UNIVERSITÀ DEGLI STUDI DI PADOVA

DIPARTIMENTO DI INGEGNERIA INDUSTRIALE

CORSO DI LAUREA MAGISTRALE IN INGEGNERIA CHIMICA E DEI PROCESSI INDUSTRIALI

Tesi di Laurea Magistrale in
Ingegneria Chimica e dei Processi Industriali

ROOTS FACING WATER DEFICIT: ABSCISIC ACID PLAYS A KEY ROLE IN MEDIATING STRESS AVOIDANCE IN POPLAR

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ANNO ACCADEMICO 2013—2014

Acknowledgements

This master thesis is the result of the six months I spent in the WSL (Swiss Federal Institute for Forest, Snow and Landscape Research) in Birmensdorf, Switzerland. This period was very interesting and rewarding, and it was a pleasure to study and work in such environment. I'm really thankful to all the people I got in contact with at WSL Institute, I always felt very well welcomed and supported.

I would like to particularly thank my supervisor, Doctor Christoph Sperisen, not only for his technical guidance, but also for the constant and helpful presence during each stage of the thesis. I thank also Doctor Matthias Arend. He taught me how to work with plants, and supported me in conducing the experiments and in interpretation of the results. I thank also Terry Menard for helping me in the microscopy part of this work, and finally Anna Bratus and Masaomi Hatakeyama, from the FGCZ (Functional Genomic Center of Zurich), for the help in RNA-seq and gene expression analysis.

This thesis is the end of my double degree project between Università degli Studi di Padova (Italy) and Instituto Superior Técnico (Portugal). I thank all the professors of these Institutions who supported me during these three years and all the colleagues who shared with me this journey until now.

Summary

Plants exposed to water deficit often respond with a strong inhibition of shoot growth, while root growth is less inhibited or even promoted. Shoot growth inhibition is well characterized, while little is known about the factors mediating root growth. In this study, abscisic acid (ABA) insensitive transgenic poplar *(Populus* x *canescens*) was used to test whether ABA is involved in the control of differential root and shoot growth responses to water deficit. Transgenic *abi1* and corresponding wild-type plants were treated with 300 mM sorbitol to simulate reduced water availability.

Plants of both genotypes responded to sorbitol with inhibition of shoot and root elongation, but wild-type plants showed a strong promotion of root radial growth, indicating that ABA positively influences root growth under water deficit.

Root tip tissue of wild-type plants treated with sorbitol inspected under the light microscope showed clearly more cells than the tissue of control plants, indicating that the sorbitol-induced increase in radial growth is a result of cell division.

To examine the role of ABA in controlling gene expression under water deficit, RNA was extracted from root tips, and the lateral root development zone, and was sequenced on an Illumina Hi-Seq 2000 instrument. The sequences were mapped to the *Populus trichocarpa* genome and then used to determine differentially expressed genes. Analyses of gene ontology terms revealed that genes related to stress were up-regulated in all tissues, except in the root tip of wild-type plants, indicating that ABA plays an important role in stress avoidance under water deficit. Identification of differentially expressed genes revealed that gibberellins and auxin are involved in ABA regulated response to drought.

Riassunto esteso

Quando una pianta si trova in situazione di stress dovuto a carenza idrica, essa risponde inibendo la crescita del germoglio, e mantenendo o addirittura promuovendo la crescita delle radici. Questa risposta permette alla pianta di bilanciare l'assorbimento e la perdita d'acqua. Recenti studi hanno dimostrato che l'inibizione della crescita del germoglio è un processo controllato dalle gibberelline e dalle proteine DELLA (Achard et al., 2006; Zawaski and Busov, 2014). I fattori che regolano la crescita delle radici, invece,sono per lo più sconosciuti. Scopo di questa tesi sperimentale è stato testare l'ipotesi che il fitormone acido abscisico (ABA) sia coinvolto nel controllo della crescita delle radici sottoposte a carenza idrica.

È stata utilizzata una specie di pioppo (*Populus* x *canescens*) insensibile all'ABA. Questa pianta transgenica esprime ectopicamente il gene mutante della pianta Arabidopsis *abi1*. Le piante *abi1* e le corrispondenti piante wild-type sono state coltivate *in vitro* con 300 mM di sorbitolo per creare condizioni simulate di carenza idrica. In assenza di sorbitolo, le piante *abi1* hanno mostrato germogli più lunghi, foglie più piccole, e un sistema di radici meno sviluppato rispetto alle piante wild-type. In entrambi i genotipi, in presenza di sorbitolo la crescita di germogli e radici è risultata inibita e le foglie sono risultate di un verde più scuro rispetto alle piante di controllo.

Nelle piante sottoposte al sorbitolo è stata osservata una riduzione significativa del potenziale idrico interno alla pianta in entrambi i genotipi (misurato con una pompa di pressione Scholander). Ciò conferma l'efficacia dell'utilizzo di sorbitolo nel ricreare una situazione di carenza idrica simulata.

Le piante di entrambi i genotipi hanno risposto alla presenza di sorbitolo riducendo l'allungamento delle radici rispetto alle piante di controllo (mezzo di coltura senza sorbitolo). Tuttavia, nelle piante wild-type e non in quelle *abi1* la crescita delle radici è aumentata considerevolmente in direzione radiale (il diametro è aumentato di un fattore 2.6) rispetto alle piante controllo. Al contrario, la crescita del germoglio risulta inibita in entrambi i genotipi, suggerendo quindi che l'ABA svolga un ruolo minore nella regolazione della crescita dei germogli. Come conseguenza dell'aumento del diametro della radice e delle scarse variazioni nella dimensione del germoglio, il rapporto in peso tra radici e germoglio risulta notevolmente maggiore per le piante wild-type,quando il sorbitolo è presente nel mezzo di cultura (Figura 3.5). Questo indica che l'ABA è coinvolto nel processo di crescita delle radici in risposta a carenza idrica.

Sono stati quindi misurati anche altri parametri relativi alle radici, quali la densità e la lunghezza delle radici laterali (§3.4.2). La densità delle radici laterali è stata utilizzata come indicatore dei processi di formazione delle stesse, mentre la lunghezza della radice laterale più sviluppata è stata utilizzata come indicatore del processo di allungamento delle radici laterali.

Non sono state osservate variazioni nella densità delle radici laterali tra piante wild-type e *abi1*, mentre in entrambi i genotipi è stato rilevato un aumento in risposta al sorbitolo, suggerendo che il processo di formazione delle radici laterali non sia dipendente dall'ABA. Nelle piante wild-type le radici laterali sono risultate significativamente più lunghe rispetto alle piante *abi1*. Non è stato osservato un effetto dovuto al sorbitolo, supportando quindi l'ipotesi che l'ABA, similmente a quanto ipotizzato per la radice primaria, sia un regolatore positivo dell'allungamento delle radici laterali in condizioni di assenza di stress.

Alcuni tessuti vegetali sono stati ispezionati al microscopio ottico per correlare le differenze osservate durante la crescita delle piante alle differenze nella struttura dei tessuti (§3.5). Sono state studiate le sezioni longitudinali della radice, le sezioni di taglio della foglia (lamina fogliare) e infine le sezioni trasversali dello stelo. Nella punta della radice della pianta wild-type trattata con sorbitolo sono state identificate chiaramente più cellule rispetto alla pianta di controllo. Questo indica che la crescita radiale in risposta al sorbitolo è soprattutto dovuta ad un aumento della divisione cellulare, piuttosto che al processo di espansione cellulare.

Non sono state osservate differenze importanti tra le foglie dei diversi genotipi. L'unica differenza riguarda la chiusura degli stomi, il cui normale funzionamento è compromesso nelle piante *abi1*, come è stato descritto in studi precedenti (Arend et al., 2009).

Nelle sezioni trasversali dello stelo sono state rilevate alcune differenze. Nelle piante wild-type in condizioni di controllo, il diametro dei vasi nello xilema è risultato più grande che nelle piante cresciute con sorbitolo, possibile indice che l'ABA potrebbe avere un impatto sulla struttura dello xilema. Inoltre, solamente nelle piante *abi l*sono stati osservati dei granuli di amido.

L'etilene prodotto dalle piante è stato misurato con un gas cromatografo per studiare la possibile interazione tra l'ABA e l'etilene nel controllo della crescita (§3.6). Nelle condizioni di controllo, l'etilene emesso dalle piante *abi1* è risultato maggiore rispetto a quello emesso dalle piante wild-type. Tuttavia, non è stata identificata una chiara risposta al sorbitolo.

Per studiare il ruolo dell'ABA nel controllo dell'espressione genica in condizioni di carenza idrica, è stato estratto l'RNA dalle punte delle radici (0.5 cm della radice principale) e dalla zona di sviluppo delle radici laterali (circa 1-2 cm della radice principale, sopra la punta). L'RNA è stato quindi sequenziato con un sequenziatore Illumina Hi-Seq 2000. Le singole sequenze sono state mappate sul genoma del *Populus trichocarpa* e quindi utilizzate per determinare le differenze di espressione genica tra i due genotipi in condizioni di carenza idrica. Il *clustering* dei geni normalizzati ha rivelato una chiara struttura gerarchica tra i vari campioni (Figura 3.13). I due tessuti analizzati hanno formato due *cluster*, seguiti poi da quattro *cluster* che rappresentano ciascuno una combinazione di tessuto e trattamento. Questo risultato sorprendente conferma l'importanza di un campionamento specifico, dal momento che i differenti tessuti rispondono in maniera molto diversa a livello di espressione genica.

Per scoprire differenze globali nell'espressione genica tra i due genotipi, è stata effettuata una analisi di arricchimento (Gene Ontology, *Biological Process* §3.7.4). Due analisi separate sono state effettuate rispettivamente per i geni sovra-espressi e sotto-espressi. Il tessuto con il più alto numero di categorie arricchite è risultato la zona di sviluppo delle radici laterali, in particolare nelle piante *abi1*. I geni coinvolti nella risposta allo stress sono stati trovati sovra-espressi in tutti i tessuti, tranne che nella punta delle radici delle piante wild-type, indicando che l'ABA svolge un ruolo importante nei meccanismi di *stress avoidance* in condizioni di carenza idrica. In tale tessuto i geni che la punta delle radici delle piante wild-type non si trova in condizioni di stress.

Per meglio caratterizzare i cambiamenti dell'espressione genica, sono stati analizzati i geni differenzialmente espressi in solo uno dei due genotipi. Per identificare questi geni, sono stati utilizzati diagrammi di Venn (§3.7.5). In entrambi i tessuti il numero di geni sovra-espressi è stato generalmente maggiore rispetto a quello misurato per i geni sotto-espressi. Nelle piante *abi1* il numero di geni differenzialmente espressi è stato molto superiore rispetto a quelli presenti nelle piante wild-type. In particolare nella zona di sviluppo delle radici laterali è stato sovra-espresso un numero di geni molto elevato (2554). Questo risultato supporta ancora l'ipotesi che la punta della radice e la zona di sviluppo delle radici laterali rispondono allo stress in modo molto diverso.

I risultati dell'identificazione selettiva di singoli geni differenzialmente espressi sono riportati nelle Tabelle 6.2 e 6.3 dell'Appendice. Per capire meglio i meccanismi molecolari regolati dall'ABA, sono stati identificati i geni coinvolti nei processi dipendenti ed indipendenti dall'ABA. Sono stati identificati inoltre i geni coinvolti nel controllo orchestrato della risposta ormonale, per scoprire meccanismi di *cross-talk* tra l'ABA e altri ormoni. L'identificazione di questi geni differenzialmente espressi suggerisce una possibile funzione dell'ABA nella regolazione delle proteine DELLA, a loro volta implicate nel controllo della crescita delle piante. L'espressione differenziale di geni coinvolti nella regolazione dei livelli dei fitormoni auxine e gibberelline in risposta al sorbitolo indica la possibile esistenza di meccanismi di compensazione per fronteggiare la carenza idrica in modo indipendente dall'azione dell'ABA. Infine, sono stati identificati alcuni tra gli *stress genes*. Tra questi, sono presenti i geni coinvolti nella disintossicazione dai ROS (*reactive oxygen species*), geni che partecipano alla biosintesi o resistenza strutturale delle pareti cellulari, e geni implicati nella trasformazione di amido a glucosio. Nella punta delle radici gli *stress genes* sono repressi nelle piante wild-type, ma indotti nelle piante *abi1*, suggerendo una correlazione tra i meccanismi di *stress avoidance* e *ensured growth*. L'identificazione di geni coinvolti nella parete cellulare conferma la grande differenza nel livello di stress provato dalla punta delle radici e dalla zona di sviluppo delle radici laterali, mentre i geni coinvolti nel metabolismo dell'amido indicano che l'ABA potrebbe influenzare la regolazione di questo processo.

Concludendo, questa tesi sperimentale ha identificato nelle radici di pioppo chel'ABA è un componente fondamentale nella regolazione genica dei meccanismi di *stress avoidance* in risposta alla carenza idrica. Questo studio pone le basi per caratterizzare ulteriormente la risposta alla carenza idrica nel pioppo, e per approfondire i cambiamenti morfologici e nell'espressione genica causati dall'ABA.

Per approfondire i cambiamenti morfologici e i processi fisiologici che accadono nelle radici che soffrono di carenza idrica saranno necessari studi al microscopio più dettagliati: che presentino più di una replica, che analizzino diversi tessuti e che evidenzino le reazioni biologiche che avvengono nei tessuti. Per proporre un modello dei meccanismi molecolari nei pioppi, saranno necessari studi con altri mutanti (*knock out mutants*), per esempio mutanti in *abi1* combinati con mutanti in proteine DELLA e in etilene.

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Symbol	Definition
ABA	abscisic acid
ABF	ABRE binding factor
abi1	mutant in ABA-insensitive 1 gene
ABRE	ABA-responsive element
ACC synthase	aminocyclopropane-1-carboxylic acid synthase
AKT1	a K^+ transporter
ANOVA	Analysis of Variance
AOX	alternative oxidase
AP2	APETALA2
APC/C	Anaphase-Promoting Complex/Cyclosome
ARCK1	a receptor-like kinase
bZIP	basic leucine zipper
Ca^{2+}	calcium ion
cADPR	Cyclic ADP-Ribose
CBL	calcineurin B-like protein
CCD	*Carotenoid Cleavage Dioxygenase*
CDK	calcium dependent protein kinase
CDKE1	Cyclin-Dependent Kinase E1
CDPK	calcium dependent protein kinase
CE	coupling element
CHLH	H subunit of the Mg Chelatase
CIPK	calcineurin B-like interacting protein kinase
CO_2	carbon dioxide
CPK	calcium dependent protein kinase
CRK36	a receptor-like kinase
DEG	Differentially Expressed Gene
ERA	Enhanced response to ABA
ERF	Ethylene Responsive Factor
FGCZ	Functional Genomic Center Zurich
GA	gibberellin
GA2 ox	GA 2-oxidase
GASA14	*GA-stimlated in Arabidopsis 14*
GO	Gene Ontology
GPCR	G-protein coupled receptor
GPCR-GTC	GPCR-type G-protein

Symbol	Definition
HDAC	histone deacetylase
HD-Zip	homeo domain-leucine zipper
K^+	Potassium ion
LEA protein	late-embiogenesis-abundant protein
LR	lateral root
MAPK	member of mitogen-activated protein kinase cascades
MAPKK	member of mitogen-activated protein kinase cascades
MAPKK	member of mitogen-activated protein kinase cascades
Mg	Magnesium
Mg	Magnesium
NAC protein	No Apical Meristem/Cut-Shaped Cotyledon protein
NADPH	oxidate form of nicotinamide adenine dinucleotide phosphate
NCED	9-cis-epoxycarotenoid dioxygenase
NO	nitric oxide
OsO_4	Osmium tetroxide
PIP	plasma membrane localized isoforms
PME	Pectine Methylesterase
PP2C	protein phosphatase 2C
Pro	Proline
PYL	a family of ABA receptors
PYR1	an ABA receptor
RCAR	an ABA receptor
RIN	RNA Integrity Number
RNA-seq	RNA-sequencing
ROS	reactive oxygen species
RPK1	a receptor-like kinase
SAUR-like family protein	Small Auxin-Up RNA-like family protein
SE	standard error
SNF1	Sucrose Non-Fermenting 1
SnRK	SNF1-type kinase
SOS1	a Na^+/H^+ antiporter involved in salt tolerance
SWI/SNF	Switch/Sucrose NonFermentable
TF	Transcription Factor
wt	wild-type
ZEP	zeaxanthin epoxidase

Chapter 1

Introduction

Due to their sessile lifestyle, plants are continuously exposed to changing environmental conditions that can potentially threaten survival. Therefore, plants have evolved complex mechanisms that enable them to accurately monitor the environment and to dynamically reprogram metabolism and growth (Claeys and Inzé, 2013). Water availability, which can be constrained by drought, salinity, or freezing, is one of the major factors limiting plant growth and development in agricultural and ecosystem settings (Boyer, 1982). The effect of water limitation will likely worsen in many regions in the coming decades due to climate change (Pennisi, 2008).

1.1 Dealing with low water availability: stress avoidance and stress tolerance

Given its importance for agriculture and ecosystems, the effects of drought on plant development have been extensively studied in the past decades. This has significantly contributed to our understanding of physiological and molecular responses of plants to water limitation, particularly in the model plant Arabidopsis and in crop plants. Mechanisms for dealing with low water availability can be divided into two major categories: stress avoidance and stress tolerance (Verslues et al., 2006; Lawlor, 2013). The aim of stress avoidance mechanisms is to balance water uptake and water loss. Water uptake is enhanced by the accumulation of solutes to lower the tissue water potential and by promoting root growth. On the other hand, water loss through evaporation is limited by closing stomata, restricting shoot growth, and accelerating leaf senescence. Stress tolerance mechanisms are aimed at protecting against cellular damage when the stress becomes too severe and stress avoidance mechanisms are no longer sufficient.

Inhibition of shoot growth, both directly through an active response and indirectly by stomatal closure, is an integral part of improving water balance and stress tolerance, aimed at ensuring plant survival by limiting water loss. However, if the stress is only temporary, limiting growth too extensively can lead to a competitive disadvantage; on the other hand, continued growth can threaten survival when water limitation turns out to be long and severe (Claeys and Inzé, 2013). Therefore, the balance between growth and survival needs to be tightly regulated, a suggestion evidenced by several observations: while altering the expression of regulators of drought responses has often succeeded in enhancing drought tolerance, at least in laboratory conditions, this usually comes at the cost of growth inhibition (Yang et al., 2010).

Moreover, genetic lines that show enhanced survival under severe stress do not exhibit improved growth under milder stress conditions, suggesting that both processes are regulated by different mechanisms (Skirycz et al., 2011b). Remarkably, most of the genes identified with a role in stress tolerance in mature tissues under severe stress conditions seem to have little effect on growth inhibition in mild drought conditions (Claeys et al., 2014).
It also has to be noted that responses to combinations of stresses cannot easily be predicted from single stress responses (Rasmussen et al., 2013). Indeed, transcriptome and metabolome responses to combined heat and severe drought, two stresses that commonly occur together, were previously found to be very different from responses to either stress alone (Rizhsky et al., 2004).

1.2 Avoidance mechanisms

In most cases, the plant's first response is to avoid low water availability. Tissue water potential and water content are maintained close to the unstressed level by increasing water uptake or limiting water loss such that the rates of water loss and water uptake remain balanced. Such a balance is achieved in the short term mainly by stomatal closure, which is a fast and actively regulated response, and not merely a consequence of altered hydraulics (Verslues et al., 2006). In the longer term, changes in root and shoot growth lead to an increased root-to-shoot ratio. Growth is much more sensitive to water limitation than photosynthesis, and as a consequence, carbohydrates often accumulate in stressed plants, showing that growth reduction is not the consequence of carbon deficit. To the contrary, growth is thought to be uncoupled from carbon availability under water-limiting conditions (Muller et al., 2011).
Tissue water storage capacity and cuticle thickness and water permeability are also of potential importance (Verslues et al., 2006).
In the case of mild water stress or water stress of a limited duration, avoidance mechanisms by themselves can be sufficient to maintain plant performance (Verslues et al., 2006). The tradeoff in this case is the loss of photosynthesis caused by reduced stomatal CO_2 uptake or a shift of resources into root growth at the expense of photosynthetic and reproductive tissue. Furthermore, these mechanisms for avoiding water loss do not themselves offer any protection from the effects of low water potential if the stress becomes more severe and the plant is no longer able to maintain a balance between water uptake and loss. In cases where low water potential cannot be avoided by altering water uptake and water loss, additional mechanisms become important in maintaining plant function (Verslues et al., 2006).

1.3 Tolerance mechanisms

If water uptake and water loss cannot be balanced, plant tissue does experience low water potential and must respond to ensure continued growth and survival. Although some „desiccation-tolerant' plants can recover from a fully air-dried state by entering in a dormant state (Oliver et al., 2000; Vicre et al., 2004), most of the plants lack this ability, thus cannot recover from a severe (approximately 50% or greater) decrease in water content. Thus, they try instead to tolerate water loss while maintaining metabolic activity. Most of the dehydration tolerance mechanisms function primarily to protect cellular structures from the effects of dehydration (Verslues et al., 2006). Cellular damage is avoided by metabolic changes and by action of protective solutes ad proteins. Several types of protective proteins, most notably dehydrins and other late-embryogenesis-abundant (LEA) proteins, are well known to accumulate in response to abiotic stress or during seed development (Close, 1997). These proteins, whose function is not fully understood, seem to act as chaperones that protect protein and membrane structures (Verslues et al., 2006). Compatible solutes can also protect protein and membrane structures under dehydration (Hincha and Hagemann, 2004). Another aspect of dehydration tolerance and of tolerance to other abiotic and biotic stresses is the control of the level of reactive oxygen species (ROS) or limitation of the damage caused by ROS (Claeys and Inzé, 2013).

Importantly, it should also not be assumed that stress avoidance and tolerance occur in a linear progression in time after the stress begins or in a linear progression from responses initiated by mild stress to those initiated by severe stress. For example, LEA and dehydrin accumulation may be initiated before significant dehydration occurs, as a way of preparing the plant for any further decrease in water content (Verslues et al., 2006).

1.4 Molecular response: the balance between growth and tolerance

Although avoidance and tolerance mechanisms are separated processes, many of the molecular events initiated by low water availability do not fit exclusively into one of the two categories. For example, accumulation of a compatible solute such as proline (Pro) may play a role in dehydration avoidance by increasing the cellular solute content and thus maintaining higher water content. At the same time, accumulation of Pro has been proposed to play a role in dehydration tolerance by protecting protein and membrane structure, regulating redox status or acting as a scavenger of ROS (Hincha and Hagemann, 2004).

Avoidance and tolerance mechanisms aim to ensure survival, but also to maintain competitiveness through continued growth: thus, there is extensive co-regulation of growth and tolerance (Claeys and Inzé, 2013).

Proline, introduced above, is an example of interplay between tolerance and growth: it is in fact transported to growing tissues to act as an energy source to support both root and shoot

growth in Arabidopsis, as Pro catabolism directly transfers electrons to the mitochondrial electron transport chain (Sharma et al., 2011). This fits the observation that an increased production or exogenous application of Pro results in higher stress tolerance and maintained growth under abiotic stress conditions (Ashraf and Foolad, 2007) and thus that mitochondria play a crucial role in orchestrating stress responses (Jacoby et al., 2011).

The role of mitochondria in regulating stress responses is also dual: alternative oxidation supplies energy for growth while maintaining redox homeostasis and thereby preventing the formation of ROS. Accordingly, plants overexpressing alternative oxidase (AOX1A) showed less growth inhibition when subjected to mild drought (Skirycz et al., 2010), while plants lacking functional AOX1A were more sensitive to combined drought and heat (Giraud et al., 2008).

Cyclin-Dependent Kinase E1 (CDKE1) was recently shown to have a role in mitochondrial retrograde signaling and AOX1a activation in response to oxidative and cold stress and was proposed to integrate environmental signals and act as a switch between growth and tolerance (Ng et al., 2013).

Furthermore, the Arabidopsis transcription factor (TF) WRKY15 regulates both cell expansion and osmotic stress tolerance through control of the mitochondrial stress response (Vanderauwera et al., 2012).

Several genes were identified that regulate both growth and tolerance to stress, with potential for independent regulation. KUP-type K^+ transporters are induced by different stresses with an osmotic component and specifically inhibit cell expansion while enhancing drought tolerance (Osakabe et al., 2013). The kinase NEK6, which is induced by severe salt stress, negatively regulates the production and signaling of the stress hormone ethylene and stimulates growth by enhancing the expression of the cyclins CYCB1;1 and CYCA3;1 while also inducing stress tolerance (Zhang et al., 2011). In rice, RSS1, a monocot-specific protein that is specifically expressed in proliferating cells and the stability of which is controlled by the Anaphase-Promoting Complex/Cyclosome (APC/C) enzyme complex, is important for maintenance of the shoot meristem under abiotic stress conditions, but is also thought to control stress tolerance responses, as its loss-of-function mutation results in the up-regulation of genes responsive to salt, drought, and cold (Ogawa et al., 2011).

The examples of coupled stress tolerance and growth modulation described here show that a flexible network of genes and processes controls the balance of survival and growth. KUP-type K^+ transporters (described above) and DELLA proteins (see §1.5.8) activate stress tolerance at the cost of growth inhibition, as it is seen in several plants (Osakabe et al., 2013). However, in order to maintain growth, other mechanisms allow more flexibility. APETALA2/Ethylene Responsive Factor (AP2/ERF)-type TFs, such as ERF6, represent nodes in the network where growth inhibition and stress tolerance diverge. At the same time,

there are factors that both promote stress tolerance and maintain growth, such as Pro, the reprogramming of mitochondrial metabolism, NEK6, and RSS1 (Claeys and Inzé, 2013).
As a final note, it should be mentioned that while many studies on stress-induced growth modulation focus on TFs, it is likely that there is also an epigenetic component to be considered here. Epigenetics is known to play a large role in the regulation of drought responses (Kim et al., 2010), partly explaining the large transcriptional reprogramming seen in response to stress.
Furthermore, microRNAs are differentially regulated by drought in proliferating and expanding leaf tissue from *Brachypodium distachyon*, a model grass species (Bertolini et al., 2013). An RNA-sequencing study of proliferating maize leaf tissue also found evidence for substantial alternative splicing, although this was in response to severe drought (Kakumanu et al., 2012).
Hormones, including abscisic acid (ABA), ethylene, and gibberellins (GAs) have been shown to play an important role in adjusting growth to water availability (Achard et al., 2006).

