Characterization of the LEAFY COTYLEDON1 regulon: Transcription factor controlled hormone cross-talk during somatic and zygotic embryogenesis

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Für meine Familie,

Björn und Lina.

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List of abbreviations

A.	*Agrobacterium tumefaciens*
A.	Arabidopsis
ABA	Abscisic acid
ABI	ABSCISIC ACID INSENSITIVE
ABRE	Abscisic acid response element
AFL	ABI3/FUS3/LEC2
AGL	AGAMOUS-LIKE
AGO2	ARGONAUTE2
AHAP3	Arabidopsis HAP3 subunit
ALF	ABI3-LIKE FACTOR
AMP1	ALTERED MERISTEM PROGRAM1
Amp^r	Ampicillin resistence gene
ANAC	ATAF1/NAM/CUC
AP2	APETALA2
ARF	Auxin response factor
ARR	Arabidopsis response regulator
ASIL1	ARABIDOPSIS 6b-INTERACTING PROTEIN LIKE1
Asp	Asparagine
At	*Arabidopsis thaliana*
ATP	Adenosine-triphosphate
att	Attachment site (Gateway)
AXR1	AUXIN RESISTANT1
BDL	BODENLOS
BEE	BR ENHANCED EXPRESSION
BEH	BES1/BZR1 homolog
bHLH	Basic helix-loop-helix
BIM	BES1-interacting Myc-like protein
Bp	Base pair
BR	Brassinosteroids
BRM	BRAHMA
BSA	Bovine serum albumine
bZIP	Basic leucine zipper
c	Carrot
CaMV	Cauliflower mosaic virus
CAND1	CULLIN-ASSOCIATED and NEDD8-DISSOCIATED1
CAS1	CYCLOARTENOL SYNTHASE1
CATMA	Complete Arabidopsis Transcript MicroArray
CBF	CCAAT-box binding factor
CCT	CO, CO-like, TIMING OF CAB EXPRESSION1
cDNA	Complementary DNA
CFP	Cyan fluorescent protein
CHD3	Chromodomain helicase DNA binding protein 3
ChIP	Chromatin immunoprecipitation
CLF	CURLY LEAF

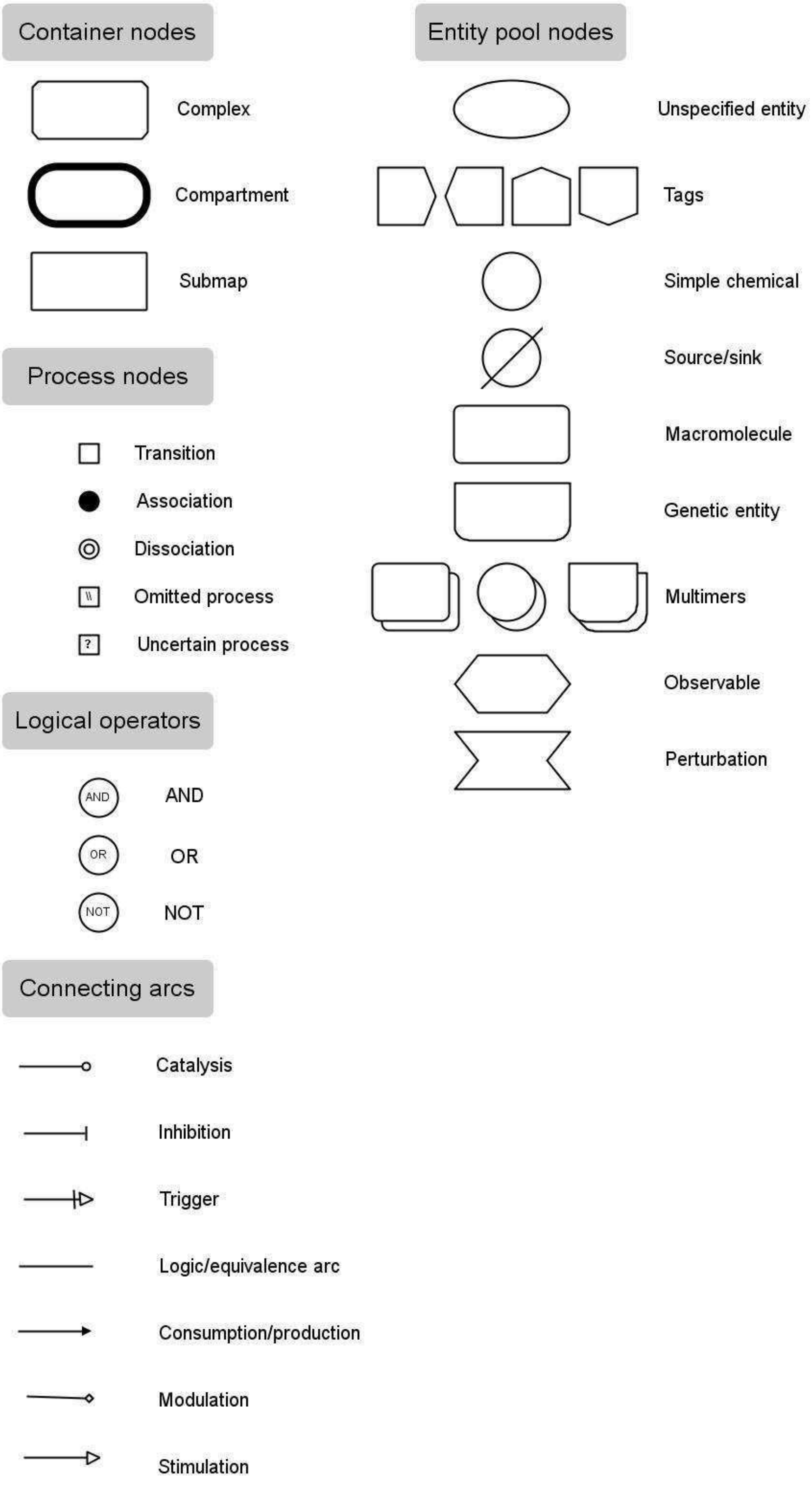
CLSM	Confocal laser scanning microscope
CLV3	CLAVATA3
CO	CONSTANS
COAR	Co-activator-co-repressor
COL	CONSTANS-like
COP1	CONSTITUTIVE PHOTOMORPHOGENIC1
Cot	Cotyledon
CP	Control protein
CPL1	C-TERMINAL DOMAIN PHOSPHATASE-LIKE 1
cRNA	Complementary RNA
CUC	CUP-SHAPED COTYLEDON
CUL1	CULLIN1
daf	Days after flowering
dd	Double destilled
DEPC	Diethyl-pyrocarbonate
dest.	Destilled
DEX	Dexamethasone
DFL1	DWARF IN LIGHT1
DNA	Desoxyribonucleic acid
DNMT	*De-novo* methyltransferases
dNTP	Desoxynucleotide-triphosphate
DOC	Sodium-deoxychelate
Dof	DNA-binding with one finger
DPBF	Daucus carota prolamin-box-binding factor
DR	Direct repeat
DRM	dsRNA-binding motifs
DRM	DOMAINS REARRANGED METHYLTRANSFERASE
DRN	DORNROESCHEN
DRNL	DORNROESCHEN-LIKE
dsRNA	Double-stranded RNA
DTT	Dithiothreitol
DWF4	DWARF4
E(Z)	ENHANCER OF ZESTE
E.coli	*Escherichia coli*
ECR1	E1 C-TERMINAL RELATED1
EDTA	Ethylendiamin-tetraacetate
EEL	Enhanced EM level
ELP	Elasthin-like polypeptide
EMF2	EMBRYONIC FLOWER2
EREBP	Ethylen-response-element-binding protein
ERF	Ethylene response factor
essp	Ectopic expression of seed storage proteins
ETC3	ENHANCER OF TRIPTYCHON AND CAPRICE 3
EtOH	Ethanol
f.c.	Final concentration
FAME	Fatty acid methyl ester
FUS3	FUSCA3

FWER	Family Wise Error Rate
g	Gramm
GA	Gibberellic acid
GA2ox6	Gibberellic-acid-2-oxidase6
Gent^r	Gentamycin-resistence-gene
GFP	Green fluorescent protein
GL2	GLABRA2
GR	Glucocorticoid receptor
GST	Gene-specific tag
GUS	ß-glucuronidase
h	Hour
H3K27me3	Trimethylation of histone H3 lysine
HAP	Heme-activated protein
HB	Homeobox
HDAC	Histone deacetylase
Hh	Head-to-head
HLS1	HOOKLESS1
hpi	Hours post imbibition
HSF	Heat shock factor
HSI	High sugar inducible
HSP	Heat shock protein
Ht	Head-to-tail
HUB1	HISTONE MONOUBIQUITINATTION1
HY5	ELONGATED HYPOCOTYL5
IAA	Indole-3-acetic acid
IBR3	IBA-RESPONSE3
IPK	Institute for Plant Genetics and Crop Plant Research
JA	Jasmonic acid
Kan^r	Kanamycin-resistence-gene
L, l	Liter
L1L	LEC1-LIKE
LEA	Late embryogenesis abundant protein
LeB4	Vicia faba Legumin
LEC	LEAFY COTYLEDON
LOB	Lateral organ boundaries
µ	Mikro
m	Milli
M	Molar
MAT	Maturation
MBD	Methyl-CpG-binding proteins
MEA	MEDEA
min	Minute
MIR	MicroRNA
ML1	MERISTEM LAYER1
MP	MONOPTEROS
mRNA	Messenger RNA
MS	Murashige und Skoog

MSG2	MASSAGU2
n	Nano
NAM	No apical meristem
NCED	9-cis-epoxycarotenoid dioxygenase
NF-Y	NUCLEAR FACTOR Y
NLS	Nuclear localization signal
nm	Nanometer
OC	Organizing centre
PAGE	Polyacrylamid gel electrophoresis
PBS	phosphate buffered saline
PCR	Polymerase Chain Reaction
PD	Process Diagram
PDK1	3-PHOSPHOINOSITIDE-DEPENDENT PROTEIN KINASE 1
PGA	Plant growth regulator
PHD	Plant homeodomain
PHE	PHERES
PHV	PHAVOLUTA
PID	PINOID
PIF5	PHYTOCHROME-INTERACTING FACTOR5
PIN	PIN-FORMED
PKL	PICKLE
PLT	PLETHORA
PP2A	Protein phosphatase 2A
PS	Photosystem
QC	Quiescent centre
qRT-PCR	Quantitative real-time PCR
RA	Root apex
RAM	Root apical meristem
RAV	Related to ABI3/VP1
RCN1	ROOTS CURL IN NAPHTHYLPHTHALAMIC ACID1
RD29	RESPONSIVE TO DROUGHTNESS29
RdDM	RNA directed DNA methylation
RNA	Ribonucleic acid
ROS1	REPRESSOR OF SILENCING1
RPD3	REDUCED POTASSIUM DEFICIENCY3
rpm	Revolutions per minute
rRNA	Ribosomal RNA
RT	Room temperature
RT	Reverse Transcription
RUB1	RELATED TO UBIQUITIN1
SA	Shoot apex
SAM	shoot apical meristem
SAP	Systemic analysis of Arabidopsis promoters
SBGN	Systems Biology Graphical Notation
SEM	Scanning electron microscopy
SHMM	Scaled transition matrix model
siRNA	Small interference RNA

SMZ	SCHLAFMUETZE
SNZ	SCHNARCHZAPFEN
SOC1	SUPPRESOR OF CONSTANS OVEREXPRESSION1
SSC	Saline sodium citrate
SSP	Seed storage protein
STM	SHOOT MERISTEMLESS
Su(Z)	SUPRESSOR OF ZESTE
SWI/SNF	Switch/Sucrose NonFermentable
SWN	SWINGER
TAG	Triacylglyceride
TAIR	The Arabidopsis Information Resource
TCH3	TOUCH3
TEM	Transmission electron microscopy
TF	Transkriptionsfaktor
TFBS	Transcription factor binding site
Th	Tail-to-head
TIGR	The Institute for Genomic Research
TIR1	TRANSPORT INHIBITOR RESPONSE1
tnp	*Turnip* mutant
TOE1	TARGET OF EAT1
TSS	Transcription start site
TT	TRANSPARENT TESTA
U	Unit
UBQ10	UBIQUITIN10
USP	Vicia faba Unknown seed protein
UTR	Untranslated region
VAL	VP1/ABI3-like
Veg	Vegetative leaf
Vp	VIVIPAROUS
VRN2	VERNALISATION2
WOX	WUSCHEL-type homeodomain
WRI1	WRINKLED1
WT	Wildtype
WUS	WUSCHEL
XVE	LexA, VP16, estradiol-receptor
ZLL/PNH	ZWILLE/ PINHEAD

SBGN Nomenclature



1. Introduction

The plant life cycle represents the alternation between sporophytic and gametophytic phases. In higher plants, the dominant sporophytic generation is initiated by a double fertilization event giving rise to embryo and endosperm, and finally the seed as a desiccated and dormant structure which will germinate once it encounters appropriate conditions. Sporophytic tissues and organs are formed, including reproductive tissues which develop from the shoot apical meristem of the adult plant. Special sets of cells of the sexual organs undergo meiosis to initiate the development of the male (pollen grains) and the female gametophyte (embryo sac). During fertilization they fuse to form again zygote, embryo and endosperm thus completing the cycle. Although these processes are well described on a morphological level, molecular mechanisms are only poorly understood.

Several key regulators of seed development have been isolated and characterized, especially factors involved in maturation and germination (Bewley 1997, Goldberg *et al.* 1989, Gutierrez *et al.* 2007, Hilhorst and Toorop 1997, Holdsworth *et al.* 2008, Koornneef *et al.* 2002, Li and Foley 1997, McCarty 1995, Santos-Mendoza *et al.* 2008) but still the complex regulatory network underlying seed development is far from being understood. For the identification of new regulators and their integration into the network, experiments in the past and in the future had/have to meet a very basic demand: choosing the appropriate model plant system. For a long time the preferred plant test model has been the small cruciferous plant *Arabidopsis thaliana* because of its small size, short life cycle, prodigious seed production, simple and established transformation methods, availability of the whole, small genomic sequence and a large array of described mutants.

1.1 Seed development

1.1.1 Early embryogenesis in Arabidopsis

Embryogenesis starts with the fertilization of egg cell and central cell of the female gametophyte by two sperm cells. During pollination, the pollen tube penetrates the ovule. One pollen nucleus fuses with the egg to produce a diploid zygote which develops into the later embryo. Another pollen nucleus fuses with two previously fused polar nuclei of the female gametophyte, giving rise to a triploid endosperm, which later delivers nutrients to the developing embryo. As a protective layer, the seed coat, derived from maternal tissue, surrounds the embryo and endosperm during embryogenesis.

Basically three major developmental processes take place during early embryo stages. First, the axis of the plant body is defined and polarity is established. Second, cell types of the earliest organs are defined such as the suspensor, provascular tissue, and shoot and root meristems. And third, cells grow and are spatially distributed giving rise to the characteristic form of the embryo and its organs.

The *Arabidopsis* embryo passes through a series of distinct morphological stages (Figure 1). In the early preglobular and globular stages the sequence of cell divisions follows a well determined pattern. The first division of the zygote gives rise to two cells, from which the apical cell will form the embryo and the basal cell leads to the formation of the suspensor (Figure 1A and B) which provides nutrients to the embryo and represents one of the first specialized organs to be formed in the plant embryo. In the course of further cell divisions the first histologically detectable tissue, namely the protoderm, forms as epidermis precursor tissue in the late globular embryo (Figure 1A and B). The uppermost cell of the suspensor differentiates into the so called hypophysis (Figure 1A and B), which will later on participate in the formation of the root apical meristem. During the globular stage, the embryo has radial symmetry. At the transition to the heart stage the symmetry becomes bilateral representing the delineation of the two major embryo organs: cotyledons and axis (Figure 1C and D). The apical domain of the embryo becomes quiescent and forms the shoot apical meristem (SAM), while divisions on its two sides results in the formation of the cotyledons and the embryo acquires a heart-like form (Figure 1E).

During early embryogenesis, several features/structures of the post-embryonic plant become apparent in the embryo, including the root meristem, the provascular tissue and the shoot apex (Figure 1E). Growth and morphogenesis continue during torpedo stage (Figure 1F), curled cotyledon stage (Figure 1G) and are completed at torpedo stage where all tissue layers will have been established (Figure 1H).

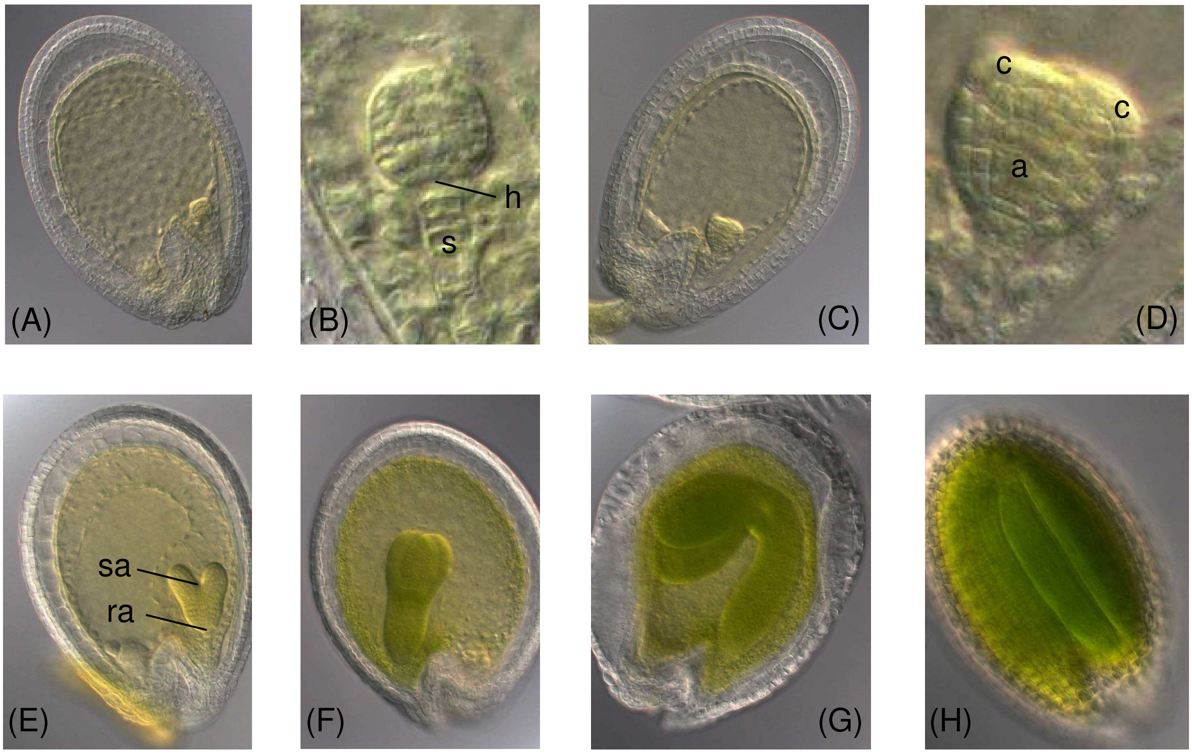


Figure 1. Arabidopsis embryogenesis.
Post fertilization: A,B) Early globular stage embryo. h, hypophysis; s, suspensor. Globular/ heart transition: C,D) Transition stage embryo. a, axis; c, cotyledons. E) Late heart stage embryo. ra, root apex; sa, shoot apex. Organ expansion and maturation: F) Linear cotyledon stage embryo. G) Curled cotyledon stage embryo. H) Mature embryo.

1.1.2 Late embryogenesis

After completion of growth and morphogenesis the embryo starts to expand until it will finally fill the whole seed. The accumulation of reserve materials starts by using up the endosperm reservoir which will only remain as single cell layer (aleurone) surrounding the expanded embryo (West and Harada 1993). The onset of the maturation program occurs and basically includes the following processes: storage compound accumulation, the suppression of precocious germination, acquisition of desiccation tolerance, desiccation, growth arrest and the entry into a dormancy period. The latter is of variable length and is broken upon germination.

During maturation seeds accumulate large amounts of different types of reserve compounds which contribute up to 90% of the seed dry weight and consist of specialized storage proteins, oils and carbohydrates whereas the proportions vary according to different species. Oils and proteins represent the main storage products in Arabidopsis.

Besides proteins with structural, regulatory or enzyme functions, the major protein fraction in seeds is represented by seed storage proteins (SSP). During germination, SSP serve as the primary source of carbon and nitrogen for the growing seedling. In developing *Arabidopsis* seeds, the most abundantly expressed storage proteins are members of the 2S albumin (named napin) and the 12S globulin (named cruciferin) protein families. Starting at torpedo stage of early embryogenesis the precursors of globulins and albumins are synthesized at the rough endoplasmic reticulum and are sorted into protein storage vacuoles (PSV), where they are assembled into the mature forms and accumulated (Müntz 1998).

Seed lipids are stored as triacylglycerols in oil bodies (oleosomes) that occupy up to 60% of the cotyledonary cell volume in mature embryos. The triacylglycerol core of an oil body is surrounded by a phospholipid monolayer and oleosins, functionally specialized proteins involved in the preservation of the oleosome structure during seed desiccation (Huang *et al.* 1994, Mansfield and Briarty 1992). They associate with lipases to initiate oleosome breakdown during germination, and degradation products contribute to cover the energy demand of the growing seedling.

With progressing maturation the seed enters a temporary dormant state, defined by arrested growth and development that enables the seed to survive harsh environmental conditions and is broken upon favourable conditions leading to seed germination. According to the time of occurrence seed dormancy can be classified into primary dormancy which is established during embryo maturation whereas secondary dormancy occurs after seed imbibition as a result of inappropriate germination conditions (Amen 1968). Besides dormancy, the significant reduction in seed water content (desiccation) also contributes to the seeds ability to survive periods of adverse conditions. Desiccation tolerance of the seed most likely results from the accumulation of carbohydrates and specialized proteins (e.g. late embryogenesis abundant proteins, LEA).

1.2 Regulation of seed development

As described in the previous sections, embryogenesis and seed formation involve a multitude of complex processes that are heavily interconnected. Therefore regulation of these processes requires an even more complex network of regulators acting strictly and with temporal and spatial specificity. It involves special genetic and epigenetic programs as well as hormonal and metabolic states, which play important roles in regulating embryogenesis and seed development (for reviews see: Brady and McCourt 2003, Santos-Mendoza *et al.* 2008, Wobus, and Weber 1999, Zhang and Ogas 2009). Genetic analyses, especially mutant screenings, have identified transcription factors as key regulators of seed development. Most of them are either involved in the control of early development (morphogenesis), or function exclusively during seed maturation.

1.2.1 Transcriptional regulation of early embryogenesis

Patterns of gene expression and the consequential pattern of cell divisions in the early embryo are largely determined by graded auxin distribution within the embryo. This in turn is achieved by asymmetric expression of certain auxin transporters such as *PIN-FORMED*1 and 7 (*PIN*1 and *PIN*7; Jenik and Barton 2005, Jenik *et al.* 2007, Weijers and Jürgens 2005). Several other genes have been implicated with the regulation of cell fate specification in the early embryo (Jenik *et al.* 2007, Nawy *et al.* 2008, Willemsen and Scheres 2004). Directly after zygotic division the expression of different *WUSCHEL*-type homeodomain (*WOX*) genes mark the domains of the early axis (Haecker *et al.* 2004). Formation of the protoderm is reflected by expression of the related homeodomain gene *MERISTEM LAYER1* (*ML1,* Takada and Jürgens 2007). Root initiation requires auxin dependent transcription of *BODENLOS* (*BDL*) and *MONOPTEROS* (*MP*) which are interacting IAA (indole- acetic acid inducible) and ARF (auxin response factor) proteins, respectively. At heart stage *BDL* and *MP* are predominantly expressed in the provascular tissue revealing a function in vascular development also (Hamann *et al.* 2002). Following auxin distribution the AP2/ERF-type transcription factors *PLETHORA1, PLETHORA2* and *PLETHORA3* (*PLT 1,2,3*) accumulate in the root meristem region (quiescent centre) and determine the stem cell proliferative potential in a dose- dependent manner (Aida *et al.* 2004, Galinha *et al.* 2007). The dynamic expression of the redundant transcription factors *CUP-SHAPED COTYLEDON1* and *2* (*CUC1* and *CUC2*) and the homeodomain transcription factors, *SHOOT MERISTEMLESS* (*STM*) and *WUS* (*WUSCHEL*), mark a small number of apical cells in the mid-globular stage embryo that are required for apical shoot meristem initiation (Aida *et al.* 1999). In later embryo stages also the expression of *CLAVATA3* (*CLV3*) can be detected in a few cells between the arising cotyledons (Fletcher *et al.* 1999). The *ZWILLE/PINHEAD* (*ZLL/PNH*) gene products are needed for a high expression of *STM* inside the apical meristem (Moussian *et al.* 1998) and for maintenance of WUS signaling (Tucker *et al.* 2008). The interaction of WUS and CLAVATA3 (CLV3) establishes a positive feedback loop required for stem cell maintenance at the shoot meristem (Brand *et al.* 2000, Schoof *et al.* 2000).

Taken together, early patterning processes involve strictly positional and cell specific hormone action as well as transcriptional control and peptide signaling. By globular stage, the developmental program is governed by factors that will also determine postembryonic patterning processes.

1.2.2 Transcriptional regulation of late embryogenesis

In comparison to the regulation of early embryogenesis and embryo patterning, the genetic program underlying seed maturation is primarily related to processes such as storage, acquisition of dormancy and development of desiccation tolerance. Molecular mechanisms which control the switch from embryogenesis to maturation are still not understood, but the analysis of mutants with alterations in seed maturation led to the identification of a group of master regulators controlling different aspects of seed development.

In Arabidopsis 4 genetic loci, *LEC1*, *LEC2*, *FUS3* and *ABI3*, are considered to regulate main processes during embryogenesis and particularly seed maturation. Regarding the mutant phenotypes, *LEC1*, *LEC2* and *FUS3* belong to the so called *LEAFY COTYLEDON* genes which, when mutated lead to the development of vegetative features in cotyledons (such as trichomes and a complex vascular pattern) and further pleiotropic effects which have been observed similarly in the *lec1-1* mutant (Lotan *et al.* 1998, Meinke 1992, West *et al.* 1994), the *lec2* mutant (Meinke *et al.* 1994, Stone *et al.* 2001) and the *fus3-1* mutant (Bäumlein *et al.* 1994, Gazzarrini *et al.* 2004, Keith *et al.* 1994, Luerssen *et al.* 1998, Tiedemann *et al.* 2008). Besides defects in specification of cotyledon identity, suspensor development is also abnormal and apical and root meristems are activated precociously. During seed maturation the *leafy cotyledon* mutants as well as the *abi3* mutant affect the accumulation of seed storage products and anthocyanin pigments and display defects in maturation maintenance due to reduced desiccation tolerance and dormancy (Giraudat *et al.* 1992, Koornneef *et al.* 1984). Despite a high similarity of the described mutant phenotypes they are not completely identical. The *abi3* mutant for example is insensitive to abscisic acid (ABA), chlorophyll is not degraded in the ripe seed and storage compound accumulation is affected more strongly than in *leafy cotyledon* mutants (Giraudat *et al.* 1992). Among *leafy cotyledon* mutants the *lec2* mutant displays the least severe phenotype with respect to desiccation intolerance and failures in storage compound accumulation.

LEC2, *FUS3* and *ABI3* encode B3-domain proteins and recognize the RY consensus sequence which is known to be implicated with seed gene expression. The RY consensus motif CATGCA(TG) is a well described *cis*-motif involved in gene expression during seed maturation (Bäumlein *et al.* 1992, Bobb *et al.* 1997, Chamberland *et al.* 1992, Chandrasekharan *et al.* 2003, Dickinson *et al.* 1988, Fujiwara and Beachy 1994, Lelievre *et al.* 1992, Reidt *et al.* 2000, Yoshino *et al.* 2006). The B3-DNA-binding domain was initially identified in the ABI3 protein and its maize homologue VP1 (Giraudat *et al.* 1992, McCarty *et al.* 1991) and is also present in other transcription factor families such as ARFs (auxin response factors, Ulmasov *et al.* 1997), RAVs (related to ABI3/VP1, Kagaya *et al.* 1999) and the HSI2/VAL (high sugar inducible/ VP1/ABI3-like) family (Suzuki *et al.* 2007, Tsukagoshi *et al.* 2007, Tsukagoshi *et al.* 2005). In contrast to *LEC2*, *FUS3* and *ABI3*, the LEC1 gene encodes one of 10

Arabidopsis homologues of the HAP3 subunits of the heterotrimeric CCAAT-box binding complex (Lee *et al.* 2003, Lotan *et al.* 1998).

ABI3, FUS3 and LEC2 constitute the AFL/B3 domain transcription factor network underlying embryo maturation and transition to dormancy whereas the single factors have partially overlapping and also distinct functions. Functional differentiation between these TF may have several reasons: temporal and spatial differences in expression patterns, co- or interaction in common expression domains and additional functions conferred by other protein domains (Suzuki and McCarty, 2008). Figure 2 shows the expression patterns of *LEC* and *ABI3* genes as analyzed by different groups and methods (modified from Santos-Mendoza *et al.* 2008). *LEC1* and *LEC2* are both expressed during early stages of embryogenesis and silent during maturation (Lotan *et al.* 1998, Stone *et al.* 2001). Their maximum expression levels preceed the expression peaks of *FUS3* and *ABI3* the transcripts of which are detectable until dry seed stages (Kroj *et al.* 2003, Parcy *et al.* 1994). With respect to spatial expression patterns within the embryo common and distinct expression domains of the single factors can be observed (Figure 2). *LEC1* is expressed in embryo and endosperm (Lee *et al.* 2003, Lotan *et al.* 1998) whereas *LEC2* expression seems to be restricted to the embryo (Kroj *et al.* 1003, Stone *et al.* 2001). *FUS3* transcript is detected mainly in the protodermal layer (Tsuchiya *et al.* 2004) and *ABI3* throughout the whole embryo, respectively (To *et al.* 2006).

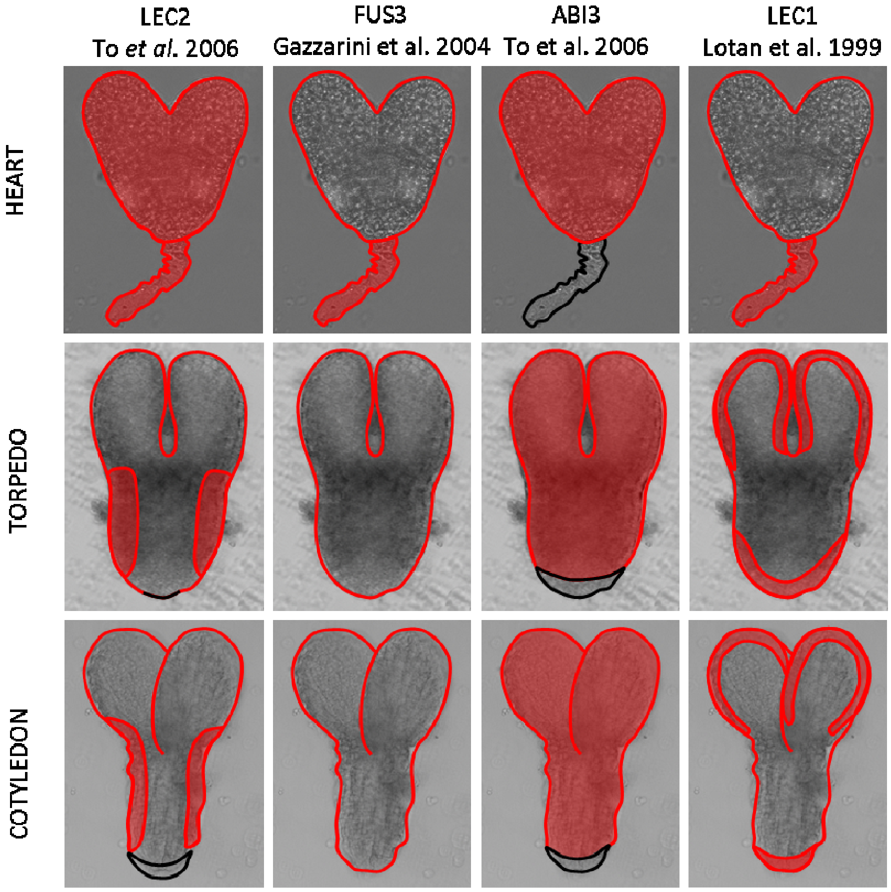


Figure 2. Expression patterns of *AFL/B3-LEC1* genes and LEC1 during embryo development (modified from Santos-Mendoza *et al.* 2008).
Components of the AFL/B3-LEC1 network display different expression domains in the Arabidopsis embryo. *LEC2* expression is found throughout the early embryo and suspensor. From torpedo stage on, *LEC2* expression is diminished in cotyledons (Kroj *et al.* 2003, To *et al.* 2006). In the case of FUS3 contradictory results have been described. Kroj *et al.* (2003) report *FUS3* to be expressed throughout the whole embryo until dry seed stages whereas according to Gazzarini *et al.* (2004) *FUS3* expression is restricted to protodermal/epidermal layers (shown here). *ABI3* expression is found throughout the whole embryo, although expression is diminished in root tips in later stages (To *et al.* 2006). During early stages of embryo development *LEC1* expression is detected in embryo and suspensor (not shown here, Lotan *et al.* 1998). From heart stage on the expression becomes restricted to the embryo periphery (especially protoderm and ground tissue, Lotan *et al.* 1998).

AFL/B3 genes and LEC1 together form a complex network of redundant and distinct regulatory pathways which have been analyzed by genetic and phenotypic analyses of mutants and various mutant combinations of the regulatory factors described above (Meinke *et al.* 1994, Parcy *et al.* 1997, Raz *et al.* 2001, To *et al.* 2006; Figure 3). Nevertheless the mode of regulatory interactions between these proteins remains to be clarified.

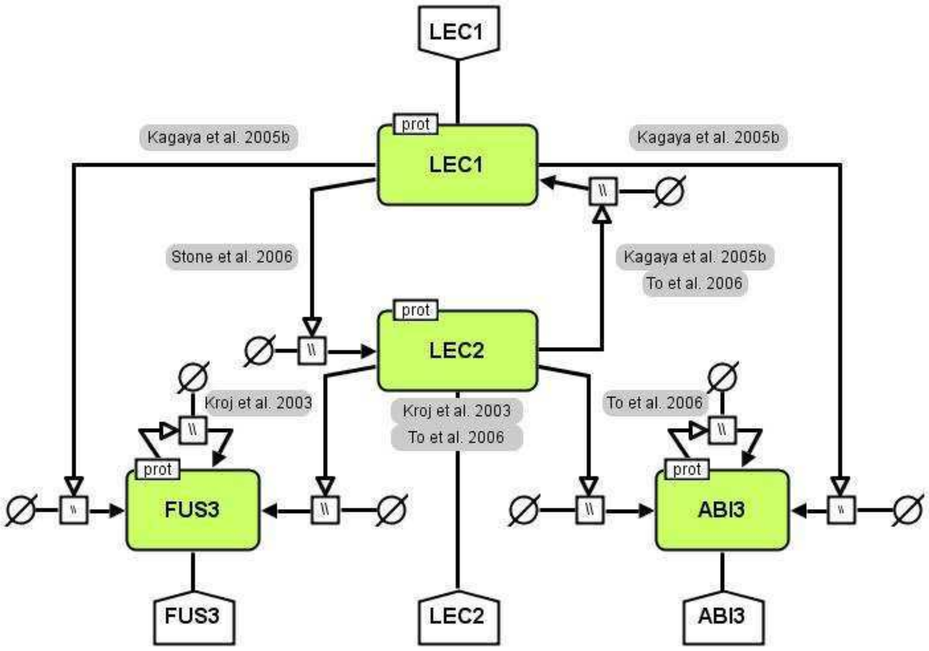


Figure 3. AFL/B3-LEC1 regulatory network underlying Arabidopsis seed development.
Regulatory interactions between components of this network have been studied comprehensively by genetic and phenotypic analyses of corresponding mutants. LEC1 seems to be hierarchically superior and acts upstream of LEC2, FUS3 and ABI3 (Kagaya *et al.* 2005b, Meinke *et al.* 1994, Stone *et al.* 2006, To *et al.* 2006) whereas LEC2 in turn acts upstream of FUS3 and ABI3 (Kroj *et al.* 2003, To *et al.* 2006). Autoregulatory feedback loops secure constant expression levels of *FUS3* and *ABI3* after silencing of *LEC1* and *LEC2* expression during seed maturation (Kroj *et al.* 2003, To *et al.* 2006).

The AFL/B3-LEC1 and following networks were drawn using the Systems Biology Graphical Notation (SBGN, www.sbgn.org, Le Novere *et al.* 2008, Available from Nature Precedings http://precedings.nature.com/ . SBGN represents an agreed-upon convention on how to draw metabolic, molecular and other kinds of interactions/network diagrams in a standardized way. Process Diagram (PD, level 1) notations were used here to display molecular interactions between components of the network(s). An overview of symbol semantic is given on page XII.

Kroj *et al.* (2001) reported that *LEC2* and *FUS3* act synergistically and in a partially redundant manner and that *LEC2* is able to regulate *FUS3* expression. *LEC1* in turn is thought to act upstream of *FUS3* and *LEC2* since most severe defects in the embryonic program are displayed in the corresponding mutant (Meinke *et al.* 1994). In a very comprehensive approach To *et al.* (2006) contributed substantial knowledge to the hierarchical organization of the above mentioned key regulators. Besides *FUS3, ABI3* is also regulated by *LEC2* since both factors can rescue most of the *lec2* mutant phenotypes. *FUS3* and *ABI3* form positive feedback loops of self-regulation which are essential for constant protein levels after the decrease of *LEC1* and *LEC2* expression during seed maturation. *ABI3* expression is additionally controlled by post-transcriptional and post-translational mechanisms. The 5’-untranslated region represses *ABI3* expression post-transcriptionally (Ng *et al.* 2004) and an ABI3-interacting protein (AIP2) is an E3 ligase targeting the ABI3 protein to the 26S proteasome (Zhang *et al.* 2005). Taken together, the data reveal a regulation of both *FUS3* and *ABI3* by *LEC1*, *LEC2* and itself, depending on the location in the embryo (To *et al.* 2006, Figures 2 and 3).

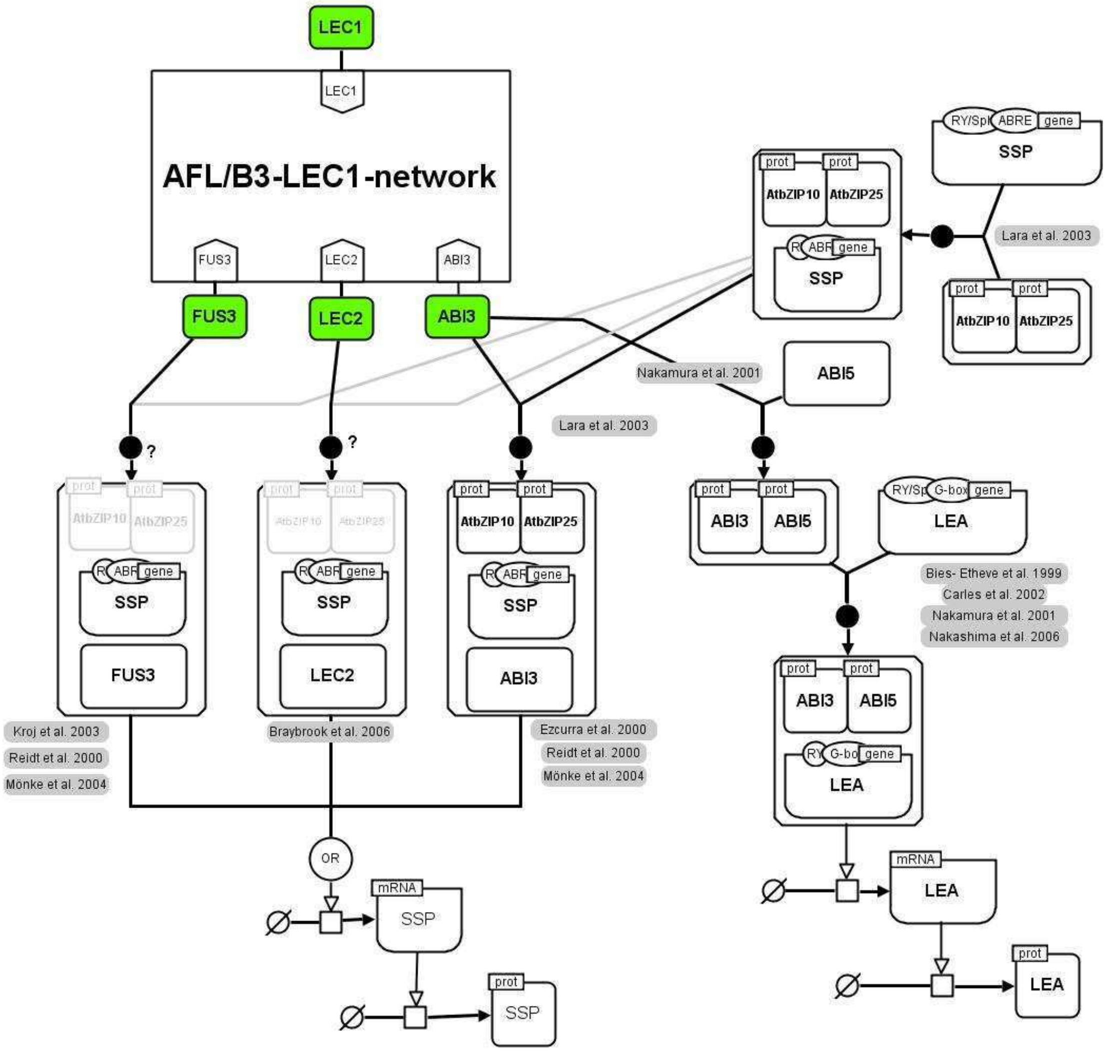


Figure 4. Regulation of maturation genes by AFL/B3-LEC1 factors.

The AFL/B3-LEC1 network is drawn simplified as submap referring to Figure 3. LEC1 regulation of maturation genes is mediated by FUS3 and ABI3 (Kagaya *et al.* 2005b). The direct interaction of LEC2, FUS3 and ABI3 via the RY element (CATGCA) has been proven by several independent approaches (Braybrook *et al.* 2006, Ezcurra *et al.* 2000, Kroj *et al.* 2003, Mönke *et al.* 2004, Reidt et al 2000). Via binding to ABRE elements (ACGT), two bZIP factors, AtbZIP10/25, participate in ABI3-mediated regulation of *SSP* genes. Physical interactions have been reported between ABI3 and bZIP proteins (Lara *et al.* 2003) and are discussed for other B3 domain proteins (LEC2 and FUS3, Vicente-Carbajosa and Carbonero 2005). *SSP* activation by LEC1 (indirectly), FUS3 and ABI3 (directly) depend on ABA (Kagaya *et al.* 2005a). ABI3 mediates ABA responsive gene expression by interaction with the bZIP protein ABI5 (Nakamura *et al.* 2001). Binding to RY and ABRE elements promotes transcription of *LATE EMBRYOGENESIS ABUNDANT* (*LEA*) and *RESPONSIVE TO DROUGHTNESS29* (*RD29*) genes (Bies-Ethéve *et al.* 1999, Carles *et al.* 2002, Nakamura *et al.* 2001, Nakashima *et al.* 2006).

SSP and *LEA* genes were first identified as direct or indirect targets of AFL/B3 and *LEC* genes (Figure 4). *LEC1* has been shown to be sufficient but not necessary for the expression of 2S and 12S seed storage proteins since this is up-regulated in 35S::LEC1 seedlings but still detectable in *lec1-1* mutant embryos (Lotan *et al.* 1998). *LEC1* regulates SSP promoters indirectly via ABI3 and FUS3 (Kagaya *et al.* 2005b). AFL/B3 genes can interact with *SSP* promoters via the RY element thus exerting direct control on their expression (Braybrook *et al.* 2006, Ezcurra *et al.* 2000, Mönke *et al.* 2004).

1.2.3 Hormonal regulation of seed development

AFL/B3 genes and *LEC1* are part of a complex network which mediates the regulation of seed gene expression and also hormone induced gene expression programs. Phytohormones are plant growth regulators such as abscisic acid (ABA), gibberellic acid (GA), ethylene, brassinosteroids (BR), cytokinin and auxin. The corresponding hormone signaling pathways are complex and only partially understood since the regulatory functions are rather achieved by a complex hormone crosstalk. As described above, auxin gradients play an important role in patterning processes in the early embryo. Connecting auxin to the AFL/B3-network, *LEC2* has been shown to activate auxin metabolism by up-regulation of auxin biosynthesis (e.g. *YUCCA4*) and auxin responsible genes (Stone *et al.* 2008, Figure 5). Auxin activation is considered to be the reason for somatic embryo formation in 35S::LEC2 seedlings. Also ABI3 is described to be involved in auxin mediated processes, namely in lateral root initiation (Brady *et al.* 2003, Suzuki *et al.* 2001) and *FUS3* expression increases upon auxin treatment (Gazzarrini *et al.* 2004). Cytokinin signaling was only shown to be affected by LEC2, which is known to down-regulate a cytokinin-responsive gene promoter (Stone *et al.* 2008, Figure 5).

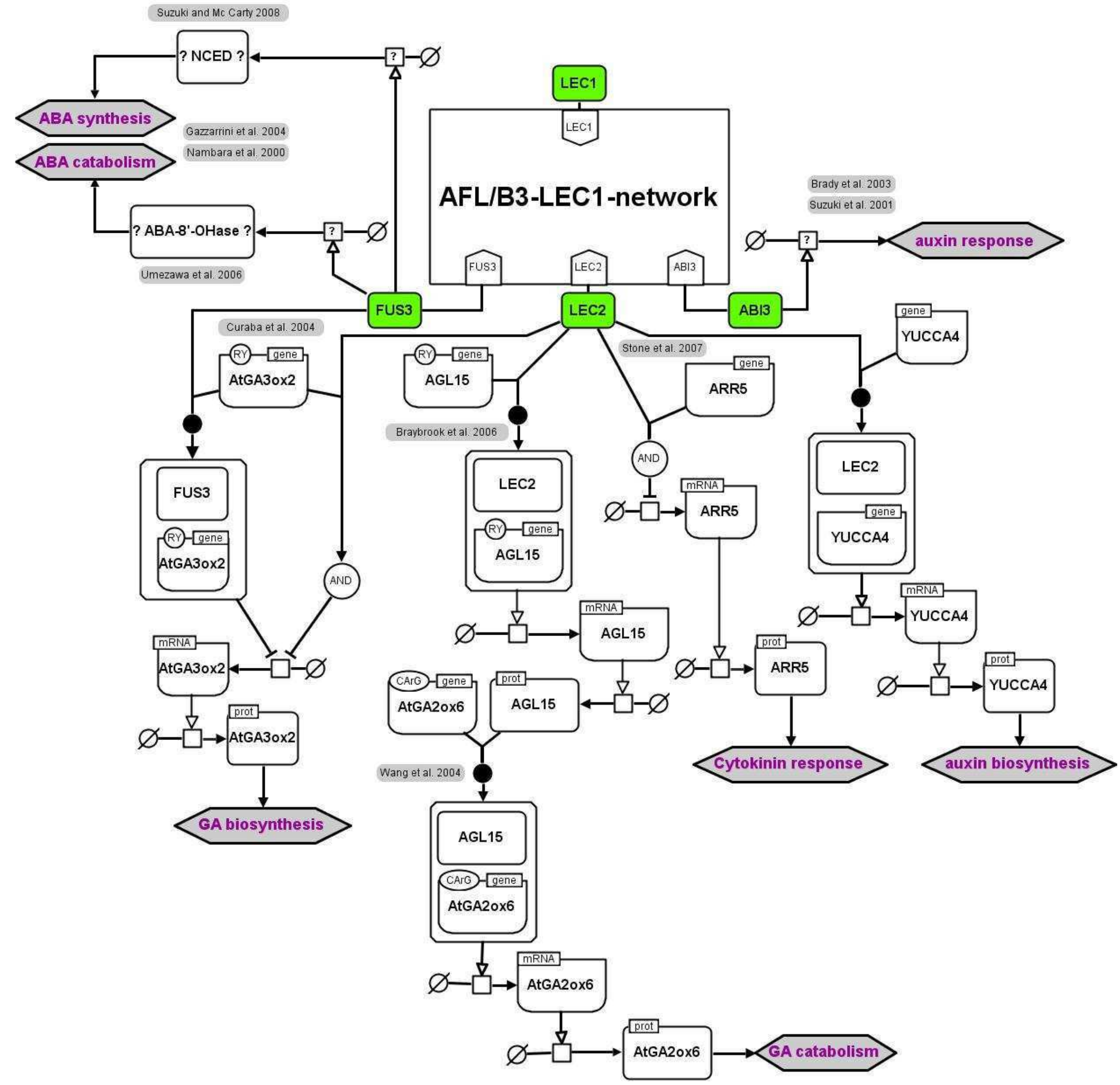


Figure 5. Interconnections between AFL/B3-LEC1 genes and different hormone biosynthetic or signaling pathways.
The ratio of ABA and GA is of utmost importance for seed maturation-related processes and germination. FUS3 is discussed to promote ABA levels by increasing ABA synthesis and decreasing ABA catabolism (Gazzarrini *et al.* 2004, Nambara *et al.* 2000, Suzuki and McCarty 2008, Umezawa *et al.* 2006). FUS3 is a negative regulator of GA biosynthesis, as it has been shown to directly down-regulate GA biosynthetic enzymes (Curaba *et al.* 2003). LEC2 affects GA levels in a similar way (Curaba *et al.* 2003) although direct binding of LEC2 to promoters of GA biosynthetic genes was not shown yet. Via direct regulation of the MADS box TF gene *AGL15*, LEC2 promotes the activation of the *GA2ox6* gene encoding a GA-degrading enzyme (Braybrook *et al.* 2006, Wang *et al.* 2004). Furthermore, a gene involved in cytokinin signaling was down-regulated by LEC2 (Braybrook *et al.* 2006). Auxin biosynthesis is positively regulated through direct activation of auxin biosynthetic genes by LEC2 (Stone *et al.* 2008). ABI3 seems to be involved in auxin response during lateral root development (Brady *et al.* 2003, Suzuki *et al.* 2001).

Seed maturation and germination are essentially determined by the ratio of ABA and GA (Brady and McCourt 2003, Debeaujon and Koornneef 2000, Dubreucq *et al.* 1996, Karssen *et al.* 1983, Koornneef *et al.* 1982, McCourt *et al.* 2005, Ogawa *et al.* 2003). The sesquiterpene hormone ABA regulates major processes such as storage compound accumulation, prevention of precocious germination, acquisition of desiccation tolerance and induction of primary dormancy (Finkelstein *et al.* 2002). GA causes the break of seed dormancy and initiates germination. GA induces genes which encode hydrolytic enzymes required for metabolite degradation in the endosperm. It is also directly stimulating embryo growth (Debeaujon and Koornneef, 2000). ABA and GA are connected to the AFL/B3 network in different ways. ABI3 and also its orthologues from other plant species are able to activate RY- containing seed gene promoters and ABA-inducible promoters (Suzuki *et al.* 1997). The mediation of ABA response is achieved by synergistic interaction of ABI3 with the ABA- and ABI3-inducible bZIP transcription factor ABI5 via the N-terminal Co-activator-co-repressor (COAR) domain (Gampala *et al.* 2002, Hobo *et al.* 1999, Lopez-Molina *et al.* 2002, Nakamura *et al.* 2001). ABI5 binds to the ABRE element (abscisic acid responsible element) ACGT to regulate ABA-inducible transcription. DNA-binding of ABI5 in turn may stabilize or facilitate the interaction of the ABI3 protein and the RY motif in the same promoter (e.g. *LEA* or *SSP* promoters; Carles *et al.* 2002, Finkelstein and Lynch 2000). The activation of *SSP* promoters by ABI3, FUS3 and indirectly by LEC1 in non-seed-tissue is completely (in the case of FUS3) or partially (in the case of ABI3) dependent on ABA (Kagaya *et al.* 2005a). Constitutively expressed ABI5 however has been shown to be able to restore the otherwise ABA-dependent activation of the *phaseolin* gene promoter by the ABI3 orthologue of *Phaseolus vulgaris* in vegetative tissues (Ng and Hall 2008). Besides effects on the gene expression program, ABA also affects protein stability as shown for ABI5 (Lopez-Molina *et al.* 2001), for ABI3 (Zhang *et al.* 2005) and FUS3 (Gazzarrini *et al.* 2004). FUS3 and LEC2 (see also above) in turn influence the level of hormone accumulation by interfering with hormone metabolism. Both TF repress the *GA3ox2* gene. The gene encoded enzyme converts an inactive precursor into active GA (Curaba *et al.* 2004). Moreover, FUS3 is described as a positive regulator of ABA synthesis (Gazzarini *et al.* 2004, Nambara *et al.* 2000). Two mechanisms are conceivable: first, the repression of genes encoding catabolic enzymes such as ABA-8'-hydroxylase (CYP707A3, Umezawa *et al.* 2006) or second, the activation of genes encoding for biosynthetic enzymes such as 9-*cis*-epoxycarotenoid dioxygenase (NCED, Suzuki and McCarty 2008).

1.2.4 Chromatin- remodeling in Arabidopsis embryogenesis

Embryogenesis of Arabidopsis is regulated at different levels. In addition to interaction of TF and hormones described above, the regulatory network also includes factors involved in chromatin changes. Figure 6 gives an overview of the regulation of AFL/B3-LEC1 and maturation genes by epigenetic, chromatin remodeling and further regulatory factors.

The factor that was first shown to repress embryonic identity during vegetative development is PICKLE, a CHD3 group chromatin-remodeling factor which contains a SWI/SNF ATPase domain (Eshed *et al.* 1999, Ogas *et al.* 1997, Ogas *et al.* 1999, Rider *et al.* 2003). The *pkl* mutant displays embryonic characteristics in the primary root meristem which due to its swollen and greenish appearance is referred to as 'pickle roots' (Ogas *et al.* 1997). Pickle roots resemble embryo-like structures in various aspects. They accumulate embryonic storage compounds, such as seed storage proteins (12S and 2S) and triacylglycerols with a fatty acid composition that is known for seeds (Ogas *et al.* 1997, Rider *et al.* 2004). The transcripts of the embryo-specific maturation regulators *LEC1*, *LEC2* and *FUS3* are elevated in pickle roots (Ogas *et al.* 1999; Rider *et al.* 2003). In addition the expression of these genes is de-repressed during germination of *pkl* seeds (Rider *et al.* 2003). These data indicate that PKL is necessary for repression of embryonic traits during post-embryonic development. Germination is defined as the time between seed imbibition and emergence of the radicle from the seed coat and PKL action seems to be restricted to a time frame before the completion of germination (24-36 hours post imbibition; Ogas *et al.* 1997, Li *et al.* 2005). Within the same time window *LEC1* expression increases in the imbibed seed (Rider *et al.* 2003) and the GA biosynthetic inhibitor Unicazole P is able to increase the penetrance of the *pkl* root phenotype from 10 to greater than 80% (Ogas *et al.* 1997). Also the induction of PKL using a dexamethasone-inducible system was only effective in the above mentioned time window (Li *et al.* 2005). De-repression of *LEC1* and other genes in the *pkl* mutant was shown to be the result of decreased trimethylation of histone H3 lysine (H3K27me3) which is normally promoted by PKL during vegetative development (Zhang *et al.* 2008). By this mechanism, PKL and the synergistic, but PKL-independent action of GA, are necessary within a narrow time window during germination for the expression of the pickle root phenotype with its embryonic features.

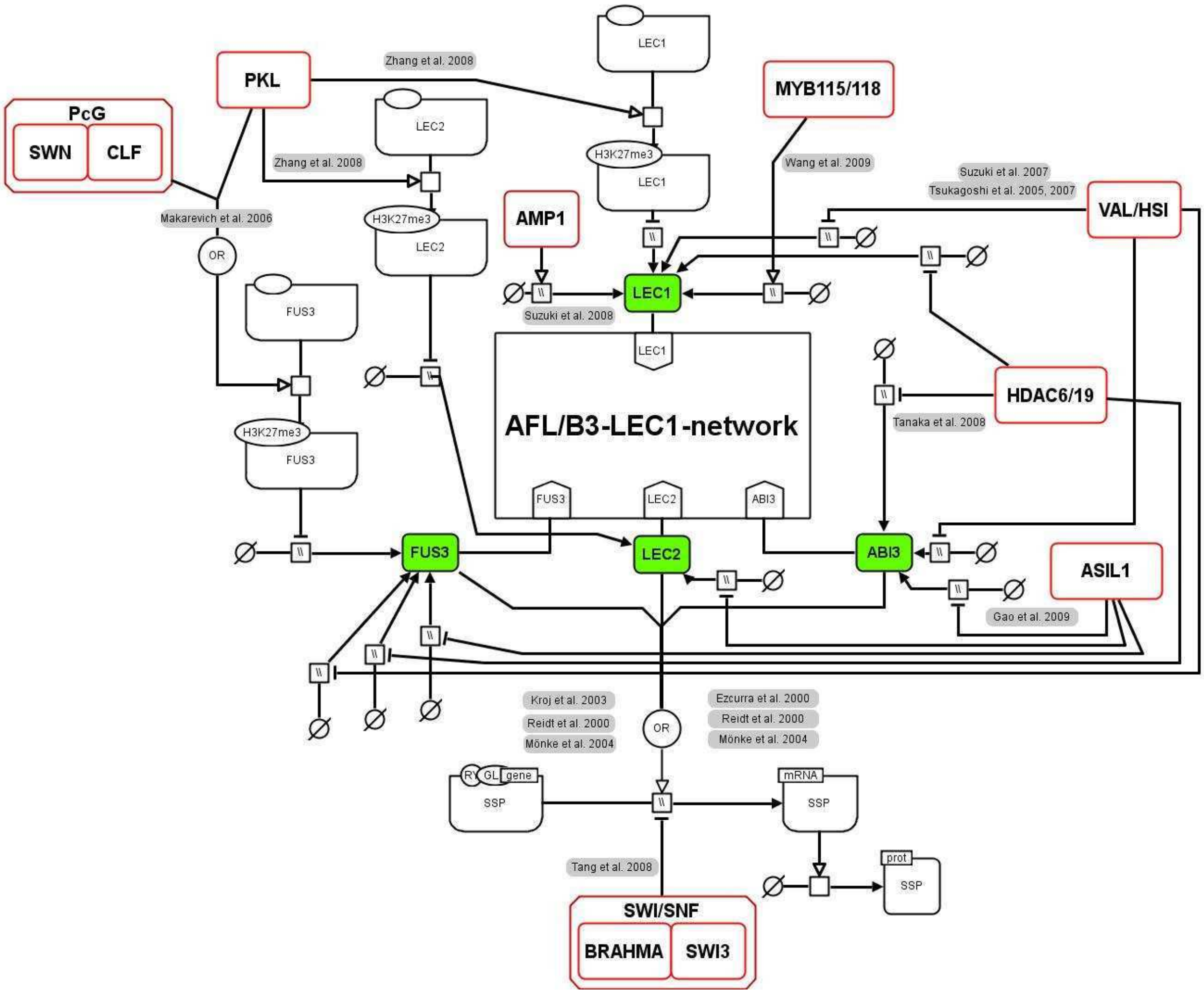


Figure 6. Regulators of the AFL/B3-LEC1 network.
The regulation of AFL/B3-LEC1 genes involves different modes of regulatory action, such as transcriptional regulation, chromatin remodeling and others. Repression of embryonic master regulators *LEC1*, *LEC2* and *FUS3* during vegetative development by the chromatin remodeling factor PKL is mediated by histone methylation (Zhang *et al.* 2008). In a similar way the Polycomb-group proteins SWN and CLF inhibit *FUS3* expression (Makarevich *et al.* 2006). As subunit of SWI/SNF multi-protein complexes BRAHMA directly represses the transcription of *SSP* genes (Tang *et al.* 2008). The membrane located peptidase AMP1 and the MYB transcription factors MYB115/118 regulate *LEC1* expression positively (Suzuki *et al.* 2008, Wang *et al.* 2009). The VAL B3 domain proteins, the histone deacetylases HDAC6/19 and the trihelix domain protein ASIL1 act as global repressors of the embryonic program and the AFL/B3-LEC1 network (Gao *et al.* 2009, Suzuki *et al.* 2007, Tanaka *et al.* 2008, Tsukagoshi *et al.* 2005, 2007).

Two histone deacetylases (HDAC), HDA6 and HDA19, have been shown to have a PKL-analogous function as repressors of embryonic properties during germination. Wildtype treatment with the HDAC-inhibitor trichostatin A leads to impaired post-germination growth and expression of embryogenesis-related genes such as *LEC1*, *LEC2*, *FUS3*, *ABI3* and seed storage protein genes. Similar effects were observed in an HDA6/HDA19 double-repression line which additionally developed embryo-like structures on true leaves (Tanaka *et al.* 2008). Although animal RPD3-HDAC-complexes (homology to yeast REDUCED POTASSIUM DEFICIENCY 3) include SWI/SNF-class chromatin-remodeling factors such as PKL (Tong *et al.* 1998, Wade *et al.* 1998, Xue *et al.* 1998, Zhang *et al.*

1998), HDA6 and HDA19 seem to act independently of PKL (Tanaka *et al.* 2008) and also no connection was found between PKL-dependent gene expression and acetylation levels (Zhang *et al.* 2008).

Three *VP1/ABI3-LIKE* (*VAL*) genes, also known as *HIGH-LEVEL SUGAR INDUCIBLE GENE 2 (HSI2)*, *HSI2-Like 1 (HSL1)* and *HSI2-Like 2 (HSL2),* encode proteins containing B3 domains similar to these of embryogenesis regulators LEC2, ABI3 and FUS3 and two other conserved domains, a CW-domain and a plant homeodomain (PHD)-like zinc finger, which are frequently found in chromatin-remodeling factors (Suzuki *et al.* 2007, Tsukagoshi *et al.* 2005, 2007). The *val1-val2* double mutant displays a phenotype reminiscient of the *pkl* mutant with embryo-like structures at apical and root meristems as well as up-regulated expression of *LEC1*, *L1L*, *ABI3* and *FUS3*. A *pkl* similar phenotype in the monogenic *val1* mutant depends on the inhibition of GA synthesis which provides even more indication of overlapping regulatory pathways. Some differences between *pkl* and *val* mutants regarding the GA-dependency, the different regions with embryonic characteristics and the PKL- or VAL-dependent gene expression may be partially explained by differing expression domains of PKL and VAL during development. VAL-dependent gene repression is probably realized by binding of its B3 domain to the RY consensus motif CATGCA which was found to be significantly enriched in promoters of VAL-regulated genes. Structural similarities of the DNA-binding domains of VAL and AFL/B3 gene products might be the reason for overlapping sets of target genes, with VAL factors acting as repressors and AFL factors as activators. Alternatively, VAL-induced changes in gene expression could be secondary effects after primary repression of AFL/B3 genes, although the functionality of chromatin-associated domains in VAL proteins would be excluded in this interpretation (Suzuki *et al.* 2007).

A mutant screen searching for mutants with ectopic expression of seed storage proteins in leaves (*essp* mutants) identified BRAHMA (BRM), a SNF2 chromatin-modeling ATPase (Tang *et al.* 2008). Transcriptome analysis of the *essp3/brm5* mutant revealed the up-regulation of genes encoding seed storage proteins, lipid transfer proteins, protease inhibitors and related seed proteins in leaves. Chromatin immunprecipitation was used to demonstrate the direct interaction between the ESSP3/BRM factor and *SSP* gene promoters. Yeast two-hybrid experiments further shows the interaction between BRM and SWI3, presumably as multi-subunit complexes similar to the yeast SWI/SNF complexes (Farrona *et al.* 2004, Hurtado *et al.* 2006). This is also supported by the observation that a *swi3c-3* mutant displays morphological and molecular phenotypes similar to that of the *essp3/brm5* mutant. Remarkably, the *VAL1/HSI2* gene has been identified as *ESSP2* in the described mutant screen and a role as adaptor or stabilizer of SWI/SNF complexes has been suggested. These complexes are also associated with HDAC, like for instance HDA6 and HDA19, known to function redundantly as post-germination repressors of embryonic properties. Similar

SWI/SNF-like repressive complexes have been described in animal systems (Tang *et al.* 2008, Harikrishnan *et al.* 2005, Ooi and Wood 2007).

Seed gene expression is also regulated by Polycomb protein complexes (Pc-G), known to influence gene expression via the modification of histone methylation patterns. The genes *MEDEA* (*MEA*), *CURLY LEAF* (*CLF*) and *SWINGER* (*SWN*) encode partially redundant histone methyltransferases (HMT), homologous to the Drosophila Pc-G protein ENHANCER OF ZESTE [E(Z)]. The *clf swn* double mutant phenotype closely resembles that of the *pkl* mutant. The apical part of *clf swn* seedlings proliferate into a colourless callus and root tips are swollen and greenish such as *pickle* roots (Chanvivattana *et al.* 2004). CLF and SWN repress target genes such as *PHERES* and *FUSCA3* by H3K27 histone methylation (Makarevich *et al.* 2006), as it was also described for PKL (Li *et al.* 2005, Zhang *et al.* 2008). The morphological and molecular similarities of *pkl* and *clf swn* mutants suggest a common pathway for the repression of embryonic traits during vegetative development. Finally, the *pkl* mutant also resembles the *emf2 vrn2* double mutant with impaired functions of the gene products EMBRYONIC FLOWER2 (EMF2), a homologue of the *Drosophila* SUPRESSOR OF ZESTE [Su(Z)], and VERNALISATION2 (VRN2, Schubert *et al.* 2005).

The AFL/B3-LEC1-regulated processes of seed maturation strongly depend on the phytohormone ABA (Kagaya *et al.* 2005a,b) and involve ABA-mediated transcriptional regulation (Chinnusamy *et al.* 2004, Himmelbach *et al.* 2003, Kim 2007, Nambara and Marion-Poll 2005, Schroeder *et al.* 2001, Wasilewska *et al.* 2008, Yamaguchi-Shinozaki and Shinozaki 2006, Zhu 2002). Several ABA-binding proteins have been described (Pandey *et al.* 2009, Shen *et al.* 2006), however their importance as ABA receptors is still controversial and the ABA signal transduction is only poorly understood. Growing evidence supports the role of ABA also for epigenetic processes such as chromatin remodeling.

The expression of the bean vicilin-like *SSP* phaseolin gene is regulated by the ABA induced ABI3-LIKE FACTOR (ALF), a homologue of the Arabidopsis ABI3. Both, ABA and ALF potentiate the activity of the phaseolin gene promoter by chromatin remodeling (Li *et al.* 1999). Characteristic changes, including acetylation of H3-K14 and methylation of H3-K4, have been found to be associated with ABA- and ALF-mediated regulation (Ng *et al.* 2006).

Moreover, the expression of ABA biosynthetic and dormancy-regulated genes was found to be altered in the *hub1* mutant (Liu *et al.* 2007b). *HISTONE MONOUBIQUITINATTION (HUB1)* encodes a C3H4 RING finger E3 ligase involved in monoubiquitination of histone H2B, a process linked to the activation of gene transcription (Sridhar *et al.* 2007).

Some histone deactylase encoding genes are known to be regulated by ABA (Sridha and Wu 2006). The HDA19 acts as a negative regulator of the embryonic program (Tanaka *et al.* 2008) and was shown to enhance the transcription repressor activity of AtERF7, a transcription factor of the AP2/EREBP family. Overexpression of AtERF7 leads to ABA hyposensitivity, whereas repression of AtERF7 causes ABA hypersensitivity (Song *et al.* 2005).

Together these results clearly demonstrate the role of ABA in epigenetic processes including histone monoubiquitination, acetylation and methylation-dependent chromatin remodeling. Further studies are required to understand the complex interaction between ABA synthesis, degradation and signaling, ABA-mediated epigenetic processes and the AFL/B3-LEC1 system of transcription factors.

1.2.5 Further regulators of Arabidopsis seed development

In addition to hormones, chromatin changes and transcriptional regulators, seed development is further controlled by several other proteins acting at a higher hierarchical level.

The maize gene *VIVIPAROUS8* (*Vp8*) encodes a membrane-located peptidase with high similarity to the Arabidopsis protein ALTERED MERISTEME PROGRAM1 (AMP1, Suzuki *et al.* 2008). The viviparous phenotype of the *vp8* mutant is probably caused by the down-regulation of the AFL/B3-LEC1 system. The ABA deficiency of the *vp8* mutant is the result of an enhanced ABA catabolism and the reduced ABA biosynthesis. The elevated cytokinin synthesis both in the *vp8* mutant of maize and the *amp1* mutant of Arabidopsis leads to a higher capacity for somatic embryogenesis (Chaudhury *et al.* 1993, Chin-Atkins *et al.* 1996, Mordhorst *et al.* 1998, Nogue *et al.* 2000). The *amp1* mutant displays very pleiotropic effects on embryonic patterning, elevated vegetative growth, constitutive photomorphogenesis and precocious flowering (Chaudhury *et al.* 1993). As an antagonist of the auxin response factor MONOPTEROS (MP), AMP1 has been shown to be involved in the regulation of meristem activity. The auxin derived MP signal determines meristematic niches by locally interfering with the differentiation-promoting activity of AMP1 (Vidaurre *et al.* 2007). Together these results suggest a function of the AMP1/VP8 as an integrator of meristem identity and seed formation.

Two MYB transcription factors, MYB115 and MYB118, have been described as promoters of the vegetative-to-embryonic transition (Wang *et al.* 2009). The gain-of-function mutant *myb118/pga37* displays ectopic formation of somatic embryos on root explants as a result of elevated *LEC1* gene expression. A similar phenotype is found in the *myb115* mutant. The activation of the *LEC1* gene by MYB115 and MYB118 seems to be indirect. A tripartite network including PKL, MYB115 and MYB118 has been suggested to control the *LEC1* homeostasis during seed development.

In contrast to the described positive regulators, the factor ARABIDOPSIS 6b-INTERACTING PROTEIN-LIKE1 (ASIL1) represents a member of the Arabidopsis trihelix DNA-binding protein family and acts as a negative regulator of embryonic gene expression programs (Gao *et al.* 2009). The *asil1*-mutant exhibits a gene expression program and metabolic profiles similar to those of late embryos. The expression of *LEC1*, *LEC2* and several other maturation-specific genes was found to be de-repressed in the *asil1* mutant and storage compounds such as SSP and seed-specific lipids are accumulated. The ASIL1-mediated gene repression seems to rely on its competitive binding to promoter elements which overlap with the binding sites of maturation-stimulating factors.

The ongoing identification of further components of the embryogenesis regulatory network clearly reflects the importance and complexity of the process. Our current view is certainly far from being complete and most likely also includes the function of small RNAs (Liu *et al.* 2007a, Reyes and Chua 2007) and other mechanisms.

