NRPB2	134	136131	2,8	1188	462,7301	923,4457	2	923,4461	-3E-04	254	262	0	37,75		36	0,0078	K	GGSSGQYIR	C	
1	KIX2	mock	AT4G21710	NRPB2	134	136131	2,8	1188	472,2691	942,5236	2	942,5247	-0,001	468	476	0	65,21		35	1,10E-05	R	AGVSQVLNR	L	
1	KIX2	mock	AT4G21710	NRPB2	134	136131	2,8	1188	802,8789	1603,743	2	1603,744	-6E-04	878	892	0	102,2		36	2,40E-09	K	TTPISQDEAQGQSSR	Y	
1	KIX2	mock	AT4G14720	PPD2	131	35056	11,4	315	515,3016	1028,589	2	1028,587	0,0021	87	95	0	68,85		36	6,00E-06	R	VTTTLIEPR	N	
1	KIX2	mock	AT4G14720	PPD2	131	35056	11,4	315	709,3802	1416,746	2	1416,746	-1E-04	18	29	0	74,01		37	2,50E-06	K	LLTEEDISQLTR	E	
1	KIX2	mock	AT4G14720	PPD2	131	35056	11,4	315	767,3803	1532,746	2	1532,747	-0,001	60	74	0	61,89		38	4,10E-05	K	ALYEPGDDSGAGILR	K	
1	KIX2	mock	AT5G54770	THI1, TZ, THI4	124	36755	7,4	349	701,9015	1401,789	2	1401,787	0,0017	181	193	0	80,16		34	2,80E-07	K	LFNAVAAEDLIVK	G	
1	KIX2	mock	AT5G54770	THI1, TZ, THI4	124	36755	7,4	349	709,8508	1417,687	2	1417,687	-1E-04	259	271	0	79		37	7,50E-07	K	ALDMNTAEDAIVR	L	
1	KIX2	mock	AT1G50600	SCL5	68	67283	3	597	397,2194	792,4243	2	792,4242	0,0001	359	366	0	50,24		36	0,00038	R	PGGPPNVR	I	
1	KIX2	mock	AT1G50600	SCL5	68	67283	3	597	528,7938	1055,573	2	1055,572	0,0008	380	389	0	53,13		34	0,00015	R	QGGLELVGQR	L	
1	KIX2	mock	AT5G52840	l-ubiquinone oxidoreductase-r	47	19338	11,2	169	511,3	1020,586	2	1020,586	-1E-04	31	39	0	41,76		32	0,0013	R	AVLIDLYSK	T	
1	KIX2	mock	AT5G52840	l-ubiquinone oxidoreductase-r	47	19338	11,2	169	530,7974	1059,58	2	1059,581	-9E-04	145	154	0	38,21	33	37	0,0094	K	TLEGLIAESK	T	
2	KIX2	mock	AT3G24150	KIX2	3010	38771	62,1	343	367,1957	732,3769	2	732,3766	0,0003	289	295	0	41,7		38	0,0046	R	IDVGSSR	A	
2	KIX2	mock	AT3G24150	KIX2	3010	38771	62,1	343	384,2731	766,5316	2	766,5317	-1E-04	54	60	0	47,39		20	1,80E-05	K	LPVVVLK	A	
2	KIX2	mock	AT3G24150	KIX2	3010	38771	62,1	343	431,7264	861,4381	2	861,4378	0,0003	272	278	0	44,76		38	0,0026	R	VCDLSLR	L	
2	KIX2	mock	AT3G24150	KIX2	3010	38771	62,1	343	464,2307	926,4468	2	926,4471	-3E-04	14	20	1	41,08		35	0,0026	K	RAWHSDR	H	
2	KIX2	mock	AT3G24150	KIX2	3010	38771	62,1	343	485,7312	969,4477	2	969,4477	0,0001	61	68	0	61,47		34	2,00E-05	K	AEEIMYSK	A	
2	KIX2	mock	AT3G24150	KIX2	3010	38771	62,1	343	523,7777	1045,541	2	1045,54	0,0004	279	288	0	48,4	33	38	0,0011	R	LGISSEPSTR	I	
2	KIX2	mock	AT3G24150	KIX2	3010	38771	62,1	343	565,3144	1128,614	2	1128,614	0,0004	85	94	0	83,61		36	2,00E-07	R	VNDAIDTIIR	R	
2	KIX2	mock	AT3G24150	KIX2	3010	38771	62,1	343	587,2874	1172,56	2	1172,561	-5E-04	35	45	0	89,29		35	4,00E-08	R	LAMEAHSAATR	K	Oxidation (M)
2	KIX2	mock	AT3G24150	KIX2	3010	38771	62,1	343	701,8576	1401,701	2	1401,701	-7E-04	15	25	1	44,28	26	37	0,002	R	AWHSDRHQPIR	G	
2	KIX2	mock	AT3G24150	KIX2	3010	38771	62,1	343	734,4319	1466,849	2	1466,85	-4E-04	49	60	1	46,54		30	0,00023	K	EWQEKLPVVVLK	A	