1.5 ABA

The phytohormone ABA is well known to serve as an endogenous messenger in the response of plants to abiotic stress, particularly to drought, cold, and salt stress (Raghavendra et al., 2010). Genetic, molecular, and biochemical studies, mainly conducted in the model plant Arabidopsis, have provided deep insights into the role of ABA in stress perception, signaling, and regulation of physiological and growth responses.

1.5.1 Biosynthesis and catabolism

ABA is a naturally occurring compound ubiquitous in plants. It is a sesquiterpenoid (15-carbon) which is partially produced via the mevalonic pathway in chloroplasts and other plastids (Finkelstein, 2013). ABA biosynthesis occurs indirectly through the production of carotenoids, which are pigments having 40 carbons produced by chloroplasts. Breakdown of these carotenoids occurs by different condensation reactions to phytoene and following desaturation steps to finally obtain lycopene. This is cyclized to either α- or β-carotene. Only β-carotene is further metabolized via zeaxanthin to violaxanthin with the key enzyme zeaxanthin epoxidase (ZEP), encoded by *ABA1*. The final plastid-localized steps in ABA biosynthesis are conversion to another C40 compound, trans-neoxanthin, isomerization of either (trans)-violaxanthin and trans-neoxanthin to their 9-cis-isomers and cleavage by 9-cis-epoxycarotenoid dioxygenase (NCED) to release the 15C compound xanthoxin. NCED is a key component as this cleavage step is rate-limiting. Thus, NCED expression is tightly regulated in response to stress or developmental signals, as well as diurnally. Xanthoxin is finally converted to ABA by a series of oxidative steps via the intermediate abscisic aldehyde.

ABA is primary synthesized in vascular tissues and transported to target tissues. This transport occurs in both xylem and phloem, permitting transport in both directions between roots and shoots (Finkelstein, 2013).
In addition to ABA synthesis, catabolism is a major mechanism for regulating ABA levels. The two major catabolism pathways known for Arabidopsis are (*i*) hydroxylation of ABA at the 8' position by P-450 type monoxygenases to give an unstable intermediate then isomerized to phaseic acid, and (*ii*) esterification of ABA to ABA-glucose ester (Finkelstein, 2013).

1.5.2 Signalling: protein phosphateses and receptors

The first Arabidopsis ABA response loci identified by mutations *ABI1* (for ABA insensitive 1) and *ABI2* were found to encode highly homologous members of the PP2C family protein phosphatases functioning as a co-receptor of the ABA receptors RCARs/PYR1/PYLs (Raghavendra et al., 2010). This family of soluble proteins is at the beginning of the so called "core ABA signaling pathway" and control ABA signaling in the cytosol and the nucleus (Raghavendra et al., 2010). Binding of ABA to RCARs/PYR1/PYLs receptors leads to inactivation of type 2C protein phosphatases such as ABI1 and ABI2 and consequently phosphorylate and activate OST1 and related Sucrose Non-Fermenting 1 (SNF1)-type kinase (SnRKs), and possibly of Ca^{2+}-dependent CPKs such as CPK23. In the presence of ABA, the phosphatase activity of the receptor is blocked. In guard cells, key targets of ABA signaling pathway are the ion channels SLAC1 and KAT1, which are activated and inhibited by OST1 action, respectively (Finkelstein, 2013). In the nucleus, key targets are the basic leucine zipper (bZIP) transcription factor ABI5 and related ABA Binding Factors (ABFs). Phosphorylated ABFs bind as dimers to the ABA-responsive cis-element (ABRE) and, in concert with other transcriptional regulators, provide the ABA-responsive transcription. ABI3 binds to ABI5 and enhances its action, whereas ABI4 and related AP2-type transcription factors target a GC-rich coupling element (CE) for optimal regulation of ABA-dependent gene expression (Raghavendra et al., 2010).
Variations in expression and affinities of the receptor and PP2C family members ultimately permit responses that vary over a wide range of ABA concentrations and cell types. In addition, the ROP11 GTPase inhibits ABA response by protecting ABI-clade PP2Cs from inactivation by the PYL9/RCAR1 receptor (Finkelstein, 2013).
The plastid-localized ABA receptor is supposed to be the H subunit of the Mg Cheletase (CHLH), which may interact with soluble proteins in the cytosol (Finkelstein, 2013). Also Mg^{2+} Chelatase complex (CHLI) contributes to ABA sensitivity, although it does not bind ABA itself. CHLH binds a group of WRKY transcription factors in the presence of ABA, thereby preventing them from moving to the nucleus where they repress expression of several

ABA-response loci, including direct repression of some ABI transcription factors (Rushton et al., 2012). However, studies on this receptor are not always consistent, suggesting that there may be complex feedback among these pathways (Finkelstein, 2013). Another class of potential receptors is G-protein coupled receptor (GPCR)-type G-proteins (GTGs), but their role is controversial (Finkelstein, 2013).

Although neither the signaling pathway initiating with the PYR/PYL/RCARs nor that for the CHLH receptors currently explains a dependence on secondary messengers for effects on gene expression, numerous studies have implicated phospholipid-derived signals, Ca^{2+}, nitric oxide (NO), cyclic ADP-ribose (cADPR), cyclic GMP, and reactive oxygen species (ROS) in ABA signaling. Several of these messengers lead to changes in cytosolic Ca^{2+} by inducing either release of Ca^{2+} from intracellular compartments or influx through plasma membrane channels. The plasma membrane channels are activated by ROS species such as H_2O_2, which is produced by nicotinamide adenine dinucleotide phosphate (NADPH) oxidases. Release from intracellular stores can be induced by phosphoinositides, sphingosine-1-phosphate, cADPR or even Ca^{2+} itself (Finkelstein, 2013).

The "core signaling pathway" described above functions in many tissues and developmental stages, but the specific receptor, phosphatase, kinase, transcription factor, etc. family members and the induced cellular responses vary with the context: none of the known loci act completely stage-specifically, and many function redundantly (Finkelstein, 2013). Furthermore, some "ABA response regulators" appear to also function in networks regulating response to sugars, salt, and most known hormones (Finkelstein, 2013).

Interestingly, the core set of repressed genes includes multiple members of the PYR/PYL/RCAR receptor family, providing mechanism to desensitize ABA response and restore homeostasis (Finkelstein, 2013).

1.5.3 Transcriptional regulators

As mentioned above, *ABI3*, *ABI4* and *ABI5* were identified as ABA response loci encoding TF of the B3-, AP2-, and bZIP- domain families, respectively (Finkelstein, 2013).

Within the AP2-domain family, ABI4 is most closely related to the Drought Response Element Binding (DREB) subfamily, but the similarity is limited to the AP2 domain. Although the DREBs mediate abiotic stress-induced expression of many of the same genes as regulated by the ABIs, DREB activity is mostly ABA-independent (Lata and Prasad, 2011; Finkelstein, 2013). Other members of ABA-independent pathways are zinc finger, homeodomain, and No Apical Meristem/Cut-Shaped Cotyledon (NAC) proteins. Additional AP2-domain family factors participating in ABA response have recently been identified on the basis of binding to a coupling element (CE1) present in many ABA-regulated promoters; most of these are most closely related to the ERF subfamily (Lee et al., 2010)

ABI5 is the only bZIP identified, but most of them are regulated by and/or mediate ABA- or stress-regulated gene expression (such as ABFs) (Choi et al., 2000).
Additional TFs involved in ABA- or stress-induced gene expression have been identified. For example, some members of the MYB and MYC (bHLH) classes (Abe et al., 1997), homeodomain-leucine zipper (HD-Zip), and WRKY factor families (Rushton et al., 2012) have also been shown to be induced by ABA or abiotic stress or to regulate stress responses.
In addition to the many TFs that participate in ABA response, numerous regulators of epigenetic effects on ABA- or stress-regulated gene expression have been identified, like histone modifying enzymes such as histone deacetylases (HDACs), polycomb group proteins and histone chaperones, modifiers of DNA methylation, and SWItch/Sucrose NonFermentable- (SWI/SNF) class regulators of nucleosome position or structure (Chinnusamy et al., 2008).

1.5.4 Other regulators

Enhanced response to ABA (ERA) is a family of negative regulators of ABA signaling. ERA1 affects meristem organization as well as ABA signaling (Andrews et al., 2010). Its potential substrates are transcription factors, GTP-binding proteins, cell cycle regulators, cell wall modifiers, and proteins implicated in cytokinin synthesis or auxin response (Galichet et al., 2008).
ERA3 is allelic to *EIN2* (Ghassemian et al., 2000), which encodes a membrane-bound putative divalent cation sensor that appears to represent a point of cross-talk between ethylene, ABA, auxin, jasmonic acid, and stress signaling (Alonso et al., 1999).
Moreover, a lot of studies on different mutants identified physiological defects phenotypes reflecting pleiotropic defects in hormonal signaling, in particular hypersensitivity to ABA.

1.5.5 Protein kinases

Several additional classes of kinases implicated in ABA and stress response have been identified: calcium dependent protein kinases (CPKs/CDPKs/CDKs) (Zhu et al., 2007), calcineurin B-like interacting protein kinases (CIPKs), and three members of mitogen-activated protein kinase cascades (MAPKs, MAPKKs and MAPKKKs) (Liu, 2012). Expression of some of these is induced by stress or ABA, whereas others are activated post-translationally. As for the SnRK, also for the CPKs and MAPKs high throughput screening has identified hundreds of potential substrates, but this is not the case of CIPKs, where only few substrates are known, such as calcium-binding regulatory partners, the calcineurin B-like proteins (CBLs), a K^+ transporter (AKT1), and a Na^+/H^+ antiporter involved in salt tolerance (SOS1) (Hashimoto et al., 2012).

Several other receptor-like kinases affecting ABA response have been identified (RPK1, ARCK1 and CRK36), but there is no evidence that any of them bind ABA (Osakabe et al., 2005; Osakabe et al., 2010; Tanaka et al., 2012).
Although the model described in §1.5.2 emphasizes inhibition of phosphatase activity and increased kinase activity, analysis of rapid ABA-induced changes in overall protein phosphorylation showed similar numbers of proteins increasing or decreasing their phosphorylation state. Whereas those with increased phosphorylation were mostly SnRK2 kinases and bZIP transcription factors, decreased phosphorylation was seen for aquaporins, several calcium related proteins, and some drought or ABA-responsive proteins (Finkelstein, 2013).

1.5.6 Gene regulation

The response to the environmental stresses drought, salinity, and cold is similar, as all these stresses impose cellular osmotic and oxidative stress. Evidence of numerous studies shows that plants respond to these stresses with major changes in gene expression. Many of these genes have been demonstrated to be regulated by ABA (Seki et al., 2002).
In particular, there are two ways of response in ABA-induced gene expression early transient (peaking at 3 hrs) and late sustained (from 10 hrs onward). Early transient response encodes regulatory proteins such as transcription factors, protein kinases and phosphatases, and an assortment of *early response to dehydration* (*erd*) genes, many of which encode proteins of unknown function (Yamaguchi-Shinozaki and Shinozaki, 2006; Fujita et al., 2011). The "late" genes are named in terms of the stresses that initially identified them, e.g. *responsive to dehydration*, *cold regulated*, *low temperature induced* and *cold induced* gene classes. These are presumed to contribute to the adaptive aspects of induced tolerance since many of them encode proteins that are structurally similar to some of the LEA proteins, while others encode proteases, presumed chaperonins, enzymes of sugar or other compatible solute metabolism, ion and water-channel proteins, and enzymes that detoxify reactive oxygen species (Ingram and Bartels, 1996). Compatible solutes mediate osmotic adjustment and contribute to stabilizing redox balance, maintaining protein folding and acting as metabolic signals (Szabados et al., 2011). Between 1-10% of the genome has been quantified as ABA-regulated in any given experiment, with the variation reflecting both differences in experimental conditions and the stringency of the criteria used to classify genes as ABA-regulated. The ABA-repressed genes also vary across experiments, but are generally enriched for those encoding proteins required for growth, such as plasma membrane, cell wall and plastid proteins (Finkelstein, 2013).
It has been demonstrated that beside of ABA-dependent signaling pathways there are also ABA-independent signaling pathways, producing a complex array of interaction (Zeller et al.,

2009). Metabolomic studies also reflect these distinct pathways such that synthesis of branched-chain amino acids, polyamines, Pro, and saccharopine is ABA-dependent, whereas production of raffinose family oligosaccharides is ABA-independent (Urano et al., 2010). Many stress induced genes contain binding sites for multiple classes of regulators, e.g. ABREs bound by bZIPs and DRE-like "coupling elements" bound by DREBs (both characterized in §1.5.3), facilitating interaction between ABA-dependent and ABA-independent regulation of these genes (Lee et al., 2010).

1.5.7 Stomata closure

Under drought conditions, apoplastic pH increases, resulting in greater apoplastic retention of ABA, which then functions as a root-to-shoot signal leading to reduced transpiration in leaves.

ABA regulates the transpiration rate via effects on the stomatal aperture both by promoting closure and inhibiting opening (Finkelstein, 2013). Although both effects result in closed stomata, they are not simple reversals of the same process in that they involve different ion channels regulated by different signaling mechanisms. In addition to this local control of guard cell physiology, recent studies suggest that ABA affects stomatal conductance by reducing hydraulic conductance of leaf vascular tissues, possibly by decreasing bundle sheath aquaporin expression or activity (Pantin et al., 2013).

As explained in the previous paragraphs, ABA perception in guard cells is mediated by multiple receptors. Several members of the PYR/PYL/RCAR receptor family mediate intracellular perception (Gonzalez-Guzman et al., 2012), GTG receptors are implicated in perception at the plasma membrane (Pandey et al., 2009), and also the plastid localized CHLH protein appears to regulate stomatal response (Shen et al., 2006).

ABA-induced stomata closure is mediated at least in part by transient increases in $[Ca^{2+}]_{cyt}$ which activate both slow-activating sustained (S-type) anion channels, permitting efflux of chloride and nitrate (Negi et al., 2008; Vahisalu et al., 2008; Geiger et al., 2011), and rapid transient (R-type) anion channels, permeable to malate and sulfate (Meyer et al., 2010). Efflux of phosphate via the PHO1 channel is also involved in the regulation of stomatal aperture (Zimmerli et al., 2012).

The resulting anion efflux depolarizes the plasma membrane, leading to activation of K^+_{out} channels permitting massive K^+ efflux, osmotic water loss and stomatal closure. K^+ is released from the vacuole by Ca^{2+}-activated K^+ channels in the tonoplast (Finkelstein, 2013).

In contrast to closure, ABA-inhibition of stomatal opening is mediated by inhibition (i) of K^+_{in} channels by SnRK2.6/OST1 (Sato et al., 2009) and (ii) of the plasma membrane H^+ ATPase, OST2 (Sutter et al., 2007).

1.5.8 Growth responses regulation

Although much is known about the role of ABA in gene regulation, the influence of ABA in growth regulation is less well characterized.
ABA may be involved in both direct growth inhibition and indirect growth stimulation (Tardieu et al., 2010; Wilkinson and Davies, 2010).
This view has arisen largely because of the typically inhibitory effect of ABA on shoot and root growth when it has been applied to well-watered plants. In some experiments, the resulting relationship between the ABA content of the tissues or xylem sap and the growth inhibition suggested that the increase in endogenous ABA in water stressed plants was sufficient to account for much if not all of the inhibition of growth that resulted from the water stress treatment (Creelman et al., 1990).
In maize, ABA has been implicated in root growth promotion under water deficit: when maize seedlings are grown at a water potential of 1.6 MPa, the ABA content of the root growth zone increases about 5-fold (Sharp et al., 2004). Studies using seedlings in which endogenous ABA levels were reduced either genetically or by inhibitors showed that accumulation of ABA is required for maintaining root growth (Sharp, 2002). In particular, three approaches have been used to study the effect on root growth of reducing the accumulation of ABA: (*i*) the inhibitor fluridone, which blocks carotenoid synthesis and, thereby, inhibits ABA synthesis although at an early step of the pathway; (*ii*) the vp5 mutant, which has a defect at the same step as that blocked by fluridone; (*iii*) the vp14 mutant, which has a defect in the synthesis of xanthoxin (Tan et al., 1997). Xanthoxin synthesis is considered a key regulatory step in water stress-induced ABA production, as explained in §1.5.1 (Qin and Zeevaart, 1999). The initial studies used fluridone and vp5 (Saab et al., 1990; Saab et al., 1992; Sharp et al., 1994), and studies of vp14 were undertaken to strengthen the conclusion that the results were due to ABA deficiency and not to other effects. The results obtained with the three approaches were very similar: at high water potential, root elongation rates (and ABA contents) were minimally affected. At low water potential, by contrast, reduced ABA accumulation was associated with more severe inhibition of root elongation than in wild-type or untreated seedlings. In all cases, root elongation rate fully recovered when the ABA content of the growth zone was restored to normal levels with exogenous ABA, confirming that the normal accumulation of ABA is necessary for root growth maintenance during water stress (Sharp et al., 1994). A follow-up study using the same system indicated that the promotive effect of ABA is a result of ABA interaction with ethylene, whereby ABA is restricting ethylene production (Spollen et al., 2000).
It seems likely that ABA and ethylene are linked to the so-called GA and DELLA protein pathway, a regulatory system known to play a key role in stress-induced growth inhibition.

GAs have been extensively studied, mainly in the shoot. It has been demonstrated that GAs play a key role in growth regulation both under optimal and stress conditions, by regulating levels of DELLA proteins, a family of nucleus localized proteins, known to inhibit growth (Achard et al., 2009). DELLA proteins were shown to be crucial integrators controlling growth and survival in response to various stresses, such as low temperature and high salinity. Under stress conditions, production of the catabolic enzymes GA 2-oxidases (GA2ox) reduces GA levels, which in turn results in DELLA protein stabilization, leading to growth repression (Magome et al., 2008). DELLA stabilization following severe salt stress results in the activation of many genes that protect cells from cellular damage, such as ROS-inactivating enzymes, and it was proposed that lowering ROS levels both enhances stress tolerance and limits cell expansion and thereby root growth (Achard et al., 2008a). Consequently, quadruple DELLA mutants of Arabidopsis, lacking the four major DELLAs, are less tolerant to severe salt stress when survival is scored, but show less growth inhibition (Achard et al., 2006). *GA-Stimulated in Arabidopsis 14* (*GASA14*) was recently suggested to be a downstream mediator of DELLAs in tolerance and growth regulation control through ROS; it is a GA-regulated gene that stimulates cell expansion and induces tolerance to severe abiotic stress by limiting ROS accumulation, potentially because the protein exhibits redox activity (Sun et al., 2013).
However, there is a level of regulation upstream of DELLAs suggesting that stress tolerance and growth responses can be separately regulated (as mentioned in §1.4): ERF6 stimulates the inactivation of GAs by 2-oxidation and thereby induces the stabilization of DELLAs, which inhibit cell proliferation and expansion (Dubois et al., 2013). However, ERF6 also activates stress tolerance genes such as WRKY33, MYB51, and STZ, and this is independent of DELLAs (Dubois et al., 2013). Additionally, ERF6 was also shown to provide a protective role against oxidative stress (Wang et al., 2013) and biotic stress (Meng et al., 2013). For cold stress, a similar pathway was established in which CBF1 is the functional equivalent of ERF6, leading to DELLA-dependent growth inhibition by up-regulation of GA2OX3 and GA2OX6 and DELLA-independent stress tolerance (Achard et al., 2008b). Similarly, in response to high salinity, DDF1 directly activates the transcription of GA2OX7, leading to a decrease in GA levels and subsequent growth inhibition, and stress tolerance genes such as RD29A (Magome et al., 2008). Finally, when AtDREB1A, a master regulator of drought tolerance, is overexpressed in soybean, up-regulation of GA2OX4 leads to a drop in GA levels and subsequent growth inhibition, which can be reversed by GA application (Suo et al., 2012).
All these observations point to a common mechanism in which stress-specific AP2/ERF-type TFs induce GA inactivation to regulate growth and independently activate stress tolerance genes (Claeys and Inzé, 2013).
Another study using transcript profiling of proliferating and expanding leaf tissue from Arabidopsis plants exposed to mild osmotic stress revealed a role for ethylene and GAs in acclimation to both short-term and long-term mild drought stress (Skirycz et al., 2010;

Skirycz et al., 2011a). This important role for GAs in growth regulation was corroborated by other studies that profiled leaf tissue at different developmental stages in *Brachypodium distachyon* and maize subjected to mild drought (Verelst et al., 2013).
The roles of ABA and ethylene are less clear, but seem to enhance the inhibitory effect of DELLA proteins on root growth, as evidenced in salt-treated Arabidopsis (Achard et al., 2006). Thus, further studies in this direction are needed.

1.6 Poplar as a model tree

Most of the studies on responses to drought stress and its regulation reported in §1.5 have been conducted in Arabidopsis or other model organisms. It is important to notice that species dependent features shape the transcriptome response to drought stress; almost none of the 27 genes reliably responsive to water stress in Arabidopsis were regulated under drought in poplar and pine (Bray, 2004; Fladung, 2006). These results can be transferred to other species only with incontestable limitation, thus studies in other species are needed.
Forest trees have tremendous economic and ecological value, as well as unique biological properties of basic scientific interest because of their long generation time. The inherent difficulties of experimenting on very large long-lived organisms motivate the development of model system for forest trees (Taylor, 2002). Poplar (*Populus)* is a well-established model for forest trees due to its rapid growth, ease of vegetative propagation, and genetic transformation. The genome of *Populus trichocarpa* is the first forest tree genome fully sequenced (Tuskan et al., 2006). Recently the genome of *Populus euphratica* was also deciphered (Ma et al., 2013). The genome of these two species turned out to be relatively small, with 485 Mbp (*Populus trichocarpa*) and 496Mbp (*Populus euphratica*), approx. 5 times larger than that of Arabidopsis. Moreover, the genus *Populus* includes a wide variety of species (about 30) from different areas around the world displaying a range of different growth characteristics and tolerance toward various stress condition.

1.7 Poplar, drought and ABA

Abiotic stress factors, especially drought, restrict plant biomass production, although our current understanding remains limited, especially for woody plants. Poplars are known to be sensitive to water deprivation as compared with other trees, but drought tolerance varies considerably between genotypes of *Populus*, suggesting that the genus provides a good model in which to investigate the molecular and genetic basis of traits associated with drought tolerance Wilkins et al. (2009).
A gradual (4 weeks) soil depletion study in *Populus euphratica* (a poplar growing in arid regions) showed early inhibition of shoot growth, whereas root growth was longer maintained

(Bogeat-Triboulot et al., 2007). This change in growth allocation in favor to roots resulted in an increase of the root-to-shoot ratio.
Another study investigated different morphological and physiological responses of two poplar species (*Populus kangdingensis* and *Populus cathayana*) to exogenous ABA application under well-watered and water-stressed conditions (Yin et al., 2004). For both species ABA application significantly decreased shoot growth, total biomass and total leaf area and significantly increased the root-to-shoot ratio for both well-watered and stressed plants.

1.7.1 Gene expression in poplar under drought and salt stress

Recently, several studies have been performed to analyze transcriptome changes of poplar species and/or genotypes exposed to drought stress and salt stress (e.g.:Wilkins et al. (2009); Cohen et al. (2010)). Although these studies revealed a number of conserved transcriptome level changes between different species and/or genotypes, there are many more changes that appear to be specific to the drought response on one or the other genotype. Morover, some studies show that drought-induced transcriptome changes are dependent on the time of the day at which they were measured (Wilkins et al., 2009).
The study by Cohen et al. (2010) provides a meta-analysis of genome-wide expression profiling in different tissues (mature leaves and root apices) across two different *Populus* genotypes. Results indicated organ-contrasting transcriptome responses to drought, and the leaf transcriptome appeared less drought-responsive than the one of root apices, which might reflect, in part, the higher sensitivity of an actively growing tissue to water deprivation. The generic response in roots involved genes that were related to ABA biosynthesis/signaling, cell rescue and/or cell redox homeostasis, and the response to hypoxia. As expected for actively growing organs, stress impacted recurrent groups of genes that were involved in expansion or in meristematic activity and cell cycle.
Additionally, poplar response to drought was found to imply cross-talk between hormonal pathways.
In this context, the DELLA pathway explained in §1.5.8 (Achard 2006) was also characterized in shoots of *Populus* (Zawaski and Busov, 2014). The authors report whole-genome microarray, physiological and transgenic evidence in hybrid poplar (*Populus tremula* x *Populus alba*) showing that gibberellin (GA) catabolism and repressive signaling mediates shoot growth inhibition and physiological adaptation in response to drought: poplar transgenic with up-regulated GA2ox and DELLA domain proteins showed hypersensitive growth inhibition and greater drought resistance in response to drought. GA2oxs are catabolic enzymes that reduce GA levels, which in turn results in DELLA protein stabilization, leading to growth repression and activation of many genes that protect cells from cellular damage, as widely discussed in §1.5.8.

This study also indicated that ABA is involved in drought-induced shoot growth inhibition, however genetic evidence has so far not been provided.

1.7.2 Growth response of ABA-insensitive poplar line under non-stress conditions

Genetic evidence that ABA is a source of growth control comes from a study analyzing a transgenic poplar line which ectopically expresses the mutant Arabidopsis *abi1* gene (Arend et al., 2009). Over-expression of this gene resulted in an ABA-insensitive phenotype revealed by a strong tendency of *abi1* plants to wilt, impaired responsiveness of their stomata to ABA, and an ABA-resistant bud outgrowth. The *abi1* plants also showed increased stomatal size, enhanced shoot growth, and retarded leaf and root development. The increased stomatal size and its reversion to the size of wild-type plants by exogenous ABA indicate a role for ABA in regulating stomatal development. Enhanced shoot growth and retarded leaf and root development support the hypothesis that ABA acts independently from drought stress as a negative regulator of growth in shoots and as a positive regulator of growth in leaves and roots. In shoots, an interaction of ABA with ethylene was observed: *abi1* plants exhibited elevated ethylene production indicating that ABA acts as negative regulator of shoot growth in nonstressed poplars by restricting ethylene production. Furthermore, it has been shown that ABA has a role in regulating shoot branching by inhibiting lateral bud outgrowth.