1.3 LEAFY COTYLEDON 1 (LEC1) as a central regulator of embryogenesis

LEC1 is one of the major regulators of the embryonic developmental program and seed development (Harada 2001). The *LEC1* gene was identified in mutant screens searching for embryo defective mutants (Meinke 1992, Meinke *et al.* 1994). The abnormalities observed in the corresponding *lec1* mutants suggest that LEC1 functions during early as well as late stages of embryo development. During morphogenesis LEC1 is required to maintain suspensor cell fate and to specify cotyledon identity. In *lec1* mutants the suspensor undergoes abnormal cell divisions and eventually gives rise to secondary embryos (Lotan *et al.* 1998). Cotyledons of *lec1* mutants possess trichomes on their adaxial surfaces, indicating the parallel occurrence of embryonic and post-embryonic traits (Meinke 1992, Meinke *et al.* 1994, West *et al.* 1994). LEC1 is also required to initiate and/or maintain the maturation phase. The *lec1* mutant displays a range of maturation defects including desiccation tolerant occasionally viviparous seeds with decreased amounts of storage compounds (Meinke 1992, Meinke *et al.* 1994, Parcy *et al.* 1997, Lotan *et al.* 1998, Vicient *et al.* 2000). In *lec1* mutants post-germinative events such as activation of the shoot apical meristem (SAM) and expression of germination-associated genes, take place during embryogenesis revealing the heterochronic nature of the mutant (Meinke *et al.* 1994, West *et al.* 1998).

LEC1 encodes a protein with similarity to the HEME ACTIVATED PROTEIN3 (HAP3) subunit of CAAT-BOX-BINDING FACTORS (CBF, Lotan *et al.* 1998). CBFs or NUCLEAR FACTOR Y (NF-Y) have been studied in mammalian and yeast systems. They consist of three (mammals) or four (yeast) subunits (HAP2, HAP3, HAP4 and HAP5; for review see: Maity and de Crombrugghe 1998, Mantovani 1999, McNabb and Pinto 2005).

In plants a trimeric CBF consisting of HAP3, HAP2 and HAP5 subunits has been shown to bind to the CCAAT box *in vitro* (Yazawa and Kamada 2007). In contrast to mammals and yeast where HAP subunits are encoded by single copy genes, in *Arabidopsis* the different subunits are represented by gene families consisting of all together 35 genes including *LEC1*. HAP subunits consist of A-, B- and C-domains whereas LEC1 shows homology to the B-domain (Lotan *et al.* 1998). Based on the amino acid sequence of the B-domain *Arabidopsis* HAP3 subunits (AHAP3) can be divided into two subgroups: LEC1-type and non-LEC1-type HAP3 (Lee *et al.* 2002). The most closely related AHAP3, LEC1-LIKE (L1L), also plays an important role in seed development although it is not functionally equal to LEC1 (Kwong *et al.* 2003).

Consistent with its role in embryogenesis, *LEC1* is specifically expressed during early and late stages of seed development. In early globular stage embryos *LEC1* transcript is detectable in embryo and suspensor. Towards the late globular stage *LEC1* expression becomes restricted to the embryo periphery or protoderm (Figure 2, Lotan *et al.* 1998). Outside the embryo, *LEC1* is described to be expressed in dark-grown, etiolated seedlings (Warpeha *et al.* 2007).

Ectopic expression of *LEC1* is sufficient to induce somatic embryogenesis and to confer embryonic identity to vegetative tissue. Seedling expressing a 35S::LEC1 construct show impaired vegetative growth of roots, hypocotyls and develop secondary cotyledons instead of vegetative leaves. Transcripts of embryo-specific genes such as seed storage protein genes were detected in embryo-like structures that emerged from vegetative cells (Lotan *et al.* 1998). Deletion of an approximately 3000bp fragment upstream of the *LEC1* transcription start site in the *turnip* (*tnp*) mutant leads to de-repression of *LEC1* and phenotypically mimics the 35S::LEC1 situation (Casson and Lindsey 2006). When fused to the ligand binding domain of the glucocorticoid-receptor and induced at seedling stage, LEC1 is able to induce transcription of seed-specific genes in the presence of ABA (Kagaya *et al.* 2005b). Also LEC1 and L1L homolgues in other species such as *Daucus carota*, *Zea mays*, *Helianthus annus* and *Theobroma cacao* have been implicated with zygotic and somatic embryogenesis (Alemanno *et al.* 2008, Fambrini *et al.* 2006, Yazawa *et al.* 2004, Zhang *et al.* 2002). The expression of *LEC1* is also implicated with asexual embryo and plantlet formation on the rim of leaves in the genus *Kalanchoe* (Garces *et al.* 2007).

Considering the roles of LEC1 during early and late embryogenesis as well as its ability to induce somatic embryogenesis suggests a general function of LEC1 in the establishment of a cellular environment which promotes embryonic development.

Several studies report on the genetic interaction between *AFL/B3-LEC1* genes (Brocard-Gifford *et al.* 2003, Parcy *et al.* 1997, Raz *et al.* 2001, To *et al.* 2006). LEC1 seems to be hierarchically superior to *AFL/B3* genes and has been shown to influence seed storage metabolism indirectly via these factors.

Ectopic expression of *LEC1* leads to induction of *LEC2*, *FUS3* and *ABI3* and consequently of *SSP* genes (Kagaya *et al.* 2005b). However, mutant analysis revealed that *AFL/B3* genes are still active in the *lec1* mutant (Parcy *et al.* 1997, Raz *et al.* 2001, West *et al.* 1994). Besides seed storage protein metabolism, LEC1 also regulates the coordinated expression of genes involved in the fatty acid biosynthesis. This function of LEC1 is in part mediated by FUS3, ABI3 and WRINKLED1 (WRI1, Mu *et al.* 2008), an AP2/EREB transcription factor involved in seed oil biosynthesis (Cernac *et al.* 2006, Cernac and Benning 2004, Focks and Benning 1998).

Taken together, LEC1 is embedded into a complex regulatory network which controls embryogenesis. Post-germination vegetative growth seems to be highly incompatible with LEC1 activity and several repressors such as PKL, HDA6, HDA19, VAL1, VAL2 and ASIL1 have been identified as repressors of *LEC1* expression during and after germination. In contrast, almost no directly regulated *downstream* genes of LEC1 are known. Therefore, the identification of LEC1 target genes is a focus of the current PhD thesis.

1.4 Aim of this work

Plant seeds are of utmost importance for human nutrition and will provide basics for a bio-based economy and energy production. Seed formation is a complex developmental process, including the synthesis of storage compounds, the acquisition of desiccation tolerance and the induction of dormancy. These processes require a precise spatial and temporal regulation of gene expression. Therefore, the trilateral project ARABIDOSEED with cooperators in Spain, France and Germany aimed to build a regulatory network during embryogenesis and seed development and to analyse its biodiversity. The establishment and use of the ChIP/chip method as well as inducible transcription factor (TF) over-expression lines were the main task for the german project partners at the IPK. The experiments for this PhD thesis have been performed in the frame of the ARABIDOSEED project.

Transcription factors such as LEC1, LEC2, ABI3 and FUS3 are key components of the regulatory network controlling embryogenesis and seed development. The role of these transcription factors has been studied extensively by genetic and molecular approaches, but genome wide analyses of target genes of these transcription factors are still rare. To identify putative target genes, inducible expression of transcription factor genes was combined with expression analysis on macro- and microarrays as well as DNA-binding studies based on chromatin immunoprecipitation and hybridisation on promoter arrays (ChIP/chip).

The main focus of the presented data is the transcription factor LEC1, as a high ranking regulator of zygotic and somatic embryogenesis. The thesis provides a contribution to the functional characterisation of the LEC1 regulon during embryogenesis of the model plant *Arabidopsis thaliana*.

2. Materials and methods

2.1 Materials

2.1.1 Plant Material

Genus	**ecotype**
Arabidopsis thaliana L.	cv. „Columbia“

2.1.2 Bacterial strains

Bacterium	**strain**	**features**
Escherichia coli	DH5α	RecA1, endA1, gyrA96, thi-1, hsdrR17, $(r_k\text{-}m_{k+})$, relA1, supE44, u80ΔlacZΔ15, Tn15 (Sambrook *et al.* 2001)
Escherichia coli	DB3.1	Invitrogen
Agrobacterium tumefaciens	pGV 2260 in C58C1	(Deblaere *et al.* 1985)

2.1.3 Plasmids

Vector	features	source
pDONR 201	Kanamycin^r , Gateway donor vector	Invitrogen
pDONR207	Gentamycin^r, Gateway donor vector	Invitrogen
pDONR223	Spectinomycin^r, Gateway donor vector	Invitrogen
p35S::R1R2::ΔGR	Kanamycin^r, Gateway binary vector for translational fusion to the glucocorticoid-receptor (GR) domain	B. Dubreucq
pMDC7	Hygromycin^r, Gateway binary vector for estradiol-inducible ectopic expression	M.D. Curtis
pMDC32	Hygromycin^r, Gateway binary vector for ectopic expression	M.D. Curtis
pMDC32::GR::myc		Nguyen Thi Minh Gudrun Mönke

2.1.4 Kits

GE Healthcare, Uppsala, Sweden	Rediprime^{TM} II DNA Labelling Kit
	Amersham Enhanced Chemiluminescence (ECL) Kit
Fermentas, Vilnius, Lithuania	Revert Aid^{TM} H Minus First Strand cDNA Synthesis Kit
	Restriction enzymes
Qiagen, Hilden, Germany	Taq DNA- Polymerase
	DNeasy® Plant Mini Kit
	RNeasy® Plant Mini Kit
	QIAquick® Gel Extraction Kit
	QIAprep® Spin Miniprep Kit
	PCR Purification Kit
Invitrogen, Groningen, Netherlands	LR- Clonase^{TM} Mix
	BP- Clonase^{TM} Mix
	Picogreen
Applied Biosystems, Foster City, CA, USA	Fast SYBR® Green Master Mix
Roche, Mannheim	DNaseI
	dNTP stock solutions

2.2 Methods

2.2.1 Cloning

General cloning techniques (ligation and restriction reactions, polymerase chain reactions and gel electrophoresis were performed according to Sambrook *et al.* (2001). Full length coding sequences from the start codon until the last codon (the stop codon was excluded) of LEC1, ABI3, FUS3, ET1, ET2, MYB44, MYB77 and GFP were obtained by reverse transcription PCR (RT-PCR) as described in Vorwieger *et al.* (2008). About 3µg of RNA was used for cDNA synthesis using the Revert Aid^{TM} H Minus First Strand cDNA Synthesis Kit (Fermentas, Vilnius, Lithuania) following the manufacturers instructions. The obtained cDNA was used for amplification of full length coding sequences using the corresponding primer pairs that contained Gateway recombination sites (Table 1). DNA fragments were then recombined into a donor vector (pDONR201/207/223) using the BP clonase enzyme mix (Invitrogen, Groningen, Netherlands), followed by recombination into an expression vector (pMDC7 or p35S::R1R2::ΔGR) with LR clonase enzyme mix (Invitrogen, Groningen, Netherlands). Recombination reactions were performed in 5µl according to the manufacturers instructions.

Table 1. Gene-specific primer sequences for Gateway cloning of seed TF.
The stop codon of every gene was replaced by a cytosine for translational fusion to the GR-domain. Every forward primer contains the attB1-recombination site ggggacaagtttgtacaaaaaagcaggct and reverse primers the attB2-recombination site ggggaccactttgtacaagaaagctgggt at the 5' ends, respectively.

gene	forward primer 5' > 3'	reverse primer 5' > 3'	Plant issue used for amplification
LEC1	ATGACCAGCTCAGTCGTAGTAGCC	GCTTATACTGACCATAATGGTCAAA	Green siliques
ABI3	ATGAAAAGCTTGCATGTGGCGGCCA	GTTTAACAGTTTGAGAAGTTGGT	Green siliques
FUS3	ATGGTTGATGAAAATGTGGAAACCAATGCC	GGTAGAAGTCATCGAGAGAGATAT	Green siliques
ET1	ATGTTCAAGAGAGACGACTACATTCGA	GAGATGTGATTCTCATCCCCTTGTG	leaf
ET2	ATGGAATTCGGCGACGGCGT	GGGTGATTCTCATTCCCTTATGCTC	leaf
MYB44	ATGGCTGATAGGATCAAAGGTCCATG	GCTCGATTCTCCCAACTCCAATTTG	leaf
MYB77	ATGGCGGATCGTGTTAAAGGTCCA	GCTCAACCTTAGGTGTTATTACTCC	leaf
GFP	ATGGTGAGCAAGGGCGAGGA	GCTTGTACAGCTCGTCCATGCCGA	plasmid

2.2.2 Transformation of Escherichia coli

Luria- Bertani- Medium (1L)

10g	Trypton
5g	Yeast extract
5,8g	Sodium chloride
2,46g	Magnesium sulphate heptahydrate
15g	Agar
add	1L ddH$_2$O

autoclave

SOC- Medium (100 ml)

1g	Trypton
0,5g	Yeast extract
200µl	5M Sodium chloride solution
250µl	1M Potassium chloride solution
add	100ml ddH$_2$O

After autoclaving add:

1ml 1M Magnesium sulphate solution

1ml 1M Magnesium chloride solution

The transformation of *Escherichia coli* was performed by heat shock according to (Cohen *et al.* 1972). Chemo-competent cells were mixed with DNA and then incubated on ice for 30 minutes. Bacteria were heat shocked for 45 seconds at 45°C, spiked with 250ml SOC-medium and afterwards incubated at 37°C for 1h under gentle shaking. Subsequently bacteria were plated on selective LB-medium and incubated over night at 37°C.

2.2.3 Transformation of Agrobacterium tumefaciens

YEB medium

5g	Bacto- beaf extract
1g	Yeast extract
5g	Peptone
5g	Saccharose
add	1L ddH$_2$O

Adjust pH7,2 with KOH

After autoclaving add: 200µl 1M Magnesium sulphate solution

The transformation of *Agrobacterium tumefaciens* was performed according to the freeze-and-thaw-method described by (Höfgen and Willmitzer 1988). One microgram plasmid DNA was added to the competent cells followed by incubation for 5 minutes on ice, 5 minutes in liquid nitrogen and finally 5 minutes at 37°C. After addition of 1ml YEB medium cells were incubated under shaking for four hours at 28°C. After a short centrifugation step the supernatant was discarded and 100µl fresh YEB medium was added. Cells were plated on plates containing selective YEB medium (with rifampicin) and incubated for one to two days at 28°C.

2.2.4 Plant growth

Media

Rich medium for Arabidopsis (1L)

4,3g	MS micro- and macrosalt mixture
10g	Sucrose
10ml	Vitamin solution
8g	Agar (0,8%; for plates)

add 1L ddH_2O

pH5,8

Vitamin stock (100ml)

10mg	Thiamin x HCl
5mg	Pyridoxine x HCl
5mg	Nicotinic acid
5g	MES
1g	Inositol

Arabidopsis thaliana plants c.v. Columbia 0 plants were grown on soil under long-day-conditions at 22°C. For sterile *in vitro* cultures Arabidopsis seeds were surface sterilized. Seeds were treated with 70% ethanol for 2 minutes and 5% sodium hypochloride solution for 10 minutes. After repeated washing steps with ddH_2O seeds were grown on rich MS medium (Murashige and Skoog 1962) in a growth chamber under conditions as described above.

For the seed staging experiment, Arabidopsis plants were grown under short day conditions and grouped according to their flowering timepoint. The first siliques at the bottom of the main shoot of 10 plants with the same flowering timepoint were sampled at 5, 7, 9, 11, 13, 15, 17 and 19 days after flowering. The developing seeds were dissected from the siliques and frozen in liquid nitrogen or used for microscopy.

2.2.5 Transformation of *Arabidopsis thaliana*

Infection medium (1L)

2,652g	MS micro- and macrosalt mixture
5g	Sucrose
10ml	*Arabidopsis* vitamin solution
add 1L ddH$_2$O	
pH5,7	
add 0,04%	Silwet L-77 (Union Carbide Chemicals and Plastics)

Transformation of Arabidopsis was done according to the protocol of (Clough and Bent 1998). Plants of *Arabidopsis thaliana* ecotype Columbia 0 were grown for three weeks under short day conditions (8 hours light, 16 hours dark) and transferred to long day (16 hours light, 8 hours dark). After three weeks, the emerging bolts were cut to induce growth of secondary bolts. Bacteria were grown till OD600>2.0, harvested by centrifugation and resuspended in three volumes of infiltration medium supplemented with 0.01% Silwet L-77 (Union Carbide Chemicals and Plastics). Inflorescences were dipped into the *Agrobacterium tumefaciens* suspension for about 1 minute. After short shaking they were incubated horizontally in covered bowls for 24 hours to keep a high humidity. After 24 hours, they were uncovered and set upright. Seeds were harvested from dry siliques, sterilized and plated onto selection plates containing the appropriate antibiotics. After two weeks, viable plants were transferred to soil, grown up and their seeds collected. Transgenic plants were analyzed by PCR or Northern hybridization.

2.2.6 Protoplast transformation

The XVE::GFP and GFP::GR plasmids were used for transient expression in tissue culture derived Arabidopsis protoplasts. The protoplast suspension was incubated with 10µg plasmid DNA for 18h in K3 medium at room temperature in dark (Reidt *et al.* 2000), containing 6-benzylaminopurine, 1-naphthaleneacetic acid and 2,4-dichlorophenoxyacetic acid, in concentration of 4,5µM, 10µM and 4.5µM, respectively. The GFP signal was localised *in vivo* using a confocal laser-scanning microscope (Zeiss, Germany). The GFP fluorophore was excited at 488nm by an argon laser and detected between 505nm and 520nm.

2.2.7 Induction experiments

For long-term induction transgenic and wild type seeds were plated on MS-plates supplemented with f.c. 10µM dexamthasone (DEX; Sigma) or f.c. 5µM estradiol (Sigma), respectively. As control treatment, seeds were plated on MS-plates with ethanol, which serves as a solvent for either DEX as well as estradiol. Ethanol was added to the same volume such as DEX or estradiol. 50mM stock solutions of DEX and estradiol were stored at -20°C.

For short-term induction experiments seedlings were grown on MS-plates for two weeks before transferring them to liquid MS-medium supplemented with f.c. 30µM DEX or 10µM estradiol. Seedlings were incubated under gentle shaking in the growth chamber until samples were taken. Tissue samples were immediately frozen in liquid nitrogen and stored at -80°C until further processing.

Treatments with abscisic acid were performed at a concentration of f.c. 10µM ABA. 50mM ethanolic ABA stock solutions were stored at -20°C.

2.2.8 Isolation of DNA

Extraction buffer

10mM	Tris	pH8,0
10mM	EDTA	pH8,0
0,1M	Sodium chloride	
2%	SDS	

Approximately 100mg leaf material was homogenized in liquid nitrogen and incubated for 2 hours at 56°C after addition of 1ml extraction buffer and 20µl proteinase K (stock 25mg/ml). After cooling down to room temperature 1ml saturated 6M sodium chloride solution was added and the pellet was discarded after 10 minutes centrifugation. The supernatant was mixed with 0.6 volumes of isopropanol and DNA was precipitated by incubation at -20°C for 30 minutes. The pellet was washed with 70% ethanol and solubilized in water.

2.2.9 Isolation of RNA

Total RNA from Arabidopsis tissues like seedlings, leaves or flowers was isolated using the RNeasy® Plant Mini Kit (Qiagen) as described in the manufacturers instructions.

RNA from Arabidopsis seeds was prepared using a method adapted from the hot-borate method described by (Wan and Wilkins 1994). Seeds from 10 siliques of different developmental stages were homogenized using a retsch-mill (Eppendorf). The powder was added to 1ml hot RNA extraction buffer (200mM sodium borate decahydrate, 30mM EGTA, 5mM EDTA, 1% SDS, 1% sodium deoxycolate, 10mM DTT, 100mM β-mercaptoethanol, 2% polyvinylpyrrolidone 40, 2% polyvinylpolypyrrolidone, pH9,0) and vortexed. Proteinase K was added to a concentration of 0.15mg ml^{-1} and incubated at 37°C for 45-60 min. Following centrifugation to remove insoluble debris, RNA was extracted with phenol/chloroform, precipitated with 1 volume isopropanol for 20 minutes on ice and the pellet solubilized in water. For purification the RNA was again precipitated with 4M LiCl overnight and then washed with 2M LiCl and ethanol (100%, 70%). After drying the pellet was resuspended in water.

2.2.10 Lipid analysis

Lipid fractions were measured by gas chromatography after esterification as described by (Borisjuk *et al.* 2005). About 2mg seedling material (induced/uninduced) was used for analysis which was performed in triplicate. The amount of total lipids was calculated as the sum of the detected fatty acid methyl esters (FAMEs).

2.2.11 Quantitative real-time PCR

Synthesis of cDNA was performed as described in chapter 2.2.1. Real-time PCR reactions were performed as described by Czechowski *et al.* (2004) In brief, reactions were run in triplicate in 384-well plates in an ABI PRISM® 7900 HT qRT-PCR instrument (Applied Biosystems,Foster City, CA, USA) using the SYBR® Green Master Mix reagent (Applied Biosystems). Reactions contained 5µl SYBR® Green solution, 1ng cDNA and 500nM of each primer to a final volume of 10µl. Primer sequences are represented in Supplementary Table S1.

2.2.12 Western blot analysis

Frozen seeds were homogenized in 3:1 (w/v) 62,5mM Tris buffer, pH6,8, containing 2% SDS, 5% β-mercaptoethanol and 10% glycerol. Protein extracts were heated for 3min before centrifugation at 10.000g for 10min. Protein content of the supernatants was measured according to Bradford and Williams (1976). SDS-PAGE and Western blotting were performed as described by (Senger *et al.* 2001). For detection of the 12S seed storage protein cruciferin the rabbit anti-cruciferin antibody 511 (diluted 1:4000) was used as primary antibody (Tiedemann *et al.* 2008). Bound antibodies were detected by an anti-rabbit IgG horseradish peroxidase conjugate (diluted 1:5000, Pierce, Rockford, Illinois, USA) by chemiluminescence signal generation using the ECL Kit (Amersham Pharmacia Biotech).

2.2.13 Macroarray hybridization

Macroarray hybridization experiments were performed by Andreas Czihal (IPK) as described in Vorwieger *et al.* (2007). In brief, messenger RNA was isolated with Dynabeads mRNA Direct Kit (Dynal Biotech) according to manufacturer's instructions. First strand cDNA was synthesized directly on the beads using AMV Reverse Transcriptase (Promega). The probes were labeled with ^{33}P-dCTP by random priming using Klenow fragment (Amersham). The membranes were prehybridized for 2 hours at 65°C in Church buffer (7% SDS, 0,5M NaPO4, 1% Albumine fraction V (Roche), pH7,2) supplemented with 40µg/ml hering sperm DNA (Stratagene). After an overnight hybridization, washing was done as follows: 2x15 minutes 2x SSC, 0,1% SDS, 2x15 minutes 1x SSC, 0,1% SDS and 2x 0,5 SSC, 0,1% SDS; all at 65 °C. The filers were exposed to Phosphoimager screen (Fuji) and scanned.

2.2.14 Microarray hybridization

Short term induction experiments were performed using transgenic LEC1::GR and non-transgenic wild type seedlings as described in chapter 2.2.7. Four induction regimes were applied to LEC1::GR seedlings (EtOH, ABA, DEX, DEX+ABA) and only two for wild type seedlings (EtOH, DEX). Two biological replicates were performed independently. After eight hours of induction, total RNA was extracted from seedlings using the RNeasy® Plant Mini Kit (Qiagen, Hilden, Germany) following the manufacturers instructions. Samples were then checked for RNA integrity with the Bioanalyzer from Agilent (Waldbroon, Germany). The cRNA synthesis, labeling, hybridizations and scanning were performed in the lab of Jean-Pierre Renou (Versailles, INRA, France) as previously reported (Lurin et al, 2004).

The microarray analysis was carried out on CATMA arrays (Crowe *et al.* 2003, Hilson *et al.* 2004) containing 24 576 gene-specific tags (GSTs) from Arabidopsis. For each genotype different treatments were compared with corresponding ethanol control treatments. For each comparison, two technical replications with fluorochrome reversal have been performed for each pool of RNA (i.e one dye swap).

The statistical analysis was based on one dye-swap per comparison (analysis per replicate). For each array, the raw data comprised the logarithm of median feature pixel intensity at wavelengths 635nm (red) and 532nm (green). No background was subtracted. According to the experimental design the log-ratios are calculated log2(red/green) or log2(green/red) and refers to the differential expression between the different treatments. An array-by-array normalisation was performed to remove systematic biases. First, spots were excluded that were considered to show badly formed features. Then a global intensity-dependent normalisation was performed using the loess procedure (Yang *et al.* 2002) to correct the dye bias. Finally, on each block, the log-ratio median was subtracted from each value of the log-ratio of the block to correct any print-tip effect on each metablock. To determine differentially expressed genes, a paired t-test was performed on the log-ratios, assuming that the variance of the log-ratios is the same for all genes. Spots displaying extremes of variance (too small or too large) were excluded. The raw P-values were adjusted by the Bonferroni method, which controls the Family Wise Error Rate (FWER, Ge *et al.* 2003). Genes were considered as being differentially expressed with a Bonferroni-corrected p-value ≤0,05 as described in Lurin *et al.* (2004).

The data were deposited in 'Complete Arabidopsis Transcriptome database (CATdb; http://urgv.evry.inra.fr/CATdb).

2.2.15 ChIP on chip

Induction experiments were performed using transgenic LEC1::GR seedlings as described in chapter 2.2.6. Seedlings were induced with DEX (and ethanol as control treatment) for 24 hours before cross-linking and chromatin isolation. Chromatin isolation and immunoprecipitation were performed by Gudrun Mönke (IPK).

Chromatin isolation

About 1g Arabidopsis LEC1::GR induced (DEX) or uninduced seedlings were used for each chromatin preparation. In order to cross-link protein-DNA complexes, plant material was vacuum infiltrated for 20min at 4°C in PBS (8mM Na_2HPO_4, 2mM KH_2PO_4, 150mM NaCl, pH7,5) plus 1% formaldehyde. The reaction was stopped by adding glycine (f.c. 125 mM) and continuing the infiltration for 10 min. All following steps were performed at 4°C and buffers were supplemented with a proteinase inhibitor cocktail (Roche). Seedlings were washed extensively with TBS and finally suspended in 15mM Tris-HCl, 2mM EDTA, 0,5mM spermin, 80mM KCl, 20mM NaCl, 15mM mercaptoethanol, 0,1% Triton X-100, pH7,5 (Dolezel *et al.* 1992). Seedlings were homogenized with a Potter homogenizer and passed through 50µm filters (Celltrics, Partec) and sedimented by centrifugation for 20min at 3000g. The pellet was resuspended in 50 mM Hepes, 140 mM NaCl, 1 mM EDTA, 1% TritonX-100, 0,1% Na-deoxycholate (DOC), 10% glycerol, pH7,5, incubated for 20min and centrifuged for 12min at 12.800g. The pellet was washed several times with the same buffer until the supernatant was colorless. After a final wash with IP buffer (50 mM Tris-HCl, 150 mM NaCl, 1% Triton X-100, pH7.9) the chromatin was suspended in 800µl IP buffer. 400µl aliquots were treated with ultrasound to reduce the average fragment size of chromatin to 500bp. After centrifugation (12min, 12.000 g) the clear supernatant was stored at -80°C. To determine chromatin yield, 50µl aliquots were incubated overnight at 65°C for breaking DNA-protein cross-links, and purified using a PCR purification kit (Qiagen, Hilden, Germany). DNA concentration was determined by UV spectrometry using a NanoDrop 1000 (Peqlab) photometer.

Anti-LEC1 antibody preparation

Antibodies specific for the transcription factor LEC1 were raised in rabbits. For antigen production, the whole gene fragment encoding LEC1 (AT1G21970) was cloned into the expression plasmid pET23a (Novagen) creating a C-terminal fusion with a His-tag and a N-terminal T7 tag. The LEC1 protein was produced in *Escherichia coli* BL21(DE3)Lys cells and purified via affinity chromatography on Ni-agarose (Qiagen). Rabbits were immunized three times at intervals of four weeks with 800µg protein per injection. Serum proteins were precipitated by saturation with ammonium sulfate. The precipitate was dissolved in PBS, dialyzed, and stored frozen at -20°C. Affinity purification of the anti-LEC1 antibody was achieved with the antigen coupled to CNBr-sepharose (Amersham). Protein concentrations were determined by Bradford assays.

Chromatin immunoprecipitation

For each immunoprecipitation 10µg chromatin of induced and uninduced samples were combined with 2µg of affinity purified anti-LEC1 antibody in 800µl of IP buffer supplemented with 0,1% DOC.

After incubation for 2h at 4°C on a rotating wheel the antibody chromatin complex was captured on 50µl of protein A coated magnetic beads (Dynal) by continuing the incubation for 2h. The beads were separated magnetically from the supernatant and washed successively with 1ml each of the following buffers: IP buffer; 50mM Tris-HCl, 0,5M NaCl, pH7,5; 50mM Tris-HCl, 0,5M LiCl, 0,5% NP 40, pH 7,5; 50mM Tris-HCl, 0,5M LiCl, 1% NP 40, 1mM EDTA, 0,7% DOC, pH 7,5, and TE buffer (20mM Tris-HCl, 1mM EDTA, pH 7,5). For each wash beads were incubated 5 min at 4°C on a rotating wheel and afterwards magnetically separated from the supernatant. The antibody-TF-DNA complex was eluted from the beads with 1% SDS at 65°C. Eluates from a first elution with 70 µl and a second elution with 30µl were combined, mixed with 7µl 3M NaCl, and incubated overnight at 65°C to reverse the cross-link between TF and DNA. Chromatin fragments were purified using the PCR Purification Kit (Qiagen) and their concentration determined using Picogreen (Invitrogen, Groningen, Netherlands) and a NanoDrop 3300 (Peqlab) fluorescence spectrophotometer.

Array construction

Macroarray spotting was performed by Urs Hähnel in the lab of Lothar Altschmied (IPK). A number of 11.904 PCR products comprising upstream regions of *Arabidopsis* genes were obtained from the European SAP-project (Systemic analysis of Arabidopsis promoters, Benhamed *et al.* 2008) and purified by reverse osmosis using MinElute plates (Qiagen, Hilden, Germany). Amplicons were resuspended in 20µl TE buffer and analyzed on 1% agarose gels before spotting. Approximately 75% of the amplicons appeared as single bands. Amplicons were mixed 1:1 (v/v) with 1M NaOH, 5M NaCl and spotted in duplicate onto positively charged Biodyne B nylon membranes (Pall, Dreieich, Germany) at a density of 140 spots/cm^2 using a Microgrid III robot (BioRobotics, Cambrigde, England). After spotting, membranes were washed with 0,5M NaOH, 1,5M NaCl for 5min, neutralized in 1,5M NaCl, 0,5M Tris-HCl, pH 7,4 for 5min, cross-linked in a UV-Stratlinker 2400 (Stratagene, Cedar Creek, United States) at 120mJ and baked for 30min at 80°C in a thermal oven.

Amplification and labeling of chromatin fragments

1 – 2ng of chromatin fragments were blunted with 5U Klenow polymerase (Fermentas, Vilnius, Lithuania) and ligated with 75pmol adapters using 15U T4 ligase at 16°C for 12h. Adapters were obtained by mixing equal amounts of the oligonucleotides oJW102 (GCGGTGACCCGGGAGATCTGA-ATTC) and oJW103 (GAATTCAGATC, Odom *et al.* 2004). Ligation reactions were terminated by heat inactivation (65°C, 10min), purified by reverse osmosis (PCR purification Kit, Qiagen, Hilden, Germany), and resuspended in 45µl water. Chromatin fragments were amplified for 40 cycles (95°C for 30s, 60°C for 60s, 72°C for 60s) using oJW102 as sole primer (10µM) in three separate reactions of 20µl (buffer: 10mM Tris-HCl, pH9,0, 50mM KCl, 0,1% (v/v) Triton X-100 containing 200µM dNTPs each and 2mM $MgCl_2$). PCR products were pooled and purified (PCR purification Kit, Qiagen, Hilden,

Germany). Labelled probes were obtained by combining three parallel random priming reactions using the Megaprime Kit (GE Healthcare, Uppsala, Sweden), 500ng amplified chromatin, and 50µCi [α-^{33}P]dCTP per reaction.

Array hybridization

Arrays were prehybridized for 1h at 65°C in Church buffer (0,5M sodium phosphate, pH7,2, 7% SDS, 1% BSA, 1mM EDTA, 10µg/ml sheared salmon sperm DNA) per cm^2 of membrane in a rotating thermal oven. Labelled probes were added and hybridization continued for 4 days. Membranes were washed twice in 0,2x SSC, 0,1% SDS for 20 min at 65°C and then placed on a moist Whatman paper, wrapped in Saran wrap and exposed to a Fuji image plate for 10 days in a lead chamber. Signals were detected using a FLA 3000 phosphoimager at 50µm resolution and 16 bit grey scale (Fuji). Spot intensities were obtained by aligning the pre-defined spotting pattern using ArrayVision software (GE Healthcare).

Processing of ChIP-chip data

Data normalization and initial statistic analysis were performed by Michael Seifert in the group of Marc Strickert (IPK). The group of LEC1_EtOH ChIP-chip experiments and the group of LEC1_DEX experiments were normalized independently. First, log-2-hybridization intensities were calculated for each experiment. Then, the median of log-2-hybridization intensities of an experiment was centered to zero. Finally, quantile normalization was applied separately to both groups resulting in normalized log-2-hybridization intensities for each experiment (Bolstad *et al.* 2003). For quality control within an experiment each intergenic region was spotted twice on the macroarray resulting in two normalized log-2-hybridization intensities per intergenic region. A filter was applied to exclude each intergenic region from an experiment if the absolute difference in normalized log-2-hybridization intensities of both spots of this intergenic region was greater than one. Separately for both groups of experiments, missing normalized log-2-hybridization intensities of an excluded intergenic region were substituted by mean log-2-hybridization intensities of this region in other experiments if this region passed the filter in at least three other experiments. Finally, the difference of the normalized mean log-2-hybridization intensities in LEC1_EtOH and LEC1_DEX was calculated for all four replicates. In order to identify putative intergenic target regions of LEC1 a Hidden Markov Model with scaled transition matrices (SHMM, Seifert *et al.* 2009) was applied. The SHMM provides a ranking of all fragments in an experiment based on log2 enrichment factors and their direct adjacency on the chromosome and was used to identify 2.000 intergenic regions with the highest scores for each of the four experiments. This threshold allowed the occurrence of one random intergenic region in at least three of four experiments in a complete random selection of 2.000 best scoring regions per experiment. The number of intergenic regions that appear in the list of 2.000 best scoring regions of three out of four biological replicates was determined. These were considered as putative intergenic target regions of LEC1 and corresponding genes represent putative LEC1::GR target genes. The criteria described above ensured a high degree of reproducibility of putative intergenic target regions for the four experiments.

2.2.16 Histology

For seed staging, seeds of different age were dissected out of the siliques, cleared by heating in chloral hydrate:water:glycerol 8:3:1 (w/v/v) until boiling and subsequently analyzed by light microscopy (Axioplan, Zeiss, Jena, Germany).

For detection GUS activity, the harvested plant material was vacuum infiltrated and incubated overnight at 37°C in the GUS staining solution containing 50 mM Na phosphate buffer pH 7,0, 10mM EDTA, 0,1% Triton X-100, 2mM potassium ferrocyanide, 2mM potassium ferricyanide, 100µg/ml chloramphenicol and 1mg/ml X-gluc. The plant material was cleared and stored in 96% ethanol and analyzed with a light microscope (Axioplan, Zeiss, Germany).

Fixations, embedding and microscopical analysis described in the following were performed in cooperation by Dr. Twan Rutten (IPK). Seeds and seedlings of transgenic and wild type *Arabidopsis thaliana* lines were chemically fixated with 0,5% glutaraldehyde and 2% formaldehyde in 50mM phosphate buffer pH7,0 supplemented with 0,05% Triton X100 for 2h. After three washing steps with buffer, probes were dehydrated in a graded ethanol series and infiltrated with HM20 resin (Tiedemann *et al.* 2008). A HM20 polymerization under UV-light at -35°C was skipped, after this procedure was found to frequently cause gas inclusions in compact storage tissues. Instead, HM20 was thermically polymerized at 60°C under nitrogen for 24h, using a Reichert-Jung AFS (Leica, Vienna, Austria). Semithin (1µm) and ultrathin (70nm) sections of embedded probes were cut on a Reichert-Jung Ultracut S (Leica, Vienna, Austria).

For light- and fluorescence microscopy semi-thin sections (1µm) were used. Histological overviews were obtained by staining sections with 1% fuchsin (Fluka) in 20% ethanol for 2min at 60°C followed by 2% crystal violet for 10sec at 60°C. Presence and distribution of starch granules was analyzed by staining with 2% iodine solution. Digital recordings were made on a Zeiss Axiovert 135 equipped with a Zeiss Axiocam (Carl Zeiss GmbH, Jena, Germany).

For immunofluorescence microscopy, sections were collected on 8 wells diagnostic slides (Carl Roth GmbH, Karlsruhe, Germany). After blocking with 3% BSA in PBS for 20min, sections were incubated with a custom made polyclonal antibody against cruciferin (Tiedemann *et al.* 2008) for 60min, washed four times 10min with PBS, incubated with Alexa488 goat anti rabbit (Molecular Probes Inc., Oregon, USA) for 45min followed by three washes with PBS. Alexa488 fluorescence was examined in a Zeiss LSM 510 Meta laser scanning microscope using a 488nm line in combination with a 505-530nm bandpass filter. The 1µm thin sections, though excellent for immunostudies, show little contrast when examined in bright field mode. To visualize tissue morphology, probes were scanned with a 364nm laser line resulting in a strong autofluorescence of (young) cell walls which was recorded with a 470-530nm band pass filter.

Transmission Electron Microscopy

Electron microscopy immunolabelling was carried out on 80nm ultrathin sections collected on 75 mesh polygonal copper grids. Blocking and labelling conditions were basically similar to those used for immunofluorescence labelling. Instead of a secondary antibody proteinA-10nm gold (British Biocell International, Cardiff, UK) was used. After final washes with PBS and two times A.D. grids were stained with 4% uranyl acetate, air dried and examined in a FEI Tecnai 20 electron microscope (Fei Company, Eindhoven, Netherlands) at 120kV acceleration voltage.

Scanning electron microscopy

For scanning electron microscopy chemically fixated and dehydrated probes were critically point dried in a Bal-Tec critical point dryer (Bal-Tec AG, Balzers, Switzerland). Dried specimen were attached onto carbon coated aluminium sample blocks and gold-coated in an Edwards S150B Sputter Coater (Edwards High Vacuum Inc., Crowley, West Sussex, UK). Probes were examined in a Hitachi S4100 SEM (Hisco Europe, Ratingen, Germany) at 5kV acceleration voltage. Digital recordings were made and saved as *tif* files.

Confocal Laser Scanning Microscopy

GFP fluorescence signals (emission peak 515nm) were studied with a Zeiss LSM 510 META confocal laser scanning microscope (Carl Zeiss, Jena, Germany). GFP was excited with a 488nm line and emission was detected with a 505-530nm band-pass filter. The identity of the GFP was confirmed by a photospectrometric analysis of the fluorescence signal with the help of the META-detector. When illuminated with a 488nm laser line, chlorophyll shows a strong reddish autofluorescence which was recorded with a 650nm long-pass filter.

3. RESULTS

3.1 Transcription factor expression profiling during Arabidopsis seed development

The ARABIDOSEED project aimed at the elucidation of transcription factor-target gene networks underlying seed development. Seed-expressed transcription factor genes have been identified using molecular and bioinformatic approaches. Transcription factor genes expressed at different stages of seed development have been selected for a more detailed analysis, including transgenic approaches, regulated gene expression, transcriptome analysis and immunprecipitation techniques.

A large scale seed staging experiment has been performed to correlate developmental stages, expression profiles and seed storage contents. For this purpose, Arabidopsis plants were staged relative to their flowering time. The first siliques of the main shoot of 10 plants with the same flowering time were sampled at 5, 7, 9, 11, 13, 15, 17 and 19 days after flowering (daf). Staged seeds were used in parallel for RNA isolation and real-time PCR as well as for microscopical inspection (Figure 7). The seed development stages correspond to heart stages (5daf), torpedo stages (7daf), cotyledon stages (9-13 days) and mature seeds with different levels of desiccation (15-19daf). For clearing, seeds were heated in chloral hydrate solution and pictures were taken using Nomarski optics.

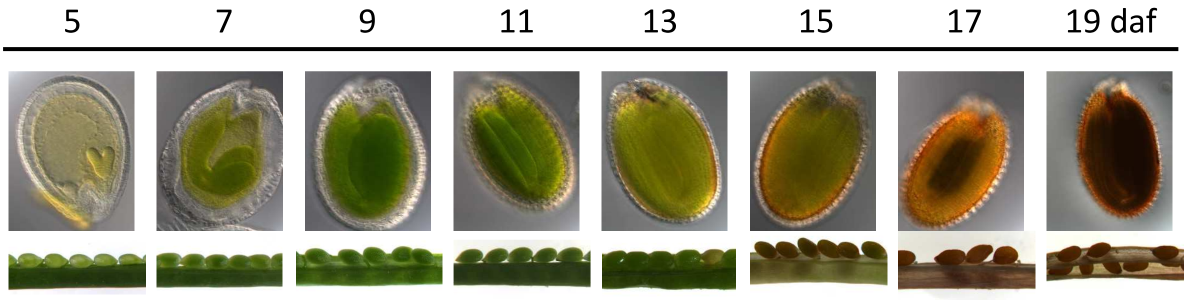


Figure 7. Microscopical analysis of Arabidopsis wild type seed development.
Seeds of different stages were collected in frame of a large scale staging experiment comprising seeds containing heart stage embryos (5daf) until dry, ripened seeds (19daf). Prior to microscopy, seeds were cleared with chlorale hydrate.

Based on the GenevestigatorV2 database (https://www.genevestigator.com/gv/index.jsp), 'seed-expressed' transcription factors were identified by the calculation of the seed index of all TAIR annotated transcription factors. The seed index is defined as the ratio between the expression in seeds or siliques *versus* the expression in all other tissues. In total, 101 transcription factors were considered to be `seed-expressed`, although their expression is not necessarily restricted to seed development (Figure 8).

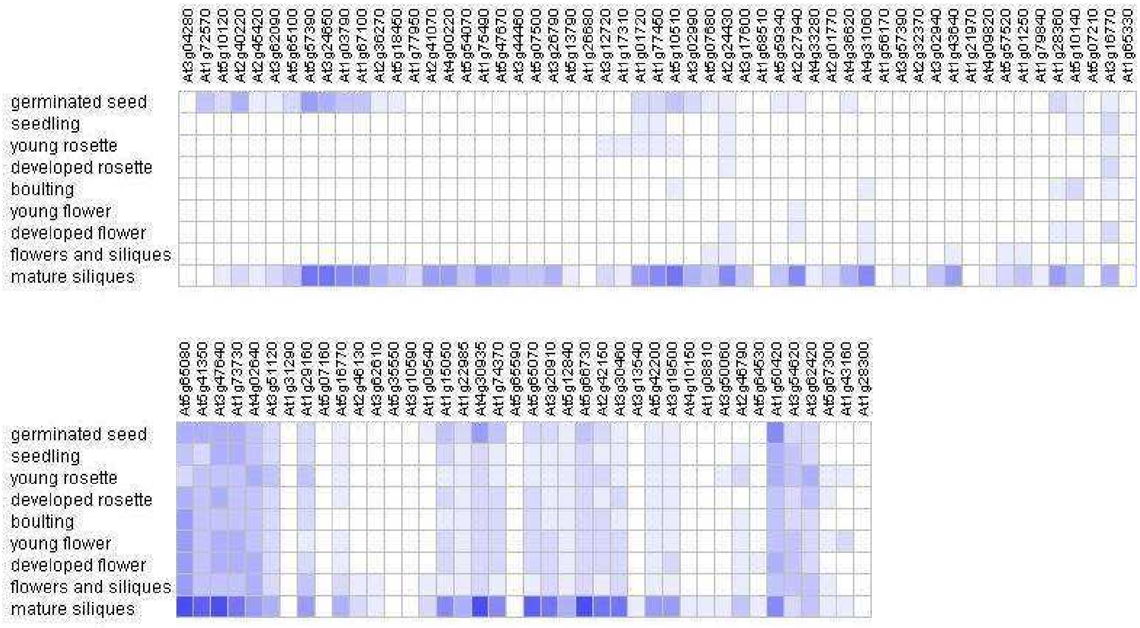


Figure 8. Seed expression of 95 Arabidopsis transcription factors.
The majority of TF are expressed exclusively in the developmental category 'mature siliques' (including seeds), some also in the category 'germinated seed'. When expression is observed in other categories, the expression maximum can be found in the categories mentioned before. Clustering analysis and visualization was performed with the corresponding tools at Genevestigator database.

'Seed-expressed' transcription factors are distributed among almost all known TF families. Most abundant TF belong to the zinc finger family (17%), the AP2/EREBP family (15%), the MYB family (12%) as well as the MADS family and bZIP family with 8% each (Figure 9). Transcription factors with known functional relevance for Arabidopsis embryogenesis and seed development like LEC1, LEC2, ABI3 and FUS3 are included in the list.

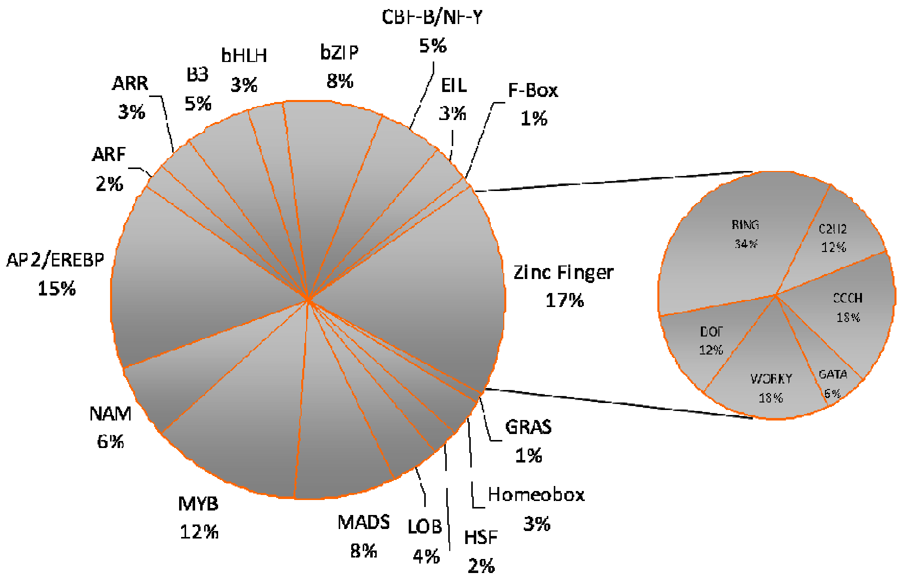


Figure 9. Proportions of transcription factor families among 'seed-expressed' transcription factors. Seed-expressed TF were classified according to different TF families (AGRIS TF database, http://arabidopsis.med.ohio-state.edu/). The biggest proportions were mapped to zinc finger (17%), AP2/EREBP (15%), MYB (12%), MADS (8%) and bZIP (8%) protein families. Among zinc finger subfamilies one third was represented by RING domain proteins, followed by CCCH and WORKY proteins with 18% each, DOF and C2H2 proteins with 12% each and the smallest proportions represented by GATA TF (6%).

TF transcripts at different seed stages were quantified using real-time PCR in cooperation with Dr. Armin Schlereth (MPIMP, Golm, Germany; Czechowski *et al.* 2004). TF expression was normalized to *UBIQUITIN10* (*UBQ10*) as a housekeeping gene. Relative expression values were calculated according to the ΔC_T method for all reactions with primer efficiency values >1.6 as estimated by the LinReg software (Ramakers *et al.* 2003). Normalized $\log_2$ ratios of the relative expression values were clustered by K-means clustering and visualized using the TM4 software (TIGR, http://www.tm4.org, Saeed *et al.* 2003). Figure 10 shows the expression profiles of 93 identified 'seed-expressed' transcription factors over 8 stages of Arabidopsis seed development. The mRNA of 8 transcription factors could not be detected. Cluster analysis revealed 6 clusters with expression profiles that correspond to different developmental stages. Supplementary Table S2 lists the genes (identifier, annotation) which are comprised by the single clusters. Cluster I corresponded to the end of early embryo morphogenesis and comprised 7 genes, whereas 4 genes show a rapid decrease in transcript level with the beginning maturation phase. These include *LEAFY COTYLEDON2* (*LEC2*), *PHERES* (*PHE*), a piwi domain-containing protein with similarity to *ARGONAUTE2* (*AGO2*) and one unknown TF. Cluster II represented the switch between morphogenesis (early embryogenesis) and maturation (late embryogenesis) and includes 26 genes including several candidates which were already implicated with this transition phase. LEC1 and LEC1-like (L1L) are described as central regulators of both early and late embryogenesis (Kwong *et al.* 2003, West *et al.* 1994). Also another HAP factor, HAP5B, is included in cluster 2. Its carrot orthologue has been shown to form a yeast-like HAP-complex together with C-LEC1 (Yazawa and Kamada 2007). The cluster also comprises five genes encoding MYB factors, including *MYB123* alias *TRANSPARENT TESTA2 (TT2)* which is responsible for proanthocyanidin accumulation in the immature embryo. Also the bHLH factor TRANSPARENT TESTA8 (TT8), which acts together with TT2 in flavonoid metabolism, belongs to the same cluster (Baudry *et al.* 2004, Nesi *et al.* 2000, Nesi *et al.* 2001). Furthermore, cluster II includes genes involved in regulation of seed lipid metabolism, like *LEC1* (mentioned above), *WRINKLED1 (WRI1)* which belongs to the AP2/EREBP family and the homeobox gene GLABRA2 (GL2, Cernac and Benning 2004, Mu *et al.* 2008, Shen *et al.* 2006). Also three MADS-box factors, AGAMOUS-LIKE 15 and 18 (AGL15/18), belong to cluster II. AGL15 has been shown to induce somatic embryogenesis when expressed constitutively (Harding *et al.* 2003) and is functionally redundant with AGL18 in flower development (Adamczyk *et al.* 2007, Lehti-Shiu *et al.* 2005). The following three clusters (III, IV, V) corresponded to seed filling and storage of compounds like seed storage proteins (e.g. 12S cruciferins). TF from these clusters included known regulators of seed storage protein synthesis such as genes encoding B3 domain proteins like ABI3 and FUS3, and bZIP family members like bZIP53, bZIP12 (EEL) and bZIP67 (DPBF4) whereas the functional role of EEL in MAT gene control is more unclear (Bensmihen *et al.* 2002). The bZIP67 factor has been shown to interact with LEC1 in the control of maturation genes (Yamamoto *et al.* 2009). Besides these more or less functionally characterized factors, several TF genes encoding zinc finger proteins, AP2/EREB proteins, MYB and MADS box family members were found to be expressed during storage phase. Finally, cluster VI included TF which are related to the LEA expression program and desiccation. TF genes show high expression from the end of the maturation phase until dry seed stages. Many TF belonging to the zinc finger and AP2/EREBP family were found in this cluster. Also the bZIP TF ABSICISIC ACID INSENSITIVE5 (ABI5), as a known regulator of LEA proteins was included here. Two seed-expressed heat shock factors, *HSFA1E* and *HSFA9*, were also expressed at late seed stages whereas *HSFA9* is known to be regulated by ABI3 whose expression precedes that of its target (Kotak *et al.* 2007). Genes in this ‘desiccation’-cluster may also partially represent putative regulators of germination the mRNA of which already accumulates during late seed development.

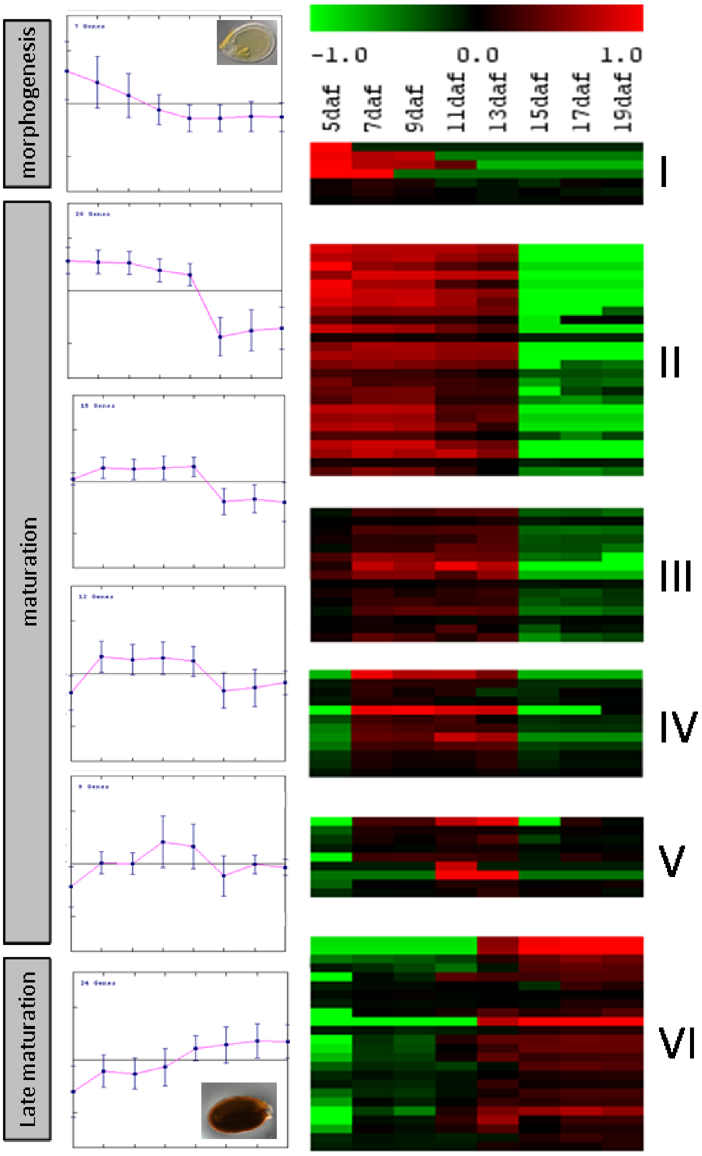


Figure 10. Expression profiling of 101 'seed-expressed' TF during *Arabidopsis* seed development. The expression profiles of 101 'seed-expressed' TF were clustered and 6 cluster groups represent different developmental stages during Arabidopsis seed development (TIGR, TM4 software). Cluster I represents the gene expression program during morphogenesis. Cluster II is associated with the transition between morphogenesis and maturation. Cluster III to V correspond to different stages of maturation whereas cluster VI represents late maturation, desiccation and possibly already germination-related gene expression.

As candidates for regulation of seed filling, the B3 domain proteins ABI3 and FUS3 were chosen for further analysis. FUS3 already has been subject of comprehensive loss-of-function analyses in the gene regulation group of the IPK (Tiedemann *et al.* 2008). As another, earlier component of the AFL/B3-LEC1-network which seems to be hierarchically superior to ABI3 and FUS3, LEC1 has been subject of further investigations. These three candidate TF, together with LEC2, are part of a complex regulatory network whereas distinct and overlapping functions have been described for each of these factors (for review see: Santos-Mendoza *et al.* 2008). Although a wealth of phenotypic and molecular data led to a complex model of genetic interactions between these factors, their precise functions remain to be elucidated and target genes of the single factors need to be identified. In the frame of this PhD work these objectives were approached by using inducible expression systems coupled to transcriptome analysis and the analysis of molecular interactions by chromatin immunoprecipitation (ChIP).

3.2 Arabidopsis lines for regulated overexpression of ‘seed-expressed’ TF

Two different inducible systems were applied for overexpression of selected TF. Gateway binary vectors for glucocorticoid- and estradiol-inducible expression were described by Baudry *et al.* (2004) and Curtis *et al.* (2002). A schematic representation of the vector cassettes is given in Figure 11. Besides several TF, the green fluorescent protein (GFP) was cloned in order to obtain transgenic control lines.

The glucocorticoid-inducible system (Figure 11A) is based on a translational fusion of the TF to the rat glucocorticoid-receptor-domain. Under the control of the 35S Cauliflower Mosaic Virus promoter (CaMV) this fusion protein is constitutively expressed in the cytosol and stabilized by heat shock proteins (HSP) acting as chaperones. After induction with the synthetic glucocorticoid-analogon dexamethasone (DEX), conformational changes lead to release of the HSP and the subsequent translocation of the fusion protein into the nucleus where the TF can bind its target promoters.

The estradiol-inducible system (Figure 11B) relies on XVE as a chimeric transcription activator, expressed under the strong constitutive promoter G10-90 and highly regulated by estradiol. It consists of three parts: the DNA binding domain of the *Escherichia coli* LexA repressor, the transactivating domain of VP16 and the regulatory region of the human estrogen receptor. The XVE element occurs as a monomer in the cytosol. After estradiol treatment the estrogen-receptor-domain dimerizes and the dimer is translocated to the nucleus where the LexA binding domain binds to the LexA operator. The VP16 transactivation domain allows the RNA polymerase to access the DNA and transcription of the candidate TF starts.

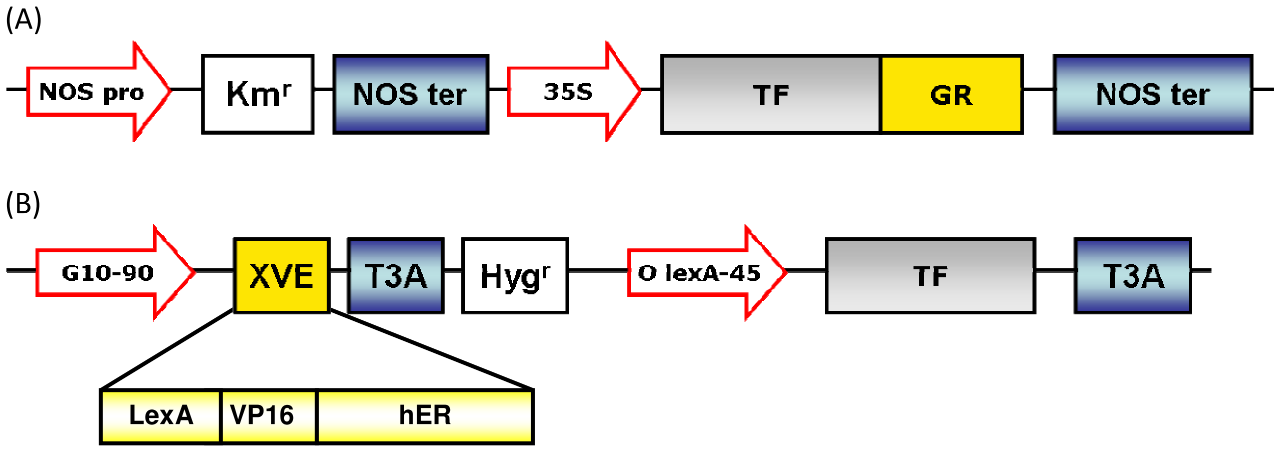


Figure 11. Gateway binary vectors for inducible overexpression of selected 'seed-expressed' TF.
(A) p35SR$_1$R$_2$ΔGR: vector for translational fusion of the TF to the rat GR-domain and DEX-inducible overexpression (Baudry *et al.* 2004). (B) pMDC7: vector for constitutive expression of XVE and estradiol-induced transactivation of TF expression (Curtis *et al.* 2002). Further explanations of the expression cassettes are given in the text.

Prior to stable transformation into Arabidopsis plants, the functionality of both inducible systems was tested transiently in Arabidopsis protoplasts. Plasmids for inducible expression of GFP (GFP::GR and XVE::GFP) were used for visualization of the induction process. Around 10µg plasmid DNA each was used for protoplast transformation. A construct for constitutive overexpression of GFP (35S::GFP) was transformed as a positive control. Two days after transformation the GFP signal was detected using a confocal laser scanning microscope (CLSM). Around 20% of the 35S::GFP-protoplasts showed a very strong signal in the cytosol and the nucleus indicating an acceptable transformation efficiency (Figure 12A left).

In the uninduced state, the protoplasts expressing the GFP in a DEX-inducible manner (GFP::GR) showed the GFP signal only in the cytosol whereas a lower transformation frequency and a much lower signal intensity were observed (Figure 12A middle). For induction DEX was added to a final concentration of 10µM. The same volume of 96% ethanol (as a solvent for DEX) was added for control (uninduced sample). 24 hours after induction protoplasts were again scanned using the CLSM. In all induced and fluorescing protoplasts the DEX-inducible GFP was translocated into the nucleus (Figure 12A right) whereas in the uninduced ones it still occured only in the cytosol.

The estradiol-inducible GFP was not visible before induction (Figure 12B middle) as its transcription would only start after addition of the inducer. Although in some rare cases a weak GFP signal was detected showing a low level of leakiness occurring in this system (Figure 12B left). 24 hours after induction with estradiol (f.c. 5µM) a very strong GFP signal was observed (Figure 12B right).

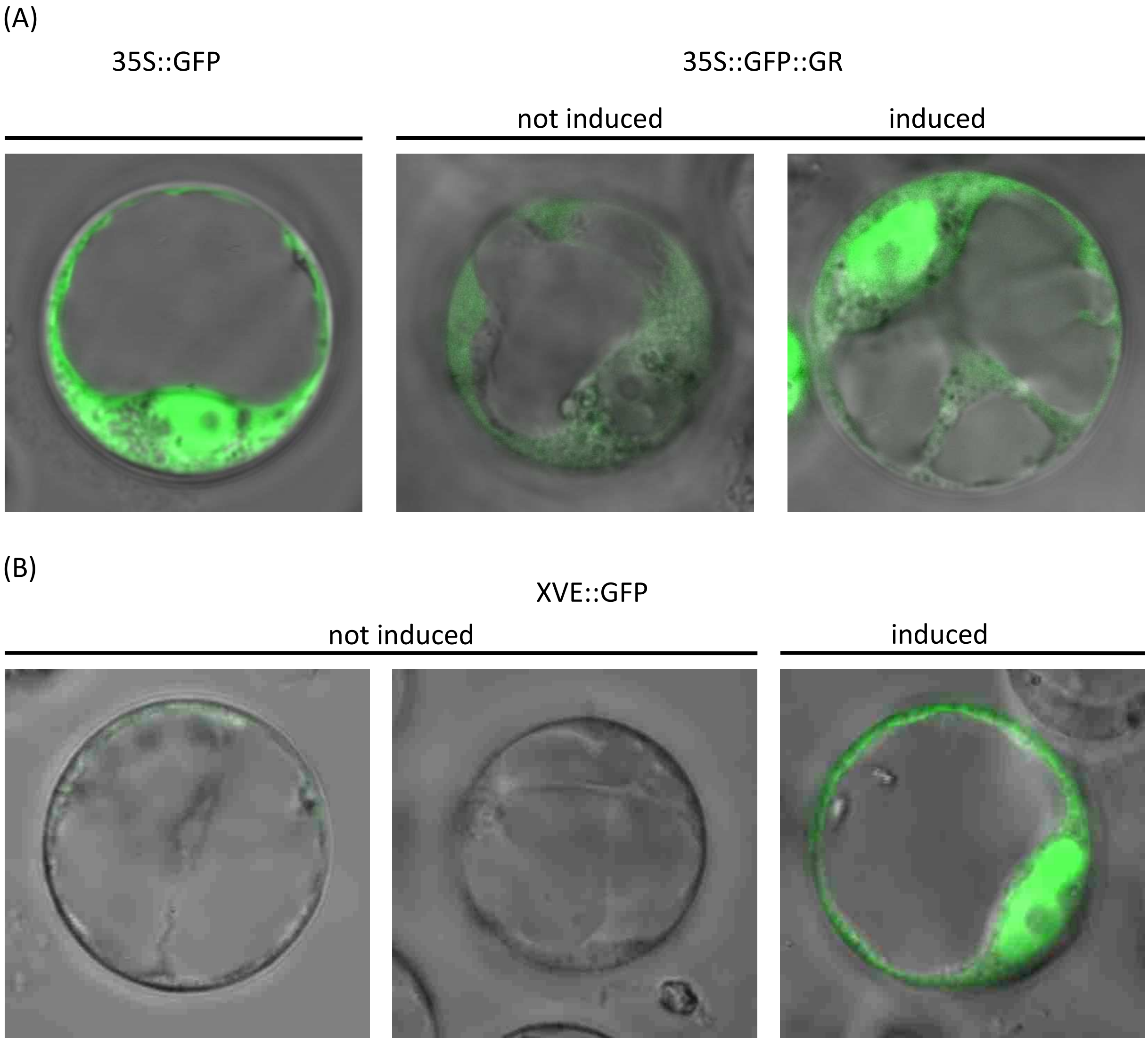


Figure 12. Protoplast transformation with inducible GFP constructs.
(A) The control construct expresses GFP constitutively throughout the whole protoplast (left). Before induction the DEX-inducible GFP occurs only in the cytosol (middle) and is translocated into the nucleus after 24 hours DEX treatment (right). (B) No (middle) or, in very rare cases (1%, left), a weak GFP signal occurred without estradiol treatment. A strong GFP signal was observed after estradiol induction (right).

Besides GFP, five candidate TF (ABI3, FUS3, LEC1, MYB44, MYB77) were cloned into both vectors and the corresponding eight constructs were transformed into *Arabidopsis thaliana* Columbia 0 plants. After selection with the appropriate antibiotic, first transformants (T0) were screened for genomic integration by PCR. Segregation analysis on selective media was performed with the offspring (T1) resulting in homozygous lines in the T2 or T3 generation. Table 2 gives an overview of all generated transgenic lines.

Table 2. Generated homozygous/heterozygous lines for inducible overexpression of selected transcription factors.
T0 transformants have been tested for genomic integration of the transgene by PCR and homozygous lines were generated by segregation analysis on media with the corresponding antibiotics. Transgene expression was tested in homozygous lines by qRT-PCR. * In the case of homozygous ABI3::GR lines the transgene expression could not be detected anymore in the T2 generation which was probably due to silencing effects. Heterozygous T1 lines were used for further analysis.

Transcription factor	*ATG number*	*DEX-inducible lines*	*Estradiol-inducible lines*
LEC1	At1g21970	3	2
ABI3	At3g24650	2*	2
FUS3	At3g26790	1	1
MYB44	At5g67300	5	2
MYB77	At3g50060	5	1

3.2.1 Estradiol induction

Arabidopsis lines expressing the inducible GFP were used to establish a standard protocol for estradiol induction experiments. Wild type and XVE::GFP seeds were sown on MS medium without supplements. After 7 days seedlings were split and transferred either to MS medium with alcohol (uninduced) or MS with estradiol (f.c. 5µM). The induction of GFP was observed using fluorescence microscopy. The GFP signal started being visible after 6 hours of induction, reached a peak at 24 hours and declined again afterwards (Figure 13).

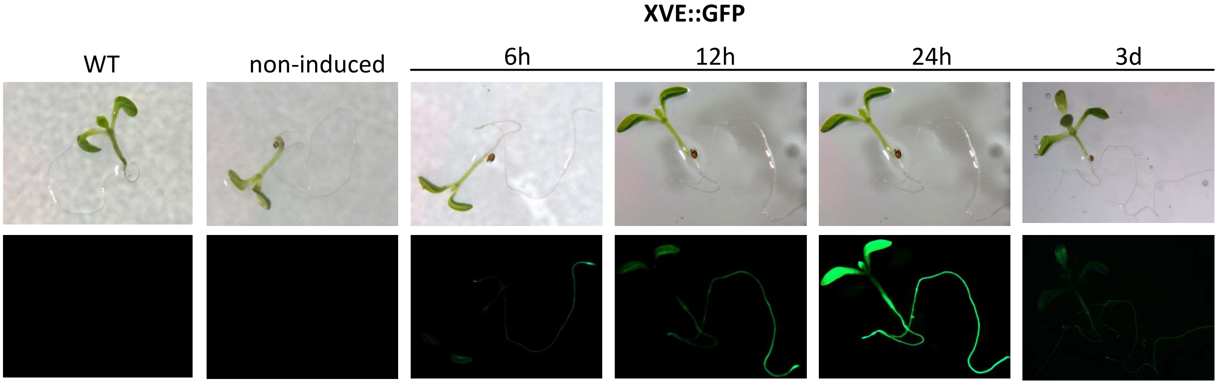
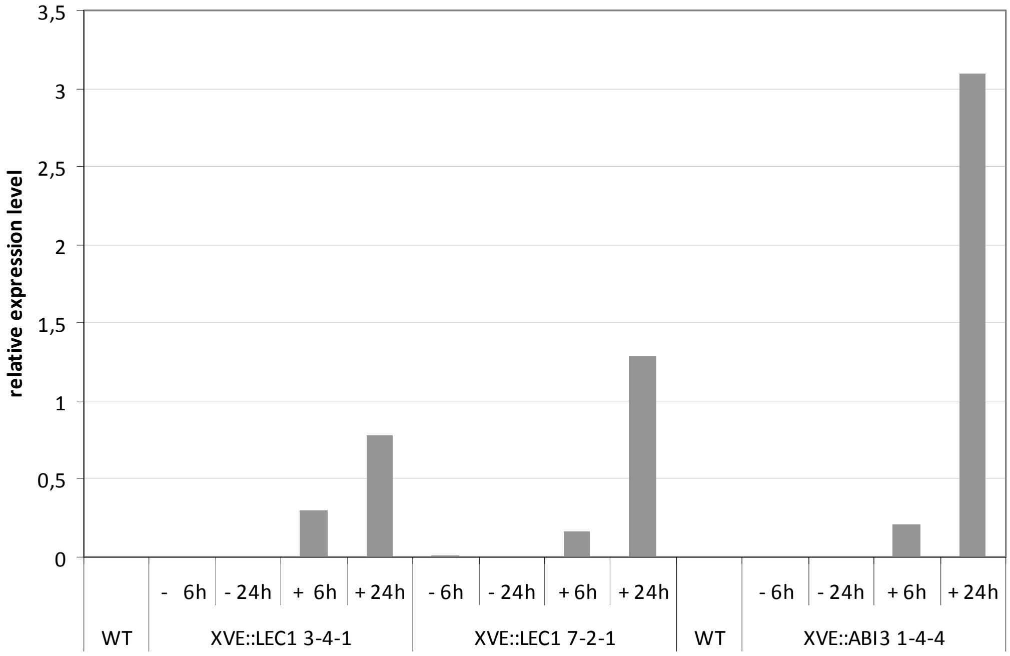


Figure 13. Estradiol- inducibility of GFP in GFP::pMDC7 seedlings.
7 days old GFP::pMDC7 seedlings were transferred to plates containing MS medium supplemented with 5µM estradiol. Using a fluorescent binocular microscope the GFP signal was detected after 6 hours of induction, reaching a maximum after 24 hours. No GFP signal was visible in uninduced XVE::GFP and in wild type seedlings.

In order to validate the estradiol-inducibility of TF, the increase in TF abundance after estradiol induction was measured by qRT-PCR. 14 days old seedlings of corresponding XVE::TF lines were induced on estradiol containing MS medium (f.c. 5µM) and samples for RNA isolation were taken at different time points after induction. Wild type seedlings of same age that were treated in the same way and uninduced transgenic samples were used as control samples. Two XVE::LEC1 lines and one XVE::ABI3 line were analyzed by qRT-PCR. The relative expression values were calculated according to the ΔC_T-method and primer efficiency was included with an ideal value of two. In comparison to the uninduced and the wildtype samples a significant increase in transcript abundance of ABI3 or LEC1 was observed after 6 hours of induction with a further increase after 24 hours (Figure 14A). The increase in FUS3 transcript after estradiol induction of XVE::FUS3 seedlings was detected by Northern blot (Figure 14B). Equal amounts of RNA isolated from induced and not induced XVE::FUS3 seedlings were hybridized with a FUS3-specific probe. The detected signal intensities prove similar induction kinetics for FUS3 as observed for ABI3 and LEC1.