2	KIX2	mock	AT3G24150	KIX2	3010	38771	62,1	343	740,361	1478,708	2	1478,708	0	301	312	0	85,33		37	1,60E-07	R	NQEELCLFAEVK	K	
2	KIX2	mock	AT3G24150	KIX2	3010	38771	62,1	343	774,4651	1546,916	2	1546,916	-1E-04	249	263	0	71,94		25	2,30E-07	R	VPEAPIIIGMPIGIK	P	
2	KIX2	mock	AT3G24150	KIX2	3010	38771	62,1	343	859,9916	1717,969	2	1717,969	-1E-04	54	68	1	61,88		31	1,00E-05	K	LPVVVLKAEEIMYSK	A	
2	KIX2	mock	AT3G24150	KIX2	3010	38771	62,1	343	895,3744	1788,734	2	1788,734	0,0002	317	331	0	98,13		28	1,00E-09	R	FEWFSNSEGQNSDSR	V	
2	KIX2	mock	AT3G24150	KIX2	3010	38771	62,1	343	966,3889	1930,763	2	1930,764	-6E-04	69	84	0	129,2		25	4,10E-13	K	ANSEEEYTDADTMWNR	V	
2	KIX2	mock	AT3G24150	KIX2	3010	38771	62,1	343	991,5129	1981,011	2	1981,012	-5E-04	167	185	0	113,5	30	37	2,50E-10	K	PSQTVQAAVPVDVLDNSNK	R	
2	KIX2	mock	AT3G24150	KIX2	3010	38771	62,1	343	713,3774	2137,111	3	2137,113	-0,002	167	186	1	75,89		36	1,20E-06	K	PSQTVQAAVPVDVLDNSNKR	V	
2	KIX2	mock	AT3G24150	KIX2	3010	38771	62,1	343	725,6423	2173,905	3	2173,905	0	314	331	1	86,14		29	2,00E-08	K	NDRFEWFSNSEGQNSDSR	V	
2	KIX2	mock	AT3G24150	KIX2	3010	38771	62,1	343	1129,527	2257,039	2	2257,04	-0,001	139	157	0	134,4		35	1,40E-12	K	IQEPVSASTNEPSYHHEYR	Q	Methyl (DE)
2	KIX2	mock	AT3G24150	KIX2	3010	38771	62,1	343	1150,077	2298,14	2	2298,14	0,0001	192	210	1	64,15	24	37	2,30E-05	R	GYPFLHESMQMHQKPLAIR	Q	Oxidation (M)
2	KIX2	mock	AT3G24150	KIX2	3010	38771	62,1	343	1224,165	2446,316	2	2446,314	0,0019	249	271	1	95,49	32	34	7,30E-09	R	VPEAPIIIGMPIGIKPSEEATER	V	
2	KIX2	mock	AT3G24150	KIX2	3010	38771	62,1	343	990,1695	2967,487	3	2967,49	-0,003	158	185	1	67,5	31	36	7,90E-06	R	QQAQQSSTKPSQTVQAAVPVDVLDNSNK	R	
2	KIX2	mock	AT5G06600	UBP12	151	131152	6,3	1116	422,7242	843,4339	2	843,4338	0,0002	838	845	0	35,15		34	0,0086	K	LGLDDPSK	L	
2	KIX2	mock	AT5G06600	UBP12	151	131152	6,3	1116	486,2686	970,5227	2	970,5236	-8E-04	1106	1113	1	37		34	0,0062	R	HAYEKPVK	I	
2	KIX2	mock	AT5G06600	UBP12	151	131152	6,3	1116	565,7707	1129,527	2	1129,527	0,0003	137	145	0	40,51		34	0,0025	K	ETQHQFNAR	E	
2	KIX2	mock	AT5G06600	UBP12	151	131152	6,3	1116	620,3142	1238,614	2	1238,614	-3E-04	253	263	0	57,7	27	37	8,80E-05	K	LQYNDTSVATK	E	

2	KIX2	mock	AT5G06600	UBP12	151	131152	6,3	1116	620,353	1238,692	2	1238,691	0,0004	688	697	1	40,43		33	0,0021	K	SKEDILLFFK	L
2	KIX2	mock	AT5G06600	UBP12	151	131152	6,3	1116	651,8479	1301,681	2	1301,683	-0,001	925	936	0	79,19		38	7,80E-07	K	QSTVGDVINELK	T
2	KIX2	mock	AT5G06600	UBP12	151	131152	6,3	1116	731,841	1461,667	2	1461,667	0,0003	848	859	0	62,93		35	1,80E-05	R	LTSHNCYSQQPK	P
2	KIX2	mock	AT1G50600	SCL5	129	67283	7,7	597	397,2193	792,4241	2	792,4242	-1E-04	359	366	0	57,91		36	7,90E-05	R	PGGPPNVR	I