1.8 Objective of the study

In this study I take advantage of the *abi1* gray poplar (*Populus* x *canescens*) line previously characterized (§1.7.2) to investigate the role of ABA in controlling growth and gene expression under water deficit conditions.

The *abi1* line is well suited for such a study for several reasons. Genetic modification of ABA levels and/or sensitivity is superior to ABA treatment experiments. However, experiments with this line is challenging, because stomata closure regulation is compromised. These plants therefore required cultivation under very humid conditions to prevent drought stress symptoms.

To examine the role of ABA in controlling growth and gene expression under water deficit, *abi1* and wild-type plants were treated with sorbitol, a osmoticum lowering the water potential of the medium, and thus making it harder for plants to take up water. Changes in gene expression were analysed through RNA-sequencing.

The following main questions were addressed:

(i) Is ABA involved in the control of differential root and shoot growth in response to water deficit?

(ii) Does water deficit induce root and shoot morphological changes and is this response ABA-mediated?

(iii) Does ABA-action result in reduced water deficit as inferred from transcriptome responses?

Chapter 2

Materials and methods

2.1 Plant Material

The Gray poplar (*Populus* × *canescens*) *abi1-1* line and the corresponding wild-type (wt) line (INRA clone 717-B4) were provided by Matthias Arend. The two genotypes were maintained *in vitro* in OS140 culture vessels (Duchefa, Haarlem, Netherlands) containing 150 ml of autoclaved one-half-strength Murashige & Skoog (1964) salts (Duchefa, Haarlem, Netherlands; pH 5.6), 1% (w/v) sucrose (Sigma-Aldrich, St. Louis, MI, USA), and 0.8% (w/v) agar (Sigma-Aldrich) (referred to as basal medium). The plants were cultivated in a shaded greenhouse equipped with daylight bulbs under a 16-h day / 8-h night regime at 23±2°C. The plants were multiplied every 6-8 weeks.

2.2 Treatment experiments

To examine growth effects of ABA under water deficit, *abi1* and wt plants were treated with 300 mM sorbitol. This artificial set-up of simulated drought has several practical advantages: it offers (*i*) tight control of stress level and onset, (*ii*) low variability, and (*iii*) the ability to grow many plants using limited space (Verslues et al., 2006; Lawlor, 2013). The optimal sorbitol concentration was determined in two preliminary experiments using growth responses and water potential as an indicator of water stress (Matthias Arend and Christoph Sperisen, personal communication). Treatment experiments were carried out under sterile conditions in 1360 ml TPP cell culture flasks (Techno Plastic Products, Trasadingen, Switzerland) with caps containing a filter of 0.22 μm pore size (TPP filter caps) for gas exchange. The culture flasks were filled with 650 ml of basal medium with and without 300 mM sorbitol (Carl Roth GmbH, Karlsruhe, Germany). Following solidification, the medium was overlaid with 150 ml of basal medium to allow root development under non-stress conditions for some days. Treatment experiments were carried out in two steps. First, cuttings containing the apex and 1–2 leaves were cultured in OS140 culture vessels containing 150 ml of basal medium to initiate root formation. Following a one-week pre-culture period, the cuttings were transferred to the treatment culture flasks, prepared the day before transfer of the plants. Each culture flask contained two plants (Figure 2.1). The plants were cultivated in a greenhouse under conditions described above for 27 days, when the largest plants reached the top of the culture flasks. At the end of the experiment, plants were carefully retrieved from the culture flasks

with tweezers and used for water potential measurements, biomass determinations, microscopy analyses, and/or RNA isolation.

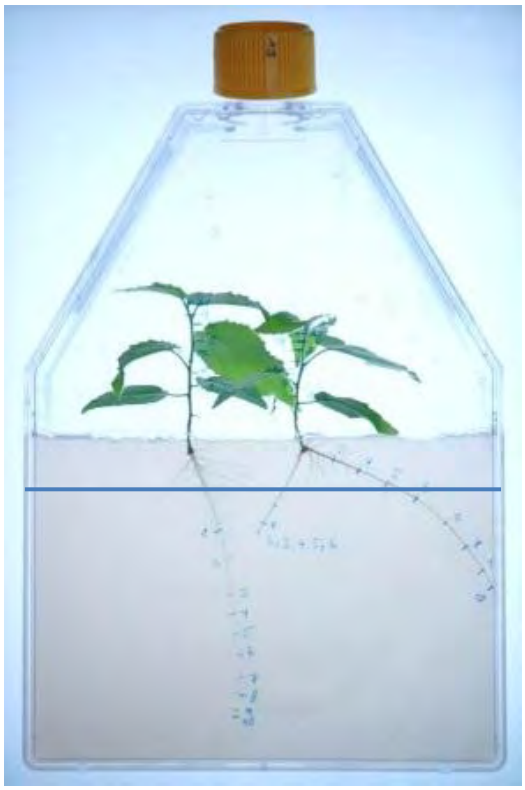


Figure 2.1. Example of TPP cell culture flask with two poplar plants. The blue line visualises the divide between the treatment medium with sorbitol (bottom) and the medium without sorbitol (top).

Three experiments were carried in this study. The goal of experiment 1 was to test whether ABA is involved in the control of altered biomass allocation to roots and shoots under osmotic stress. The experiment was carried out by Christoph Sperisen and Matthias Arend in October 2013. It is included in this study because the data are analysed jointly with those of the other experiments. The experiment was composed of a total of 24 culture vessels representing six biological replicates for each genotype and treatment. Experiment 2 served for microscopy (§2.3) and molecular analyses (§2.4). It was carried out in February 2014 and included seven biological replicates per genotype and treatment. In experiment 3, it was tested whether ABA influences the emission of ethylene. The experiment was carried out in April 2014 and included seven biological replicates per genotype and treatment. In each of the experiments, various growth parameters were assessed, which are summarized in Table 2.1.

Table 2.1. Summary of experiments carried out in this study and the parameters assessed.

	Experiment 1	**Experiment 2**	**Experiment 3**
Aim of experiment	to test effect of ABA on biomass allocation to roots and shoots under osmotic stress	RNA-seq and microscopy analyses	to test effect of ABA on ethylene emission
Biological replicates (culture flasks)	6 (24)	7 (28)	7 (28)
Duration of treatment (d)	27	27	27
Treatment	300 mM sorbitol	300 mM sorbitol	300 mM sorbitole
Water potential (MPa)	yes	n.a.	yes
Biomass (mg)	yes	n.a.	yes
Shoot elongation rate (mm/d)	yes	yes	yes
Primary root elongation rate (mm/d)	yes	yes	yes
Length of shoot (mm)	yes	yes	yes
Length of longest primary root (mm)	yes	yes	yes
Length of longest lateral root (mm)	yes	yes	yes
Lateral root density (mm)	yes	yes	yes
Ethylene emission (ppm dry weigt^{-1}h^{-1})	n.a.	n.a.	yes

2.2.1 Water Potential measurement

To verify the effectiveness of the sorbitol treatment, midday water potentials were measured in excised shoots using a Scholander pressure bomb (Scholander et al., 1964). Its application is schematically presented in Figure 2.2.

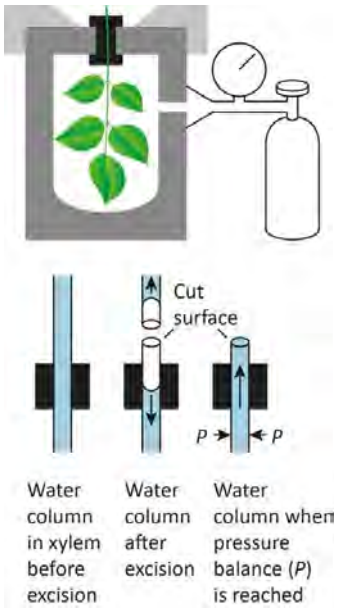


Figure 2.2. Draft of Scholander pressure bomb (top) and graphical explanation of water potential calculation (bottom) (redrawn by Christoph Sperisen from Taiz & Zeiger, Plant Physiology online)

Shoots were separated from the roots with a razor blade and fitted in the rubber compression gland in the lid of the pressure cylinder. Pressurised nitrogen was slowly added to the pressure cylinder until sap was forced out of the xylem, visible at the cut end of the stem. The pressure that was required to do so is equal and opposite to the water potential of the shoot.

2.2.2 Growth analyses

During the course of the treatment, root and shoot elongation was monitored at two day intervals through marking the position of the root and shoot apex on the surface of the culture flasks (starting at day 11 from the beginning of the treatment). Elongation rates were estimated by dividing length increments by the time elapsed. Other growth parameters were assessed at the end of the experiment, either directly on the culture flasks or on digital images taken the day before harvesting. The parameters assessed were shoot length, length of longest primary root, diameter of longest primary root measured 0.5 cm from the root tip, length of the longest lateral root, and lateral root density. The latter parameter was calculated by dividing the number of lateral roots by the length of the root zone with lateral roots (Dubrovsky and Forde, 2012). Length measurements on the culture flasks were carried out with a ruler, those on images with the software *WinRhizo Tron* v. 2012b software (Regent Instruments, Sainte-Foy-Sillery-Cap-Rouge, Quebec, Canada) (Table 2.2). Prior to biomass determination, agar that adhered to the roots was carefully manually removed using gloves. Roots, leaves, and the stems were analysed separately. Dry weight was measured by placing the tissues into folded weighing papers or small aluminium foil pouches. Samples were dried in an oven at 60 °C for two days. To avoid differences in moisture absorption during weighing, all samples were allowed to absorb air moisture for two days. The tissues were then weighed on a laboratory scale AE240 balance (Mettler Toledo, Greifensee, Switzerland).

Table 2.2. Techniques used for measuring growth parameters. Culture flask stands for measurement with ruler (in millimetres) on the culture flask's surface; image stands for measurement on digital photographs of culture flasks using *WinRhizo Tron* software in millimetres.

Growth parameters	Experiment 1	Experiment 2	Experiment 3
Length increments of shoot	culture flask	culture flask	culture flask
Length increments of primary roots	culture flask	culture flask	culture flask
Shoot length	image	image	culture flask
Length of longest primary root	image	image	culture flask
Diameter of longest primary root	image	image	image
Length of longest lateral root	image	image	culture flask
Number of lateral roots	image	culture flask	culture flask
Length of root zone with lateral roots	image	culture flask	culture flask

2.2.3 Ethylene emission

Samples for ethylene quantification were collected two days before the end of experiment 3. Culture flasks were ventilated in a laminar flow through evacuation of the gas with a sterile pipette connected to a vacuum pump (for 30 sec per culture flask). Following ventilation, the culture flasks were closed with an air tight cap (Techno Plastic Products), in which a septum was installed (taken from 11 ml Vacutainer tubes; BD, Franklin Lakes, NJ USA), and the plants were incubated for 24 hours under the conditions described above to let ethylene accumulate. Using a 100 ml syringe, 50 ml of gas was collected, and half of that volume was transferred to a second 100 ml syringe. The gas of each syringe was then injected into pre-evacuated containers and analysed for ethylene and methane in co-operation with Pascal Niklaus of the Institute of Evolutionary Biology and Environmental Studies (University of Zurich) using an Agilent 7890 gas chromatograph with a flame ionization and an electron-capture detector (Agilent Technologies, Santa Clara, CA, USA). The analysis ran with a 12 ft Porapak Q column and helium as carrier gas, isotheric at 80 °C. Ethylene was quantified in ppb relative to methane present in the atmosphere (1.8 ppm). Ethylene emission was expressed as the rate of ethylene emitted per dry weight of the entire plant and hour.

2.2.4 Statistical analysis

Statistical analyses were carried out with means of the two plants cultivated in the same culture vessel unless one of the two plants was excluded from the analyses for the following reasons: (*i*) plant did not root, (*ii*) plant formed two or more shoots, (*iii*) root growth started significantly later (> 5 days) than all other plants or stopped without forming a new primary root. All comparisons of parameter means were analysed with ANOVA after testing normality (Shapiro-Wilk and Kolmogorov-Smirnov tests) and homogeneity of variances (Levene test). Differences between means were considered significant when the *p*-value was smaller than 0.05, and Tukey post-hoc tests were performed to investigate significant differences. If homogeneity of variances was violated, Welch ANOVA was performed and, in case of significant differences, Games-Howell post-hoc tests were run. All statistical analyses were carried out with *SPSS* v. 22 software (IBM, Armonk, NY, USA.), and were run separately for each experiment. If significant differences between experiments were found, effect size was calculated to investigate the biological meaning of the variability between experiments.

2.3 Microscopy

Microscopy analyses were carried out with plants of experiment 2. The analyses were carried out with the support of Terence Menard (WSL). The tips of primary roots, squared portions of leaf surfaces, and stem sections of each genotype and treatment were fixed in phosphate

buffered (Sörensen, pH 7) glutaraldehyde (2.5%), rinsed in phosphate buffer (Sörensen, pH 7; 6 times for 15 min), and post fixed overnight in a mixture composed of equal volumes of phosphate buffer and 2% OsO_4. Rinsed samples were gradually dehydrated in ethanol (25%, 50%, 70%, and 90% ethanol each for 20 min, absolute ethanol three times for 30 min), embedded in Epon according to the protocol of Crèvecoeur (see Appendix: §6.1, provided by Terence Menard), and then sectioned on an Ultracut microtome (Leica Microsystems, Wetzlar, Germany) at a thickness of 1.50 µm. Cross sections of stem tissue and longitudinal sections of root tips and leaf blades were then stained in 1% Toluidin blue. The sections were photographed on a Leica Leitz DMRB Infinity 2 microscope.

2.4 Transcriptome analyses

2.4.1 RNA isolation

RNA was isolated from plants of experiment 2. The sampling was done all in one day between 10 a.m. and 1:30 p.m. to minimize possible effects of diurnal fluctuations in ABA content and signalling that allow plants to anticipate timed fluctuations in light, temperature and water status (Finkelstein, 2013). Roots separated from the shoot were rinsed in autoclaved basal medium lacking agar. Each RNA sample was composed of tissue from two plants, cultivated in the same culture flask. Using tweezers with their tips wrapped with silicon band (to prevent sticking of the tissues to the tweezers), the following tissues were sampled: (*i*) root tip (first 0.5 cm of the primary root), (*ii*) lateral root formation zone (approx. 1-2 cm, depending on the genotype and treatment), with both tissues collected in sterile Eppendorf tubes. One (*iii*) fully unfolded leaf (collected in aluminium foil), (*iv*) shoot apex (in a sterile Eppendorf tube), and (*v*) shoot stem (in aluminium foil) were also sampled, but not considered for RNA-seq analyses at the moment, as ABA mainly affects root growth. All tissues were immediately frozen in liquid nitrogen and stored at -80°C until RNA isolation. RNA extraction and purification were performed with the Agilent Plant RNA Mini Isolation Kit with modifications (Appendix: §6.3). Total RNA was quantified with Nanodrop 1000 Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and Qubit 1.0 Fluorometer (Life Technologies, CA, USA), and the physical integrity of the RNA was examined on Agilent Bioanalyzer 2100 (at WSL) and Agilent 2200 TapeStation (at Functional Genomic Center Zurich, FGCZ) instruments, with no signs of RNA degradation detected (Appendix: §6.4). Three biological replicates for each genotype and treatment combination were selected for further steps, based on the RNA Integrity Number (RIN), RNA concentration, and the time it took for sampling root tissues until freezing.

2.4.2 Construction of cDNA libraries

Complementary DNA (cDNA) libraries were constructed by Anna Bratus (FGCZ) using the TruSeq Standed mRNA HT Sample Prep Kit (Illumina, Sant Diego, CA, USA). Total RNA (500 ng) isolated from 24 samples (12 from root tips and 12 from lateral root development zones) was polyA-enriched and then reverse-transcribed into double-stranded cDNA. The cDNA samples were fragmented, end-repaired, and polyadenylated, followed by ligation of TruSeq adapters containing indexes for multiplexing. Fragments containing TrueSeq adapters on both ends were selectively enriched with PCR. The enriched libraries were quantified on a Qubit 1.0 Fluorometer and their quality was examined on the Agilent 2200 TapeStation, with all libraries banding at 260 bp. The individual libraries were adjusted to 10 nM concentrations and pooled according to Illumina's instructions.

2.4.3 Cluster generation and sequencing

The TruSeq PE Cluster Kit v3-cBot-HS (Illumina) was used for cluster generation using 10 pM of pooled libraries. The index-coded libraries were spread over three Illumina HiSeq 2000 lanes to assure the same instrument variation for the entire experiment. Sequencing was performed on an Illumina HiSeq 2000 instrument using the TrueSeq SBS Kit v3-HS (Illumina) with 101 bp paired-end reads.

2.4.4 Quality check and trimming of HiSeq reads

Quality checks of the Illumina HiSeq reads and subsequent bioinformatic analyses (§2.4.4-§2.4.7) were carried out by Masaomi Hatakeyama (FGCZ). The quality of the reads was examined with *FastQC* v.0.10.1 (bioinformatics.babraham.ac.uk/projects/fastqc/). Given their high quality, all reads were kept for further analyses. *FastQC* was also used to trim adapter sequences. In addition, the first left nucleotide was trimmed since HiSeq Illumina data have a GC bias in the first nucleotide, with C more often added (unpublished data). *FastqScreen* v.0.4.2 (bioinformatics.babraham.ac.uk/projects/fastq_screen/) was used for identifying rRNA contamination. Three of the samples had some more rRNA contaminants than the others, revealed by *STAR* v. 2.3.0 (Dobin et al., 2013) mapping results.

2.4.5 Mapping HiSeq reads to Populus trichocarpa genome

The trimmed HiSeq reads were mapped to the *Populus trichocarpa* genome sequence v.3.0 (phytozome.jgi.doe.gov/pz/portal.html#!info?alias=Org_Ptrichocarpa; (Tuskan et al., 2006)) using *STAR* v.2.3.0(Dobin et al., 2013). *STAR* was preferred over other RNA sequencing aligners due to its mapping speed and high sensitivity settings. The software was configured as follows: the minimum number of matched bases (outFilterMatchNmin) was set to 30,

maximum number of mismatches (outFilterMismatchNmax) was 5 in order to account for sequence differences between *Populus* × *canescens* and *Populus trichocarpa*, maximum ratio of mismatches to mapped length (outFilterMismatchNoverLmax) was 0.05, and for each read multiple mappings of up to 50 were allowed. For all other parameters default settings were used. The htseq-count command from the *HTSeq v.05.4p5* software (Anders et al., 2014) was used to count the reads that uniquely mapped to a given *Populus trichocarpa* gene model. Reads that were assigned to more than one gene model were discarded.

2.4.6 Differential gene expression analyses

Differential expression analyses were performed in *Bioconductor* (Gentleman et al., 2004) using the *edgeR v.3.6.4* package (Robinson et al., 2010). Differentially expressed genes were determined for the following pairs of experimental treatments: (*i*) wt root tips treated with sorbitol over control wt root tips, (*ii*) *abi1* root tips treated with sorbitol over control *abi1* root tips, (*iii*) wt lateral root development zone treated with sorbitol over control wt lateral root development zone, and (*iv*) *abi1* lateral root development zone treated with sorbitol over control *abi1* lateral root development zone. To normalize the read counts according to the library size of each sample, the trimmed mean of M-values (TMM) normalization method was used. After fitting the data to a generalized linear model (GLM), contrasts between experimental treatments (see above) were defined and tested for significant expression differences using a likelihood ratio test. Genes whose total read count was less than 10 in each treatment pair were discarded. To correct for multiple testing, false discovery rate (FDR) corrected *p*-values were computed using Benjamini-Hochberg method. Genes which had an FDR-adjusted *p* value equal or less than 0.05 and a fold change equal or higher than 2.00 were considered as differentially expressed in a given pair of treatments.

2.4.7 Gene ontology enrichment analyses

To obtain a global view of gene expression changes, gene ontology (GO) enrichment analyses were carried out using *goseq* v.1.14.0 (Young et al., 2010). Gene annotations and associated GO terms were taken from the *Populus trichocarpa* genome sequence v.3.0 database (Ptrichocarpa_210_v3.0.gene.gff3). The GO terms are automated results from *interpor2go* and thus not empirically derived (Ptrichocarpa_210_v3.0.readme.txt). Differentially expressed genes were split into up- and down-regulated groups and a separate *goseq* enrichment test was applied for each set of pairwise experimental treatments (see above). *P* values were obtained by approximating Wallenius' non-central hypergeometric distribution, compensating for over-detection of differential expression for long and highly expressed transcripts. The resulting *p* values were FDR corrected using the Benjamini-Hochberg

method. Significantly enriched GO terms were identified as those that showed a corrected *p* value equal or smaller than 0.05

Chapter 3

Results

In each experiment of this study, wild-type (wt) and *abi1* plants were treated with and without 300 mM of sorbitol for 27 days, when the largest plants reached the top of the culture flasks (Figure 3.1).

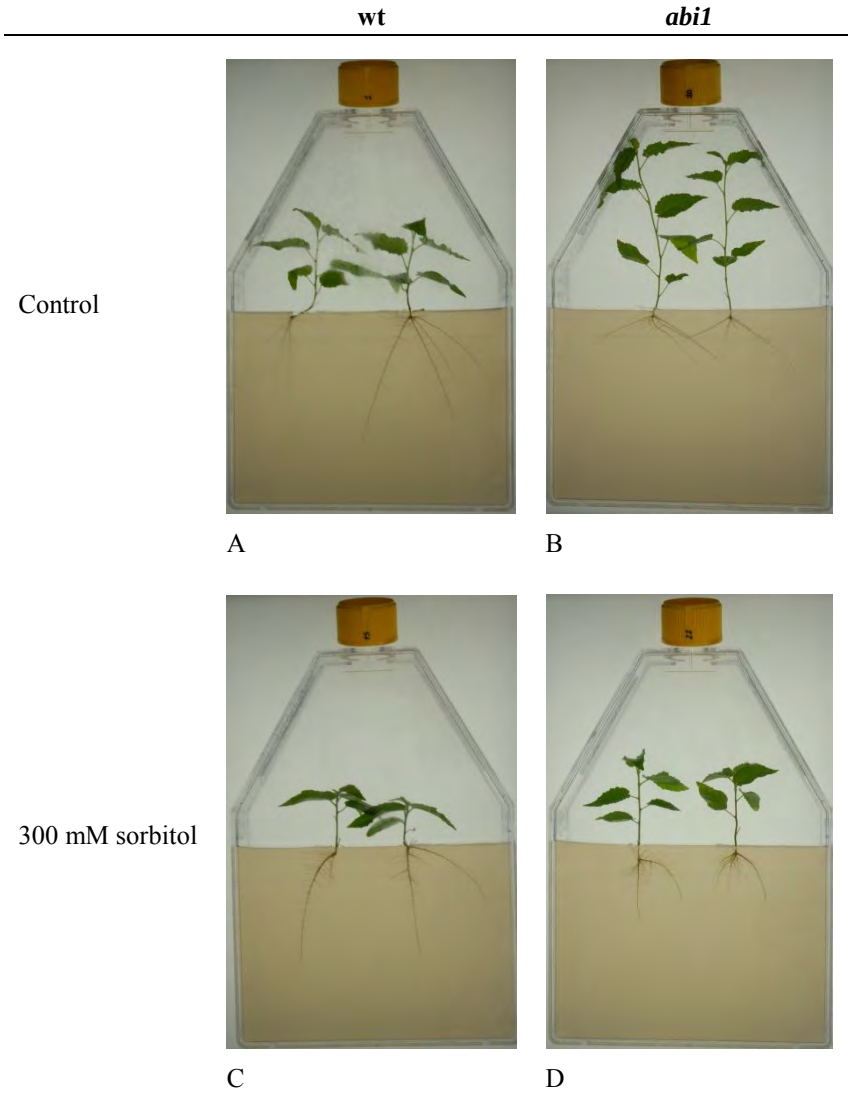


Figure 3.1. Wild-type (wt) and *abi1* plants treated with and without 300 mM sorbitol. Pictures were taken after 27 days of treatment.

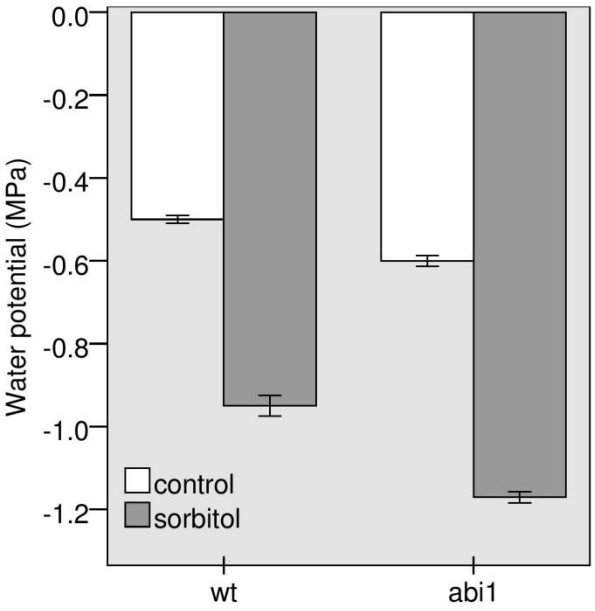
Visual inspection of the plants showed that under control condition, *abi1* plants had longer shoots (with longer internodes), smaller leaves, and a smaller root system than wt plants. Under sorbitol, the shoot growth was inhibited and the leaves were darker green in both

genotypes. The root growth was also inhibited in both genotypes, yet the roots of wild-type plants seemed to be thicker.