(A)



(B)

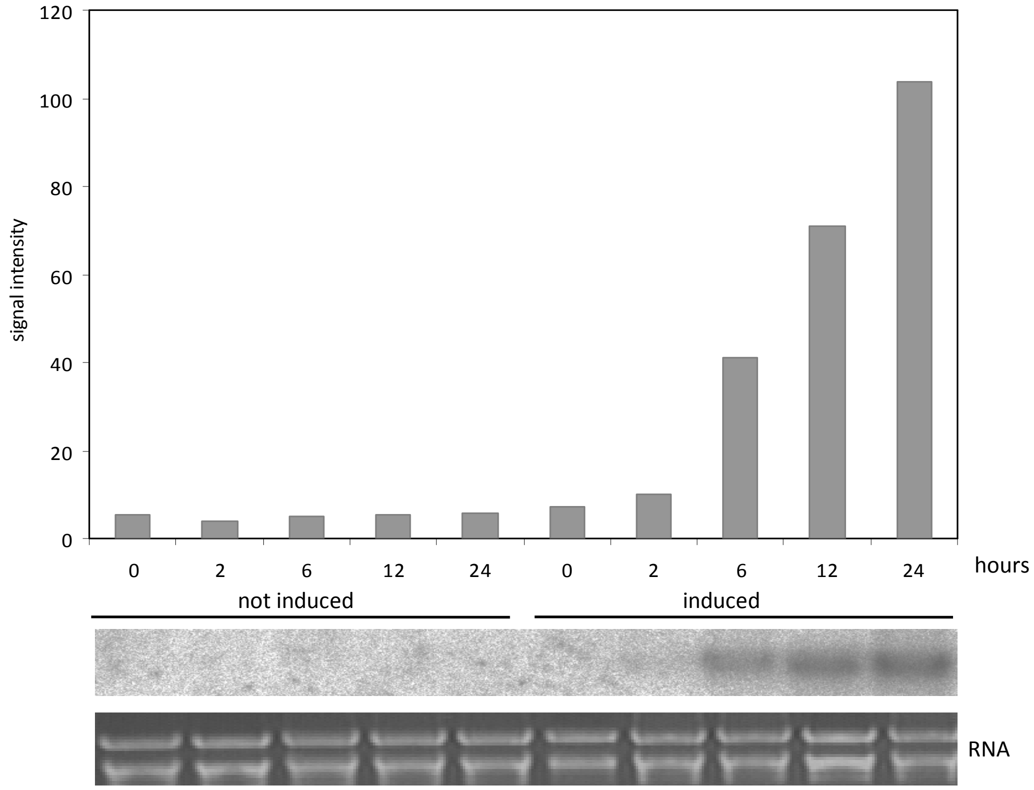


Figure 14. Relative TF expression in estradiol- inducible overexpression lines.
(A) The expression of different TF in induced vs. uninduced seedlings of several estradiol-inducible *LEC1* and *ABI3* overexpression lines was measured by qRT-PCR in comparison to wild type samples. Each column represents the mean value of three technical replicates. (B) *FUS3* expression in an estradiol-inducible *FUS3*-overexpression line after induction vs. the uninduced controls as detected by Northern hybridization. Signal quantification was done using the TINA program.

3.2.2 Dexamethasone induction

The induction with DEX rather relies on translocation of the TF::GR fusion protein to the nucleus than on transcriptional activation. Thus it was possible to analyze transgene expression without induction. Several homo- and heterozygous inducible TF::GR lines have been tested for transgene expression by quantitative real-time PCR using TF specific primers and primers for UBQ10 as a housekeeping gene. 14 days old seedlings of DEX-inducible lines grown on MS medium without supplements were sampled for RNA isolation. Almost all analyzed transgenic lines showed an increased expression of the corresponding TF in comparison to the wild type (Figure 15). For not exclusively 'seed-expressed 'TF (MYB44 and MYB77) low expression was detected in the wild type seedlings. Strictly seed specifically expressed TF like FUS3, ABI3 and LEC1 were not expressed at all in wild type seedlings.

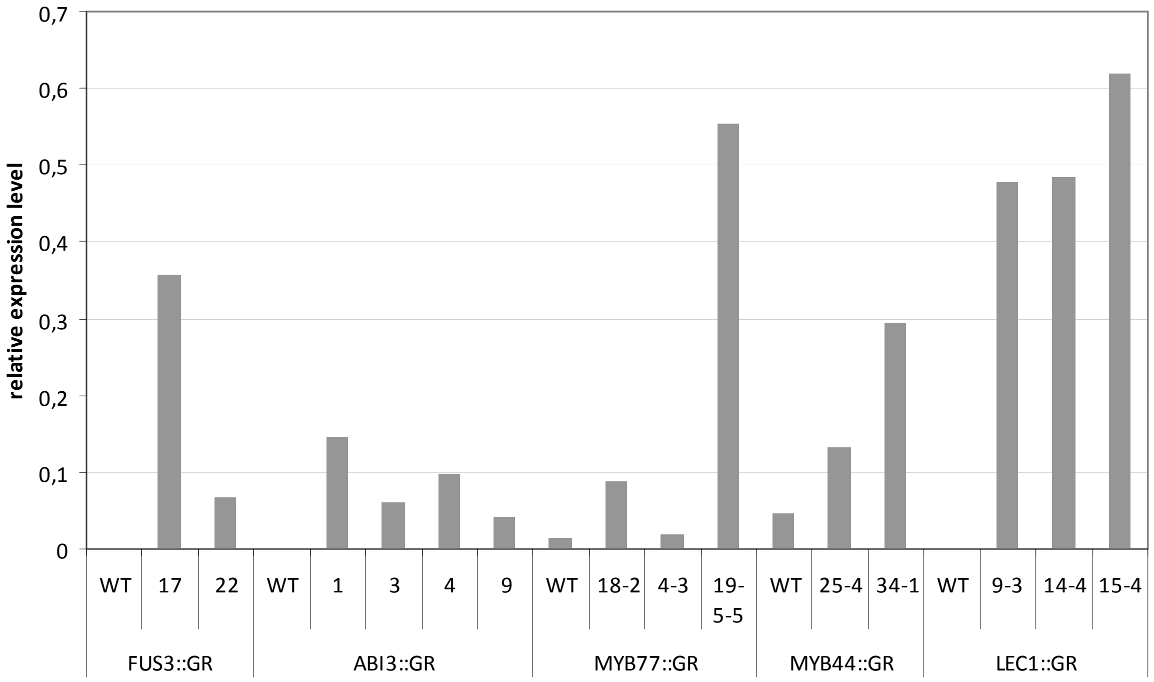


Figure 15. Relative TF expression in DEX- inducible overexpression lines.
The expression of different TF genes was measured by qRT-PCR in 14 day old seedlings of several homozygous/heterozygous T1/T2 DEX-inducible overexpression lines in comparison to wild type samples. Each column represents the mean value of three technical replicates. In the case of FUS3, the homozygous offspring of analyzed T1 plant lines was used further analyses (line 17-1-1). Due to non-detectable transgene expression in homozygous T2 lines, heterozygous T1 ABI3::GR plants (line 4) were further analyzed.

The GFP::GR control line was not suitable for functional validation of the GR fusion *in planta*. No GFP signal could be detected using fluorescence microscopy before or after induction of GFP::GR seedlings. This was probably due to low transgene expression.

In order to detect TF::GR fusion proteins, Western blot analysis was performed using anti-TF antibodies (ABI3, FUS3, LEC1). No fusion proteins could be detected in several Western blot experiments. However by application of a functionality-mediated approach on the basis of a transient co-transformation system, protein expression could be validated for the ABI3::GR protein.

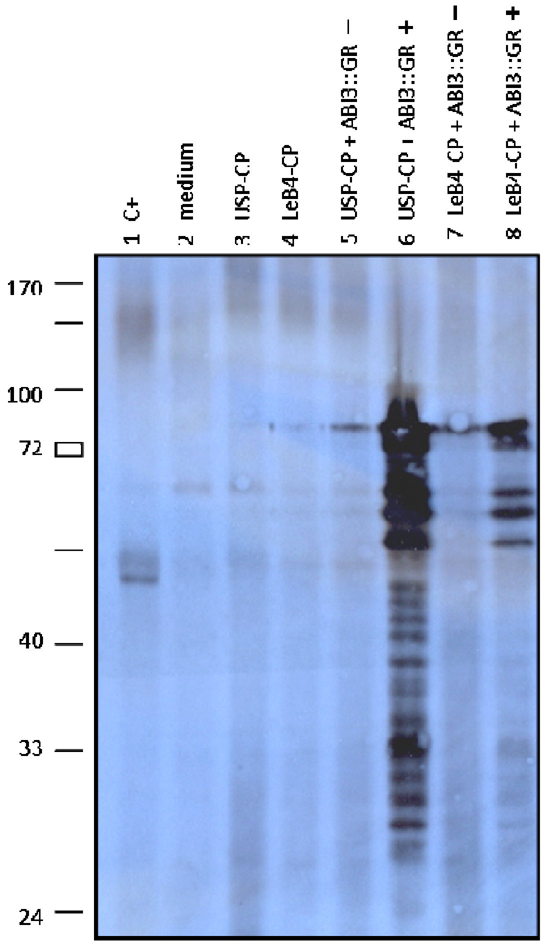


Figure 16. ABI3::GR is a functional protein and activates USP and LeB4 promoters.
Agrobacterium cultures carrying the ABI3::GR were co-infiltrated with cultures carrying the USP/LeB4::CP plasmids. Leaf discs of infiltrated tobacco leaves were induced with DEX (indicated with +) for three days and then used for protein isolation. Only after induction, the ABI3::GR protein was able to activate USP and to a lesser extent the LeB4 promoter (lanes 6 and 8). In uninduced control samples (lanes 5 and 7) and after infiltration of only medium (lane 2) or single Agrobacterium cultures (lanes 3 and 4) no protein CP was detected.

Agrobacterium tumefaciens cultures carrying the ABI3::GR plasmid were infiltrated into tobacco (*Nicotiana tabacum*) leaves together with another Agrobacterium culture carrying a plasmid containing a control protein (CP) behind a seed-specific promoter. Both seed-specifc promoters (*UNKNOWN SEED PROTEIN*, USP; *LEGUMIN*, LeB4) contain RY motifs and are known to be activated by ABI3 (Mönke *et al.* 2004, Reidt *et al.* 2000).

As control treatments, the Agrobacterium suspension carrying the plasmids with control protein and different seed-specific promoters was infiltrated alone or the pure medium was injected. Three days after co-transformation leaf discs were excised and transferred to plates containing MS medium supplemented with DEX (f.c. 30µM). In addition, leaf discs were superficially treated with DEX solution. After three days of induction samples were taken and Western blot analysis was performed. Figure 16 shows that a functional ABI3::GR fusion protein is translocated into the nucleus after DEX treatment. There it is able to activate USP and LeB4 promoters that drive the expression of the control protein which is detected using an anti-CP antibody. The control protein is fused to 100 times elastin-like polypeptides (ELP) which are degraded during polyacrylamid gel electrophoresis (PAGE, Meyer and Chilkoti 1999).

For the FUS3 protein, functionality could not be confirmed, although it is described to act on USP and LeB4 promoters in similar ways such as ABI3. Due to missing knowledge about target promoters of LEC1, MYB44 and MYB77 it was not possible to check the protein functionality by the method described above.

3.3 Phenotypic analysis of inducible LEC1 overexpression lines

All generated inducible lines were analyzed phenotypically after permanent induction. Seeds were sown on medium containing 10µM DEX or 5µM estradiol and constantly observed using the binocular microscope. Besides the MYB77::GR lines which showed dwarf growth only the LEC1-inducible lines of both systems displayed clear and homogenous phenotypes. These lines will be in the focus of the next sections.

3.3.1 Macroscopical observations

Two weeks old, permanently induced seedlings of two homozygous XVE::LEC1 lines (3-4 and 7-2) and wild type Col 0 were subject of phenotypic analysis. Both transgenic XVE::LEC1 lines had similar transgene expression level in response to the inductor (Figure 14A). Nevertheless, clear phenotypic differences between these lines could be observed which may be due to influences of the location of genomic integration. The majority of line 3-4 induced seedlings were indistinguishable from wild type (Figure 17A – C) or uninduced seedlings (Figure 17D and E). Only some seedlings displayed a long-hypocotoyl-phenotype with closed cotyledons, therefore resembling etiolated seedlings (Figure 17F). The process of germination was clearly delayed in induced XVE::LEC1 line 7-2 seeds in comparison to the corresponding controls and line 3-4. Four days after imbibition only the radicula of XVE::LEC1 seedlings grown in the presence of estradiol protruded the seed coat whereas wild type and non-induced XVE::LEC1 seedlings had already developed green cotyledons, hypocotyls and the primary root (Figure 17A-C; G).

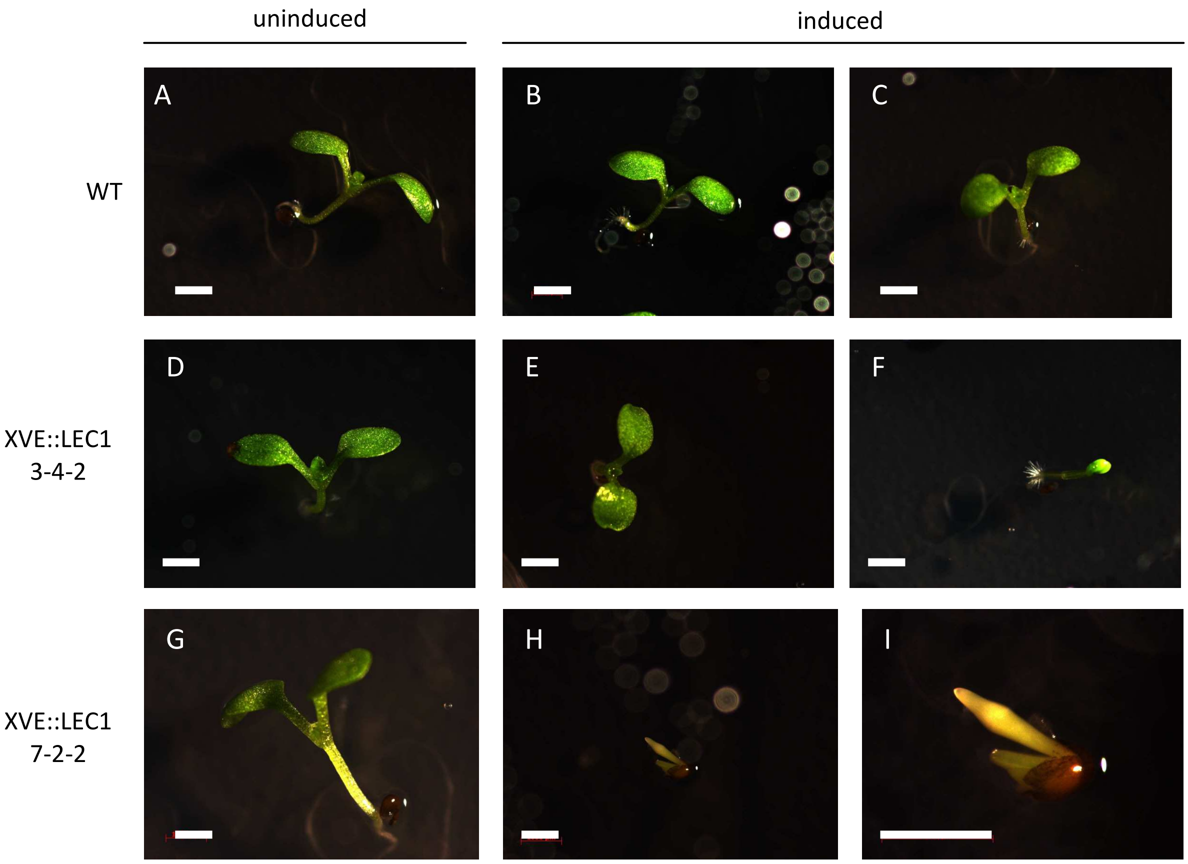


Figure 17. Phenotypic analysis of permanently induced XVE::LEC1 seedlings.
Seeds of XVE::LEC1 lines 3-4-2 and 7-2-2 as well as wild type were sown on MS medium with and without 5µM estradiol. WT seedlings developed normal under both growing conditions (A,B,C). Uninduced transgenic seedlings resembled WT seedlings (D,G). After induction, XVE::LEC1 seedlings of line 3-4-2 mostly developed similar to WT (E). In rare cases seedlings were smaller and cotyledons did not expand (F). Induced seedlings of line 7-2-2 displayed a homogenous phenotype characterized by growth retardation, stocky and thickened organs and light greenish appearance (H,I). Bars represent 1mm.

Line 7-2 seedlings displayed a very homogenous phenotype. They developed stocky and thickened cotelydons, roots and hypocotyls with a light greenish appearance (Figure 17H and enlarged in I) therefore resembling the 35S::LEC1 seedlings described by Lotan *et al.* (1998). Further development was impaired and no vegetative structures were formed.

Permanently induced LEC1::GR seedlings displayed complex morphological abnormalities that never occured in non-induced LEC1::GR or wild type seedlings (Figure 18A and B). Germination and seedling growth were similarly delayed and inhibited as described before for XVE::LEC1 line 7-2. Two weeks after imbibition two phenotypic categories (phenotype A, Figure 18C and phenotype B, Figure 18D and E) could be clearly distinguished at a reproducible ratio of 40% to 60%. Comparable results were obtained with three independent transgenic lines (9/3; 14/4 and 15/4). Results presented in the following were obtained with line 15/4.

Phenotype A seedlings displayed identical phenotypic features as observed before for estradiol-inducible seedlings of line 7-2. They only developed very small, swollen organs of light greenish appearance (Figure 18C). Root development was completely abolished. Phenotype B (Figure 18D, and E) was similar to phenotype A with respect to development of apical structures (hypocotyls and leaves) but first formed a normal primary root that underwent abnormal development later on.

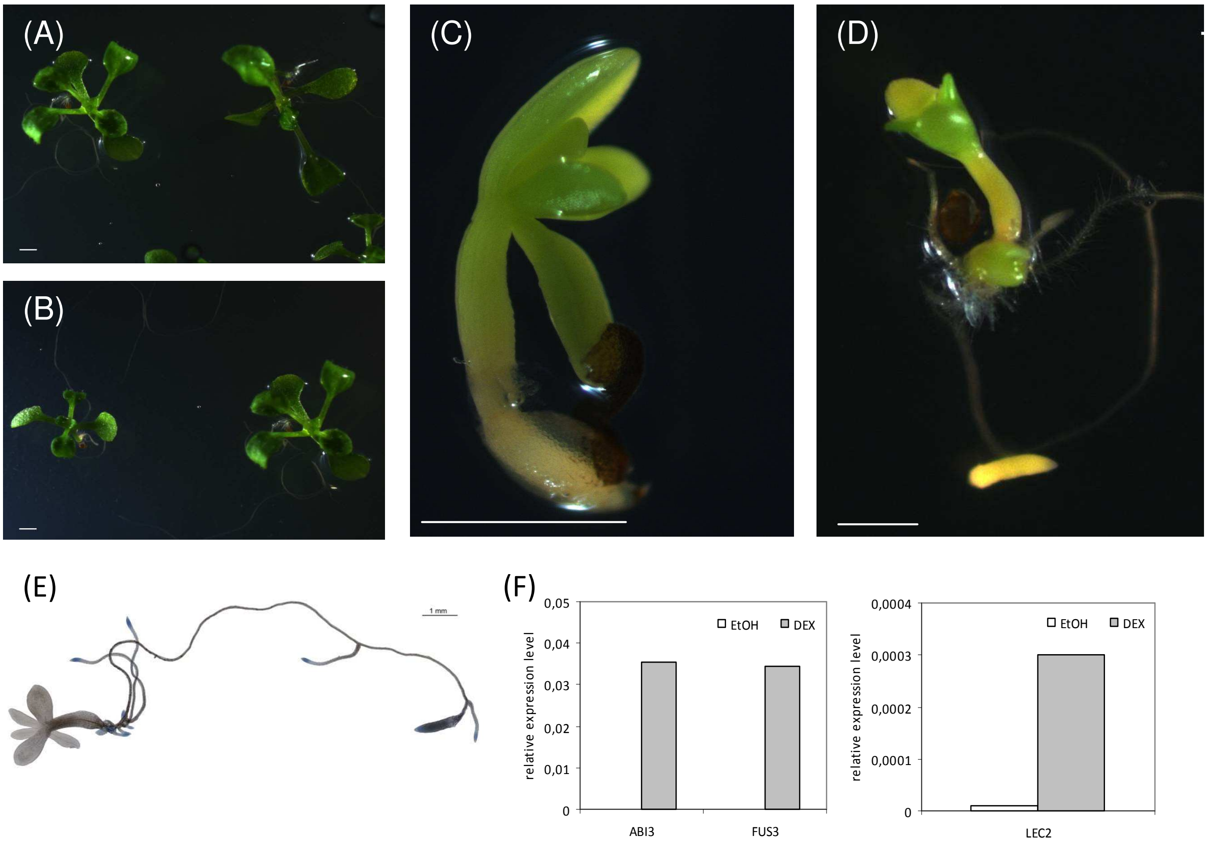


Figure 18. Permanent DEX- induction of LEC1::GR seedlings.
LEC1::GR seeds were sown on MS medium containing 10µM DEX. After two weeks of induction two phenotypic categories can be clearly distinguished among the induced seedlings. These phenotypes never occurred in WT seedlings (A) and uninduced seedlings (B). 40% of the induced seedlings display phenotype A (C) with thickened organs and secondary cotyledons. 60% showed phenotype B (D, E) which is equal to phenotype A with respect to apical organs but develops a normal primary root. After 10 days the primary root tip becomes swollen and greenish, referred to as *pkl* roots (D, E). The expression of seed-expressed AFL/B3 genes was analyzed by qRT-PCR in type-B seedlings. *ABI3* and *FUS3* are activated to higher levels than *LEC2*. Expression of these genes was (almost) not detectable in uninduced LEC1::GR seedlings (F). Bars represent 1mm.

In order to prove embryonic identity of LEC1-induced structures, the expression of three factors of the AFL/B3 network (*ABI3*, *FUS3*, *LEC2*) was analyzed in B-type seedlings by qRT-PCR. These TF genes are naturally seed-expressed factors and were found to be highly expressed in LEC1-induced embryonic structures in comparison to uninduced LEC1::GR seedlings (Figure 18F). *LEC2* expression was much lower than expression of *ABI3* and *FUS3*. This corresponds to the natural situation in embryos where *LEC2* transcript levels are also much lower (ATGeneExpress, http://csbdb.mpimp-golm.mpg.de).

Apical, basal and central domains of the LEC1-induced seedling were analyzed in detail. Cotyledon-like leaves that were observed in both phenotypic categories had less or no trichomes compared to control seedlings (Figure 19A and B). In addition, the leaf nervature of secondary leaves showed a very simple pattern therefore morphologically rather resembling tissue of embryonic origin (cotyledons) than vegetative tissue (Figure 19C-F).

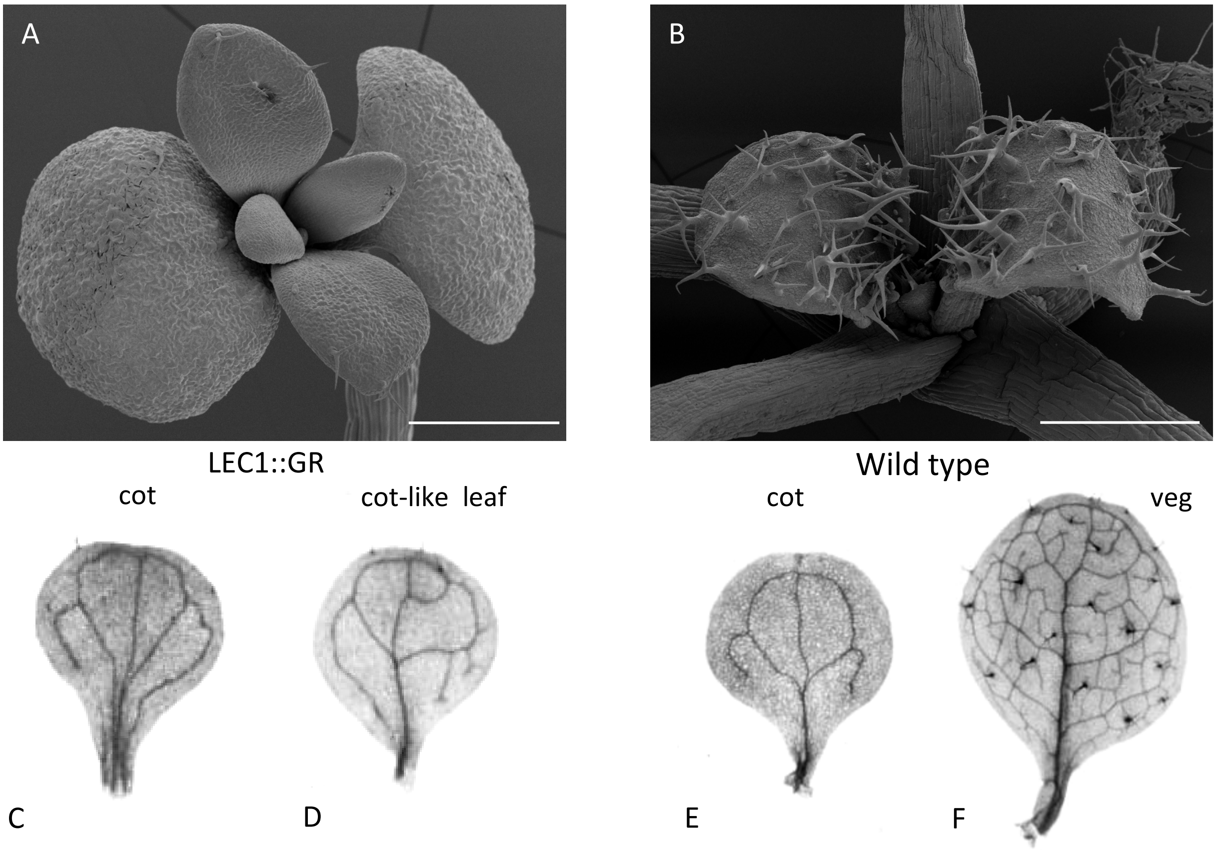


Figure 19. Development of secondary cotyledons after permanent DEX- induction of LEC1.

After two weeks of induction LEC1::GR seedlings did not possess any vegetative leaf but had developed cotyledon-like leaves (cot-like leaf) instead (A). These embryonic leaves have no or only few trichomes as shown by transmission electron microscopy (TEM) and show a very simple venation pattern similar to that of cotyledons (C; D). WT seedlings developed normal vegetative leaves with numerous trichomes (B). Vegetative leaves (veg) of the WT seedling display a complex venation pattern in comparison to cotyledons (E, F). Bars represent 700µm.

Ten days after imbibition the primary root tip of induced LEC1::GR seedlings (phenotype B) started swelling and turned greenish. This phenomenon closely resembled the previously described *pkl* mutant (Ogas *et al.* 1997) and is therefore referred to as *pickle* roots (Figure 18D and E, Figure 20A). When cut off and transferred to B5 regeneration medium, these LEC1-induced *pkl* root tips exhibit, after some callus formation, the potential to regenerate into new plants.

In comparison to wild type root tips with 150-200µm in diameter, *pkl* root tips dramatically increased in size of about 600 µm in diameter (Figure 20A). To distinguish whether the increased size is caused by cell division or cell expansion, cells in epidermis, cortex and endodermis have been counted in wild type and four independently induced seedlings. In wild type root tips cortex and endodermis constantly consist of 8 cells (Scheres *et al.* 2002) and the number of epidermal cells varies between 21 and 35 (Lin and Schiefelbein 2001). As shown in Figure 20B, there is no difference in cell number between wild type and LEC1-induced *pkl* roots in the cortex and the endodermis, and the number of epidermal cells is slightly increased in *pkl* roots. Together, this indicates that the increased size of *pkl* roots is mainly due to cell expansion.

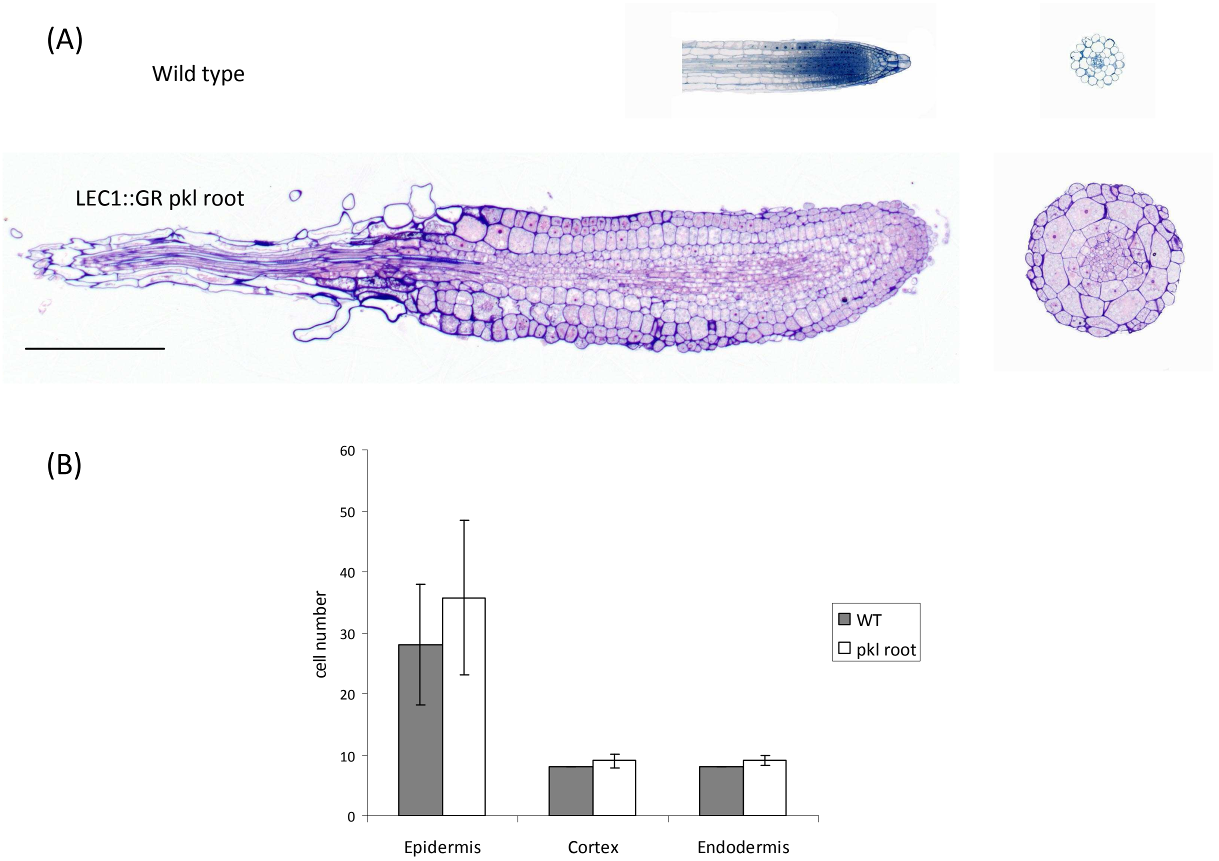


Figure 20. Longitudinal and transverse sections of *pkl* roots in comparison to a WT root tip.
After ten days of permanent induction the primary root tip of induced LEC1::GR seedlings becomes swollen and greenish, referred to as *pkl* roots. (A) *Pkl* roots (lower panel) are about three or four times more voluminous than WT root tips (upper panel). Bar represents 500µm. (B) The cell number of epidermis, cortex and endodermis in 4 independent *pkl* root transverse sections was counted. With regard to cortex and endodermis cell number, *pkl* roots are identical to wild type root tips with around 8 cells per layer. The number of epidermal cells ranges from 21 to 35 cells in wildtype root tips (Lin and Schiefelbein 2001), whereas in *pkl* roots it was observed to be slightly higher, although with similar fluctuations.

The seedlings never continued growing apically but they formed callus-like tissue at the transition between primary root and hypocotyl which later on was the initial point for somatic embryo formation (Figure 21A-C). Heart stage (Figure 21A) and torpedo stage embryos (Figure 21B) could clearly be distinguished. After 'germination' green plantlets regenerated (Figure 21C and D).

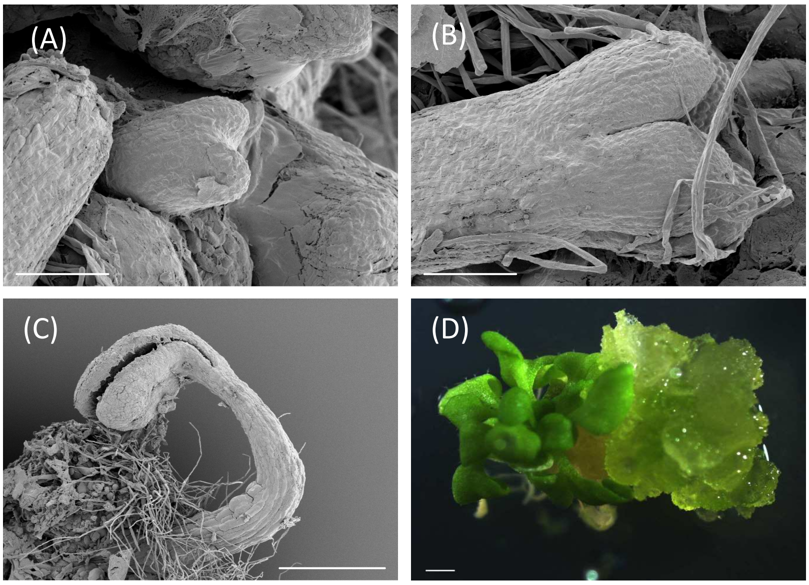


Figure 21. Callus formation, somatic embryogenesis and plantlet regeneration after permanent LEC1 induction.
After ten days of permanent induction LEC1 seedlings started to develop a callus at the junction between root and hypocotyl (collet). This callus was starting point for somatic embryo formation. (A) Heart stage embryo. (B) Cotyledon stage embryo. These embryos showed the potential for regeneration of new plants. Bars = 70µm (C) Early seedling. Bar = 600µm (D) Regenerated plant. Bar = 1mm.

Permanent induction of LEC1 led to impaired vegetative development and morphological alterations in all green parts of the seedling, with cotyledons, leaves and hypocotyl resembling embryonic tissues. LEC1-induced embryonic structures at the primary root tip with showed the potential for de- and re-differentiation into vegetative tissue via somatic embryogenesis.

Taken together, two independent transgenic systems for the either estradiol- or dexamethasone-regulated expression of the transcription factor LEC1 have been established. The functionality of both systems was proven based on the occurrence of similar phenotypic changes. Further experiments were performed with the LEC1::GR line 15-4.

3.3.2 Ultrastructural and immuno-histochemical observations

LEC1 induced phenotypes were analyzed macroscopically within previous sections. The present section presents results of ultrastructural and immunohistochemical analyses of LEC1-induced embryonic structures.

In wild type, storage parenchyma cells of non-hydrated seeds are densely filled with lipid droplets, visible as translucent structures (Figure 22C), surrounding large protein bodies (Figure 22A and C) containing the seed storage protein cruciferin as shown by immuno-histochemical staining (Figure 22B). The dense packing of the lipid droplets gives the endomembrane system its characteristic reticulate pattern (Figure 22C). In Arabidopsis lipids and proteins are major storage compounds and no starch granules can be found. Upon germination the storage components stored in cotyledons and hypocotyl are primary energy source to the heterotrophic seedling. Reserves will be exhausted and cotyledons may turn green and start photosynthesis until the first vegetative leaves can produce energy for plant growth.

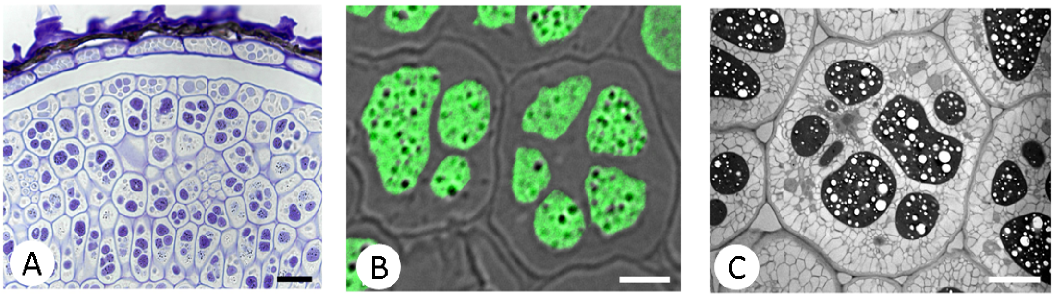


Figure 22. Microscopical analysis of an Arabidopsis wild type seed.
Parenchyme cells of wild type seeds contain large rectangular cells with protein bodies (A) containing seed storage proteins like cruciferin as shown by immuno-histochemical analysis (B). Lipid droplets surround protein bodies and appear translucent as revealed by TEM (C). Bar = 20µm in A, 5µm in B, C.

Histological analysis of 14 day old, permanently induced LEC1::GR seedlings revealed features of storage organs in LEC1-induced structures. Figure 23 shows that the cells of the cotelydons, hypocotyl and *pkl* roots were largely filled with storage components. Also at the ultrastructural level they exhibited remarkable similarity to the pre-germination condition (compare figures 22A-C and 23).

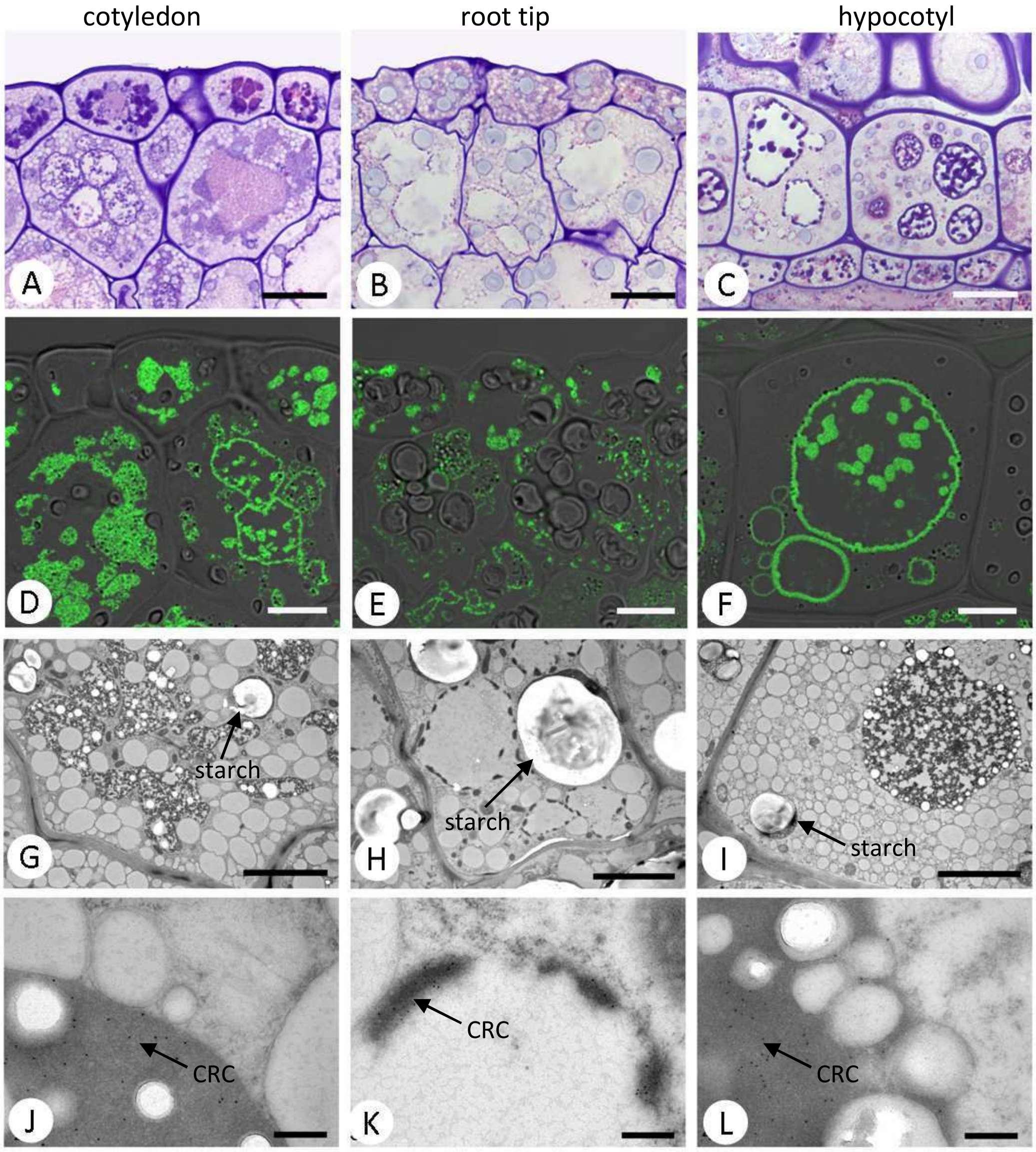


Figure 23. Ultrastructural and immuno-histochemical analysis of permanently induced LEC1::GR seedlings.
Cotyledon-like leaves, swollen root tips and hypocotyls of permanently induced LEC1::GR seedlings were analyzed light microscopy after crystal violet staining (A-C), fluorescence microscopy after immuno-histochemical staining for cruciferin (D-F), transmission electron microscopy (TEM, G-I) and TEM after immuno-gold-labeling detecting cruciferin (J-L). Cells of all tissues of permanently induced LEC1::GR seedlings contained vacuoles with marginal cruciferin accumulations (CRC, dark structures, D-F, J-L) and starch grains (round, light structures, G-I). Bars represent 5µm in A-I and 0,5µm in J-L.

The thickened organs of LEC1::GR seedlings after permanent induction, for example cotyledons and cotyledon-like leaves (Figure 23A, D, G, J), root tip (Figure 23B, E, H, K) and stem (Figure 23C, F, I, L) beared features of storage compartments. The cells making up these tissues are subrectangular to spherical in shape and cruciferin-rich protein accumulations in their vacuoles (Figures 22 and 23A-F). Interesting was the presence of starch granules which were absent from dormant seed cotyledons (compare Figure 22C and 23G). Arabidopsis mainly stores proteins and lipids. Therefore the presence of considerable amounts of starch in LEC1 induced embryos was unexpected and indicated an influx or re-shifting of storage components in the transgenic cotyledons. Electron microscopy studies confirmed the dense packing of storage components and underlined the similarity to the storage parenchyme of the cotyledon (compare Figures 22C and 23G-L).

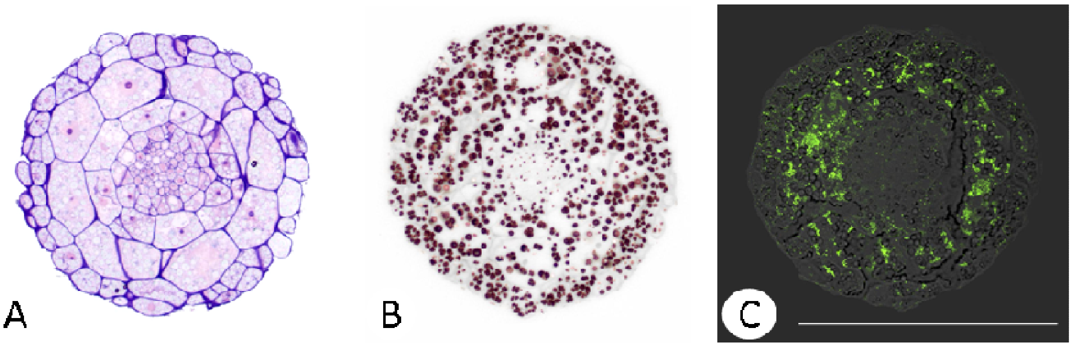


Figure 24. Ultrastructural and immuno- histochemical analysis of transverse sections of *pkl* root tips.
Swollen root tips after LEC1 induction were analyzed by light microscopy after crystal violet and iodine staining (A, B) and fluorescence microscopy after immuno- histochemical staining for cruciferin (C). Bar represents 500µm.

Transverse sections of swollen root tips of induced LEC1::GR seedlings were further analyzed in more detail (Figure 24). The extensive deposition of starch in embryonic pkl roots was proven by iodine staining (Figure 24B). Cruciferin accumulations were detected with different intensity throughout all cell layers of pkl root tips. Epidermal and stele cells contained less cruciferin than endodermis and cortex cells (Figure 24C).

Taken together, embryonic identity of LEC1-induced structures was further supported by ultrastructural and immuno-histochemical analysis. The data reveal a high level of similarity between LEC1-induced embryonic structures and wild type embryos.

3.3.3 Inducibility of embryonic/*pkl*-root phenotypes

The previous section described phenotypic changes observed after permanent induction of LEC1 function. The following section addresses the question whether the *pkl* root phenotype is also inducible at later stages of development.

First, the *pkl* root inducibility was analyzed in seedlings during or shortly after germination whereas germination is defined as the time between seed imbibition and emergence of the radicle from the seed coat. LEC1::GR seeds were sown on MS medium without supplements and transferred to induction medium containing DEX (f.c. 10µM) after different time points between 24 and 96 hours (4 days) after seed imbibition (hpi). As a control, LEC1::GR seeds were permanently induced leading to the characteristic distribution of phenotype A and B of 40% and 60%, respectively (see also Figure 18). Three independent replicates were performed with 50 seedlings transferred at every time point. Phenotypic characterization was done 14 days after seed imbibition. Figure 19 displays the percentage of the different phenotypes after induction at 0/24/28/32/48/72/96 hours after seed imbibition. The data demonstrate a rather narrow time window of about 48 hours of sensitivity for LEC1-induction of the *pkl* root phenotype (Figure 25A, white bars). Interestingly, a third phenotypic category was observed, referred to as phenotype C (Figure 25A, blue bars). These seedlings had prolonged hypocotyls and even formed apical hooks, therefore resembling etiolated seedlings (Figure 25B). Leaves mostly appeared wild type-like and *pkl* root tips occurred occasionally. This phenotype occurred to very low percentages when seedlings were induced between 24 and 48hpi. Nevertheless, when induced after 72hpi, almost no type-A- and B-seedlings were observed and the type-C-phenotype was the only abnormal phenotype. The induction after 96 hpi and later did not lead to phenotypic alterations and 100% of the induced LEC1::GR seedlings appeared WT-like (Figure 25A).

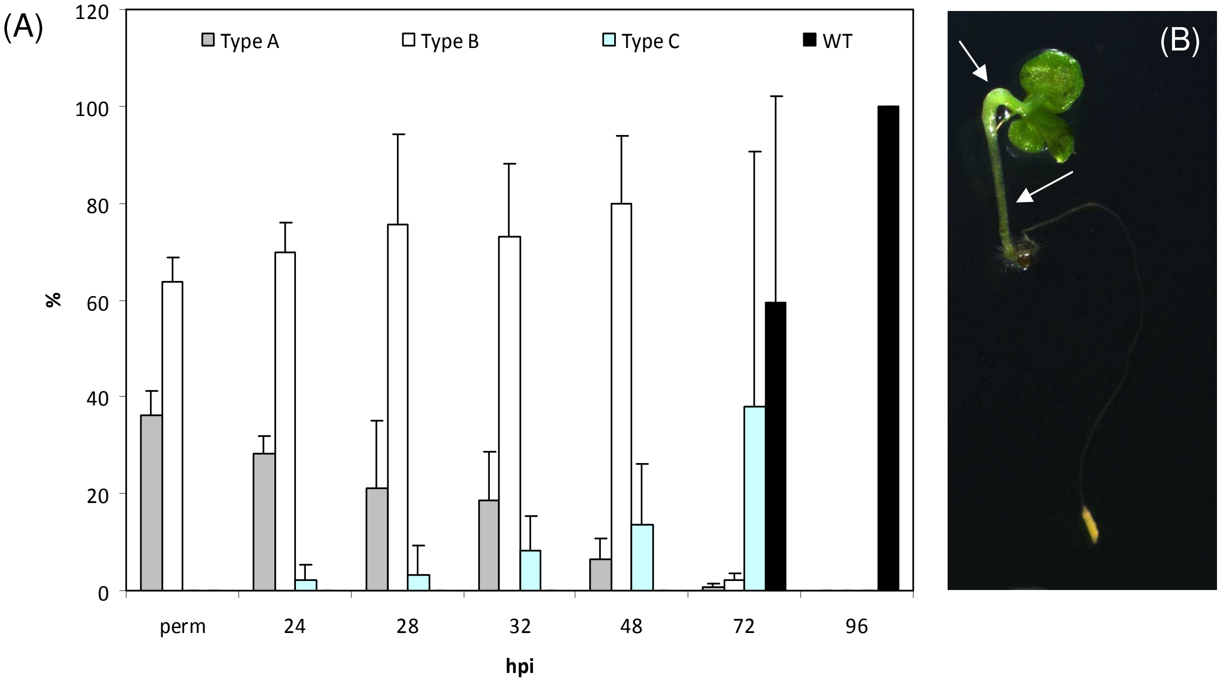


Figure 25. Inducibility of the pkl root phenotype during and shortly after germination.
LEC1::GR seedlings were sown on MS and transferred to induction medium at different timepoints after seed imbibition. Permanently induced seedlings serve as a control (perm). Two weeks after seed imbibition phenotypic evaluation was carried out. The *pkl* root phenotype is only inducible by LEC1 when induced up to 48 hpi. The novel phenotype C occurred mostly after 72hpi and was characterized by long hypocotyls and apical hook formation (B). About 100% of the seedlings induced at 96 hpi showed WT-like phenotypes.

Second, the occurrence of the *pkl* root phenotype was analyzed with respect to the length of the induction time. LEC1::GR seeds were sown on MS without supplements and transferred to induction medium (10µM DEX) after 24 hpi. After a period of 4 to 72 hours of induction the seedlings were re-transferred to MS without DEX and phenotypes were analyzed after 14 days post imbibition. Figure 26 shows the percentage of phenotype A-, B-, C- and the WT-like phenotypes after different induction duration. When induced for 4 to 12 hours, the majority of LEC1::GR seedlings displayed phenotype C and WT-like phenotypes. The longer the induction duration the less WT-like seedlings were observed. After 24 hours of induction approximately equal numbers of seedlings with embryonic structures (type A and B) and type-C-LEC1::GR-seedlings were counted. Only more than 48 hours of induction lead to predominant occurrence of the *pkl* root phenotype. As a result, LEC1 induction of the *pkl* root phenotype is not a hit- and- run mechanism but rather requires LEC1 action over at least 48 hours. In contrast, *LEC1* expression about 4 hours was sufficient for triggering phenotype-C-development.

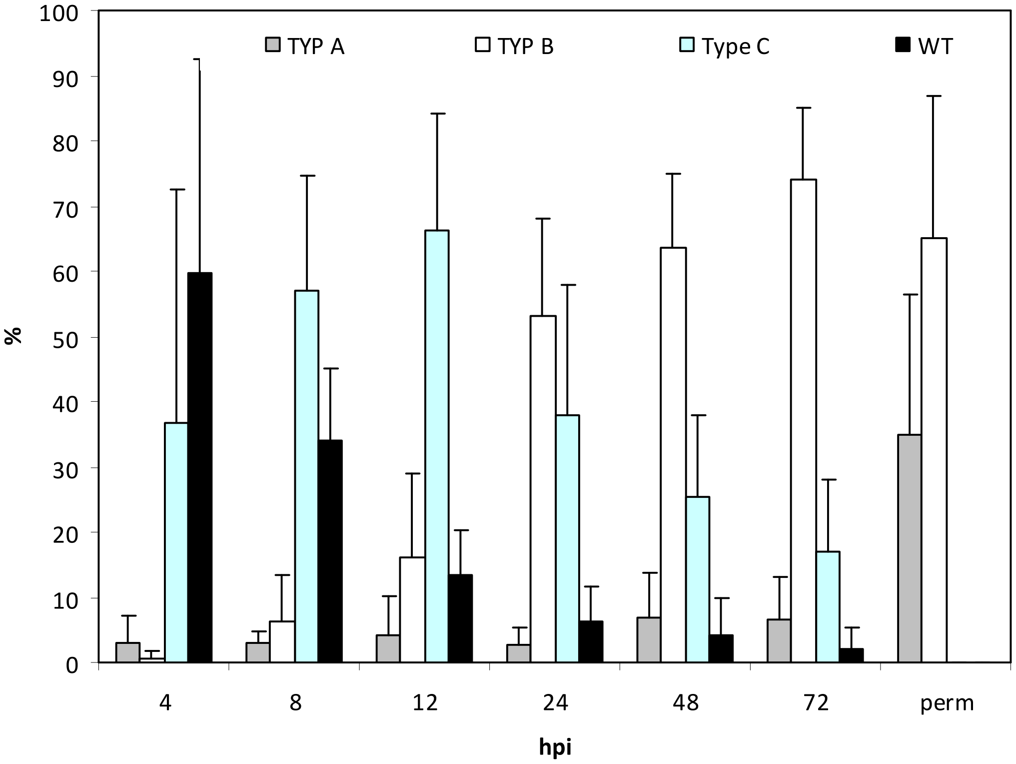


Figure 26. Influence of induction duration on development of the pkl root phenotype.
LEC1::GR seedlings were sown on MS without DEX, transferred to DEX-containing medium after 24hpi and then re-transferred to non-inductive medium after variable length of induction. Short induction times were sufficient for type-C-development in a considerable number (4h) or the majority (8 and 12h) of induced seedlings. Less than 24 hours of induction of LEC1 did not trigger the formation of the *pkl* root phenotype. About 24 hours of induction led to equal numbers of *pkl* root and type-C-phenotype. The number of phenotype B seedlings exceeded that of WT-like or type-C seedlings only when induced more than 48 hours.

Third, the development of the *pkl* root phenotype was analyzed in fully developed, vegetative Arabidopsis seedlings. For this reason, LEC1::GR and WT seedlings were grown for 10 days on non-inductive MS medium and then transferred to DEX-containing medium (f.c.10µM). As shown before, the *pkl* root phenotype was only inducible by DEX in LEC1::GR seedlings during and shortly after germination (until 48hpi, Figure 25A). In accordance with this, no *pkl* root formation was observed after DEX treatment of vegetative LEC1::GR seedlings (Figure 28Z).

Several lines of evidence support a role of the phytohormone abscisic acid (ABA) to establish and maintain a 'seed milieu' on the molecular level which allows the accumulation of seed storage compounds, desiccation and dormancy (McCourt *et al.* 2005). Moreover the activation of maturation-related genes has been shown to be ABA-dependent (Kagaya *et al.* 2005a, Kagaya *et al.* 2005b, Suzuki *et al.* 2001). As putative candidate target genes of LEC1, the expression of *FUS3* and *ABI3* was analyzed after induction of LEC1+ABA-treatment. Vegetative LEC1::GR seedlings were treated with DEX+ABA (and single hormones as control) and the transcript levels of *FUS3* and *ABI3* were measured after 6, 12, 24, 48, 72, 96 hours and 7d of induction.

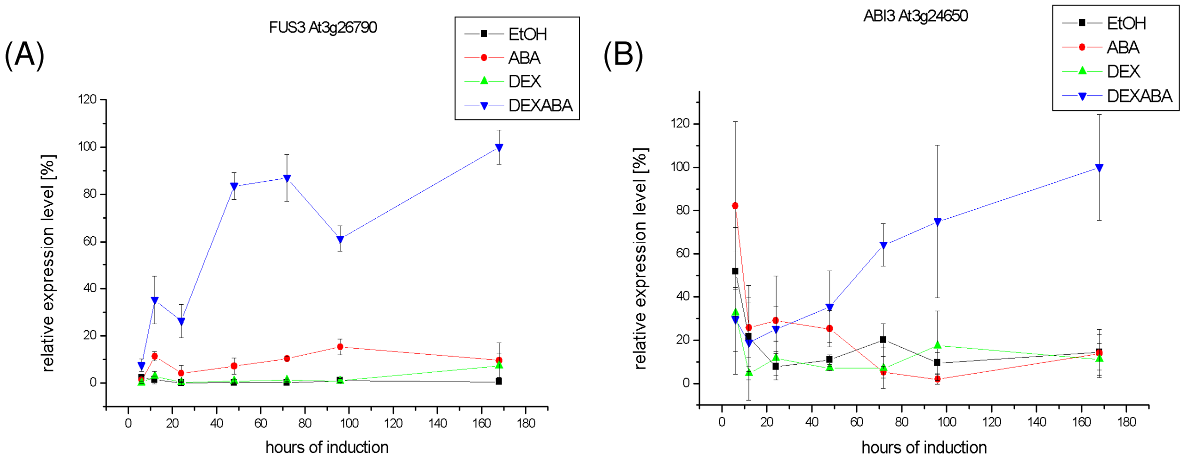


Figure 27. ABA-dependent activation of *FUS3* and *ABI3* by LEC1.
Uninduced LEC1::GR seedlings were treated with different hormone combinations (ABA, DEX, DEX+ABA) for 6, 12, 24, 48, 72, 96 hours and 7d. Ethanol treatment served as control. Induction was performed in liquid medium and under gentle shaking. The expression of *FUS3* (A) and *ABI3* (B) was analyzed at indicated timepoints by qRT-PCR. The expression relative to the housekeeping gene *UBQ10* is given here in percent of the highest value measured among comparable samples. *FUS3* expression could be rapidly activated by DEX+ABA-treatment (A) whereas activation of *ABI3* expression by DEX+ABA was much slower (B).

FUS3 expression was found to be rapidly up-regulated by LEC1+ABA whereas no induction was observed after treatment with ABA or DEX alone and ethanol (Figure 27A). In contrast to *FUS3*, the LEC1+ABA-mediated activation *ABI3* expression followed much slower induction kinetics (Figure 27B). Only after 72 hours of induction the expression of *ABI3* was considerably higher in DEX+ABA-treated seedlings compared to control seedlings (Figure 27B). These data are contradictory to the previously reported ABA-independent activation of *FUS3* and *ABI3* by LEC1 (Kagaya *et al.* 2005b) but indicate FUS3 as a putative direct target of LEC1 regulation. Activation of *ABI3* seems to be mediated by further factors. Moreover, these results indicate ABA as the missing factor to induce embryogenesis during vegetative development.

Therefore phenotypic alterations were analyzed in LEC1::GR seedlings after induction with ABA alone and a combination of DEX+ABA. Seedlings were grown and induced as described above and ABA was added to a final concentration of 10µM. After ten days of induction with DEX and ABA all LEC1::GR seedlings had developed *pkl* roots at primary and secondary root tips (Figure 28AB). These root structures were identical to swollen root tips observed after permanent induction and neither occurred in wild type seedlings after DEX+ABA-treatment nor in LEC1::GR seedlings after induction with ABA or DEX alone (Figure 28Y-AB). In addition to the development of embryonic structures at root tips after DEX+ABA-treatment, different morphological alterations have been observed at the apical meristem of wild type and LEC1::GR seedlings after different treatments. Wild type and LEC1::GR seedlings treated with ethanol, as a dissolvent for DEX and ABA, develop vegetative leaves with numerous trichomes (Figure 20A and E) and floral meristems already started to emerge (Figure 28I; M; Q) as revealed by transmission electron microscopy (TEM). The same is true for DEX-treated wild type seedlings (Figure 28B and J). Delayed growth of DEX- treated LEC1::GR seedlings is revealed by the absence of floral meristems (Figure 28N and R). Moreover, these seedlings show severely mis-shaped leaves that possess less trichomes (Figure 28F). The absence of the seed maturation marker cruciferin in these leaves excludes a embryonic identity (Figure 28V). ABA effects on seedling morphology were observed independent of the genetic background. Growth of ABA- treated wild type and LEC1::GR seedlings and DEX+ABA treated wild type seedlings was inhibited in similar ways such as in DEX-treated LEC1::GR seedlings (Figure 28C, D, G, K, L, O and S). After ABA-treatment leaves still developed several trichomes and also maintained the normal round leaf shape (Figure 28C, D and G).

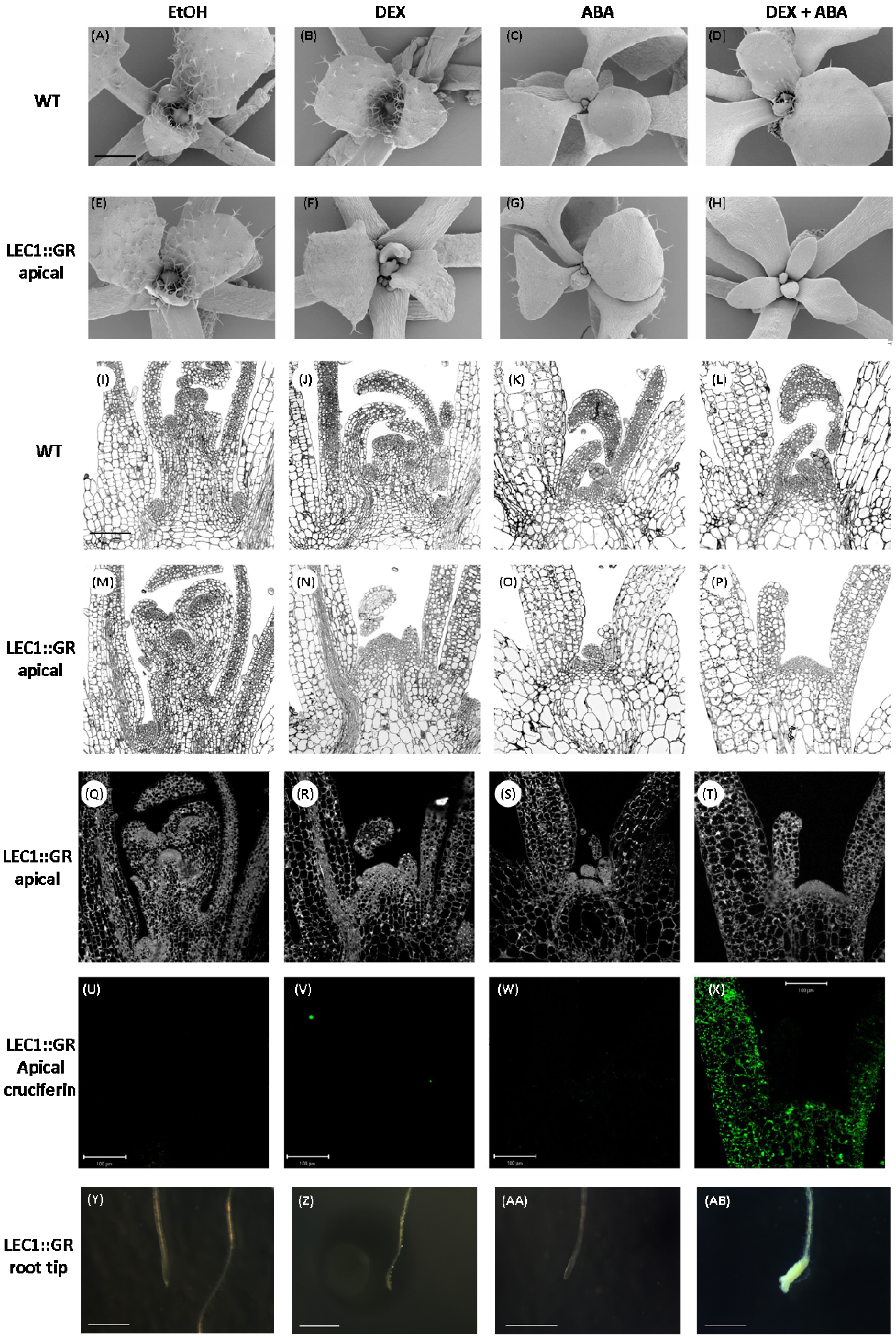


Figure 28. Morphological and immuno- histochemical analysis of transiently induced LEC1::GR seedlings under different induction regimes.

Ten day old, uninduced LEC1::GR and WT seedlings were transferred to MS supplemented with different hormone combinations (DEX, ABA, DEX+ABA). Alcohol treatment served as a control treatment. After one week of induction seedlings were fixed and embedded for histological analyses including TEM (A-H), light microscopy (I-T) and fluorescence microscopy after immuno-histochemical staining for cruciferin (U-X). After alcohol treatment no differences were observed between WT and LEC1::GR seedlings (A-Y).

Continued from page -64-

DEX treatment did not lead to phenotypic alterations in WT seedlings (B, J), although mis-shaped leaves developed in LEC1::GR seedlings (F, N, R) that did not accumulate cruciferin (V). No *pkl* roots were observed after induction with DEX alone (Z). ABA treatment affected WT and LEC1::GR seedlings in a similar way. Leaves of both genotypes possessed less trichomes (C, D, G) but did not accumulate cruciferin (W). Root tips remained unchanged (AA). Embryonic structures at SAM (cotyledon-like leaves: H, P, T, X) and RAM (*pkl* roots: AB) only developed after DEX+ABA treatment. As a proof of embryonic identity, cruciferin accumulation was detected in cotyledon-like leaves (X). Bars represent 700µm in A-H, see A; 100µm in I-L, see I; 100µm in M-X, see U and 1mm in Y-AB.

Similar to *pkl* root development at the RAM, LEC1::GR seedlings developed embryonic structures at the apical meristem (SAM) only after treatment with DEX and ABA. Several small, but perfectly round leaves developed from the apical meristem that did not possess any trichom (Figure 28H). As another striking cotyledonary feature, the presence of large amounts of cruciferin was detected in these cotyledon-like leaves after LEC1 induction and ABA treatment (Figure 28X). This was never found in LEC1::GR seedlings after treatment with DEX or ABA alone.

A detailed view on cruciferin accumulation in cotyledon-like leaves is given in Figure 29. The amount of cruciferin correlated well with leaf age. The youngest leaf (L1) did not show any cruciferin signal, the second youngest leaf (L2) started to accumulate cruciferin in some cells and the oldest leaf (L3) gave a strong cruciferin signal in all cells. The apical meristem was visible as light structure, consisting of numerous small cells and cruciferin accumulation started in cells below the stem cell area.

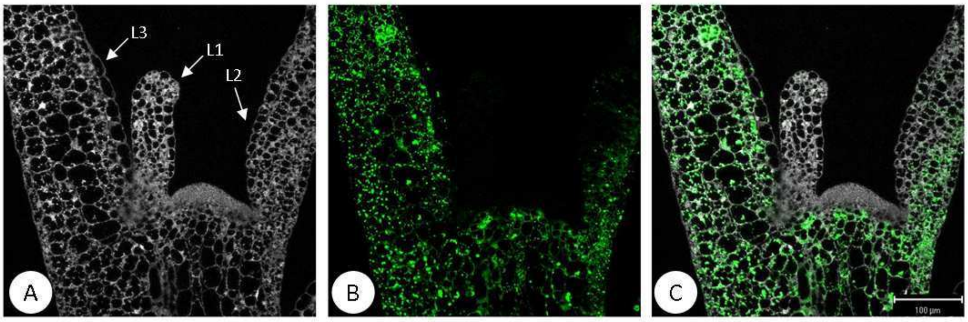


Figure 29. Cruciferin accumulation below the apical meristem and in cotyledon-like leaves after transient induction of LEC1 seedlings with DEX+ABA.
An overlay picture of the contrast-rich brightfield image (A) and the fluorescence image (B) of the SAM region of transiently induced LEC1::GR seedlings indicated that cruciferin accumulation in LEC1+ABA-induced cotyledon-like leaves depended on leaf age and was not detected in meristematic regions (SAM). This region was marked by the aggregation of numerous small cells (C).

Taken together, these results revealed LEC1 as a trigger of the formation of embryonic structures emerging from shoot and root apical meristems. After completion of germination (>48 hpi) the induction of embryonic development by LEC1 strictly depended on ABA. LEC1+ABA-induced structures resembled storage organs with respect to morphology and ultrastructure and contained seed storage compounds such as cruciferin and starch. The embryonic *pkl* root tips have been suggested to be the result of LEC1 de-repression in the *pkl* mutant (Ogas *et al.* 1997). Results of the present study demonstrated that embryo formation in the *pkl* mutant is directly triggered by *LEC1* overexpression. Furthermore, LEC1-mediated formation of embryonic structures was extended from the RAM (*pkl* roots) to the SAM where LEC1+ABA-induced the formation of cotyledon-like leaves bearing embryonic features. Obviously, LEC1+ABA-induced embryonic tissue always originated from meristematic areas at root and shoot whereas already differentiated plant tissues or cells are not affected. Stem cell populations seemed to be maintained (at least for some time) after LEC1-induction and *de novo* developed organs and tissues were differentiated into embryonic structures.

3.3.4 Analysis of embryogenesis and stem cell markers during LEC1-induced development

In order to gain more insight into cellular and molecular processes of LEC1-induced development of embryonic structures, several marker lines were crossed with the homozygous LEC1::GR 15/4 line. Table 3 comprises all marker lines that were used for crossing, corresponding marker genes and their natural expression patterns. Marker lines express promoter – reporter-gene – constructs whereas different reporter genes were used for visualization of promoter activity (e.g. green fluorescent protein, GFP; β - glucuronidase, GUS; cyan fluorescent protein, CFP). Before crossing with LEC1::GR, the marker lines were analyzed for reporter-gene expression during seed development using fluorescence microscopy or GUS staining. Promoter activity was detected as described in corresponding publications (Table 3). Missing pictures indicate work in progress. In the following section, used marker genes are indicated in bold.

For comparative analysis of LEC1-induced embryogenesis and zygotic embryogenesis, marker lines were selected the promoters of which are active during early embryogenesis and pattern formation as well as late embryogenesis and storage metabolism. In the early two-celled embryo the asymmetric expression of *PIN7*, encoding an auxin efflux facilitator, leads to the establishment of an apical auxin maximum which largely influences the development of the apical/basal embryo axis (Friml *et al.* 2003). At the globular stage, the auxin maximum shifts to hypophysis and suspensor as achieved by switches of PIN protein localization (Friml *et al.* 2002). Auxin accumulation can be visualized using the synthetic auxin inducible promoter **DR5** (Ulmasov *et al.* 1997). The indole acetic acid (IAA) protein BODENLOS (BDL) is degraded upon auxin signaling, thus releasing its interaction partner, the auxin response factor 5 (ARF5) or **MONOPTEROS** (**MP**) which is then able to activate its target genes (Hamann *et al.* 2002). BDL-/ MP-mediated auxin signaling is required for normal root and vascular development in the embryo (Hamann *et al.* 2002, Hardtke and Berleth 1998). In addition, the activation of the AP2 domain containing ***PLETHORA1*** and ***2*** genes by BDL-/ MP-mediated auxin signaling is a prerequisite for root stem cell development (Aida *et al.* 2006, Blilou *et al.* 2005). After asymmetric division of the hypophysis in the globular embryo the upper daughter cell expresses the auxin- inducible QC- marker gene ***WUSCHEL-HOMEOBOX 5*** (***WOX5***), revealing that the identity of the root stem cell niche is already established at this stage. The *PLETHORA* gene products together with WOX5 are responsible for root stem cell maintenance also during vegetative development. Similar to the RAM, also the shoot apical meristem is established in early globular embryos as marked by the expression of ***WUSCHEL*** (***WUS***) in four subapical cells which will develop into the organizing centre (OC), as the apical stem cell niche (Laux *et al.* 2004). In later embryo stages also the expression of ***CLAVATA 3*** (***CLV3***) can be detected in a few cells between the arising cotyledons (Fletcher *et al.* 1999). The interaction of WUS and CLAVATA 3 (CLV3) establishes a positive feedback loop required for stem cell maintenance at the shoot meristem (Brand *et al.* 2000, Schoof *et al.* 2000).