2	KIX2	mock	AT1G50600	SCL5	129	67283	7,7	597	405,7062	809,3979	2	809,3919	0,006	572	577	0	40,91		36	0,0033	K	YTLEER	D
2	KIX2	mock	AT1G50600	SCL5	129	67283	7,7	597	506,769	1011,524	2	1011,524	-2E-04	279	288	0	47,19		35	0,00072	R	LASSGSSIYK	A
2	KIX2	mock	AT1G50600	SCL5	129	67283	7,7	597	528,7941	1055,574	2	1055,572	0,0013	380	389	0	53,7		34	0,00011	R	QGGLELVGQR	L
2	KIX2	mock	AT1G50600	SCL5	129	67283	7,7	597	646,8552	1291,696	2	1291,696	0,0001	267	278	0	67,07	31	37	9,90E-06	R	LGAYMLEGLVAR	L
2	KIX2	mock	AT5G54770	THI1, TZ, THI4	106	36755	7,4	349	701,9023	1401,79	2	1401,787	0,0034	181	193	0	65,06		34	9,20E-06	K	LFNAVAAEDLIVK	G
2	KIX2	mock	AT5G54770	THI1, TZ, THI4	106	36755	7,4	349	709,8509	1417,687	2	1417,687	0,0002	259	271	0	76,38		37	1,40E-06	K	ALDMNTAEDAIVR	L
2	KIX2	mock	AT5G06290	2-cysteine peroxiredoxin B	99	29932	15,8	273	620,83	1239,645	2	1239,646	-5E-04	74	85	0	73,11		35	1,90E-06	K	AQADDLPLVGNK	A
2	KIX2	mock	AT5G06290	2-cysteine peroxiredoxin B	99	29932	15,8	273	743,4249	1484,835	2	1484,835	0,0001	189	202	0	40,69		34	0,0024	K	SFGVLIPDQGIALR	G
2	KIX2	mock	AT4G14720	PPD2	94	35056	6,3	315	515,3008	1028,587	2	1028,587	0,0004	87	95	0	54,04		36	0,00017	R	VTTTLIEPR	N
2	KIX2	mock	AT4G14720	PPD2	94	35056	6,3	315	607,8586	1213,703	2	1213,703	-4E-04	49	59	0	75,56		35	9,60E-07	K	SQAIQQVLSLK	A
2	KIX2	mock	AT4G21710	NRPB2	81	136131	2,9	1188	462,7299	923,4453	2	923,4461	-7E-04	254	262	0	39,59		36	0,0053	K	GGSSGQYIR	C
2	KIX2	mock	AT4G21710	NRPB2	81	136131	2,9	1188	600,8714	1199,728	2	1199,728	0,0004	270	279	0	31,09		30	0,0084	R	TEIPIIIVFR	A
2	KIX2	mock	AT4G21710	NRPB2	81	136131	2,9	1188	802,8792	1603,744	2	1603,744	0,0001	878	892	0	78,3		36	6,20E-07	K	TTPISQDEAQGQSSR	Y

Annex 5. List of genes retrieved in the PPD2 DNA-binding microarray with 0.95 confidence

AGI (TAIR10)	Type	Short_description
AT1G01290	protein_coding	cofactor of nitrate reductase and xanthine dehydrogenase 3
AT1G01790	protein_coding	K+ efflux antiporter 1
AT1G01920	protein_coding	SET domain-containing protein
AT1G07490	protein_coding	ROTUNDIFOLIA like 3
AT1G08050	protein_coding	Zinc finger (C3HC4-type RING finger) family protein
AT1G10360	protein_coding	glutathione S-transferase TAU 18
AT1G10690	protein_coding	
AT1G11610	protein_coding	cytochrome P450, family 71, subfamily A, polypeptide 18
AT1G11620	protein_coding	F-box and associated interaction domains-containing protein
AT1G18570	protein_coding	myb domain protein 51
AT1G22720	protein_coding	Protein kinase superfamily protein
AT1G26870	protein_coding	NAC (No Apical Meristem) domain transcriptional regulator superfamily protein
AT1G32713	mirna	MIR829a; miRNA
AT1G35500	protein_coding	
AT1G35900	protein_coding	
AT1G38240	transposable_element_gene	transposable element gene
AT1G46696	protein_coding	Protein of unknown function, DUF601