In the three experiments performed, 20 of the totally 212 treated plants were excluded from analysis (for criteria see Materials and Methods §2.2.4). It has to be noted that 14 of the excluded plants were grown in sorbitol (Table 6.1 of Appendix). Most of these plants were excluded due to poor root formation.

3.1 Water Potential

To examine the effectiveness of the sorbitol treatment, midday water potentials were measured using the Scholander bomb. The water potential measured after 27 days showed a clear treatment effect (Figure 3.2). Exposure to sorbitol resulted in a significant decrease in water potential in both genotypes, confirming the effectiveness of the treatment. Data is presented for experiment 1. The results of experiment 3, in which the same parameter was measured, were comparable.



Descriptive statistics		N	Mean	SE
wt	control	6	0.5	0.009
	sorbitol	6	0.95	0.025
abi1	control	6	0.6	0.013
	sorbitol	6	0.8	0.014

Tests		*p* value
Normality (Kolmogorov-Smirnov)		> 0.05
Homogenity of variances (Levene)		0.025
Robust ANOVA (Welch)	F(3,10.691) = 546,673	< 0.0005

Post hoc (Games-Howell)	Difference	95% CI	*p* value
wt_sor - wt_con	0.45	0.36-0.54	0.000
*abi1*_sor - *abi1*_con	0.57	0.51-0.63	0.000
*abi1*_con - wt_con	0.1	0.05-0.15	0.001
*abi1*_sor - wt_sor	0.22	0.13-0.31	0.000

Figure 3.2. Water potential (MPa) measured for wild-type (wt) and *abi1* plants under control (white) and sorbitol (grey) conditions. Values are means ± SE. Only significant post hoc tests are reported ($p < 0.05$).

A small, but significant difference between the two genotypes under both conditions was observed: the water potential of *abi1* plants was slightly lower than that of wt plants. Given the strong treatment effect, this difference was considered acceptable for subsequent analysis.

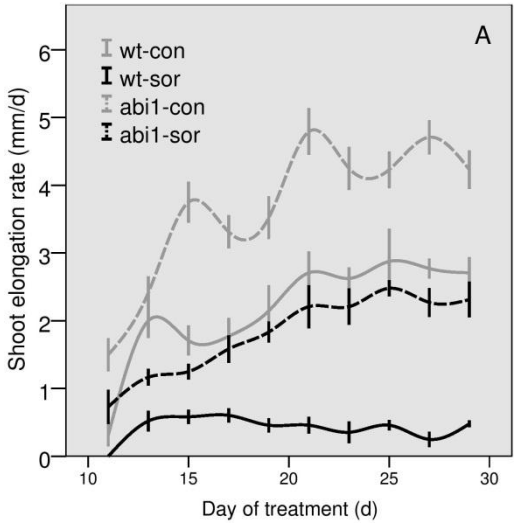
3.2 Growth dynamics

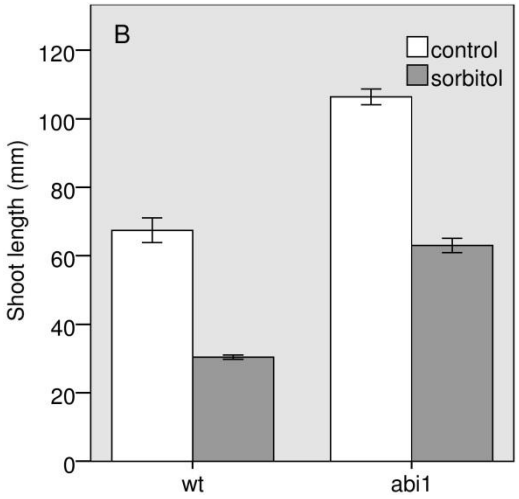
To characterize shoot and root growth during the treatment period, shoot and root elongation was measured every two days. Length increments were used to determine elongation rates (mm/day). The results are shown in Figure 3.3A-C.

Shoot elongation rates generally increased and were higher in *abi1* plants than in wt plants (Figure 3.3A). Growth rates of sorbitol treated plants were lower than those of control plants, independent of the genotype. The same trend of shoot elongation rate was visible in experiment 2 and 3, but with a bigger difference in elongation rates between control and sorbitol treated *abi1* plants. After 27 days of treatment, the shoots of *abi1* control plants were significantly longer than those of wt control plants (Figure 3.3B), consistent with the previous description of the *abi1* phenotype (Arend et al., 2009). Sorbitol treatment resulted in a strong reduction of shoot length in both genotypes. The statistical analysis did not show any significant interaction between genotype and treatment, suggesting a similar response to water deficit for wt and *abi1* plants. In experiment 3, the interaction was significant, but the effect size of the two experiments was very comparable (0.95 for experiment 1 and 0.92 for experiment 3).

Elongation rates of primary roots fluctuated at the beginning of the treatment and then slightly decreased under all experimental conditions (Figure 3.3C). Sorbitol treated plants showed lower root growth rates than control plants, particularly in the case of *abi1* plants. After 27 days of treatment, the roots of wt control plants were clearly longer than those of *abi1* control plants (Figure 3.3C). Sorbitol treatment resulted in a significant decrease in the root length of wt plants but not in that of *abi1* plants. Experiments 2 and 3 showed the same trend, but with a less pronounced growth of *abi1* plants under control conditions and a less pronounced decrease in root length of wt plants.

In summary, the results support previous conclusions, indicating that under non-stress conditions ABA is a negative regulator of shoot elongation, but a positive regulator of root elongation (Arend et al., 2009). Under sorbitol conditions, ABA seems to be involved only in the control of root elongation; shoot growth inhibition induced by sorbitol seems to be ABA-independent.

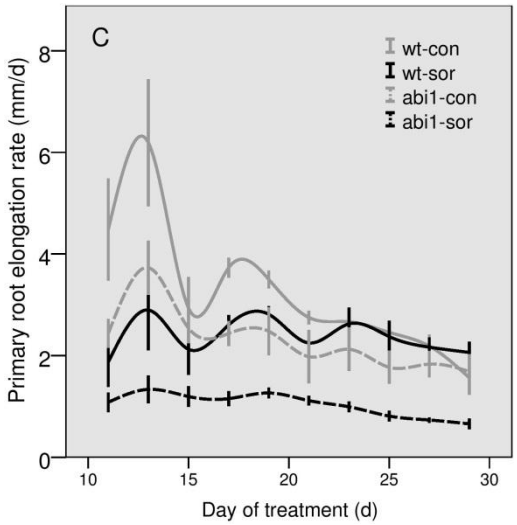


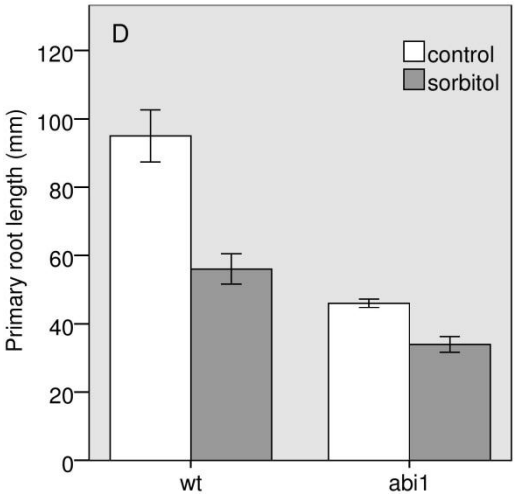


Descriptive statistics		N	Mean	SE
wt	Control	6	67.44	3.61
	Sorbitol	6	30.41	0.59
abi1	Control	6	106.39	2.29
	Sorbitol	6	62.98	2.10

Tests	*p* value
Normality (Shapiro-Wilk)	> 0.05
Homogenity of variances (Levene)	0.152

ANOVA	*p* value
Genotype	0.000
Treatment	0.000
Interaction	0.199





Descriptive statistics		N	Mean	SE
wt	control	4	95.04	7.63
	sorbitol	6	56.05	4.46
abi1	control	6	45.99	1.22
	sorbitol	6	33.97	2.29

Tests	*p* value
Normality (Shapiro-Wilk)	> 0.05
Homogenity of variances (Levene)	0.216

ANOVA	*p* value
Genotype	0.000
Treatment	0.000
Interaction	0.003

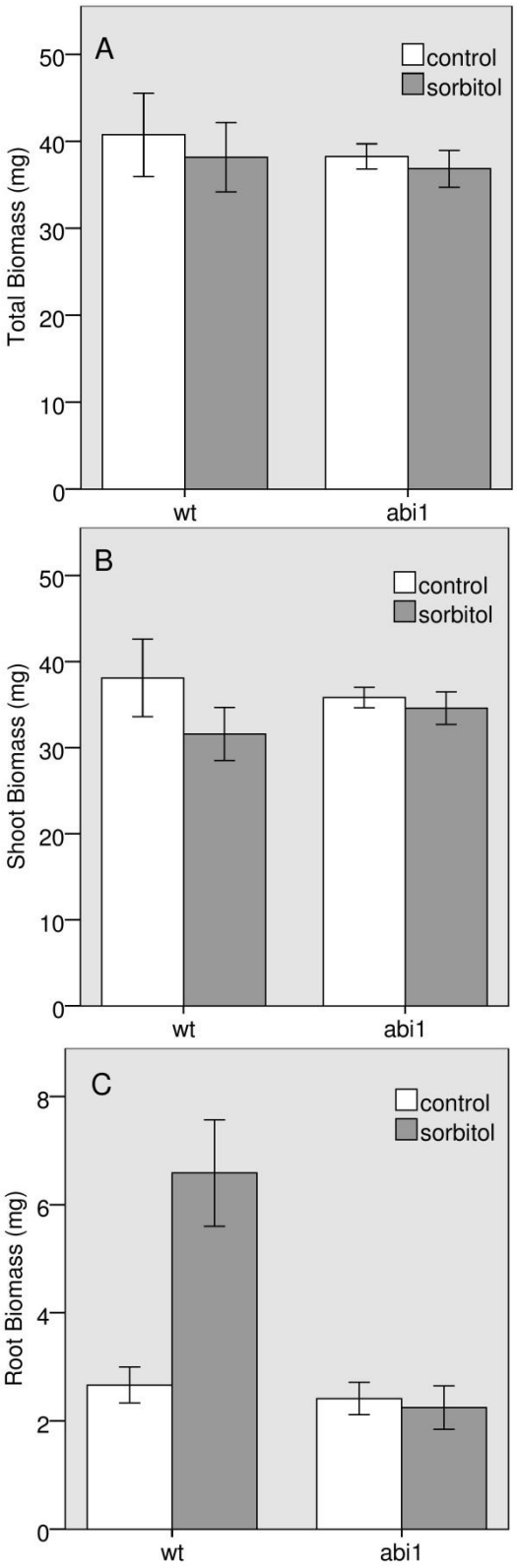
Post hoc (Tukey)	Difference	95% CI	*p* value
wt_con - wt_sor	38.99	22.36-55.62	0.000
wt_con - *abi1*_con	49.04	32.41-65.68	0.000
wt_sor - *abi1*_sor	22.08	7.20-36.96	0.003

Figure 3.3. Shoot (A) and root (C) elongation rates (mm/day) measured for wild-type (wt, continuous line) and *abi1*(dashed line) plants under control (white) and sorbitol (grey) conditions during the experiment. Measurements were started 11 days after transfer of the plants to the treatment media. Shoot (B) and primary root (D) length (mm) after 27 days of treatment, measured for wild-type (wt) and *abi1* under control (white) and sorbitol (grey) conditions. Values are means ± SE. Only significant post hoc tests are reported ($p < 0.05$).

3.3 Biomass allocation

To examine the effect of ABA on the allocation of biomass to roots and shoots, shoot and root material were separated, dried, and weighed for each plant. Total biomass, shoot biomass and root biomass are presented in Figure 3.4.

Total biomass and shoot biomass did not show any significant difference and interaction between genotypes and treatments. Experiment 3 confirmed this result. The situation was very different when looking at the root system (Figure 3.4.C). Under control conditions, the root biomass was comparable between the two genotypes. Under sorbitol, on the other hand, wt but not *abi1* plants showed a significant increase (3-fold) in root biomass. The same trends were apparent also in experiment 3, but here *abi1* plants also tended to allocate biomass to the roots, but to a much lower extent than wt plants. The reason for this discrepancy is not clear, but may be related to the high light conditions during the experiment (for most of the experiment there was sunshine). Although the greenhouse was shaded, sun irradiated the culture flasks, particularly in the mornings and evenings, and may have affected plant growth, particularly that of *abi1* plants, whose growth seemed to be more affected than in experiment 1. Additional experiments will be necessary to obtain more conclusive results.



Descriptive statistics		N	Mean	SE
wt	control	6	40.75	4.79
	sorbitol	6	38.17	4.00
abi1	control	6	38.25	1.45
	sorbitol	6	36.83	2.12

Tests	*p* value
Normality (Shapiro-Wilk)	> 0.05
Homogenity of variances (Levene)	0.364
ANOVA	***p* value**
Genotype	0.577
Treatment	0.560
Interaction	0.865

Descriptive statistics		N	Mean	SE
wt	control	6	38.08	4.50
	sorbitol	6	31.58	3.10
abi1	control	6	35.83	1.19
	sorbitol	6	34.58	1.89

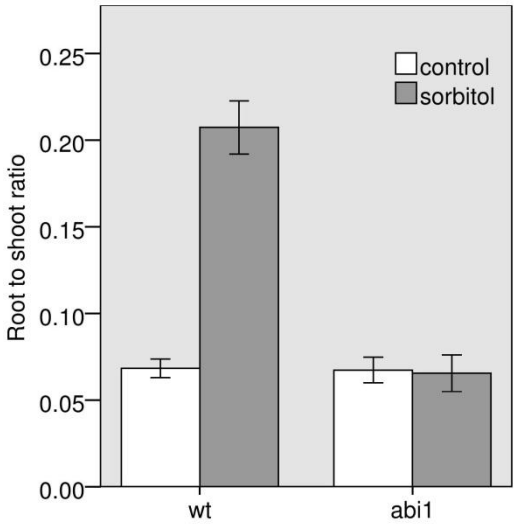
Tests	*p* value
Normality (Shapiro-Wilk)	> 0.05
Homogenity of variances (Levene)	0.269
ANOVA	***p* value**
Genotype	0.900
Treatment	0.204
Interaction	0.385

Descriptive statistics		N	Mean	SE
wt	control	6	2.67	0.33
	sorbitol	6	6.58	0.99
abi1	control	6	2.42	0.30
	sorbitol	6	2.25	0.40

Tests			*p* value
Normality (Shapiro-Wilk)			> 0.05
Homogenity of variances (Levene)			0.099
ANOVA			***p* value**
Genotype			0.001
Treatment			0.004
Interaction			0.002
Post hoc (Tukey)	**Difference**	**95% CI**	***p* value**
wt_sor - wt_con	3.92	1.63-6.21	0.001
wt_sor – *abi1*_sor	4.33	2.04-6.62	0.000

Figure 3.4. Biomass allocation in *abi1* and wild-type (wt) plants treated with and without 300 mM sorbitol. Total biomass (A), shoot biomass (B) and root biomass (C) (mg) after 27 days of treatment, measured for wt and *abi1* plants under control (white) and sorbitol (grey) conditions. Values are means ± SE. Only significant post hoc tests are reported ($p < 0.05$).

To quantify differences in biomass allocation between roots and shoots, the root-to-shoot ratio was calculated. The results are shown in Figure 3.5. The graph was similar to that of the root biomass, because the shoot biomass does not change between different conditions. The root-to-shoot-ratio of control wt and *abi1* plants was comparable. On the other hand, wt but not *abi1* plants responded to sorbitol with an increase in the root-to-shoot ratio, suggesting a role of ABA in the control of this response.



Descriptive statistics		N	Mean	SE
wt	control	6	0.068	0.0053
	sorbitol	6	0.207	0.02
abi1	control	6	0.067	0.0075
	sorbitol	6	0.066	0.0106

Tests	*p* value
Normality (Shapiro-Wilk)	> 0.05
Homogenity of variances (Levene)	0.077

ANOVA	*p* value
Genotype	0.000
Treatment	0.000
Interaction	0.000

Post hoc (Tukey)	Difference	95% CI	*p* value
wt_sor - wt_con	0.014	0.10-0.18	0.000
wt_sor – *abi1*_sor	0.015	0.10-0.18	0.000

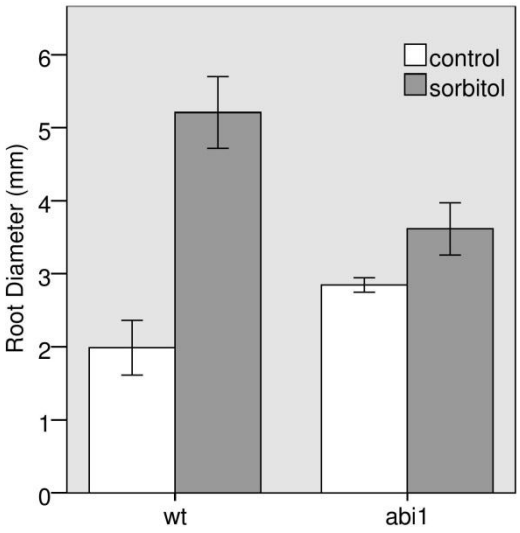
Figure 3.5. Root-to-shoot ratio (without dimension) measured for wild-type (wt) and *abi1* plants under control (white) and sorbitol (grey) conditions. Values are means ± SE. Only significant post hoc tests are reported ($p < 0.05$).

3.4 Root structure

Because growth dynamics and biomass allocation show that ABA mainly affects root growth, additional parameters of the root system were investigated: diameter of the primary root and density and length of lateral roots.

3.4.1 Diameter

The diameter of primary roots was measured at 0.5 cm from the root tip. The results are shown in Figure 3.6. Under non-stress conditions, the root diameter of *abi1* plants tended to be larger than that of wt plants, but the difference was not significant. Exposure to sorbitol resulted in a strong and significant increase of the root diameter in wt plants (2.5 fold increase), while the observed increase in *abi1* plants was not significant, suggesting a very different response to sorbitol between wt and *abi1* plants.



Descriptive statistics		N	Mean	SE
wt	control	4	1.99	0.37
	sorbitol	4	5.21	0.49
abi1	control	6	2.85	0.10
	sorbitol	6	3.61	0.36

Tests	***p* value**
Normality (Shapiro-Wilk)	> 0.05
Homogenity of variances (Levene)	0.153

ANOVA	***p* value**
Genotype	0.000
Treatment	0.000
Interaction	0.002

Post hoc (Tukey)	**Difference**	**95% CI**	***p* value**
wt_sor - wt_con	3.22	1.73-4.72	0.000
wt_sor - *abi1*_sor	1.59	0.23-2.96	0.020

Figure 3.6. Diameter (mm) measured for wild-type (wt) and *abi1* plants under control (white) and sorbitol (grey) conditions. Diameter was measured at 0.5 cm from the tip of the primary root after 27 days of treatment,. Values are mean ± SE. Only significant post hoc tests are reported ($p < 0.05$).

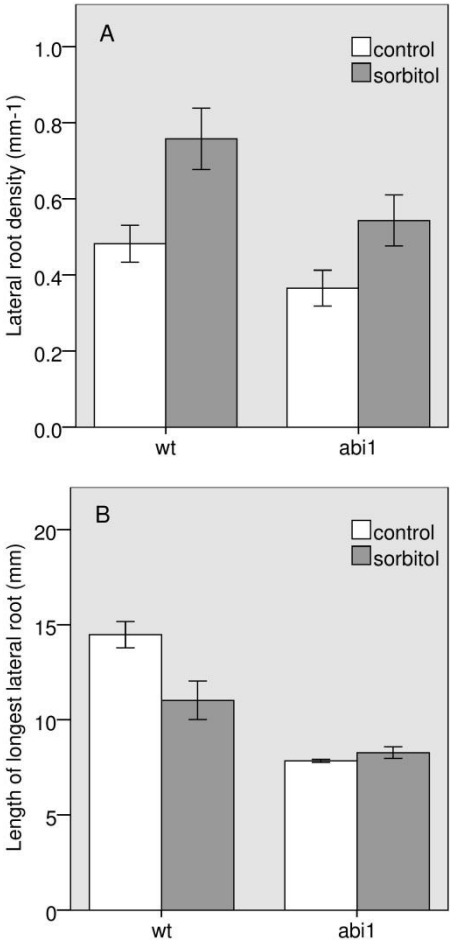
Measurements of the root diameter were very consistent among the three experiments, supporting the conclusion that wt plants respond to water stress by promoting radial growth, resulting in increased root biomass. As this response is not observed in *abi1* plants, this straightforward result strongly suggests that ABA is a key factor in regulating root growth under water deficit.

3.4.2 Lateral roots

Initiation, emergence, and elongation of lateral roots are complex biological processes well known to be regulated by several plant hormones, such as auxin and ethylene (Ruzicka et al., 2007). To assess whether these processes are also influenced by ABA, the following parameters were investigated. The density of lateral roots was used as an indicator of initiation and emergence processes (Figure 3.7A), and the length of the longest lateral root as an indicator of the lateral roots elongation process (Figure 3.7B).

The density of lateral roots was comparable between wt and *abi1* plants and increased in both genotypes in response to sorbitol, though not significantly. In the other two experiments, the treatment effect was significant, but there was no significant interaction between treatment and genotype, suggesting that the control of lateral root density is ABA-independent.

The length of the longest lateral root was significantly larger in wt control than in *abi1* control plants. No sorbitol effect was observed.



Descriptive statistics		**N**	**Mean**	**SE**
wt	control	4	0.48	0.05
	sorbitol	5	0.76	0.08
abi1	control	6	0.36	0.05
	sorbitol	6	0.54	0.07

Tests	***p* value**
Normality (Shapiro-Wilk)	> 0.05
Homogenity of variances (Levene)	0.342
ANOVA	***p* value**
Genotype	0.020
Treatment	0.003
Interaction	0.458

Descriptive statistics		N	**Mean**	**SE**
Wt	control	4	14.47	0.69
	sorbitol	5	11.03	1.02
abi1	control	6	7.85	0.09
	sorbitol	6	8.28	0.30

Tests		***p* value**
Normality (Shapiro-Wilk)		> 0.05
Homogenity of variances (Levene)		0.011
Robust ANOVA (Welch)	F(3,7.195) = 28.13	< 0.0005
Post hoc (Games-Howell)	**Difference**	**95% CI**	***p* value**
wt_con – *abi1*_con	6.19	3.17-9.22	0.007

Figure 3.7. Density of the lateral roots (mm^{-1}, A) and length of the longest lateral root (mm, B) after 27 days of treatment, measured for wild-type (wt) and *abi1* plants under control (white) and sorbitol (grey) conditions. Values are mean ± SE. Only significant post hoc tests are reported ($p < 0.05$).

The results of all three experiments were consistent, supporting the hypothesis that ABA, similar to root elongation, is a positive regulator of lateral root elongation under non-stress conditions.

3.5 Tissue structure

To assess whether growth changes observed in the previous paragraphs are related to changes in the tissue structure, sections of root, leaf, and stem tissues were inspected under the light microscope (Experiment 2). Each genotype and treatment combination was represented by a single plant. Accordingly, data were treated carefully.

In the case of roots, longitudinal cuttings of root tips were analyzed to visualize the root apex and the cells of the root elongation zone (Figure 3.8).

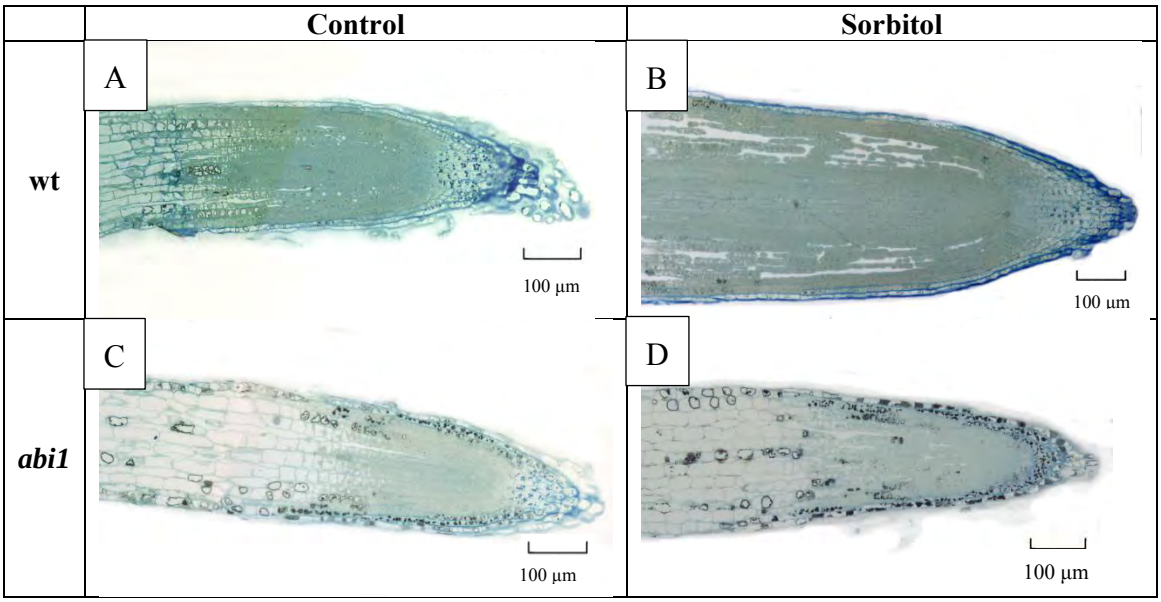


Figure 3.8. Pictures of longitudinal cuttings (10x) of root tips for wild-type (wt, A, B) and *abi1* (C, D) plants. Left column shows control conditions (A, C), right sorbitol conditions (B, D).

No clear differences were obvious between the root tip of the wt and *abi1* control plants. As expected, the root tip of the sorbitol treated wt plant was much thicker than that of the *abi1* plant. In the wt plant, more cells were visible, particularly in the center of the root tip (Figure 3.8B), indicating that the sorbitol-induced increase in radial growth is primarily a result of cell division, rather than of cell enlargement. Since this phenomenon is not observed in the *abi1* plants, this response seems to be ABA-dependent.