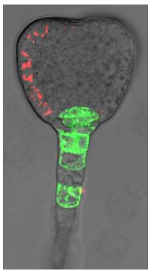
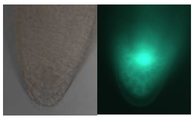
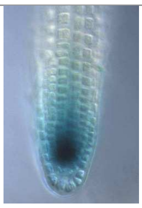
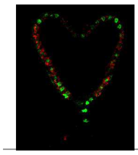
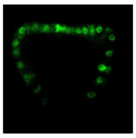
The homeodomain-GLABRA2 (HD-GL2) transcription factor **MERISTEM LAYER1** (**ML1**) represents an early marker for the development of apical/basal and radial axis of the embryo is. The expression of the corresponding gene is confined to the protodermal cell layer and the suspensor of early embryos. During later embryogenesis and vegetative development it marks the L1 layer of the shoot apical meristem where it is required for epidermis specification (Abe *et al.* 2003, Lu *et al.* 1996, Takada and Jürgens 2007). In contrast to the full *AtML1* promoter, a shorter version (**ΔNP**) restricts reporter gene expression to the apical cell lineage (Takada and Jürgens 2007).

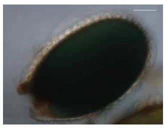
The promoter of the *Vicia faba* ***UNKNOWN SEED PROTEIN*** (***USP***) gene has been shown to be only active during seed development. In *Arabidopsis* the activity is restricted to embryos from globular stage until mature embryos (Bäumlein *et al.* 1991a).

As a marker for seed maturation and storage metabolism, the activity of the *Vicia faba* **LEGUMIN** promoter is mainly restricted to seeds (embryo and endosperm) containing embryos that completed the morphogenesis phase (Bäumlein *et al.* 1991b).

Table 3. Embryo and stem cell marker lines used for crossing with LEC1::GR.

Several embryo and stem cell marker lines were collected from different labs. The name of the marker line is associated with ATG numbers of the corresponding marker genes the promoters of which have been cloned. The wild type expression of the reporter genes in different marker lines was analyzed prior to crossing and in most cases matched with published data. Missing pictures indicate work in progress.

marker line	gene name ATG number	Expression in WT		reference
DR5::GFP	synthetic promoter		marks auxin maxima early embryo: apical suspensor cell and hypophysis post- embryo: root tip (QC) and sites where organ primordial will emerge	(Ulmasov *et al.* 1997)
pMP::SV40-3xGFP	MONOPTEROS; ARF5; AT1G19850		vascular tissue in embryo and later organ primordia	(Hardtke and Berleth 1998)
pPLT1::eCFP	PLETHORA1; AT3G20840		QC of the root meristem	(Aida *et al.* 2004)
pPLT2::eCFP	PLETHORA2; AT1G51190			
pWOX5::GFP *AKS 50*	WUSCHEL-RELATED HOMEOBOX5; At3G11260		embryo: hypophysis post- embryo: QC of the root meristem	(Haecker *et al.* 2004)
pWOX5::GUS *AKS 34*				
pWUS::GUS *IB39-6*	WUSCHEL; At2G17950		OC of the shoot apical meristem (embryo and post- embryo)	(Mayer *et al.* 1998)
pCLV3::GFP *ML 362.2*	CLAVATA3; AT2G27250		outer layers of the shoot apical meristem	(Fletcher *et al.* 1999)
pCLV3::GUS *MT 194.3*				
pATML1::NLS::GFP	MERISTEM LAYER 1; AT4G21750		embryo: epidermis and suspensor	(Abe *et al.* 2003b, Lu *et al.* 1996)
p ΔNP::NLS::GFP			embryo: epidermis	(Takada and Jürgens 2007)

USP::GUS *472 30p::GUS*	*Vicia faba* UNKNOWN SEED PROTEIN		whole embryo globular - mature	(Baumlein *et al.* 1991a)
LEG::GUS *472 leg:: GUS*	*Vicia faba* LEGUMIN		whole embryo cotyledon – mature	(Baumlein *et al.* 1991b)

All marker lines (Table 3) have been crossed into LEC1::GR and analysis of marker expression in the LEC1::GR background is in progress.

The AtML1 promoters (full and Δ NP) were not found to be activated in the process of LEC1-induced embryogenesis. In the following, results will be presented that were achieved with the DR5::GFP x LEC1::GR, USP::GUS x LEC1::GR and leg::GUS x LEC1::GR lines.

LEC1::GR x DR5::GFP (lines 14, 16, 20) and wild type seeds were permanently induced on MS medium containing 10µM DEX. Seedlings treated only with ethanol served as controls. After 4, 7 and 19 days of induction seedlings were analyzed by confocal laser scanning microscopy (CLSM) to detect the GFP fluorescence. All three independent lines showed similar, reproducible results. An auxin maximum at the tip of each cotyledon was detected in a similar way in control and induced seedlings (Figure 30A-D). This signal was constantly detected over the analyzed time period whereas an increase in GFP signal was observed in the whole apical part of the seedlings, except cotyledon-like leaves, after 19 days of induction (Figure 30D). An auxin maximum at the root tip, which marks the root stem cell niche, was detected in root tips of uninduced seedlings (Figure 30E). In root tips, during the process of *pkl* root formation, starting after ~7 days of induction and completing by 19 days of induction, the GFP signal was detected mainly unchanged (Figure 30F-H). This suggests that the RAM stem cell population, as maintained by auxin accumulation, and the expression of fate determinants in response to auxin signaling were unaffected by RAM-surrounding embryonic structures.

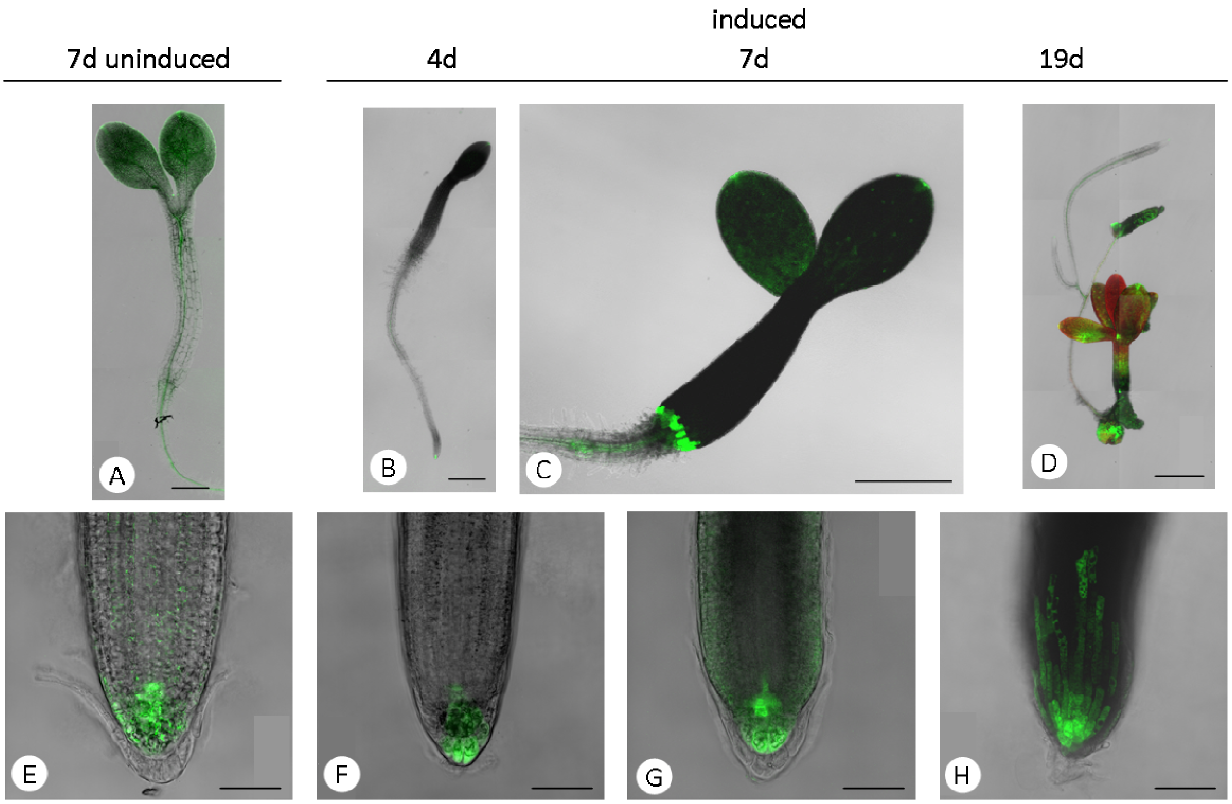


Figure 30. Auxin maxima in embryonic structures of LEC1::GR seedlings after permanent induction. Permanently induced LEC1::GR x DR5::GFP seedlings were analyzed by fluorescence microscopy at 4, 7 and 19 days post imbibition. Control seedlings grew on MS supplemented with ethanol and showed GFP signals in the tips of the cotyledons, vascular tissue (A) and the root tip (E). GFP signals at the cotyledon tips remained unchanged during induction with DEX (A-D). Also during the process of *pkl* root formation (starting at day 7 and completed at day 19) auxin maxima as indicated by GFP signals were constantly detected at the root tips (E-H). Prior to callus formation at root-hypocotyl-junction (collet, D), visible at day 19 post imbibition, an unusual auxin maximum was detected after 7 days of induction in this collet region (C). Bars represent 500µm in A-C, 1mm in D, 50µm in E-G and 100µm in H.

As already described in previous sections, 60% of the permanently induced LEC1::GR seedlings showed phenotype B. One characteristic feature of phenotype B was callus formation at the junction between an embryonic hypocotyl and a normal primary root (Figure 30D). Figure 31 gives a detailed view on the collet region of uninduced compared to induced seedlings prior to callus formation. A vegetative hypocotyl appeared as translucent structure (Figure 31A) whereas the induced, embryonic hypocotyl was untransparent due to storage of large amounts of seed storage compounds (Figure 31 E). The junction between hypocotyl and root was clearly marked by chlorophyll autofluorescence which defines the basal end of the hypocotyl and the apical end of the root in induced and control seedlings (Figure 31B and G). Using the DR5::GFP marker it was possible to detect a high, local auxin accumulation in the lowermost epidermal cell layer of the hypocotyl (Figure 31F and H). This auxin maximum was detected after 7 days of induction, prior to callus formation and was never visible in uninduced LEC1::GR seedlings (Figure 31B and D). With the starting callus formation, the GFP signal in this cell layer decreased and epidermal cells of the whole hypocotyl showed a weak GFP fluorescence (Figure 30D).

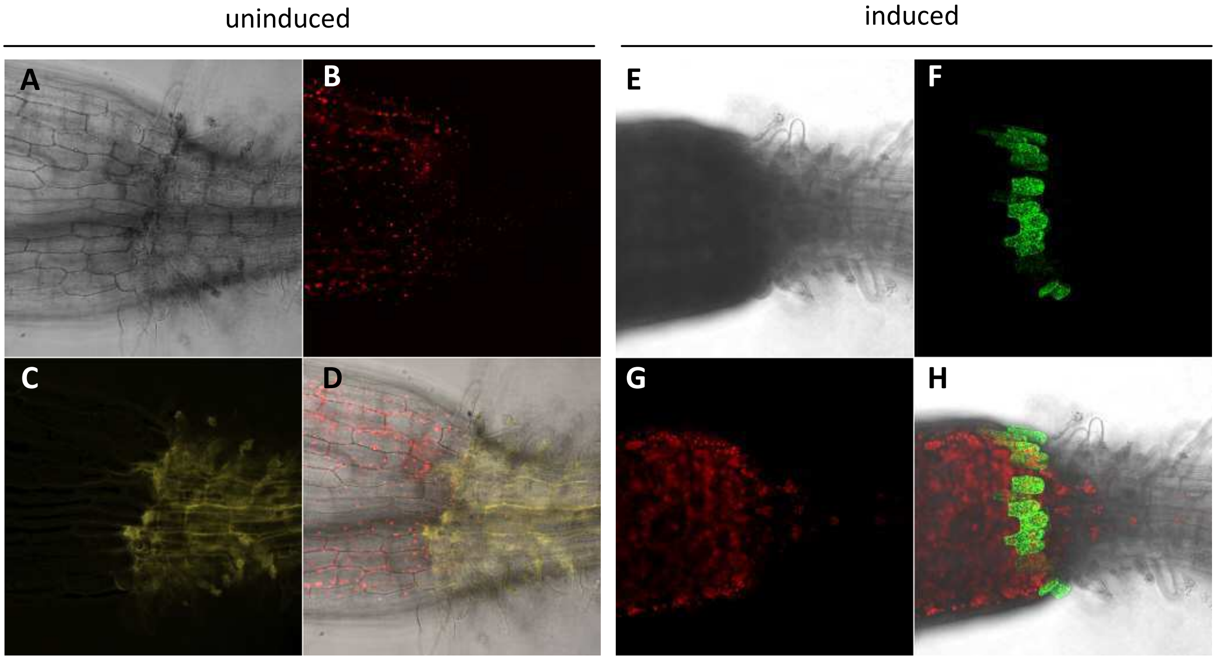


Figure 31. Auxin maximum at the junction between hypocotyl and primary root of permanently induced LEC1::GR seedlings.
The collet region of permanently induced LEC1::GR x DR5::GFP seedlings was analyzed by fluorescence microscopy at day 7 post imbibition. Uninduced seedlings served as controls (A-D). The junction between hypocotyl and root tissues was clearly marked by presence and absence of chlorophyll autofluorescence in control and induced seedlings (A, B, E, G). In control seedlings no GFP signal was detected in the collet region (B, D). A ring of GFP-expressing epidermal cells was observed in induced seedlings at the root-hypocotyl-junction (F, H).

Taken together, the data reveal the maintenance of auxin maxima at cotyledon and root tips during the process of LEC1-induced embryogenesis. Due to auxin-dependent expression of RAM identity genes *PLT1* and *2*, this data suggests that RAM meristem identity is unaffected by surrounding LEC1-induced embryonic differentiation. This has to be further proven by analysis of the expression of RAM stem cell identity markers during LEC1-mediated embryogenesis. Furthermore, a novel auxin-maximum was found in the lowermost epidermal cell layer at the root-hypocotyl-junction after LEC1 induction which later on serves as initial point of callus formation and somatic embryogenesis (SE). This suggests a function of LEC1 as inducer of SE which is mediated by LEC1-activation of auxin signalling and metabolism.

The activity of two seed-specific promoters of *Vicia faba* was investigated during the process of LEC1-induced embryogenesis. The promoter of the *UNKNOWN SEED PROTEIN* (*USP*) gene is active during early and late embryogenesis (globular to mature embryos) whereas the LEGUMIN promoter is only activated during seed maturation (Bäumlein *et al.* 1991a/b). LEC1::GR x USP::GUS and LEC1::GR x leg::GUS seedlings were permanently induced on MS containing 10µM DEX. Ethanol-treated seedlings served as controls.

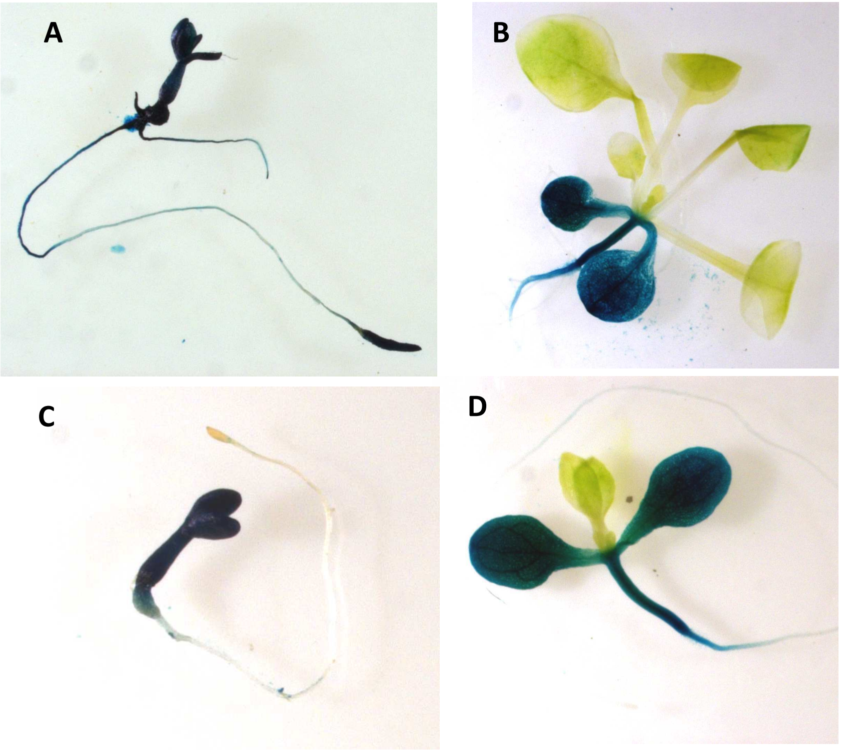


Figure 32. Activity of the *Vicia faba* USP and legumin promoters in LEC1-induced embryonic structures.
LEC1::GR x USP::GUS (A,B) and LEC1::GR x leg::GUS (C,D) seedlings were permanently induced on MS supplemented with 10µM DEX. Ethanol treatment served as control. GUS staining was performed in induced and control seedlings after 14 (LEC1::GR x USP::GUS) or 7 days post imbibition (LEC1::GR x leg::GUS). The USP promoter was active throughout the whole induced LEC1::GR seedling with exception of the root (A). The legumin promoter was active only in apical parts (hypocotyl and leaves) of LEC1::GR induced seedlings (C). Activity of both promoters was also detected in cotyledons and hypocotyl of uninduced control seedlings (B, D).

Induced and uninduced LEC1::GR x USP::GUS and LEC1::GR x leg::GUS seedlings of the phenotype B (see chapter 3.3.1, Figure 18) were used for GUS-staning after 14 and 7 days post imbibition, respectively. In induced LEC1::GR x USP::GUS seedlings, GUS staining was detected throughout the whole seedling, from *pkl* root tip to cotyledon-like leaves (Figure 32A). The signal was weaker in root tissue, supporting its vegetative character. The GUS signal was excluded from vegetative leaves and roots of uninduced LEC1::GR x USP::GUS seedlings (Figure 32B). A positive GUS signal in uninduced hypocotyls and cotyledons indicicated their embryonic origin (Figure 32B). Induced LEC1::GR x leg::GUS seedlings showed GUS staining in the whole apical part of the seedling (hypocotyl and leaves, Figure 32C). The root and the root tip, which just started swelling, were not GUS positive (Figure 32C). This might have been due to the early seedling stage that was analyzed. In addition the legumin promoter represents maturation-specific gene expression which is probably not activated in early stages of *pkl* root development. GUS staining was also observed in hypocotyls and cotyledons of uninduced control seedling (Figure 32D).

Taken together, depending on the stage of LEC1-induced embryogenesis early and late embryogenesis markers were activated. All embryonic parts of LEC1-induced seedlings showed GUS staining, indicating the expression of embryogenesis markers. Weak or missing GUS signals in phenotype B roots proved their apparent vegetative character.

3.4 Identification of TF target genes

The establishment of a transcriptional regulatory network underlying Arabidopsis embryogenesis and seed development was the major goal within the ARABIDOSEED project. Therefore, in frame of the present PhD work, main regulators of seed development, namely components of the AFL/B3-LEC1-network have been ectopically expressed using two different inducible regimes. The complex genetic interactions of ABI3, FUS3, LEC2 and LEC1, which have been studied in detail by forward and reverse genetic approaches, are in the focus of several reviews (Santos-Mendoza *et al.* 2008, Vicente-Carbajosa and Carbonero 2005). Nevertheless only few direct target genes, mostly implicated with seed storage have been identified yet. In the frame of this PhD work the described inducible lines for *ABI3*, *FUS3* and *LEC1* overexpression were used for target gene detection by transcriptome analysis and the analysis of molecular interactions with DNA by chromatin immunoprecipitation (ChIP) coupled to macroarray hybridization. The identification of LEC1 target genes will be in the focus of the following sections. All results were obtained with the LEC1::GR DEX-inducible overexpression line 15-4.

3.4.1 Macroarray analysis

LEC1 seems to be hierarchically superior to AFL/B3 genes and is supposed to mediate its action by triggering the transcription of other regulatory genes such as transcription factors. Therefore a macroarray hybridization approach was performed using the REGIA filter which represents about 1200 Arabidopsis transcription factors (Paz-Ares and the REGIA Consortium 2002). LEC1::GR seedlings were grown on MS medium without supplements for two weeks. The induction was performed in liquid MS with a) 0.1% ethanol as control, b) 10µM ABA, c) 30µM DEX and d) 10µM ABA and 30µM DEX. Samples were taken after 8 hours of induction and subsequently frozen in liquid nitrogen. Messenger RNA was isolated from whole induced seedlings, radioactively labelled with ^{33}P and hybridized to the REGIA filter. Two independent replicates were performed. TF genes were considered as putative LEC1 target gene when they were activated or repressed more than three times in both biological replicates. Obtained candidate target genes were classified into treatment categories. These were defined as follows: ABA-inducible genes were regulated after single ABA treatment and combined DEX+ABA treatment (comparison: EtOH *vs*. ABA and EtOH *vs*. DEX+ABA). DEX-regulated genes were activated or repressed after single DEX treatment and combined DEX+ABA treatment (comparison: EtOH *vs*. DEX and EtOH *vs*. DEX+ABA). The most important genes are DEX+ABA-regulated TF genes the expression of which was only altered after DEX+ABA treatment (comparison: EtOH *vs.* DEX+ABA).

Table 4. TF genes up- or down-regulated by DEX+ABA-treatment in LEC1::GR seedlings.
TF genes were considered as putative LEC1 target when they were induced or repressed more than three times in both biological replicates. Fold change values of two biological replicates are given.

ATG number	annotation		
		induction factor exp 1	induction factor exp 2
At3g54990	SMZ (SCHLAFMUETZE)	4,85	6,96
At3g21270	ATDOF2 (Arabidopsis dof zinc finger protein 2)	7,39	6,11
At1g43160	RAP2.6 (related to AP2.6)	24,26	3,21
		repression factor exp 1	repression factor exp 2
At5g08330	TCP family transcription factor, putative	3,53	3,11
At3g17609	HYH (HY5-HOMOLOG)	11,24	5,52
At1g28370	ATERF11 (ERF domain protein 11)	16,55	6,12
At1g80840	WRKY40 (WRKY DNA-binding protein 40)	21,79	9,19
At3g16870	zinc finger (GATA type) family protein	4,56	3,19

Table 4 lists all transcription factor genes that were up- or down-regulated more than three times in both biological experiments after induction with DEX+ABA. Three/five transcription factor genes were found to be up/down-regulated, respectively. In an independent third induction experiment the up-regulation of three TF was confirmed by quantitative RT-PCR after 6 hours of induction (Figure 33A). *UBIQUITIN10* (*UBQ10*) was used as reference gene and relative expression was calculated according to the ΔC_T method. Only after DEX+ABA treatment an increased amount of TF transcript was detectable whereas in two cases a slight induction was observed after single ABA treatment (SMZ and RAP2.6, Figure 33A). Two up-regulated TF genes encode for proteins related to APETALA 2 (AP2). SCHLAFMÜTZE (SMZ) has been shown to repress flowering together with its homolog SCHNARCHZAPFEN (SNZ, Schmid *et al.* 2003). Both of them contain a MIR172 complementary site and are down-regulated during early flowering. SMZ expression during seed development starts after the LEC1 expression peak in the globular embryo (ATGeneExpress, http://csbdb.mpimp-golm.mpg.de, Figure 33C). The same was observed for RAP2.6 which is expressed at much higher levels than RAP2/SMZ at the end of seed morphogenesis (Figure 33C). DOF2 belongs to a plant specific TF family and encodes a protein with one zinc finger (DNA binding with one finger, Yanagisawa 2004). Its expression precedes the LEC1 expression peak and stays constantly low during later stages of embryo morphogenesis (Figure 33C). This expression pattern is contradictory to a positive regulation of *DOF2* by LEC1.

The repression of three out of five DEX+ABA down-regulated TF genes was analyzed again in an independent third induction experiment after 6 hours of induction by qRT-PCR. The decrease in transcript abundance of all three down-regulated TF could not be confirmed as DEX+ABA-dependent but rather depended on ABA alone (Figure 33B). Only the down-regulation of *HYH* by DEX+ABA was analyzed after 24 hours of induction and the data revealed a decrease in HYH transcript levels after ABA treatment. Nevertheless, after DEX+ABA-treatment HYH transcript abundance was decreased even more than after ABA-treatment.

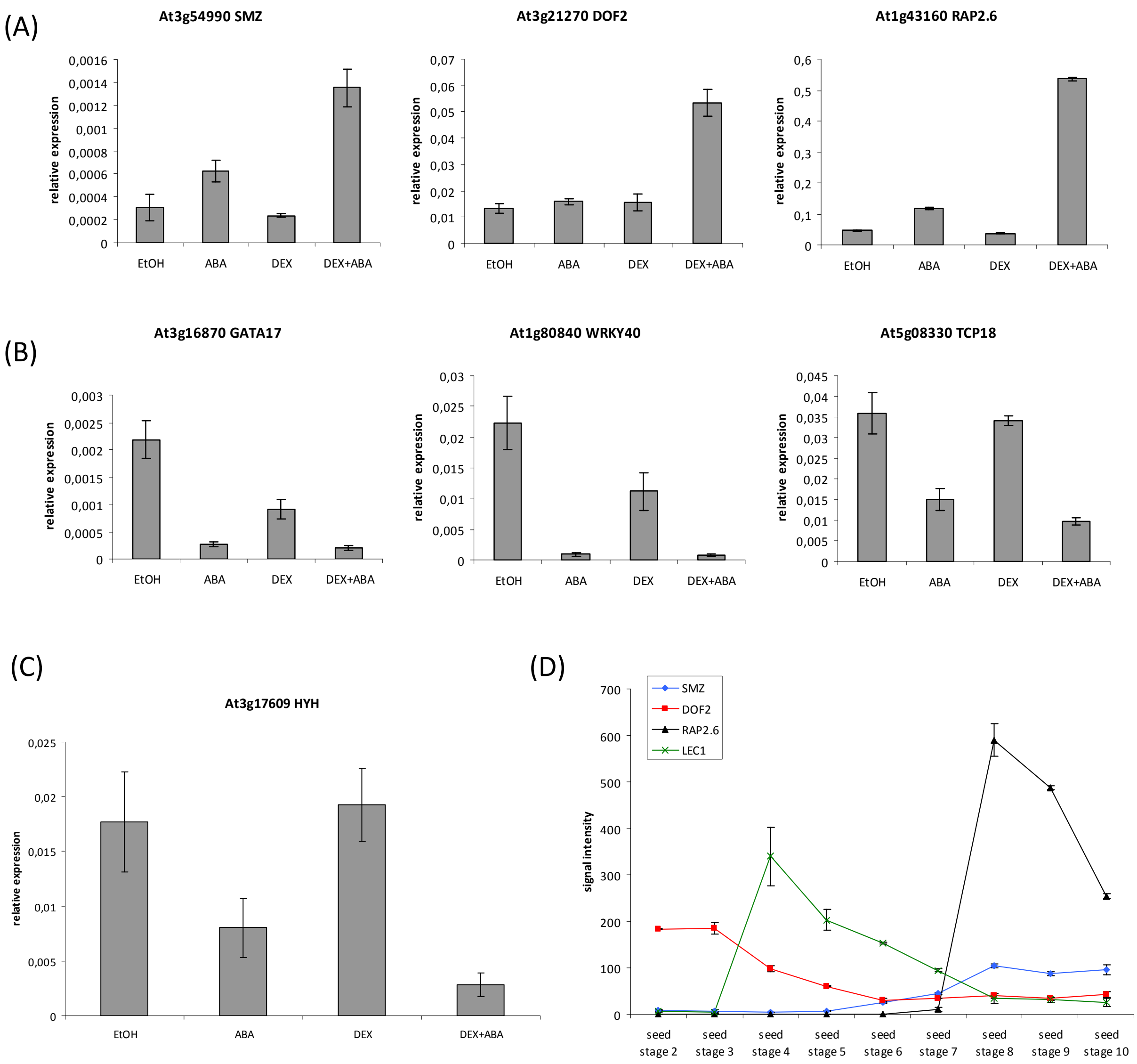


Figure 33. Confirmation of DEX+ABA-regulated TF genes by qRT-PCR.
The activation of TF genes by LEC1+ABA was confirmed in an independent third induction experiment and qRT-PCR. All three up-regulated TF genes were found to be activated within 6 hours of induction (A). The expression of three out of five analyzed down-regulated TF genes was decreased in dependence of ABA after 6 hours of induction (B). The down-regulation of *HYH* by DEX+ABA could be confirmed by qRT-PCR after 24 hours of induction, although some decrease in *HYH* transcript was already detected after ABA-treatment alone (C). Expression of *LEC1* and its putative TF target genes was analyzed during seed development. The expression peak of *LEC1* preceded these of *SMZ* and *RAP2.6* supporting their positive regulation by LEC1 (D). *DOF2* expression is decreasing during seed development thus contradicting the positive regulation by LEC1 (D). Seed expression data was generated in frame of the ATGeneExpress in the lab of Dr. Bernd Weisshaar. For details about the seed staging and experimental design see http://www.genomforschung.uni-bielefeld.de/GF-research/AtGenExpress-SeedsSiliques.html. Seed stages correspond to: 1 – globular, up to four cells; 2 – early globulat to mid globular; 3 – mid globular to early heart; 4 – early heart to late heart; 5 – late heart to mid torpedo; 6 – mid torpedo to late torpedo; 7 – late torpedo to early walking stick; 8 – walking stick to early curled cotyledons; 9 – curled cotyledons to early green cotyledons; 10 – green cotyledons.

After DEX treatment no TF were found to be up- or down-regulated in both biological experiments. Although, several ABA-regulated TF genes were identified that have been either up- or down-regulated after ABA+DEX- and ABA-treatment (Table 5).

Table 5. ABA-regulated TF genes.
TF genes were considered as putative LEC1 target when they were induced or repressed more than three times in both biological replicates. ABA- regulated genes showed altered expression after single ABA- and combined ABA+DEX-treatment.

AGI	annotation	induction factor			
		ABA exp 1	ABA exp 2	DEX+ABA exp 1	DEX+ABA exp 2
At1g45249	ABF2 (ABSCISIC ACID RESPONSIVE ELEMENTS-BINDING FACTOR 2)	3,54	5,38	4,51	7,53
At5g49450	ATBZIP1 (ARABIDOPSIS THALIANA BASIC LEUCINE-ZIPPER 1)	3,46	3,40	6,05	4,02
At1g22640	MYB3 (myb domain protein 3)	7,84	10,66	5,00	7,60
At2g46680	ATHB-7 (ARABIDOPSIS THALIANA HOMEOBOX 7)	39,29	45,44	42,01	35,28
At4g34000	ABF3/DPBF5 (ABSCISIC ACID RESPONSIVE ELEMENTS-BINDING FACTOR 3) transcription activator	6,86	5,91	9,63	6,84
At1g52890	ANAC019 (Arabidopsis NAC domain containing protein 19)	25,20	19,85	15,84	16,74
At2g47190	MYB2 (myb domain protein 2)	8,06	47,84	5,10	28,93
		repression factor			
		ABA exp 1	ABA exp 2	DEX+ABA exp 1	DEX+ABA exp 2
At2g47520	AP2 domain-containing transcription factor, putative	4,65	32,92	13,36	25,87
At4g31800	WRKY18 (WRKY DNA-binding protein 18)	3,48	3,38	30,29	14,33
At1g08810	MYB60 (myb domain protein 60)	7,26	5,50	3,62	4,96
At2g40340	DREB2C, AP2 domain-containing transcription factor	6,35	9,21	39,76	21,94
At3g23250	MYB15 (myb domain protein 15)	7,72	10,35	19,13	39,86
At2g38470	WRKY33 (WRKY DNA-binding protein 33)	4,11	13,94	10,52	6,09
At5g07580	DNA binding / transcription factor	3,76	3,09	21,52	13,62
At1g63040		4,18	8,27	4,37	6,12

ABA mediates a wide range of responses such as response to dehydration and responses to different kinds of abiotic stress (salt and cold stress; Bartels and Sunkar 2005, Himmelbach *et al.* 2003, Leung and Giraudat 1998). Seven TF genes were found to be up-regulated by ABA alone (Table 5). Among them several TF are known to be implicated with ABA signaling, like for instance the ABRE (abscisic acid responsive element) binding factors ABF2 and ABF3 (Kang *et al.* 2002, Uno *et al.* 2000). *AtbZIP1* is connected to ABA in different ways. First, it has been reported to be up-regulated by cold and salt stress in roots. Second, it is supposed to have a function during seed maturation, a process largely influenced by ABA levels (Weltmeier *et al.* 2009). The homeodomain leucine-zipper gene *ATHB7* is known to be transcribed in an ABA-dependent manner and the protein has been shown to mediate growth response after drought stress (Olsson *et al.* 2004). *ANAC19* and *MYB3* have already been described to be up-regulated by ABA and abiotic stress (Tran *et al.* 2004) and both proteins interact with C-TERMINAL DOMAIN PHOSPHATASE-LIKE 1 (CPL1) via its C-terminal double-stranded RNA (dsRNA)-binding motifs (DRMs, Bang *et al.* 2008). MYB2 functions as transcriptional activator in ABA- and drought-inducible gene expression (Abe *et al.* 2003a, Yoo *et al.* 2005). Thus, the ABA-inducibility of seven TF as revealed by macroarray analysis confirms former studies and ATGeneExpress expression data (Figure 34A).

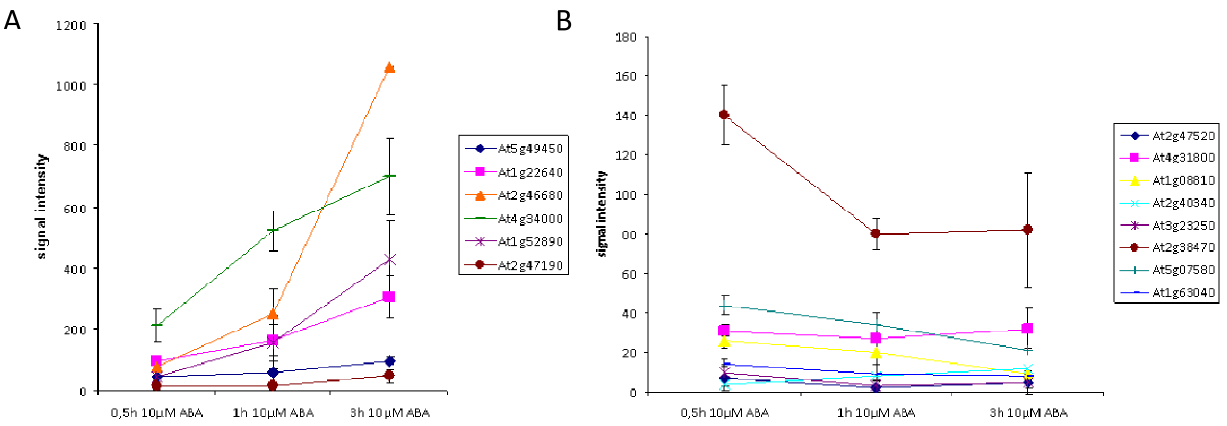


Figure 34. ABA-induction or repression of TF genes is confirmed by microarray data.
The ABA-inducible/repressed expression of TF genes (macroarray) was confirmed by publically available expression data. The ABA-induciblity was confirmed for all TF genes (A) whereas the ABA-repression was not definitely clear for all TF genes in the database (B). Expression data was generated within the ATGeneExpress project and is available at http://csbdb.mpimp-golm.mpg.de.

Eight TF genes have been shown to be down-regulated in response to ABA treatment. For six out of eight TF the ABA-induced down-regulation is confirmed by microarray expression data (ATGeneExpress; http://csbdb.mpimp-golm.mpg.de; Figure 34B) although only few have been directly implicated with ABA signaling in abiotic stress response or seed development. DREB2C has been described in connection with heat stress and dehydration. It was shown to bind the dehydration responsive element/C-repeat (DRE/CRT; A/GCCGAC) motif (Lim *et al.* 2007). DREB2C repression by ABA has not been reported yet. The expression of MYB60 is negatively regulated by drought and ABA (Cominelli *et al.* 2005). For MYB15 contradictory results have been published, as it has been shown to be up-regulated by cold stress (Agarwal *et al.* 2006). Also WRKY33 is implicated with ABA signaling and salt tolerance (Jiang and Deyholos 2009).

3.4.2 Microarray analysis

The macroarray described in the previous section is restricted to transcription factor genes. In order to screen putative LEC1 target genes on a nearly genome-wide level, microarray analysis was performed in cooperation with Jean-Pierre Renou at the URGV (Plant Genomics Research Unit, Evry Cedex, France) using the Complete Arabidopsis Transcript MicroArray (CATMA, Crowe *et al.* 2003, Hilson *et al.* 2004, Sclep *et al.* 2007). CATMA arrays contain 24 576 gene-specific tags (GSTs) from *Arabidopsis*. Plant growth and induction were performed as described in the previous section. Two biological replicates of the LEC1::GR line treated with a) 0.1% ethanol, b) 10µM ABA, c) 30µM DEX and d) 10µM ABA + 30µM DEX and of wild type treated with a) 0.1% ethanol and b) 30µM DEX were performed. ABA, DEX and DEX+ABA treatments were compared with the ethanol control treatment by simultaneous hybridization of the Cy5-labelled treated sample and the Cy3-labelled control sample. For each comparison, two technical replications with fluorochrome reversal have been performed for each pool of RNA (dye swap). RNA was extracted from 14 days old seedlings after 8 hours of induction and cRNA synthesis, labeling, hybridizations and scanning were performed as previously reported (Lurin *et al.* 2004). Statistical analysis of the microarray data was done according to Lurin *et al.* 2004 and genes were considered to be significantly differentially expressed with a Bonferroni-corrected p-value ≤0.05.

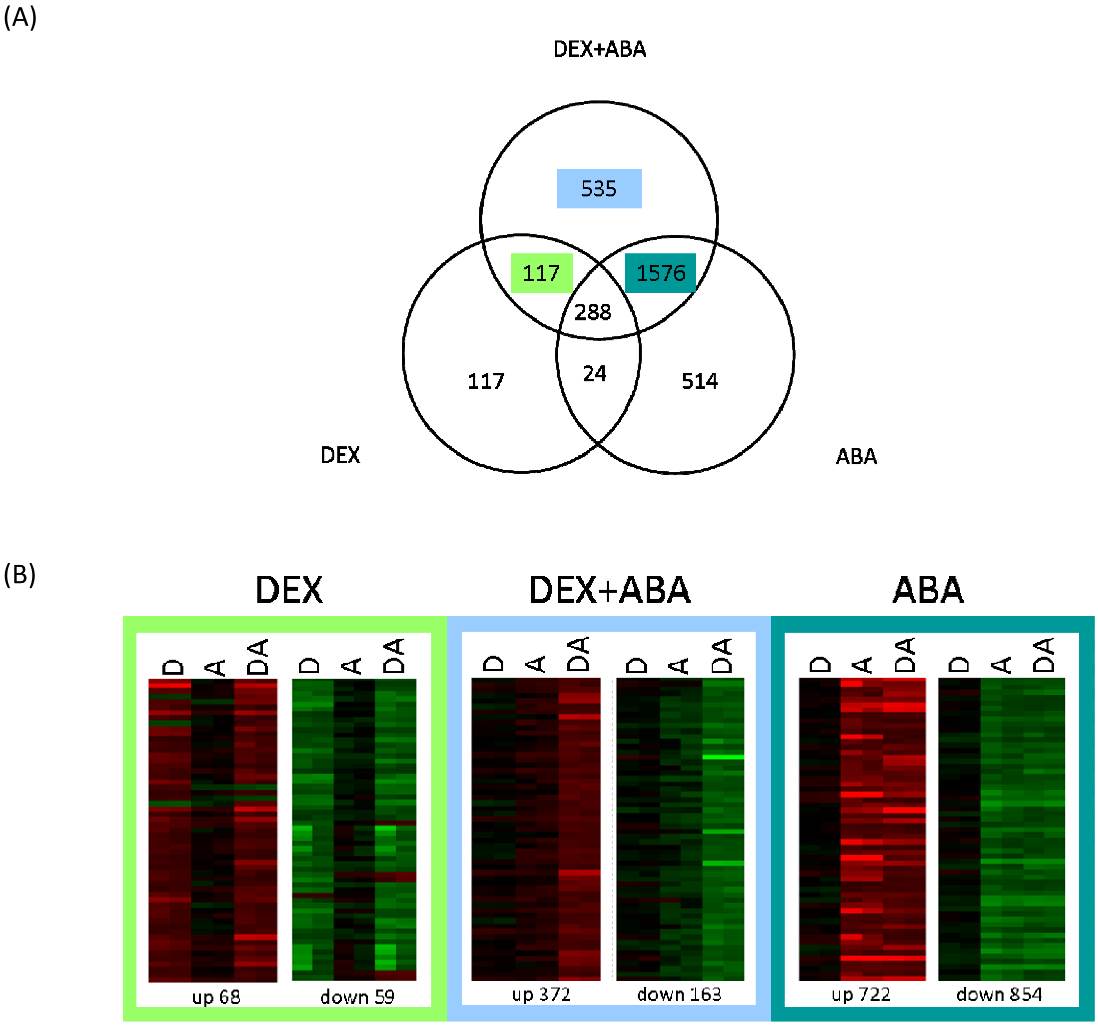


Figure 35. Overview of differentially expressed genes, treatment categories and the numbers of up/down-regulated genes in each category.
After data normalization and statistics a number of 3171 genes were identified to be significantly differentially expressed after treatment in comparison to control (ethanol) in both replicates. Treatment categories were defined as described before for macroarray anaylsis (A). A number of 535, 117 and 1567 genes belonged to biologically important treatment categories DEX+ABA-regulated, DEX-regulated and ABA-regulated genes, respectively (A). The numbers of up- and down-regulated genes are equally distributed among genes in the treatment categories 'DEX-regulated' and 'ABA-regulated' whereas up-regulated genes prevail among DEX+ABA-regulated genes (B). D: DEX, light green. A: ABA, dark blue. DA: DEX+ABA, light blue.

A total of 3171 genes were detected as significantly differentially expressed in at least one treatment in both biological replicates (Figure 35). These genes were grouped according to their expression in response to different induction regimes. Three biologically important treatment categories were defined as described in the chapter 3.4.1 'Macroarray analysis'. A number of 117 DEX-/LEC1-regulated genes showed significantly altered expression after treatment with DEX alone and a combination of DEX and ABA (Figure 35A+B, light green, Supplementary Table S3.2). DEX+ABA-treatment led to altered expression of 535 genes that did not show altered expression after single DEX- or ABA-treatment (Figure 35A+B, light blue, Supplemetary Table S3.1). Most genes (1576) were found to be regulated by ABA as revealed by significant differences in expression after ABA- and combined DEX+ABA- treatment (Figure 35A+B, dark blue, Supplemetary Table S3.3).

Table 6. Overlap between macro- and microarray results.
Three DEX+ABA-regulated and 5 ABA-regulated genes were confirmed by macro- and microarray. These numbers correspond to a number of 35% of macroarray targets that have been confirmed independently by microarray analysis. Fold change values of induction/repression in macro- and microarray analysis are given.

	AGI	name	macroarray				microarray			
			ABA 1	ABA 2	DEX+ABA 1	DEX+ABA 2	ABA 1	ABA 2	DEX+ABA 1	DEX+ABA 2
DEX+ABA	At3g54990	SMZ	-	-	4,85	6,96	-	-	2,64	2,65
	At3g16870	GATA type	-	-	-4,56	-3,19	-	-	-2,07	-1,73
	At1g28370	ERF11	-	-	-16,55	-6,12	-	-	-1,95	-1,80
ABA	At1g52890	ANAC19	25,20	19,85	15,84	16,74	9,91	22,42	7,42	9,17
	At5g49450	bZIP1	3,46	3,40	6,05	4,02	3,54	2,80	5,16	5,15
	At1g22640	MYB3	7,84	10,66	5,00	7,60	4,66	6,11	2,73	2,63
	At2g46680	HB-7	39,29	45,44	42,01	35,28	20,32	23,07	21,15	21,28
	At4g31800	WRKY18	-3,48	-3,38	-30,29	-14,33	-2,27	-3,23	-4,17	-4,14

To support the significance of the obtained microarray results, target gene lists were first matched to putative targets as identified by macroarray analysis. As shown in Table 6, three of eight DEX+ABA-regulated transcription factor genes (macroarray) could be confirmed by microarray analysis. These include one DEX+ABA-upregulated AP2 TF (At3g54990; SMZ) and two DEX+ABA-downregulated TF (At3g16870; GATA type zinc finger and At1g28370; ERF11). Regarding ABA-regulated transcription factor genes (macroarray), many ABA-upregulated TF (four out of seven) could be confirmed by microarray analysis whereas only one out of eight ABA-downregulated TF was verified. Taken together 35% (8 of 23 genes) of the macroarray LEC1- targets were independently confirmed as putative LEC1 target genes by microarray analysis.

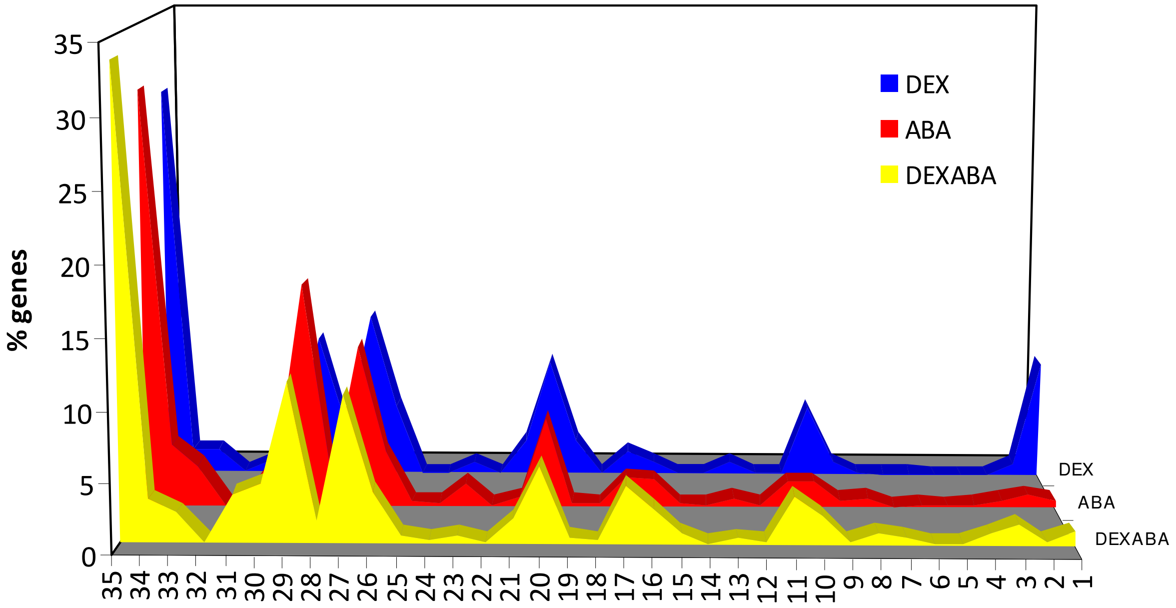


Figure 36. Functional classification of DEX+ABA regulated genes.
Differentially expressed genes of three biologically important treatment categories were mapped to 35 MapMan functional categories (BINs, Thimm *et al.* 2004). The comparison of gene distributions between functional categories and treatment categories indicates high similarity.

The Mapman software tool (Thimm *et al.* 2004) was applied in order to achieve a functional classification of significantly differentially expressed genes of all three treatment categories (DEX, ABA, DEX+ABA). Figure 36 gives an overview of all 35 BINs (Thimm *et al.* 2004), representing main functional categories, and the percentage of genes of each treatment category that mapped to each BIN. Surprisingly, distributions of genes of different treatment categories among the functional categories resembled each other (Figure 36). About one third of the genes in every treatment category (DEX: 29,1%; ABA: 30,3%; DEX+ABA: 33,5%) could not be assigned (BIN35). Except for not assigned genes, most genes in all three treatment categories mapped to BIN29 ‘protein’ (DEX: 10,3%; ABA: 16,3%; DEX+ABA: 11,3%) and BIN27 ‘RNA’ (DEX: 11,9%; ABA: 11,7%; DEX+ABA: 10,3%).

In spite of similarities between the distributions of genes in the different functional categories, only DEX+ABA-treatment led to the development of embryonic structures. These structures never appeared after treatment with DEX or ABA alone (see previous sections). These differences in phenotype occurrence were reflected on transcriptome level since significantly over-represented functional categories could be identified among DEX+ABA-regulated genes in comparison to DEX- or ABA-regulated genes (Table 7). Three over-represented functional categories have been identified for DEX-regulated genes. In the following, I will only concentrate on over-represented categories found among DEX+ABA- regulated genes, BIN11 ‘lipid metabolism’, BIN17 ‘hormone metabolism’ and BIN31 ‘cell’.

Table 7. Over-representation analysis.
Significantly over-represented functional categories were identified in different treatment categories (Fisher's Exact test, p<0,05) in comparison to all regulated genes. Categories indicated in bold were significantly enriched among genes in the corresponding treatment category. Categories additionally indicated in red will be subject of further considerations.

No.	Functional category	**ABA** 1576 genes	p-value	**DEX** 117 genes	p-value	**DEX+ABA** 535 genes	p-value
1	Photosystem (PS)	9 (0,57%)	0.999	**10 (8,55%)**	**1,15e-04**	5 (0,94%)	0.715
10	Cell wall	30(1,89%)	0.999	**6 (5,13%)**	**0.033**	11 (2,07%)	0.595
11	Lipid metabolism	30 (1,89%)	0.943	0	-	**18 (3,38%)**	**0.025**
17	Hormone metabolism	34 (2,15%)	0.984	2 (1,71%)	0.819	**22 (4,14%)**	**0.011**
19	Tetrapyrrole synthesis	4 (0,25%)	0.978	**3 (2,56%)**	**0.009**	2 (0,37%)	0.672
31	Cell	22 (1,39%)	0.995	1 (0,85%)	0.892	**18 (3,38%)**	**0.0039**

The category cell (BIN31) was represented by 18 DEX+ABA-regulated genes. About 66,7% (12 genes) of these genes mapped into the sub-category cell organisation whereas 11 of them were found to be up-regulated. Most of these genes are involved in actin and tubulin organization and will not be subject of further consideration.

A number of 18 genes were mapped to 'lipid metabolism' (Table 8), among them 6 genes encoding lipid biosynthesis enzymes and 2 oleosin genes were found to be up-regulated. Two genes are implicated with phospholipid metabolism, two further genes with the metabolism of exotic lipids such as brassinosteroids and sphingolipids, one gene is connected to glycerol metabolism and the remaining four genes mapped to lipid degradation. Also *WRINKLED1* (*WRI1*) which was described as main regulator of seed oil biosynthesis was found to be up-regulated by DEX+ABA-treatment (Cernac and Benning, 2004).

Table 8. DEX+ABA- regulated genes that were mapped to category 11 ‘lipid metabolism’. About 18 DEX+ABA-regulated genes were mapped to BIN11 ‘lipid metabolism’. The activation of four genes by DEX+ABA was confirmed in another independent induction experiment by qRT-PCR. Another three genes were independently confirmed by studies of Mu *et al.* (2008) where these genes have been shown to be induced after permanent estradiol-induction of *LEC1*. Due to the use of a different ontology tool, more genes have been connected to ‘lipid metabolism’ by Mu *et al.* (2008). Thus, further five DEX+ABA-regulated genes were confirmed by Mu *et al.* (2008) although they have not been mapped to MapMan BIN11 but to other categories. For microarray data, the mean fold change value of both replicates is given. For qRT-PCR analysis the fold change values represent only one biological experiment that has to be repeated.

AGI	**Name**	**microarray DEX+ABA**	**qRT-PCR DEX+ABA**	**confirmed by Mu *et al.***
		fold change	fold change	fold change
11 lipid metabolism				
At5g16390	BCCP1/CAC1	2,14	-	-
At5g46290	KAS1	2,09	2,92	-
At2g05990	MOD1	2,12	5,37	1,63
At2g17650	AMP-dependent synthetase	2,19	-	-
At2g47240	long-chain-fatty-acid--CoA ligase	-1,57	-	-
At2g26640	beta-ketoacyl-CoA synthase	1,57	-	-
At2g34590	transketolase	2,27	4,65	1,22
At1g74320	choline kinase	1,60	-	-
At4g30340	ATDGK7	-1,54	-	-
At2g25890	glycine-rich protein / oleosin	2,74	-	-
At5g51210	OLEO3	4,38	18,19	8,81
At5g40610	glycerol-3-phosphate dehydrogenase	1,82	-	-
At5g58980	ceramidase	1,66	-	-
At2g07050	CAS1	2,16	-	-
At1g18460	lipase	2,29	-	-
At3g06810	acyl-CoA dehydrogenase-related	1,80	-	-
At4g14430	enoyl-CoA hydratase/isomerase	1,67	-	-
At1g01710	acyl-CoA thioesterase	1,55	-	-
other functional categories				
8 TCA/ org. transformation			-	
AT3G16950	LPD1	1,70	-	3,15
16 Secondary metabolism			-	
AT2G38040	CAC3	2,30	-	1,94
18 Co-factor and vitamine metabolism			-	
AT5G08415	lipoic acid synthase	1,72	-	2,30
35 not assigned			-	
AT1G80950	phospholipid/glycerol acyltransferase	1,58	-	1,12
AT1G52670	biotin/lipoyl attachment domain-containing protein	2,46	-	2,23

The up-regulation of three lipid-related genes by LEC1 was confirmed by Mu *et al.* (2008) who studied the activation of lipid biosynthesis after permanent *LEC1*-induction with estradiol. Due to the use of different gene ontology databases, different sets of genes were mapped to lipid biosynthetic pathways. Further five DEX+ABA-regulated genes were confirmed by Mu *et al.* (2008) that were not mapped to the MapMan category ‘lipid metabolism’ but to other functional categories (Table 8).

The DEX+ABA-inducibility of three genes encoding lipid biosynthetic enzymes, *WRI1* and one oleosin gene was validated by qRT-PCR in another independent induction experiment (Figure 37). Diagrams in Figure 37 compare log ratios obtained by microarray analysis (grey bars) with qRT-PCR results (blue lines). Compared with the oleosin gene, *WRI1* and lipid biosynthetic genes were only mildly up-regulated after DEX+ABA treatment (Table 8 and Figure 37). Moreover, an increase in transcript of *WRI1* and three lipid biosynthetic genes was only detectable after 24 hours of induction (Figure 37). However, the up-regulation of the oleosin gene was already detected after four hours of induction (Figure 37). Due to fast induction kinetics, this gene might be interpreted as direct LEC1 target gene. This suggests different mechanisms to be responsible for activation of genes involved in either lipid storage or lipid biosynthetic genes. In addition, the latter genes showed a slight ABA-inducibility (Figure 37) which was not the case for the oleosin gene.

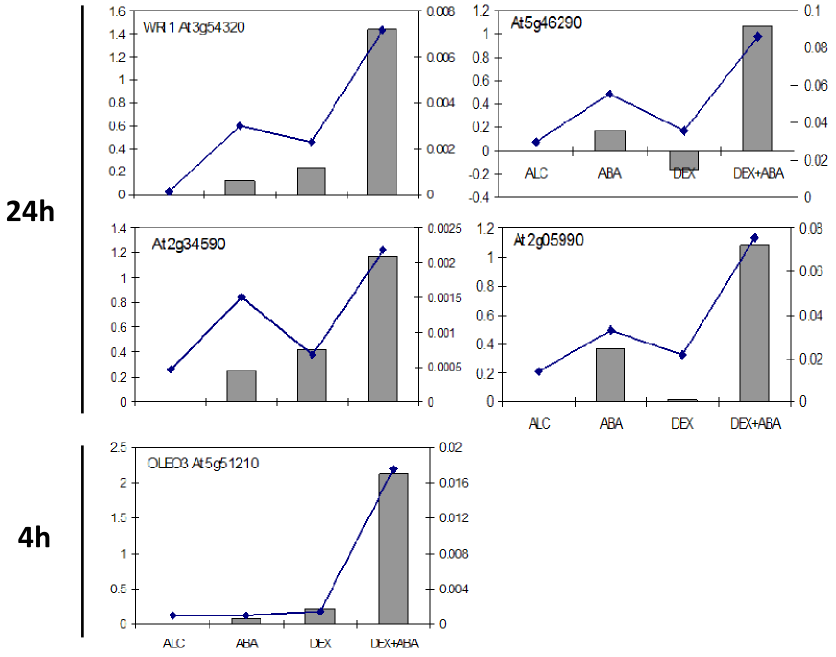


Figure 37. Activation of genes involved in lipid biosynthesis, its regulation and lipid storage by LEC1 and ABA.
The DEX+ABA-inducibility of *WRI1*, three lipid biosynthetic genes and *OLEO3* was confirmed in another independent induction experiment by qRT-PCR. Grey bars represent log ratios of the microarray experiment (left Y-axis) and blue lines represent relative expression levels as measured by qRT-PCR (right Y-axis). *WRI1* and lipid biosynthetic genes were only found to be activated by DEX+ABA after 24 hours of induction and were also slightly induced by ABA-treatment alone. The expression of OLEO3 was increased substantially already after 4 hours of induction and ABA did not have any effect.

In order to confirm LEC1 activation of lipid metabolism, lipid content and fatty acid composition were analyzed by gas chromatography in 14 day old, permanently induced LEC1::GR seedlings (root and apical part) in comparison to uninduced seedlings (cooperation with Silke Schiebold and Hardy Rolletschek, IPK). In the absence of the inducer LEC1::GR seedlings accumulated 15,8±2,5mg total lipids per gram dry weight (mg/g DW) and 22,9±2,3mg/g DW in roots and apical parts, respectively. In contrast, LEC1::GR seedlings grown on 10µM DEX accumulated much higher amounts of total lipids, 69,1±2,7mg/g DW and 171,1±2,6mg/g DW in roots (including pkl root tips) and apical parts, respectively. This corresponded to a 4/7 fold increase in total lipid accumulation in roots and apical parts of induced LEC1::GR seedlings.

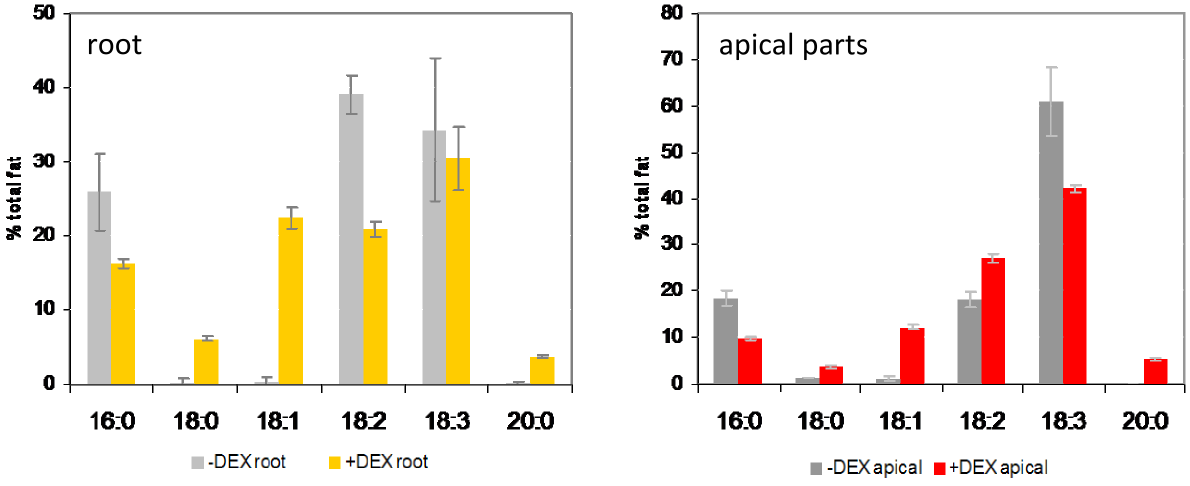


Figure 38. Fatty acid composition in roots and apical parts of induced and uninduced LEC1::GR seedlings.
The proportions of different fatty acid fractions among total lipids were analyzed by gas chromatography in permanently induced LEC1::GR seedlings and uninduced control seedlings. Embryonic root structures (*pkl* roots, left) and embryonic apical structures (cotyledon-like leaves and swollen hypocotyls, right) were analyzed independently in comparison to corresponding parts of uninduced seedlings. Increased levels of 18:0, 18:1 and 20:0 fatty acids species were measured in all embryonic structures such as roots (yellow) and apical parts (red) *vs.* uninduced structures (grey).

With regard to different fatty acid fractions, the increased total lipid content in induced LEC1::GR seedlings was mainly caused by elevated levels of saturated (18:0, stearic acid; 20:0 arachidic acid) and less unsaturated fatty acids (18:1, oleic acid, Figure 38). These fractions were almost undetectable in uninduced seedlings (Figure 38). The portions of major fatty acid species in roots and apical parts (18:3, linolenic acid; 18:2, linoleic acid and 16:0, palmitic acid) were unaffected or slightly decreased after LEC1 induction.

Taken together these results reveal LEC1- induced lipid accumulation during seedling development after germination, therefore pointing to a role of LEC1 as a regulator of seed oil biosynthesis. Due to different induction kinetics of lipid-related genes, this LEC1 function seems to involve several activation mechanisms or mediating factors.

The third functional category that was found to be significantly over-represented among DEX+ABA-regulated genes was BIN 17 ‘hormone metabolism’ including 22 genes. According to the Mapman ontology similar numbers of genes were mapped to the sub-categories auxin metabolism (4 genes), brassinosteroid (BR) metabolism (4 genes), ethylene (5 genes) and gibberellic acid metabolism (4 genes). ABA metabolism, cytokinin metabolism and jasmonic acid metabolism were only represented by 2, 2 and 1 gene, respectively (Table 9). After additional manual annotation of DEX+ABA-regulated genes six more genes were assigned to the sub-category auxin metabolism. These genes are implicated with auxin signaling, auxin metabolism as well as myo-inositol metabolism.

DEX+ABA-regulated genes that were implicated with ethylene- and GA metabolism include genes encoding biosynthetic enzymes (EAT1 for ethylene and GA3, GA20ox2 for GA) and also encoding components of the corresponding signaling pathways (ethylene response factors and gibberellin-regulated proteins). The majority of genes in these sub-categories was found to be down-regulated by DEX+ABA treatment (ethylene 4 out of 5; GA 3 out of 4). Opposite regulation of two GA biosynthetic genes (GA3 and GA20ox2) by DEX+ABA might have been caused by feedback regulations. In contrast to down-regulation of genes in the GA and ethylene sub-category, all genes that were mapped to brassinosteroid metabolism were up- regulated by DEX+ABA-treatment. These include one biosynthetic gene encoding CYCLOARTENOL SYNTHASE 1 (CAS1) and three genes involved in BR signaling. Among them one putative LEC1 target gene encoded a putative leucine-rich repeat transmembrane protein kinase with similarity to the brassinosteroid receptor BRASSINOSTEROID INSENSITIVE1 (BRI1, Li and Chory 1997). *BES1-interacting Myc-like protein1* (BIM1) encodes a bHLH transcription factor that, in complex with BES1, binds to the E-box in many BR-induced promoters (Yin et al. 2005). Besides with BES1, BIM1 has been reported to interact with embryonic patterning genes DORNROESCHEN (DRN) and DORNROESCHEN-LIKE (DRNL) as well as PHAVOLUTA (PHV, Chandler et al. 2009). DRN and DRNL were described as *upstream* components of auxin transport and signaling (Chandler et al. 2007). These data suggest LEC1-regulated hormone interplay between BR and auxin during embryogenesis.

A strong link between LEC1 and auxin is described during induction of somatic embryogenesis (Casson and Lindsey 2006, Gaj et al. 2005). Interestingly, 10 DEX+ABA- regulated genes are connected to auxin metabolism and auxin signaling (Table 9). Seven of these genes play a role in auxin signal transduction. These include TRANSPORT INHIBITOR RESPONSE 1 (TIR1) which is up-regulated by DEX+ABA and has been described as auxin receptor. The TIR1 protein mediates auxin response and auxin induced transcriptional changes by recruiting Aux/IAA transcriptional repressors for ubiquitination (Gray et al. 2001, Kepinski and Leyser 2005, Tan et al. 2007). Four further DEX+ABA up-regulated genes that mapped to this category encode an indole-3-acetic acid inducible factor (IAA8), two auxin-responsive proteins and a kinase gene encoding a 3-phosphoinositide-dependent protein kinase with similarity to 3-PHOSPHOINOSITIDE-DEPENDENT PROTEIN KINASE 1 (PDK1). PDK1 has an important function as activator of PINOID (PID, Zegzouti et al. 2006) which in turn is responsible for localisation of auxin transport facilitators and auxin distribution (Friml et al. 2004).

Table 9. DEX+ABA-regulated genes assigned to BIN 17 'hormone metabolism'.
About 22 DEX+ABA-regulated genes were mapped to BIN17 'hormone metabolism'. A number of six further genes were manually annotated to this category. The activation of most hormone-related genes by DEX+ABA after 24 hours of induction was confirmed in another independent experiment by qRT-PCR. In contradiction to the microarray results, DEX+ABA-down-regulated genes were found to be activated in a third independent induction experiment. For microarray data, the mean fold change value of both replicates is given. For qRT-PCR analysis the fold change values represent only one biological experiment and that has to be repeated.

Hormone	Class	AGI	Name	microarray	qRT-PCR	
				DEX+ABA	DEX	DEX+ABA
ABA	signaling	AT5G65310	HB-5	-1,83	-	-
ABA	signaling	AT1G52340	ABA2	1,61	-	-
Auxin	signaling	At1g31340	RUB1	-1,56	3,20	1,20
Auxin	signaling	At2g22670	IAA8	1,83	3,17	4,69
Auxin	signaling	At2g46690		1,90	2,87	12,04
Auxin	signaling	At3g10540	putative PDK1	1,61	2,82	2,92
Auxin	signaling	At3g62980	TIR1	1,81	0,95	1,96
Auxin	signaling	At5g19140		1,86	4,31	11,81
Auxin	signaling	At5g25890	IAA28	-1,66	2,84	7,96
Auxin	metabolism	At3g06810	IBR3	1,80	2,34	5,05
Auxin	myo-inositol	At4g18010	IP5PII	-1,99	4,00	3,98
Auxin	myo-inositol	At4g39800	MI1PS	2,22	1,54	1,29
BR	signaling	At1g74360	BRI1-LIKE	1,71	-	-
BR	signaling	At5g02820	RHL2	1,67	1,49	1,73
BR	signaling	At5g08130	BIM1	1,54	1,16	2,21
BR	metabolism	At2g07050	CAS1	2,16	3,00	6,97
Cytokinin	signaling	AT1G59940	ARR3	-1,84	-	-
	signaling	AT2G41310	ATRR3	1,74	2,67	6,38
Ethylene	signaling	At1g28370	ERF11	-1,87	-	-
	signaling	At3g20310	ERF7	1,57	-	-
	signaling	At5g07580		-2,60	-	-
	signaling	At5g25190		-1,73	-	-
	metabolism	At1g05010	EAT1	-2,60	-	-

GA	signaling	At2g14900		-1,68	-	-
	signaling	At3g02885	GASA5	-2,07	-	-
	metabolism	At5g25900	GA3	2,70	-	-
	metabolism	At1g30040	GA20ox2	-1,60	-	-
JA	signaling	At1g52100	jacalin	2,17	-	-

The expression of two auxin signaling genes, IAA28 and RUB1, was found to be repressed by DEX+ABA-treatment whereas qRT-PCR results revealed both genes to be activated by DEX+ABA-treatment. Similar to ubiquitin, RUB1 is conjugated to proteins like for example CULLIN1 (AtCUL1) which is a component of the ubiquitin protein ligase complex SFC^{TIR} (del Pozo and Estelle 1999). The role of RUB1 modification on AtCUL1 and the effects on ubiquitin protein ligase complex SFC^{TIR} are still unclear. Another DEX+ABA up-regulated gene is involved in auxin allocation and encodes the acyl-CoA dehydrogenase IBA-RESPONSE3 (IBR3) which is known to convert indole-3-butyric acid to the active auxin indole-3-acetic acid in a process similar to fatty acid beta-oxidation (Zolman *et al.* 2007). Genes encoding two enzymes of the inositol-phosphate metabolism have been identified as putative LEC1 target genes. One up-regulated gene encodes the myo-inositol-1-phosphate synthase which catalyzes the conversion of glucose-6-phosphate to inositol-3-phosphate (Mitsuhashi *et al.* 2008). This reaction represents the first essential step in inositol-6-phosphate synthesis whereas this compound is reported to bind the TIR1 protein thus supporting it to sense auxin (Tan *et al.* 2007). The gene encoding the INOSITOL-POLYPHOSPHATE-5-PHOSPHATASE II, the enzyme catalyzing the dephosphorylation of inositol-6-phosphate was down-regulated by DEX+ABA-treatment, although activation was observed in the confirmation experiment as measured by qRT-PCR.

ABA-dependent LEC1-induced phenotypic alterations were reflected on transcriptome level by different over-represented functional categories. The enrichment of genes involved in lipid and hormone metabolism seem to have highest functional significance. A function of LEC1 as an indirect regulator of seed oil biosynthesis has been reported recently (Mu *et al.* 2008). Regarding hormone metabolism, most genes have been implicated with auxin signaling. The phytohormone auxin, indole-3-acetic acid (IAA), is one of the most important plant growth regulators and influences cell division, elongation and differentiation. Auxin signaling is mediated by a complex sensing and signal transduction mechanism which involves protein modification (ubiquitination) and degradation (26S proteasome) of Aux/IAA transcriptional repressors. Auxin distribution throughout the plant, especially polar auxin transport is determined by the location of several auxin efflux facilitators (*PINFORMED*). In this study many genes connected to auxin signaling have been identified as putative LEC1 targets. This suggests LEC1 induced embryonic development to be mediated by auxin signaling. Further experiments are necessary to confirm the direct regulation of auxin-related genes by LEC1.