AT1G49245	protein_coding	Prefoldin chaperone subunit family protein
AT1G53780	protein_coding	peptidyl-prolyl cis-trans isomerases;hydrolases;nucleoside-triphosphatases;ATP binding;nucleotide binding;ATPases
AT1G61080	protein_coding	Hydroxyproline-rich glycoprotein family protein
AT1G71160	protein_coding	3-ketoacyl-CoA synthase 7
AT1G74730	protein_coding	Protein of unknown function (DUF1118)
AT1G79790	protein_coding	Haloacid dehalogenase-like hydrolase (HAD) superfamily protein
AT1G79810	protein_coding	Pex2/Pex12 N-terminal domain-containing protein / zinc finger (C3HC4-type RING finger) family protein
AT1G80030	protein_coding	Molecular chaperone Hsp40/DnaJ family protein
AT2G01020	ribosomal_rna	rRNA
AT2G01370	protein_coding	DNA-binding storekeeper protein-related transcriptional regulator
AT2G03600	protein_coding	ureide permease 3
AT2G03690	protein_coding	coenzyme Q biosynthesis Coq4 family protein / ubiquinone biosynthesis Coq4 family protein
AT2G06140	transposable_element_gene	transposable element gene
AT2G06904	protein_coding	nucleic acid binding;zinc ion binding
AT2G06906	protein_coding	
AT2G06930	transposable_element_gene	transposable element gene
AT2G07483	transposable_element_gene	transposable element gene
AT2G07689	protein_coding	NADH-Ubiquinone/plastoquinone (complex I) protein
AT2G07743	pre_trna	pre-tRNA
AT2G07779	protein_coding	
AT2G09840	protein_coding	
AT2G11780	transposable_element_gene	transposable element gene
AT2G11830	transposable_element_gene	transposable element gene
AT2G13440	protein_coding	glucose-inhibited division family A protein
AT2G14560	protein_coding	Protein of unknown function (DUF567)
AT2G15110	protein_coding	Protein of unknown function, DUF601
AT2G29490	protein_coding	glutathione S-transferase TAU 1
AT2G29510	protein_coding	Protein of unknown function (DUF3527)
AT2G30450	pre_trna	pre-tRNA
AT2G30780	protein_coding	Tetratricopeptide repeat (TPR)-like superfamily protein

AT2G32160	protein_coding	S-adenosyl-L-methionine-dependent methyltransferases superfamily protein
AT2G32380	protein_coding	Transmembrane protein 97, predicted
AT2G33080	protein_coding	receptor like protein 28
AT2G34050	protein_coding	
AT2G35130	protein_coding	Tetratricopeptide repeat (TPR)-like superfamily protein
AT2G35980	protein_coding	Late embryogenesis abundant (LEA) hydroxyproline-rich glycoprotein family
AT2G38544	protein_coding	
AT2G45100	protein_coding	Cyclin/Brf1-like TBP-binding protein
AT2G45110	protein_coding	expansin B4
AT2G46980	protein_coding	
AT3G01160	protein_coding	
AT3G02540	protein_coding	Rad23 UV excision repair protein family
AT3G05660	protein_coding	receptor like protein 33
AT3G05670	protein_coding	RING/U-box protein
AT3G05710	protein_coding	syntaxin of plants 43
AT3G06270	protein_coding	Protein phosphatase 2C family protein