In the case of the leaf, sections of leaf blades were analyzed to visualize stomata and different cell layers (Figure 3.9). In *abi1* but not in wt plants, stomata were open under both treatment conditions, consistent with the impaired control of stomata closure in *abi1* plants (Arend et al., 2009). Apart from this observation, no clear differences in leaf morphology were identified.

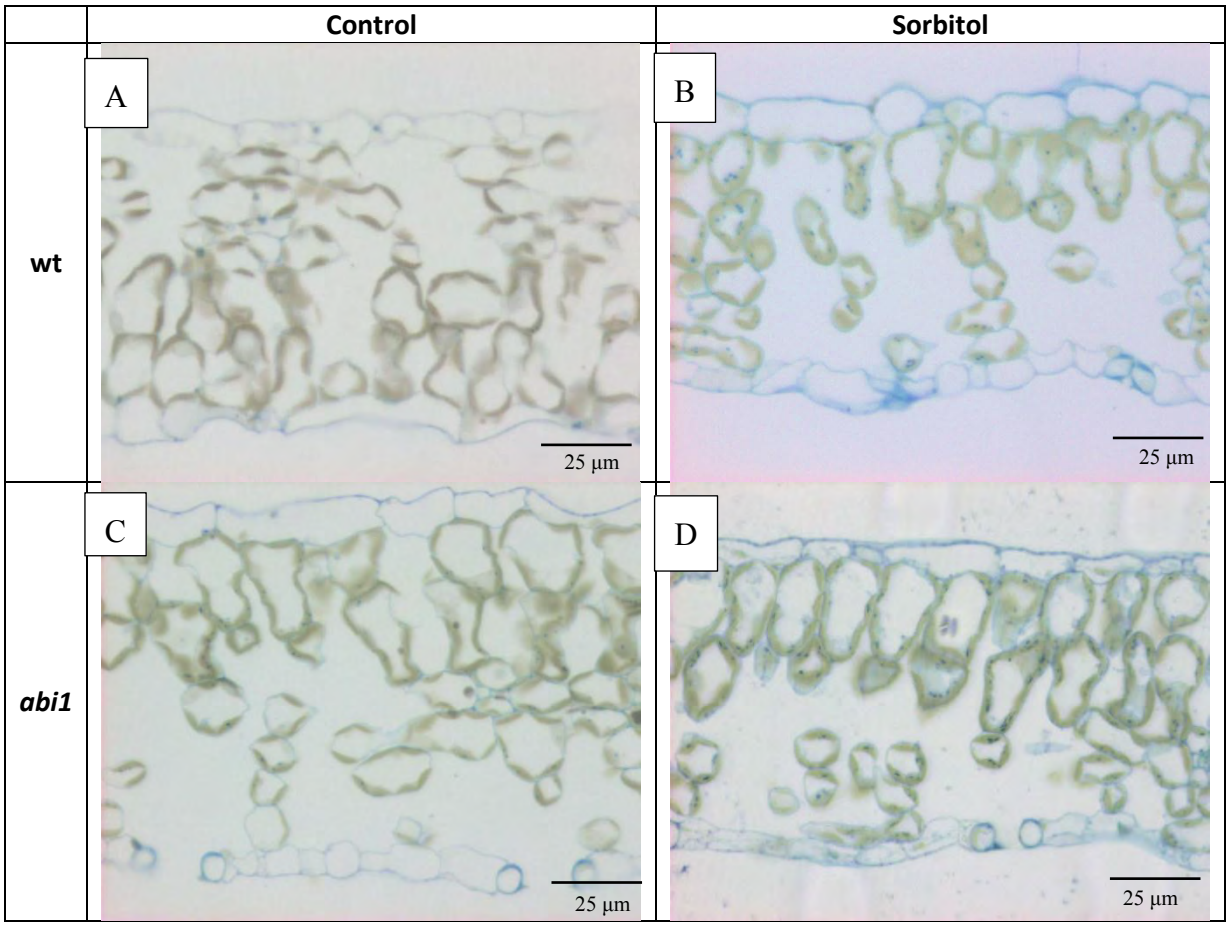


Figure 3.9. Pictures of leaf blades (40x) for wild-type (wt, A, B) and *abi1* (C, D) plants. The left column shows control conditions (A, C), the right sorbitol conditions (B, D).

Differently, stem sections indicated several morphological differences (Figure 3.10).

In the wt control plant, the diameter of xylem vessels seems to be larger than in the sorbitol treated plant, a tissue pattern not observed in *abi1* plant, possibly indicating that ABA has an impact on the structure of the xylem. Similarly, in the sorbitol treated wt but not in the *abi1* plant, the cell walls were thicker, probably due to lignification or other differences in cell wall components.

On the other hand, in the sorbitol treated *abi1* plant, but not in the wt plant, starch granules (visible as small dark dots in the cells) were observed, a response typical for plants exposed to water deficit (Finkelstein, 2013).

Clearly, analysis of more plants is necessary to confirm these results

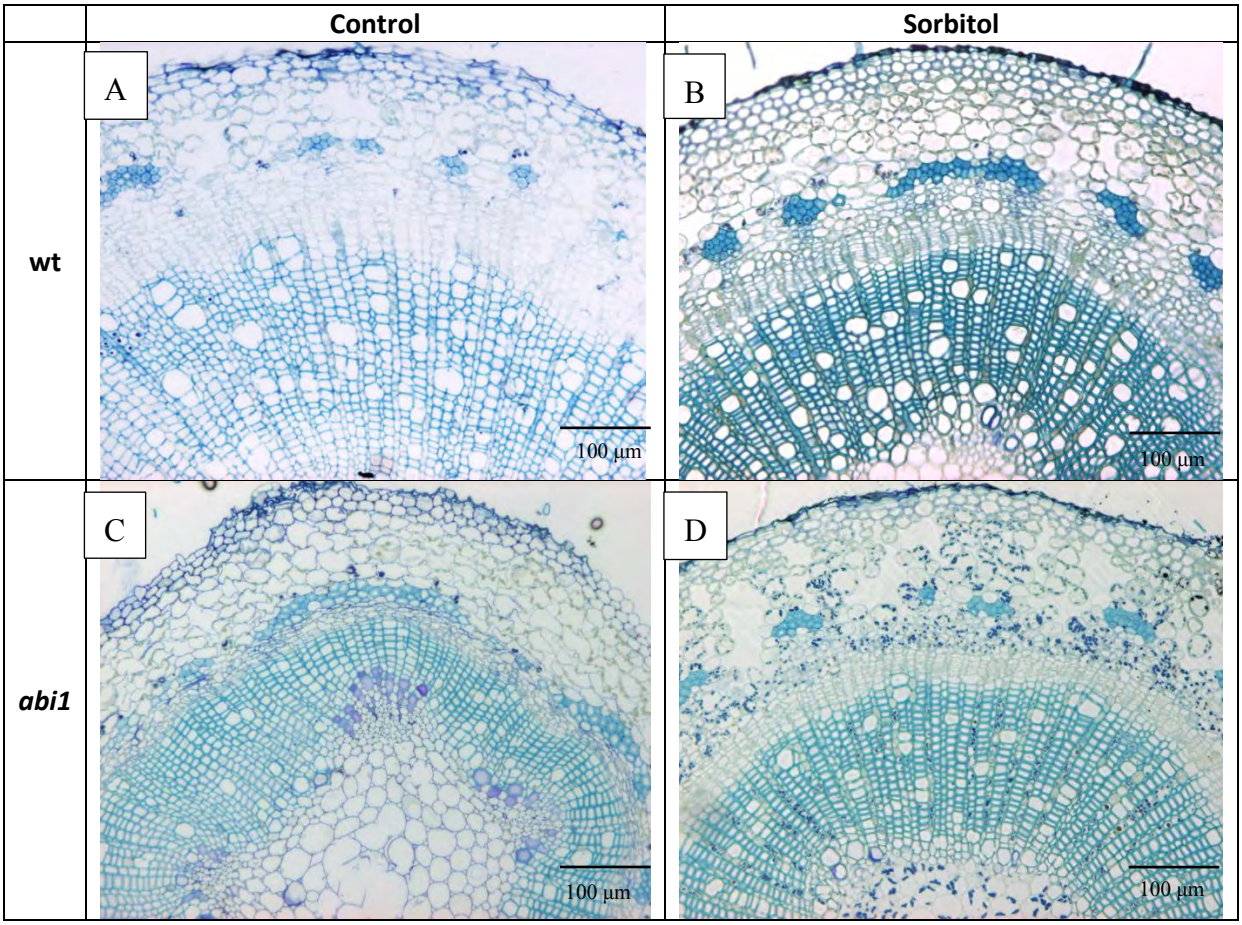
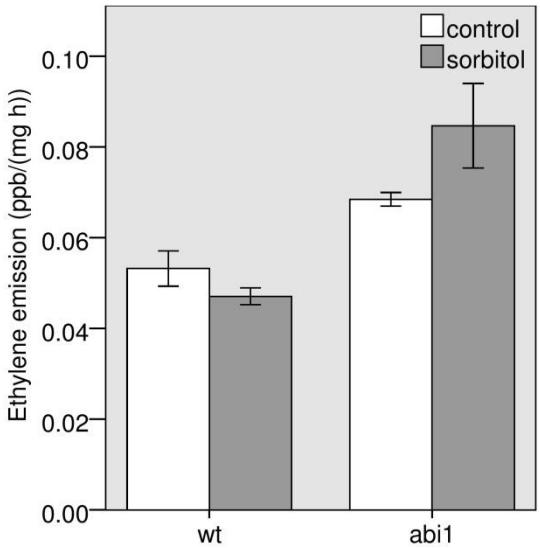


Figure 3.10. Pictures of stem cross sections (10x) for wild-type (wt, A, B) and *abi1* (C, D) plants. First column shows control conditions (A, C), second column shows sorbitol conditions (B, D).

3.6 Ethylene Production

Ethylene production was measured to investigate the possible interaction between ABA and ethylene in growth control. Ethylene was sampled at the end of experiment 3. As a starting point, the culture flasks were ventilated and then closed with an air tight cap equipped with a septum. The plants were then incubated for two days to let ethylene accumulate. The ethylene concentration of each culture flask was quantified by gas-chromatography. To estimate the rate of ethylene emission, ethylene concentrations were expressed as ppb per gram dry weight of the two plants present in each culture flask and per hour (Figure 3.11). Ethylene emission under control conditions was higher in *abi1* plants than in wt plants, consistent with previous conclusions (Arend et al., 2009) reporting that *abi1* plants exhibit elevated ethylene emission rates (2-3 times higher) when compared to wt plants. Under sorbitol conditions only *abi1* plants responded with an increase in ethylene emission, yet the effect was statistically not significant.



Descriptive statistics		N	Mean	SE
Wt	control	6	0.053	0.0039
	sorbitol	6	0.047	0.0018
abi1	control	5	0.068	0.0015
	sorbitol	3	0.085	0.0093

Tests		*p* value
Normality (Shapiro-Wilk)		> 0.05
Homogenity of variances (Levene)		0.017
Robust ANOVA (Welch)	F(3,6.34) = 25.228	< 0.0005

Post hoc (Games-Howell)	Difference	95% CI	*p* value
*abi1*_con – wt_con	0.015	0.001-0.029	0.036

Figure 3.11. Ethylene emission rate (ppb/(mg h)) measured for wild-type (wt) and *abi1* under control (white) and sorbitol (grey) conditions. Values are means ± SE. Only significant post hoc tests are reported ($p < 0.05$).

3.7 Gene expression

3.7.1 RNA isolation

RNA for gene expression analyses was extracted from two root tissues: root tip (first 0.5 cm of the primary root), and lateral root (LR) development zone (approx. 1-2 cm, depending on the genotype and treatment). Both of these tissues are characterized by cell division and/or cell elongation, and thus should be suitable for studying growth responses to water deficit at the molecular level. Differentiated tissues are expected to show different responses (Dinneny et al., 2008; Skirycz et al., 2010; Duan et al., 2013; Verelst et al., 2013).

All the RNA isolated in this study were of high quality. The RNAs used for RNA sequencing (three biological replicates for each genotype and treatment combination) had a RIN above 8.5, their yield ranged from 223 to 3254 ng, and the time that took for sampling the tissues until freezing was less than 8 min (Table 3.1).

Table 3.1. RNA samples selected for further analysis. Sample ID, A for root tip and B for lateral root development zone. Number of the cell culture flask, sampling time until freezing, number of pooled root tips, fresh weight of the pooled sample, RNA concentration, RNA Integrity Number (RIN), and the 260/280 ratio is provided.

	Condition	ID	Culture	Sampling (min)	n° of root tips	fresh weight (g)	RNA conc. (ng/µL)	RIN	260/280
A (Root tip)		2A	2	5	5	1.7	18.99	9.7	1.92
	wt; control	5°	5	5	5	1.9	24.77	9.7	2.06
		6°	6	5	4	1.3	55.98	9.8	1.98
		8°	8	8	8	2.1	39.73	9.3	2
	***abi1*; control**	10°	10	7	9	1.9	50.95	9.7	2.12
		11°	11	5	8	2.0	31.71	9.8	1.98
		15°	15	5	3	4.7	98.6	9.2	2.03
	wt; sorbitol	16°	16	4	3	5.1	52.15	9	2.04
		19°	19	4	4	3.5	95.46	8.5	1.99
		22°	22	6	6	14.2	94.02	8.8	2.06
	***abi1*; sorbitol**	24°	24	6	10	17.6	72.87	9.3	2.05
		25°	25	6	8	9.1	63.47	9.3	2.03
B (Lateral root development zone)		2B	2	5	-	5.2	23.87	8.8	1.84
	wt; control	5B	5	5	-	5.3	21.64	9	1.96
		6B	6	5	-	7.1	54.49	9.4	2
		8B	8	8	-	6.1	39.12	8.7	2.09
	***abi1*; control**	10B	10	7	-	10.9	42.63	9.1	2.11
		11B	11	5	-	6.0	38.16	9.2	1.91
		15B	15	5	-	17.6	69.45	9	2.08
	wt; sorbitol	16B	16	4	-	24.7	26.99	8.9	1.92
		19B	19	4	-	6.3	26.07	9.3	2.09
		22B	22	6	-	15.4	74.32	8.7	2.04
	***abi1*; sorbitol**	24B	24	6	-	260	51.23	8.6	2.15
		25B	25	6	-	10.8	66.43	8.7	1.98

3.7.2 Sequencing and Mapping

The cDNA libraries were sequenced on an Illumina HiSeq 2000 instrument with 101 bp paired-end reads. Due to high quality, all Hiseq reads were further processes. Their number ranged from 9.8 to 20.9 Million per sample, with no clear effects of either genotype, treatment, and tissue (Table 3.2).

Table 3.2. Total number and genome coverage of reads for the 24 samples analyzed. Coverage is expressed as the percentage of genes identified by reads mapping to a single gene model relative to the total number of gene models identified in the *Populus trichocarpa* genome (v3.0).

Sample	Total number of reads	Coverage (%)
wt-control-root-tips-2A	16395891	57
wt-control-root-tips-5A	15143743	79
wt-control-root-tips-6A	20812983	80
abi1-control-root-tips-8A	17371803	70
abi1-control-root-tips-10A	10555578	75
abi1-control-root-tips-11A	15411653	78
wt-sorbitol-root-tips-15A	13588641	79
wt-sorbitol-root-tips-16A	16806877	81
wt-sorbitol-root-tips-19A	13799791	81
abi1-sorbitol-root-tips-22A	14724363	78
abi1-sorbitol-root-tips-24A	10864850	78
abi1-sorbitol-root-tips-25A	12069404	78
wt-control-root-dev-2B	9780790	77
wt-control-root-dev-5B	12981961	78
wt-control-root-dev-6B	12350960	74
abi1-control-root-dev-8B	11236846	76
abi1-control-root-dev-10B	14104004	79
abi1-control-root-dev-11B	13682752	78
wt-sorbitol-root-dev-15B	16732519	78
wt-sorbitol-root-dev-16B	17491134	79
wt-sorbitol-root-dev-19B	13483767	79
abi1-sorbitol-root-dev-22B	16015463	77
abi1-sorbitol-root-dev-24B	14814259	75
abi1-sorbitol-root-dev-25B	16121905	78

Reads that aligned to more than one gene model of the Populus trichocarpa genome (v3.0) were discarded (Figure 3.12).The remaining reads identified 32051 genes, corresponding to 77.5% of the 41336 gene models identified in the Populus trichocarpa genome.

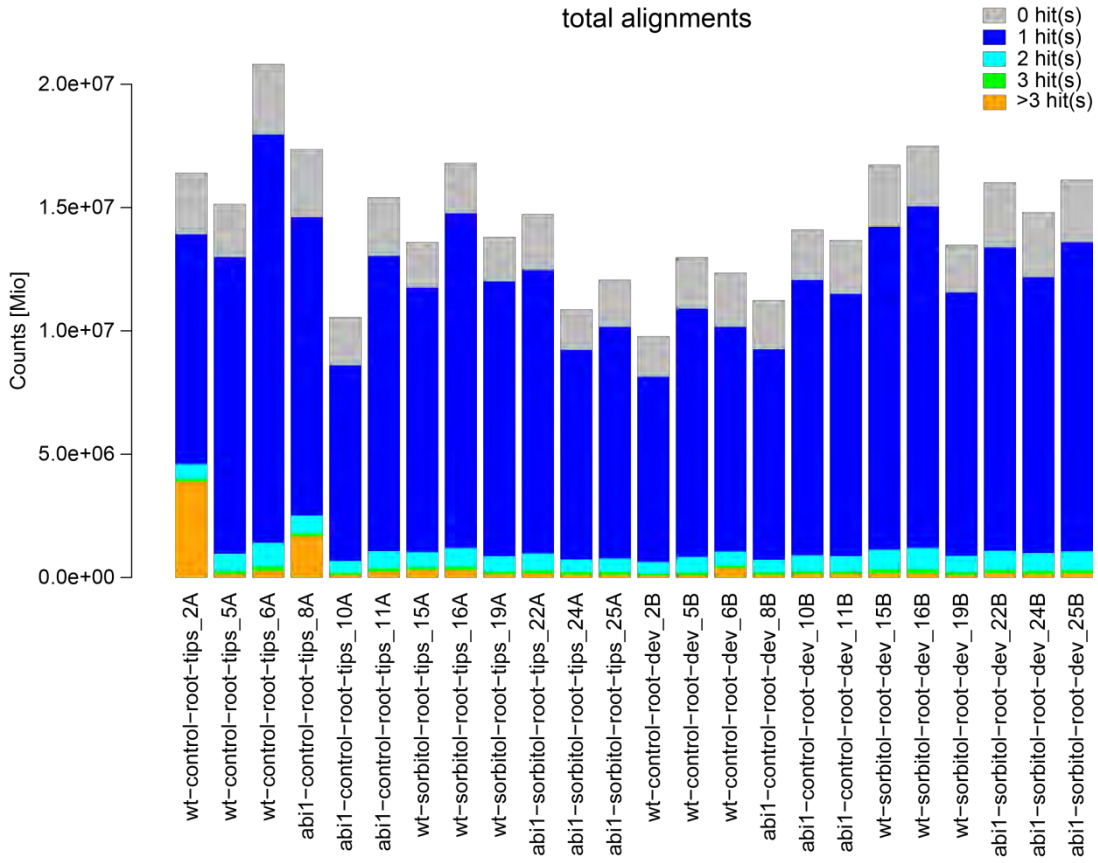


Figure 3.12. Allignements of reads to the *Populus trichocarpa* genome (v3.0) for the 24 samples analyzed

3.7.3 *Heat map of differentially expressed genes*

Clustering of normalized genes revealed a clear hierarchical structure of the samples (Figure 3.13). The two tissues analyzed formed two clusters, followed by four clusters representing each tissue and treatment combination. The three biological replicates of each genotype treated with and without sorbitol generally clustered together. This striking result confirms the importance of a tissue specific sampling and tissue specific analysis of gene expression.

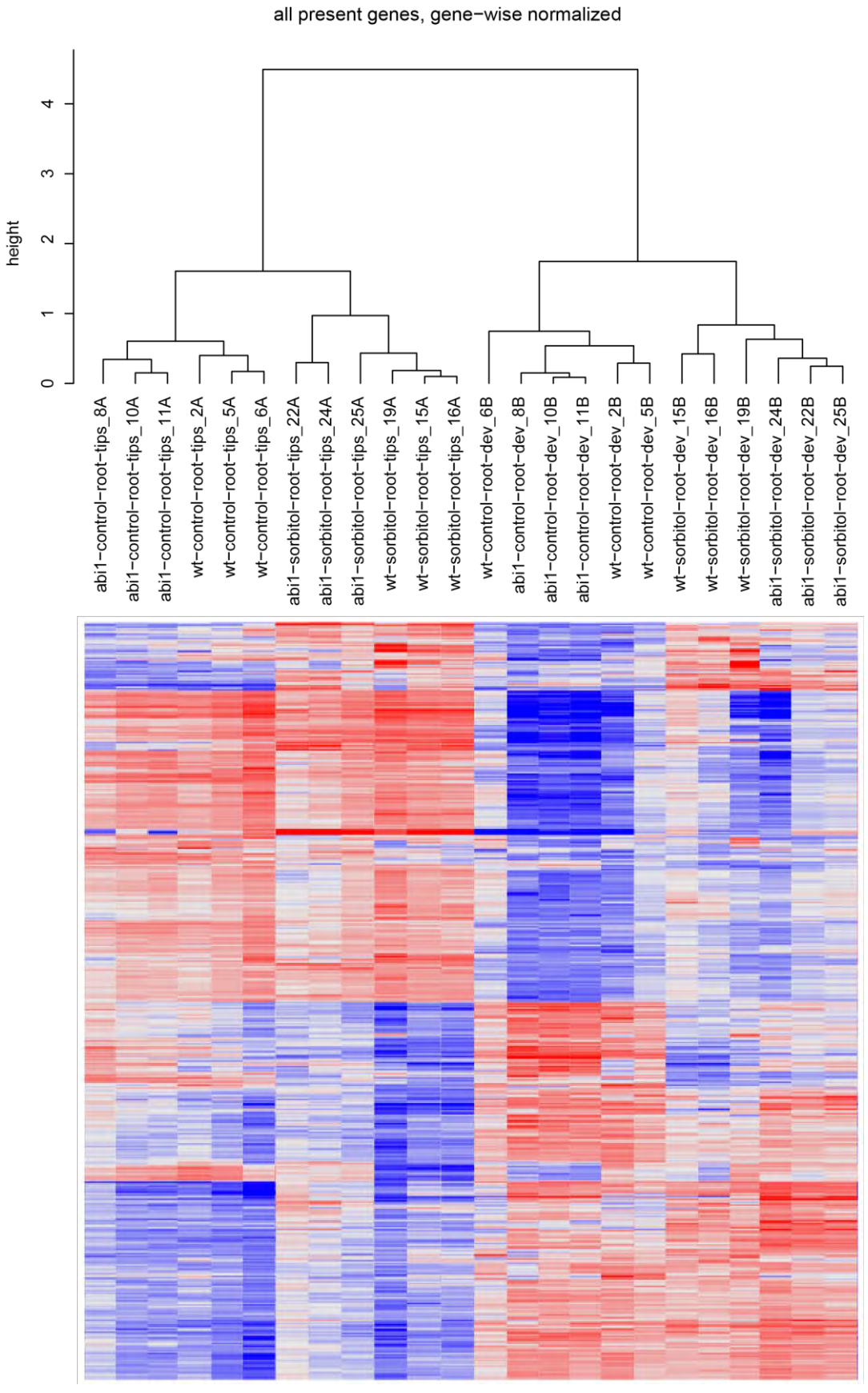


Figure 3.13. Heatmap and clustering of total number of genes, gene-wise normalized for the 24 samples analyzed.

3.7.4 Gene Ontology enrichment analysis

To analyse the effect of water deficit on gene expression in wt and *abi1* plants, differentially expressed genes were determined for the following pairs of experimental treatments: (*i*) wt root tips treated with sorbitol over control wt root tips, (*ii*) *abi1* root tips treated with sorbitol over control *abi1* root tips, (*iii*) wt LR development zone treated with sorbitol over control wt LR development zone, and (*iv*) *abi1* LR development zone treated with sorbitol over control *abi1* LR development zone. Genes which had an FDR-adjusted *p* value equal or less than 0.05 and a fold change equal or higher than 2.00 were considered as differentially expressed in a given pair of treatment. To uncover global differences in gene expression in the two genotypes, GO enrichment analyses were carried with up- and down-regulated genes. Totally, the differentially expressed genes were associated with 721 GO biological process terms, of which 101 were significantly over-represented (FDR corrected *p* value equal or smaller than 0.05) for up-regulated genes and 52 for down-regulated genes Table 3.3. The LR development zone of *abi1* plants was the tissue with the highest number of enriched categories, suggesting important physiological changes caused by water deficit in this tissue.

Table 3.3- Number of enriched GO biological process terms in wild-type and *abi1* plants. Two tissues were analyzed, root tip and lateral root development zone (root-dev)

	Up-regulated genes	**Down-regulated genes**
wt-root-tips	19	19
abi1-root-tips	37	22
wt-root-dev	16	15
abi1-root-dev	84	24
Total enriched GO terms	101	52

A graphical overview of the top 10 enriched GO terms for each genotype and tissue is presented in Figure 3.14. The complete list of enriched GO terms is given in §0 and §6.7 of the Appendix. Not surprisingly, in the group of up-regulated genes, genes related to gene expression, translation, and metabolic processes are enriched in both genotypes and tissues. Genes involved in ribosome biogenesis and rRNA processing, on the other hand, are only enriched in root tips of wt plants, probably reflecting a strong growth activity in this tissue. Conversely, genes associated with stress responses were enriched under all conditions, except in root tips of wt plants, suggesting that the root tips of wt plants experiences little stress under water deficit.