3.4.3 ChIP on chip

In order to identify direct LEC1 target genes, the interaction of the LEC1 protein with DNA was analyzed by chromatin-immunoprecipitation (cooperation with Gudrun Mönke, IPK) and following hybridization of precipitated DNA fragments to the SAP (Systemic analysis of Arabidopsis promoters) promoter array (cooperation with Urs Hähnel and Lothar Altschmied, IPK, Benhamed *et al.* 2008). Two week old uninduced LEC1::GR seedlings were incubated in liquid MS induction medium containing DEX (30µM) for 24 hours. After sampling, seedlings were fixed and the chromatin fraction was isolated from nuclei. Promoter DNA fragments were precipitated using a rabbit anti-LEC1 antibody. After linear amplification of precipitated DNA-fragments putative LEC1 targets were identified by hybridization of the DNA on the SAPv1 macroarray containing 8471 transcriptionally active promoters (intergenic regions, Benhamed *et al.* 2008). Four biological replicates were performed, whereas the ethanol- treated sample served as a control (LEC1_EtOH vs. LEC1_DEX).

After data normalization and statistical analysis, in total 770 promoter regions were identified to be enriched in induced LEC1::GR seedlings after chromatin-immunoprecipitation with anti-LEC1 antibody (Supplemetary Table S4.1). This number was corrected by taking into account that several promoter amplicons, present on the SAP macroarray represent divergent promoters depending on the direction of the adjacent genes (+/- DNA strand). Theoretically, four genomic constellations of neighbouring genes are conceivable (head-to-head; tail-to-tail; head/tail-to-tail/head), considering the promoter as head of the corresponding gene. Only three have to be taken into account in ChIP data analysis. The intergenic region of genes in tail-to-tail constellation does not represent a promoter region and is therefore not represented on the SAP filter. The remeinaing three constellations were assumed to occur in equal parts (33%). The problem of divergent promoters had to be considered only for genes in ‘head-to-head’ constellation. In this genomic constellation, ChIP does not discriminate between a uni- or bidirectional promoter function of the intergenic region. Additional experiments such as qRT-PCR are required to identify the true target promoter. In total 422 LEC1::GR putative target genes were found in head-to-head constellation with the adjacent genes (Figure 39). This fraction corresponded to 54,8% of all LEC1::GR targets, thus exceeding the theoretically expected ratio of 33%. All putative LEC1 target genes that occured in other genomic constellations than head-to-head were considered as ‘candidate LEC1 targets’ (348 genes, Figure 39).

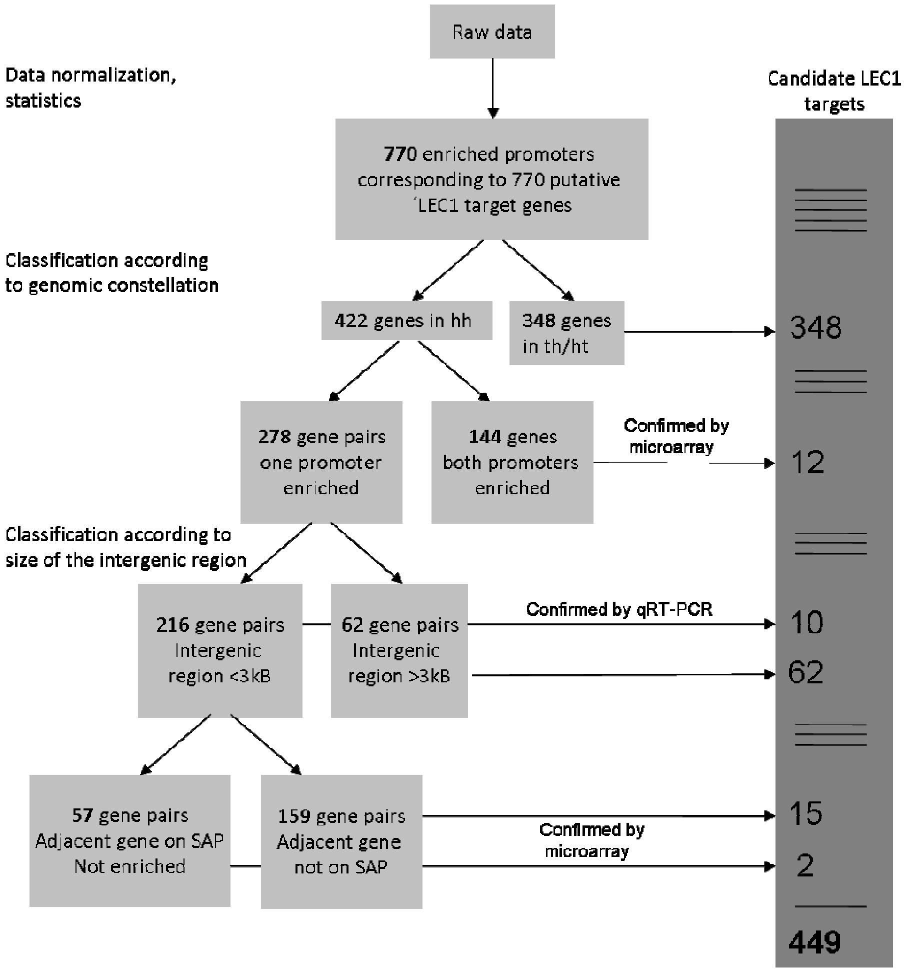


Figure 39. Analysis pipeline for ChIP/chip results obtained with SAP promoter filters.
Data normalization and statistical methods identified about 770 LEC1 target promoters. After classification of target promoters according to genomic constellation of corresponding genes, only 348 were considered as candidate target promoters because corresponding genes are located in tail-to-tail, head-to-tail or tail-to-head constellations. Due to the problem of divergent promoters for genes in head-to-head constellation (hh, 422 genes), these had to be analyzed in more detail. In the case of 72 hh-gene-pairs (144 target genes) both corresponding promoters were found to be enriched. Candidate targets have to be identified by further experiments. This was the case for 12 genes that were confirmed by microarray analysis. The remaining 278 gene pairs, where only the promoter of one gene was found to be bound by LEC1, were classified according to the size of the intergenic region. With an intergenic region >3kB overlapping regions of two SAP promoter fragments were supposed to exclude regions of TF binding and corresponding genes were considered as candidate targets (62 genes). A number of 10 hh-genes with an intergenic region < 3kB were confirmed independently by qRT-PCR. A number of 17 (15+2) hh-genes with an intergenic region < 3kB were confirmed by microarray analysis whereas in two cases the confirmed gene was not represented on the SAP filter.

For 72 head-to-head gene pairs both adjacent genes (144 of 770 genes) have been identified as putative LEC1 target genes. For 10/2 gene pairs the expression of one of both adjacent genes was found to be altered in response to DEX+ABA/DEX treatment, respectively, in the microarray experiment. These independently confirmed target genes were considered as candidate targets. Another 278 putative LEC1 targets as identified by ChIP/chip are located in head-to-head to its adjacent gene whereas this was not in the list. These genes were classified according to the size of the intergenic region. This size determines the overlap of spotted promoter fragments of two adjacent genes. The bigger the intergenic region, the bigger are gene-specific promoter regions near to TSS/ATG. Thus, for small intergenic regions, it is not clear whether the LEC1-binding site is covered by both promoter fragments or if it is located in gene specific parts of the spotted fragments. SAP promoter amplicons are limited to a size of less than 2.5kB. Therefore a threshold size of 3kB intergenic region was assumed to exclude an overlap in regions containing putative LEC1 binding sites (500bp upstream of ATG). For 62 of these gene pairs the intergenic region (from stop to ATG) was bigger than 3kb. The corresponding 62 genes in the list of LEC1 target genes were considered as candidate targets due to their large gene interspace. The remaining 216 head-to-head genes with an intergenic region of less than 3kb were supposed to share important promoter regions with their neighbour gene. Although the promoter fragments of 57 of these genes were supposed to show substantial overlap, the adjacent gene was not found in the list of LEC1 targets. Among these 57 gene pairs, for two gene pairs one gene has been identified as putative LEC1 target by microarray analysis revealing them as candidate targets. Candidate targets among remaining 55 gene pairs have to be confirmed by independent methods. Finally, for about 159 putative LEC1 targets that are located in head-to-head in the genome the adjacent genes were not represented on the SAP macroarray (Figure 39). In 15 out of 159 gene pairs, one gene was confirmed as a candidate LEC1 target by microarray analysis (Figure 39). In 7 cases the confirmed gene was the originally identified ChIP target, whereas the remaining 8 confirmed genes represent the adjacent gene that was not represented on the SAP filter.

Taken together the list of candidate LEC1 ChIP/chip targets contains all genes the promoter fragments of which have been found to be enriched after ChIP using the anti-LEC1 antibody and 1) are not located in head-to-head constellation in the genome, 2) are located in head-to-head constellation with an intergenic region >3Kb or 3) are located in head-to-head constellation and one of the adjacent genes has been identified as LEC1 target by microarray analysis or qRT-PCR. All together this list actually contains 449 candidate genes that will be used for further analyses (functional classification and promoter analysis, Figure 38, Supplemetary Table S4.2). With more validation experiments (qRT-PCR) more candidates among the head-to-head-gene pairs will be identified thus increasing the number of candidate LEC1 targets.

To compare target genes identified by either microarray or ChIP/chip technology one needs to consider the relevant differences between both approaches. Microarrays measure transcript abundancies whereas ChIP/chip monitors DNA-protein interactions. ChIP/chip identifies primary target genes of LEC1 whereas microarray analysis cannot discriminate between primary and secondary targets. Of 3171 genes identified as differentially expressed by microarray analysis, about 70% (2207) were represented by corresponding promoter fragments on the SAP filter. Among these differentially expressed genes, 146 gene promoters (6.6%) were found to be bound by the LEC1::GR transcription factor.

Chromatin for the ChIP/chip experiments was isolated from uninduced and DEX-induced LEC1::GR seedlings. In accordance with this, the highest fraction of LEC1-targets identified by both methods (13 out of 79, 16%) was found in the treatment category `DEX-regulated genes` (Figure 40). No ABA was applied for the ChIP/chip experiment assuming that the hormone is not directly involved in the binding of LEC1::GR to the target promoters. This is further confirmed for the treatment category `DEX+ABA-regulated genes` with an overlap of 44 out of 354 genes (12%) between microarray analysis and ChIP/chip technology (Figure 40). Unexpectedly, about 4% of genes found by microarray to be regulated by ABA treatment alone, also appear in the list of ChIP/chip identified targets (Figure 40). This number is currently interpreted as the false positive rate among the ChIP/chip identified target genes.

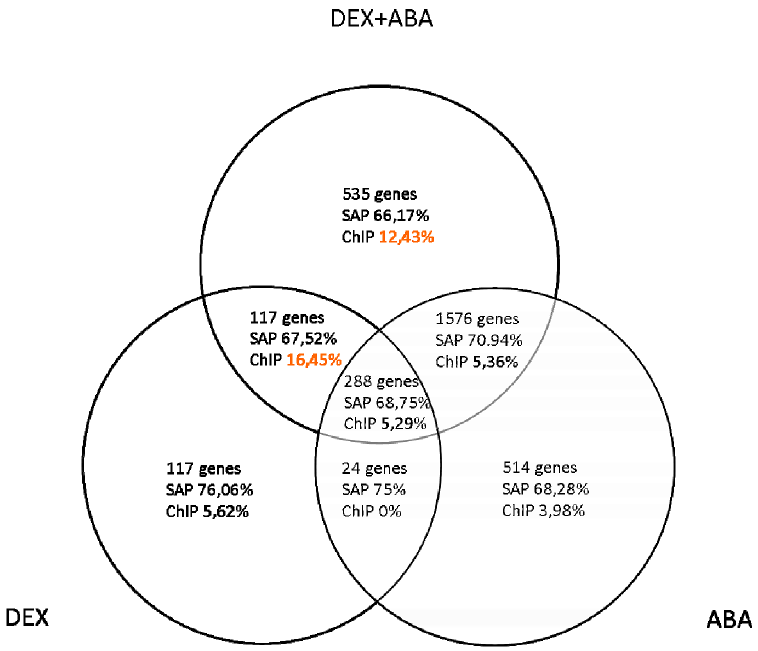


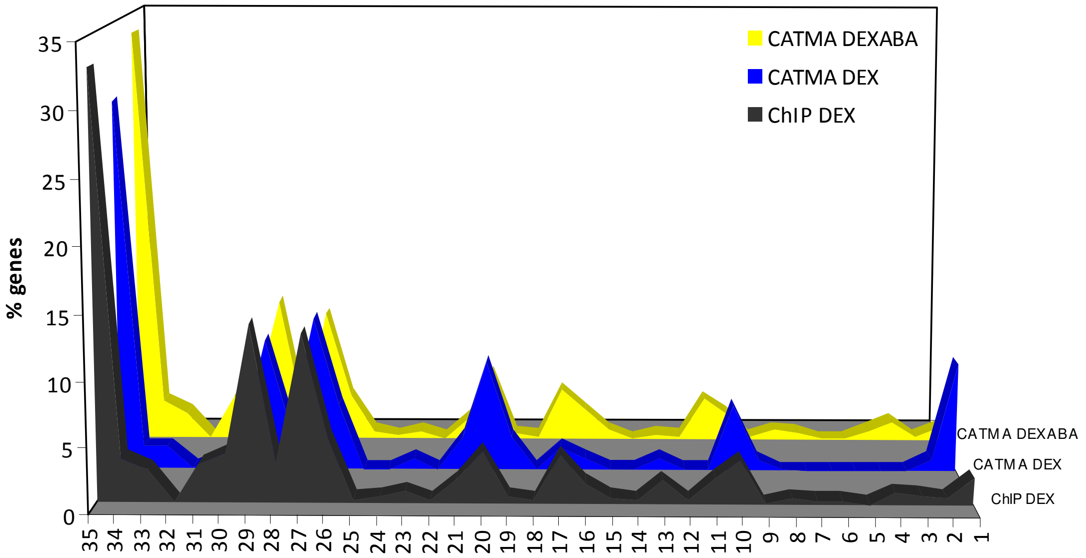
Figure 40. Mapping of ChIP/chip candidate genes to treatment categories defined for microarray analysis.
The overlap between ChIP/chip targets and microarray candidate genes in different treatment categories was analyzed. For the ChIP experiment, induction of LEC1::GR seedlings was performed with DEX alone. In accordance with this, the highest overlap was found between ChIP targets and DEX-regulated genes. It was supposed that ABA might not influence LEC1-binding to its target promoters. This was confirmed by susbstantial overlap between ChIP targets and DEX+ABA-regulated genes. SAP: percentage of genes of the treatment category that are represented on the SAP filter. ChIP: percentage of genes of the treatment category the promoters of which were bound by LEC1. Numbers indicated in orange show highest overlaps between microarray and ChIP/chip candidates in the treatment categories ‘DEX-regulated’ and ‘DEX+ABA-regulated’.

Although only a small fraction of LEC1 target genes have been identified by both microarray and ChIP/chip, both candidate gene lists showed good correlation/conformance after functional classification. Candidate genes are distributed similarly among all MapMan BINs (Figure 41A). No significant over-represented functional categories were identified when comparing ChIP/chip candidate targets and (microarray) DEX+ABA- or DEX-regulated candidate gene lists using the Gene-Classifier tool (http://mapman.mpimp-golm.mpg.de; Usadel *et al.* 2006).

In a genome wide comparison to all annotated Arabidopsis genes (TAIR 7) several functional categories have been found to be over-represented among the LEC1 ChIP target genes (Figure 41B). A Fishers exact test was performed (Gene-Classifier tool; http://mapman.mpimp-golm.mpg.de) comparing the genome-wide occurrence of genes in a certain functional category (counts genome) with the re-presentation in the list of putative LEC1 target genes (counts LEC1 ChIP). The enrichment value is considered to be significant below a Benjamini-Hochberg-corrected critical p-value of $1{,}35\text{x}10^{-03}$.

In agreement with the microarray results, numerous putative LEC1 target genes mapped to the MapMan category 17 ‘hormone metabolism’. Although general hormone-related genes were not found to be over-represented (p-value 0,015), genes in the subcategory ‘Brassinosteroid metabolism’ (MapMan BIN 17.3) were significantly enriched among LEC1 target genes ($8{,}83\text{x}10^{-05}$). Also several auxin-related genes were identified among hormone-related LEC1 targets. Non-significance of the sub-category ‘auxin metabolism’ was probably due to incomplete MapMan annotation, since several more genes could be manually mapped to auxin metabolism and signaling. Table 10 gives an overview of hormone-related genes the promoter fragments of which have been found to be enriched after ChIP with anti-LEC1 antibody. Besides genes that are implicated in auxin and brassinosteroid signaling pathways, also metabolism and signaling of other phytohormones was affected to some extent.

(A)



(B)

No.	Functional category	Counts LEC1 ChIP	Counts genome	Enrich-ment	p-value
17.3	Hormone metabolism. Brassinosteroid	6	52	8,2	$8{,}83 \times 10^{-05}$
17.3.2.2	Hormone metabolism. Brassinosteroid. Signal transduction. BZR	3	6	35,54	$5{,}36 \times 10^{-05}$
27.3	RNA. Regulation of transcription	56	2555	1,56	$1{,}10 \times 10^{-03}$
27.3.7	RNA. Regulation of transcription. C2C2(Zn) CO-like, Constans-like zinc finger family	4	34	8,36	$1{,}28 \times 10^{-03}$
27.3.26	RNA. Regulation of transcription. MYB-related transcription factor family	7	45	7,89	$4{,}14 \times 10^{-04}$
28.1.3	DNA. Synthesis/Chromatin structure. Histone	5	54	6,58	$9{,}67 \times 10^{-04}$

Figure 41. Functional classification of putative LEC1 target genes identified by ChIP/chip.
Genes corresponding to LEC1-bound promoters identified by ChIP/chip were mapped to 35 MapMan functional categories (BINs, Thimm *et al.* 2004). The comparison of gene distributions between microarray functional categories 'DEX-regulated' and 'DEX+ABA-regulated' indicated good correlation (A). Over-represented functional categories were identified by Fishers exact test (Gene-Classifier tool; http://mapman.mpimp-golm.mpg.de) comparing the genome-wide occurrence of genes in a certain functional category (counts genome) with the re-presentation in the list of putative LEC1 target genes (counts LEC1 ChIP, B).

All auxin-related LEC1 target genes are involved in auxin signaling with the exception of *DWARF IN LIGHT1* (*DFL1*) which encodes an IAA-conjugating protein. DFL1, also called GH3.6, is one of 19 members of the Arabidopsis GH3 family of early auxin response genes (Hagen and Guilfoyle 2002, Staswick *et al.* 2002) and has been shown to conjugate asparagine to free IAA (Staswick *et al.* 2005). IAA-Asp is no IAA storage form but rather removes IAA from the active pool by tagging it for oxidative degradation (Ostin *et al.* 1998).

Table 10. Hormone- related genes the promoters of which have been bound by LEC1::GR.
A number of 29 putative LEC1 target genes encode proteins involved in hormone signaling pathways and biosynthesis. The mean fold change enrichment values of three biological ChIP replicates with an anti-LEC1 antibody are given. For microarray data, the mean fold change value of both biological replicates is given. Validation experiments (24 hours of induction) using qRT-PCR were only performed once and fold change values of activation are given. No gene was found to be repressed by LEC1. Several LEC1 targets were activated in ABA-independent manner. TIR1 and ATRR3 were confirmed by ChIP/chip and microarray.

Hormone	**Class**	**AGI**	**Name**	**ChIP/Chip**	**microarray**	**qRT-PCR**	
				DEX	DEX+ABA	DEX	DEX+ABA
ABA	signaling	At5g15960	KIN1	2,62	-	-	-
ABA	signaling	At5g15970	KIN2	2,89	-	-	-
Auxin	signaling	At1g25490	RCN1	2,71	-	3,32	4,76
Auxin	signaling	At1g71090	auxin efflux carrier	3,66	-	1,85	3,11
Auxin	signaling	At2g41100	TCH3	2,16	-	2,52	0,58
Auxin	signaling	At3g04730	IAA16	2,04	-	29,23	64,5
Auxin	signaling	At3g43120	auxin responsive	2,97	-	-	-
Auxin	signaling	At3g62980	TIR1	3,43	1,72	0,95	1,96
Auxin	signaling	At3g50060	MYB77	2,68	-	1,62	3,34
Auxin	signaling	At4g36800	RCE1	2,38	-	3,29	5,33
Auxin	signaling	At5g01990	auxin efflux carrier	2,04	-	3,12	14,63
Auxin	metabolism	At5g54510	DFL1	2,04	-	10,09	23,42
Auxin+BR	signaling	At1g15580	IAA5	2,97	-	12,67	128,34
Auxin+BR	signaling	At3g15540	IAA19	3,86	-	5,69	8,40
Auxin+BR	signaling	At5g62000	ARF2	2,68	-	3,53	5,12
Auxin+BR	signaling	At1g18400	BEE1	2,04	-	4,89	0,88
BR	signaling	At4g33430	BAK1/SERK3	2,45	-	1,13	3,74
BR	signaling	At1g19350	BES1	3,25	-	1,63	1,50
BR	signaling	AT1G69010	BIM2	2,85	-	2,16	1,71
BR	metabolism	At2g36800	DOGT1	3,84	-	1,96	9,18
BR	metabolism	At3g50660	DWF4	2,14	-	4,36	4,24
BR	signaling	At1g78700	BEH4	3,51	-	4,03	2,44
BR	signaling	At3g61460	BRH1	3,14	-	5,07	2,73
BR	signaling	At4g36780	BEH2	3,86	-	3,35	4,08
Cytokinin	metabolism	At2g36800	DOGT1	3,84	-	1,96	9,18
	signaling	At2g41310	ATRR3	1,99	1,71	2,67	6,38
Ethylene	signaling	At3g19500	ethylene-resp.	3,03	-	-	-
	signaling	AT5G64050	ERS	2,11	-	2,74	4,38
GA	metabolism	At1g79460	GA2	5,17	-	-	-
JA	metabolism	At1g76680	OPR1	2,35	-	-	-

As important auxin signaling component, the auxin receptor TIR1 was one of two hormone-related, putative LEC1 targets that have been confirmed by microarray and ChIP/chip. TIR1 is an essential constituent of the SCF^{TIR1} complex that is responsible for ubiquitinylation of AUX/IAA transcriptional repressors during auxin response. For assembly of the SCF^{TIR1} complex CULLIN1 (CUL1) binding to CULLIN-ASSOCIATED and NEDD8-DISSOCIATED1 (CAND1) is released and RELATED TO UBIQUITIN1 (RUB1) is conjugated to CUL1 by a heterodimeric RUB-activating enzyme that is composed of the E1 enzymes AUXIN RESISTANT1 (AXR1) and E1 C-TERMINAL RELATED1 (ECR1) and the RUB-conjugating E2 enzyme called RCE1 (del Pozo *et al.* 2002, del Pozo and Estelle 1999, Dharmasiri and Estelle 2002, Petroski and Deshaies 2005). RUB1 and RCE1 were identified as LEC1 targets by microarray analysis or ChIP/chip, respectively. Inactivation of one of the described components/enzymes of SCF^{TIR1} lead to severe perturbations in auxin signaling (del Pozo and Estelle 1999, Gray *et al.* 2002) that can manifest during early embryo development (Dharmasiri *et al.* 2003).

Three genes that encode for proteins involved in auxin transport and its regulation were identified as putative LEC1 targets. Besides two auxin efflux carrier genes, the *TOUCH3* (*TCH3*) gene encodes a calmodulin-related, calcium-binding protein (Braam and Davis 1990). It interacts with the PINOID (PID) kinase and regulates its activity in response to calcium levels (Benjamins *et al.* 2003). PID has been shown to be responsible for localization of PINFORMED (PIN) auxin transport facilitators and auxin distribution (Friml *et al.* 2004).

Another putative LEC1 target gene, *ROOTS CURL IN NAPHTHYLPHTHALAMIC ACID1* (*RCN1*) encodes one of three Arabidopsis A subunits of the heterotrimeric serine/threonine protein phosphatase 2A (PP2A) complex (Zhou *et al.* 2004). RCN1 has been shown to be implicated with several phytohormone pathways. It functions as transducer of ABA signals (Kwak *et al.* 2002) and as negative regulator of ethylene signaling (Larsen and Cancel 2003, Larsen and Chang 2001). RCN1 induced changes in basipetal auxin transport that are likely to be the result of altered localization of PINFORMED1 (PIN1, Michniewicz *et al.* 2007), influence gravity response (Muday *et al.* 2006) and compromise the organization of stem cells at the RAM (Blakeslee *et al.* 2008).

Taken together the data reveal the LEC1 induced activation of genes that are involved in auxin perception, auxin transport and auxin response, thus affecting several developmental processes including embryogenesis and meristem formation and maintenance.

Biological and developmental processes are affected by overlapping hormone action and hormone response is rather the result of hormone interactions (Teale *et al.* 2008). The multitude of auxin- and brassinosteroid-related genes that were found to be regulated by LEC1 points to auxin-BR-interplay during embryogenesis. Synergism between these two phytohormones has been described regarding a variety of physiological processes (Hardtke 2007, Hardtke *et al.* 2007, Nemhauser *et al.* 2004) and both signaling pathways seem to meet on the level of gene expression (Goda *et al.* 2004). Interestingly, several both auxin- and BR-responsive genes were identified as putative LEC1 target genes by ChIP/chip. Figure 42 displays BR- and auxin-related putative LEC1 target genes in the context of overlapping signal transduction pathways.

The repressor AUXIN RESPONSE FACTOR2 (ARF2) and two indole-3-acetic acid inducible factors, IAA5 and IAA19 are known to be activated by IAA and brassinolide (BL) and represent a possible point of intersection between both hormone pathways (Nakamura *et al.* 2003). IAA19 is also called MASSAGU2 (MSG2) and has been shown to bind to AUXIN RESPONSE FACTOR7 (ARF7, Tatematsu *et al.* 2004). MYB77, a putative LEC1 target, represents another interaction partner of ARF7 thus being important for auxin mediated lateral root development (Shin *et al.* 2007).

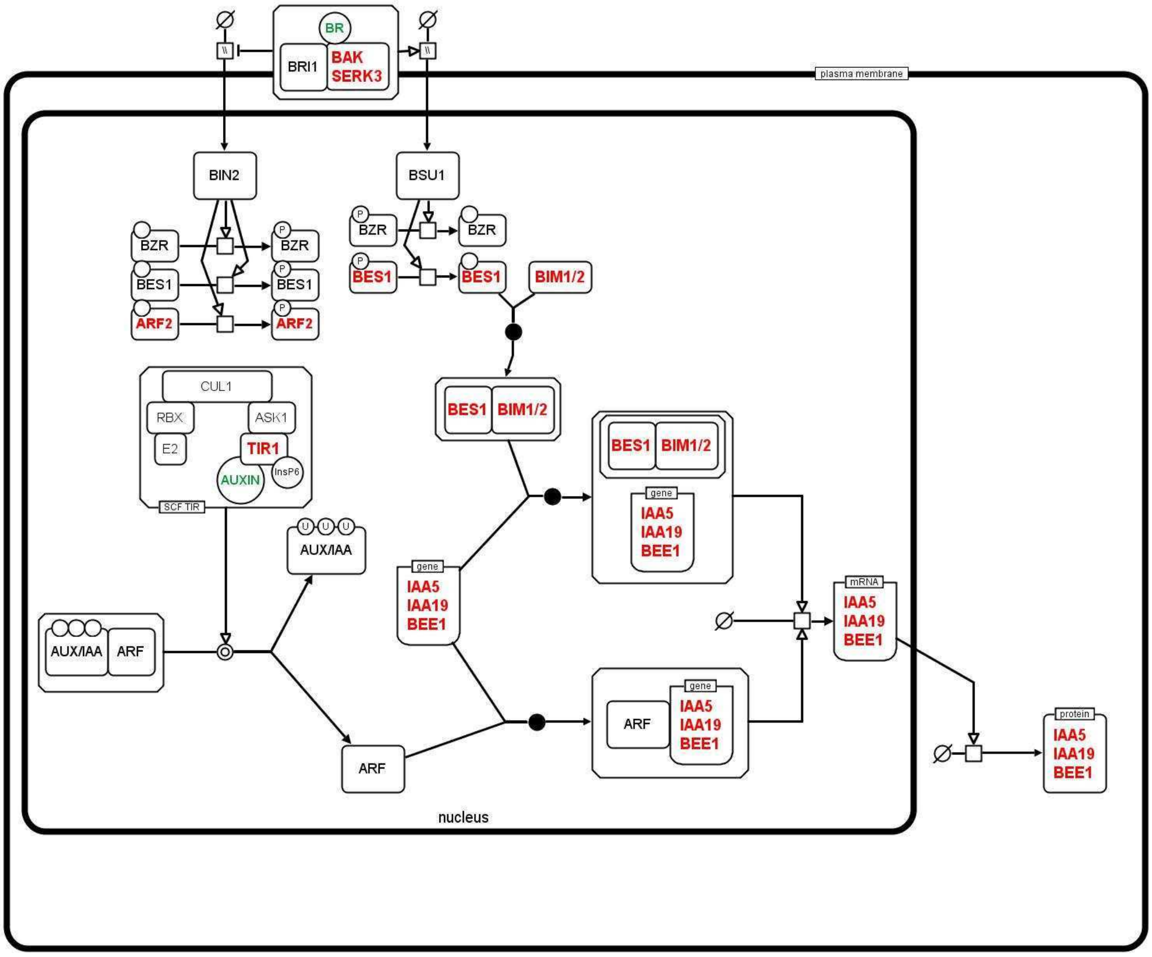


Figure 42. Auxin and BR signaling pathways meet on the level of gene expression.
Proteins that are encoded by putative LEC1 target genes are marked in red. BR perception is realized by the membrane-localized receptor kinase BRI1 that binds BR with high affinity (Wang *et al.* 2001). BRI1 binds to BRI1 ASSOCIATED RECEPTOR KINASE1 (BAK1, SERK3, Nam and Li 2002). *Downstream* of the BR receptors, BRASSINOSTEROID INSENSITIVE2 (BIN2) negatively regulates BR signaling (Li and Nam 2002) whereas *bri1* SUPRESSOR1 (BSU1) positively regulates BR signaling (Mora-Garcia *et al.* 2004). BIN2 phosphorylation of ARF2 abolishes its DNA binding thus de-repressing the transcription of auxin-induced genes (Vert *et al.* 2008). Upon BR perception, hypophosphorylated BRI1-EMS-SUPPRESSOR1 (BES1)/ BRASSINAZOLE-RESISTANT1 (BZR1) proteins accumulate in the nucleus and regulate transcription in response to BR (He *et al.* 2002, Wang *et al.* 2002, Yin *et al.* 2002).

Continued from page -98-

BES1-binding to target promoters is enhanced by interaction with BES1-INTERACTING MYC-LIKE PROTEIN1 and 2 (BIM1/2, Yin *et al.* 2005). TIR1 represents the auxin receptor and inositol-6-phosphate (InsP6) supports auxin binding to TIR1 (Kepinski and Leyser 2005, Tan *et al.* 2007). TIR1 is an essential constituent of the SCF^{TIR1} complex that is responsible for ubiquitinylation of AUX/IAA transcriptional repressors during auxin response (Gray *et al.* 2001). These AUX/IAA proteins under low auxin conditions dimerize with ARF proteins, thereby blocking its activity (Tiwari *et al.* 2003, Tiwari *et al.* 2001, Ulmasov *et al.* 1997). Upon auxin reception, ARF factors are released and regulate auxin responsive gene expression (Gray *et al.* 2001). Several genes are described to be up-regulated upon auxin and BR treatment. IAA5 and 19 and BEE1 represent such candidates which integrate LEC1, auxin and BR signaling pathways (Friedrichsen *et al.* 2002, Goda *et al.* 2004, Nakamura *et al.* 2003, Nemhauser *et al.* 2004).

BR ENHANCED EXPRESSION1 (*BEE1*) is an early BR response gene that encodes a basic helix-loop-helix (bHLH) transcription factor (Friedrichsen *et al.* 2002). BEE1 expression is down-regulated by ABA (Friedrichsen *et al.* 2002). This was nicely confirmed by qRT-PCR experiments here (Table 9). Moreover, it seems also to be involved in auxin signaling pathways (Nemhauser *et al.* 2004). The BRI1-ASSOCIATED RECEPTOR KINASE1 (BAK1) and BRI1-EMS-SUPPRESSOR1 (BES1) represent important component of BR signaling (Vert and Chory 2006), the corresponding genes of which were identified as putative LEC1 target. After BR perception by BRI1 and its co-receptor BAK1 (Nam and Li 2002), a series of (de)phosphorylation events finally results in dephosphorylated BES1 which is able to dimerize and to activate BR response genes (Vert and Chory 2006). BES1 has been shown to interact with two bHLH transcription factors, BES1-INTERACTING MYC-LIKE PROTEIN1 and 2 (BIM1 and BIM2) that are functionally redundant and facilitate BES1-DNA interaction (Yin *et al.* 2005). Both BIM1 and BIM2 have been found to be regulated by LEC1 in microarray and ChIP/chip, respectively. According to the qRT-PCR results of validation experiments the activation of BES1, BIM1 and BIM2 by LEC1 or LEC1 in combination with ABA was rather moderate and has to be confirmed in further experiments (Table 9 and 10). The promoters of two BES1/BZR1 homolog proteins (BEH2 and BEH4) and a brassinosteroid-inducible RING-H2 gene (BRH1) have also been found to be bound by LEC1.

Besides the described BR signaling components also two genes encoding BR metabolic enzymes were found as putative LEC1 targets. Besides the BR biosynthetic gene *DWARF4* (*DWF4*), a DON-glucosyltransferase is involved in BR homeostasis by conjugating glycosyl groups to brassinolide. Overexpression of this gene results in BR deficient phenotypes suggesting BL glyocosylation as a means of regulating hormone activity (Poppenberger *et al.* 2005).

Taken together LEC1 affects auxin and BR homeostasis by interfering with signaling and biosynthesis of either hormone. Several signaling components that have been identified as LEC1 target are involved in a variety of physiological processes, including embryogenesis, meristem function, hypocotyl elongation and general (overlapping) hormone responses. The results suggest LEC1 controlled hormone interplay during somatic and early zygotic embyrogenesis.

LEC1 putative direct target genes as identified by ChIP/chip are enriched for genes involved in regulation of transcription (p-value 1,10x10^{-03}). About 56 genes out of 449 candidateLEC1 target genes encode for transcription factors and related proteins (12,5%). LEC1 is considered to be hierarchically superior among key regulators of Arabidopsis seed development. This is confirmed by the initiation of transcriptional cascades by LEC1 through activation of many transcription factor genes. Table 11 gives an overview of LEC1-regulated TF genes including information about fold change of enrichment after ChIP, fold change activation by LEC1 (qRT-PCR), annotation and classification into TF families.

Table 11. LEC1 regulated genes in the functional category 27.3 'RNA-regulation of transcription'. A number of 56 putative LEC1 target genes encode transcription factors and other proteins involved in regulation of transcription by chromatin remodeling. TF genes are classified according to TF families. The mean fold change enrichment values of three biological ChIP replicates with an anti-LEC1 antibody are given. Validation experiments (24 hours of induction) using qRT-PCR were only performed once and fold change values of activation are given. No gene was found to be repressed by LEC1. Several LEC1 targets were activated in ABA-independent manner. Except ANAC19, no LEC1-regulated TF gene was activated by ABA alone.

AGI	**name**	**ChIP/chip**	**qRT-PCR**	
Classical transcription factors				
AP2/EREB			DEX	DEX+ABA
At2g28550	RAP2.7; TOE1 (TARGET OF EAT1 1)	1,72	2,43	1,84
At3g14230	RAP2.2	3,39	-	-
At4g23750	CRF2 (CYTOKININ RESPONSE FACTOR 2)	1,99	-	-
ARF				
At5g62000	ARF2 (AUXIN RESPONSE FACTOR 2)	2,68	3,53	5,12
ARR				
At2g41310	ATRR3 (RESPONSE REGULATOR 3)	1,99	2,67	6,38
bHLH				
At1g18400	BEE1 (BR ENHANCED EXPRESSION 1)	2,04	6,47	1,16
At1g69010	BIM2 (BES1-INTERACTING MYC-LIKE PROTEIN 2)	2,85	2,16	1,71
At3g59060	PIF5; PIL6 (PHYTOCHROME-INTERACTING FACTOR 5; PIF3-LIKE 6)	3,51	-	-
At3g61950	bHLH protein	2,60	-	-
At5g15160	bHLH family protein similar to PRE1 (PACLOBUTRAZOL RESISTANCE1)	2,62	-	-
At5g46690	bHLH071 (BETA HLH PROTEIN 71)	1,73	-	-
zinc finger C2C2 CO-like				
At1g68190	zinc finger (B-box type) family protein	3,36	-	-
At1g68520	COL6 (CONSTANS-LIKE 6)	2,08	-	-
At5g15850	COL1 (CONSTANS-LIKE 1)	3,14	-	-
At5g24930	COL4 (CONSTANS-LIKE 4)	2,57	-	-

zinc finger CCCH				
At1g19860	zinc finger (CCCH-type) family protein	2,66	-	-
At2g02160	zinc finger (CCCH-type) family protein similar to DEAD box RNA helicase	2,48	4,89	6,38
At2g25900	ATCTH (Arabidopsis thaliana Cys3His zinc finger protein)	3,18	0,53	3,27
At2g41900	zinc finger (CCCH-type) family protein	3,03	-	-
zinc finger RanBP2				
At1g70650	zinc finger (Ran-binding) family protein	3,89	-	-
zinc finger AN1				
At4g22820	A20 and AN1 domain-containing stress-associated protein 9 (SAP9)	2,62	-	-
zinc finger RING				
At1g49780	U-box domain-containing protein	2,28	-	-
At3g18710	U-box domain-containing protein	2,39	-	-
CCAAT				
At5g47670	L1L (LEAFY COTYLEDON 1-LIKE; NFYB6)	2,77	3,34	4,06
At1g07980	histone-like transcription factor (CBF/NF-YC)	1,65	3,73	4,92
homeodomain				
At1g75410	BEL1-like homeodomain 3 (BLH3)	1,72	-	-
At3g54390	similar to transcription factor	4,47	-	-
MADS				
At2g45660	SOC1; AGL20 (SUPPRESSOR OF CONSTANS OVEREXPRESSION 1; AGAMOUS-LIKE 20)	1,65	-	-
MYB				
At3g09370	MYB3R-3 (myb domain protein 3R-3)	2,22	-	-
At3g50060	myb family transcription factor	2,68	1,62	3,34
At4g01060	CPL3; ETC3 (CAPRICE-LIKE MYB3; ENHANCER OF TRY AND CPC 3)	1,77	-	-
At1g74840	myb family transcription factor	3,07	-	-
At2g38090	myb family transcription factor	2,69	-	-
At5g17300	myb family transcription factor	1,69	19,88	206,26
At5g47390	myb family transcription factor	2,23	-	-
NAC				
At1g01720	ATAF1 (Arabidopsis NAC domain containing protein 2)	3,71	-	-
At1g52890	ANAC019 (Arabidopsis NAC domain containing protein 19)	3,05	2,40	33,32
At1g77450	ANAC032 (Arabidopsis NAC domain containing protein 32)	4,38	-	-
At5g24590		3,48	-	-
TCP				
At4g18390	TCP family transcription factor	3,14	1,98	2,33
bZIP				
At1g42990	ATBZIP60 (BASIC REGION/LEUCINE ZIPPER MOTIF 60)	7,67	1,44	5,83
At4g01120	GBF2 (G-BOX BINDING FACTOR 2)	2,45	130,10	577,74
At1g19490	bZIP transcription factor family protein	2,71	-	-
At5g11260	HY5 (ELONGATED HYPOCOTYL 5)	2,68	-	-
LOB				
At2g45420	Lateral organ boundaries (LOB) domain protein 18 (LBD18)	2,01	-	-

IAA				
At1g15580	IAA5 (indoleacetic acid-induced protein 5)	2,97	12,67	128,34
At3g04730	IAA16 (indoleacetic acid-induced protein 16)	2,04	292,39	645,05
At3g15540	IAA19 (indoleacetic acid-induced protein 19)	3,86	6,80	10,21
TFIIa				
At1g07470	transcription factor IIA large subunit	2,89	-	-
Trihelix				
At3g14180	transcription factor	3,53	-	-
Chromatin-associated factors				
bromodomain				
At3g60110	DNA-binding bromodomain-containing protein	2,22	-	-
methyl transferase				
At3g17310	methyltransferase family protein, similar to DRM2 (DOMAINS REARRANGED METHYLTRANSFERASE 2)	2,04	-	-
At5g14620	DRM2 (DOMAINS REARRANGED METHYLTRANSFERASE 2)	1,96	-	-
histone deacetylase				
At5g61060	HDA5 (HISTONE DEACETYLASE5)	2,36	-	-
methyl-CpG-binding				
At1g15340	MBD10 (methyl-CpG-binding domain 10)	3,32	-	-
HMG				
At1g20693	HMGB2 (HIGH MOBILITY GROUP B 2)	2,79	-	-
helicase				
At3g58570	RH52 (DEAD-box ATP-dependent RNA helicase 52)	1,91	-	-
Pentatricopeptide Repeat				
At4g16390	chloroplastic RNA-binding protein P67	1,51	-	-

Many hormone-related TF genes that have been described above are again included in the list of LEC1-regulated TF (ARF, IAA, bHLH). Largest proportions are represented by the MYB-, NAC- and zinc finger families of transcription factors. The family of MYB transcription factors is significantly over-represented among LEC1 target genes (p-value 4,14x10^{-04}). Most of the identified MYB TF have not been functionally characterized yet. The ENHANCER OF TRIPTYCHON AND CAPRICE 3 (ETC3) encodes a single-repeat R3 MYB protein that is involved trichome formation on inflorescence stems and pedicles (Tominaga *et al.* 2008, Wang *et al.* 2008). In functional redundancy with other R3 MYB family members ETC3 acts in suppression of trichome formation on siliques and cotyledons (Wang *et al.* 2008). In addition it has been shown to be involved in flower development, possibly *via* repression of SOC1 and FT, and epidermal cell size by regulation of endoreduplication (Tominaga *et al.* 2008).

Among zinc fingers the sub-category 27.3.7 ‘RNA-Regulation of transcription-C2C2(Zn) CO-like, Constans-like zinc finger family’ was found to be over-represented among LEC1 ChIP/chip target genes (p-value 1,36x10^{-03}). CONSTANS, its early target *SUPPRESSOR OF CONSTANS*

OVEREXPRESSION1 (*SOC1*) and the *COL* genes have only been implicated with photoperiodic flowering (Putterill *et al.* 1995) and light signaling yet (Zobell *et al.* 2005). Interestingly, it has been shown that DNA-binding of COL proteins is mediated by the CCAAT-binding, trimeric HAP complex (Ben-Naim *et al.* 2006, Wenkel *et al.* 2006). The regulation of these genes by LEC1 and the function of potential COL-HAP complexes during embryogenesis will be discussed.

LEC1 was found to bind the promoter of another gene that has been implicated with photoperiodic flowering. TARGET OF EAT1 (TOE1) belongs to the APETALA2 family of TF and is part of a CO-independent pathway in flowering control. Together with SCHLAFMUETZE (SMZ), TOE1 is a target of the miR172 which itself is regulated *via* GIGANTEA-mediated photoperiod-dependent RNA-processing (Jung *et al.* 2007). TOE1 and SMZ are known to repress flowering (Jung *et al.* 2007, Schmid *et al.* 2003). Flowering control/induction is tightly linked to light signaling as determined by light quality, intensity and duration per day (photoperiod; Samach and Coupland 2000, Simpson *et al.* 1999). Besides induction of flowering the photomorphogenetic response of plants to light also includes seed germination, inhibition of hypocotyl elongation, cotyledon and leaf expansion, pigment (chlorophyll, anthocyanin) synthesis and stem elongation (Schäfer and Nagy 2006). Two important components of light signaling, PIF5 and HY5, have been identified as putative LEC1 target genes. The HY5 HOMOLOG (HYH) gene was found to be repressed by LEC1+ABA in macroarray analysis (Table 4 and Figure 33). Its implications in LEC1 induced processes during embryogenesis will be discussed.

Several members of the plant-specific NAC-domain TF family have been identified as putative LEC1 targets. The Arabidopsis genome encodes numerous NAC proteins (Riechmann *et al.* 2000) which are implicated with a number of developmental processes such as meristem formation, defense and stress responses (for review see: Olsen *et al.* 2005). Only few NAC factors have been functionally characterized. ATAF1 and ANAC19 function in wounding and ABA mediated abiotic stress response such as drought and dehydration (Collinge and Boller 2001, Lu *et al.* 2007, Tran *et al.* 2004). ABA-inducibility of ANAC19 was confirmed in the present study by qRT-PCR and macroarray analysis (Table 5). Although this puts into question the identity as LEC1 target gene, LEC1 may be functionally connected to NAC factors which are described in conjunction with an undifferentiated state of cells. ATAF1 (At1g01720) and ANAC19 (At1g52890) are up-regulated in pluripotent protoplast cells in comparison to differentiated leaf cells (Avivi *et al.* 2004). Up-regulation of these genes is due to chromatin-reorganization (hypomethylation) of regions of Arabidopsis chromosome I (Avivi *et al.* 2004). Another putative LEC1 target gene encoding a third NAC domain factor is also located on chromosome I (At1g77450). These findings support the contribution of chromatin remodeling in acquisistion of pluripotentiality and a possible role of LEC1 in de-differentiation which is a pre-requisite for somatic embryogenesis.

Several further LEC1 target genes encoded epigenetic factors involved in histone modification and DNA methylation. DNA methylation is responsible for inactivation of transposable elements in the genome and regulating the expression of single genes (Chan *et al.* 2005, Martienssen and Colot 2001, Zhang *et al.* 2006, Zilberman and Henikoff 2007). With respect to promoter DNA methylation, the methylated state is considered as transcriptionally inactive. DNA methylation patterns are established in a biphasic process. The (1) initial methylation of unmethylated DNA sequences (de-novo methylation) is (2) maintained by replication of preexisting methylation. Two members of the DOMAINS REARRANGED METHYLTRANSFERASE (DRM) family responsible for *de-novo* methylation (Cao and Jacobsen 2002) have been identified as putative LEC1 target genes. DRM2 methylation is known to be guided by siRNAs (RNA directed DNA methylation, RdDM, Chan *et al.* 2004). DRM2-mediated hypermethylation at CpG sites was found in promoters of genes specifically repressed in callus cells (Berdasco *et al.* 2008). This suggests hypermethylation as another epigenetic process involved in establishment or maintenance of an undifferentiated state in cells. As a proof of genetic interactions between DNA methylation and demethylation, DRM2 was shown to be required for expression of *REPRESSOR OF SILENCING1* (*ROS1*, Penterman *et al.* 2007). ROS1 was found to be up-regulated after DEX+ABA-treatment (microarray) and encodes a DEMETER-like DNA glycosylase (Gong *et al.* 2002). ROS1-mediated demethylation relies on erasure of 5-methylcytosine through a base excision repair process (Morales-Ruiz *et al.* 2006).

The inhibition of transcription by promoter DNA methylation can be mediated by two mechanisms. First, cytosine methylation inhibits the access of TF to their target binding motif and second, methyl-CpG-binding proteins (MBD) activate co-repressors to silence transcription (Klose and Bird 2006). The *MBD10* gene encoding one of 13 members of the MBD family of Arabidopsis was found as LEC1 target gene. Together with DRM2, MBD10 has been shown to be involved in large-scale silencing of rRNA loci in nucleolar dominance in genetic hybrids (Preuss *et al.* 2008).

One gene encoding a histone deacetylase (HDA5) was found among putative LEC1 targets. Histone deacetylases catalyze histone deacetylation leading to transcriptional repression (Kadosh and Struhl 1998, Rundlett *et al.* 1998). In mammals de-novo methyltransferases (DNMT) and MBD proteins are described to associate with HDAC proteins in order to repress transcription (Fuks *et al.* 2000, Jones *et al.* 1998, Nan *et al.* 1998).

Taken together, LEC1 seems to be involved in several signaling cascades which mediate a wide range of physiological and developmental processes. As a high ranking regulator LEC1 seems to integrate various transcriptional cascades. Moreover, the data suggest its involvement in regulation of chromatin structure.

LEC1-induced activation of auxin and BR signaling as well as a broad range of transcriptional cascades is supposed to play an important role in early patterning processes and embryo morphogenesis. The integration of various signaling pathways by LEC1 will be discussed.

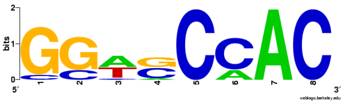
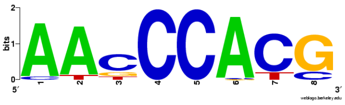
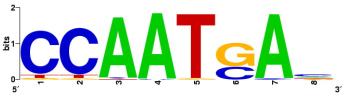
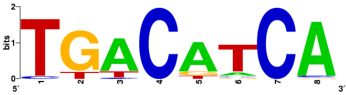
3.4.4 Promoter analysis

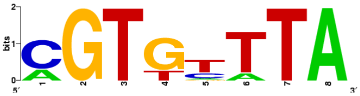
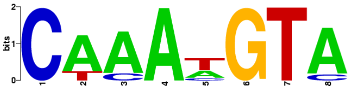
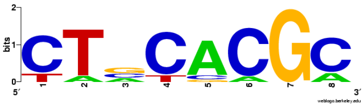
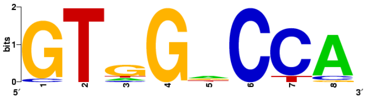
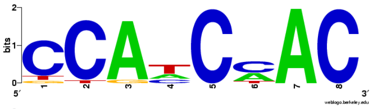
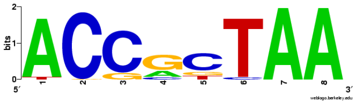
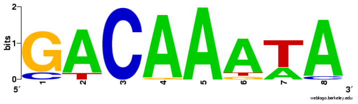
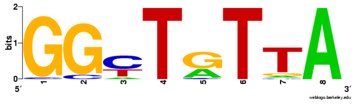
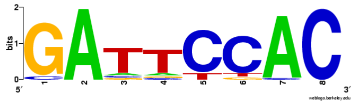
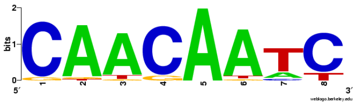
Transcriptional regulatory networks consist of TF and a battery of target genes which are supposed to share a common TF binding site (TFBS) in their promoter regions. Promoter analysis is an important step in identification of known and *de-novo* TFBS. The binding-site-searching software 'Amadeus' provides a platform for genome-scale detection of novel motifs (Linhart *et al.* 2008). This tool was employed in order to detect promoter motifs in putative LEC1 target genes as identified by microarray and ChIP/chip. Five candidate gene lists were analyzed. The 500bp promoter regions upstream of the annotated transcription start site (TSS) of 1) 117 DEX-regulated genes (microarray), 2) 535 DEX+ABA-regulated genes (microarray), 3) 449 ChIP/chip candidate target genes, 4) 13 genes overlapping between DEX-regulated genes and ChIP/chip candidate targets and 5) 44 genes overlapping between DEX+ABA-regulated genes and ChIP/chip candidate targets have been screened for over-represented motifs. Motifs were classified by similarity to Transfac annotated TFBS (Biobase GmbH; http://www.biobase-international.com/pages/index.php?id=transfac). The calculated similarity score is smaller, the higher the similarity observed between the identified *de-novo* motif and the corresponding similar Transfac annotated TFBS.

The ChIP/chip method relies on protein binding to DNA sequences that contain certain motifs or motif combinations providing the basis for regulatory interactions. Microarray analysis eventually does not discriminate between primary and secondary target genes, thereby diluting genes the promoters of which contain a certain TFBS. Therefore ChIP/chip candidates for regulation by LEC1 are preferentially considered in this section. Table 12 lists motif logos (http://weblogo.berkeley.edu/logo.cgi), enrichment values and p-values of 24 motifs that were identified to be significantly enriched in LEC1 bound promoters (ChIP/chip candidate targets). In addition, the most similar Transfac plant TFBS, the corresponding IDs, the binding TF family and the similarity score are given. Out of 449 candidate LEC1 target promoters, 12 promoters were not analyzed due to high redundancy with other gene promoters.

LEC1 encodes a CCAAT-box binding factor (Lee *et al.* 2003). Among 24 binding motifs, two CCAAT-box containing motifs were found to be over-represented in promoters of ChIP/chip candidate targets (Table 12, motif #7 and #23). This motif was not found to be over-represented among putative LEC1 target genes identified by microarray. As given by the similarity score (Table 12), especially motif #7 resembled very much CCAAT-box motifs in the Transfac database. No plant-derived CCAAT-box was found to be annotated in Transfac (TRANSFAC, Release 7.0). The score of CCAAT-boxes in LEC1-bound promoters was based on sequence similarity to corresponding viral, yeast or vertebrate motifs. Motif #7 occurred at least once in 150 promoters of 437 LEC1 secure target promoters (ChIP/chip). The location of 150 identified CCAAT-boxes relative to the annotated TSS is displayed in Figure 42. CCAAT motifs are distributed equally within 500bp upstream regions of the TSS, although an enrichment could be detected in TSS-near regions (-70 to -200bp). By manual annotation, two further motifs were identified which show similarity to a CCAAAT-box found in the promoter of the seed-specifically expressed *phaseolin* gene (Bustos *et al.* 1989, Li and Hall 1999, Table 12, motif #12 and #19).

Table 12. Promoter motifs in putative LEC1 target genes identified by ChIP/chip.
Over-representation of promoter motifs was analyzed using the Amadeus software (Linhart *et al.* 2008). A number of 24 over-representated motifs were identified (see enrichment- and p-values) and compared to Transfac annotated TFBS. Given motif logos display the occurrence of nucleotides at each position of the motif. Similar motifs, corresponding IDs and the TF class known to bind these motifs are given.

#	Similar motif	Transfac ID (similarity score)	TF	motif logo	enrich-ment	p-value
1	G-box	M00399 (0,0873); M00400 (0,1649); M00401 (0,1132)	bZIP		4,53	6,3E-82
2	G-box	M00371 (0,209); M00366 (0,2361)	bZIP		1,76	6,0E-18
3	C/G-box	M00356 (0,2062); M00441 (0,2257); M00400 (0,2315)	bZIP		2,66	2,7E-16
4	MYB	M00004 (0,2319)	MYB		1,87	3,7E-15
5	G-box	M00366 (0,2285)	bZIP		2,94	6,0E-15
6	unknwon				2,08	6,8E-15
7	**CCAAT-box**	M00309 (0,1187); M00288 (0,1719); M00254 (0,2027)	HAP/ NF-Y		1,80	1,8E-14
8	C/G-box	M00357 (0,2005); M00197 (0,2045)	bZIP		2,15	6,2E-14
9	unknown				2,16	1,7E-12
10	unknown				1,85	7,8E-12

11	unknown				1,95	1,2E-11
12	unknown CCAAAT	-	HAP/ NF-Y		1,75	2,0E-11
13	unknown				2,35	2,7E-11
14	G-box	M00358 (0,1722)	bZIP		1,92	3,5E-11
15	MYB?	M00226 (0,23)	MYB		1,95	5,4E-11
16	unknown				2,75	7,6E-11
17	unknown				2,27	8,7E-11
18	unknown				2,07	9,8E-11
19	ARF CCAAAT	M00438 (0,1613) -	ARF HAP/ NF-Y		1,56	1,1E-10
20	unknown				2,12	1,3E-10
21	G-box	M00441 (0,1739)	bZIP		2,00	1,4E-10
22	unknown				2,30	1,9E-10
23	**CCAAT-box**	M00200 (0,2273)	HAP/ NF-Y		1,50	4,3E-10
24	unknown				2,20	4,6E-10

Besides four motifs with similarity to CCAA(A)T-box motifs, 20 further motifs were significantly enriched in promoters of putative LEC1 targets identified by CHIP/chip. Twelve motifs were designated as 'unknown' due to marginal similarity to known plant TFBS in the Transfac database. About seven motifs were found to be similar to different types of G-box. G-box-like motif #1 had the highest similarity score when compared to related motifs in Transfac and comprised the complete ABRE core element ACGT. Two motifs showed weak similarity to MYB factor binding sites.

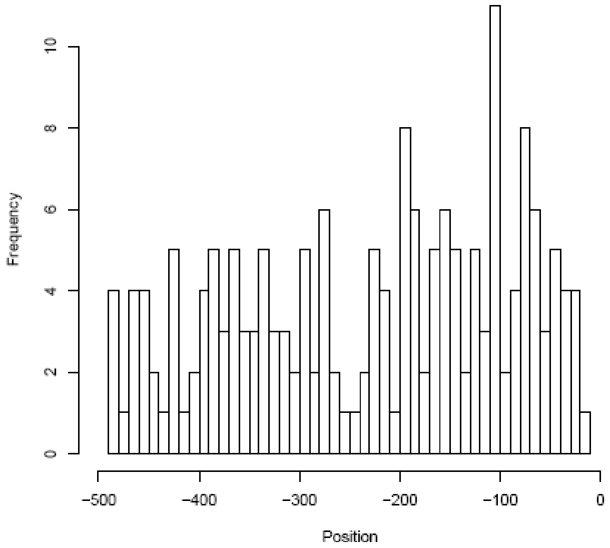


Figure 43. Location of CCAAT-box-motif (# 7) in LEC1 target promoters upstream of the TSS.
The histogram displays the location of 150 identified CCAAT-box motis (motif #7) in the promoters of putative LEC1 target genes. The highest occurrence of motifs was found in the regions between -70 and -200bp upstream of the annotated TSS.

Taken together, two motif classes, G-box and CCAA(A)T-box, were found to be over-represented in promoters of direct LEC1 target genes. The CCAA(A)T-box motif is known to be the binding site for NF-Y/HAP factors in mammals and yeast. The over-representation of CCAAT-box motifs in promoters of putative LEC1 target genes was shown here for the first time. These results prove the identity of ChIP candidates as LEC1 target genes and further support the CCAAT-box-binding activity of Arabidopsis HAP factors.

4. Discussion

The CCAAT-box binding transcription factor LEAFY COTYLEDON1 (LEC1) represents an essential regulator of Arabidopsis zygotic embryogenesis. It controls aspects of early embryogenesis such as suspensor differentiation and cotyledon identity as well as processes of late embryogenesis including seed maturation processes such as synthesis and accumulation of storage compounds as well as the acquisition of desiccation tolerance and dormancy (Lotan *et al.* 1998, West *et al.* 1994). The repression of LEC1 functions during and after germination is an essential prerequisite for the progression of the vegetative seedling development. Several repressors of LEC1 functions, such as PICKLE (PKL), VAL and HDAC have been identified (Ogas *et al.* 1997, Suzuki *et al.* 2007, Tanaka *et al.* 2008), whereas only few, maturation-related LEC1-regulated *downstream* factors have been described yet.

Constitutive expression of *LEC1* causes the development of ectopic somatic embryos (Lotan *et al.* 1998). The processes of zygotic and somatic embryogenesis resemble each other with respect of morphology, metabolism and the underlying molecular regulatory processes (Zimmerman 1993). Due to this similarity the LEC1-induced somatic embryogenesis provides an excellent experimental system to analyze the function of LEC1 in zygotic embryogenesis.

Therefore, the present thesis describes the construction and application of transgenic Arabidopsis lines with inducible *LEC1* expression. The obtained results contribute to the understanding of cellular and molecular processes during induction and progression of somatic embryogenesis and its implications for zygotic embryogenesis. Furthermore, the experiments describe LEC1-regulated target genes as a contribution to define and characterize the LEC1 regulon.

4.1 Histology of LEC1-induced embryogenesis

4.1.1 LEC1 expression during germination suppresses vegetative development

Regulated expression of *LEC1* was achieved by the application of two inducible systems. The dexamethasone-inducible GR-system is only suitable for transcription factors. The induction is based on a translocation of the TF::GR fusion protein to the nucleus (Baudry *et al.* 2004, Lloyd *et al.* 1994). The estradiol-inducible system makes use of the human estrogen receptor that confers estradiol-inducibility to the artificial XVE transcription factor. The corresponding XVE protein transactivates transcription of the gene of interest (Curtis and Grossniklaus 2003, Zuo *et al.* 2000).

Both inducible expression systems led to similar phenotypes. Only the DEX-inducible LEC1::GR lines were used for more detailed histological and molecular analyses. The phenotypes observed after permanent induction of LEC1 (Figures 17 and 18) resembled previously described phenotypes observed in constitutive LEC1 over-expression lines. This demonstrates the principal suitability of the inducible systems. Moreover, inducible systems provide additional experimental advantages. Lethal effects and propagation problems encountered by constitutive LEC1 expression (Lotan *et al.* 1998) can be avoided with the inducible system. Inducible expression also bypasses possible secondary or higher order effects of developmental or metabolic adaptations that might have occurred in constitutive over-expression lines or stable mutants (Zuo and Chua 2000).

Permanent induction of LEC1 was sufficient to suppress vegetative development in almost all parts of the transgenic seedling plant. Induced seedlings were severely disturbed in growth and development and displayed thickened, carnose and light greenish structures in all apical parts (cotyledons and hypocotyl, Figure 18C and D). In contrast, uninduced LEC1::GR seedlings appeared wild type-like (Figure 18A and B). In induced LEC1::GR seedlings, developing cotyledon-like leaves showed embryonic characteristics like the absence of trichomes and simple venation patterns (Figure 19). All organs were transformed into embryonic structures with cells resembling storage parenchyma and containing large protein storage vacuoles, lipid bodies as well as starch granules (Figure 23). Embryogenesis markers like the *Vicia faba* legumin and *USP* gene promoters were found to be active in LEC1-induced swollen structures (Figure 32). These observations confirmed experiments with constitutive LEC1 overexpression lines where similar phenotypes have been described. Constitutive *LEC1* overexpression lines produced small, fleshy seedlings with cotyledons and roots that failed to expand. Secondary leaves showed cotyledon-like features. Accumulation of cruciferin and oleosin mRNAs demonstrated excessive storage of proteins and lipids (Lotan *et al.* 1998). Also seedlings of the LEC1 gain-of-function mutant *turnip* displayed similar phenotypes and stored large amounts of lipids and starch in swollen hypocotyl regions (Casson and Lindsey 2006). In contrast to lipids and proteins, starch represents a minor storage compound in *Arabidopsis* wild type. The massive storage of starch in LEC1-induced embryonic structures may be the result of overloaded lipid and protein synthetic pathways. Starch synthesis has been discussed as default storage pathway (Lin *et al.* 1999).

Permanent *LEC1* gene expression caused two different categories of root phenotypes (Figure 18). In about 40% of the induced seedlings the growth of the primary root was completely impaired (Figure 18C). The shortened and swollen roots lacked root hairs and the cells exhibited features of storage organs (Figure 18C). This phenotype was designated as type A. Approximately 60% of the induced seedlings first developed a normal primary root (Figure 18D and E). About ten days after induction the root tip showed swelling and turned green, whereas the remaining root kept its vegetative state (Figure 18D and E). This phenotype was designated as type B. Swollen root tips were restricted to primary roots and contained large amounts of storage compounds (cruciferin, lipids and starch, Figure 18D and E, Figure 24). With respect to morphology, cell fate and storage compound composition these structures resembled very much embryonic root tips as observed in the *pickle* mutant (Ogas *et al.* 1997, Rider *et al.* 2004). PKL encodes a CHD3 chromatin remodeling factor that represses *LEC1* during germination and vegetative development by promoting trimethylation of histone H3 lysine (H3K27me3; Henderson *et al.* 2004, Ogas *et al.* 1999, Zhang *et al.* 2008). The observed, striking similarities between *pkl* and LEC1::GR phenotypes prove LEC1 as trigger of embryonic development in the *pkl* mutant. Moreover, the LEC1-induced embryonic phenotypes (A and B) indicate that LEC1 almost completely abolishes vegetative development. This suggests that repression of LEC1 after germination is the prerequisite for vegetative development.

4.1.2 LEC1+ABA-induced embryonic differentiation of meristematic cells during vegetative development

The described phenotype B can only be induced during a narrow time window of up to 48 hours after seed imbibition (hpi, Figure 25A). When induced after 72hpi, most seedlings displayed the long-hypocotyl phenotype (C, Figure 25B) or appeared wild type-like and no severe embryonic phenotypes (A or B) were observed. At later developmental stages no striking phenotypic alterations could be induced by DEX application anymore (Figure 25A). This confirms similar results obtained with the *pkl* mutant. The penetrance of the embryonic *pkl* phenotype can only be increased by addition of unicazole, a gibberellic acid (GA) biosynthetic inhibitor, before germination is completed (36hpi, Ogas *et al.* 1997). GA is known to have multiple functions during plant development, promoting seed germination and transition to vegetative growth. The GA antagonist abscisic acid (ABA) functions in maintenance of dormancy and therefore occurs at very high levels in dormant seeds. ABA accumulation shows a biphasic course with the first peak in mid-maturation (expression of seed storage protein genes) and a second peak during late maturation (expression of *LEA* genes, Karssen *et al.* 1983). After seed imbibition ABA levels decrease and increased GA synthesis shifts the ABA/GA ratio in favour of GA. Inhibition of GA synthesis during germination would lead to an increase in the ABA/GA ratio which, in the *pkl* mutant background, supports the development of embryonic structures (Ogas *et al.* 1997). In accordance with this, LEC1 is able to promote embryonic development in vegetative seedlings when ABA is provided.

Two week old uninduced, wild type-like LEC1::GR seedlings were treated with DEX+ABA. After 7 to 9 days post induction all root tips became swollen and *pkl* roots developed (Figure 28). No phenotypic alterations were observed in seedling roots of control treatments (EtOH, DEX, ABA, Figure 28).

With respect to apical regions of induced seedlings, single ABA-treatment affected development of seedlings in a genotype-independent manner. Leaves of ABA-treated wild type and LEC1::GR seedlings displayed enhanced chlorophyll degradation and possessed few trichomes (Figure 28C and G). No cruciferin was detected (Figure 28W). After treatment with DEX alone severly mis-shaped leaves appeared that seemed to be defective in cellular organization and leaf identity (Figure 28F). This phenomenon was not visible in wild type seedlings after DEX treatment indicating a clear LEC1 effect (Figure 28B and F). Histological analysis revealed that only in DEX+ABA-treated LEC1::GR seedlings, cotyledon-like leaves had developed after 14 days of induction (Figure 28H). Already differentiated, old leaves did not change their fate. Cruciferin accumulation was detectable in newly formed, cotyledon-like leaves and gave evidence for the embryonic identity of these LEC1+ABA-induced structures (Figure 28X). These data confirm the activation of genes encoding seed storage proteins (SSP, 12S cruciferin and 2S napin) by LEC1 and ABA which is discussed to be mediated by ABI3 and FUS3 (Kagaya *et al.* 2005). In the present study the transcript abundance of *LEC2*, *ABI3* and *FUS3* was found to be increased in apparent embryonic structures after permanent LEC1 induction in LEC1::GR seedlings (Figure 18F). Rapid induction of these genes by LEC1+ABA as shown by Kagaya *et al.* (2005b) was only shown for FUS3 (Figure 27A). *FUS3* expression was elevated about 4 times after 6 hours of induction with DEX+ABA and further increased during the observed time window (Figure 27A). ABI3 and FUS3 are known to regulate *SSP* accumulation (Mönke *et al.* 2004, Parcy *et al.* 1994, Suzuki *et al.* 1997), and FUS3 represents a good candidate to be involved in the regulatory cascade between LEC1 and maturation genes.

Taken together, these results demonstrate that *LEC1* gene expression combined with ABA-treatment is sufficient to induce the formation of embryonic structures during vegetative development. These embryonic structures, as cotyledon-like leaves and embryonic root tips, develop from the shoot and root apical meristems, respectively. Their embryonic identity is for instance revealed by the accumulation of storage compounds. As a very rare event, Lotan *et al.* (1998) described LEC1-induced embryonic structures emerging from vegetative cells on the leaf surface. Currently it is not clear, whether these embryonic structures derive from remaining meristematic cells or result from a trans-differentiation process initiated on differentiated leaf cells. This phenomenon was never observed after the activation of the LEC1::GR construct. The results shown here rather suggest that LEC1 competent cells are mainly located in the shoot and root meristems, and they represent undifferentiated cells which are able to initiate embryonic differentiation under the combined influence of LEC1+ABA. LEC1 might serve as trigger and ABA in turn as a maintainer of embryonic differentiation.

4.1.3 LEC1 does not affect stem cell identity

Assuming that embryo- and LEC1-competent cells are located in SAM and RAM, detailed histological analyses of apical and root meristem were performed. Embryogenesis and stem cell marker lines (for overview see Table 3) were used in order to define regions or cells that are the initial point for LEC1-induced embryo development. Most of these experiments are still in progress.

The SAM is divided into three regions: the central zone (CZ), the peripheral zone (PZ) and the rib zone (RZ). Slowly dividing stem cells are located in the CZ and their daughter cells in the PZ and RZ will be recruited into newly developing aerial organs (stem and leaves). Stem cells are maintained by signals from the organizing centre, a group of *WUSCHEL* (*WUS*)-expressing cells underlying the CZ (Laux *et al.* 1996, Mayer *et al.* 1998). Stem cells produce the CLAVATA3 (CLV3)-peptide (Fletcher *et al.* 1999, Ito *et al.* 2006, Kondo *et al.* 2006) which, due to its diffusible nature, is able to repress WUS by activation of transmembrane receptor kinases (Brand *et al.* 2000, Schoof *et al.* 2000). The KNOX gene *SHOOT MERISTEMLESS* (*STM*) represents another critical regulator of meristem formation and maintenance by influencing levels of cell division and repressing differentiation (Barton and Poethig 1993, Clark *et al.* 1996, Endrizzi *et al.* 1996, Gallois *et al.* 2002, Lenhard *et al.* 2002). Promoter-reporter-lines for WUS, CLV3 and STM have been crossed with LEC1::GR plants in order to analyze their expression domains after LEC1-induction and ABA-treatment. The analysis of these lines is still in progress.

Histological analysis of SAM regions in induced LEC1::GR seedlings indicates that the meristematic character is maintained during development of cruciferin accumulating embryonic structures. The fluorescence signal indicating SSP accumulation was not detected in the SAM region and was just visible in underlying cell layers and cotyledon-like leaves (Figure 29). Moreover, the meristematic region still appears as aggregation of numerous small cells (Figure 29). These results suggest that

SAM cells did not undergo embryonic differentiation. The expression domains of WUS/CLV3 stem cell markers during LEC1-induced embryogenesis have still to be analyzed.

In comparison to the SAM, RAM architecture is more simple. Root stem cells, also called root initial cells, are located around few mitotically inactive cells which constitute the quiescent centre (QC). Root initials follow a defined division pattern whereas one daughter cell remains initial and the second daughter cell differentiates into one of the different cell lineages of the root (vasculature, pericycle, endodermis, cortex or epidermis). The presence of the QC cells is necessary for the stem cell character of root initials (van den Berg *et al.* 1995). The WUS homolog *WOX5* is exclusively expressed in QC cells and functions in inhibition of columella differentiation. Interchangeability of WUS and WOX5 suggest common mechanisms underlying stem cell niche maintenance in root and shoot (Sarkar *et al.* 2007). Additionally, QC identity is maintained by overlapping signals from the GRAS transcription factors SCARECROW (SCR) and SHORTROOT (SHR) as well as the AP2 TF PLETHORA1 and 2. In its function in radial patterning of the root, SHR moves from vascular cells of the stele into neighbouring cells. There it activates SCR which in turn is responsible for differentiation of stem cell daughters (Helariutta *et al.* 2000, Nakajima *et al.* 2001, Wysocka-Diller *et al.* 2000). In the QC, SHR activation of SCR overlaps with *PLT* expression and both signals are important for positioning of the stem cell niche (Sabatini *et al.* 2003). *PLT* expression is also found in stem cells surrounding the QC and is controlled by auxin (Aida *et al.* 2004, Blilou *et al.* 2005). A continuous directed auxin flow in direction to the root tip is necessary for QC position, as after QC ablation redistribution of auxin is the first step of QC regeneration (Xu *et al.* 2006). Auxin accumulation can be visualized using the highly auxin inducible synthetic DR5 promoter that consists of direct repeats of the auxin responsive element (Ulmasov *et al.* 1997). Two NAC domain TF, FEZ and SOMBRERO (SMB) are expressed in root cap stem cells and their daughters and control cell division frequency and orientation to ensure the accurate root cap developmental program (Willemsen *et al.* 2008).