AT3G07310	protein_coding	Protein of unknown function (DUF760)
AT3G09730	protein_coding	
AT3G13205	pseudogene	
AT3G13225	protein_coding	WW domain-containing protein
AT3G13275	protein_coding	
AT3G13277	other_rna	other RNA
AT3G17668	protein_coding	DnaJ/Hsp40 cysteine-rich domain superfamily protein
AT3G17845	protein_coding	
AT3G19870	protein_coding	
AT3G20362	protein_coding	
AT3G21040	transposable_element_gene	transposable element gene
AT3G21060	protein_coding	Transducin/WD40 repeat-like superfamily protein
AT3G21950	protein_coding	S-adenosyl-L-methionine-dependent methyltransferases superfamily protein
AT3G24495	protein_coding	MUTS homolog 7
AT3G27040	protein_coding	Meprin and TRAF (MATH) homology domain-containing protein
AT3G28970	protein_coding	Domain of unknown function (DUF298)
AT3G32465	transposable_element_gene	transposable element gene
AT3G33118	transposable_element_gene	transposable element gene
AT3G33130	transposable_element_gene	transposable element gene
AT3G33163	transposable_element_gene	transposable element gene
AT3G33201	transposable_element_gene	transposable element gene
AT3G36411	transposable_element_gene	transposable element gene
AT3G41979	ribosomal_rna	rRNA
AT3G42353	transposable_element_gene	transposable element gene
AT3G42356	transposable_element_gene	transposable element gene
AT3G43444	transposable_element_gene	transposable element gene
AT3G43447	transposable_element_gene	transposable element gene
AT3G43520	protein_coding	Transmembrane proteins 14C
AT3G45930	protein_coding	Histone superfamily protein
AT3G46320	protein_coding	Histone superfamily protein
AT3G50020	protein_coding	Serine protease inhibitor, potato inhibitor I-type family protein
AT3G53500	protein_coding	RNA-binding (RRM/RBD/RNP motifs) family protein with retrovirus zinc finger-like domain
AT3G55830	protein_coding	Nucleotide-diphospho-sugar transferases superfamily protein
AT3G59490	protein_coding	

AT3G61198	other_rna	other RNA
AT4G00651	protein_coding	
AT4G03415	protein_coding	Protein phosphatase 2C family protein
AT4G03500	protein_coding	Ankyrin repeat family protein
AT4G04000	transposable_element_gene	transposable element gene
AT4G07502	transposable_element_gene	transposable element gene
AT4G08967	protein_coding	
AT4G09595	transposable_element_gene	transposable element gene
AT4G13610	protein_coding	DNA (cytosine-5-)-methyltransferase family protein
AT4G14368	protein_coding	Regulator of chromosome condensation (RCC1) family protein
AT4G17160	protein_coding	RAB GTPase homolog B1A
AT4G17510	protein_coding	ubiquitin C-terminal hydrolase 3
AT4G20340	protein_coding	Transcription factor TFIIE, alpha subunit
AT4G20740	protein_coding	Pentatricopeptide repeat (PPR-like) superfamily protein
AT4G21890	protein_coding	