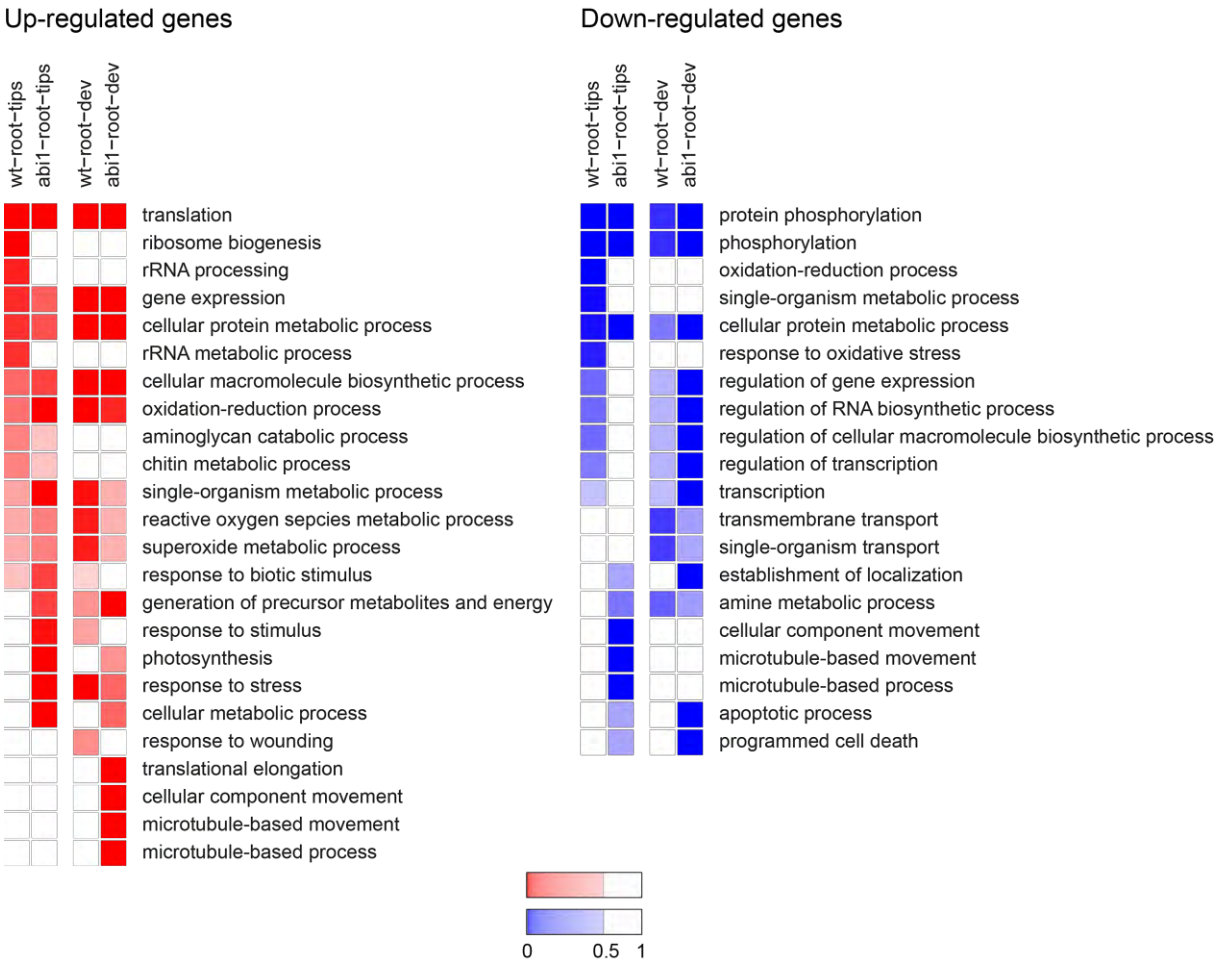


Figure 3.14. Heat map of the top 10 enriched GO biological process terms of up- and down-regulated genes in root tips and lateral root development zone (root-dev) of wild-type (wt) and *abi1* plants.

In the group of down-regulated genes, genes involved in phosphorylation were enriched in both genotypes and tissues. Interestingly, in the root tip of wt plants, genes functioning in the response to oxidative stress were over-represented. Similarly, genes related to microtubule-based processes were enriched only in the root tips of *abi1* plants. Other genes involved in microtubule-based processes, but up-regulated, were enriched in the root development zone of *abi1* plants. In the same tissue, down-regulated genes involved in programmed cell death were highly enriched.

3.7.5 Venn diagrams

To further characterize gene expression changes, genes differentially expressed in only one of the two genotypes were analyzed. To identify such genes, Venn diagrams were generated (bioinfogp.cnb.csic.es/tools/venny/) (Figure 3.15).

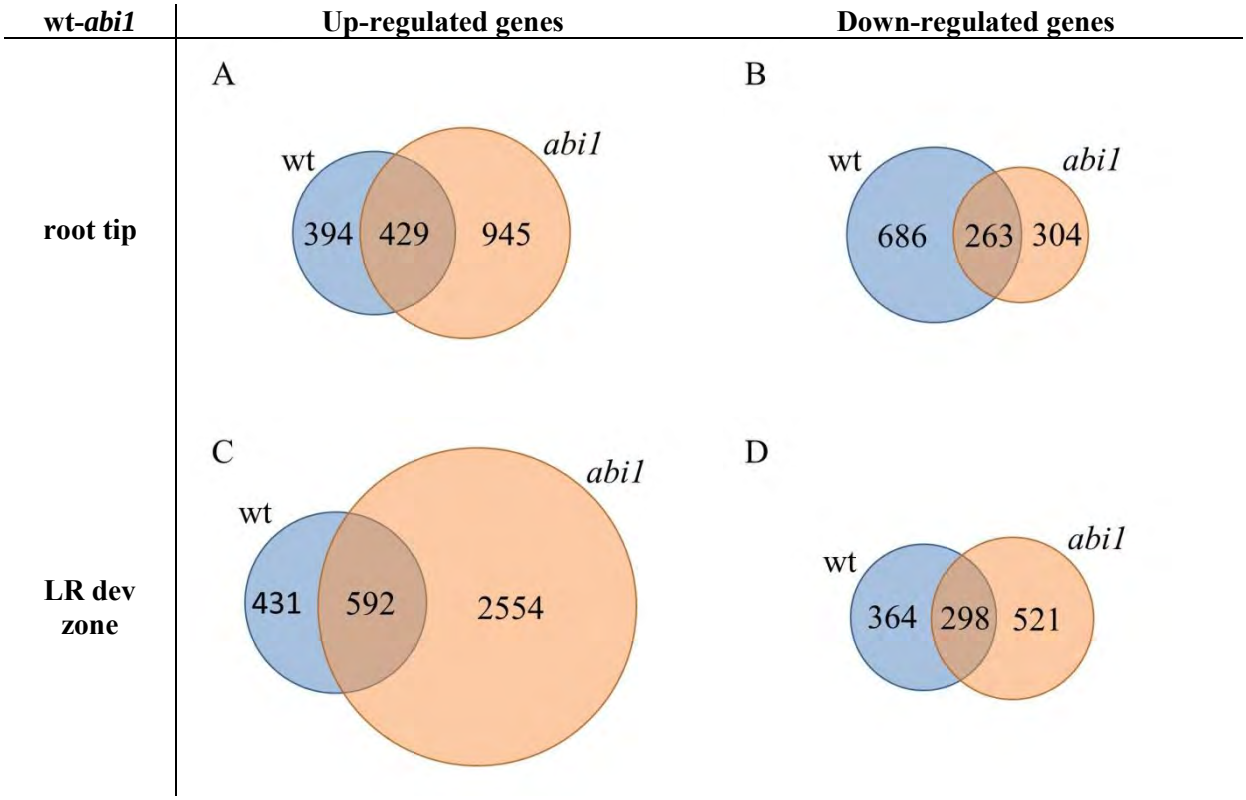


Figure 3.15. Venn diagrams of up (A, C) and down (B, D) regulated genes for root tip (A, B) and LR development zone. Number of DEGs specific for the two genotypes and commonly regulated are also represented by proportional areas of the circles (Redrawn from bioinfogp.cnb.csic.es/tools/venny/).

In both tissues, the number of up-regulated genes (A, C) was generally higher than that of the down-regulated genes (B, D). In *abi1* plants, there were more regulated genes than in wt plants, with the exception of the down-regulated genes in the root tip (B). In particular, a very high number of genes (2554) was up-regulated in the lateral root development zone. This result further supports the suggestion that the root tip and the LR development zone respond to water deficit in very different ways.

To examine the molecular mechanisms underlying ABA-regulated growth and physiological root responses to water deficit, genes related to ABA and other plant hormones were further analyzed. In addition, genes related to stress were inspected, to test whether ABA plays a role in stress avoidance. Selected genes differentially expressed in root tips and in the lateral root development zone are given in Table 6.2 and Table 6.3 of the Appendix.

To better understand ABA-regulated responses, genes related to ABA-dependent and ABA-independent pathways are reported. There are then genes involved in the orchestrated

hormonal regulation to investigate the cross-talk between ABA and other hormones. Finishing, the genes involved in stress response include ROS detoxification and stress genes, cell wall genes and starch accumulation related genes.

3.7.6 ABA pathway

It was hypothesized that genes functioning in ABA biosynthesis and catabolism are differentially expressed not only in wt plants but also in *abi1* plants, because *abi1* is mainly impaired in ABA signaling. Genes functioning in ABA signaling, on the other hand, were predicted to be differentially expressed primarily in wt plants. The set of regulated genes related to ABA biosynthesis and catabolism included one *Nine-Cis-Epoxycarotenoid Dioxygenase 3* (*NCED3*) and two members of the *CYP707A* gene family. NCED3 is a key enzyme of ABA biosynthesis and a well-known marker for drought stress (Finkelstein, 2013). This gene was induced in both genotypes and tissues and suggests ABA biosynthesis. *CYP707A* family members encode ABA-8'hydroxylases (Kushiro et al., 2004) and are considered markers for ABA catabolism. In root tips, one of the two identified CYP707A members was induced in wt plants, and the other in *abi1* plants.

The set of regulated genes involved in ABA signaling included four putative ABA receptor PYR1 genes. In wt plants, all four genes were repressed, both in root tips and in the LR development zone. One of the genes was also down-regulated in *abi1* plants, in the LR development zone. Down-regulation of PYR1 genes is also observed in Arabidopsis, and may reflect feedback mechanisms to desensitize ABA response and restore homeostasis (Finkelstein, 2013). One of the genes was down-regulated also in the lateral root development zone of *abi1* plants. Other putative components of the ABA signaling pathways were PP2C family protein phosphatase genes. Some of them are induced in both genotypes (6 in root tip and 4 in lateral root development zone), while others are regulated solely in wt (9) and *abi1* (11) plants, suggesting a complex regulatory network of phosphorylation.

Additional genes with putative functions in mediating plant responses to ABA were calcineurin B-like interacting (CBL) genes, whose proteins are part of the CBL-CIPK signaling pathway (Hashimoto et al., 2012). Two of the genes are regulated in root tips of wt plants. A third gene is induced in the root tips of *abi1* plants.

3.7.7 ABA-independent pathways

The set of regulated genes involved in ABA-independent stress response included six No Apical Meristem/Cut-Shaped Cotyledon (NAC) genes, transcriptional regulators involved in ABA-independent gene expression. NAC genes were found to be regulated only in *abi1* plants, suggesting a reinforced ABA-independent response in the ABA insensitive genotype.

3.7.8 Cross-talk between ABA and other hormones

Genes related to the metabolism and response to other hormones indicate that there is a cross-talk between ABA and the hormones gibberellic acid and auxin. For example, of the 8 gibberellin oxidases identified, one is regulated only in wt plants, while three were regulated solely in *abi1* plants. Similarly, of the two RGA DELLA proteins detected, one was down-regulated in wt plants, and the other up-regulated in *abi1* plants. Small auxin-up RNA (SAUR)-like family proteins genes, involved in response to auxin, are regulated almost exclusively in *abi1* plants, both in root tips and in the lateral root development zone.
On the other hand, interaction between ABA and ethylene is not clear: an aminocyclopropane-1-carboxylic acid (ACC) synthase gene, involved in ethylene biosynthesis (Wang et al., 2002), was down-regulated in the root tip of both genotypes, but two ACC synthase genes were downregulated in LR development zone of *abi1*.

3.7.9 Stress response

Differentially expressed genes related to plant stress included genes involved in ROS detoxification and cell rescue, cell wall structure, and starch accumulation.
Genes involved in ROS detoxification and cell rescue included several superoxide dismutases, glutathione S-trasferases, and late embryogenesis abundant (LEA) proteins. Genes encoding superoxide dismutases were induced and typically occurred in both genotypes. Genes encoding LEA proteins showed a similar pattern. Glutathione S-tranferase genes, on the other hand, included several members that were specific for one genotype. In root tips, they were repressed in wt plants, but induced in *abi1* plants, suggesting a correlation between stress avoidance and ensured growth. In the lateral root development zone, they were induced in both genotypes.
Genes involved in cell wall changes included pectin methylesterases (PME), pectin methylesterase inhibitors, and expansins. Proteins encoded by these genes are important key enzymes in carbohydrate metabolism (Hothorn et al., 2004), determinants of the cell wall solidity. The identified genes indicate a strong difference in stress level experienced by root tip and LR development zone. PME genes are not regulated in root tip of wt, while 23 of the total 64 cell wall genes identified are upregulated only in LR development zone of *abi1*.
Genes involved in starch accumulation indicate that ABA influences the regulation of sugar and starch levels. The set of genes included 3 plant glycogenin-like starch initiation genes and 9 genes encoding amylases, enzymes involved in hydrolysis of starch into sugar.
All the three plant glycogenin-like starch initiation genes are up-regulated only in *abi1*.
Of the 9 amylases identified, three are repressed only in wt plants, while two are induced solely in *abi1* plants.

Chapter 4

Discussion and Conclusion

This study was designed to examine the role of ABA in regulating shoot and root growth of poplar exposed to water deficit. Based on the results presented, the following major conclusions can be drawn

(i) Poplar responds to water deficit with a strong inhibition of shoot growth, while root growth is maintained through radial growth, resulting in an increased root-to-shoot ratio in biomass.
(ii) Water deficit-induced promotion of root growth is ABA dependent, while ABA plays a minor role (if at all) in shoot growth inhibition.
(iii) ABA-regulated radial root growth promotion under water deficit involves several other plant hormones, including auxin, ethylene, gibberellic acid, as well as components of the DELLA protein pathway.
(iv) As a result of ABA action, roots experience little stress, particularly the root tip.
(v) Hence, ABA is suggested to be a key component of the regulatory network mediating water stress avoidance in root of poplar.

4.1 Poplar roots respond to water deficit through promoting radial growth

To simulate water deficit conditions, wt and *abi1* plants were treated with sorbitol, an often used osmoticum which lowers the water potential of the medium (Claeys et al., 2014). The poplar plants were treated with 300 mM of sorbitol, a concentration that enabled plants still to growth, and thus allowed growth responses to be studied. Exposure to sorbitol resulted in inhibition of root elongation, but radial growth was promoted (diameter increased by factor 2.6). On the other hand, shoot growth was inhibited, resulting in an increased root to shoot ratio (3-fold increase).

Water deficit-induced inhibition of shoot growth while root growth is maintained (or even promoted at mild water deficit) is a phenomenon described for a number of plant species, including herbaceous and perennial woody plant species (Bogeat-Triboulot et al., 2007; Comas et al., 2013). Two contrasting root growth responses are described: (*i*) maintenance or promotion of growth through root elongation and (*ii*) promotion of radial root growth.

The majority of the studies describe effects on root elongation (Comas et al., 2013). For example, in *Capsicum annuum* (Kulkarni and Phalke, 2009; Shao et al., 2010) it was shown that the length of the primary root was even greater in drought-stressed plants than in control

plants at the expense of root thickening. In contrast, in peanuts, cotton and maize, although they all respond with an increase of the root-to-shoot ratio when exposed to water deficit, root elongation is inhibited in case of severe drought stress (Bengough et al., 2011). A well known example for promotion of radial growth is rice: it has been shown that genotypes with larger root were more tolerant to drought. The increase in root diameter was a consequence of higher cell numbers per cortex layer and stele of developing roots were more drought tolerant (Jeong et al., 2013). Although poplar also has shown to respond to water deficit with an increase in the root-to-shoot ratio (Bogeat-Triboulot et al., 2007) specific root growth patterns were not analysed.

The physiological relevance of the two growth responses is not clear, as there are rather suggestions than experimental evidence: root elongation may enable roots to penetrate to lower and more humid soil layers. On the other hand, root radial growth may prevent water loss and/or more efficient water uptake through increase root surface, and/or enable more efficient water transport due to increased xylem size. A study conducted with rice (Sibounheuang et al., 2006) found that water tolerant genotypes have a larger diameter and a larger xylem when compared with water sensitive genotypes. Thus, xylem size needs to be examined also in poplar to correlate morphological changes in roots with physiological processes.

4.2 ABA is a key regulator of water deficit-induced root growth promotion in poplar.

Radial root growth promotion under water deficit was observed only in wt plants: *abi1* plants were unable to respond to the treatment with growth stimulation, strongly suggesting that ABA is a key regulatory component in water deficit-induced root growth. On the contrary, shoot growth was inhibited in both genotypes, suggesting a minor role of ABA in shoot growth regulation.

A key role of ABA in maintaining root growth under water deficit was previously suggested for maize (Sharp et al., 2004): plants in which ABA levels were reduced either genetically or by inhibitors showed a reduced root growth under low water potentials, demonstrating that ABA is required for maintaining root growth. However, unlike in poplar, maize responds to water deficit with maintaining root elongation. In addition, these studies were carried out only with plants under water deficit, and therefore did not allow direct comparisons of non-stress and stress conditions.

Lateral roots are among the most active portions of the root system in water uptake (Comas et al., 2013). In the present study it was tested whether ABA is also involved in potential growth changes of lateral roots. The results indicate that poplar responds to water deficit with an increase in the density of lateral roots. However, this response seems to be ABA-independent.

On the other hand, the results indicate that ABA plays a role in lateral root elongation, but this mechanism is not influenced by drought. This observation is in contrast with studies in Arabidopsis reporting that lateral root formation is a process dependent by crosstalk of different hormones, with ABA acting as a negative regulator (Moriwaki et al., 2011; Duan et al., 2013). These findings suggest that ABA can play contrasting roles in the control of formation and growth of lateral roots in different plant species.

4.3 ABA-regulated radial root growth promotion under water deficit involves several other plant hormones

The transcriptome analysis revealed that several plant hormones are involved in the ABA regulated response to water deficit, including, GA and auxin.

Interactions among various phytohormones integrate the diverse input signals and readjusting growth as well as acquiring stress tolerance. The presence of multiple and often redundant signaling intermediates for each phytohormone appears to help in this crosstalk.

Ethylene biosynthesis reduction is in accordance with previous studies (Sharp, 2002). However, while these study identify interaction between ABA and ethylene, in the present study ethylene regulation seems to be ABA-independent.

Ethylene measurement with GC revealed that ethylene emission is maintained under drought stress. This result is in contrast with the increase in ethylene as a consequence of stress found in Arabidopsis (Achard et al., 2006). By contrast, *abi1* plants are not able to regulate ethylene emission. This can also be related with a different level of stress felt by the two genotypes.

The crucial role of DELLA proteins in root growth inhibition was studied with mutants in Arabidopsis (Achard et al., 2006) and poplar (Zawaski and Busov, 2014). The analysis of the transcriptome in the present study confirmed the importance of hormonal crosstalk in root growth control, and suggests a role of ABA in regulating DELLA proteins level.

Moreover, the finding that auxin level was regulated almost only in *abi1* plants, in both tissues, suggest an important role of this hormone in stress response, and the presence of compensation mechanisms to deal with water deficit in an ABA-independent way.

4.4 The root tip experiences little stress under water deficit, whereas the LR development zone experiences stress.

Transcriptome analysis and clustering of gene expression revealed a tissue specific response to low water availability. Thus, most of the genes involved in response to stress are induced only in the LR development zone, suggesting different levels of stress for the different tissues. The Venn Diagrams confirm that many genes are upregulated in the LR development zone, expecially in *abi1* plants (Figure 3.15C). Moreover, in the present study is reported down-regulation of stress responsive genes in root tip, which was not described so far (to our best of

knowledge). The GO enrichment analysis (Figure 3.14) show that the genes involved in response to oxidative stress are even down-regulated under stress compared with control conditions.

4.5 ABA is suggested to be a key component in regulatory network mediating water stress avoidance in roots.

So far, studies of water stress avoidance were mainly focused on shoot, and only little was known on ABA-mediated response to drought in roots. It has been shown (in the same poplar genotype of this study) that gibberellin GA catabolism, its repressive signaling and crosstalk between hormones mediate shoot growth inhibition and physiological adaptation in response to drought (Zawaski and Busov, 2014). Anyway they don't provide any information about mechanisms occurring in the roots. The present study proposes an ABA regulated mechanism of water stress in roots, involving interaction with other hormones in the gene expression response. The low level of stress experienced by wild-type plants suggests a key role of ABA in regulatory network mediating water stress avoidance.

4.6 Technical conclusions

Beside of the important biological conclusions, several conclusions can be drawn regarding technical aspects of this study:

(i) The sorbitol treatment to induce drought stress was effective, as confirmed by water potential measurement, and, because of its artificial set-up, permits a tight control of stress level and has low variability (Claeys et al., 2014). The different level of stress experienced by the two genotypes could be improved by adjusting the sorbitol concentration to have the same water potential in both genotypes.

(ii) The present study reveals that the tissue sampling is a key decision in gene expression analysis because the response to drought stress is highly tissue specific.

(iii) De novo assembly strategy was not necessary because of the high number of genes identified in the trascriptome (more than 2/3 of the entire genome). For more detailed studies of expression of gene families (for example the PYR genes), genome sequence would anyway be required.

4.7 Outlook

The present study opens the possibility to further characterize the drought stress response in poplar, and to give more insights in ABA-induced morphological changes and gene expression.

In particular, to draw a model of molecular mechanisms poplar plants with additional mutant genes (including knock out mutants) will be necessary. For example *abi1* mutants combined with DELLA protein mutants, and/or ethylene.

Moreover, given the striking result of down-regulation of stress genes in root tips, with further studies it would be possible to determine if this response is only related to water stress or also to other abiotic stresses.

A further microscopic analysis is needed to characterize the physiological processes occurring in roots under water stress conditions. In particular, observation of the xylem size of primary root could give insights in the physiological mechanisms of water stress avoidance and tolerance in poplar roots. In fact, a bigger xylem size could be related with a more efficient water uptake.

With further microscopic analysis of stem sections it would be possible to evaluate the effect of ABA on reducing xylem vessels size in sorbitol treated plants: this response could increase conservative water use and reduce risk of cavitation limiting hydraulic conductivity. (Comas et al., 2013).

Moreover, microscopic observation of lateral roots primordia could separately assess the consecutive development mechanisms of lateral roots to characterize the adaptation of the root system to drought stress. Density of lateral roots in fact depends on two different biological processes, initiation and emergence of lateral roots, which are highly susceptible to distinct genetic and environmental factors (Dubrovsky and Forde, 2012).

It would be interesting to further investigate also some results inferred by GO enrichement analysis: in *abi1* the processes related with the movement of cellular component are up-regulated in the LR development zone, but down-regulated in the root tip. Recent studies in roots of *Zea mays* tested the possible role of microtubules in the induction of ABA biosynthesis (Lu et al., 2007). It is suggested that changes in microtubule dynamics would trigger maize root cells to biosynthesize ABA, and interactions between osmotic stress and microtubule dynamics would have an effect on ABA accumulation in root cells, although the exact mechanism is still not clear (Lu et al., 2007). Apoptotic processes are down-regulated in *abi1*. Contrastingly, other studies in maize reproductive and leaf meristem tissue showed that drought caused activation of programmed cell (Kakumanu et al., 2012).

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Appendix

6.1 Dehydration and embedding protocol: Method M. Creuecoeur

Tissue is post fixed in OsO_4; dehydrated and subsequently embedded in Epon.