Marker lines of several of the described genes (*WUS, WOX5, PLT1, PLT2, FEZ* and *SMB* as well as the DR5 marker) that specify the root stem cell niche were crossed into LEC1::GR plants. Analysis of most of these lines is in progress.

Using the DR5::GFP x LEC1::GR line, the auxin maximum in wild type and uninduced LEC1::GR root tips was located in the QC and underlying cell layers. During DEX+ABA-induced *pkl* root development, auxin flow into the root tip and its accumulation remained more or less stable (Figure 30). The expression of *PLT1/2* as meristem identity genes depends on auxin and is supposed to be realized when auxin can be detected. The expression of other above mentioned meristem identity genes also is expected to stay unaltered during *pkl* root formation. This indicates that the root stem cell niche is maintained during *pkl* root formation and suggests that LEC1 can only interfere with normal development after root initials or their daughter cells received signals that allow differentiation. Under the influence of LEC1+ABA, cells that have abandoned stem cell identity, differentiate into embryonic cells rather than into different root cell lineages such as epidermis and endodermis.

4.2 Towards the characterization of the LEC1 regulon

Induction of LEC1 and ABA-treatment are sufficient for switching vegetative developmental patterns into embryogenic differentiation in special parts of the plant. LEC1-competent cells are located in meristematic regions of shoot (SAM) and root (RAM). To get more insight into LEC1-induced embryonic processes, knowledge about *downstream* components of the LEC1 signaling pathway is of utmost importance. Several different methodical approaches have been taken to identify factors that act *downstream* of LEC1 in LEC1-induced embryogenesis. For this purpose, four experimental approaches have been applied: macroarray hybridization, microarray hybridization, chromatin immunoprecipitation combined with promoter array hybridization (ChIP/chip) as well as quantitative real-time PCR (qRT-PCR).

Although all these methods are principally suited to detect target genes, they clearly differ in several aspects. Whereas macro- and microarray are hybridization-based methods that measure transcript levels, chromatin-immunoprecipitation detects protein-DNA-interactions *in vivo*. Candidate genes identified by these methods have been validated using quantitative real-time PCR, an amplification-based method for exact quantification of transcript abundance. Only candidates that were confirmed by at least two out of four methods were considered as putative LEC1 target genes. According to this criterium, a final list of putative LEC1-regulated genes (Table S5) contained 114 genes. This list represents the status quo of putative LEC1 target genes. For different reasons, it should be considered as partial and biased rather than a comprehensive list. First, macroarray (1200 TF), microarray (24 576 GSTs) and ChIP/chip analyses (8471 promoters) were not performed on a full genome scale (33.282 Arabidopsis genes) and qRT-PCR analysis will reveal more candidate genes as activated or repressed by LEC1. Second, the composition of the list is certainly influenced by type I (exclusion of true positives) and type II (inclusion of false positives) errors that are often unavoidable in large-scale analyses (Oh *et al.* 2009). Third, the final list of candidate genes is biased due to the enrichment of genes of over-represented functional categories. Independent of the confirmation of genes by different large-scale methods (macro-, microarray and ChIP/chip), genes from over-represented functional categories (lipid and hormone metabolism) have preferentially been validated by qRT-PCR. Fourth, list composition was further influenced by overlapping and divergent results that were obtained by different methods (Table 6 and Figure 39). Principal technical differences and, particularly, the discrimination between activation of target genes (arrays, qRT-PCR) and binding to the promoter of a target gene (ChIP) might have caused divergent results. Moreover, induction times and regimes applied to LEC1::GR seedlings differed between the employed approaches. Macro- and microarray experiments were performed using seedling material that was induced for eight hours. These analyses therefore could eventually not discriminate between primary and secondary target genes. The time window for detection of primary targets is not clearly defined, although sometimes they are considered to be detected after up to 4 hours of induction (Braybrook *et al.* 2006). The discrimination of putative primary and secondary LEC1 (microarray) targets was shown exemplary for genes involved in lipid metabolism and storage (see following section). ChIP/chip analyses are based on the detection of protein-DNA binding, and therefore identify primary target genes of the factor of interest. In the present study, ChIP/chip was performed using seedling material that was induced only with DEX for 24 hours. In accordance with this, several candidate genes were shown to be activated by LEC1 (independent of ABA) after 24 hours of induction, as measured by qRT-PCR. Due to such a slow activation of *per definitionem* primary target genes, microarray and ChIP/chip analyses were hardly comparable. This fact, together with rather low fold change values, might have been due to an activation which is restricted to certain tissues or cells, e.g. LEC1-competent cells in meristematic regions. In order to reassess this assumption, the activation of candidates by LEC1+ABA was analyzed after induction about 24 hours in root tips in comparison to leaves (data not shown). The activation was observed to be similar in all analyzed tissues.

Although, all experimental approaches applied in this work aim at the detection of target genes, method-specific features have to be considered. Together with certain peculiarities of the biological system the approaches have been applied to, this partially explains the observed divergence in results obtained with the different methods.

4.3 LEC1 acts via the CCAA(A)T-box

In the present work, different large scale approaches/techniques have been applied to the same biological system. This allows a direct comparison of the used experimental approaches. After data evaluation and additional analyses, particularly motif analysis, the ChIP/chip technique clearly turned out to be the most attractive method for detection of LEC1 target genes.

Using the ‘Amadeus’ software, two CCAAT-box-containing or similar motifs were found in LEC1 ChIP/chip target genes by *de-novo* motif search (Table 12, motifs #7 and #23). The LEC1 gene encodes a protein with high similarity to the HAP3 subunit of the eukaryotic heterotrimeric heme-activator protein complex (HAP, yeast), also known as nuclear factor Y (NF-Y, vertebrates) or CCAAT-box-binding complex (CBF, planta; Lotan *et al.* 1998, Mantovani 1999). This complex binds CCAAT-sequences that are present in around 25% of all eukaryotic promoters (Bucher 1990, Gelinas *et al.* 1985, Romier *et al.* 2003) and are located between -100 to -60 bp upstream of the transcription start site (TSS, Mantovani 1998). In planta, the CCAAT-box-binding activity of nuclear extracts has been reported (Kusnetsov *et al.* 1999) and, recently, using the carrot system, HAP-complex-formation and its binding to CCAAT-boxes was proven *in vitro* (Yazawa *et al.* 2007). Although, in promoters activated by another Arabidopsis HAP3 subunit (HAP3b), CCAAT-boxes were not found to be enriched (Cai *et al.* 2007).

Interestingly, two CCAAAT-like motifs, containing an additional A, were found to occur in LEC1-bound promoters (Table 12, motifs #12 and #19). With regard to seed development, a CCAAAT-box has been identified in the β-phaseolin promoter of *Phaseolus vulgaris.* In cooperation with other *cis*-motifs, this might be responsible for activation of *phaseolin*, encoding the major storage protein in bean, during seed development (Bustos *et al.* 1989, Li and Hall 1999). The inactivity of the *phas* promoter during vegetative development is due to chromatin structure (Frisch *et al.* 1995). LEC1 binding to CCAAAT-boxes could be involved in the activation of chromatin structure during embryogenesis (e.g. deacetylation). Through physical interactions with histone acetyltransferase (HAT) enzymes, the mammalian NF-Y complex has been shown to possess HAT activity (Currie 1998).

Several motifs were found to be over-represented in LEC1-bound promoters that showed similarity to abscisic acid response elements (ABRE), also called G-box, C-box or G/C-box hybrids (Table 12). The ABRE core element is represented by the four nucleotides ACGT. Only one ABRE/G-box in LEC1-target promoters exactly matched this sequence (Table 12, motif #1). The remaining six ABRE/G-box-like motifs displayed only weak similarity. G-box like motifs are bound by several bZIP proteins, many of which are involved in ABA mediated processes during seed and vegetative development (for review see: Jakoby *et al.* 2002). Interestingly, LEC1 was shown to interact with a seed specific ABRE-binding bZIP factor, bZIP67 (Yamamoto *et al.* 2009). The occurrence of a CCAAT/G-box combination in LEC1-bound promoters might reflect interactions between LEC1 and bZIP proteins during regulation of *downstream* targets.

Taken together, the over-representation of CCAA(A)T-box motifs in putative target promoters of Arabidopsis HAP3 subunits was shown here for the first time. The data suggest the ChIP/chip approach as the method of choice and demonstrate the identity of candidates as LEC1 target genes. The over-representation of ABRE/G-box and similar motifs in LEC1-bound promoters supports findings about HAP/bZIP interactions in the control of seed genes and could account for ABA-dependent induction of target genes by LEC1.

4.4 The LEC1 regulon is involved in a variety of physiological processes

4.4.1 LEC1 is a regulator of lipid metabolism

Besides seed storage proteins, triacylglycerides (TAGs) represent main storage compounds of Arabidopsis seeds. The regulation of fatty acid synthesis mainly occurs at the transcriptional level (Baud and Lepiniec 2009, Ohlrogge and Jaworski 1997).

In the present study, the functional category 'lipid metabolism' (BIN11) was found to be over-represented among DEX+ABA-regulated genes obtained by microarray analysis (Table 7). These genes were found to be involved in the regulation of seed TAG synthesis (WRI1), TAG synthesis (KAS1, CAC1, CAC3), TAG storage (oleosins) and other sub-categories (Table 8). The obtained results are supported by a report of Mu *et al.* (2008) that describes LEC1 as key regulator of TAG synthesis.

By studying mutants with reduced amounts of seed TAGs, several factors have been identified as important regulators in developmental control of seed oil biosynthesis. The AP2/EREB transcription factor WRINKLED1 (WRI1) was first described as regulator of seed oil accumulation (Cernac and Benning 2004, Focks and Benning 1998). Seeds of the corresponding *wri1* mutant display a shrunken and wrinkled phenotype and contain reduced amounts of TAGs (Focks and Benning 1998). This phenotype seems to be the result of deficiencies in seed lipid and carbohydrate metabolism and many genes encoding central fatty acid and glycolytic enzymes are targets of WRI1 (Andre and Benning 2007, Baud *et al.* 2007a, Baud *et al.* 2007b, Ruuska *et al.* 2002). The up-regulation of *WRI1* expression by LEC1 as shown in this work had been previously reported for the *turnip* mutant where LEC1 is de-repressed (Casson and Lindsey, 2006).

Besides WRI1, three of four components of the AFL/B3-LEC-network, namely LEC1, LEC2 and FUS3, have been implicated with the regulation of seed oil content. As a direct target of LEC2, WRI1 mediates LEC2-induced fatty acid biosynthesis in developing leaves (Baud *et al.* 2007a, Santos-Mendoza *et al.* 2005). LEC2-controlled oleosin synthesis is required for oil body development which is a prerequisite for TAG storage (Braybrook *et al.* 2006). In contrast to LEC2, FUS3 directly influences the expression of lipid biosynthetic genes (Wang *et al.* 2007). LEC1 was already known as another key regulator of seed oil biosynthesis (Mu *et al.* 2008). In accordance with this, about 17% of lipid-metabolism-associated genes that were found to be up-regulated after permanent induction of LEC1 (Mu *et al.* 2008) could be confirmed in the present study by microarray after short-term induction (Table 8). In contrast, the promoter of only one gene encoding a lipid biosynthetic enzyme (SSI2) was identified as putative direct target of LEC1 by ChIP/chip. Some candidate genes involved in lipid biosynthesis were validated by qRT-PCR where the activation by LEC1+ABA was only detectable after

24 hours of induction (Figure 37). Nevertheless, one oleosin gene was found to be activated within 4 hours, thus representing a potential direct target of LEC1 regulation (Figure 37). Slow induction kinetics of lipid biosynthetic genes and no enrichment of corresponding promoters after ChIP indicate that yet unknown factors mediate LEC1-induced activation of lipid biosynthesis. FUS3 represents a potential candidate for this function. In the present work, it was shown to be highly expressed in embryonic structures after permanent LEC1-induction (Figure 18F). Moreover, its expression was found to be up-regulated by LEC1 in an adequate, narrow time window during vegetative development (within 4-8 hours after induction, Figure 27A).

Higher lipid contents of LEC1-induced embryonic structures support a function of LEC1 in control of seed fatty acid metabolism. The total lipid content in apical embryonic structures as well as *pkl* root tips of permanently induced LEC1::GR seedlings was elevated between 4 to 7 times when compared to uninduced control seedlings. Lipid composition of LEC1-induced embryonic structures resembled very much the composition found in Arabidopsis seeds (Figure 38). Induced LEC1::GR seedlings had increased levels of 18:0, 18:1 and 20:0 fatty acid fractions whereas levels of short chain fatty acids (16:0) and linolenic acid (18:3) were decreased (Figure 38). In accordance with this, a comparison of lipid profiles of wild type seeds and seedlings reveals that seedlings contain higher levels of short chain fatty acids (16:0, 16:1 and 16:3) and linolenic acid (18:3) whereas levels of oleic acid (18:1) and long chain fatty acids (20:0 and 20:1) are elevated in seeds (Mu *et al.* 2008).

The regulation of seed lipid biosynthesis by LEC1+ABA represents a late, maturation-related function of LEC1. Supportively, the peak in LEC1 expression during seed development (heart stage embryo) precedes the sharp increase in seed fatty acid content in seeds after 7 days after flowering (torpedo stage, Baud *et al.* 2002).

All known regulators of seed lipid metabolism activate either TAG synthesis (WRI1, FUS3) or, on a higher hierachical level, promote the expression of *downstream* regulators (LEC2 > WRI1) and oleosins which encode structural constituents of oil bodies (LEC2). In contrast, LEC1 seems to affect all levels by activating the *downstream* regulator WRI1, genes encoding lipid biosynthetic enzymes (KAS1, CAC1, CAC3) and genes encoding proteins involved in lipid storage (oleosins). Besides FUS3, further intermediate factors that act *downstream* of LEC1 in activation of seed fatty acid metabolism remain to be identified.

4.4.2 LEC1 activates a number of genes implicated with flower development and flowering time

Surprisingly, several putative LEC1 target genes are implicated with regulation of flowering time. The expression of *SCHLAFMÜTZE* (*SMZ*) was found to be up-regulated by LEC1+ABA in macro- and microarray analysis (Table 4 and Table 13). The *AINTEGUMENTA-LIKE5* (*AIL5*) transcript abundance was found to be elevated after DEX+ABA- treatment in microarray analysis (Table 13). Short-term activation of *SMZ* and *AIL5* by LEC1+ABA could be confirmed by qRT-PCR (Figure 33A). The promoters of *SUPPRESSOR OF CONSTANS OVEREXPRESSION1* (*SOC1*) and *TARGET OF EAT1 1* (*TOE1*) were demonstrated to be bound by LEC1 and activation of *TOE1* transcription could be detected after 24 hours of induction with DEX (Table 11). The family of CONSTANS-LIKE (COL) was found to be over-represented among LEC1 ChIP/chip targets (Figure 40B), but transcripitonal activation of these genes has not been validated by qRT-PCR yet.

Three flowering-related transcription factors that represent putative LEC1 targets belong to the AP2/ERF family. This TF family represents one of the largest families in Arabidopsis (Riechmann *et al.* 2000, Riechmann and Meyerowitz 1998). AIL5 and TOE1 are assigned to the AP2 subfamily whereas SMZ belongs to the 'others' subfamily representing members with only one AP2 domain but resembling members of the AP2 subfamily (Sakuma *et al.* 2002, Schmid *et al.* 2003). Within the AP2 subgroup (including genes with one AP2-domain such as SMZ), TOE1 and SMZ belong to the AP2-like group whereas AIL5 shows high similarity to AINTEGUMENTA (ANT, Nole-Wilson *et al.* 2005).

Similar to ANT, overexpression of *AIL5* leads to larger floral organs suggesting a function in organ growth control (Nole-Wilson *et al.* 2005). Besides several AIL genes, the ANT-like group also includes root meristem identity genes *PLETHORA1* and *PLETHORA2* as well as *BABYBOOM* (*BBM*). All of these genes are highly expressed in undifferentiated callus tissue (Nole-Wilson *et al.* 2005). *AIL5* expression is also detectable in embryos, especially during later stages of morphogenesis (CSB.DB, http://csbdb.mpimp-golm.mpg.de/, ATGeneExpress data). On the one hand, a function of AIL5 in promoting the un-differentiated state of cells would support the inducing role of LEC1 during initiation of SE. The organ growth promoting activity of AIL5, on the other hand, indicates a possible function of LEC1 in preparing the embryo for maturation.

SOC1, SMZ and TOE1 are involved in different pathways of photoperiodic flowering. The duration of the daily light period (photoperiod) is one of the most important factors in the control of flowering time. Photoperiodic signaling is mediated by factors under circadian regulation producing rhythmic outputs in dependence of light signals. CONSTANS (CO) and *upstream* of it GIGANTEA (GI) represent such factors (Mizoguchi *et al.* 2005, Park *et al.* 1999, Suarez-Lopez *et al.* 2001, Yanovsky and Kay

2002) which in turn regulate *downstream* factors such as the flowering promoting MADS TF SOC1 (Onouchi *et al.* 2000, Samach *et al.* 2000). SMZ and TOE1 are part of a GI-mediated, but CO-independent pathway in photoperiodic flowering (Jung *et al.* 2007). The abundance miR172 is regulated photoperiodically by GI-mediated RNA-processing. The mRNAs of *SMZ* and *TOE1* as well as other related genes (*APETALA2, TARGET OF EAT1 2, SCHNARCHZAPFEN*) are targets of miR172-mediated mRNA-cleavage (Schwab *et al.* 2005). SMZ and TOE1 function as floral repressors and the GI-mediated miR172 pathway promotes flowering when SMZ/TOE1 transcript levels decrease below a certain threshold. No embryo-related function of SMZ and TOE1 has been described yet. Floral repression nevertheless is associated with meristem inactivity. The shoot apical meristem (SAM) is precociuosly activated in the *lec1* mutant embryo (Lotan *et al.* 1998). This might be due to silencing of SMZ and the consequent missing repression of meristem activity.

Five members of the COL-family of zinc finger proteins have been identified as primary LEC1 targets by ChIP/chip (Table 11). *CONSTANS-LIKE* (*COL*) genes constitute a large gene family with 17 members in Arabidopsis (Robson *et al.* 2001) including CONSTANS (CO) which is known to promote flowering in response to long photoperiods (Putterill *et al.* 1995, Suarez-Lopez *et al.* 2001). *COL* genes are under circadian regulation (Ledger *et al.* 2001) and have mostly been implicated with photoperiodic flowering (Putterill *et al.* 1995) and light signaling (Zobell *et al.* 2005). The flax seed-specifically expressed *COL1* gene is discussed in functioning as a coupling link between photoperiodic regulation and seed development (Gutierrez *et al.* 2006). At*COL1* shows high expression during late embryogenesis (CSB.DB, ATGeneExpress data) but does not display seed-specific expression. In a similar way, the remaining four Arabidopsis *COL* genes that were identified as LEC1 targets in the present study are expressed during embryo and vegetative development (CSB.DB, ATGeneExpress data, Ledger *et al.* 2001). No functional implications are reported for COL genes during Arabidopsis embryogenesis, nevertheless a connection between COL proteins and LEC1 rather exists on the level of protein-protein-interactions. LEC1 and other HAP3 subunits as well as some HAP5 subunits of the HAP complex have been shown to interact with the CCT (CO, CO-like, TIMING OF CAB EXPRESSION 1) domains (Strayer *et al.* 2000) of CO and COL genes (Ben-Naim *et al.* 2006, Wenkel *et al.* 2006). The CCT domain is partially homologous to subdomains of HAP2 subunits which are responsible for protein interaction and DNA-binding (Wenkel *et al.* 2006). Through interaction with HAP subunits, COL factors are recruited to CCAAT-box motifs (Ben-Naim *et al.* 2006). Due to the large number of HAP3/5 subunits and CCT-domain containing factors encoded by the Arabidopsis genome (26 and 45, respectively), a great variety in possible complex combinations exists. Specific (co-)expression patterns may indicate the common regulation of processes. It is possible that COL-HAP complexes have functional importance during photoperiodic control of flowering only. COL proteins as well as several COL-interacting HAP subunits have also been implicated with regulation of the flowering timepoint (Cai *et al.* 2007, Chen *et al.* 2007, Ben-Naim *et al.* 2006, Wenkel *et al.* 2006). Nevertheless, co-expression of *COL* genes and *LEC1* during embryo morphogenesis supports a synergistic function in this process. The LEC1-mediated regulation of genes encoding its own interaction partners could either represent positive or negative feedback mechanisms depending on whether LEC1 acts as activator or repressor. During seed development, COL proteins might also act independent of LEC1, possibly by recruiting other regulatory factors. The *COL1* gene for example is expressed during early embryogenesis, but much higher during seed maturation when *LEC1* expression is already diminished or even silent. This indicates LEC1 as transcriptional activator of *COL1* whereas the COL1 protein performs its functions independent of LEC1. LEC1-connected COL functions and underlying mechanisms during embryogenesis remain to be elucidated.

4.4.3 LEC1 induces somatic embryogenesis via increased auxin sensitivity and signaling

Permanently induced LEC1::GR seedlings that represented phenotype B possessed characteristic embryonic structures in the apical domain and at the root tip (Figure 18D and E). In addition, callus outgrowth was observed starting in the collet region at the root-hypocotyl-junction (Figure 21). The collet region represents the transition zone between hypocotyl and root which is characterized by ectopic root hair formation. The epidermal tissue in the collet region is different from root and hypocotyl epidermis (Lin and Schiefelbein 2001). Using the introgressed DR5 marker, a ring-like auxin maximum was detected in the lowermost epidermal cell layer of the hypocotyl of induced LEC1::GR seedlings. This phenomenon was visible in permanently DEX-induced LEC1::GR seedlings as well as in transiently DEX+ABA-induced plants (Figure 31). The observed auxin maximum overlapped with regions of later callus formation. The auxin maximum disappeared after callus outgrowth was initiated and visible (Figure 30D).

The results of the present study revealed the partially ABA-dependent function of LEC1 in the activation of several hormone signaling pathways. The functional category ‘hormone metabolism’ was found to be over-represented among LEC1+ABA-regulated genes obtained by microarray analysis. The majority of genes in this category mapped to sub-categories ‘auxin and brassinosteroid metabolism’ (Table 9). Genes involved in brassinosteroid signaling were significantly over-represented among primary LEC1 targets as identified by CHIP/chip (Figure 41B). Also the promoters of several auxin-related genes were found to be bound by LEC1 (Table 10). Among these *TIR1*, which encodes an auxin receptor protein, was confirmed by microarray and ChIP (Table 13). The (in part

ABA-dependent) activation of a number of auxin- and BR-related candidate genes by LEC1 was validated by qRT-PCR (Tables 9 and 10).

Plant growth regulators (phytohormones) play important roles in the process of somatic embryogenesis (SE), whereas different stages have to be distinguished. During induction of SE somatic cells acquire embryonic competence. This phase mostly involves a de-differentiation step and callus formation. In most species auxins alone or a combination with cytokinins is used to induce SE (Gaj 2004). A pulse of auxin is able to induce somatic embryogenesis in a variety of plant species including Arabidopsis (Mordhorst *et al.* 1998). ABA and GA were described as inducers of SE in very rare cases and depending on the species (Nishiwaki *et al.* 2000, Rudus *et al.* 2002). ABA treatments promote SE in carrot cells (*Daucus carota,* Nishiwaki *et al.* 2000), and mutations in several ABA signaling genes compromise SE in Arabidopsis (Gaj *et al.* 2006). BR can induce SE under certain instances (Malik *et al.* 2008) while ethylene is known to inhibit the induction of SE (Jimenéz and Thomas 2000). During progression of SE proembryos are formed that pass through morphological stages known from zygotic embryogenesis. Somatic embryos undergo maturation and dessication until they 'germinate'. After auxin induction SE proceeds in the absence of this hormone which, in constantly high concentrations, would possibly prevent apical-basal embryo patterning. The roles of cytokinins and GA during this stage of SE are controversial. Similar to zygotic embryogenesis, ABA treatment has a positive effect on maturation of somatic embryos (Mauri and Manzanera 2004) and especially in conifers it is required for the progression of proembryo development (Stasolla *et al.* 2002).

The role of brassinosteroids in the process of SE is rather ambiguous. Do LEC1-induced BR signaling components have a function during LEC1-induced embryogenesis? BR function as inducer of SE has only been reported once (Malik *et al.* 2008), although BR signaling seems to be essential for acquisition of embryogenic competence. The gene encoding the SOMATIC EMBRYOGENESIS RECEPTOR-LIKE KINASE3 (SERK3), also called BRI1-ASSOCIATED KINASE1 (BAK1), has been identified as a direct target of LEC1 by ChIP/chip. SERK3 is part of multiple protein complexes that also contain the SERK1 and BRASSINOSTEROID-INSENSITIVE1 (BRI1, Karlova *et al.* 2006). This membrane-located, leucine-rich repeat receptor-like kinase is responsible for BR perception by BR-binding to its extracellular domains (Kinoshita *et al.* 2005, Wang *et al.* 2001b). Among 5 members of the SERK subfamily only SERK1 and 3 have been shown to function in BRI1-mediated BR-signaling (Albrecht *et al.* 2008). SERK1 is known to play an essential role in somatic embryogenesis in several plant species (Nolan *et al.* 2009 and references therein) as its expression marks regions with embryogenic competence (Hecht *et al.* 2001). Both *serk1* and *bri1* mutants are impaired in SE initiation suggesting a role of BR signaling in this process (Kwaaitaal and de Vries 2007). Nevertheless, SERK3/BAK1 has not been implicated with SE yet and independent of BR it has been demonstrated to control innate immunity and cell death control (Chinchilla *et al.* 2007, Kemmerling *et al.* 2007). In complex with BRI1 it may mediate BR responses like cell expansion, vascular differentiation, hypocotyl elongation and others.

In contrast to BR, auxin has well established functions during induction of SE. Also the *LEC1* gene has been implicated with the induction of somatic embryos. *LEC1* expression has been shown to be able to substitute the auxin pulse during induction of SE and to induce SE without hormone treatment (Lotan *et al.* 1998). The *lec1* mutant is strongly impaired in their embryogenic response (Gaj *et al.* 2005). LEC1 and its homolog LEC1-LIKE (L1L) are known to be implicated with SE in different plant species such as Arabidopsis (Gaj *et al.* 2005, Ikeda-Iwai *et al.* 2002), carrot (Yazawa *et al.* 2004) and maize (Zhang *et al.* 2002). Moreover, the overlap of the expression domain of L1L, the promoter of which was shown here to be bound by LEC1 (Table 11), and auxin accumulation marks regions with embryonic competence in epiphyllous leaves of *Helianthus annuus* x *H. tuberosus* (Chiappetta *et al.* 2009). Given that LEC1 regulates TIR1 and other auxin signaling components, LEC1 induced somatic embryogenesis is supposed to be realized through activation of auxin metabolism and especially by increasing auxin sensitivity. Hhistological data of the present study revealed the LEC1-induced high auxin levels in regions of the root-hypocotyl-junction which served as initial point for callus formation (Figure 31). Although, no genes involved in auxin biosynthesis were found to be induced by LEC1. Two explanations are conceivable. First, the LEC1 induced activation of auxin signaling could affect auxin transport in a way leading to the observed auxin maximum at the root-hypocotyl-junction. Inherent feedback connections exist between auxin signaling and transport (Leyser 2006). Second, LEC1 could indirectly induce auxin biosynthesis *via* other factors. Similar to *LEC1*, *LEC2* expression can induce somatic embryo formation which is mediated by the activation of auxin biosynthesis (Stone *et al.* 2008). It is conceivable that LEC1 directly increases auxin sensitivity whereas it indirectly affects auxin biosynthesis *via* LEC2. Supportively, *LEC2* expression was found to be up-regulated in LEC1-induced embryonic structures (Figure 18F).

The data suggest that, besides meristematic regions (RAM and SAM, see previous sections), the hypocotyl seems to host LEC1-competent cells also. Supportively, hypocotyl explants are known to have highest embryonic competence, after explants derived from embryonic tissue (Fehér 2006). Also in the *tnp* mutant, *LEC1* de-repression leads to abnormal structures at the root-hypocotyl-junction which displays alterations in epidermal cell identity and resembles storage tissue (Casson *et al.* 2006). The expression of auxin signaling components is increased in abnormal hypocotyl regions and auxin, as well as auxin transport inhibitor treatments enhance the *tnp* phenotype (Casson *et al.* 2006). The results of the present study confirm the hypothesized increased auxin levels in the root-hypocotyl-junction after *LEC1* expression (Figure 31).

Epidermal cell fate in root and hypocotyl is largely determined by the position-dependent expression of the homeodomain transcription factor *GLABRA2* (*GL2*) in hairless epidermal cells (DiCristina *et al.* 1996, Hung *et al.* 1998, Masucci *et al.* 1996). *GL2* expression is significantly reduced in epidermal cells at the root-hypocotyl-junction in comparison to root and hypocotyl, starting in torpedo stage embryos. Also seedlings lack *GL2* expression in this area resulting in ectopic root hair formation (Lin *et al.* 2001). GL2 together with TRANSPARENT TESTA GLABRA1 (TTG1) is described to negatively regulate ethylene and auxin signaling in differentiating hairless epidermal cells (Masucci and Schiefelbein 1996). At the root-hypocotyl-junction missing *GL2* expression promotes auxin signaling independent of position leading to ectopic root hair differentiation. The LEC1-induced auxin maximum in this region promotes callus formation and seems to be independent of GL2. GL2 was not found to be down-regulated by LEC1. Nevertheless, cells of the root-hypocotyl-junction might be more competent to LEC1 action and somatic embryogenesis due to a naturally higher level of auxin which is even enhanced by LEC1.

LEC1 also seems to be important during progression of SE. Its expression has been detected in carrot auxin-induced somatic embryos starting before induction (removal from auxin containing medium) until 7 to 10 days after induction (Yazawa *et al.* 2004). The role of ABA during SE induction is unclear, although it is necessary for LEC1-activation of TIR1 and other auxin signaling components. During SE progression, LEC1+ABA are important regulators of maturation aspects like lipid storage and desiccation tolerance (Shiota *et al.* 1999), respectively. The LEC1- and ABA-induced activation of lipid metabolism represents a ‘late’ LEC1 function and part of the general maturation-supporting function of ABA. These functions are directly transferable to zygotic embryogenesis (see chapter 4.4.1).

Taken together, the present studies reveal that LEC1 confers embryonic competence to somatic cells of the hypocotyl *via* the enhancement of auxin perception and synthesis. The hypocotyl seems to be predisposed for embryogenesis by the persisting auxin level in this tissue due to the lack of *GL2* expression. LEC1 primarily increases auxin sensitivity, whereas the LEC+ABA-induced auxin maximum in the root-hypocotyl-junction (collet) might be an indirect effect on auxin synthesis mediated by LEC2.

4.4.4 LEC1 integrates auxin and brassinosteroid signaling in the Arabidopsis embryo

Cross-talk between phytohormones has been implicated with zygotic embryogenesis and seed development (for review see Brady and McCourt 2003). Auxin is responsible for embryonic patterning during early morphogenesis and the balance between ABA and GA triggers the transition between dormancy and germination. Jasmonic acid acts -like ABA- as an inhibitor of germination

(Wilen *et al.* 1991). Ethylene has been described to support the function of GA in dormancy break-up. Cytokinins act as antagonists of auxin in RAM establishment during early morphogenesis (Müller and Sheen 2008) and affect seed germination and seed size (Riefler *et al.* 2006). The role of BR in seed development is ambigious. GA analogous functions are discussed since BR can rescue GA deficient germination phenotypes (Steber and McCourt 2001). In early embryogenesis, components of the BR signaling pathway seem to be involved in embryo patterning (Chandler *et al.* 2009).

The following chapter will focus on the LEC1-controlled function of auxin and BR in hypocotyl and suspensor formation, since auxin- and BR-related as well as light signaling genes have been identified as target genes of LEC1 using microarrray and ChIP/chip approaches.

The hypocotyl

The data described in the present thesis suggest the importance of a fourpartite network integrating LEC1 with auxin, BR and light signaling in Arabidopsis embryogenesis. Interestingly, BR and auxin signaling have been described to interact in the control of various physiological processes (Hardtke 2007, Hardtke *et al.* 2007, Nemhauser *et al.* 2004). Synergistic promoting action of both hormones is proposed for hypocotyl elongation (Clouse 1996, Romano *et al.* 1995). Exogenous treatment of excised hypocotyl parts with either hormone results in elongation of these segments (Grove *et al.* 1979, Mandava 1988).

In the present study, LEC1 was able to induce hypocotyl elongation and hook formation (phenotype C, Figure 25B). In consistent contrast to this, lec1 mutant embryos have been described to be defective in hypocotyl elongation and apical hook formation (Meinke *et al.* 1994). Moreover, dark-grown lec1 seedlings show de-etiolated growth with short hypocotyls (Brocard-Gifford *et al.* 2003). The presented data -combined with previously described observations- suggest a function of LEC1 in the regulation of hypocotyl elongation and bending (hook formation) *via* the integration of light and hormone signaling pathways. This suggestion is mainly based on the identification of auxin-, BR- and light- related LEC1 target genes, which are implicated with hypocotyl elongation and hook formation. These target genes include auxin-related genes such as IAA19 and ARF2, genes which encode BR signaling components such as BIM1, BIM2, BES1, BEH2, BEH4 and the BR biosynthetic gene DWF4 as well as components of light signaling such as PIF5 and HY5 (Tables 9, 10 and 11).

The *INDOLE ACETIC ACID19* (*IAA19*) gene, also called *MASSAGU2* (*MSG2*), represents a possible point of intersection between auxin and BR signaling pathways (Nakamura *et al.* 2003). Highest expression of IAA19/MSG2 is found in the hypocotyl of etiolated seedlings (Tatematsu *et al.* 2004). Interestingly, outside the embryo, LEC1 expression was detected in etiolated seedlings (Warpeha *et al.* 2007). IAA19/MSG2 interacts with the NONPHOTOTROPIC HYPOCOTYL4/AUXIN RESPONSE FACTOR7

(NPH4/ARF7) and this IAA/ARF pair is known to be implicated with differential growth responses observed in hypocotyls (curvature response, gravitropism, phototropism, apical hook formation, Tatematsu *et al.* 2004) and primary root tips (root gravitropism, Weijers *et al.* 2005). The repressor AUXIN RESPONSE FACTOR2 (ARF2) has also been shown to integrate auxin and BR signaling pathways. Its activity is directly controlled by phosphorylation through BIN2 GSK3 kinase (Vert *et al.* 2008). Further targets for BIN2 phosphorylation are BES1 and BZR1 which are responsible for transcriptional activation in response to BR (He *et al.* 2002, Vert and Chory 2006). BIN2 phosphorylation of ARF2 abolishes its DNA binding thus de-repressing the transcription of auxin-induced genes (Vert *et al.* 2008). Furthermore, as a *downstream* target of HOOKLESS1 (HLS1), ARF2 is responsible for cell elongation in the hypocotyl and proper hook formation (Li *et al.* 2004).

Most of the BR signaling components that have been identified as putative LEC1 targets are implicated with hypocotyl elongation. The typical dwarf phentoype of BR deficient seedlings is displayed by *bim1/bim2/bim3* mutants as well as plants with reduced expression of *BES1* (Yin *et al.* 2005, Yin *et al.* 2002). BES1 and its homologues BES1/BZR1 HOMOLOG2 and 4 proteins (BEH2/4) seem to perform redundant functions in BR signaling (Yin *et al.* 2005).The *DWARF4* (*DWF4*) gene encodes a cytochrome P450 monooxygenase that catalyzes hydroxylation steps in BR biosynthesis (Choe *et al.* 1998). *DWF4* expression correlates with occurrence of high amounts of bioactive BR in roots, collet region (Kim *et al.* 2006) and hypocotyl where it is directly responsible for hypocotyl length (Choe *et al.* 2001).

Interestingly, also two important components of light signaling, PIF5 and HY5, have been identified as putative LEC1 target genes by ChIP/chip. Hypocotyl elongation and hook formation are components of the photomorphogenic response of plants. Therefore these factors may be involved in a hormone-TF regulatory network that acts *downstream* of LEC1 in hypocotyl elongation. PHYTOCHROME-INTERACTING FACTOR5 (PIF5) belongs to the subfamily 15 of bHLH TF proteins (Bailey *et al.* 2003) and is involved in photomorphogenic response (Fujimori *et al.* 2004). PIF5 is inactivated through the 26S proteasome in response to light but its expression recurs during the night in response to circadian clock regulation (Nozue *et al.* 2007, Shen *et al.* 2007). In the light, PIF5 interaction with the photoactivated phytochrome B (Khanna *et al.* 2004) leads to co-degradation of the photoreceptor protein. By this mechanism PIF5 functions as a negative regulator of phyB signaling and phyB-mediated inhibition of hypocotyl elongation (Khanna *et al.* 2007). Overexpression of *PIF5* leads to the formation of exaggerated apical hook and shorter hypocotyls, whereas the pif5 mutant seedlings have more open hooks (Khanna *et al.* 2007). The basic leucine zipper (bZIP) TF ELONGATED HYPOCOTYL5 (HY5) plays an important role in seedling adaptation from skotomorphogenesis (dark) to photomorphogenesis (light; Chattopadhyay *et al.* 1998, Koornneef *et al.* 1980, Oyama *et al.* 1997). The corresponding *hy5* mutant displays features of etiolated seedlings, especially with respect to loss of light-induced inhibition of hypocotyl elongation (Oyama *et al.* 1997). HY5 is responsible for light-induced transcription (Ang *et al.* 1998, Chattopadhyay *et al.* 1998) and itself is controlled by CONSTITUTIVE PHOTOMORPHOGENIC1 (COP1)-mediated targeting for degradation in the dark (Osterlund *et al.* 2000). Together with its homolog HY5 HOMOLOG (HYH), HY5 is implicated with auxin signaling. Interestingly the expression of HYH was found to be down-regulated by LEC+ABA in the macroarray analysis (Table 4 and Figure 33). The *hy5/hyh* double mutant is strongly impaired in auxin signaling and phenotypic features of auxin response mutants can be observed in the embryos (fused cotyledons and alterations in cotyledon vasculature, Sibout *et al.* 2006) as well as seedlings (alterations in gravitropic response and root growth; Oyama *et al.* 1997). A high number of auxin-related genes are missexpressed in *hy5/hyh* plants (Sibout *et al.* 1006). These data suggest HY5 as an integrator of auxin and light signaling thereby affecting hypocotyl growth. As promoter of photomorphogenesis, *HY5* is expected to be down-regulated by LEC1, in a similar way such as *HYH*. This has still to be analyzed.

Post-germinative hypocotyl elongation in the dark has been shown to be influenced by endospermic fatty acid reserves (Penfield *et al.* 2004). Removal of the endosperm results in reduced hypocotyl elongation in dark-grown wild type seedlings (Penfield *et al.* 2004). LEC1 is expressed in embryo and endosperm (Lee *et al.* 2003, Lotan *et al.* 1998) as well as dark-grown, etiolated seedlings (Warpeha *et al.* 2006). Moreover, results described in this study revealed a function of LEC1 in the control of seed fatty acid biosynthesis. These data suggest LEC1 to be implicated with embryonic and post-germinative hypocotyl elongation. Thus, the LEC1-dependent lipid synthesis and accumulation in embryo and endosperm provide resources for the post-germinative hypocotyl elongation.

The described physiological aspects provide the basis to understand hypocotyl development from the embryonic elongation stage to the post-germination elongation stage. The embryonic hypocotyl originates from the central domain of the embryo (Jürgens 2001, West and Harada 1993) as part of the apical-basal axis. During early embryo growth, the embryonic hypocotyl elongates. At a later stage, in *Brassicacea* the embryonic hypocotyl bends immediately below the cotyledons resulting in the development of an apical hook. The hook is formed by enhanced cell elongation at the convex side and reduced cell expansion at the concave side of the embryonic hypocotyl (Silk and Erickson 1978). After germination two conditions have to be distinguished concerning hypocotyl elongation. In the dark, skotomorphogenic growth is characterized by enhanced hypocotyl elongation, apical hook maintenance and closed cotyledons. Hypocotyl elongation is the result of regulated cell expansion (Gendreau *et al.* 1997). Obviously, the apical hook protects the SAM from damage during the penetration of the overlying soil layer (Harpham *et al.* 1991). In the light, the photomorphogenic program leads to the inhibition of hypocotyl elongation, hypocotyl straightening and cotyledon expansion as well as the initiation of photosynthesis.

The results of the present study suggest comparable LEC1-mediated mechanisms underlying both, the embryonic hypocotyl elongation and hook formation as well as the mentioned triade of skotomorphogenic growth. Given the regulation of hypocotyl-related auxin and BR signaling components by LEC1, the impaired hypocotyl elongation and hook formation in embryos of the *lec1* mutant (Meinke *et al.* 1994) and the de-etiolated, short hypocotyl-phenotype of dark-grown *lec1* seedlings (Brocard-Gifford *et al.* 2003) it is well conceivable that all these distortions are the result of perturbations of auxin and BR functions as well as of light signaling (Figure 44). *Vice versa*, it explains the long-hypocotyl-phenotype as it is observed after the induction of LEC1 expression (Figure 25B).

The suspensor

Besides effects on hypocotyl elongation, auxin and BR interactions have also been described during embryonic patterning. BIM1, as component of the BR signaling pathway, has been identified as LEC1 target gene by microarray analysis. The BIM1 protein has been demonstrated to interact with two AP2/ERF transcription factors, DORNROESCHEN (DRN) and DORNROESCHEN-LIKE (DRNL), that are acting *upstream* of auxin transport and response (Chandler *et al.* 2007, Chandler *et al.* 2009). These findings suggest hormone crosstalk between auxin and BR on transcription factor level during early embryogenesis. The *drn*, *drnl* and *bim1* mutants display severe embryo patterning defects such as aberrant cotyledon numbers, abnormal cell divisions in the hypophysis region as well as the suspensor, the latter leading to the occurrence of double and triple suspensor cell files (Chandler *et al.* 2007, Chandler *et al.* 2009). Interestingly, a similar suspensor phenotype is observed in the *lec1* mutant (Lotan *et al.* 1998). Unlike DRN, DRNL and BIM1 that are only expressed in apical domains of the embryo (Chandler *et al.* 2007, Chandler *et al.* 2009), LEC1 has been shown to be expressed in the suspensor (Lotan *et al.* 1998). Partial overlapping phenotypes in *lec1*, *drn*, *drnl* and *bim1* mutant embryos and BIM1 regulation by LEC1 further support the hypothesis of auxin- and BR-mediated LEC1 functions during suspensor differentiation (Figure 44).

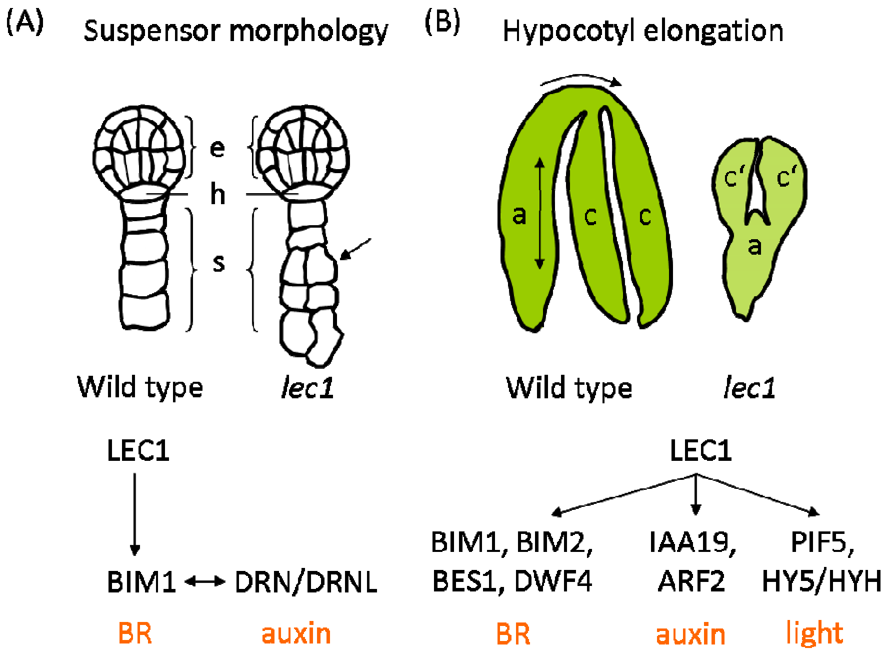


Figure 44. A four-partite network of LEC1, BR, auxin and light controls processes during early zygotic embryogenesis.
(A) LEC1 controls suspensor differentiation. The *lec1* mutant embryo displays defects in suspensor formation (Lotan *et al.* 1998). Abnormal cell divisions occur that lead to more than one suspensor cell file. Similar phenotypes are described for the *bim1* mutant with *BIM1* as a LEC1 target gene. The interaction of BIM1 and DRN/DRNL represents a point of cross-talk between BR and auxin signaling *downstream* of LEC1. (e,embryo; h, hypophysis, s, suspensor) (B) LEC1 is responsible for embryonic hypocotyl elongation. Supportively, *lec1* mutant embryos are impaired in hypocotyl elongation (Lotan *et al.* 1998, Meinke *et al.* 1994) and LEC1 has been shown to be involved in similar processes during etiolation (Brocard-Gifford *et al.* 2005, Warpeha *et al.* 2007). Several LEC1 target genes are implicated with BR, auxin and light signaling, all of which are known to be important factors during hypocotyl elongation (a, axis, c, cotyledon, c', leafy cotyledon).

Taken together, the integration of auxin, BR and light signaling, represents a novel function of LEC1 which is required for suspensor differentiation and hypocotyl elongation. Embryonic hypocotyl formation as well as post-germinative hypocotyl elongation are critical steps in the plant life cycle, since they assure the proper transition from skotomorphic heterotrophic to photomorphic autotrophic growth.

4.3 The role of ABA

The phytohormone abscisic acid was first discovered in the 1960s. Since then numerous studies pointed out the roles of ABA in plant growth and development (Finkelstein *et al.* 2002). First, the probably best documented function of ABA is during seed development, supporting seed maturation, desiccation and dormancy and antagonizing GA in promotion of germination (Brady *et al.* 2003). Second, ABA plays important roles during vegetative growth such as conferring tolerance to abiotic environmental stresses (drought and salinity; Swamy and Smith 1999, Wilkinson and Davies 2002), affecting stomatal conductance (Acharya and Assmann 2009, Jones and Mansfield 1970). Third, it is implicated with the transition from vegetative to reproductive phase (Chinnusamy and Zhu 2008).

An ABA-shifted ratio of GA/ABA in seeds preserves the ‘status quo’ state of late zygotic embryogenesis (McCourt *et al.* 2005) and the expression of several maturation-associated genes (*SSP*, *LEA*) depends on ABA (Kagaya *et al.* 2005a, Kagaya *et al.* 2005b, Parcy *et al.* 1994). In accordance with this, in the present study, the activation of lipid biosynthesis, as a maturation process, by LEC1 was shown to require ABA-treatment. ABA has not been implicated with early patterning processes and morphogenesis during zygotic embryogenesis yet. Accordingly, the first, embryo-derived peak of ABA biosynthesis is observed when the embryo had more or less completed morphogenesis.

Regarding somatic embryogenesis, ABA treatments can promote SE in carrot cells, and mutations in several ABA signaling genes compromise SE in Arabidopsis (Gaj *et al* 2006, Nishiwaki *et al.* 2000). ABA performs similar supporting functions in progression of SE such as in late seed development, particularly in conifer species (Mauri and Manzanera 2004, Stasolla *et al.* 2002).

How far early processes in LEC1-induced embryogenesis depend on ABA is unclear. However, many primary LEC1 target genes are activated independent of ABA. This suggests a model in which initial, probably hormone-related processes during LEC1 induced SE do not require ABA whereas LEC1 induced maturation traits (lipid accumulation) depend on intermediate factors (e.g. FUS3) as well as ABA.

The signaling cascades that underlie ABA-mediated processes are highly complex, and far from being entirely understood. ABA perception seems to be realized by ABA-binding proteins. Two receptor proteins have been described so far. The H subunit of the larger trimeric Mg^{2+}-chelatase complex is encoded by the *GUN5* gene in Arabidopsis and has dual functions: binding to Mg^{2+}-protoporphyrin in chlorophyll synthesis and binding to ABA (Shen *et al.* 2006). Just recently, two novel G-protein-coupled receptors (GPRC), GTG1 and GTG2, have been identified as membrane-localized ABA receptors (Pandey *et al.* 2009).

G-proteins are involved in several aspects of ABA signaling (Coursol *et al.* 2003, Pandey and Assmann 2004, Pandey *et al.* 2006, Wang *et al.* 2001a). Interestingly, LEC1 has been shown to be part of a G-protein-mediated signaling cascade *downstream* of ABA (Warpeha *et al.* 2007). GTG1, GTG2 and GCR1 which represents a third known GPRC, have been demonstrated to bind to the sole G-protein α subunit (GPA1) in Arabidopsis (Pandey *et al.* 2009, Pandey and Assmann 2004). GPA1 in turn interacts with PIRIN1 (Lapik and Kaufman 2003), a protein that is shown to interact with CCAAT-box binding factors in mammals (Wendler *et al.* 1997) and particularly with the Arabidopsis LEC1

(Warpeha *et al.* 2007). This signal transduction chain is responsible for regulation of gene transcription in etiolated seedlings (Warpeha *et al.* 2007) and seems to be implicated in ABA-mediated delay in seed germination (Lapik *et al.* 2003, Warpeha *et al.* 2007). The data indicate the existence of ABA- and LEC1-involving regulatory cascades underlying hypocotyl elongation. How far G-protein mediated signaling is involved in ABA-dependent, LEC1-induced embryogenesis is unclear.

In the present study, besides CCAAT-box motifs, a G-box motif, comprising the ABRE core element ACGT, and several G-box-similar motifs were found to be over-represented in putative LEC1 target promoters (Table 12). This suggests that LEC1 may confer ABA inducibility to part of its target genes by recruiting additional factors that interact with ABA-response elements (ABRE) in the corresponding promoters. This hypothesis is supported by a recent report about the interaction of LEC1 and L1L with the ABRE-binding factor bZIP67 (Yamamoto *et al.* 2009). LEC1 activation of the *cruciferin* gene was shown to be either dependent on ABA or on the presence of bZIP67. The *bZIP67* gene is seed-specifically expressed and was therefore included in the TF profiling of seed development which was performed in frame of this work. The expression profile of *bZIP67* was similar to that of other TF genes which are known to be involved in regulation of seed filling and storage of compounds like seed storage proteins (Figure 10). These include for example *ABI3* and *FUS3* as well as a gene encoding another bZIP factor, bZIP12 (Supplemetary Table S2). This factor has been demonstrated to control the expression of the late embryogenesis abundant protein *AtEM1* (Bensmihen *et al.* 2002) and to behave similar to bZIP67 with regard to interaction with LEC1 and L1L and the activation of the cruciferin promoter (Yamamoto *et al.* 2009). Neither *bZIP12* nor *bZIP67* have been found to be up-regulated by ABA treatment in macro- and microarray analysis. Due to seed-specific expression of *bZIP12* and *bZIP67*, their interaction with LEC1 in the induced LEC1::GR seedling seems to be unlikely. Nevertheless, several other bZIP factors were ABA inducible (Table 3 and 4) which might act in complex with LEC1 to regulate its *downstream* genes. Alternatively, bZIP proteins can be activated by phosphorylation (Wasilewska *et al.* 2008). Cooperative action of LEC1 and bZIP factors might be required for activation of candidate genes and possibly accounts for the ABA-dependency of LEC1-induced processes.

Several lines of evidence suggest that ABA regulates chromatin structure and modifies DNA or histones in order to change gene expression programs in response to certain developmental or environmental stimuli (for reviews see: Chinnusamy and Zhu 2008, Chinnusamy and Zhu 2009). ABA-mediated changes in chromatin architecture might facilitate the access of LEC1 to its target promoters. *Vice versa*, LEC1 could account for chromatin rearrangements prior to ABA-mediated transcriptional activation. Several factors involved in DNA methylation and de-acetylation have been identified as LEC1 targets by ChIP/chip (Table 11). NF-Y factors are reported to possess histone deacetylase activity by recruiting HDA proteins (Currie 1998). Studies on the *Phasoelus vulgaris* *phaseolin* SSP gene promoter exemplify this scenario. Promoter potentiation involves PvALF- (ABI3 orthologue) mediated chromatin remodeling (Li *et al.* 1999) and activation is achieved by an ABA mediated signal cascade including ABI5 (Ng *et al.* 2008). CCAAT-box binding factors like LEC1 are discussed to be involved in chromatin remodeling over a CCAAAT-box region in the *phas* promoter. The data indicate chromatin-related mechanisms underlying LEC+ABA-mediated activation or repression of transcription. Further experiments are required to reveal a possible function of LEC1 in chromatin remodeling in regions of its target promoters.

In guard cell signaling, protein phosphorylation is known to constitute an essential component of ABA signal transduction (Himmelbach *et al.* 2003, Leung and Giraudat 1998, Schmidt *et al.* 1995, Sokolovski *et al.* 2005). Two SnRK3 family members (SnRK3.8, CIPK10 and SnRK3.13, CIPK8) and one CDPK (CDPK6) have been identified as putative LEC1 target genes by microarray and ChIP/chip (Tables 11 and 13). The expression of several members of the Sucrose Non-Fermenting Related Kinase2 (SnRK2) family is known to be ABA-inducible (Boudsocq *et al.* 2004). The corresponding proteins phosphorylate certain motifs in ABRE-binding bZIP TF proteins thus mediating ABA response (Furihata *et al.* 2006). Some bZIP factors have been shown to get phosphorylated by calcium-dependent protein kinases (CDPK) (Choi *et al.* 2005). It is conceivable that LEC1-regulated kinases might activate bZIP factors which in turn interact with LEC1 in controlling the expression of its target genes. How far SnRK3 family members exert similar functions such as SnRK2 kinases remains to be elucidated. The role of these proteins in ABA-mediated LEC1 responses is unclear.

Finally, ABA has been described to have a protein stabilizing function. Protein stability of two components of the AFL/B3 network, FUS3 and ABI3, as well as ABI5 has been shown to be positively affected by ABA (Gazzarini *et al.* 2004, Lopez-Molina *et al.* 2001, Zhang *et al.* 2005). How far ABA can stabilize the LEC1::GR or putative interacting proteins remains to be elucidated.

Taken together, ABA responses are mediated by several mechanisms including G-protein signaling, transcriptional activation, epigenetic modifications, protein phosphorylation and post-translational protein stability. The mechanisms underlying the ABA-dependency of LEC1 target genes have to be further investigated.

4.4 Conclusions

Comprehensive histological and molecular analyses of LEC1-induced embryogenesis have been performed in frame of the present work. This section summarizes the main conclusions drawn from the obtained results.

- LEC1 induces somatic embryogenesis. Progression from embryonic into vegetative development requires the repression of *LEC1* during germination and after etiolation.
- *LEC1* expression is sufficient to switch on embryonic differentiation during vegetative development at RAM (*pkl* root tips) and SAM (cotyledon-like leaves) in dependence on ABA.
- Stem cell identity is not impaired during LEC1-induced embryogenesis at RAM and SAM. Embryonic structures originate from differentiation-competent stem cell daughters that are prone for LEC1(+ABA) action.
- LEC1-induced callus formation and somatic embryogenesis at the collet (root-hypocotyl-junction) is primarily mediated by LEC1-enhanced auxin sensitivity and signaling. A secondary high, local auxin accumulation in this region seems to be an indirect effect of LEC1-mediated activation of LEC2.
- LEC1 is responsible for hypocotyl elongation in the embryo where LEC1-activated auxin, BR and light signaling ensure proper embryo growth.
- During post-germinative seedling etiolation, LEC1-mediated integration of hormone and light signaling pathways as well as the LEC1-controlled seed lipid synthesis, are the prerequisite for elongation of the hypocotyl as a transition stage between heterotrophic and autotrophic growth.

4.5 Outlook

The wealth of data that are presented in this thesis led to several conclusions and uncovered new functions of LEC1 during embryogenesis as well as during post-germinative growth. Nevertheless, numerous questions remain to be answered and are partially investigated in on-going experiments.

In order to histologically analyze initial points/cells of LEC1-induced embryogenesis, the expression of stem cell markers will be investigated at early stages of LEC1-triggered formation of embryonic structures.

Further experiments will be performed to elucidate mechanisms that underlie the LEC1-induced auxin maximum in the collet region. Possible changes in auxin transport will be proven by the application of auxin transport inhibitors. Additionally, the expression of *LEC2* and auxin biosynthetic genes (*YUCCA*) will be measured in the collet region after induction of LEC1.

In order to achieve a classification of ABA-dependent and ABA-independent LEC1 target genes, the activation of more putative candidates by LEC1+ABA/LEC1 will be analyzed by qRT-PCR and the presented qRT-PCR results will be repeated.

Assuming that the activation of LEC1 candidate targets occurs with spatial restriction to certain regions/tissues/cells, promoter-reporter-lines of these candidates will be established.

In the present study, the ChIP/chip method represented the method of choice for detection of LEC1 target genes. The used SAP macroarray only contains promoter fragments of one third of all *Arabidopsis* genes. In order to define the complete LEC1 regulon, this analysis will be extended to the genome-wide scale.

Finally, returning to the ARABIDOSEED project, the established inducible over-expression lines for the AFL/B3 genes *FUS3* and *ABI3* will be used for genome-wide, expression analysis. Comparative analysis of the ABI3-, FUS3- and LEC1-regulon will be used to establish a transcriptional regulatory network underlying *Arabidopsis* embryogenesis and seed development.

5. Summary (english)

Plant seeds are of utmost importance for human nutrition and will provide basics for a bio-based economy and energy production. Seed formation is a complex developmental process requiring a network of spatially and temporally precise regulation of gene activity.

The transcription factor (TF) LEAFY COTYLEDON1 (LEC1) acts as an essential regulator of Arabidopsis embryogenesis and seed development. It controls aspects of early embryogenesis such as cotyledon identity and suspensor morphology, as well as seed maturation processes such as storage compound accumulation, acquisition of desiccation tolerance and dormancy. Ectopic expression of LEC1 leads to the induction of somatic embryogenesis. Several upstream regulators of LEC1 have been identified, including chromatin remodeling factors such as PKL, VAL/HIS and HDAC6/19, as well as other transcription factors such as ASIL1 and MYB115/118.

To identify *downstream* components of the LEC1 regulon, dexamethasone- and estradiol-regulated expression of LEC1 were applied and revealed the abscisic acid (ABA)-dependent switch from vegetative growth to an embryonic developmental program. Shoot apical meristem (SAM)-derived cotyledon-like leaves and embryo-like structures derived from the root apical meristem (RAM) were shown to store seed storage proteins, lipids and starch. LEC1-triggered embryonic differentiation seemed to originate from differentiation-competent stem cell daughters in SAM and RAM leaving stem cell identity unaffected. A LEC1- and ABA-induced auxin maximum at the root-hypocotyl-junction (collet) led to callus formation and subsequent somatic embryogenesis.

Microarray and CHIP/chip analysis revealed the over-representation of hormone-related, lipid biosynthetic and transcription factor genes among LEC1 target genes. LEC1-activated auxin signaling is thought to initialize callus formation at the collet whereas LEC1 primarily increases auxin sensitivity. The observed auxin accumulation seemed to be the result of a signaling cascade involving LEC2 and YUCCA factors/enzymes *downstream* of LEC1. The activation of auxin as well as brassinosteroid signaling by LEC1 provides novel insights into TF-hormone cross-talk during early zygotic embryogenesis and indicates extra-embryonic functions of LEC1 during post-germinative seedling etiolation. The LEC1-induced long-hypocotyl-phenotype and impaired embryonic as well as post-germinative hypocotyl elongation observed in the *lec1* mutant support a model of LEC1-controlled integration of the synergistic, hypocotyl-growth-promoting activities of auxin and BR.

6. Zusammenfassung (deutsch)

Pflanzliche Samen sind die Grundlage menschlicher und tierischer Ernährung sowie einer biologisch basierten Energie- und Rohstoffproduktion. Die Entwicklung des Samens erfordert ein komplexes Netzwerk zeitlich und räumlich präziser Regulation von Genen.

Der Transkriptionsfaktor LEAFY COTYLEDON1 (LEC1) ist ein zentraler Regulator der Embryogenese und Samenentwicklung in Arabidopsis. Er ist sowohl an der Regulation früher Prozesse wie Keimblatt-Identität und Suspensor-Morphologie, als auch in der Regulation später Prozesse der Samenreifung wie zum Beispiel der Speicherstoff-Akkumulation und dem Erwerb von Austrocknungstoleranz sowie Dormanz beteiligt. Die ektopische Expression von *LEC1* fördert die Bildung von somatischen Embryonen. Eine Vielzahl von *upstream* Regulatoren von LEC1 ist bekannt. Dazu gehören Chromatin-Remodeling Faktoren wie zum Beispiel PKL, VAL/HIS und HDAC6/19 und auch andere Transkriptionsfaktoren wie ASIL1 und MYB115/118.

Mit dem Ziel *downstream*-regulierte Zielgene von LEC1 zu identifizieren, wurden zwei induzierbare Systeme (Dexamethason- und Östrogen-Induktion) für die regulierte Expression von LEC1 etabliert. Die Induktion der LEC1-Aktivität ermöglichte -in Anwesenheit des Pflanzenhormons Abscisinsäure (ABA)- die kontrollierte Umschaltung von vegetativen auf embryonale Differenzierungsprogramme. Ausgehend von Sproß- und Wurzelapikalmeristem bildeten sich dabei Kotyledonen-ähnliche Blätter bzw. wurzelbürtige Embryonen, die Samenspeicherstoffe wie Proteine, Lipide und Stärke akkumulierten.

Ausgangspunkt für die LEC1-gesteuerte embryonale Entwicklung waren differenzierungskompetente Stammzell-Tochterzellen, wobei die Stammzellidentität an sich nicht beeinträchtigt wurde. Am Übergang zwischen Hypokotyl und Wurzel, der so genannten *Collet*-Region, löste die Induktion von LEC1 und ABA-Behandlung eine hohe, lokale Akkumulation von Auxin aus, die anschließend zur Kallusbildung und somatischer Embryogenese führte.

Für die Definition und Charakterisierung des LEC1-Regulons wurden Mikro- und Makroarrayanalysen sowie Chromatin-Immunopräzipitations-Experimente durchgeführt. Unter den identifizierten LEC1-Zielgenen sind Gene angereichert, die im Zusammenhang mit Phytohormon-Signaltransduktion, Lipidsynthese und Transkriptionsregulation stehen.

Die LEC1-vermittelte Erhöhung der Auxin-Sensitivität wird als der primäre Auslöser der somatischen Embryogenese am Übergang zwischen Hypokotyl und Wurzel interpretiert. Die beobachtete Auxin-Akkumulation in dieser Region ist wahrscheinlich das Ergebnis einer durch LEC1 ausgelösten

Signaltransduktion, die -vermittelt durch LEC2- zur Aktivierung von Genen der Auxin-Biosynthese führt.

Die Integration von Auxin- und Brassinosteroid-Signaltransduktion durch LEC1 belegt die komplexe Wechselwirkung von Phytohormonen und Transkriptionsfaktoren während der Embryogenese, als eine Voraussetzung für die embryonale Verlängerung des Hypokotyls sowie die für *Brassicaceae* typische Krümmung des Embryos. Die LEC1-induzierte Verlängerung des Hypokotyls sowie die für die *lec1*-Mutante beschriebene Störung des Hypokotylwachstums unterstützen diese Hypothese und weisen außerdem auf eine Funktion von LEC1 außerhalb des Embryos hin.

7. References

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8.1 Wissenschaftliche Laufbahn

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10/2002 – 09/2003	**Leibniz Institut für Pflanzengenetik und Kulturpflanzenforschung (IPK)**, Gatersleben, Deutschland Diplomandin in der Gruppe von Helmut Bäumlein (Gene Regulation) *Iron deficiency and transcription factor controlled synthesis of riboflavin*

8.2 Akademische Laufbahn

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10/2000 – 11/2000	**CCS Haryana Agricultural University**, Hisar, India Praktikum in der Abteilung Plant Breeding, Prof. R.K. Behl *In vitro screening for salt tolerance of soybean*
07/1998	Abitur, Note 1.4, von-Bülow-Gymnasium, Neudietendorf, Deutschland

8.3 Publikationen

Journal Publikationen

Tiedemann J, Rutten T, Mönke G, **Vorwieger A**, Rolletschek H, Meissner D, Milkowski C, Petereck S, Mock HP, Zank T, Bäumlein H (2008) Dissection of a complex seed phenotype: Novel insights of FUSCA3 regulated developmental processes. ***Developmental Biology*** 317, 1-12.

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09/2008 **Junker A**: Towards a molecular understanding of LEC1 functions. 1st International PhD School of Plant Development, Retzbach – Würzburg, Deutschland

06/2006 **Vorwieger A**: Transcriptional regulatory networks underlying Arabidopsis seed development. Brookhaven National Laboratory, Upton, New York, USA

06/2005 **Vorwieger A**: Target genes of seed specific transcription factors. 1st IPK Student Conference (ISC), Gatersleben, Deutschland

Poster

09/2007 **Vorwieger A**, Rutten T, Tiedemann J, Bäumlein H: Regulated expression of LEC1 in *Arabidopsis thaliana* seedlings gives rise to *pkl* root phenotype. 4th Tri-National Arabidopsis Meeting (TNAM), Wien, Österreich

07/2006 **Vorwieger A**, Czihal A, Tiedemann J, Tran ML, Mönke G, Conrad U, Altschmied L, Grosse I, Bäumlein H: Arabidopsis seed development: Towards a regulatory network of transcription factors and target genes. 19th International Congress on Sexual Plant Reproduction (ICSPR), Budapest, Ungarn

05/2006 **Vorwieger A**, Czihal A, Tiedemann J, Tran ML, Mönke G, Conrad U, Altschmied L, Grosse I, Bäumlein H: Arabidopsis seed development: Towards a regulatory network of transcription factors and target genes. 2nd ISC, Gatersleben, Deutschland

10/2005 **Vorwieger A**, Gryczka C, Saalbach I, Czihal A, Tiedemann J, Mock HP, Labrenz M, Borris R, Jacoby M, Bäumlein H: Iron assimilation and transcription factor controlled synthesis of riboflavin in roots. Fachtagung “Wirtschaftskraft Pflanze – Zukunft durch Innovationscluster” Hannover, Deutschland

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10. Erklärungen

GEMÄß § 5 ABS. 2 DER PROMOTIONSORDNUNG VOM 17.09.1998

Ich erkläre hiermit, dass ich mich mit der vorliegenden wissenschaftlichen Arbeit erstmals um die Erlangung des Doktorgrades bewerbe. Ich habe die Arbeit selbständig und ohne fremde Hilfe verfasst, und nur die von mir angegebenen Quellen und Hilfsmittel benutzt. Wörtlich oder inhaltlich entnommene Stellen aus anderen Werken habe ich als solche kenntlich gemacht.

Gatersleben, Juni 2009

APPENDIX

Table S1. Gene-specific qRT-PCR primer sequences.

Arabidopsis identifiers (TAIR), the gene names if available and the forward/reverse primer sequences are given. Primers for several TF (red) were described by (Czechowski *et al.* 2004). Primers were designed to have an optimal melting temperature of 60°C and to amplify a fragment of 80 to maximum 140 bp length.