AT4G23320	protein_coding	cysteine-rich RLK (RECEPTOR-like protein kinase) 24
AT4G26675	pre_trna	pre-tRNA
AT4G26680	protein_coding	Tetratricopeptide repeat (TPR)-like superfamily protein
AT4G28100	protein_coding	
AT4G28360	protein_coding	Ribosomal protein L22p/L17e family protein
AT4G28362	pre_trna	pre-tRNA
AT4G31770	protein_coding	debranching enzyme 1
AT4G31780	protein_coding	monogalactosyl diacylglycerol synthase 1
AT4G35731	pseudogene	
AT4G36060	protein_coding	basic helix-loop-helix (bHLH) DNA-binding superfamily protein
AT4G36100	protein_coding	Sec1/munc18-like (SM) proteins superfamily
AT4G36840	protein_coding	Galactose oxidase/kelch repeat superfamily protein
AT4G39364	small_nucleolar_rna	snoRNA
AT4G39366	small_nucleolar_rna	snoRNA
AT4G39370	protein_coding	ubiquitin-specific protease 27
AT5G04560	protein_coding	HhH-GPD base excision DNA repair family protein
AT5G05110	protein_coding	Cystatin/monellin family protein
AT5G07590	protein_coding	Transducin/WD40 repeat-like superfamily protein
AT5G08540	protein_coding	
AT5G15390	protein_coding	tRNA/rRNA methyltransferase (SpoU) family protein
AT5G16750	protein_coding	Transducin family protein / WD-40 repeat family protein
AT5G18340	protein_coding	ARM repeat superfamily protein
AT5G22100	protein_coding	RNA cyclase family protein
AT5G27927	transposable_element_gene	transposable element gene
AT5G28929	transposable_element_gene	transposable element gene
AT5G29070	protein_coding	
AT5G30410	transposable_element_gene	transposable element gene
AT5G31496	transposable_element_gene	transposable element gene
AT5G31758	transposable_element_gene	transposable element gene
AT5G32520	transposable_element_gene	transposable element gene
AT5G33175	transposable_element_gene	transposable element gene
AT5G33898	protein_coding	Protein of unknown function (DUF3287)
AT5G34841	transposable_element_gene	transposable element gene
AT5G34880	transposable_element_gene	transposable element gene
AT5G40740	protein_coding	

AT5G43110	protein_coding	pumilio 14
AT5G46105	pre_trna	pre-tRNA
AT5G49560	protein_coding	Putative methyltransferase family protein
AT5G50080	protein_coding	ethylene response factor 110
AT5G52000	protein_coding	importin alpha isoform 8
AT5G53440	protein_coding	
AT5G56580	protein_coding	MAP kinase kinase 6
AT5G56590	protein_coding	O-Glycosyl hydrolases family 17 protein
AT5G57440	protein_coding	Haloacid dehalogenase-like hydrolase (HAD) superfamily protein
AT5G59900	protein_coding	Pentatricopeptide repeat (PPR) superfamily protein
AT5G62150	protein_coding	peptidoglycan-binding LysM domain-containing protein
AT5G66980	protein_coding	AP2/B3-like transcriptional factor family protein
ATMG00110	protein_coding	cytochrome C biogenesis 206
ATMG01060	protein_coding	
ATMG01070	pre_trna	