1. Rinse the glutaraldehyde fixed samples in the same buffer (i.e. phosphate) 6 x 15 mins
2. Post fix in 1:1 2% OsO_4 and phosphate buffer (over night i.e. approx. 15 hrs)
3. rinse out the Osmium with the phosphate buffer
4. Dehydrate in ethanol
   - 25% 20 mins
   - 50% 20 mins
   - 70% 20 mins
   - 90% 20 mins
   - 100% 3 x 30 mins

   total 170 mins
5. EPON
   - propylene oxide / 100% ethanol 1:1 30 mins
   - propylene oxide 2 x 30 mins
   - propylene oxide / epon 3:1 30 mins
   - propylene oxide / epon 1:1 30 mins
   - propylene oxide / epon 1:3 30 mins
   - epon without *accelerator at 60 °C 2 x 15 mins

   total 210 mins
6. Cure at 60 °C for 48 hrs

Preparation of Epon (solutions can be refrigerated at -20 °C)

Solution A:	Epon 812	31 g
	DDSA	50 g
mix for 1 hr		

Solution B:	Epon 812	50 g
	NMA	44.5 g
mix for 1 hr		

Final mix:	Solution A	11.25 g
	Solution B	13.75 g
	*DMP (accelerator)	0.375 g

6.2 Stain for Toluidin blue / P-phenylenediamine

For a 1% solution of Toluidin blue dissolved in dist. water

1. heat 150 ml of Toluidin blue (enough to cover the slide rack) in a suitable container to 40 °C.
2. Prepare two baths with dist. water.
3. Moisten slides by immersing them in 40 °C dist water for 2 mins.
4. Immerse slides in the Toluidin blue stain for 8 mins at 40 °C
5. Rinse in water baths (shake carefully)
6. Dry on fluff-free wipes

6.3 Agilent Plant RNA Isolation Mini Kit

Protocol: Product Number 5188-2780

Rod creaning: Wash with ammoniaca, then tap water, then Milli-Q water; dry carefully with paper

Preparation of Aliquotes:

- Washing solution 9 mL add 45.5 mL of ethanol to the Wash Solution in stock
- Extraction buffer 4mL+40µL ME 10µL of β-mercaptoethanol (ME)/mL of Extraction Solution; Agitate and heat until the solution is clear
- Isopropanol 5 mL
- Nuclease-free water 350 µL

RNA extraction

Roots recovery

- Plant extraction from agar
- Roots cutting in culture medium with razorblade and tweezer with Teflon
- Roots weight (balance sensitive to µg)
- Roots freezing in liquid nitrogen

RNA extraction

- Lysis with liquid nitrogen and prechilled plastic rod
- Addition of extraction buffer (10 µL of extraction buffer / mg of tissue)
- Lysis with plastic rod
- Omogenization: vortex 3 times; then vortex occasionally (do it immediately)

Purification

- Loading of filtration column (natural color; max column volume: 600 µL)
- Centrifugation (3 mins ; 16000 g)
- Recovery of the eluate on the opposite side of the pellet

Polishing

- Addition of iso-propanol for RNA precipitation (equal volume of eluate)
- Mix by hand all tubes, than for each tube: mix again, immediately load the column
- Loading of mini isolation column
- Incubation 5 mins
- Centrifugation (0.5 min ; 16000 g)
- Wash with 300 µL of Wash Solution
- Centrifugation (0.5 min ; 16000 g)
- Wash with 300 µL of Wash Solution
- Centrifugation (0.5 min ; 16000 g)
- Wash with 300 µL of Wash Solution

- Centrifugation (1 min ; 16000 g)
- Centrifugation (1 min ; 16000 g)

Final recovery

- Transfer column in a new tube provided
- Add 30 µL of free-nuclease water (recommended between 10-50 µL)
- Incubate approx. 2 mins
- Centrifugation (1 min ; 16000 g)

Make aliquots (3 µL for quality check, 15 µL for WSL, 15 µL for FGCZ)

Freeze tubes at -80 °C

6.4 Agilent RNA 6000 Pico; Bioanalyzer

Manual part number: G2938-90046 Rev. B

Samples have to be diluted to concentration of 50-5000 pg/µL (water)

Preliminary Operations

- Start the software
- Equilibrate all reagents for 30 mins
- Adjust the syringe
- Ladder preparation: Dilute with 90 µL of RNase-free water; denaturate at 70 °C – 2 mins
- Keep samples and ladder on ice
- Heat denaturate RNA samples at 70 °C – 2 mins
- Clean the Electrodes with 350 µL of fresh RNase-free water

Gel-dye mix preparation

- Filter with provided column 550 µL of RNA 6000 Pico gel matrix
- Centrifuge 10 mins 1500 g
- Vortex RNA 6000 Pico dye concentrate for 10 s; spin down
- Add 1 µL of RNA 6000 Pico dye concentrate to a 65 µL aliquot of filtered gel
- Vortex the Gel-dye mix
- Centrifuge 10 mins 13000 g

Chip preparation

- Pipet 9 µL of gel-dye mix in the G hole (white on black)
- Press the plunger; wait 30 s; release the plunger; wait 10 s; pull back the plunger to 1 mL
- Pipet 9 µL of gel-dye mix in each G hole (black on white)
- Pipet 9 µL of RNA 6000 Pico conditioning solution in CS hole
- Pipet 5 µL of RNA 6000 Pico marker in ladder (//) and all 11 samples holes
- Pipet 1 µL of deionized water in the unused samples holes
- Pipet 1 µL of diluted ladder in "//" hole
- Pipet 1 µL of each sample in each sample wells
- Vortex the chip for 60 s at 2400 rpm

Run the chip (30 mins)

6.5 Table 6.1. Exclusion of plants from analysis for the three experiments.

	Experiment 1	**Experiment 2**	**Experiment 3**	**Total**
wt; control	1	0	2	**3**
wt; sorbitol	2	3	2	**7**
abi1; control	0	0	3	**3**
abi1; sorbitol	2	1	4	**7**
Total number of excluded plants	**5**	**4**	**11**	**20**
Total number of plants	48	56	108	212
Percentage of excluded plants	10%	7%	10%	9%

6.6 GO terms enrichment analysis of up-regulated genes

Significant categories (p <0.05 FDR corrected) are highlighted in grey.

Category	**wt root tip**	***abi1* root tip**	**wt LR dev. zone**	***abi1* LR dev. zone**
ribosome biogenesis	2.6E-07	1.0E+00	1.0E+00	6.6E-01
translation	2.6E-07	2.5E-08	1.2E-13	4.3E-92
rRNA processing	2.0E-05	1.0E+00	1.0E+00	1.0E+00
gene expression	3.3E-05	3.2E-04	3.8E-09	2.5E-67
cellular protein metabolic process	4.0E-05	1.7E-04	3.6E-08	2.8E-80
rRNA metabolic process	4.0E-05	1.0E+00	1.0E+00	1.0E+00
cellular macromolecule biosynthetic process	4.9E-04	8.8E-05	5.8E-09	5.9E-83
oxidation-reduction process	6.9E-04	1.1E-09	3.6E-08	2.5E-05
aminoglycan catabolic process	1.5E-03	2.6E-02	1.3E-01	4.2E-01
chitin metabolic process	1.5E-03	2.6E-02	1.3E-01	4.2E-01
chitin catabolic process	1.5E-03	2.6E-02	1.3E-01	4.2E-01
glucosamine-containing compound catabolic process	1.5E-03	2.6E-02	1.3E-01	4.2E-01
single-organism metabolic process	6.8E-03	2.5E-08	1.0E-05	1.2E-02
superoxide metabolic process	8.8E-03	1.4E-03	1.7E-05	1.2E-02
reactive oxygen species metabolic process	8.8E-03	1.4E-03	1.7E-05	1.2E-02
ribonucleoprotein complex biogenesis	1.6E-02	1.0E+00	1.0E+00	6.4E-02
ncRNA processing	2.1E-02	1.0E+00	1.0E+00	1.0E+00
response to biotic stimulus	2.4E-02	8.8E-05	4.9E-02	4.3E-01
carbohydrate metabolic process	2.4E-02	9.1E-01	1.0E+00	1.4E-03
organic substance metabolic process	7.6E-02	4.4E-02	1.0E+00	9.4E-03
generation of precursor metabolites and energy	1.1E-01	8.8E-05	3.2E-03	4.7E-06
primary metabolic process	1.6E-01	7.3E-01	1.0E+00	3.8E-02
protein targeting to mitochondrion	2.1E-01	1.4E-01	5.0E-01	8.8E-04
mitochondrial transport	2.1E-01	1.4E-01	5.0E-01	8.8E-04
intracellular protein transmembrane import	2.1E-01	1.4E-01	5.0E-01	8.8E-04
establishment of protein localization to mitochondrial membrane	2.1E-01	1.4E-01	5.0E-01	8.8E-04
response to stimulus	2.9E-01	1.0E-05	6.6E-03	9.1E-01
photosynthesis	4.7E-01	3.0E-08	1.0E+00	3.2E-03
response to stress	4.8E-01	5.1E-07	2.3E-07	4.5E-04
photosynthesis	4.8E-01	8.8E-05	1.8E-01	8.9E-03
photosynthesis	4.8E-01	8.8E-05	2.9E-01	3.1E-02
cation transport	4.8E-01	4.4E-02	1.8E-01	1.0E+00
nucleoside metabolic process	6.1E-01	2.7E-01	2.6E-01	2.5E-02
nucleobase-containing small molecule metabolic process	6.1E-01	2.7E-01	2.6E-01	2.5E-02
glycosyl compound metabolic process	6.1E-01	2.7E-01	2.6E-01	2.5E-02
glycolysis	6.3E-01	4.2E-01	6.8E-02	5.9E-04
carbohydrate derivative metabolic process	7.3E-01	3.5E-01	3.3E-01	4.8E-02
cellular glucan metabolic process	7.3E-01	3.0E-02	1.0E+00	1.2E-02
glucan metabolic process	7.3E-01	3.0E-02	1.0E+00	1.2E-02
cellular polysaccharide metabolic process	7.3E-01	3.0E-02	1.0E+00	1.2E-02

Category	wt root tip	*abi1* root tip	wt LR dev. zone	*abi1* LR dev. zone
glucose catabolic process	8.1E-01	7.1E-01	1.3E-01	3.5E-03
inner mitochondrial membrane organization	8.1E-01	1.2E-01	1.8E-01	6.3E-04
protein import into mitochondrial inner membrane	8.1E-01	1.2E-01	1.8E-01	6.3E-04
establishment of protein localization to mitochondrion	8.1E-01	1.2E-01	1.8E-01	6.3E-04
mitochondrion organization	9.6E-01	1.9E-01	2.6E-01	2.4E-04
ATP biosynthetic process	1.0E+00	2.2E-01	8.8E-01	5.8E-03
cellular metabolic process	1.0E+00	1.9E-06	1.0E+00	4.3E-04
protein targeting	1.0E+00	4.6E-02	4.2E-02	4.2E-04
electron transport chain	1.0E+00	1.2E-02	1.0E+00	3.7E-01
tetraterpenoid biosynthetic process	1.0E+00	3.0E-02	1.3E-01	8.0E-01
carotenoid metabolic process	1.0E+00	3.0E-02	1.3E-01	8.0E-01
carotenoid biosynthetic process	1.0E+00	3.0E-02	1.3E-01	8.0E-01
single-organism biosynthetic process	1.0E+00	1.0E+00	1.0E+00	2.7E-05
response to oxidative stress	1.0E+00	4.8E-01	1.7E-01	2.4E-04
isocitrate metabolic process	1.0E+00	1.0E+00	1.0E+00	7.3E-03
tricarboxylic acid metabolic process	1.0E+00	1.0E+00	1.0E+00	7.3E-03
organic substance biosynthetic process	1.0E+00	1.0E+00	1.0E+00	2.0E-04
translational elongation	1.0E+00	4.7E-01	1.0E+00	3.2E-09
cell wall modification	1.0E+00	1.0E+00	1.0E+00	4.3E-02
cell wall organization	1.0E+00	1.0E+00	1.0E+00	4.3E-02
steroid biosynthetic process	1.0E+00	7.5E-01	1.0E+00	1.3E-02
steroid metabolic process	1.0E+00	7.9E-01	1.0E+00	9.2E-03
protein folding	1.0E+00	1.0E+00	1.0E+00	1.0E-02
monocarboxylic acid biosynthetic process	1.0E+00	1.0E+00	9.7E-01	1.7E-04
lipid biosynthetic process	1.0E+00	1.0E+00	1.0E+00	2.5E-03
fatty acid metabolic process	1.0E+00	1.0E+00	1.0E+00	1.8E-04
cellular process	1.0E+00	8.2E-01	1.0E+00	1.6E-02
energy coupled proton transport	1.0E+00	3.1E-01	1.0E+00	5.0E-05
ATP synthesis coupled proton transport	1.0E+00	3.1E-01	1.0E+00	5.0E-05
anion transport	1.0E+00	1.0E+00	1.0E+00	9.0E-03
fatty acid biosynthetic process	1.0E+00	1.0E+00	1.0E+00	3.2E-04
cellular component movement	1.0E+00	1.0E+00	1.0E+00	6.3E-10
microtubule-based movement	1.0E+00	1.0E+00	1.0E+00	6.3E-10
GTP metabolic process	1.0E+00	1.0E+00	1.0E+00	3.5E-03
cellular protein complex assembly	1.0E+00	1.0E+00	1.0E+00	2.4E-02
extracellular polysaccharide biosynthetic process	1.0E+00	1.0E+00	1.0E+00	7.3E-03
extracellular polysaccharide metabolic process	1.0E+00	1.0E+00	1.0E+00	7.3E-03
microtubule-based process	1.0E+00	1.0E+00	1.0E+00	2.3E-09
nucleosome assembly	1.0E+00	1.0E+00	1.0E+00	4.5E-04
chromatin assembly	1.0E+00	1.0E+00	1.0E+00	4.5E-04
nucleosome organization	1.0E+00	1.0E+00	1.0E+00	4.5E-04
protein-DNA complex assembly	1.0E+00	1.0E+00	1.0E+00	4.5E-04
protein complex assembly	1.0E+00	1.0E+00	1.0E+00	4.5E-04
transition metal ion transport	1.0E+00	3.7E-02	9.7E-01	2.1E-02

Category	**wt root tip**	***abi1* root tip**	**wt LR dev. zone**	***abi1* LR dev. zone**
mitochondrial electron transport	1.0E+00	5.1E-03	3.6E-02	7.3E-03
GTP catabolic process	1.0E+00	1.0E+00	1.0E+00	1.2E-02
purine ribonucleotide catabolic process	1.0E+00	1.0E+00	1.0E+00	3.2E-03
purine ribonucleoside triphosphate catabolic process	1.0E+00	1.0E+00	1.0E+00	3.2E-03
response to wounding	1.0E+00	1.9E-01	2.4E-03	2.7E-01
chlorophyll biosynthetic process	1.0E+00	1.0E+00	1.0E+00	4.4E-02
respiratory electron transport chain	1.0E+00	2.0E-04	7.0E-02	1.6E-02
mitochondrial ATP synthesis coupled electron transport	1.0E+00	5.1E-03	3.6E-02	7.3E-03
regulation of ion transport	1.0E+00	1.0E+00	1.0E+00	1.2E-02
regulation of anion transport	1.0E+00	1.0E+00	1.0E+00	1.2E-02
cellular protein catabolic process	1.0E+00	4.8E-01	1.0E+00	6.1E-03
protein polymerization	1.0E+00	1.0E+00	1.0E+00	3.4E-02
proteolysis involved in cellular protein catabolic process	1.0E+00	4.8E-01	1.0E+00	6.1E-03
guanosine-containing compound catabolic process	1.0E+00	1.0E+00	1.0E+00	1.2E-02
cellular iron ion homeostasis	1.0E+00	3.3E-02	5.4E-01	7.3E-03
cellular transition metal ion homeostasis	1.0E+00	3.3E-02	5.4E-01	7.3E-03
iron ion homeostasis	1.0E+00	3.3E-02	5.4E-01	7.3E-03

6.7 GO terms enrichment analysis of down-regulated genes

Significant categories (p <0.05 FDR corrected) are highlighted in grey.

Category	wt root tip	*abi1* root tip	wt LR dev. zone	*abi1* LR dev. zone
protein phosphorylation	7.8E-08	4.2E-24	3.5E-05	1.5E-09
phosphorylation	7.8E-08	3.3E-23	3.5E-05	2.8E-09
oxidation-reduction process	1.0E-06	1.0E+00	6.5E-02	1.0E+00
single-organism metabolic process	6.4E-06	1.0E+00	1.1E-01	1.0E+00
cellular protein modification process	8.7E-06	4.2E-24	9.7E-04	2.6E-10
response to oxidative stress	1.8E-05	4.9E-01	8.7E-01	1.0E+00
regulation of gene expression	4.8E-04	1.4E-01	1.3E-02	1.4E-15
regulation of RNA biosynthetic process	4.8E-04	1.4E-01	1.3E-02	1.4E-15
regulation of cellular macromolecule biosynthetic process	4.8E-04	1.5E-01	1.3E-02	1.4E-15
regulation of transcription	1.1E-03	1.1E-01	1.3E-02	1.4E-15
response to stress	1.2E-03	1.0E+00	6.4E-01	1.0E+00
lipid metabolic process	1.9E-02	1.0E+00	1.0E+00	1.0E+00
single-organism biosynthetic process	1.9E-02	1.3E-01	1.0E+00	1.0E+00
fatty acid metabolic process	2.0E-02	3.5E-01	1.0E+00	1.0E+00
monocarboxylic acid biosynthetic process	2.4E-02	1.8E-01	1.0E+00	1.0E+00
transcription	2.4E-02	1.2E-01	1.9E-02	1.4E-15
response to biotic stimulus	2.9E-02	1.0E+00	5.0E-02	1.0E+00
organic substance biosynthetic process	3.6E-02	1.8E-01	1.0E+00	1.0E+00
fatty acid biosynthetic process	4.5E-02	2.8E-01	1.0E+00	1.0E+00
trehalose biosynthetic process	1.2E-01	1.8E-01	2.1E-01	5.0E-03
transmembrane transport	1.9E-01	1.2E-01	6.3E-05	5.0E-03
disaccharide biosynthetic process	2.1E-01	2.8E-01	3.3E-01	1.2E-02
single-organism transport	2.2E-01	1.2E-01	6.8E-05	7.5E-03
trehalose metabolic process	2.4E-01	2.6E-01	3.2E-01	9.5E-03
establishment of localization	3.1E-01	6.7E-03	1.1E-01	1.7E-06
cell wall modification	5.1E-01	1.2E-01	4.3E-02	1.0E+00
cell wall organization	5.1E-01	1.2E-01	4.3E-02	1.0E+00
cation transport	7.7E-01	9.6E-01	1.0E-01	9.5E-03
amine metabolic process	8.8E-01	8.3E-04	3.2E-04	4.6E-03
cell recognition	8.8E-01	2.1E-02	3.3E-01	1.4E-01
pollen-pistil interaction	8.8E-01	2.1E-02	3.3E-01	1.4E-01
recognition of pollen	8.8E-01	2.1E-02	3.3E-01	1.4E-01
cellular process involved in reproduction	1.0E+00	2.9E-02	3.8E-01	1.5E-01
beta-glucan biosynthetic process	1.0E+00	2.0E-02	1.3E-01	1.0E+00
transport	1.0E+00	7.5E-02	1.0E+00	1.7E-02
oligopeptide transport	1.0E+00	1.0E+00	1.9E-02	1.0E+00
peptide transport	1.0E+00	1.0E+00	1.9E-02	1.0E+00
ion transmembrane transport	1.0E+00	1.0E+00	1.2E-01	7.2E-04
cytokinin metabolic process	1.0E+00	1.5E-02	6.5E-02	6.8E-01
cellular hormone metabolic process	1.0E+00	1.5E-02	6.5E-02	6.8E-01

Category	**wt root tip**	***abi1* root tip**	**wt LR dev. zone**	***abi1* LR dev. zone**
apoptotic process	1.0E+00	6.7E-03	3.7E-01	2.6E-10
programmed cell death	1.0E+00	6.7E-03	3.7E-01	2.6E-10
microtubule-based process	1.0E+00	1.3E-07	1.0E+00	1.0E+00
DNA replication	1.0E+00	9.0E-03	1.0E+00	1.0E+00
cellular component movement	1.0E+00	7.9E-10	1.0E+00	1.0E+00
microtubule-based movement	1.0E+00	7.9E-10	1.0E+00	1.0E+00
ARF protein signal transduction	1.0E+00	5.0E-02	1.0E+00	1.0E+00
regulation of Ras protein signal transduction	1.0E+00	5.0E-02	1.0E+00	1.0E+00
cellular potassium ion transport	1.0E+00	1.0E+00	1.0E+00	1.2E-02
potassium ion transmembrane transport	1.0E+00	1.0E+00	1.0E+00	1.2E-02
protein localization	1.0E+00	2.1E-02	1.0E+00	2.2E-02
macromolecule localization	1.0E+00	2.1E-02	1.0E+00	2.2E-02

6.8 Table 6.2. Selected differentially expressed genes (DEGs) in root tip tissue.

Genes were considered differentially expressed with $log_2 < -1$ (grey) or $log_2 > 1$, and were significant for FDR < 0.05.
The three columns present genes differentially expressed only in wild-type plants (wild-type), in plants of both genotypes (common) and only in *abi1* plants (*abi1*). Each gene is presented with Identifier and Annotation according to the *Populus Trichocarpa* genome (www.phytozome.net; v3.0) and the logarithmic ratio of gene expression between sorbitol and control conditions (log_2 Ratio).
DEGs involved in the ABA pathway, in the ABA-independent pathway, in the hormonal regulation, and in the stress response were selected.

ROOT TIP

wild-type			**common**				***abi1***		
Identifier	**Annotation**	**log_2 Ratio**	**Identifier**	**Annotation**	**log_2 Ratio** wt	*abi1*	**Identifier**	**Annotation**	**log_2 Ratio**
				ABA pathway					
Potri.004G235400	cytochrome P450. family 707. subfamily A. polypeptide 1	1.39	Potri.011G112400	nine-cis-epoxycarotenoid dioxygenase 3	4.39	5.16	Potri.T093800	CBL-interacting protein kinase 21	1.23
Potri.018G082600	ABI-1-like 1	1.06	Potri.017G094500	ABI five binding protein 3	1.80	1.86	Potri.004G140900	cytochrome P450. family 707. subfamily A. polypeptide 4	3.67
Potri.001G092100	highly ABA-induced PP2C gene 3	5.33	Potri.012G002700	highly ABA-induced PP2C gene 3	2.37	2.05	Potri.001G393800	nine-cis-epoxycarotenoid dioxygenase 3	2.54
Potri.010G121600	Protein phosphatase 2C family protein	1.12	Potri.009G037300	highly ABA-induced PP2C gene 2	2.21	3.06	Potri.015G026700	SLAC1 homologue 3	1.13
Potri.006G263500	CBL-interacting protein kinase 25	1.79	Potri.T137100	highly ABA-induced PP2C gene 3	1.88	1.31	Potri.008G106700	basic region/leucine zipper motif 53	2.56
Potri.008G073400	PYR1-like 6	-2.94	Potri.012G131800	Protein phosphatase 2C family protein	1.38	1.42	Potri.010G142900	basic leucine-zipper 1	1.54
Potri.010G183900	PYR1-like 6	-2.35	Potri.008G059200	protein phosphatase 2CA	1.12	1.05	Potri.002G136400	homeobox-leucine zipper protein 3	1.45
Potri.016G125400	PYR1-like 4	-1.67	Potri.012G139300	Homeobox-leucine zipper family protein / lipid-binding START domain-containing protein	2.73	2.90	Potri.008G168400	Protein phosphatase 2C family protein	1.52
Potri.006G104100	PYR1-like 4	-1.59	Potri.002G115900	basic leucine-zipper 4	1.78	1.31	Potri.015G018800	highly ABA-induced PP2C gene 3	1.59
Potri.010G002500	CBL-interacting protein kinase 1	-1.66	Potri.006G199400	calcium-dependent protein kinase 20	2.96	4.42	Potri.001G245200	highly ABA-induced PP2C gene 2	1.47
			Potri.013G099400	Protein phosphatase 2C family protein	-1.14	-1.25	Potri.006G238500	carotenoid cleavage dioxygenase 8	-1.52

ROOT TIP

wild-type			common				*abi1*		
Identifier	**Annotation**	**log_2 Ratio**	**Identifier**	**Annotation**	**log_2 Ratio** wt	*abi1*	**Identifier**	**Annotation**	**log_2 Ratio**
				ABA pathway					
			Potri.007G075200	GRAM domain-containing protein / ABA-responsive protein-related	-2.68	-2.29	Potri.005G088400	GRAM domain-containing protein / ABA-responsive protein-related	-3.72
			Potri.007G075700	GRAM domain-containing protein / ABA-responsive protein-related	-1.82	-1.81	Potri.001G184100	HD-ZIP IV family of homeobox-leucine zipper protein with lipid-binding START domain	-1.24
							Potri.003G052400	HD-ZIP IV family of homeobox-leucine zipper protein with lipid-binding START domain	-1.14
							Potri.019G071600	Protein phosphatase 2C family protein	-2.36
				ABA-indipendent pathway					
Potri.014G066200	C2H2-type zinc finger family protein	-3.27					Potri.010G229400	C2H2-type zinc finger family protein	1.15
							Potri.001G404100	NAC (No Apical Meristem) domain transcriptional regulator superfamily protein	1.89
							Potri.011G123300	NAC (No Apical Meristem) domain transcriptional regulator superfamily protein	1.46
				Hormonal regulators					
Potri.001G176600	gibberellin 3-oxidase 1	1.54	Potri.011G134000	gibberellin 2-oxidase 8	6.20	6.26	Potri.006G247700	gibberellin 3-oxidase 1	3.83
Potri.001G242000	SCARECROW-like 14	1.49	Potri.008G145300	gibberellin 2-oxidase 8	3.37	1.70	Potri.014G117300	gibberellin 2-oxidase 6	1.56
Potri.001G378400	Arabidopsis thaliana gibberellin 2-oxidase 1	-1.03	Potri.002G022600	GAST1 protein homolog 1	1.63	3.92	Potri.005G065400	gibberellin 20 oxidase 2	1.55
Potri.007G133000	GRAS family transcription factor family protein	-1.52	Potri.002G024300	SAUR-like auxin-responsive protein family	1.26	1.53	Potri.015G134600	gibberellin 20 oxidase 2	1.35
Potri.003G065400	GRAS family transcription factor	-1.42	Potri.001G004700	related to AP2 11	2.20	7.49	Potri.017G083000	GAST1 protein homolog 4	1.19
Potri.014G164400	GRAS family transcription factor	-1.29	Potri.003G220200	related to AP2 11	2.08	5.48	Potri.007G051300	Gibberellin-regulated family protein	-1.43
Potri.005G175300	GRAS family transcription factor	-1.07	Potri.002G181600	AP2/B3-like transcriptional factor family protein	-3.33	-1.36	Potri.014G025200	related to AP2 1	1.21
Potri.005G095100	RGA-like 1	-3.00	Potri.011G149700	AP2/B3-like transcriptional factor family protein	-2.63	-1.68	Potri.003G162500	related to AP2 6l	1.03