ATG	gene name	forward primer 5' > 3'	reverse primer 5' > 3'
AT1G01470	LEA14	GATCAGTTTCACTTTCCACAGC	ATCAAGAGCCGTCATGTCCT
AT1G07980	CBF/NF-Y	TGCCTCTTTCCCATTCCTCCAACG	TCCTTGCAGATAGTGTTCCCGAGA
AT1G13640	PI-3- and 4-kinase family protein...	CCCGCTTAGCGTAAACTTTG	GCCGAGTAAAGCAACTCCTG
At1g15580	IAA5	TTCCGCTCTGCAAATTCTGTTCG	CGATCCAAGGAACATTTCCCAAGG
At1g18400	BEE1	TCACTGCAGCAAGTTCGTTT	CTCCCCATCTCCACTGTCTC
At1g19350	BES1	CTGAAGCTGGTTGGGTTGTT	TTGCTCGAGAAGATGATCCA
At1g19650	SEC14	AGAGATGGCTTCACCGAAAA	AAAACAGTAGGACTTATGTTACCATTG
AT1G21970	LEC1	TTTCACGGCCCATCTCATGGCCTA	AGTACCGACCACCTCCCATAACCA
AT1G23740		TAATGGTGCCGGAGGAGTAG	CGCAGTTGCAGCTACTTTTG
At1g23750		TGATCGTCTTTACCGCAAGA	ACATGTCGATTTTCGCATTG
At1g25490	RCN1	CAACTGTCGAGTCTACCTTTTTG	TCAACAGCCAATAGTCTCACAGA
AT1G28370		TGAAGGACGACGTGTGGTTTTGGA	CAGTTCTCAGGTGGAGGAGGGAAA
At1g31340	RUB1	CGCGAAGAAGCACATTGAT	AGCAGCCTTGTTGAAGTTTTTC
AT1G42990	bZIP60	TGGCTAAAAAACGAAGAAGGAGAG	TCAAGCATACGTCCTAGTCTCAAG
AT1G43160	RAP2.6	GCTGTGACTAAAGAATGTGAAAGC	CCTTGTGTGGGTCTCGAATCTC
At1g52890	ANAC019	CGTTGTTGCTGAATTCTCTAGCAT	TCGTAGAAACGGAAGCACTAAGTT
At1g56330	SAR1	TGATTTGGGTGGTCATCAGA	TCCACAAGGTAGACAACAGCA
At1g58340	ZF14	TCTCAAAACTCGAAACCTGTGA	CGCCTTCACTTCCTCTAAGC
At1g69010	BIM2	TGCTTGCGACAGATCAATGCCT	CTCTAGTGCCTACTCGCATGAGTT
AT1G69800		TTGGGGAAAGATTTCTATGAAGTT	AAAAGGAGCCCATCTAAACGA
At1g71090	auxin efflux	TGTTGTGCCTTTGTTGAAGC	GAGCCTAAACGTGGCTCTTG
At1g72610	GLP1	CATTACCGCTGGGTTTGTCT	CTGTGGGAAAACCATGACCT
At1g74310	HSP101	GGAGAAGGATAAAGCCAGCA	TTCATCGTGAGAGGCTGAAG
At1g74320		CGAAGAGTTCCTCCATGCAC	AGACCATGAAACTCCTTCATCC
At1g74360	BRI1 LIKE 2	ACGGAGCTGAGCAAATGACT	TCATGTTTGGTCTTGCCTGA
At1g75040	PR5	CTCTTCCTCGTGTTCATCACA	ACGGTGGTAGGGCAATTGTT
At1g77690	amino acid permease	GCCACCAACATTCTCTACACC	CTTGAACTTTTGCGGTTTCC
At1g78700	BEH4	TCTGTAACGAAGCTGGTTGG	CTGAACCACCACCTATCTCCA
AT1G80080	TMM	TGTGTTAACCGGGACAGTGA	GTCTCGACGTTTCTGCATCA
AT1G80840	AtWRKY40	TGCGAGTTGAAGAAGATCCACCGA	TCCGAGAGCTTCTTGTTCTCAGCA
AT2G02160		GGAACACCGATTGCGTTTAC	GGGTTCATACGCGCATACTC
At2g05935		GAGTGCTGATTGCTGGAACA	TGTTGTGGTCTCCACGTCAT
At2g05990		TCAGGGCTTCCAATTGATTT	AGATTTTGCTATGGCCCAAC
At2g07050	CAS1	GAATGAAGCACGCAACCTTT	ATGAAGTGATGCCCAAAGAA
At2g22670	IAA8	GCCAAGGCACAGGTTGTTGGTT	TCCATGCTCACCTTCACAAACAGA
At2g25880	ATAUR2	CCCAATATTCTGCGGCTCTA	AAAGCTCACCCCTTACAGCA
AT2G25890		TGTTGGTACCGGCAGTGATA	AATCCACGTTAACGCCGTAG
AT2G25900		AACGGCCACAATCTCTTCAC	GAGAAGCGAAGGAGTCGAGA
AT2G27250	CLV3	TGGATTCGAAGAGTTTTCTGC	TCCTTGAACGTGAGCATGAG
AT2G28550	TOE1	GCCGAGGGAAGAGCAACAGAAAAG	TCTGCCATCCCCAGTTACTCATCA
At2g30040	MAPKKK14	ATCGGAACTCGGGAGAGATT	GCTGCAAGAGCTGATCACAG
At2g30140		CTCCTTCTTCCTCCACTCTGA	GGAGATAAACCGGGGACGTA
At2g34590		TGCTCCTTCTCGCTCTTCTC	CGAGCTACGAGGCTAGAACC
At2g34650	PID	TATTGCTCCGGTGGTGATTT	ACTAGAACTTCGGCGGCATA
AT2G36530	LOS2	TGTTGGAGCTGCTTCTTTCA	CATCCTGGCCGTACTTCTTC

At2g36800	DOGT1	CCTAAACCGTGCCATTGAGT	CCTTCTTGCAAACCAGCTTC
AT2G37170	PIP2B	TTTTGGTGGCATGATCTTCA	CGGGCTAAGAACAAACCAAA
AT2G38280	FAC1	CCGAGATGGAACATATTTGACC	CTGCATGAACATCCAAAAGG
AT2G38820		CTGAATGTTCTTCTGGTGAAGC	GTCAGCAAATTCCTCACACG
AT2G39270		GCGTTCCTCATATTGCCACT	TTTCCATGGTTAACAAGCTCCT
AT2G39290	PGPS1	TTGCAGCAGCCATTACAGAC	GATCCAAAAAGGCACCAAAC
At2g41100	TCH3	GAAGCCTTCCGCGTATTC	GCTTTTGTTTGGGTTTCACC
AT2G41310	ATRR3	TGCACTCTCTACATCGCCACAAAT	ACTTCTAAACGCCGCTGATTCCTT
At2g41430	ERD15	TTTGACACTGATGCTGCTGA	CCATCTCACCATTCTTTCCAA
At2g43820	UGT74F2	TCGAAAAAGCTGATTTCGTTC	CAGGACAAGCTTTCGACCAC
At2g45490	ATAUR3	TGGTGAGCTCTATGGTGTGC	CAATAGGCCAGTGCCTGACT
At2g46600	sim PBP1	GGAAGATGCTCAAGGAATGG	GGCTTAACCGAACCATGAGA
At2g46690		CCTTGTCACGTGGAGGAGTT	ACGGCCACCATGTCTATGAT
At3g01970	WRKY45	GAAGTACGGCCAAAAAGCAG	CCTCTGCACTTGCTTCTTCA
AT3G02220		GAATGCTAGGGAAAGAGATCG	CACTCCTTGACGCTTCTTCA
AT3G02230	RGP1	GTGGCTCAACATCCCTGATT	GATGGTCATGACAGCATCCA
At3g02310	SEP2	GACAAAGCTCTGTTGGCATCAAGC	TGAAGCAAGTTCGCTGCATCAAGA
AT3G02570	MEE31	CAGAGATGGCTTTGGCCTAT	TCCGGAATAGCTCGAATGAC
At3g02580	STE1	GTATCTCCATGCCACCCATC	ATCCCGTCTACTGGGTGAAA
At3g04730	IAA16	CGGACATGACGTTCTTGCGGAAAG	AAACCACCAGCCAAGGCACAA
At3g06810	IBR3	GATGCCATTGGTCTTGAGAA	CCTTCACTTGTTGAAGCCAAA
At3g10540	put sim PDK1	AATATGGGACTGATACATCGAGA	CATTGGCTTTACGCTACCAAA
AT3G15280		GGTTATGCTCTCCGACAAGC	TCGTCGTGGACTTATCGTCA
AT3G15290		TCCATTACTCGTCTTGCATCTG	TCATTATTGGAGGAGGGTTCA
At3g15540	IAA19	TCGGTGTGGCCTTGAAAGATGG	TGCATGACTCTAGAAACATCCCCC
At3g15630		CTCTTTTTGGCATGTCATCG	CGCAGAGACCTCTTTTCAGC
AT3G15640		ATGTAATGCCCATTGCGACT	AAGGACCTTCAGGGAAGTCAA
AT3G16050	A37	AAGCAGATGGGTAGGGTTCC	ACCCCATCACAACCTAGCTG
AT3G16060	kinesin	GCTGTTGCAAAGATTAAAGTCG	TGGCATGGGTGTCAACAATA
At3g16870	GATA 17	ACAATGACTTTGGTCCGGCAG	GACTTGCGTTGATTGCGGAA
AT3G17609	AtbZIP64	TCCCACAAGAAGCACAAAACTGC	CATTTTGAGTCTGGTCAAGCTCCG
At3g19700	HAIKU2	AACGAGATTTCTCGGCTCCT	TGTACACGTCGCTCTTCTCG
AT3G21270	ADOF2	TTGGAGGAGGAGGGATCATGCTTG	TCAAACCCAAACCCATACCCGGAT
AT3G24650	ABI3	GGCAGGGATGGAAACCAGAAAAGA	GGCAAAACGATCCTTCCGAGGTTA
AT3G26790	FUS3	TCCCTTCCTTGCATTCAAGTGCC	ATGATACTCCCGAAGAAAGCCGC
At3g43120	auxin responsive	GTGACTCGGACGAGGAAACT	GAGCTCGGGTCCAACATAAA
AT3G46540		TCCGATGAGTTTCAAGGTGA	TTTCTAACAGCTAATCCCCAAT
At3g50060	MYB77	GTAAAACCGCCGGAAATCGGCA	ATTCCGTCACCGTCGTCTCCTGTT
At3g50660	DWF4	CGGTGATCTCAGCCGTACAT	ATGACGCTCCGTTGTTTTG
AT3G51800	ATG2	ACTGCAGCTGATGTTGCTCT	GCTGCAGCTACCTTCTGAAT
At3g51810	AtEM1	AGCAAGGAGAGACCGTCGT	GTCTGCCCTCCCTTGCTC
At3g54320	WRI1	CCCGACGCAGCTCTATCTAC	TCTGAATCGAATTCCAAGAGC
AT3G54990	SMZ	TGGGCGGGTTTGATACTGCTTACG	TGTCTGCATCGAGACCACGGAATT
At3g58990		CGAGTTTCTCGCGATCTTCT	ACAACATCGACACCGATCAA
At3g61460	BRH1	CACCGTTTGTTCCAGATGAG	TCACGGGACAGTGAAAATCA
AT3G62260		TTGAGTTGCCGAAGCCTAGT	CCTAATGGCATTTTCCCGTA
AT3G62600		ACGGGAGGGATACTTTGTGA	TCACCGTCAAGAATGGGTTC
At3g62610	MYB11	CGTGGAGTTTACGGCTGAGATGAG	ACCAGGTGGTCAACAATTGCGAG
At3g62980	TIR1	GCTTCTCCACCGATGGTCT	GCCACTAACGTCGTCAACAT
AT4G01120	GBF2	TCGCCAATGATGGCTCCTTATGGA	GTGGTTGTGAGCCCATTTGAACAC
AT4G01120	GBF2	TCGCCAATGATGGCTCCTTATGGA	GTGGTTGTGAGCCCATTTGAACAC
At4g01130		TCAGACGGTCGTCTTATCATTG	ATGTCGAAAATCCGATCCAA
At4g03050	AOP3	TTTTGGAGAAAGCTCACGAA	TTTCTCAGCATACAGTTGGATCA
At4g05320	UBQ10	CACACTCCACTTGGTCTTGCGT	TGGTCTTTCCGGTGAGAGTCTTCA
At4g14130	XTR7	GGCACCGTCACTGCTTACTA	CATAAGGCTTCCCTGTCTCG

AT4G14540	HAP3	GGGAGACAAGGCGATAAGGAAGGT	TAGTCACCATGCCACCACCGTACA
At4g18010	IP5PII	TCGAGATTGAAGATTGGCTCT	ACGTTTCCAGCGTTTAATGG
AT4G18390		GGACCCTTCAGTCCAATTCA	TGTGGACCTCCTCCACTTTC
AT4G23100	RML1	CGTGCTGGTCTTGCTTTACA	AAATCCGTTTGGCTTTCCTT
AT4G23650	CPK3	AAGATGTTCGCCGTGAAGTC	TCGTAGGCTCCCTTCAAGTC
AT4G23930		CAATTCTCCGCCGTTAGAAA	TAACCGATCCGGTTTCCATA
At4g23940		CTCTGACGCCTCATGGCTA	CTTTCTCCATGGTGGTGGTT
AT4G24190	SHD	CCATAGCAAAATCTGGAACG	AATCCAACTCCAAATTGTCCA
AT4G24390		CATGCTCAAGCCCATCATAA	GCCACTGCCTGTAGTCCTTC
AT4G24400	CIPK8	CGAACATGTAAACCTGGATGA	TGGACCTGTGTCTCTTGTTCC
At4g33430	BAK1	AATGGAAGTGTTGCCTCCTG	CGCAATACGCTGTCTCTTTG
AT4G33440		CACATCAGCCTCATTCATGG	CACATTTTCCCCTGTCCATC
AT4G35090	CAT2	ATTTCATGCACAGGGACGA	CAGGCGGAGTTGGATACTTC
AT4G36490	SEC14	CTCGTCTTCAAAAGGTCGATG	AATCCTGAGCCAGCATTGAT
At4g36780	BEH2	AAGCTCTCTGTCTCGAAGCTG	CTGATGCTGGTGGCTAAACC
At4g36800	RCE1	TGCTACTAGAGGAGGAGCTTCTGT	TGAACAAGAGCTAGGAAGGTTCA
At4g36930	SPT	TCTTCCGTTGGAGCTAGTGG	TGACTTGGAAGAGGGAGCTT
At4g39800	MI-1-P SYNTHASE	TTCGTGTCGGATCTTTTAACG	ATCCCATCCTCCAAACACAA
At5g01990	auxin efflux carrier	AGGGACACTTCCAACCCTTT	ATAGAGGATGATGGCACCAA
At5g02820	RHL2	TCTGCCTCAGGAACATTCAC	CATCATCCAACACAGCATCA
AT5G03520	RabE1d	TTGAGCAACATGCCTCAGAT	CTTGGCCCTTTGCTGTTG
AT5G07020		TGGTTGGACTGGCTATGTTG	CTGAGCTAATCGCGCTCTCT
At5g07580		TGTGATGGTTTCATGGGAGA	CCGGATCAAGAACTGGAGAA
At5g08130	BIM1	AGTTGCTGCAACATCAGCTCTCAT	TGGGAAGAAGGGACAGACAAGGAA
At5g08330	TCP18	GTCGAGCAGACGTCAAACAA	TCCTTCTTCCTCTTCCGTCA
AT5G11260	HY5	GGCGACTGTCGGAGAAAGTCAAAG	TCAACAACCTCTTCAGCCGCTTG
At5g13080	WRKY75	AGGCCGTCAAGAACAACAAG	TGCACTTGCTTCTTCACATTG
At5g13630	GUN5	AGGCCGAGGATCGATGTAGT	CGCCACCATCTTGATAGCTC
At5g16750	TOZ	TTCAGCTGTGACTTCAATTGCT	GAAGGTCCCACAAGTTCACAA
At5g16760	inositol 1,3,4-trisphosphate 5/6-kinase	GCGAGAAATGTCCTGGTGTT	CCTCAAGCATCGAAACCCTA
AT5G17300		TGCTCTTACAACCACTGCAAA	TTGATCGAGCTCTCCCTGTT
AT5G17430	BBM/AIL2	ACTCGCAAAGGAAGACAAGGAGGT	GTGCGGCTAAATCGTAAGCCCTA
At5g19140		GCTTGCTTACAGCCATCACA	GAGCCTTGGAACAAGCAGAA
At5g25890	IAA28	GCTCCTCCTTGTCACCAATTCACT	ACTGGAGCTACCTCAACCCTGTTA
At5g46290		GAAAGATGCCTGGAAGATGC	CAGCAAGATCACCAGCAAGA
AT5G47120	BI1	ATCTTTGGTGGCTCTGCATC	TCTTGTGTGTCCACCACCAT
AT5G47390		TCCTTTCTTTCTCTCGCGGCTAGA	GGACGGAGGAAGAACACAAGCTA
At5g47670	L1L	TTGTACCTCCACCGCTACAGAGAG	GTCATACTAACGGACCCAGCACTG
At5g51210	OLEO3	CGCCTTCTCTTGGCTCTACA	GCACTCTGCTTCCAACCTTC
At5g54160	ATOMT1	GGTGTTTCCATTGCTGCTCT	ATGGAATCCCACCATCAAGA
At5g54510	DFL1	GATCAACAGAATCGCCAATG	CTCCACCAGAAGTCCCAGAA
AT5G57390	AIL5	AAGTTTAGGACCGCCGGGGTATA	TTCTCCAGTCGAACGGCAAGATGG
AT5G58380	CIPK10	AAGCCGGAGAACCTATTGCT	GAGGCCATCTTGTCTCTTGC
At5g62000	ARF2	TTGGACCCTGAGGCTGCTCTTTAC	TCGTCTTGTCTAGGAACCGTCACA
AT5G64050	ATERS/ERS/OVA3	TCGATGCTTTTGCTCAAGTG	GAAGCTGTTGCCCATTTCC
AT5G67300	MYB44	TTCAAGCCTGTGCCTAGACCTGGT	AGACGACGTTTCGATAGGAAGCGG
AtCg01120	RPS15	TCACATTTGGAATTGCACAGA	AGCAGTCGTTGACGTTTTCC

Table S2. Transcription factor expression profiling of Arabidopsis seed development. The expression of seed-'expressed' TF was measured by qRT-PCR at different stages of seed development. Cluster analysis was performed corresponding to the expression profiles of analyzed TF genes using the TM4 software.

ATG	**Annotation**	**TF family (AGRIS)**
cluster 1		
AT2G32370	DNA binding / transcription factor	Homoeobox
AT1G31290	PAZ domain-containing protein / piwi domain-containing protein	na
AT1G28300	LEC2__LEC2 (LEAFY COTYLEDON 2); transcription factor	B3
AT1G65330	PHE1_AGL37__PHE1 (PHERES1); DNA binding / transcription factor	MADS
AT1G50420	SCL3__SCL3 (SCARECROW-LIKE 3); transcription factor	GRAS
AT3G50060	MYB77__MYB77; DNA binding / transcription factor	MYB
AT3G16770	ATEBP_RAP2.3__ATEBP/ERF72/RAP2.3 (RELATED TO AP2 3); DNA binding / protein binding / transcription activator/ transcription factor	AP2/EREBP
cluster 2		
AT3G17600	IAA31__IAA31 (indoleacetic acid-induced protein 31); transcription factor	ARF
AT5G50080		AP2/EREBP
AT3G54320	WRI1_ASML1_WRI__WRI1 (WRINKLED 1); DNA binding / transcription factor	AP2/EREBP
AT5G07210	ARR21__ARR21 (ARABIDOPSIS RESPONSE REGULATOR 21); transcription factor/ two-component response regulator	ARR
AT3G04280	ARR22__ARR22 (ARABIDOPSIS RESPONSE REGULATOR 22)	ARR
AT1G26680	transcriptional factor B3 family protein	B3
AT4G09820	TT8__TT8 (TRANSPARENT TESTA 8); DNA binding / transcription factor	bHLH
At3g54620	ATBZIP25_BZO2H4__ATBZIP25/BZO2H4 (ARABIDOPSIS THALIANA BASIC LEUCINE ZIPPER 25)	bZIP
AT1G21970	LEC1_EMB212_EMB 212__LEC1 (LEAFY COTYLEDON 1); transcription factor	CBF-B/NF-Y
AT1G56170	HAP5B__HAP5B (Heme activator protein (yeast) homolog 5B)	CBF-B/NF-Y
AT5G47670	CCAAT-box binding transcription factor family protein / leafy cotyledon 1-related (L1L)	CBF-B/NF-Y
AT1G79840	GL2__GL2 (GLABRA 2); DNA binding / transcription factor	Homoeobox
AT2G45420	LBD18__LBD18 (LOB DOMAIN-CONTAINING PROTEIN 18)	LOB
AT1G68510	LBD42__LBD42 (LOB DOMAIN-CONTAINING PROTEIN 42)	LOB
AT3G57390	AGL18__AGL18 (AGAMOUS-LIKE 18); transcription factor	MADS
AT5G13790	AGL15__AGL15 (AGAMOUS-LIKE 15); DNA binding / transcription factor	MADS
AT1G08810	AtMYB60_MYB60__MYB60 (myb domain protein 60); DNA binding / transcription factor	MYB
AT3G62610	AtMYB11__AtMYB11 (myb domain protein 11); DNA binding / transcription factor	MYB
AT1G09540		MYB
AT3G13540	AtM2_AtMYB5_ATMYB5__ATMYB5 (MYB DOMAIN PROTEIN 5); DNA binding / transcription factor	MYB
AT5G35550	AtMYB123_TT2_MYB123__TT2 (TRANSPARENT TESTA 2); DNA binding / transcription factor	MYB
AT3G27780		na
AT1G32510		NAM
AT5G57520	ZFP2__ZFP2 (ZINC FINGER PROTEIN 2); nucleic acid binding / transcription factor/ zinc ion binding	Zinc Finger
AT5G65590	Dof-type zinc finger domain-containing protein	Zinc Finger
AT5G42200	zinc finger (C3HC4-type RING finger) family protein	Zinc Finger

cluster 3		
AT3G44460	ATBZIP67_DPBF2__DPBF2 (BASIC LEUCINE ZIPPER TRANSCRIPTION FACTOR 67); DNA binding / transcription factor	bZIP
AT2G40220	ABI4_GIN6_ISI3_SAN5_SIS5_SUN6__ABI4 (ABA INSENSITIVE 4); DNA binding / transcription factor	AP2/EREBP
AT5G10510	AIL6__AIL6 (AINTEGUMENTA-LIKE 6)	AP2/EREBP
AT3G26790	FUS3__FUS3 (FUSCA 3); DNA binding / transcription factor	B3
AT5G60830		bZIP
At3g62420	ATBZIP53__ATBZIP53 (BASIC REGION/LEUCINE ZIPPER MOTIF 53); DNA binding / protein heterodimerization/ sequence-specific DNA binding / transcription factor	bZIP
AT1G73730	EIL3_SLIM1__EIL3 (ETHYLENE-INSENSITIVE3-LIKE3); transcription factor	EIL
AT4G00220	JLO_LBD30__JLO/LBD30 (JAGGED LATERAL ORGANS)	LOB
AT1G67100	LBD40__LBD40 (LOB DOMAIN-CONTAINING PROTEIN 40)	LOB
AT5G07680	ANAC079_ATNAC4_ANAC080__ANAC079/ANAC080/ATNAC4 (Arabidopsis NAC domain containing protein 79, Arabidopsis NAC domain containing protein 80); transcription factor	NAM
AT2G01770	VIT1__VIT1 (VACUOLAR IRON TRANSPORTER 1); iron ion transmembrane transporter	unknwon
AT5G07500	PEI1__PEI1; nucleic acid binding / transcription factor	Zinc Finger
AT1G29160	Dof-type zinc finger domain-containing protein	Zinc Finger
AT2G46130	WRKY43__WRKY43 (WRKY DNA-binding protein 43); transcription factor	Zinc Finger
AT4G10150	zinc finger (C3HC4-type RING finger) family protein	Zinc Finger
AT1G72570	DNA binding / transcription factor	AP2/EREBP
AT1G01250	AP2 domain-containing transcription factor, putative	AP2/EREBP
AT5G57390	AIL5__AIL5 (AINTEGUMENTA-LIKE 5); DNA binding / transcription factor	AP2/EREBP
AT3G24650	ABI3__ABI3 (ABA INSENSITIVE 3); DNA binding / transcription activator/ transcription factor	B3
AT2G41070	EEL_ATBZIP12_DPBF4__EEL (ENHANCED EM LEVEL); DNA binding / transcription factor	bZIP
AT3G20910	CCAAT-binding transcription factor (CBF-B/NF-YA) family protein	CBF-B/NF-Y
AT5G10140	FLC_AGL25_FLF_FLC__FLC (FLOWERING LOCUS C)	MADS
AT3G02940	AtMYB107_MYB107__MYB107 (myb domain protein 107); DNA binding / transcription factor	MYB
AT5G16770	AtMYB9__AtMYB9 (myb domain protein 9); DNA binding	MYB
AT2G24430	ANAC038_ANAC039__ANAC038/ANAC039; transcription factor	NAM
AT5G41570		Zinc Finger
AT5G41350	zinc finger (C3HC4-type RING finger) family protein	Zinc Finger
cluster 4		
AT5G41570		Zinc finger
AT2G41070	EEL_ATBZIP12_DPBF4__EEL (ENHANCED EM LEVEL); DNA binding / transcription factor	bZIP
AT5G41350	zinc finger (C3HC4-type RING finger) family protein	Zinc finger
AT5G10140	FLC_AGL25_FLF_FLC__FLC (FLOWERING LOCUS C)	MADS
AT3G02940	AtMYB107_MYB107__MYB107 (myb domain protein 107); DNA binding / transcription factor	MYB
AT5G16770	AtMYB9__AtMYB9 (myb domain protein 9); DNA binding	MYB
AT2G24430	ANAC038_ANAC039__ANAC038/ANAC039; transcription factor	NAM
AT1G72570	AIL1__AIL1 (AINTEGUMENTA-LIKE 1); DNA binding / transcription factor	AP2/EREBP
AT1G01250	AP2 domain-containing transcription factor, putative	AP2/EREBP
AT5G57390	AIL5__AIL5 (AINTEGUMENTA-LIKE 5); DNA binding / transcription factor	AP2/EREBP
AT3G24650	ABI3__ABI3 (ABA INSENSITIVE 3); DNA binding / transcription activator/ transcription factor	B3
AT3G20910	CCAAT-binding transcription factor (CBF-B/NF-YA) family protein	CCAAT

cluster 5		
AT1G17310	MADS-box protein (AGL100)	MADS
AT2G46790	APRR9_PRR9_TL1__APRR9 (PSEUDO-RESPONSE REGULATOR 9); transcription regulator	APRR
AT4G33280	DNA binding / transcription factor	B3
AT3G47640	basic helix-loop-helix (bHLH) family protein	bHLH
AT5G10120	ethylene insensitive 3 family protein	EIL
AT5G65070	AGL69_MAF4_FCL4__MAF4 (MADS AFFECTING FLOWERING 4)	MADS
AT5G67300	AtMYB44_AtMYBr1_MYBR1__ATMYB44/ATMYBR1/MYBR1 (MYB DOMAIN PROTEIN 44); DNA binding / transcription factor	MYB
AT3G12720	AtMYB67_AtY53__AtMYB67/AtY53 (myb domain protein 67); DNA binding / transcription factor	MYB
AT3G51120	zinc finger (CCCH-type) family protein	Zinc Finger
cluster 6		
AT1G43160	RAP2.6__RAP2.6 (related to AP2 6); DNA binding / transcription factor	AP2/EREBP
AT1G28360	ATERF12_ERF12__ATERF12/ERF12 (ERF domain protein 12); DNA binding / transcription factor/ transcription repressor	AP2/EREBP
AT5G18450	AP2 domain-containing transcription factor, putative	AP2/EREBP
AT1G75490	DNA binding / transcription factor	AP2/EREBP
AT1G22985	AP2 domain-containing transcription factor, putative	AP2/EREBP
AT1G15050	IAA34__IAA34 (indoleacetic acid-induced protein 34); transcription factor	ARF
AT2G36270	ABI5_EEL_GIA1__ABI5 (ABA INSENSITIVE 5); DNA binding / transcription activator/ transcription factor	bZIP
At4g02640	ATBZIP10_BZO2H1__BZO2H1 (ARABIDOPSIS THALIANA BASIC LEUCINE ZIPPER 10); DNA binding / transcription factor	bZIP
AT5G12840	EMB2220_ATHAP2A_HAP2__HAP2A (EMBRYO DEFECTIVE 2220); transcription factor	CBF-B/NF-Y
AT5G65100	ethylene insensitive 3 family protein	EIL
AT3G02990	ATHSFA1E_HSFA1E__ATHSFA1E (Arabidopsis thaliana heat shock transcription factor A1E); DNA binding / transcription factor	HSF
AT5G54070	AT-HSFA9_HSFA9__AT-HSFA9 (ARABIDOPSIS THALIANA HEAT SHOCK TRANSCRIPTION FACTOR A9); DNA binding / transcription factor	HSF
AT1G77950	AGL67__AGL67	MADS
AT5G65080	AGL68_MAF5__AGL68/MAF5 (MADS AFFECTING FLOWERING 5)	MADS
AT2G42150	DNA-binding bromodomain-containing protein	MYB
AT1G01720	ANAC002_ATAF1__ATAF1 (Arabidopsis NAC domain containing protein 2); transcription factor	NAM
AT1G77450	ANAC032__ANAC032 (Arabidopsis NAC domain containing protein 32); transcription factor	NAM
AT1G74370	zinc finger (C3HC4-type RING finger) family protein	Zinc Finger
AT3G30460	zinc finger (C3HC4-type RING finger) family protein	Zinc Finger
AT4G30935	WRKY32__WRKY32 (WRKY DNA-binding protein 32); transcription factor	Zinc Finger
AT5G66730	zinc finger (C2H2 type) family protein	Zinc Finger
AT4G36620	zinc finger (GATA type) family protein	Zinc Finger
AT1G03790	zinc finger (CCCH-type) family protein	Zinc Finger
AT2G27940	zinc finger (C3HC4-type RING finger) family protein	Zinc Finger

nd	
AT4G31060	AP2 domain-containing transcription factor, putative
AT5G07160	DNA binding / transcription factor
At5g59340	WOX2__WOX2 (WUSCHEL-related homeobox 2); transcription factor
AT5G64530	ANAC104_XND1__ANAC104/XND1 (Arabidopsis NAC domain containing protein 104); transcription factor
AT1G43640	AtTLP5__AtTLP5 (TUBBY LIKE PROTEIN 5); phosphoric diester hydrolase/ transcription factor
AT3G10590	myb family transcription factor
AT3G19500	ethylene-responsive protein -related
AT3G62090	PIL2_PIF6__PIL2 (PHYTOCHROME INTERACTING FACTOR 3-LIKE 2)

Table S3.1. Microarray analysis: LEC1+ABA-regulated genes.

The expression of 535 genes was found to be significantly changed (p<0,05) by LEC1+ABA in comparison to the ethanol treated control. Log-ratios of two biological replicates are given.

ATG	dex aba 1	dex aba 2
AT1E57620	-0,718434463	-0,819339914
AT1E59250	-1,011828935	-0,784841355
AT1G01160	0,74144905	0,791693685
AT1G01225	0,82172816	0,760044141
AT1G01420	1,581429746	1,589500986
AT1G01430	0,906017715	0,906552467
AT1G01710	0,670760131	0,600251474
AT1G03600	0,634645764	0,608065345
AT1G03610	0,892729848	0,866542907
AT1G03800	1,369761107	1,330650676
AT1G04660	0,652076807	0,805490646
AT1G05000	-1,032326585	-0,984726162
AT1G05010	-1,413600706	-1,345407589
AT1G05420	-0,716202496	-0,680458615
AT1G06040	0,775013517	0,569451938
AT1G06180	1,021431586	0,883825693
AT1G06690	0,821662962	0,820125234
AT1G07030	-0,635819166	-0,583359454
AT1G07110	0,780466413	0,68038677
AT1G07640	0,786044988	0,754889816
AT1G07650	-0,817584489	-1,016374085
AT1G07870	1,286680516	1,175730413
AT1G07890	-0,79556276	-0,830703173
AT1G07890	-0,75353937	-0,806134376
AT1G09000	0,746316938	0,718924483
AT1G09510	0,776742758	0,818611925
AT1G10150	1,086308484	1,108094713
AT1G11860	0,782088133	0,729846764
AT1G11930	0,830986892	0,731484179
AT1G12230	0,64866534	0,707970624
AT1G13700	1,861352788	1,772514601
AT1G13900	0,73517608	0,696594947
AT1G13960	0,715022967	0,669308656
AT1G14580	0,743933585	0,60787415
AT1G14700	0,795018249	0,862676562
AT1G14810	-0,716425964	-0,584876391
AT1G15310	0,801271847	0,819622183
AT1G15960	1,848125932	1,70946374
AT1G16370	-0,670690654	-0,853080297
AT1G16560	0,845452882	0,736467124
AT1G17500	-0,748881363	-0,642657642
AT1G17830	1,253923883	1,161351078
AT1G18210	-0,680763611	-0,712630025
AT1G18460	1,211981848	1,17867346
AT1G18840	0,934662313	0,631960225
AT1G19180	0,85973379	0,665422441
AT1G19380	-0,650638208	-0,657329742
AT1G19440	-0,837097554	-0,845809056
AT1G19650	1,061555095	1,268278768

AT1G21480	0,719399596	0,585849288
AT1G21670	0,972344876	1,064596014
AT1G22590	0,738404837	0,681357162
AT1G23750	1,034104864	1,029805198
AT1G24490	-0,640093017	-0,599558895
AT1G26665	0,93235387	0,760168091
AT1G27050	-0,637368511	-0,61358537
AT1G27435	-0,857087684	-0,768611923
AT1G28100	0,779305139	0,797660174
AT1G28370	-0,958903184	-0,850761695
AT1G28400	-0,868111833	-0,768293823
AT1G29660	-1,630576014	-1,384450291
AT1G30040	-0,671855502	-0,694952651
AT1G30220	0,703607818	0,853997977
AT1G31340	-0,667502725	-0,620125765
AT1G31780	0,781815766	0,578671037
AT1G31830	1,263082936	1,193634879
AT1G32080	0,678120635	0,649397905
AT1G32170	0,985016209	0,922767596
AT1G32330	0,772011129	0,657192039
AT1G33390	-0,835072253	-0,683423129
AT1G35140	-1,107493001	-0,948515805
AT1G35560	-0,647950228	-0,579169322
AT1G41830	0,750215729	0,616251965
AT1G42960	1,235738015	0,847677657
AT1G44000	0,760539576	0,75122592
AT1G49570	-1,048885851	-1,036422382
AT1G50170	-0,654462411	-0,710088423
AT1G51390	0,67482692	0,802263037
AT1G51690	0,626287272	0,605969447
AT1G52100	1,14547211	1,091402495
AT1G52340	0,653307895	0,719561911
AT1G52670	1,371293315	1,220777834
AT1G53170	1,087412778	0,877243541
AT1G54290	0,865371133	0,760928073
AT1G54860	1,873187763	1,883402853
AT1G55000	0,631779213	0,631450664
AT1G55340	0,811291196	0,800079592
AT1G55360	1,127976725	1,089379092
AT1G57770	1,189093563	1,183507822
AT1G58170	-1,014760511	-0,861323999
AT1G59940	-0,980660582	-0,770352307
AT1G60220	0,702528243	0,623141161
AT1G60770	-0,824651422	-0,703931897
AT1G60950	0,646847688	0,596718576
AT1G61255	1,254042963	1,070254013
AT1G61340	-0,786714908	-0,574179376
AT1G61730	-0,849535921	-0,768352449
AT1G64440	0,822742829	0,623796332
AT1G64690	1,144016887	0,977623925
AT1G64780	-0,899519693	-0,761030874
AT1G65230	0,823852372	0,713669264
AT1G65320	0,799717318	0,686203446
AT1G65420	0,811502499	0,70025104
AT1G66900	0,660567946	0,678473779
AT1G67750	1,740778703	1,779385031

AT1G69010	1,158843464	1,126294409
AT1G69410	1,27502183	1,278991835
AT1G69430	0,708925668	0,656100201
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AT5G47120	0,720877198	0,592676509
AT5G47210	-0,720244498	-0,591899058
AT5G47390	0,751887487	0,579307486
AT5G47770	0,860833068	0,674483525
AT5G48230	0,725017576	0,637028104
AT5G48290	-0,929457509	-1,040244339
AT5G48450	-0,716714725	-0,668244474
AT5G49460	0,795821534	0,650316845
AT5G49650	1,139959878	1,028491451
AT5G51210	2,146696225	2,113439031

AT5G51970	0,709800181	0,769361195
AT5G52420	0,677726094	0,668708889
AT5G52440	0,852024876	0,638451331
AT5G52550	0,705740893	0,660620901
AT5G53750	-1,647813016	-1,601638908
AT5G53860	0,804300731	0,671007384
AT5G54930	0,920150886	0,774319254
AT5G54940	0,856517152	0,735850318
AT5G55140	-0,723133005	-0,69729839
AT5G55990	0,868101251	0,676451786
AT5G56010	-0,686750436	-0,782079442
AT5G56750	0,659408361	0,594819224
AT5G57270	0,783220888	0,733097442
AT5G57280	-0,841212894	-0,863819161
AT5G57390	1,36779849	1,42283744
AT5G57630	0,807287912	0,583453183
AT5G58320	0,787986029	0,819282793
AT5G58350	1,312388213	1,320146316
AT5G58380	0,905601255	1,000357197
AT5G58660	2,206801683	2,148445004
AT5G58870	0,641699324	0,632456351
AT5G58980	0,817838997	0,65136008
AT5G59890	1,043132656	0,937059149
AT5G60300	-0,6548921	-0,782964287
AT5G60580	1,19959513	0,996283172
AT5G60600	1,010772779	1,000917355
AT5G60840	-0,647995547	-0,88830996
AT5G62090	0,741022042	0,724441277
AT5G62500	0,639961491	0,579267563
AT5G63530	2,1207569	2,135816398
AT5G63560	0,7435301	0,829932927
AT5G63620	0,77899264	0,601037067
AT5G64510	1,048129849	1,306152445
AT5G65310	-0,881342825	-0,859439201
ATMG00160	-0,862740105	-0,834771759
ATMG01370	-0,868291098	-0,67990535
Nogene	-0,795110951	-0,695043761
Nogene	-0,788456801	-0,657055674
Nogene	0,774512113	0,82792197

Table S3.2. Microarray analysis: LEC1-regulated genes.

The expression of 117 genes was found to be significantly changed (p<0,05) by LEC1 and LEC1+ABA in comparison to the ethanol treated control. Log-ratios of two biological replicates are given.

ATG	dex 1	dex 2	dex+aba 1	dex+aba 2
AT1G01520	0,791654694	0,641581106	1,288387124	1,054018706
AT1G10070	-0,650515697	-0,656369834	0,964395438	0,962148273
AT1G12900	0,803922612	0,620647866	0,83421995	0,80560027
AT1G13390	-0,754586306	-0,644483407	-0,952600096	-0,828342356
AT1G15415	-1,100880952	-1,070015203	-0,665652336	-0,67686412
AT1G18740	0,774998775	0,832725516	0,943621804	0,842952814
AT1G19530	-1,03125084	-1,036475726	-0,808102736	-0,777729279
AT1G21500	1,677667818	1,452077808	1,153675214	0,902992291
AT1G21750	0,859606199	0,738352244	0,690510556	0,607118496
AT1G26770	-0,963557518	-0,836682079	-1,216998589	-1,227351666
AT1G28480	-1,207650188	-0,904241794	-1,312282957	-0,995965664
AT1G29070	0,674943029	0,590413302	0,703737114	0,589991685
AT1G30870	-1,142305065	-1,254952049	-1,266240238	-1,35367619
AT1G32060	0,734893269	0,65148831	1,518765416	1,519539696
AT1G33110	-1,580264004	-1,574596295	-1,117258807	-0,805973554
AT1G34680	-1,665410895	-0,781437775	-1,729845132	-0,841001127
AT1G35420	0,591920122	0,648926213	1,066857659	1,061021771
AT1G64510	0,874905428	0,728914278	0,832132701	0,611864666
AT1G72520	-1,342520366	-1,056410429	-1,304049932	-0,965099393
AT1G73120	2,73119529	2,867696307	2,066770368	2,147057963
AT1G74450	0,984248053	1,006464906	1,325823224	1,37144251
AT1G76590	-0,584433674	-0,656600596	1,074124068	1,14764216
AT1G78850	-0,874325708	-0,924990961	0,834584179	0,91170768
AT2G03090	0,89997088	0,868007038	1,331216499	1,283560717
AT2G03720	-0,689987994	-0,613323059	-1,052350857	-0,964169394
AT2G07739	-1,134143463	-0,67647199	-1,345637879	-1,086478622
AT2G25000	-0,860710347	-0,888944497	-0,743495116	-1,007857054
AT2G26300	-1,161908822	-0,888056817	-1,124162984	-0,960804664
AT2G27310	-0,786006261	-0,672541985	-0,924681533	-0,785465204
AT2G31725	0,918542733	0,71292063	0,640699405	0,588167218
AT2G33180	0,950854235	0,719885999	0,796511249	0,573730268
AT2G33585	-0,822432971	-0,686447617	-0,971316256	-0,879584474
AT2G34420	1,813369026	1,674933711	1,849293703	1,618342409
AT2G37970	-1,071321766	-0,978577875	0,855882751	1,046631626
AT2G38140	0,890327099	0,67224063	0,739589601	0,595604142
AT2G38240	-1,415656071	-1,276768118	0,635015828	0,99254111
AT2G38940	-0,950050159	-0,793835727	-1,205100063	-1,044851157
AT2G39570	-0,725730969	-0,724899671	0,658300225	0,812581821
AT2G44110	-0,633629183	-0,676570595	-0,723671599	-0,739513524
AT3G05730	1,296599663	1,123180153	1,40872059	1,041655278
AT3G06435	-0,868668215	-0,784894707	-1,122922218	-1,015418251
AT3G08940	1,321702697	1,289816297	0,74932744	0,575101613
AT3G12050	-0,766127413	-0,744578948	-0,97177292	-0,903369629
AT3G13510	0,694159521	0,67186707	0,718293078	0,685288868
AT3G14930	0,602268856	0,593719995	0,844961744	0,74207805
AT3G16670	0,819637936	0,672625321	1,239666158	0,732599241
AT3G18080	0,591186491	0,671185536	0,909732119	1,015250485
AT3G18830	-1,183251628	-1,100173487	-0,968492633	-0,908326623
AT3G20860	-0,788509172	-0,952870548	-1,392472379	-1,300266667
AT3G26650	1,076012897	0,890548212	1,050655529	0,94370989

AT3G26690	-0,828541831	-0,794877529	-0,823091728	-0,929477597
AT3G29320	0,689734249	0,704920664	0,850101404	0,883350965
AT3G46220	-1,366594931	-1,166781498	-1,073202814	-0,925772081
AT3G47460	1,633380404	1,432501389	1,050606742	1,169533201
AT3G47720	-0,933433267	-0,860784259	-0,820679482	-0,662479887
AT3G50900	-1,030846124	-0,711281183	-1,423582941	-1,22781503
AT3G51660	0,618339457	0,645920849	-0,983446277	-1,192316334
AT3G52150	1,153302897	0,998001407	0,904760476	0,699640043
AT3G54050	0,921494674	0,800716303	0,751171268	0,634598756
AT3G54210	1,001296271	0,676836119	0,832966149	0,669907768
AT3G55250	1,128084812	1,057258792	0,935546473	0,728453819
AT3G55800	0,901667464	0,837912554	0,648392866	0,576948696
AT3G60530	0,853683343	0,703511135	1,037372464	0,755044344
AT3G61490	-0,879969022	-0,581863291	-1,226355428	-0,953268617
AT4G01120	0,787902747	0,679357063	1,240474232	1,25896961
AT4G02770	1,370918551	1,18641274	0,9515895	0,602381757
AT4G08850	0,744838912	0,799620085	-0,694836388	-0,833368891
AT4G13180	-0,978845526	-0,703458883	-0,82485988	-0,658580773
AT4G15110	0,596897984	0,589675258	0,701094047	0,616755184
AT4G19450	-0,662708117	-0,5954588	-0,659807679	-0,634535221
AT4G23820	0,663135636	0,723650528	-0,699487544	-0,713037463
AT4G24190	0,971518674	0,735288623	0,921677032	0,71238896
AT4G25080	0,798097738	0,710326778	0,930186258	0,742829332
AT4G25820	-1,440967674	-1,537659743	-1,043371514	-1,279116983
AT4G26010	-0,992882171	-0,972038904	-1,239627589	-1,150622367
AT4G27840	0,629782552	0,592379781	0,696009215	0,728204971
AT4G28850	-1,459908577	-1,334312253	-1,603410228	-1,486222552
AT4G30320	-0,883160932	-0,931248884	-1,174760496	-1,117314104
AT4G30530	-0,756874178	-0,792780685	-1,139571575	-1,150784222
AT4G31860	-1,146952046	-1,182708755	-0,760971829	-0,670242307
AT4G34110	-1,453644222	-1,355781208	-0,854351164	-0,799821536
AT4G34220	-1,148539309	-0,946838831	-0,796875694	-0,795611564
AT4G34590	-0,894597328	-0,951122631	0,993437923	1,019202511
AT4G37295	-1,225097645	-0,920696411	-1,502906753	-1,308952251
AT4G38620	0,614869738	0,769572106	0,744313428	0,912464379
AT5G01075	1,418851339	1,362675592	2,377727749	2,104321178
AT5G01530	0,776509061	0,619183286	0,872043915	0,705382226
AT5G03545	-1,15354265	-1,016683531	-1,257058936	-1,453591163
AT5G05500	-1,214165912	-1,280698662	-1,317543678	-1,399225486
AT5G18270	-0,931926654	-0,976460248	-0,904659006	-0,761566972
AT5G18600	0,959170294	0,753421137	2,349403062	2,211289653
AT5G19110	-0,661612873	-0,621261522	-0,911161569	-0,730220347
AT5G19940	1,168306944	1,010407751	0,736698648	0,696710837
AT5G23210	1,082288202	0,915978866	1,304234247	1,093036514
AT5G24314	0,798728734	0,695203702	0,885630529	0,720603346
AT5G24490	1,222761487	0,896771908	1,738893327	1,699925691
AT5G33290	-0,563978184	-0,725487842	-0,643159775	-0,83287698
AT5G38410	0,716235911	0,715834031	1,152284619	1,086537933
AT5G38520	1,089888091	0,894020619	1,750951318	1,667094947
AT5G44580	0,921883502	0,870584153	0,990835405	0,931287486
AT5G45930	1,00605997	0,688978622	0,852789104	0,670418037
AT5G52960	0,775140349	0,600314898	0,705397937	0,629944409
AT5G57660	0,648731495	0,715807266	0,700977903	0,59091751
AT5G57930	0,74992833	0,721225724	0,828066044	0,671195773
AT5G58070	-1,004121422	-0,910301608	0,676352287	0,763439765
AT5G59530	-0,831739373	-0,698299149	-0,89566733	-0,769548285

AT5G59820	-0,688337753	-0,67422147	-1,387035679	-1,141646368
AT5G59870	1,127560306	1,024125639	0,734004287	0,574422903
AT5G61890	-0,899674996	-0,852131726	-0,902566063	-0,830248196
ATCG00065	-1,59587542	-0,969210485	-2,172427584	-1,071112955
ATCG00210	-2,174030608	-0,79468391	-2,403409761	-1,182606044
ATCG00330	-1,893075156	-0,75028659	-1,791505242	-0,753901238
ATCG00430	-1,697987276	-0,788263062	-1,693561613	-0,83584719
ATCG00740	-1,548243066	-0,592333345	-1,992680515	-0,982318255
ATCG00790	-1,675380791	-0,844551252	-1,525375742	-0,778685038
ATCG01070	-1,581094861	-0,610012241	-2,123661474	-1,065766887
ATMG01320	-1,505433503	-0,692873451	-1,216172285	-0,804116341

Table S3.3. Microarray analysis: ABA-regulated genes.

The expression of 1576 genes was found to be significantly changed ($p<0,05$) by ABA and LEC1+ABA in comparison to the ethanol treated control. Log-ratios of two biological replicates are given.

ATG	aba 1	aba 2	dex+aba 1	dex+aba 2
AT1E27540	1,119647286	1,098811462	3,016582215	3,125662173
AT1G01010	-0,756512163	-0,618636136	-0,799507957	-0,73208539
AT1G01120	1,967623518	1,496080293	0,693259837	0,664444844
AT1G01140	1,342574257	0,840562153	0,719251305	0,727746787
AT1G01240	1,776441196	1,316560802	2,039097587	2,148956941
AT1G01250	1,444503203	1,077714493	1,742659349	1,6237005
AT1G01470	2,177119659	1,676397024	1,198654153	1,179795802
AT1G01830	-0,816991715	-0,603077371	-0,734372302	-0,760535406
AT1G01940	-1,151128046	-0,839364465	-0,835785646	-0,997253301
AT1G02205	4,493339509	4,272721509	2,405674944	2,389548136
AT1G02305	1,119997718	1,269594958	1,697588016	1,591510498
AT1G02310	1,401819777	1,474845995	2,229377316	2,236698303
AT1G02360	-1,085959985	-0,972167386	-0,936175537	-1,076238288
AT1G02660	1,621339729	1,532212929	2,141176887	2,16412532
AT1G02816	2,406463759	2,089432581	2,278907055	2,221384001
AT1G02870	-1,153842177	-0,941950807	-0,649210315	-0,837123668
AT1G03030	1,093753494	0,762860461	0,772634288	1,099650402
AT1G03360	-1,795515282	-1,41701571	-1,237391145	-1,27937787
AT1G03530	-1,104839405	-0,946310084	-1,03428642	-0,950083269
AT1G03790	1,704417163	1,472348589	2,657960919	2,777907771
AT1G03830	-0,955374882	-0,770314327	-0,710497697	-0,665075368
AT1G03860	-1,037427671	-0,875884507	-0,871035494	-0,979248201
AT1G03870	-3,185941779	-2,910306585	-3,412713386	-3,489415507
AT1G04120	0,716881487	0,736441466	1,406295807	1,647529289
AT1G04190	-0,602351634	-0,647634168	-0,952001434	-0,790549792
AT1G04210	3,693962732	3,708064336	3,14432759	3,060532035
AT1G04310	0,810757361	0,653354282	2,106882678	2,253946834
AT1G04350	0,980183024	0,982904452	1,000294557	1,156210076
AT1G04400	0,704699738	0,803993974	1,128799105	1,037723906
AT1G04430	-0,759599399	-0,609267373	-0,867655193	-0,876495677
AT1G04800	-0,739904875	-0,855544729	-0,776709187	-0,769240069
AT1G04870	-1,209678753	-1,057813523	-1,116941296	-0,940436779
AT1G04940	-1,679591012	-1,4524389	-1,542101237	-1,523026046
AT1G05300	-2,376358316	-2,369756659	-3,036131311	-3,049046428
AT1G06400	1,278750314	1,154400855	1,447474744	1,511918892
AT1G06400	1,11612333	0,962042475	1,257024169	1,381579441
AT1G06430	1,255451764	1,317755372	0,939477686	0,942266897
AT1G06670	-0,699329412	-0,641195249	-0,865649598	-0,691229391
AT1G06840	-0,815456672	-0,66584284	-1,224179974	-1,143678756
AT1G07000	-0,85297817	-0,737699305	-0,900541373	-1,036070212
AT1G07070	-1,190010971	-0,92842515	-0,805040133	-0,940142064
AT1G07080	0,761845467	0,814975952	0,883522482	0,896834028
AT1G07090	-1,0453192	-0,9391697	-0,958640435	-0,87796166
AT1G07210	-0,846638867	-0,817969369	-1,110886582	-0,957660204
AT1G07280	1,070322929	0,877430398	0,904452099	0,624281911
AT1G07570	-0,73919262	-0,696747622	-0,679365611	-0,688883003
AT1G07620	-0,998826103	-0,775839609	-0,65181687	-0,74279651
AT1G07720	2,195199981	1,920306153	1,566098889	1,639692442

AT1G07830	-1,403184026	-1,146988293	-0,874013489	-0,972406176
AT1G07840	-0,801725176	-0,656727941	-0,663303553	-0,790389777
AT1G07930	-0,927595596	-0,972995088	-0,903746344	-0,958011952
AT1G07940	-1,085053945	-0,991456405	-0,835887408	-0,91929518
AT1G07985	2,791029689	2,466886269	3,300321184	3,132815729
AT1G08040	0,646124606	0,774587956	0,818163154	0,800191512
AT1G08230	1,879474009	1,732739329	1,474244487	1,522393397
AT1G08410	-1,470634286	-1,343246115	-1,427125545	-1,362586626
AT1G08580	-1,589339372	-1,306762739	-1,529291512	-1,495892287
AT1G08630	1,25713872	0,754309124	1,055278099	1,090303057
AT1G08845	-0,954340037	-0,998164324	-1,194556587	-1,076088399
AT1G09130	1,288926465	0,749735562	0,711064868	0,775915934
AT1G09430	0,623489776	0,673979323	0,725588539	0,760000341
AT1G09530	1,15721618	1,133689045	1,955216029	1,810891552
AT1G09640	-0,938014749	-0,842554808	-0,807084868	-0,806879244
AT1G09690	-0,869834474	-0,790783872	-0,690340745	-0,692619939
AT1G09815	-1,088354555	-0,927443018	-0,872412893	-0,979019362
AT1G09830	-0,927707505	-1,031857598	-1,116367786	-1,070254432
AT1G09850	1,027819686	1,114283042	1,261278109	1,285962988
AT1G10070	0,737526356	0,879076005	1,097640711	1,044705045
AT1G10230	-0,975828962	-0,698161962	-0,748791445	-0,922968419
AT1G10270	-1,016101446	-1,04982807	-1,458296202	-1,321129968
AT1G10340	-1,326992404	-0,817379506	-0,690900668	-1,00882151
AT1G10450	-1,118784519	-0,922822029	-0,653629632	-0,791030309
AT1G10500	1,197528111	1,101802968	1,005900234	0,905123912
AT1G10522	-1,383078685	-1,271111254	-1,06490721	-1,026599644
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AT1G11280	-0,874153898	-0,72105109	-0,662166818	-0,651863505
AT1G11475	-1,413879102	-1,039616258	-1,264739058	-1,412414315
AT1G11840	1,241426131	1,167286797	1,161497048	1,047303552
AT1G11910	1,221857622	1,301805545	1,356599353	1,357297182
AT1G12640	1,095125417	0,667345791	0,666152379	0,706591288
AT1G12830	-1,31787743	-1,176885932	-1,120633266	-0,977939703
AT1G12845	3,334612342	2,759757244	1,561090749	1,54718889
AT1G12920	-0,835021345	-0,720656943	-0,915193157	-0,784571278
AT1G13160	-1,553434891	-1,284838273	-1,236186103	-1,279855087
AT1G13280	1,101580663	0,948585871	1,06348511	1,003885554
AT1G13360	1,306523482	0,78637409	0,817833696	0,924396069
AT1G13380	-0,80043045	-0,693981116	-0,628711438	-0,705197852
AT1G13740	0,998941055	0,642801417	1,041533862	1,136306403
AT1G14060	-1,139257713	-0,890113531	-1,159197173	-1,260918669
AT1G14300	-1,630567963	-1,26318916	-1,370280336	-1,387723563
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AT1G14620	-1,626321639	-1,400102494	-1,277547015	-1,472144368
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AT1G14980	-0,968137623	-1,001647201	-1,280952972	-1,26353212
AT1G15200	-0,725231104	-0,673614828	-0,852962726	-0,793753416
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AT1G15440	-1,250590398	-1,03167309	-0,838824332	-0,923842614
AT1G15480	-1,553174444	-1,447081039	-1,574167662	-1,464664838
AT1G15740	1,547400588	1,611875939	1,493066195	1,560826028
AT1G15870	-1,414152743	-0,964149171	-1,339728349	-1,306046054
AT1G15930	-1,066534175	-0,891537044	-0,621939782	-0,704051213
AT1G16180	0,674767469	0,615237163	0,796229741	0,596584943
AT1G16350	-1,259156552	-1,00651144	-0,712155383	-0,765221905

AT1G16400	-0,80022354	-0,651238151	-1,313924602	-1,476579867
AT1G16445	-1,064317019	-0,77944749	-0,879173874	-0,894362822
AT1G16640	-1,134729632	-0,964774194	-1,149440997	-1,258802472
AT1G16650	-1,12651249	-0,959732463	-0,9969648	-1,078158562
AT1G16830	-1,209270159	-1,084965207	-1,160341111	-1,156059301
AT1G16840	1,742087311	1,006846842	1,021564162	0,814244884
AT1G16850	4,518452785	4,124744117	3,645082466	3,64434605
AT1G17020	2,081209683	1,931460483	2,076495705	2,019938145
AT1G17100	0,842340131	0,687857332	1,521998539	1,714888227
AT1G17560	-1,44270467	-1,274705076	-1,345172266	-1,451474923
AT1G17745	1,693390354	1,708136864	1,544492673	1,589796121
AT1G17840	1,104378276	1,054282005	0,744094186	0,823228016
AT1G18100	1,410322933	1,006672329	1,167753683	1,063844037
AT1G18480	-1,151067634	-1,003473383	-0,868253358	-0,780934702
AT1G18590	-0,841582231	-0,63607686	-1,09615084	-1,029764054
AT1G18630	-0,859315964	-0,715744949	-0,772301565	-0,724846567
AT1G18800	-1,0132876	-0,766721676	-0,979074182	-1,025616543
AT1G18850	-1,538609127	-1,195753448	-1,399596805	-1,409423416
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AT1G19050	-1,723174764	-1,413303329	-2,320261663	-2,493451252
AT1G19120	-0,868261774	-0,644140073	-0,749380239	-0,610060343
AT1G19200	2,056606072	1,793318247	1,710655628	1,738389858
AT1G19230	-1,332023858	-1,18856393	-1,247092404	-1,336073018
AT1G19525	-1,10756266	-0,938073122	-1,146954649	-1,058899779
AT1G19600	-1,141070098	-0,945184827	-0,665631904	-0,665629674
AT1G19970	2,501301456	2,357427988	2,639006103	2,539853366
AT1G20220	-0,883393309	-0,890398666	-1,078279706	-0,919115255
AT1G20225	0,886401841	0,78670758	0,933144517	0,811439313
AT1G20510	-0,957283515	-0,737615279	-1,47839091	-1,459187726
AT1G20900	-0,745543857	-0,716133989	-1,136657066	-1,121891504
AT1G20910	-0,920338882	-0,747866385	-0,667380544	-0,636733341
AT1G21050	-0,865833014	-0,792856466	-1,006514713	-1,065407817
AT1G21270	-2,220246835	-1,679558889	-2,216995141	-2,328090837
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AT1G21460	2,768770069	2,235264045	1,99419761	1,770626977
AT1G21520	-0,591386475	-0,632113773	-2,776398897	-2,89045717
AT1G21790	1,742762107	1,695252143	1,081469414	1,132604438
AT1G21910	-1,512860305	-1,427943044	-1,129722375	-1,29079965
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AT1G22640	2,610499026	2,415269681	1,447188274	1,396145064
AT1G22690	-2,832581549	-2,717742975	-2,364402526	-2,360238874
AT1G22780	-0,922402135	-0,817986822	-0,833687561	-0,791004835
AT1G22930	0,912342499	0,949358352	1,74500319	1,755854829
AT1G23040	1,086707541	0,956179564	1,453529332	1,067285754
AT1G23120	0,926844787	0,749203584	1,059202685	0,99758509
AT1G23190	1,347878815	1,411042044	1,293005232	1,318240595
AT1G23205	0,899117967	1,072313203	2,876280846	2,8527981
AT1G23280	-1,783637244	-1,362157696	-1,282213421	-1,461836859
AT1G23730	1,087191829	1,065970804	1,175461101	1,103810282
AT1G23800	2,376021869	2,483395846	3,022151496	3,020854583
AT1G24470	1,270693723	1,164472218	0,721868045	0,61290589
AT1G24600	2,178564345	2,066294074	1,132678549	1,211426667
AT1G25260	-1,402960279	-1,228699982	-1,184763768	-1,267819242
AT1G26340	-0,845727759	-0,828282172	-0,821558027	-0,864087312
AT1G26450	1,122221458	1,099786475	1,651285863	1,784111605
AT1G26470	-0,804990663	-0,649343508	-0,721501077	-0,853194354

AT1G26560	0,627334404	0,884965697	0,950781425	0,766337039
AT1G26670	0,746392795	0,825766784	0,650427786	0,616406754
AT1G26740	-1,180744577	-1,059642614	-1,03101652	-1,05206918
AT1G26880	-1,032758507	-0,886473929	-0,785605342	-0,99474709
AT1G26910	-0,937309283	-0,933021681	-1,092129805	-1,028684493
AT1G26920	1,705825054	1,358254139	0,788219043	0,684400608
AT1G27060	-1,346135002	-1,161236052	-1,118250071	-1,245626257
AT1G27130	-0,836390296	-0,799456629	-1,121116816	-1,149429297
AT1G27210	-1,130551203	-0,890535765	-0,629308171	-0,86579989
AT1G27290	2,149205104	1,573724182	1,429277517	1,413332128
AT1G27400	-1,214171417	-0,991022914	-0,620859008	-0,743079284
AT1G27470	-1,658873856	-1,357237472	-1,392615064	-1,402735228
AT1G28010	-1,150297274	-0,826549147	-1,158975088	-1,118631849
AT1G28210	-0,987226864	-0,698850346	-1,22436612	-1,028423215
AT1G28260	1,336142211	1,100514285	1,478100158	1,456705685
AT1G28290	-0,876900651	-0,815463347	-0,955653771	-0,94092119
AT1G28380	-1,414386117	-1,229304563	-1,527471743	-1,644827753
AT1G28520	1,362554894	0,648614041	0,765441103	1,018544175
AT1G28960	1,402618224	1,265908719	1,381936634	1,45153976
AT1G29250	-1,508299724	-1,409041653	-1,273911542	-1,408792088
AT1G29280	-1,035652421	-0,855090764	-0,902218818	-0,905802112
AT1G29330	1,319945487	0,879309062	0,905292241	0,891109828
AT1G29690	-1,108253894	-0,89652668	-0,716967774	-0,80774295
AT1G29880	-1,185736746	-0,809013526	-0,622174276	-0,638960011
AT1G29940	-1,886900687	-1,330068159	-1,593095228	-1,592206243
AT1G30120	1,131215927	1,001751636	1,782077287	1,777086097
AT1G30130	1,01688526	0,824194815	1,157902793	1,019375185
AT1G30230	-0,927423817	-0,687998321	-0,737071785	-0,693738136
AT1G30240	-1,123746335	-0,742939381	-0,740199824	-0,820846204
AT1G30360	1,082750302	0,987871291	0,884695477	0,833464876
AT1G30370	-0,801354838	-0,756807334	-0,788263935	-0,92217434
AT1G31660	-1,185489322	-1,076840339	-1,141367478	-1,213765089
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AT1G31820	1,70112784	1,62042275	1,686311141	1,700322478
AT1G32400	0,757665616	0,675287331	0,839927612	0,802653577
AT1G32410	0,923372496	0,764447152	1,249420239	1,258190281
AT1G32450	1,903156432	2,184041959	1,662385215	1,653616148
AT1G32520	0,775395834	0,702101703	0,735301723	0,741007697
AT1G32580	-1,299164265	-1,080051172	-1,180662594	-1,321693102
AT1G33480	1,828184204	1,79648182	1,993412493	2,023596909
AT1G33600	-1,156958261	-0,920800689	-0,663790766	-0,668142714
AT1G34370	-1,268654245	-0,948797964	-0,816288627	-0,82550407
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AT1G36160	1,057946138	1,030913905	1,198412931	1,269230676
AT1G36310	-0,842195414	-0,675549726	-0,752555734	-0,727493057
AT1G36380	1,753767237	1,434024371	1,656687685	1,545745301
AT1G42440	-1,191794541	-0,994807983	-0,93543356	-1,063432686
AT1G43860	-1,328681926	-0,920262452	-0,781651534	-0,938864588
AT1G44170	1,131935197	1,129816276	0,990275217	0,838856068
AT1G44800	1,476289203	1,454124452	1,306843451	1,332468286
AT1G47840	-1,388919498	-0,944677091	-1,165487948	-1,047418676
AT1G47960	3,088520007	2,265247659	2,532495647	2,189832734

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AT1G48100	1,518286273	1,46265401	1,248823179	1,360830902
AT1G48320	0,709504701	0,848691143	1,092610811	0,783461652
AT1G48370	1,050847943	0,702307497	1,528058255	1,54318589
AT1G48460	-1,300448724	-1,254224876	-1,028989778	-0,967010894
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AT1G48750	3,210112277	2,682379588	1,443094899	1,630539288
AT1G48920	-0,912124572	-1,103160592	-1,652653873	-1,313706501
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AT1G49540	-1,169155143	-1,081183404	-1,09323378	-1,018671862
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AT1G49670	2,147192865	1,344582926	1,367429638	1,599109544
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AT1G50020	1,201035518	0,869304996	1,338979044	1,316974717
AT1G50110	-1,156576403	-1,055555449	-0,719310808	-0,878488893
AT1G50260	0,72475613	0,870583072	1,510486659	1,458823219
AT1G50480	2,214027978	1,076411786	0,674448318	0,76131295
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AT1G50920	-1,634436242	-1,404313686	-1,493845169	-1,363880782
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AT1G51380	-1,53175389	-1,403964628	-1,706945224	-1,549585268
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AT1G51620	-1,148445129	-1,478320158	-1,739860258	-1,437857343
AT1G51805	-0,702375488	-0,596834773	-0,7102781	-0,644969448
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AT1G52050	-0,984529849	-0,743195233	-0,667218811	-0,671749906
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AT1G52160	-1,266053933	-0,925603899	-0,986365463	-1,072883507
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AT1G53560	3,190383557	2,526931916	1,61187467	1,476476327
AT1G53580	2,781292967	2,131238407	2,350580681	2,219655802
AT1G53645	-1,363339781	-1,176038964	-1,449109071	-1,319398662
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AT1G54000	-0,938992195	-1,041859873	-1,819542176	-1,591896887
AT1G54100	2,489417327	2,004950097	2,345345674	2,438059208
AT1G54120	1,187138427	0,97097295	1,53992135	1,480724051
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AT1G54310	-0,698461672	-0,795614085	-0,926318742	-0,78707224
AT1G54340	0,738239817	0,793877776	0,793714598	0,735090201
AT1G54380	-0,67456076	-0,722187252	-0,843249698	-0,808202915

AT1G54575	1,169457486	1,063964605	0,63953008	0,590087139
AT1G54680	-0,996359871	-0,734575501	-0,772354537	-0,904331276
AT1G54740	0,920494327	0,922814145	0,791866194	0,649255284
AT1G55240	0,842169605	0,84450573	0,988289592	0,975391835
AT1G55450	-2,07375731	-1,713309864	-1,237281074	-1,356435092
AT1G55960	1,116736378	0,760267102	1,05411917	0,917550998
AT1G56110	-1,376498564	-1,414747262	-2,296020964	-1,920025037
AT1G56140	-1,757703811	-1,413127787	-1,394255557	-1,421124961
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AT1G56580	1,202466698	1,10745577	0,808120774	0,702111002
AT1G56700	1,297809253	1,365700219	0,919573812	0,879164369
AT1G58180	0,697391725	0,814826718	1,790075987	1,735007764
AT1G58200	0,942981963	1,201161877	1,256609185	1,197624839
AT1G58270	4,954889824	3,248169421	2,007533741	2,088912577
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AT1G59590	-1,326110871	-1,130569259	-1,32061425	-1,26561209
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AT1G61360	-1,29442025	-0,996046068	-1,327531505	-1,470415272
AT1G61380	-2,067035371	-1,909200724	-1,975996671	-2,002720429
AT1G61570	-1,121451226	-0,935996589	-1,385825563	-1,247042069
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AT1G62350	-1,04426011	-0,960621227	-0,911131019	-0,932191686
AT1G62380	-1,835172333	-1,326473055	-1,41971798	-1,62860954
AT1G62540	3,182142052	3,075068381	1,304481952	1,392391558
AT1G62560	0,668853886	0,78258229	1,672571828	1,680301003
AT1G62710	1,562015546	1,541224302	1,004511274	1,107502634
AT1G63420	0,639084046	0,825230788	0,929523885	0,903057341
AT1G63660	-1,784934854	-1,475275921	-1,40214222	-1,35082927
AT1G63780	-1,109272803	-0,973981452	-0,955628933	-1,03530998
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AT1G63840	1,661575912	0,959384067	0,835329982	0,998575201
AT1G64140	-1,028776638	-0,756425151	-0,860118082	-0,798987256
AT1G64170	-1,499257691	-1,431656554	-1,768964419	-1,790044185
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AT1G64490	-1,290450641	-0,949887338	-1,053583744	-1,155688091
AT1G64600	-1,274892238	-1,190097823	-1,294836498	-1,243908855
AT1G64850	1,112612531	1,276910678	1,358728657	1,258299361
AT1G64880	-1,354664252	-1,125692757	-1,092315281	-1,119426576
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AT1G65030	-1,819534162	-1,54636818	-1,608024346	-1,66730671
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AT1G65230	-1,295476217	-1,140657375	-1,070921234	-1,103989711
AT1G65370	-0,913291082	-0,663490947	-0,735230784	-0,777113608
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AT1G66890	1,174325761	0,816984667	0,718364712	0,716698382
AT1G67110	-0,880139057	-0,792752765	-0,901254205	-0,933417756
AT1G67120	-1,310179924	-1,037165814	-1,041081957	-1,174763432
AT1G67300	1,011088835	0,959133732	0,77175925	0,767077058
AT1G67850	-0,932319717	-0,654525883	-1,017171529	-1,092980963
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AT1G68500	1,626110997	1,54028337	1,292952225	1,341031706
AT1G68550	-0,625404325	-0,663399297	-1,050111895	-1,008915502
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AT1G69200	-1,132745882	-0,8958492	-0,781352393	-0,799975506
AT1G69260	3,356461297	3,175349137	3,518463879	3,379772073
AT1G69295	1,740837434	1,555538461	1,672916964	1,773925431
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AT1G70140	-0,769567574	-0,690370328	-0,64986087	-0,640895522
AT1G70580	0,988260138	1,015438022	1,674759921	1,628772174
AT1G70610	0,693215184	0,767927917	0,831787209	0,786414167
AT1G70730	0,856152369	0,980810533	1,140325067	1,070279711
AT1G70990	-1,982943508	-1,705756412	-1,579998415	-1,818267882
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AT1G71260	-1,377512835	-1,09128513	-0,735217163	-0,977617094
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AT1G72430	-2,232354066	-1,744549438	-1,349389726	-1,695265702
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AT1G73330	-1,120190254	-0,811431499	-1,085822015	-1,05643854
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AT1G73810	0,881064086	0,797335127	-0,666035283	-0,726798904
AT1G73920	1,059995997	1,163354147	1,428023863	1,525528525
AT1G74000	1,245857259	1,075715646	1,505110116	1,57549022
AT1G74020	3,344351741	1,761000659	1,89621999	1,978630187
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AT1G74270	-0,776737845	-0,698992742	-0,656619819	-0,792722812
AT1G74330	-1,287108603	-0,872666969	-0,947805522	-1,11285687
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AT1G74590	-0,945390831	-0,712445871	-1,1309401	-1,115903936
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AT1G75900	2,074097891	1,195479952	2,103096141	2,005679909
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AT1G76300	-1,248037125	-1,112775618	-1,160034274	-1,233716408
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AT1G76990	-1,883452856	-1,663560899	-2,249939227	-2,05559831
AT1G77000	1,73207023	1,705107946	1,284097989	1,342673244
AT1G77260	0,668492734	0,725566528	0,883689207	0,888140775
AT1G77380	-1,200573799	-1,26835219	-2,398921824	-2,330461267
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AT1G77750	-0,782808424	-0,821866078	-1,123774198	-1,065599269
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AT1G77930	0,739758125	0,714374321	0,718594823	0,642130397
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AT1G78230	1,875830689	1,918212038	2,805154623	2,665955359
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AT1G78860	-1,354468194	-1,088313741	-1,195284948	-1,254907331
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AT1G79040	0,985698089	0,704660659	0,984095395	1,027355774
AT1G79140	-1,762251769	-1,535229924	-1,834284527	-1,797707399
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AT1G79320	0,79375609	0,868129783	0,961280109	0,860129177
AT1G79340	0,634969372	0,625457074	0,699639888	0,639751577
AT1G79400	-0,983428709	-1,129721841	-1,266444543	-1,063960507
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AT1G80110	1,011145037	0,777103988	1,370125845	1,280949606
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AT1G80150	-1,110981684	-0,870388786	-1,03185496	-1,066969796
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AT2G01490	1,498354842	1,670765469	1,180844238	1,139502658
AT2G01540	1,218046183	0,849051694	1,376826299	1,444453601
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AT2G01620	0,661125521	0,603652736	1,043999356	1,002652439
AT2G01820	0,764515245	0,851227739	1,165660565	1,144033733
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AT2G03820	-0,751006903	-0,916586748	-1,169289907	-1,068699002
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AT2G04030	-1,348456287	-1,317455081	-1,715003015	-1,48133826
AT2G04160	1,930884968	1,932332089	1,091054656	1,340689157
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AT2G04795	-0,903691493	-0,821174467	-0,640069471	-0,706470987
AT2G05520	1,042935413	0,73045454	0,88307281	0,771420067
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AT2G15620	-0,844228055	-0,736252348	-1,033006991	-1,01949191
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AT2G16510	0,80532978	0,85210579	0,862193911	0,7511555
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AT2G16900	-1,392683852	-1,300745615	-1,16994273	-1,040039152
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AT2G17510	-0,841589175	-0,704929099	-1,015218981	-0,849186581
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AT2G17740	-1,938023717	-1,527878511	-1,704984419	-1,745031522
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AT2G18220	-1,738678763	-1,637036497	-2,020969928	-1,856402243
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AT2G18520	-1,484995685	-1,401668929	-1,344086989	-1,298681767
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AT2G19860	1,067805846	1,172638051	0,81397867	0,783240993
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AT2G19870	-0,934353339	-0,747553467	-0,628082437	-0,718866597
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AT2G20770	-0,949955073	-0,785795066	-0,842234303	-0,857785751
AT2G21045	-1,027949827	-0,787451922	-1,966760402	-1,838641856
AT2G21140	2,246722865	2,216503857	1,921134398	1,324444697
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AT2G21820	1,08720996	1,031828678	0,754168234	0,8184151
AT2G21960	1,452742812	1,319659706	1,647213024	1,669564507
AT2G22230	0,700007951	0,855032362	1,407027755	1,447330843
AT2G22400	-0,945530879	-0,726849695	-0,811175515	-0,886417792
AT2G22430	1,754517101	1,60219571	0,928683225	1,04715272
AT2G22510	1,661733813	1,398845547	1,636698975	1,495231943
AT2G22660	1,510186141	1,463829201	1,829671664	1,986249743
AT2G23120	1,691262793	1,697092858	2,260455703	2,162461734
AT2G23150	1,84761184	1,425705915	1,174533677	1,386396734
AT2G23150	1,293961527	0,801871483	1,201166531	1,103589675
AT2G23430	0,975115534	0,903388762	0,673376336	0,682079012
AT2G23840	0,871422606	1,124267381	1,051521142	0,9958058
AT2G23910	1,858459871	1,631507685	1,578537971	1,50571231
AT2G23930	-1,039115798	-0,924063733	-1,07164552	-1,14274981
AT2G24040	1,811198479	1,601115373	1,308014184	1,18897549
AT2G24500	-1,565656105	-1,333515286	-1,314654135	-1,261678011
AT2G24600	-2,656584024	-2,352426987	-2,658698287	-2,843621483
AT2G24730	-1,40971367	-1,022126712	-0,669688185	-0,777032637
AT2G24820	1,547444458	1,151733802	1,148118904	1,207524737
AT2G25450	2,439435954	2,477071218	2,437746647	2,393800274
AT2G25610	0,755835813	0,749808727	0,689918153	0,65695135
AT2G26440	-3,540132986	-3,392226072	-3,285381582	-3,234793431
AT2G26640	-1,080960746	-0,715823655	-1,012813711	-0,995309911
AT2G26670	0,669894036	0,848654304	0,848659459	0,643550576
AT2G27150	0,794241949	0,892795818	1,197951861	1,149843936
AT2G27590	-1,049255163	-0,948260174	-1,057583723	-1,069379266
AT2G27775	-1,242528346	-0,978544807	-0,897229455	-1,030831761
AT2G27830	1,130360106	0,906570028	1,666816635	1,726470195
AT2G27840	-1,896590282	-1,705698671	-2,00211424	-1,942881236
AT2G28110	1,301172547	1,250405013	1,008180514	1,057484067
AT2G28200	1,180773222	1,208359299	0,672336505	0,762027404
AT2G28400	2,265239827	1,280587373	0,72861573	0,705147928
AT2G28470	0,818158854	0,802392678	1,38528642	1,381023781
AT2G28540	-0,912775024	-0,694972474	-0,688830653	-0,627912104
AT2G28600	-1,583075337	-1,389774377	-1,529786431	-1,35873109
AT2G28930	-1,062983495	-0,967057646	-1,053826688	-1,022817688
AT2G29350	3,21827844	2,850371014	3,108396717	3,068051344
AT2G29450	2,294631907	2,342934451	1,90987666	1,811943298
AT2G29540	-0,949622327	-0,805279339	-0,85394052	-0,863623996