ROOT TIP

wild-type			common				*abi1*		
Identifier	**Annotation**	**log_2 Ratio**	**Identifier**	**Annotation**	**log_2 Ratio** wt	*abi1*	**Identifier**	**Annotation**	**log_2 Ratio**
				Hormonal regulators					
Potri.005G054100	DREB2A-interacting protein 2	2.60	Potri.001G099400	1-aminocyclopropane-1-carboxylic acid (acc) synthase 6	-1.37	-1.01	Potri.006G211000	SAUR-like auxin-responsive protein family	3.76
Potri.001G452200	AP2/B3-like transcriptional factor family protein	-1.25					Potri.012G102700	SAUR-like auxin-responsive protein family	1.68
Potri.013G056700	related to AP2 11	-1.11					Potri.001G306300	SAUR-like auxin-responsive protein family	1.56
Potri.016G084500	related to AP2.7	-1.08					Potri.011G143400	SAUR-like auxin-responsive protein family	1.46
Potri.010G216200	related to AP2.7	-1.01					Potri.006G137200	SAUR-like auxin-responsive protein family	1.19
Potri.003G071000	SAUR-like auxin-responsive protein family	1.55					Potri.006G137000	SAUR-like auxin-responsive protein family	1.17
Potri.012G023400	SAUR-like auxin-responsive protein family	-1.04					Potri.004G164300	SAUR-like auxin-responsive protein family	1.07
Potri.T155100	GAST1 protein homolog 3	-3.53					Potri.001G060400	SAUR-like auxin-responsive protein family	1.03
				Stress response					
Potri.T111300	Late Embryogenesis Abundant 4-5	5.09	Potri.011G140600	glutathione S-transferase TAU 22	2.19	3.10	Potri.011G113300	glutathione S-transferase TAU 25	4.38
Potri.016G046400	Late Embryogenesis Abundant 4-5	3.58	Potri.010G002600	late embryogenesis abundant domain-containing protein / LEA domain-containing protein	5.79	2.88	Potri.011G112900	glutathione S-transferase TAU 25	2.89
Potri.017G138800	glutathione S-transferase phi 12	-3.50	Potri.005G122400	Late embryogenesis abundant (LEA) protein	4.79	4.93	Potri.011G113000	glutathione S-transferase TAU 19	2.36
Potri.010G035500	Glutathione S-transferase family protein	-1.23	Potri.004G046000	Late embryogenesis abundant protein (LEA) family protein	4.39	4.47	Potri.006G024200	glutathione S-transferase TAU 8	1.47
Potri.010G032800	Glutathione S-transferase family protein	-1.09	Potri.015G002400	Late embryogenesis abundant (LEA) hydroxyproline-rich glycoprotein family	3.39	2.75	Potri.016G118500	glutathione S-transferase tau 7	1.38
Potri.004G067100	Late embryogenesis abundant (LEA) hydroxyproline-rich glycoprotein family	-2.46	Potri.011G054200	Late embryogenesis abundant protein (LEA) family protein	3.06	2.55	Potri.T178900	glutathione S-transferase TAU 25	1.34

ROOT TIP

wild-type			common				*abi1*		
Identifier	**Annotation**	**log_2 Ratio**	**Identifier**	**Annotation**	**log_2 Ratio** wt	*abi1*	**Identifier**	**Annotation**	**log_2 Ratio**
				Stress response					
Potri.011G133900	Late embryogenesis abundant (LEA) hydroxyproline-rich glycoprotein family	-1.71	Potri.014G090800	Late embryogenesis abundant protein	1.51	1.94	Potri.008G175100	glutathione S-transferase tau 7	1.07
Potri.001G200700	Late embryogenesis abundant (LEA) hydroxyproline-rich glycoprotein family	-1.37	Potri.013G031100	copper/zinc superoxide dismutase 1	1.36	1.43	Potri.T035300	glutathione S-transferase 6	1.04
Potri.008G031700	lactate/malate dehydrogenase family protein	1.09	Potri.005G044400	copper/zinc superoxide dismutase 1	1.01	1.23	Potri.015G148200	Late embryogenesis abundant (LEA) hydroxyproline-rich glycoprotein family	1.27
Potri.011G044300	beta-galactosidase 8	1.58	Potri.015G110400	Fe superoxide dismutase 2	-1.81	-1.03	Potri.004G216700	copper/zinc superoxide dismutase 2	1.12
Potri.007G099800	beta-galactosidase 10	1.10	Potri.003G111200	Lactate/malate dehydrogenase family protein	2.59	3.61	Potri.009G005100	copper/zinc superoxide dismutase 2	1.04
Potri.001G401700	expansin 12	2.90	Potri.001G119300	Plant invertase/pectin methylesterase inhibitor superfamily protein	4.66	5.63	Potri.010G164100	Early-responsive to dehydration stress protein (ERD4)	-1.06
Potri.001G240900	expansin A4	1.28	Potri.015G128900	Plant invertase/pectin methylesterase inhibitor superfamily protein	1.49	3.57	Potri.015G127700	Plant invertase/pectin methylesterase inhibitor superfamily	1.69
Potri.019G101900	expansin B3	-1.40	Potri.001G112900	expansin A7	2.75	6.63	Potri.014G044100	Plant invertase/pectin methylesterase inhibitor superfamily protein	1.36
Potri.001G148900	chloroplast beta-amylase	-2.87	Potri.013G154700	expansin A8	1.40	1.90	Potri.002G145800	Plant invertase/pectin methylesterase inhibitor superfamily protein	1.36
Potri.008G204200	beta-amylase 3	-1.04	Potri.002G145500	Plant invertase/pectin methylesterase inhibitor superfamily	-6.68	-3.75	Potri.006G137800	Plant invertase/pectin methylesterase inhibitor superfamily protein	1.30
Potri.T151500	pectin methylesterase 3	-1.32	Potri.003G086500	Plant invertase/pectin methylesterase inhibitor superfamily protein	-4.49	-3.32	Potri.016G135200	expansin A8	5.14
			Potri.014G067100	Plant invertase/pectin methylesterase inhibitor superfamily	-2.95	-1.85	Potri.004G181700	expansin-like A2	1.59

ROOT TIP

wild-type			common				*abi1*		
Identifier	**Annotation**	**log_2 Ratio**	**Identifier**	**Annotation**	**log_2 Ratio** wt	*abi1*	**Identifier**	**Annotation**	**log_2 Ratio**
				Stress response					
			Potri.014G127000	Plant invertase/pectin methylesterase inhibitor superfamily	-1.98	-1.70	Potri.010G202500	expansin A4	1.13
			Potri.008G174100	beta-amylase 1	1.41	1.18	Potri.002G202600	Plant invertase/pectin methylesterase inhibitor superfamily	-3.51
							Potri.001G162500	pectin methylesterase 3	-1.21
							Potri.T101200	beta-amylase 5	2.46
							Potri.005G033500	plant glycogenin-like starch initiation protein 5	1.62

6.9 Table 6.3. Selected differentially expressed genes (DEGs) in lateral root development zone tissue.

Genes were considered differentially expressed with $log_2 < -1$ (grey) or $log_2 > 1$. and were significant for FDR < 0.05.
The three columns present genes differentially expressed only in wild-type plants (wild-type). in plants of both genotypes (common) and only in *abi1* plants (*abi1*). Each gene is presented with Identifier and Annotation according to the *Populus Trichocarpa* genome (www.phytozome.net; v3.0) and the logarithmic ratio of gene expression between sorbitol and control conditions (log_2 Ratio).
DEGs involved in the ABA pathway. in the ABA-independent pathway. in the hormonal regulation. and in the stress response were selected.

LATERAL ROOT DEVELOPMENT ZONE

wild-type			common				*abi1*		
Identifier	**Annotation**	**log_2 Ratio**	**Identifier**	**Annotation**	**log_2 Ratio** wt	*abi1*	**Identifier**	**Annotation**	**log_2 Ratio**
				ABA pathway					
Potri.018G044100	carotenoid cleavage dioxygenase 8	-1.52	Potri.011G112400	nine-cis-epoxycarotenoid dioxygenase 3	3.75	2.65	Potri.006G238500	carotenoid cleavage dioxygenase 8	-2.41
Potri.001G092100	highly ABA-induced PP2C gene 3	7.92	Potri.001G393800	nine-cis-epoxycarotenoid dioxygenase 3	1.47	1.85	Potri.015G141800	Homeobox-leucine zipper family protein / lipid-binding START domain-containing protein	3.27
Potri.015G018800	highly ABA-induced PP2C gene 3	1.50	Potri.004G235400	cytochrome P450. family 707. subfamily A. polypeptide 1	1.58	1.20	Potri.001G381000	Protein phosphatase 2C family protein	-2.51
Potri.001G245200	highly ABA-induced PP2C gene 2	1.45	Potri.017G094500	ABI five binding protein 3	1.52	1.42	Potri.010G187000	phosphatase 2C5	-2.17
Potri.008G168400	Protein phosphatase 2C family protein	1.50	Potri.006G104100	PYR1-like 4	-2.00	-1.11	Potri.018G059800	Protein phosphatase 2C family protein	-1.12
Potri.013G012200	Protein phosphatase 2C family protein	1.44	Potri.012G002700	highly ABA-induced PP2C gene 3	2.70	2.06	Potri.013G099400	Protein phosphatase 2C family protein	-1.02
Potri.018G150800	Protein phosphatase 2C family protein	1.18	Potri.009G037300	highly ABA-induced PP2C gene 2	2.18	3.49	Potri.013G090800	SNF1 kinase homolog 10	-1.40
Potri.008G059200	protein phosphatase 2CA	1.14	Potri.T137100	highly ABA-induced PP2C gene 3	1.62	1.15	Potri.001G381000	Protein phosphatase 2C family protein	-2.51
Potri.008G073400	PYR1-like 6	-3.02	Potri.005G072600	SNF1-related protein kinase 2.7	3.55	1.42	Potri.018G059800	Protein phosphatase 2C family protein	-1.12
Potri.010G183900	PYR1-like 6	-2.12	Potri.007G075700	GRAM domain-containing protein / ABA-responsive protein-related	-3.43	-2.68	Potri.013G099400	Protein phosphatase 2C family protein	-1.02

LATERAL ROOT DEVELOPMENT ZONE

wild-type			common				*abi1*		
Identifier	**Annotation**	**log_2 Ratio**	**Identifier**	**Annotation**	**log_2 Ratio** wt	*abi1*	**Identifier**	**Annotation**	**log_2 Ratio**
				ABA pathway					
Potri.016G125400	PYR1-like 4	-1.33	Potri.007G075200	GRAM domain-containing protein / ABA-responsive protein-related	-3.26	-2.89	Potri.005G088700	GRAM domain-containing protein / ABA-responsive protein-related	-2.13
Potri.003G052400	HD-ZIP IV family of homeobox-leucine zipper protein with lipid-binding START domain	-1.13	Potri.012G139300	Homeobox-leucine zipper family protein / lipid-binding START domain-containing protein	4.86	4.12	Potri.011G116700	Protein phosphatase 2C family protein	2.22
Potri.001G184100	HD-ZIP IV family of homeobox-leucine zipper protein with lipid-binding START domain	-1.02	Potri.012G131800	Protein phosphatase 2C family protein	1.82	1.36	Potri.010G047600	Protein phosphatase 2C family protein	1.66
Potri.015G026800	SLAC1 homologue 3	2.02					Potri.018G034200	Protein phosphatase 2A regulatory B subunit family protein	1.06
Potri.015G026700	SLAC1 homologue 3	1.88					Potri.009G149400	histone deacetylase 3	1.45
Potri.006G263500	CBL-interacting protein kinase 25	1.77							
Potri.003G181900	CBL-interacting protein kinase 25	1.55							
Potri.T093800	CBL-interacting protein kinase 21	1.04							
				ABA-indipendent pathway					
Potri.009G004800	C2H2-type zinc finger family protein	-1.18					Potri.001G267900	C2H2-type zinc finger family protein	3.25
							Potri.010G229400	C2H2-type zinc finger family protein	2.22
							Potri.014G066200	C2H2-type zinc finger family protein	1.58
							Potri.016G098100	C2H2-type zinc finger family protein	1.13
							Potri.003G205000	C2H2-type zinc finger family protein	1.06
							Potri.010G176600	NAC (No Apical Meristem) domain transcriptional regulator superfamily protein	4.02
							Potri.008G080000	NAC (No Apical Meristem) domain transcriptional regulator superfamily protein	2.38
							Potri.002G178700	NAC (No Apical Meristem) domain transcriptional regulator superfamily protein	1.30

LATERAL ROOT DEVELOPMENT ZONE

wild-type			common				*abi1*		
Identifier	**Annotation**	**log_2 Ratio**	**Identifier**	**Annotation**	**log_2 Ratio** wt	*abi1*	**Identifier**	**Annotation**	**log_2 Ratio**
				ABA-indipendent pathway					
							Potri.005G069500	NAC (No Apical Meristem) domain transcriptional regulator superfamily protein	-1.30
				Hormonal regulators					
Potri.015G134600	gibberellin 20 oxidase 2	1.52	Potri.008G145300	gibberellin 2-oxidase 8	2.31	2.44	Potri.001G350600	Gibberellin-regulated family protein	4.68
Potri.012G132400	gibberellin 20 oxidase 2	1.26	Potri.006G199400	calcium-dependent protein kinase 20	2.72	2.72	Potri.007G051300	Gibberellin-regulated family protein	4.23
Potri.002G022600	GAST1 protein homolog 1	1.45	Potri.011G095600	Arabidopsis thaliana gibberellin 2-oxidase 1	-1.25	-1.39	Potri.006G247700	gibberellin 3-oxidase 1	1.87
Potri.001G242000	SCARECROW-like 14	1.28	Potri.017G025900	GRAS family transcription factor	-1.39	-1.29	Potri.017G083000	GAST1 protein homolog 4	2.00
Potri.T155100	GAST1 protein homolog 3	-2.34	Potri.001G242100	SCARECROW-like 14	-1.64	-1.50	Potri.001G109400	GRAS family transcription factor	2.45
Potri.014G164400	GRAS family transcription factor	-1.30	Potri.001G067600	related to AP2 6l	1.17	1.01	Potri.003G122400	GRAS family transcription factor	2.34
Potri.014G025200	related to AP2 1	1.13	Potri.003G071000	SAUR-like auxin-responsive protein family	2.77	3.28	Potri.005G095100	RGA-like 1	5.68
Potri.011G149700	AP2/B3-like transcriptional factor family protein	-1.78					Potri.002G185200	rotamase CYP 4	1.88
Potri.002G181600	AP2/B3-like transcriptional factor family protein	-1.26					Potri.004G168800	rotamase CYP 3	1.64
Potri.012G102700	SAUR-like auxin-responsive protein family	2.38					Potri.009G130100	rotamase CYP 1	1.57
Potri.002G024500	SAUR-like auxin-responsive protein family	1.36					Potri.006G200600	calcium dependent protein kinase 1	1.03
							Potri.001G176600	gibberellin 3-oxidase 1	-1.19
							Potri.001G415200	GRAS family transcription factor	-1.22
							Potri.016G143900	GRAS family transcription factor	-1.14
							Potri.007G053500	GRAS family transcription factor	-1.12
							Potri.007G026300	GRAS family transcription factor	-1.05
							Potri.006G016200	SCARECROW-like 13	-1.14
							Potri.001G409500	scarecrow-like 5	-1.02
							Potri.018G146300	acetyl Co-enzyme a carboxylase biotin carboxylase subunit	1.42
							Potri.001G004700	related to AP2 11	5.22
							Potri.009G103300	AP2/B3-like transcriptional factor family protein	2.16

LATERAL ROOT DEVELOPMENT ZONE

wild-type			common			*abi1*		
Identifier	**Annotation**	**log_2 Ratio**	**Identifier**	**Annotation**	**log_2 Ratio** wt	*abi1*	**Identifier**	**Annotation**	**log_2 Ratio**
				Hormonal regulators					
							Potri.014G031600	AP2/B3-like transcriptional factor family protein	2.08
							Potri.011G054000	AP2/B3-like transcriptional factor family protein	1.87
							Potri.014G031700	AP2/B3-like transcriptional factor family protein	1.79
							Potri.010G163900	related to AP2 11	1.77
							Potri.001G041500	ARIA-interacting double AP2 domain protein	1.58
							Potri.009G103100	AP2/B3-like transcriptional factor family protein	1.56
							Potri.007G035500	AP2/B3-like transcriptional factor family protein	1.11
							Potri.007G090600	related to AP2 4	-1.31
							Potri.018G109200	AP2/B3 transcription factor family protein	-1.28
							Potri.006G186300	AP2/B3 transcription factor family protein	-1.10
							Potri.006G208100	AP2/B3-like transcriptional factor family protein	-1.05
							Potri.016G084500	related to AP2.7	-1.04
							Potri.001G099400	1-aminocyclopropane-1-carboxylic acid (acc) synthase 6	-1.19
							Potri.003G132300	1-aminocyclopropane-1-carboxylic acid (acc) synthase 6	-1.22
							Potri.006G278100	SAUR-like auxin-responsive protein family	4.42
							Potri.018G063400	SAUR-like auxin-responsive protein family	2.82
							Potri.002G000600	SAUR-like auxin-responsive protein family	2.73
							Potri.004G164400	SAUR-like auxin-responsive protein family	2.30
							Potri.002G024300	SAUR-like auxin-responsive protein family	1.50
							Potri.004G164300	SAUR-like auxin-responsive protein family	1.46

LATERAL ROOT DEVELOPMENT ZONE

wild-type			common				*abi1*		
Identifier	**Annotation**	**log_2 Ratio**	**Identifier**	**Annotation**	**log_2 Ratio** wt	*abi1*	**Identifier**	**Annotation**	**log_2 Ratio**
				Hormonal regulators					
							Potri.001G306300	SAUR-like auxin-responsive protein family	1.36
							Potri.009G125900	SAUR-like auxin-responsive protein family	1.13
							Potri.007G112000	SAUR-like auxin-responsive protein family	-1.88
							Potri.003G113100	SAUR-like auxin-responsive protein family	-1.35
				Stress response					
Potri.011G113100	glutathione S-transferase TAU 25	2.17	Potri.002G226800	ERD (early-responsive to dehydration stress) family protein	1.82	2.18	Potri.014G156100	ERD (early-responsive to dehydration stress) family protein	1.45
Potri.006G024200	glutathione S-transferase TAU 8	2.45	Potri.019G130500	glutathione S-transferase TAU 25	3.13	1.20	Potri.011G140600	glutathione S-transferase TAU 22	5.32
Potri.017G140900	microsomal glutathione s-transferase. putative	1.73	Potri.T178900	glutathione S-transferase TAU 25	2.85	1.19	Potri.014G132200	Glutathione S-transferase family protein	3.99
Potri.016G118500	glutathione S-transferase tau 7	1.37	Potri.011G140400	glutathione S-transferase TAU 19	2.38	6.96	Potri.011G140700	glutathione S-transferase TAU 19	2.74
Potri.016G104500	glutathione S-transferase TAU 8	1.32	Potri.011G113000	glutathione S-transferase TAU 19	2.28	2.09	Potri.010G035500	Glutathione S-transferase family protein	2.21
Potri.001G431700	glutathione S-transferase TAU 19	1.15	Potri.008G175000	glutathione S-transferase tau 7	2.12	1.45	Potri.010G032800	Glutathione S-transferase family protein	1.71
Potri.006G204300	Late embryogenesis abundant (LEA) hydroxyproline-rich glycoprotein family	1.15	Potri.010G061400	glutathione S-transferase tau 7	1.95	1.53	Potri.002G015100	glutathione S-transferase F11	1.19
Potri.017G138800	glutathione S-transferase phi 12	-2.18	Potri.008G174900	glutathione S-transferase tau 7	1.95	1.20	Potri.010G070900	glutathione S-transferase tau 7	1.09
Potri.002G145700	Plant invertase/pectin methylesterase inhibitor superfamily	4.58	Potri.011G114000	glutathione S-transferase TAU 19	1.85	2.53	Potri.T035100	glutathione S-transferase 6	1.08
Potri.001G119300	Plant invertase/pectin methylesterase inhibitor superfamily protein	3.63	Potri.010G060900	glutathione S-transferase tau 7	1.69	1.11	Potri.004G067100	Late embryogenesis abundant (LEA) hydroxyproline-rich glycoprotein family	2.13
Potri.015G127700	Plant invertase/pectin methylesterase inhibitor superfamily	1.67	Potri.008G175100	glutathione S-transferase tau 7	1.68	1.55	Potri.004G046000	Late embryogenesis abundant protein (LEA) family protein	2.12
Potri.002G145500	Plant invertase/pectin methylesterase inhibitor superfamily	-5.20	Potri.010G061700	glutathione S-transferase tau 7	1.67	1.20	Potri.014G094400	Late embryogenesis abundant (LEA) hydroxyproline-rich glycoprotein family	1.31

LATERAL ROOT DEVELOPMENT ZONE

wild-type			common				*abi1*		
Identifier	**Annotation**	**log_2 Ratio**	**Identifier**	**Annotation**	**log_2 Ratio** wt	*abi1*	**Identifier**	**Annotation**	**log_2 Ratio**
				Stress response					
Potri.003G086500	Plant invertase/pectin methylesterase inhibitor superfamily protein	-2.49	Potri.010G061100	glutathione S-transferase tau 7	1.60	1.23	Potri.009G019600	Late embryogenesis abundant (LEA) hydroxyproline-rich glycoprotein family	1.06
Potri.003G002800	pectin methylesterase 3	-1.87	Potri.019G090300	late embryogenesis abundant domain-containing protein / LEA domain-containing protein	5.44	6.42	Potri.005G089600	Fe superoxide dismutase 3	1.37
Potri.001G162500	pectin methylesterase 3	-1.66	Potri.005G122400	Late embryogenesis abundant (LEA) protein	4.69	5.46	Potri.001G376500	Lactate/malate dehydrogenase family protein	1.36
Potri.001G162600	pectin methylesterase 3	-1.59	Potri.010G002600	late embryogenesis abundant domain-containing protein / LEA domain-containing protein	4.41	3.18	Potri.005G232600	beta-galactosidase 3	2.11
Potri.T151500	pectin methylesterase 3	-1.53	Potri.016G046400	Late Embryogenesis Abundant 4-5	3.34	2.87	Potri.007G099800	beta-galactosidase 10	1.97
Potri.006G134600	Plant invertase/pectin methylesterase inhibitor superfamily	-1.51	Potri.017G108500	Late embryogenesis abundant protein (LEA) family protein	3.00	3.07	Potri.008G011100	Plant invertase/pectin methylesterase inhibitor superfamily	7.09
Potri.015G128300	Plant invertase/pectin methylesterase inhibitor superfamily protein	-1.47	Potri.T111300	Late Embryogenesis Abundant 4-5	2.27	2.53	Potri.006G137800	Plant invertase/pectin methylesterase inhibitor superfamily protein	4.80
Potri.003G072800	pectin methylesterase 3	-1.26	Potri.014G090800	Late embryogenesis abundant protein	2.21	8.78	Potri.002G202600	Plant invertase/pectin methylesterase inhibitor superfamily	4.35
Potri.003G113600	Plant invertase/pectin methylesterase inhibitor superfamily protein	-1.19	Potri.011G054200	Late embryogenesis abundant protein (LEA) family protein	2.12	5.16	Potri.014G067500	Plant invertase/pectin methylesterase inhibitor superfamily protein	3.60
Potri.012G014500	Plant invertase/pectin methylesterase inhibitor superfamily	-1.12	Potri.015G002400	Late embryogenesis abundant (LEA) hydroxyproline-rich glycoprotein family	1.74	2.17	Potri.001G162700	pectin methylesterase 1	3.16
Potri.003G113700	pectin methylesterase inhibitor 1	-1.12	Potri.014G106100	Late embryogenesis abundant (LEA) hydroxyproline-rich glycoprotein family	-2.27	-2.23	Potri.012G127400	Plant invertase/pectin methylesterase inhibitor superfamily protein	2.98
Potri.019G101900	expansin B3	-1.76	Potri.013G031100	copper/zinc superoxide dismutase 1	2.65	1.83	Potri.005G061500	Plant invertase/pectin methylesterase inhibitor superfamily	2.61

LATERAL ROOT DEVELOPMENT ZONE

wild-type			common				*abi1*		
Identifier	**Annotation**	**log_2 Ratio**	**Identifier**	**Annotation**	**log_2 Ratio** wt	*abi1*	**Identifier**	**Annotation**	**log_2 Ratio**
				Stress response					
Potri.001G148900	chloroplast beta-amylase	-1.58	Potri.004G216700	copper/zinc superoxide dismutase 2	2.49	1.98	Potri.015G127800	Plant invertase/pectin methylesterase inhibitor superfamily	2.51
			Potri.009G005100	copper/zinc superoxide dismutase 2	2.09	1.49	Potri.002G202500	Plant invertase/pectin methylesterase inhibitor superfamily	1.77
			Potri.005G044400	copper/zinc superoxide dismutase 1	1.74	1.61	Potri.014G127000	Plant invertase/pectin methylesterase inhibitor superfamily	1.76
			Potri.019G057300	manganese superoxide dismutase 1	1.15	1.13	Potri.015G128700	Plant invertase/pectin methylesterase inhibitor superfamily protein	1.57
			Potri.015G110400	Fe superoxide dismutase 2	-2.73	-1.42	Potri.014G067100	Plant invertase/pectin methylesterase inhibitor superfamily	1.54
			Potri.003G111200	Lactate/malate dehydrogenase family protein	3.16	4.45	Potri.001G162400	pectin methylesterase 3	1.31
			Potri.001G200400	beta galactosidase 1	1.15	1.32	Potri.006G134500	pectin methylesterase 44	1.14
			Potri.012G126900	Plant invertase/pectin methylesterase inhibitor superfamily	1.55	1.60	Potri.001G112900	expansin A7	7.53
			Potri.004G181700	expansin-like A2	1.31	1.92	Potri.002G017900	expansin 11	7.26
			Potri.001G240900	expansin A4	1.08	1.34	Potri.014G066300	expansin B2	6.16
			Potri.003G083200	expansin-like B1	-2.48	-2.00	Potri.019G057500	expansin A8	5.23
			Potri.008G174100	beta-amylase 1	1.57	1.59	Potri.001G001100	expansin A15	3.21
			Potri.010G062900	beta-amylase 1	1.05	1.25	Potri.013G060800	expansin A15	3.06
			Potri.008G204200	beta-amylase 3	-1.19	-1.78	Potri.008G088300	expansin A1	1.70
							Potri.004G208300	expansin A20	1.17
							Potri.009G031800	expansin A4	1.04
							Potri.002G126300	alpha-amylase-like	1.04
							Potri.005G061600	plant glycogenin-like starch initiation protein 1	1.13
							Potri.005G033500	plant glycogenin-like starch initiation protein 5	1.11