AT2G29740	-1,079155058	-0,799917138	-0,908418508	-0,910798654
AT2G30250	-1,155044566	-0,987508115	-1,177142229	-1,372365886
AT2G30360	1,387827143	1,436974043	0,971683043	0,968097772
AT2G30690	1,729539776	1,199324816	1,181428607	1,093574498
AT2G30720	-1,314730825	-1,050563665	-1,098394158	-1,188306368
AT2G30780	-0,898849205	-0,826050481	-1,769827108	-1,946779703
AT2G30790	-1,181363086	-1,021015286	-0,966948249	-0,966327092
AT2G30830	-1,045366396	-1,034919633	-1,287878111	-1,148780943
AT2G30990	-1,128855608	-1,065807319	-1,084686528	-1,030678021
AT2G31010	1,427020093	0,856184871	0,861869967	0,929028794
AT2G31250	-0,722920219	-0,721939983	-0,803808747	-0,756588553
AT2G31370	-1,774544722	-1,425406468	-1,832840121	-1,788900969
AT2G31380	1,945848466	0,970530041	0,772304808	0,926183608
AT2G31400	-1,317252605	-1,22316116	-1,125046288	-1,21116263
AT2G31660	2,522222338	2,436669615	2,303596469	2,194408175
AT2G31865	-1,457825632	-1,195932066	-1,193769744	-1,208138699
AT2G31880	-1,191501213	-0,868571495	-1,068102751	-1,155197449
AT2G31945	1,091668292	0,641125513	0,928522755	0,833808317
AT2G31990	0,593104801	0,847087716	0,740080282	0,748168518
AT2G32060	-1,027668766	-1,126524633	-0,963386886	-0,918393248
AT2G32220	-1,413574726	-1,150937102	-0,972407191	-1,1996808
AT2G32300	0,578365815	0,634886865	1,126168735	1,191303106
AT2G32400	-1,091454848	-0,897195123	-0,972027483	-0,973856387
AT2G32470	0,648232923	0,844458403	1,034975794	0,975130802
AT2G33210	-1,599454431	-1,472631041	-1,945191118	-1,700533414
AT2G33330	-1,025414774	-0,99232911	-0,875879117	-0,981100096
AT2G33370	-1,523114044	-1,536400419	-1,042370404	-1,051968578
AT2G33840	-1,198462585	-0,922446185	-1,15334415	-1,123608205
AT2G34260	-0,953475947	-0,826655682	-1,21257369	-0,896557039
AT2G34310	1,117538499	1,171784152	1,284897858	1,133438853
AT2G34357	-1,962063068	-1,728592646	-1,68062384	-1,751261451
AT2G34570	-1,569040917	-1,346002817	-1,335276566	-1,44594035
AT2G34600	0,897025712	1,122076427	1,897077248	1,831237772
AT2G34610	1,495707575	1,368103928	2,052394371	2,00738309
AT2G34750	-0,961665069	-0,781370946	-0,752286443	-0,75938879
AT2G34810	1,821647584	2,020216215	2,58477111	2,498925248
AT2G35040	-0,877413308	-0,857772253	-1,051348054	-0,943446855
AT2G35240	-1,046005994	-0,841311492	-0,927489071	-0,895346377
AT2G35300	1,323403361	0,927819481	1,110411838	0,806781991
AT2G35390	-0,845222763	-0,642176149	-0,628168828	-0,589780806
AT2G35670	0,712826925	0,711785372	1,392984324	1,226356959
AT2G35940	1,195517892	1,251519084	0,95401657	0,940186851
AT2G36080	1,520034295	1,507397134	1,831616419	1,74095336
AT2G36160	-0,7145584	-0,786525571	-0,838445666	-0,842423112
AT2G36340	-0,709980604	-0,831726018	-0,780485452	-0,687928413
AT2G36620	-0,770311385	-0,658757869	-0,666103159	-0,686817906
AT2G36630	-1,512920142	-1,222942697	-0,813895626	-0,826188327
AT2G36830	1,667767352	1,213807474	0,859477296	0,692327584
AT2G36895	0,721822167	1,002771416	1,197989764	1,092414782
AT2G36930	-1,282102871	-1,093159296	-1,032638724	-1,09154479
AT2G37020	-0,76807561	-0,688447369	-0,669277682	-0,829448191
AT2G37230	-1,092466768	-0,787406127	-0,666823639	-0,769057328
AT2G37250	-0,941788055	-0,689023616	-1,013849797	-1,051995396
AT2G37330	-0,718643267	-0,678083959	-0,694935841	-0,738730618
AT2G37400	-1,111042574	-0,920247379	-1,06767331	-1,045685522
AT2G37430	-0,979814374	-0,802135488	-1,002862596	-1,105093027

AT2G37600	-1,014358361	-0,840219672	-0,636689224	-0,713328971
AT2G37770	2,659646883	2,057293678	1,97345513	1,989316703
AT2G37870	2,635816006	1,74823073	1,515704073	1,094737737
AT2G37990	-1,386562169	-1,233600428	-1,188548417	-1,259760479
AT2G38290	-2,008464404	-1,565272364	-2,39272982	-2,154891237
AT2G38310	-3,736227182	-3,78039246	-4,01845251	-3,914235942
AT2G38400	1,344554914	1,645833654	3,763130042	3,833764774
AT2G38480	0,731538793	0,798675124	0,700806599	0,692853972
AT2G38540	2,959134856	2,538349673	2,313222545	2,226944717
AT2G38750	1,677617653	2,145167707	1,132059558	0,938022476
AT2G38770	-0,825424887	-0,682711347	-0,710479651	-0,693593475
AT2G39030	3,362998723	3,275750898	0,708028342	0,777415855
AT2G39050	1,79578243	1,819507175	1,215764567	1,195897474
AT2G39250	1,082570155	0,906989352	1,309249711	1,282918607
AT2G39530	-1,915797184	-1,517915793	-1,351867038	-1,620129603
AT2G39630	0,940852528	0,810908504	0,629607022	0,685555219
AT2G39670	-1,286464254	-1,173130109	-0,622242177	-0,652492703
AT2G39725	1,458296607	1,362866029	0,641494371	0,624724636
AT2G39780	0,852276564	0,909744475	0,846410943	0,792096779
AT2G39795	-1,781531844	-1,613490394	-1,610499005	-1,579322716
AT2G39800	3,389052771	3,450735162	3,7393352	3,762341752
AT2G40010	-0,775926211	-0,753613046	-0,969565849	-0,864658829
AT2G40140	-1,263258527	-1,03174092	-1,025378769	-1,013762485
AT2G40330	-3,173650973	-2,728060599	-3,528581178	-3,373118478
AT2G40360	-1,837943469	-1,612921445	-1,64553721	-1,764512744
AT2G40420	1,33775243	1,259326696	1,209331709	1,17357371
AT2G40700	-1,538815001	-1,225110172	-1,117173858	-1,204107952
AT2G40700	-1,282906898	-0,899334214	-0,815331395	-0,998119541
AT2G40780	-0,671318999	-0,609424296	-0,795850211	-0,741987143
AT2G40830	0,79396716	0,757038	0,870773495	0,65605481
AT2G40840	0,896931383	1,064229231	0,69923063	0,726318574
AT2G41090	-1,804341438	-1,730869654	-1,011197868	-1,025434996
AT2G41190	3,919661248	3,5961415	3,691920152	3,888188196
AT2G41470	1,946277441	1,65823022	0,731910822	0,765294857
AT2G41540	0,851598005	0,887117228	0,670531631	0,603613773
AT2G41650	-1,238792043	-1,104676273	-1,213804918	-1,194650445
AT2G41660	1,698113401	1,081576463	0,640181056	0,771601253
AT2G41670	-1,554195504	-1,270729419	-1,266519893	-1,322450229
AT2G41835	-1,250873744	-0,853396417	-0,635605277	-0,814666518
AT2G41870	1,705667939	1,422533391	0,71220419	0,741161908
AT2G42060	-1,005717874	-0,592960251	-0,674381598	-0,7971349
AT2G42650	-1,114029074	-0,682612919	-1,002616021	-0,885913228
AT2G42740	-1,25355967	-1,074496099	-0,692958199	-0,847215799
AT2G42750	0,836642722	0,837013106	1,12540716	1,043434196
AT2G42790	2,075987068	2,06111151	2,546177409	2,43963737
AT2G42910	-0,968070463	-0,869304803	-1,047796763	-1,076965251
AT2G42975	0,770277436	0,890308177	0,951608416	1,119440298
AT2G43020	0,899729727	0,878393759	1,036336808	0,991538273
AT2G43330	1,343828505	1,23333351	1,216050598	1,182309863
AT2G43340	1,284229693	1,030124535	1,173776773	1,273410739
AT2G43530	2,264537934	2,481665164	0,999537675	1,012537309
AT2G43570	2,083047691	1,759404429	1,138296846	1,005603962
AT2G43620	-2,373003433	-2,235779385	-1,6602379	-1,869083919
AT2G43650	-1,453327409	-1,281673937	-1,212016892	-1,278019596
AT2G44060	1,888488333	0,993993464	0,834086076	0,833457619
AT2G44120	-0,84570716	-0,928155251	-0,871855853	-0,805723059

AT2G44180	-0,912177698	-0,716011572	-0,700771569	-0,817089817
AT2G44370	-2,333801564	-2,017736163	-2,221218052	-2,424871296
AT2G44530	-0,84942698	-0,733883989	-0,84333191	-0,874027983
AT2G44790	-0,761918272	-0,868028297	-1,132011532	-1,165051532
AT2G44860	-2,021823346	-1,726461651	-1,670867839	-1,774638361
AT2G44970	0,692682265	0,825223212	1,038172244	0,868102822
AT2G45000	-0,882208462	-0,750934648	-0,908414447	-0,802001032
AT2G45030	-0,980555892	-0,714163563	-0,77200607	-0,86974233
AT2G45210	0,689487942	0,592414088	0,89891976	0,724741936
AT2G45460	-1,117788262	-0,892537842	-0,749516091	-0,749246591
AT2G45550	2,427450347	2,220302232	2,485669834	2,818524771
AT2G45570	1,569936996	1,433804112	2,652003996	2,488912223
AT2G45630	0,680127817	0,794370038	0,754456807	0,658386056
AT2G45630	0,666788655	0,69504429	0,689566471	0,611311477
AT2G45660	0,901885444	0,961507457	1,128723596	1,228252418
AT2G45710	-0,9639118	-0,846368805	-0,978741369	-0,981414694
AT2G45740	2,041249617	1,817690424	2,154258682	2,024181825
AT2G46030	1,365797945	1,48327655	1,664331189	1,432880213
AT2G46100	1,40422522	1,394577075	0,827988022	0,782800212
AT2G46450	-1,115116579	-0,88298445	-1,322684504	-1,2900269
AT2G46680	4,527842114	4,43618672	4,402730236	4,411503183
AT2G46740	-1,85594486	-1,322688654	-1,51866433	-1,583270973
AT2G46800	0,624570831	0,873925834	0,865782897	0,708326774
AT2G47020	1,166773462	1,029580205	0,721095238	0,623603849
AT2G47270	1,415012334	1,606257771	0,891236565	0,941560422
AT2G47420	-1,62153336	-1,293323662	-1,215911563	-1,244032115
AT2G47610	-0,64940518	-0,63001213	-0,991021273	-0,774095399
AT2G47640	-0,762367137	-0,763693981	-0,849790375	-0,880187014
AT2G47770	4,516809323	4,119059781	4,342992738	4,343735231
AT2G47780	-0,986167039	-1,118442049	-1,608747654	-1,322682206
AT2G47980	-1,424230258	-1,336137802	-1,809849551	-1,617437491
AT2G48020	0,637583459	0,723824376	1,113700561	1,040192321
AT2G48130	1,564982203	1,291094081	0,770740079	0,745589237
AT3E15070	-1,674462296	-1,405383263	-1,654498385	-1,747423293
AT3E16060	2,318294187	2,320323777	1,116863139	1,148127179
AT3G01090	0,978779718	0,727603096	0,884865261	0,889625874
AT3G01100	0,835928719	1,078258076	1,026244119	0,943225349
AT3G01170	1,372257555	0,952653919	0,786437168	0,716432749
AT3G01190	-2,034010765	-1,622558573	-1,22242351	-1,318173973
AT3G01260	-0,870740978	-0,759416736	-0,938987118	-1,014663135
AT3G01420	2,539102853	2,270456307	2,689747764	2,726583595
AT3G01720	-1,183763522	-0,792759063	-0,668161132	-0,624312373
AT3G01790	-1,21115765	-0,968787526	-1,067393657	-1,106683786
AT3G01820	-0,935485386	-0,985030076	-1,51906592	-1,445910127
AT3G02140	1,320892976	0,902903215	0,70455247	0,679473149
AT3G02150	0,981661929	1,10525389	0,738312382	0,639613997
AT3G02190	-1,120935251	-0,971075955	-1,11182052	-1,150273148
AT3G02220	-1,213131153	-1,04393076	-0,887037565	-1,0396216
AT3G02470	3,991611464	1,646677126	0,9034301	1,191348663
AT3G02480	6,803347266	6,255545531	6,361281507	6,243066712
AT3G02790	-1,430219919	-1,292563672	-0,856729056	-0,958667104
AT3G02910	1,41291579	1,07296849	0,82027884	0,837535198
AT3G03420	-0,883571261	-0,649688152	-0,799463574	-0,799830203
AT3G03470	2,27034943	1,791872644	2,305203492	2,208057152
AT3G03855	-1,296811141	-1,010676906	-0,816884411	-0,77496088
AT3G03920	-1,959340396	-1,814078422	-1,954174497	-1,947301821

AT3G03960	-1,350816504	-1,113260296	-0,956787334	-0,883053382
AT3G03990	2,406142046	1,444701323	1,207193169	1,550416094
AT3G04040	0,853158626	0,83644693	0,904453507	0,982291326
AT3G04070	1,795178634	1,622202598	1,137841005	1,111570767
AT3G04210	-1,427955611	-1,100268617	-1,656341578	-1,531303799
AT3G04230	-0,693266888	-0,812354768	-1,079316078	-1,007175194
AT3G04240	0,746186146	0,687617937	0,683871593	0,638827645
AT3G04770	-1,822301082	-1,743081851	-1,893320653	-1,758436563
AT3G04840	-1,031200806	-0,937649753	-0,625901069	-0,818327671
AT3G04870	0,633177325	0,916807178	1,453482799	1,327323117
AT3G05060	-1,32671716	-1,15990938	-1,270050713	-1,300981054
AT3G05180	0,578045618	0,665242298	0,890104684	0,861536376
AT3G05560	-0,957359478	-0,749123055	-0,66167388	-0,737633305
AT3G05590	-1,049076642	-0,929181827	-1,051402059	-1,024027848
AT3G05640	2,478792391	1,952592487	2,594536411	2,669193311
AT3G05810	-0,854277209	-0,625425104	-1,105271043	-1,220340811
AT3G05880	3,063311583	2,8480518	2,258228732	2,141018295
AT3G05910	0,941886335	1,037450896	1,772205374	1,799839586
AT3G06320	-0,794840367	-0,671839859	-0,871589348	-1,016351519
AT3G06420	0,982731192	1,18784244	1,010273939	0,979454531
AT3G06460	-0,720990433	-0,737095598	-0,830303746	-0,671085138
AT3G06483	0,792219517	0,968931033	1,257048544	1,139457751
AT3G06510	1,578471851	1,622235808	1,777146772	1,776984954
AT3G06530	-2,216557717	-1,934273685	-2,283680549	-2,123405644
AT3G06610	-0,822501407	-0,6552544	-0,695062775	-0,763404852
AT3G06790	-0,956924463	-0,813579521	-0,654121794	-0,69230517
AT3G06930	-1,150751797	-0,92788952	-0,980248093	-0,999034239
AT3G07530	-1,151602804	-0,857470859	-0,632820574	-0,648319882
AT3G07580	-0,621062602	-0,609339817	-0,822087523	-0,760424975
AT3G07590	-1,36211885	-1,266116205	-1,409061592	-1,354137463
AT3G07750	-1,351443089	-1,253337109	-1,462143492	-1,346517583
AT3G07770	-1,577899793	-1,318142777	-1,840450921	-1,741829755
AT3G08640	-0,789294419	-0,803250905	-1,181566319	-1,047921255
AT3G08943	-0,987042334	-1,142410664	-1,27938362	-1,181445397
AT3G08950	-1,13232461	-0,857487994	-0,687238264	-0,939979557
AT3G09250	0,687782177	0,812763736	0,88852938	0,879446537
AT3G09390	1,380469857	1,286927771	1,373528026	1,383062521
AT3G09630	-0,993623768	-0,947159994	-0,89830426	-0,860317579
AT3G09735	-1,030810619	-0,886253597	-0,914015367	-0,888055515
AT3G10040	-1,403685162	-1,295766987	-1,650119245	-1,79577631
AT3G10160	-1,218680697	-1,020082433	-1,014425443	-1,160338909
AT3G10190	3,147849082	3,007495909	3,674168529	3,628597581
AT3G10330	-1,641547806	-1,235765815	-1,173701302	-1,277755204
AT3G10360	2,163965222	2,042417913	2,521604932	2,453461912
AT3G10480	-1,122896339	-1,032934523	-0,900862728	-0,984316568
AT3G10530	1,087733234	0,993981578	1,575058104	1,542491694
AT3G10910	0,65672526	0,630417604	1,055705256	1,061542338
AT3G10940	0,944650146	1,061099358	0,858635018	0,837250118
AT3G11170	-0,852896846	-0,728640115	-0,812406839	-0,795660057
AT3G11280	-0,88037541	-0,689081797	-1,440143473	-1,40800826
AT3G11410	3,754548672	2,522727873	2,29050877	2,313260451
AT3G11420	1,488575893	1,601550521	1,923260677	1,752242247
AT3G11500	-0,706466173	-0,632407466	-0,62874977	-0,623403125
AT3G11600	1,751750378	1,452188606	1,010864649	1,008780155
AT3G11660	2,275512673	2,199558067	1,809754283	1,809123757
AT3G11780	0,622996085	0,717381493	0,9325531	0,681224726

AT3G11820	-1,240504222	-1,191103817	-1,26912416	-1,238437419
AT3G12020	2,01121628	2,024683127	2,065398929	1,994401005
AT3G12120	1,40053865	1,115066103	1,491845053	1,528813784
AT3G12145	-1,270786816	-1,311973611	-1,324392332	-1,293647097
AT3G12440	0,947313738	0,833736202	0,715723142	0,685808838
AT3G12530	0,625150737	0,745791522	1,049516931	1,04951215
AT3G12630	-2,17898595	-2,181104063	-2,925485375	-3,006973973
AT3G12720	-0,869308532	-0,8644953	-0,943472275	-0,888834372
AT3G12775	-0,775941464	-0,683892855	-0,864471268	-0,764219104
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AT3G12990	-0,858590605	-0,645506794	-0,669566477	-0,657654395
AT3G13090	-0,776985798	-0,591813304	-0,715832239	-0,882639428
AT3G13160	-1,143550045	-1,106214256	-0,965146549	-1,062603829
AT3G13230	-1,948876869	-1,482119691	-1,415410273	-1,573035906
AT3G13450	1,155157536	1,122698232	1,522404051	1,589542911
AT3G13470	-0,963084863	-0,777351884	-1,016906697	-1,008519508
AT3G13670	2,660802388	2,528570754	2,763248806	2,713456321
AT3G13720	1,686369663	1,469436416	1,229059832	1,188166637
AT3G13940	-1,958576777	-1,557433603	-1,434220906	-1,584958547
AT3G14067	3,352347655	2,928324096	2,101943105	2,316828836
AT3G14280	2,406227876	2,311136493	2,113609062	1,859075446
AT3G14310	-1,278160049	-1,438809852	-1,644120464	-1,55153957
AT3G14900	-1,07899071	-0,962273151	-0,93461449	-0,860269439
AT3G15000	-1,488504617	-1,320053397	-1,1936591	-1,200417961
AT3G15060	-1,594164695	-1,315559197	-0,9825204	-1,17677271
AT3G15080	-1,712925945	-1,214452128	-1,185688877	-1,355606127
AT3G15350	1,328067396	1,172267951	1,532110277	1,409765865
AT3G15357	-1,257402925	-1,265978341	-1,515211425	-1,640821521
AT3G15450	0,618717802	0,708643392	2,491722522	2,359716597
AT3G15500	2,022900293	1,75572466	1,229989334	1,224034117
AT3G15670	1,849130111	2,080900832	1,610980792	1,458658419
AT3G15790	0,621305184	0,679522667	1,572791505	1,503315135
AT3G15900	1,339854898	1,160331502	0,706831234	0,778122077
AT3G16080	-0,789686615	-0,820502342	-0,878111172	-0,992625648
AT3G16310	-1,595634619	-1,328497529	-1,389319201	-1,375127391
AT3G16310	-1,053220928	-1,008555868	-0,921038832	-0,961472419
AT3G16650	-0,846241538	-0,697407613	-0,764210947	-0,619446473
AT3G16810	-1,536212642	-1,404045096	-1,386039233	-1,380196534
AT3G16990	2,096090412	2,122960409	1,683975869	1,781296718
AT3G17000	1,747849485	1,793697534	1,44291737	1,395344037
AT3G17380	-1,374829423	-1,33663227	-1,297235318	-1,284125558
AT3G17465	-1,578164078	-1,386091819	-1,452678829	-1,41403322
AT3G17520	4,549428775	4,174784539	4,48899537	4,355055094
AT3G17770	0,883236351	1,035129116	1,333163373	1,377009191
AT3G17790	2,070697696	2,096390958	2,04527862	1,967208866
AT3G17790	0,75701691	0,797356362	1,450532255	1,441348294
AT3G17810	2,277914696	2,375535116	3,001860891	2,958206908
AT3G17940	0,780123731	0,763129038	0,726855228	0,707830906
AT3G18130	-1,272841196	-1,29380631	-1,554613948	-1,330219414
AT3G18190	-0,712091723	-0,604956361	-0,694013915	-0,743225009
AT3G18280	0,650140222	0,851881836	0,952074085	1,060248928
AT3G18600	-2,07468053	-1,805004531	-2,276168362	-2,191529728
AT3G18740	-0,675312584	-0,607942606	-0,718696419	-0,661828154
AT3G18760	-1,124813823	-0,873758517	-0,986475818	-0,966725941
AT3G19030	1,547929207	1,272560031	1,248226055	1,218836205

AT3G19100	1,956322666	1,827170306	1,993948334	2,008110409
AT3G19120	-0,788550689	-0,813870377	-0,648196704	-0,641151166
AT3G19130	-1,111968225	-1,010289622	-0,982396037	-0,88429278
AT3G19160	-1,086657956	-1,171956188	-0,893483425	-0,950577601
AT3G19360	-1,460487254	-1,249089099	-1,291226411	-1,265598468
AT3G19440	-1,017069424	-0,794383419	-0,688579929	-0,82558683
AT3G19580	1,445773616	1,403446423	1,840593745	1,721312816
AT3G19620	0,63545593	0,685410448	0,997065473	1,153353243
AT3G19900	2,207089612	1,799186391	0,98466396	0,996312539
AT3G20000	-0,950179759	-0,933099019	-1,113179788	-1,095230035
AT3G20050	-0,810239115	-0,774167511	-0,904406939	-0,821611436
AT3G20330	-1,201347933	-1,09081782	-1,008902338	-0,985106765
AT3G20460	0,637397331	0,64334496	0,871224964	0,665986393
AT3G20470	1,078753778	1,277763641	1,354887926	1,030811454
AT3G20500	1,440434725	1,127292267	1,150952443	1,306632996
AT3G20510	-0,937755825	-0,84562075	-0,904935796	-0,984320742
AT3G21520	2,085682246	1,81528944	1,993621718	2,064364098
AT3G21540	-1,111044793	-0,82911921	-0,903482446	-0,839223419
AT3G21750	1,083477773	0,763516207	0,954392641	1,039494375
AT3G21790	1,055024546	0,619035289	0,6340899	0,655697995
AT3G22200	1,568468253	1,641356159	1,45034109	1,441638852
AT3G22440	0,628121439	0,837254245	0,833138278	0,705938251
AT3G22620	2,186313695	2,094225259	1,300008436	1,155448533
AT3G22660	-1,500165311	-1,568479839	-1,918247697	-1,77818601
AT3G22830	1,51425976	1,777766502	1,247005263	1,302867272
AT3G22840	2,477691193	2,289358218	4,764015658	4,774086674
AT3G23030	-1,522968922	-0,984009567	-0,922942736	-0,906791708
AT3G23150	-1,067539516	-0,901351342	-1,080326208	-1,072021847
AT3G23630	-1,196772781	-0,942340839	-1,023221302	-1,111293146
AT3G23830	-1,480781121	-1,326272314	-1,649165375	-1,619979175
AT3G23920	2,711517871	2,657021876	2,155955692	2,247499905
AT3G23940	-1,836262049	-1,554835282	-1,431926642	-1,515348042
AT3G23990	-1,383402573	-1,197368416	-1,405741135	-1,349373807
AT3G24160	0,70410439	0,788821325	0,824402751	0,743023525
AT3G24320	-0,993207232	-0,737448614	-0,691666986	-0,783753011
AT3G24520	1,114912816	0,886659401	0,719622204	0,679906792
AT3G24760	0,910363755	0,849625092	0,831055456	0,706272418
AT3G25110	0,843940476	0,866196286	1,878436015	1,857646914
AT3G25430	-1,15281238	-1,096655949	-0,939846581	-0,928376208
AT3G25620	0,926395898	1,07885425	0,672963413	0,696702525
AT3G25730	-1,497520868	-1,604763921	-2,102154139	-1,883422235
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AT3G25882	-0,965455379	-0,974997215	-1,17050134	-1,172643933
AT3G25910	-0,920839072	-0,72586171	-0,90995762	-0,820039502
AT3G25940	-1,398511765	-1,135313237	-1,476623651	-1,471998425
AT3G26380	0,756315537	0,856955104	0,874800195	0,920033363
AT3G26410	-1,61294868	-1,315929069	-0,975936467	-1,14384803
AT3G26420	-0,596928663	-0,615419158	-0,68710134	-0,710073872
AT3G26470	-1,315654792	-0,983578633	-1,075107528	-1,250899581
AT3G26520	1,557618813	1,414220921	0,869245769	0,645370624
AT3G27020	0,979129049	1,121578372	1,333610157	1,233894414
AT3G27550	-0,823671295	-0,734891208	-0,819743019	-0,848554191
AT3G27820	1,127068475	1,250437697	1,716812239	1,735948083
AT3G28130	0,982787264	1,098109714	0,71143983	0,839090539
AT3G28180	-1,090843654	-0,947299757	-0,900645645	-0,8376065
AT3G28300	1,80038212	1,301513321	1,754660132	1,82014483

AT3G28300	1,858834232	1,36199777	1,906401168	1,802116916
AT3G28480	-0,911281382	-0,787389844	-1,041724988	-0,96923345
AT3G43440	1,117753627	1,171926929	1,411768934	1,397951261
AT3G43740	-1,009639423	-0,745478716	-0,621778865	-0,728737901
AT3G43960	-0,877323563	-0,853739073	-0,982417483	-0,850329525
AT3G44100	1,228657637	1,344923375	1,501285908	1,538601846
AT3G44310	1,683227229	1,849011862	1,258000298	1,290849549
AT3G44590	-1,069166147	-0,999177398	-0,675380739	-0,746104055
AT3G44750	-1,775658298	-1,772914852	-1,863421828	-1,893655212
AT3G44880	2,554660803	2,205954294	2,056307878	2,05541971
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AT3G46000	0,728939735	0,64585386	0,798916592	0,758693791
AT3G46040	-1,09257233	-0,925492553	-0,932749664	-0,91969087
AT3G46210	-1,718455878	-1,546013709	-1,676069998	-1,68637692
AT3G46280	-1,913732542	-1,629480439	-1,205003512	-1,30431815
AT3G46560	-0,990177361	-0,922460431	-1,453250165	-1,373426064
AT3G47080	1,328127895	1,029489052	1,354798666	1,217871123
AT3G47160	0,671979717	0,860843333	1,117127064	0,977614932
AT3G47295	0,996937954	1,012309295	0,899188479	0,648003455
AT3G47370	-1,049940788	-0,997146146	-1,020227568	-1,092904396
AT3G47520	-0,883585678	-0,832032742	-0,746996417	-0,644756932
AT3G47950	1,372230507	1,598322375	1,585322705	1,654784631
AT3G48250	-1,237450869	-1,102301012	-0,990026457	-1,011791606
AT3G48350	2,41023645	2,236064806	1,04853752	1,001875379
AT3G48870	-1,659432013	-1,350564035	-1,064694006	-0,985319063
AT3G48930	-0,871573603	-0,836100126	-0,694113352	-0,809181049
AT3G49040	-0,586856023	-0,590638313	-0,713776527	-0,718055201
AT3G49110	-1,656833149	-1,513108575	-0,942230194	-1,103582204
AT3G49180	-1,34597415	-1,105968222	-1,158809504	-1,158336956
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AT3G49590	1,15939178	1,422410222	1,103238819	1,045985945
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AT3G49940	-1,023207925	-0,968276787	-1,479066654	-1,535937788
AT3G50685	1,377261119	0,918505642	0,819580162	1,006925818
AT3G50740	-2,324853066	-2,213412103	-2,536601237	-2,75129479
AT3G50830	1,591579877	1,466268338	1,051576144	0,970189984
AT3G51130	0,763154894	0,672912793	0,713742024	0,816567269
AT3G51190	-0,830596194	-0,799579057	-0,750542866	-0,763317484
AT3G51330	-1,018825619	-0,634847627	-0,728054266	-0,856561108
AT3G51510	0,714296141	0,597541348	0,650542274	0,617715342
AT3G51730	2,128760208	2,046775205	1,843423806	1,879470238
AT3G51750	2,237480892	1,856995877	2,317963652	2,442930019
AT3G51800	-1,190013896	-1,004044976	-0,911090124	-1,004248394
AT3G51830	0,922010243	0,951508844	1,079688491	0,993172441
AT3G51840	1,654086561	1,672728803	1,81609375	1,9460011
AT3G51860	2,211948313	1,40158433	0,860234329	0,611941074
AT3G51920	-1,30791869	-1,089970673	-1,140901339	-1,392834858
AT3G52030	-1,280597923	-0,90403371	-0,916562206	-1,019022978
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AT3G52580	-1,040030044	-0,928283382	-0,784678706	-0,850843347
AT3G52840	1,301682225	1,262860341	1,394042805	1,371280392
AT3G52870	-1,710620278	-1,498150237	-1,866972491	-1,953843874
AT3G52880	0,617739477	0,752642568	0,939543047	0,918747131
AT3G52920	1,535642713	1,536730686	1,193588585	1,204797207

AT3G53020	-0,62672375	-0,632824597	-0,808577983	-0,784370375
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AT3G53980	2,52247415	2,378794021	1,641170438	1,744944244
AT3G53990	1,014346112	0,690727573	0,717830931	0,63582094
AT3G54040	-1,067552524	-1,080481401	-1,753002981	-1,904096605
AT3G54090	-1,260596633	-1,125845664	-0,646636222	-0,672307537
AT3G54360	1,44891352	1,029699423	1,113236213	1,074742015
AT3G54470	-1,138663089	-0,970823609	-0,901821594	-0,889952626
AT3G54640	-0,896507583	-0,976263923	-1,004227425	-0,910330605
AT3G54820	1,699388069	1,605710227	1,991157832	2,215100607
AT3G55010	-0,793166854	-1,088872071	-1,417381351	-1,249016177
AT3G55280	-0,84640959	-0,76499491	-0,676950443	-0,818971567
AT3G55340	-1,092881028	-0,753579134	-0,797248076	-0,861466721
AT3G55400	-1,348028631	-1,093826746	-0,77045274	-0,807312625
AT3G55450	-1,402635574	-1,270813503	-1,515487937	-1,54506405
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AT3G55510	-1,663921038	-1,279996756	-1,19689921	-1,379139564
AT3G55605	-1,491460455	-1,21975912	-1,383454273	-1,451837761
AT3G55610	1,258751872	0,627917193	0,67874929	0,759134091
AT3G55610	1,022156735	1,106094857	1,823919361	1,786383698
AT3G55690	-0,859881447	-0,724990435	-0,693224877	-0,73882163
AT3G56310	1,151717468	1,170049238	0,821575942	0,876448853
AT3G56410	-0,842898964	-0,803287446	-0,673713681	-0,773805434
AT3G56460	1,299500714	0,670422189	0,635759046	0,618899323
AT3G56710	-1,471650691	-1,518254511	-1,705445093	-1,737048744
AT3G56940	0,617297531	0,717936652	0,890150471	0,887857599
AT3G56990	-1,397938745	-1,345156217	-1,541881632	-1,452927142
AT3G57000	-0,83326051	-0,800034057	-1,073088786	-1,129802526
AT3G57010	3,06889094	3,21881978	2,992124678	3,084237535
AT3G57020	1,873016289	2,076861212	1,479662533	1,706226111
AT3G57150	-1,443623436	-1,542752811	-1,757555272	-1,583062605
AT3G57320	0,656173976	0,874665717	1,161712383	1,072817015
AT3G57330	-1,447278938	-1,09040171	-1,469662552	-1,576244665
AT3G57390	-1,160594059	-1,031166663	-0,662576136	-0,599784223
AT3G57490	-0,697994896	-0,725353967	-0,899666204	-0,833109992
AT3G57520	2,212011499	1,947292793	2,969344897	2,828899087
AT3G57550	-1,699259858	-1,380332031	-0,762521018	-0,942537599
AT3G57610	-1,199945697	-1,069093457	-0,787831744	-0,859399292
AT3G57660	-1,779402801	-1,467323704	-1,497173808	-1,533557214
AT3G58270	-0,88985487	-0,815821387	-0,696340396	-0,652467047
AT3G58450	1,186890103	1,121726195	0,844847025	0,951051838
AT3G58660	-0,991501586	-1,072755384	-1,581855984	-1,508419324
AT3G58740	0,908917936	1,09879142	0,976566851	0,968325169
AT3G58930	-0,83413021	-0,714951501	-0,759832197	-0,694354118
AT3G59080	-1,30819413	-1,022786145	-1,346628013	-1,504863499
AT3G59210	0,853430034	0,907079887	0,967403962	0,932441435
AT3G59650	-0,841080107	-0,788709344	-0,635391991	-0,688330264
AT3G59880	-0,649345321	-0,689993415	-1,047158895	-1,182725692
AT3G59900	-0,929571286	-0,745339931	1,270186783	1,015016524
AT3G60130	1,709620991	1,875803715	1,97074193	1,977880484
AT3G60245	-0,751474979	-0,771100205	-0,761464315	-0,754870893
AT3G60320	-0,802746116	-0,665199526	-0,81837404	-0,747611107
AT3G60360	-1,811908357	-1,545628403	-1,37212164	-1,416344669
AT3G60500	-1,254312711	-1,146053747	-0,981641099	-1,026150268
AT3G61070	0,68797837	0,706985378	0,897208168	0,870841667
AT3G61100	-1,555869812	-1,423281735	-1,472497065	-1,561857304

AT3G61240	-0,953686165	-0,819699687	-0,833713247	-0,788055591
AT3G61300	-1,015083317	-0,881250511	-0,757586398	-0,733569807
AT3G61420	0,800919463	1,067990183	1,885059051	1,699421202
AT3G61620	-1,393482164	-1,178987667	-0,964712948	-1,002222581
AT3G61630	-1,667513357	-1,76879887	-1,623154797	-1,398298305
AT3G61640	-1,432845277	-1,419510714	-1,415768446	-1,483946337
AT3G61680	0,742551501	0,741453522	0,945407785	1,298190025
AT3G61860	-0,707393107	-0,647793748	-1,004043439	-0,935584135
AT3G61890	4,500542759	4,51886465	3,779014872	3,746843953
AT3G62090	1,165784837	1,021234747	0,902491112	0,990262781
AT3G62460	-0,973934828	-0,856095047	-0,832213206	-0,900832055
AT3G62650	0,821085798	0,866366064	0,680859644	0,594695586
AT3G62720	-0,701484226	-0,65482158	-0,7624404	-0,743421344
AT3G62730	0,915248541	0,641499648	0,755124313	0,915767634
AT3G62870	-1,114183622	-0,967446471	-0,926565958	-0,905552269
AT3G63090	-1,492951116	-1,346765568	-1,458710832	-1,391322853
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AT4E37260	1,712040265	1,489233735	1,401911307	1,286013372
AT4G00026	-0,901265845	-0,793036059	-0,808835649	-0,747988801
AT4G00300	-0,981206941	-0,872241442	-0,81674964	-0,721248936
AT4G00620	-1,585981252	-1,368017586	-1,320774327	-1,380204756
AT4G00752	0,855374598	0,996776678	0,77910698	0,914128743
AT4G00780	-2,024183967	-1,826797564	-1,987142792	-2,047054131
AT4G00810	-0,611235756	-0,672072825	-0,645860009	-0,658594014
AT4G00895	0,773021338	0,785736634	1,08551095	0,791140006
AT4G01430	0,909073077	1,127940737	0,755354627	0,868966605
AT4G01610	2,030544648	1,914673282	1,611635981	1,793958727
AT4G01630	-0,878828232	-0,719876453	-0,686959572	-0,758912284
AT4G01935	0,966028962	0,953927733	1,109004186	1,167401215
AT4G01990	-1,116329617	-0,936079186	-0,985085165	-1,070224156
AT4G02220	-1,094319264	-1,064951394	-1,099191828	-1,037367999
AT4G02230	-0,878865991	-0,81963049	-0,675458537	-0,653412154
AT4G02280	1,808410416	1,324788252	0,872223769	1,053665942
AT4G02370	1,268816808	1,108152578	1,066587824	1,249902397
AT4G02400	-1,406682035	-1,254793241	-1,384810966	-1,29543229
AT4G02520	-1,579044559	-1,295070864	-0,972161813	-1,070896259
AT4G02630	0,892925686	0,999486803	0,65648058	0,682463034
AT4G03190	-1,160857526	-0,950727658	-0,841005986	-1,025244202
AT4G04180	-0,838628968	-0,740924949	-0,74154768	-0,758718652
AT4G04540	-1,121814394	-0,720149222	-1,002990725	-1,168129158
AT4G04700	-1,51010288	-1,09979585	-0,711439005	-0,976690211
AT4G04920	-1,260657314	-0,9857214	-1,152944257	-1,200563483
AT4G05420	-1,301018083	-1,179367538	-0,931385367	-1,072871569
AT4G05450	-1,207189169	-1,045752264	-0,737318113	-0,776309726
AT4G05530	1,102701434	1,057200089	0,896694054	0,836547279
AT4G08150	-0,896124295	-0,756779727	-0,906019725	-0,903041535
AT4G08300	1,383545959	1,209412817	0,780005055	0,872187688
AT4G08950	-1,522528713	-1,637200639	-2,425760512	-2,52712544
AT4G09490	1,14788913	0,941038157	0,954588669	0,897113951
AT4G09600	1,348709211	1,461743583	1,516980084	1,684680557
AT4G09730	-1,197920354	-1,07326843	-1,266008316	-1,139425737
AT4G09890	0,597392117	0,602171313	0,920268504	0,976669068
AT4G10150	1,755812673	1,761984462	2,280923491	1,992876067
AT4G10770	-1,246897545	-0,899887236	-0,917523382	-0,893142797
AT4G11130	1,914251739	1,943728475	2,215023875	2,344873339
AT4G11230	-0,851570476	-0,750559091	-0,749628995	-0,752150966

AT4G11570	1,892481709	1,54136115	1,119347385	1,346370074
AT4G12000	0,571547472	0,833403195	1,366014284	1,374273124
AT4G12130	0,672640919	0,713473873	1,402276076	1,438138534
AT4G12600	-1,399365568	-1,341295949	-1,702739666	-1,633855547
AT4G12730	-1,45574388	-1,469376736	-1,900359237	-1,961198309
AT4G13170	-1,038619423	-0,953167671	-0,810984371	-0,960958195
AT4G13270	0,808315616	0,700597325	0,769472332	0,603687695
AT4G13430	-0,645095278	-0,639800707	-0,860619627	-0,783080302
AT4G13510	-1,677915454	-1,533278686	-1,566204434	-1,534851182
AT4G13530	1,479027865	1,414900601	1,215940377	1,232290837
AT4G13540	0,942740703	0,934426696	1,807120038	1,847245484
AT4G13850	-1,258155014	-0,990719466	-1,004278538	-1,173386736
AT4G14010	0,935391281	0,942619763	0,932845267	0,940164304
AT4G14270	1,550817239	1,420605202	1,081866581	1,104079988
AT4G14320	-0,889892569	-0,678118356	-0,733581946	-0,740283501
AT4G14365	-2,885185363	-2,620381582	-3,116064474	-3,099325765
AT4G14365	-1,006011177	-0,970648055	-1,179843092	-1,138338522
AT4G14950	0,636330146	0,759534041	0,81152605	0,85251981
AT4G15530	1,49338756	1,550933554	3,398460611	3,320422648
AT4G15530	1,952458258	1,155692321	2,67108242	2,582495853
AT4G15640	-1,59180899	-1,453148239	-1,39381354	-1,475722834
AT4G15770	-0,947520373	-1,127994718	-1,371794252	-1,537124728
AT4G15850	-1,520400747	-1,241032745	-1,004009904	-1,137063976
AT4G15940	1,179184846	1,281465069	1,887256947	1,788963561
AT4G16190	2,847507859	1,837635903	1,250483098	1,248566986
AT4G16520	0,961064575	1,010496314	1,269214226	1,190727814
AT4G16690	1,855986419	1,807110184	2,880295528	2,867077138
AT4G17030	2,028646055	2,125579298	2,591139044	2,445088721
AT4G17270	0,972902007	1,048014061	2,154542833	2,149005122
AT4G17460	-0,904080984	-0,80953387	-0,816373075	-0,785361103
AT4G17520	-1,132931134	-0,899328977	-0,782606729	-0,778626157
AT4G17610	-0,83094822	-0,67406336	-0,717614795	-0,630900671
AT4G17660	-0,852029668	-0,717179477	-1,291134618	-1,351653713
AT4G17720	-0,814064743	-0,661405612	-0,659738556	-0,794375215
AT4G17870	-1,621348662	-1,332347958	-1,255038878	-1,240278977
AT4G17880	-0,689714517	-0,634942283	-1,036203267	-0,983664065
AT4G18205	-2,287396572	-1,938140746	-2,233880254	-2,455431718
AT4G18210	0,769119515	0,719970023	0,786154479	0,860881429
AT4G18430	-1,189780471	-0,817743469	-0,795144097	-0,963504331
AT4G18440	-1,003741448	-0,833932694	-1,297414398	-1,218191241
AT4G18810	1,202406277	1,17981973	0,7907327	0,869297704
AT4G18980	1,315587332	1,171764786	1,352962303	1,494416949
AT4G19050	0,833782006	0,847811475	1,124144803	1,099104912
AT4G19160	0,751939841	0,710671411	0,793948855	0,839350055
AT4G19390	1,785343482	1,53158647	0,802607809	0,757501697
AT4G19860	0,772799998	1,129006395	1,489235026	1,344591917
AT4G20000	-2,561111388	-2,102298203	-2,373448969	-2,648157697
AT4G20410	0,589650281	0,649720776	0,868191005	0,686445202
AT4G20440	-0,94701689	-0,994596407	-1,007549626	-0,818509915
AT4G20780	-1,370118977	-1,107316268	-1,309241545	-1,469887119
AT4G20930	1,236998089	1,213710989	0,897465881	0,952518282
AT4G21140	-1,121484745	-0,957445904	-1,033815751	-1,003600568
AT4G21320	0,949341079	0,809856873	0,857512936	1,021092606
AT4G21410	-2,277476628	-2,094947924	-2,121815331	-2,102478298
AT4G21470	1,152676996	0,921255466	0,723000643	0,730739761
AT4G21570	2,130858546	1,841417758	1,355673751	1,425093036

AT4G21960	0,579251576	0,702703191	1,019738771	0,917409715
AT4G22240	1,780376207	1,785667556	1,729053342	1,694882033
AT4G22380	-1,383937983	-1,202960395	-1,289653778	-1,278277498
AT4G22490	-0,939154032	-1,409065698	-0,626001327	-0,601632254
AT4G22753	1,613765081	1,370037056	1,563872555	1,529974447
AT4G22820	1,735168924	1,42140576	1,180643034	1,261057062
AT4G23050	1,493459952	1,562207363	1,544067414	1,626128778
AT4G23130	-1,25536438	-1,077350853	-1,202422047	-1,43477566
AT4G23180	-0,784023196	-0,641568349	-0,685191964	-0,715832822
AT4G23190	-1,943318513	-1,690602371	-1,298484071	-1,282515448
AT4G23400	1,175131155	0,96128272	1,593082431	1,567762776
AT4G23500	1,594687257	0,789719459	0,925838021	0,856575273
AT4G23550	0,889963453	0,708819664	1,117452438	1,085351753
AT4G23670	2,210012354	2,07981505	1,144263237	1,420811878
AT4G23690	1,85310476	1,465451864	0,881177943	1,105929634
AT4G23800	-0,833034807	-0,755086211	-0,751989503	-0,991635454
AT4G23800	-1,199283508	-1,304392579	-1,769004391	-1,721018177
AT4G23880	0,745118129	0,880239249	0,672593086	0,632993082
AT4G23890	1,109457789	0,993975309	1,028100934	1,022381694
AT4G24130	2,178084225	2,129718022	1,09107641	1,012012793
AT4G24220	2,173274784	1,655665689	1,157088775	1,139077223
AT4G24270	-1,443477112	-1,110983536	-1,052622241	-1,128810031
AT4G24690	-1,229742674	-0,94557251	-0,905834242	-0,907734531
AT4G24830	-1,279048297	-1,034079043	-1,04150326	-0,94833001
AT4G24940	-0,98763713	-0,797415508	-0,747182744	-0,693473983
AT4G25000	3,122163407	3,148487017	3,003355487	2,904858938
AT4G25110	-1,082645669	-0,73085023	-1,263352717	-1,177712063
AT4G25170	0,751852841	0,840651825	0,731864343	0,727328192
AT4G25300	-0,888264971	-0,611558337	-0,708464511	-0,717953091
AT4G25340	-1,411404067	-1,415780799	-1,868130042	-1,707158415
AT4G25570	0,938212567	0,854650192	1,329144621	1,328254858
AT4G25630	-2,365777213	-2,257832377	-2,512367256	-2,454406828
AT4G25710	-0,901092481	-0,77497136	-1,046190247	-0,938794446
AT4G25730	-2,146798109	-1,916685278	-2,407860515	-2,199512206
AT4G25740	-0,894055972	-0,95747047	-1,014241212	-0,977082517
AT4G25890	-0,826824407	-0,913722731	-1,017044156	-1,056032039
AT4G25910	0,978785158	0,718966375	0,634097952	0,646338147
AT4G26110	-1,532265946	-1,333315582	-1,467143501	-1,428548872
AT4G26930	-0,799130329	-0,67832807	-0,964205567	-0,978842815
AT4G27070	1,169261252	1,264357698	0,92699992	0,947843647
AT4G27080	3,19682659	3,144123062	1,927608034	1,9011654
AT4G27120	1,198752687	1,080373031	1,421240903	1,424899534
AT4G27380	1,028774409	0,887624721	1,147860848	1,163876071
AT4G27800	0,85911605	0,964339816	0,991594197	1,076479708
AT4G28030	0,689702132	0,659354835	1,426666715	1,356473019
AT4G28088	1,038600813	1,048191888	2,026507406	2,122995796
AT4G28190	-0,781250499	-0,719642267	-0,682746675	-0,724519916
AT4G28210	-1,09334009	-0,951167323	-0,762423926	-0,731577495
AT4G28290	1,337430922	1,385419454	1,219583706	1,129404315
AT4G28400	-0,915033026	-0,619998237	-0,681230058	-0,796338301
AT4G28450	-1,738895259	-1,593289465	-1,615087694	-1,63317603
AT4G28510	-1,758275198	-1,592595324	-1,681258776	-1,689625605
AT4G28730	1,75480872	1,264064142	0,828714729	0,716653768
AT4G28740	0,977893584	0,746940421	0,629473049	0,640537242
AT4G29130	0,841617963	0,928999577	0,809146974	0,779449282
AT4G29160	0,614835791	0,774521039	1,312559144	1,169457065

AT4G29410	-1,426705514	-1,068055742	-0,734721865	-0,881331117
AT4G29510	-1,565953332	-1,37785106	-1,398133824	-1,455386828
AT4G29690	-0,819126655	-0,608120079	-0,800580186	-0,697461747
AT4G30220	-1,033337962	-0,928364159	-1,036272861	-1,083149188
AT4G30270	2,673357953	1,491595726	0,85531747	0,736139467
AT4G30450	0,843639316	0,839459804	0,778388159	0,86350173
AT4G30470	1,786881009	1,574772298	1,304726644	1,430508229
AT4G30480	0,754259649	0,919411988	2,050156	1,779837719
AT4G30660	1,38029764	1,398253161	0,943533811	0,871213206
AT4G30690	-0,934385306	-0,640412682	-0,689245145	-0,7153578
AT4G30800	-1,720255958	-1,510326826	-1,614527615	-1,785992288
AT4G30930	-1,443859194	-1,199761282	-1,130898465	-1,19420038
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AT4G30993	1,7776726	1,084472688	0,833811315	0,799608025
AT4G31500	-1,158825418	-0,95153933	-0,911331561	-0,863908243
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AT4G31790	-1,628882876	-1,348517188	-1,437972473	-1,439504755
AT4G31800	-1,687858739	-1,435467358	-2,057839991	-2,046399852
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AT4G32250	2,452062525	2,548378163	1,831801524	1,94476308
AT4G32295	0,734096881	0,870216381	0,921002233	0,993546821
AT4G32320	0,59583799	0,649942683	0,660191848	0,647526169
AT4G32400	-0,964669022	-0,770656221	-0,873200916	-0,874351238
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AT4G32460	-1,089344292	-0,921169572	-0,737851535	-0,78414794
AT4G32520	-1,322407264	-1,138315702	-0,951741949	-1,093646878
AT4G32770	2,111633928	2,16842663	2,290096854	2,406313486
AT4G32810	2,109081676	2,141847556	2,011627371	1,811104474
AT4G32930	0,898594001	0,961078152	0,885103761	0,777034651
AT4G33050	-2,274107542	-1,890199927	-2,677617419	-2,832452492
AT4G33090	1,023217481	1,030364411	1,017889178	0,794641407
AT4G33300	-1,780112302	-1,431322907	-1,504775341	-1,603640713
AT4G33700	0,607472545	0,716235963	1,178605204	1,077662125
AT4G33760	-1,731502733	-1,581066086	-1,555987094	-1,416525784
AT4G33920	-0,647168218	-0,73508369	-0,900154312	-0,913368432
AT4G34030	0,758628335	0,749380591	1,340780377	1,256085368
AT4G34170	-0,77968809	-0,641877666	-0,777626672	-0,717054435
AT4G34190	-1,115998072	-0,936729795	-0,991512525	-1,036321044
AT4G34670	-1,269261862	-0,989479318	-0,729198886	-0,771777012
AT4G34720	0,834813676	0,733871873	0,898096606	0,878404371
AT4G34740	-1,610331209	-1,520048548	-1,276290149	-1,330018293
AT4G34770	-1,689104472	-1,540685002	-1,012058227	-1,23449388
AT4G34850	1,49144175	1,479970401	0,781770796	0,698295673
AT4G34920	1,165319831	1,163314716	1,320992336	1,222945055
AT4G34950	-0,717707603	-0,633668158	-0,857682695	-0,808459367
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AT4G35490	-0,756811432	-0,701347369	-0,647449556	-0,756385247
AT4G35730	-1,105313955	-0,805788338	-0,733963448	-0,594440213
AT4G36020	-1,149523436	-1,271722857	-1,376785336	-1,252119305
AT4G36040	1,1493914	1,059737922	2,159063173	2,328391329
AT4G36090	-1,36932551	-1,067229275	-0,985063144	-0,966451723
AT4G36130	-1,170222747	-1,094903199	-1,222584075	-1,13696018
AT4G36670	-2,056217907	-1,876370066	-2,034784633	-1,972278181
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AT4G36760	2,919366704	1,50641569	1,263368783	1,314606379

AT4G37200	0,843088278	0,892543007	1,500607001	1,513094011
AT4G37220	1,046848983	0,6766025	1,834574779	1,774427434
AT4G37260	1,446748912	1,396339635	1,389292459	1,351716183
AT4G37430	2,228308115	2,395813712	2,789757534	2,701119333
AT4G37470	1,936811945	1,862739894	1,04746923	0,922518629
AT4G37660	-0,954036144	-0,844140893	-0,97062435	-0,915190763
AT4G38100	-0,99908725	-0,941561354	-0,899256936	-0,902714938
AT4G38440	-0,905163548	-0,784373799	-0,713032414	-0,702895966
AT4G38550	-1,816417418	-1,770428056	-2,096023022	-2,244449336
AT4G39260	-0,691896588	-0,77199545	-1,235100501	-1,088466856
AT4G39350	-1,300573919	-1,02803602	-0,996222599	-1,062904567
AT4G39730	1,341612786	1,32557458	1,365493475	1,327391839
AT4G39740	-1,124312912	-0,845488077	-0,63480375	-0,627637271
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AT5G01210	-1,553682355	-1,25673257	-1,347453498	-1,433066079
AT5G01260	0,74894069	1,004505815	1,045712592	0,935268442
AT5G01290	1,325233661	1,121168058	3,34175721	3,256271045
AT5G01350	-0,8663238	-0,640381938	-0,830074823	-1,00636561
AT5G01520	2,450505298	1,805208309	2,65458793	2,531400939
AT5G01540	-1,361846259	-0,985631472	-1,569359713	-1,547448305
AT5G01820	1,626160714	1,536566253	0,999016374	1,02519955
AT5G01950	-0,991376247	-0,777866964	-0,874664918	-0,797935426
AT5G01990	0,8332028	0,759506296	0,8355928	0,848925311
AT5G02230	1,504789639	1,286949117	1,204695105	1,082485642
AT5G02280	1,228531276	0,807280789	0,660077072	0,683543051
AT5G02420	1,137554606	0,898444569	0,998155486	0,984560102
AT5G02480	0,93269841	0,632688184	0,700528148	0,700828903
AT5G02540	-0,789578337	-0,797180267	-1,008719329	-1,088737259
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AT5G03210	3,000884991	2,347154696	0,713280142	0,601467525
AT5G03460	1,129493825	0,819163766	0,670038969	0,784732234
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AT5G04230	-0,727791846	-0,679464901	-0,805274368	-0,728759151
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AT5G04260	1,791917766	1,170131852	0,799280224	0,865952174
AT5G04370	0,944165753	1,095440268	2,038920983	2,15566184
AT5G04600	-0,855260949	-0,820892763	-0,889690421	-0,740164078
AT5G05220	1,116444748	0,829486364	1,804376274	1,869136102
AT5G05270	1,209620822	0,905213195	1,341278685	1,275752047
AT5G05460	-1,067114664	-0,840908288	-0,786700141	-0,90254037
AT5G05470	-1,387726371	-1,108465939	-1,128194576	-1,087219294
AT5G05540	-1,216224089	-1,050314149	-1,245957736	-1,216455811
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AT5G06180	0,982622508	0,955630026	0,640693858	0,758150786
AT5G06280	1,778876711	1,354556416	1,145572316	1,067908011
AT5G06360	-1,064206667	-0,813686731	-0,657543856	-0,783379701
AT5G06370	1,163519574	1,032543983	0,984131743	1,128476116
AT5G06550	-0,87366322	-1,06045803	-1,372988767	-1,305177687
AT5G06760	3,308861464	2,95039076	2,417070848	2,400510726
AT5G07460	-0,902675023	-0,650973145	-1,140461205	-1,227919346
AT5G07680	1,276271647	1,379390414	0,943533043	0,969522489

AT5G07900	-0,872497439	-0,768884016	-0,635364558	-0,716648065
AT5G08180	-1,385812091	-1,277575673	-1,351710705	-1,350495985
AT5G08180	-1,372615425	-1,199378915	-1,168167657	-1,307788215
AT5G08330	-0,628516674	-0,710629201	-0,929956739	-0,926873253
AT5G08410	2,005542866	1,415933071	1,432978731	1,32690767
AT5G08590	0,834447615	0,919988679	1,210839639	1,305875704
AT5G08620	-1,885676136	-1,746534534	-1,766891196	-1,76962532
AT5G09220	-0,652232545	-0,637432039	-1,434838421	-1,263634183
AT5G09270	-1,33958191	-1,131330548	-1,199752291	-1,278640547
AT5G09380	-0,836918684	-0,901920768	-0,862080944	-0,754290494
AT5G09420	-1,746206069	-1,624165765	-1,835536927	-1,970997047
AT5G09510	-0,787060266	-0,683513203	-0,620237144	-0,631237597
AT5G09530	4,300535306	3,612213354	1,601267045	1,789489637
AT5G09590	-1,295872655	-0,877453246	-1,37199519	-1,380689347
AT5G09620	1,450497129	1,384979204	1,724090609	1,658197331
AT5G09995	1,141515167	0,642569292	0,718059181	0,590230983
AT5G10160	-2,025573527	-1,885019159	-1,202861683	-1,394046527
AT5G10230	1,11701765	1,476784081	1,21487347	1,087089229
AT5G10300	1,67920624	1,875218581	3,802507557	3,786930546
AT5G10480	2,340379095	1,218600928	0,684198015	0,636076074
AT5G10540	0,731360317	0,789589305	0,712393274	0,707799488
AT5G10860	0,967522513	0,967392462	0,800856818	0,76187474
AT5G10930	1,39064012	1,34665591	1,907235676	2,034974145
AT5G11010	-0,980809058	-0,840655867	-0,629731243	-0,73191867
AT5G11070	2,26230843	1,817628355	1,792199455	1,830089875
AT5G11160	-0,946003207	-0,815780299	-1,662442999	-1,543972498
AT5G11310	-0,943152527	-0,927716503	-1,166251731	-1,055138515
AT5G11520	1,176466922	0,909115425	1,065655185	0,928283405
AT5G11670	-1,396958261	-1,166502593	-0,995048127	-0,918065353
AT5G12200	0,868706598	1,005223438	1,078019046	1,03485035
AT5G12220	-1,355417508	-1,221404305	-1,418883806	-1,356846267
AT5G12970	-0,71035533	-0,671857355	-0,763364158	-0,673907518
AT5G13030	0,805098416	0,728291488	0,784699171	0,652652516
AT5G13140	0,954570537	0,754380546	1,181277253	1,020682723
AT5G13200	2,283973911	1,903967902	2,216180809	2,076303713
AT5G13680	-1,14840525	-0,817940101	-0,863065271	-0,818430458
AT5G13740	0,883647404	0,750026515	1,810606042	1,876575735
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AT5G13830	-1,317258886	-0,964471673	-0,709801659	-0,896422535
AT5G14105	-0,809838758	-0,835660413	-1,298269306	-1,20172007
AT5G14180	1,107625524	1,398139084	3,021317508	3,074772137
AT5G14230	-1,45465849	-1,24919929	-0,929359369	-1,188046403
AT5G14280	0,790502727	0,697474245	1,062255082	1,025219071
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AT5G14440	-1,576611501	-1,365713793	-1,12503687	-1,20909756
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AT5G14600	-0,930312521	-0,820263085	-0,637581108	-0,823130573
AT5G14700	1,297758065	1,300982995	0,8564761	0,941218777
AT5G14800	-1,426371872	-1,246649123	-0,872314089	-0,841382965
AT5G14930	-0,75326008	-0,62694793	-0,916511299	-1,015572417
AT5G15230	-0,934641394	-0,825692221	-0,860949836	-1,051094685
AT5G15240	1,52290165	1,580583223	2,517378291	2,477600882
AT5G15350	-0,787412847	-0,699943284	-0,939773639	-1,114987296
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AT5G15730	-0,977602519	-0,815716047	-0,915957826	-0,812257931
AT5G15750	-1,525782161	-1,231372731	-1,317293604	-1,431768303
AT5G16120	1,027781703	1,310614812	1,066126045	1,042425768
AT5G16360	1,18887524	1,260721152	0,784307741	0,758123944
AT5G16950	-0,793621361	-0,698050794	-0,852360293	-0,774101224
AT5G17190	1,018167791	0,820676863	1,07264415	1,103282998
AT5G17270	-1,436120733	-1,020275728	-1,191470781	-1,221951909
AT5G17330	-1,706038222	-1,126071429	-0,859394763	-0,976265874
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AT5G17930	-0,870152306	-0,787094422	-0,638506824	-0,716337817
AT5G18130	1,834376337	1,493740869	1,158251537	1,128921419
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AT5G18400	-1,3629961	-1,197436659	-1,383139468	-1,367468226
AT5G18440	-1,052425606	-0,675255664	-0,789614904	-0,824472141
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AT5G19540	0,601824377	0,763418514	1,1204894	0,99963679
AT5G20160	-1,529117433	-1,426538018	-1,598228197	-1,716533814
AT5G20250	-1,058724141	-0,628562118	0,96961929	0,899690033
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AT5G20590	-1,246455992	-1,097826531	-1,070168338	-1,003738773
AT5G20650	0,743960639	0,718103148	0,944211683	0,891312275
AT5G20720	-0,679742875	-0,645938494	-0,645563315	-0,636145169
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AT5G22690	-1,214639302	-0,995885785	-1,052106192	-1,138214535
AT5G23020	-1,94772597	-1,646415309	-2,636968346	-2,423025111
AT5G23220	3,383907781	3,360655341	0,915984607	0,982349
AT5G23235	1,578287595	1,730774617	1,774515417	1,640027963
AT5G23250	-0,856029719	-0,814386104	-0,84059741	-0,782205399
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AT5G23660	1,565943686	1,570918424	0,882684264	0,90459257
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AT5G23860	-0,672863317	-0,637893563	-0,771304456	-0,822574218
AT5G23880	-0,643887102	-0,596826558	-0,650890113	-0,595022943
AT5G23920	0,747964044	0,693582237	0,789392932	0,650762694
AT5G24040	-1,061189049	-0,861623561	-0,829127893	-0,904170503
AT5G24830	-1,018810193	-0,91669134	-0,913466832	-0,953311942
AT5G24850	0,825293013	0,814245772	1,080877514	1,085285203
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AT5G24930	2,231739343	1,547377117	1,600414527	1,69839731
AT5G25110	0,642212611	0,782086731	0,698213155	0,752757315
AT5G25170	-1,128140245	-1,00149645	-1,127774462	-1,200010926
AT5G25220	1,195158149	1,070582891	1,096492801	1,029874663
AT5G25450	-1,386850659	-0,989713047	-0,708777981	-1,03685397
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AT5G25840	-1,115216759	-1,051029502	-1,102940982	-1,027570102

AT5G26180	-1,219026908	-0,87627864	-0,939727378	-0,971216268
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AT5G26940	1,442394472	1,539700392	0,91292557	0,826868403
AT5G27150	2,839538649	2,133930365	1,328619099	1,218677176
AT5G27280	0,898510877	0,807934468	0,964088426	0,893965559
AT5G27330	-1,44010048	-1,013227924	-1,261801917	-1,212623885
AT5G27380	-1,077630969	-0,800141223	-0,765515343	-0,936364505
AT5G27390	-0,758659068	-0,720267678	-0,754601429	-0,888553649
AT5G27520	0,849190005	0,778514442	0,86867645	0,684560936
AT5G27770	-1,009143478	-0,908537536	-0,691448832	-0,749375278
AT5G28050	0,944410717	0,829893285	0,819186235	0,743001265
AT5G34850	0,940007687	1,079610756	1,497530914	1,328611746
AT5G35180	0,752716042	0,801309934	0,778961629	0,700569989
AT5G35460	1,329315472	1,038424063	1,162057248	1,040828005
AT5G35530	-0,722340828	-0,673328311	-0,645835744	-0,669872603
AT5G35630	2,609181262	1,269678545	0,67251892	0,648423234
AT5G35980	1,178601968	0,719493979	0,809376839	0,802341103
AT5G37350	-1,161515063	-0,987743864	-0,990034234	-0,969345975
AT5G37360	1,548619147	1,422338449	0,882207301	0,862845607
AT5G37440	-0,943926339	-0,902221507	-0,92487305	-0,949951622
AT5G37475	-0,853357126	-0,763897589	-0,718390647	-0,614443287
AT5G37500	1,18792489	1,393906135	1,064400891	1,030610222
AT5G37550	1,178461011	1,140895477	1,847553269	1,715795549
AT5G37770	-0,988836678	-0,907286272	-1,088488132	-1,203207111
AT5G37850	-1,190831233	-0,877346239	-0,898691098	-0,900350998
AT5G38720	-0,819573604	-0,693914848	-0,862605381	-0,756180517
AT5G38890	-0,634366804	-0,616687	-0,668830884	-0,710130119
AT5G38895	0,921013442	0,705392602	0,797536042	0,761095583
AT5G38980	1,835511199	1,784711662	0,917531472	0,851281433
AT5G39520	4,236304788	3,297455396	2,901755871	2,673040731
AT5G39580	-1,161609749	-1,507028886	-1,777951684	-1,701320781
AT5G39610	1,322986369	1,09203354	1,107113899	1,016441899
AT5G39730	0,95250708	1,062467061	0,923102797	0,789139228
AT5G39840	-1,531434483	-1,222673864	-0,910961999	-1,113083315
AT5G40450	-1,125939835	-0,747020217	-0,978277406	-1,062617745
AT5G40480	-1,919636193	-1,560145568	-1,687898572	-1,787308731
AT5G40670	0,994363013	1,045577122	0,983603595	0,926375516
AT5G40770	-1,235293014	-1,200530942	-1,495563451	-1,370607577
AT5G40780	-1,172384763	-0,728215244	-1,010629486	-0,936431714
AT5G40960	0,669745645	0,688385604	0,854960116	0,665520476
AT5G41010	-1,021165866	-0,876671841	-1,128893223	-0,98879789
AT5G41020	-1,008527053	-0,790615656	-0,70686762	-0,742292808
AT5G41670	-2,316579312	-1,922334628	-1,90280918	-2,059992657
AT5G42050	1,418849161	0,792664706	0,924387563	0,783222928
AT5G42150	-1,469171656	-1,158384563	-1,165937859	-1,304194421
AT5G42820	-0,980567368	-0,790833539	-0,882330757	-0,792007233
AT5G42890	0,782811539	0,859661468	0,862030416	0,744515069
AT5G43060	2,101258141	2,045919802	2,414989264	2,419394238
AT5G43260	1,54988265	1,303471056	1,844712295	1,703062031
AT5G43420	-1,244997622	-0,787970787	-1,136157	-1,108579652
AT5G43430	0,776137399	0,816704615	0,87235208	0,677532763
AT5G43780	0,741089535	0,850747129	0,975734241	0,964168332
AT5G43830	1,075343193	0,958708913	1,611548879	1,434852605
AT5G43850	2,021491669	1,851837507	2,405090254	2,210812335
AT5G43940	1,243058885	0,653517634	0,756195908	0,886443836
AT5G44070	-0,943770666	-0,778995295	-0,893502032	-0,972754415

AT5G44130	-0,71457195	-0,823614125	-1,587328608	-1,662807124
AT5G45080	-1,113389576	-0,775781215	-0,971741063	-1,097695321
AT5G45310	1,124139732	1,139763049	2,933815227	2,966396022
AT5G45340	1,244199225	1,166458517	1,652351154	1,719200358
AT5G45490	-1,514805482	-1,180317126	-0,999038478	-1,196409731
AT5G45510	-0,915086788	-0,672806188	-0,68095795	-0,745324808
AT5G45930	0,861667132	1,060463161	0,952378746	0,769110379
AT5G45950	3,563934657	3,081358343	2,907540501	2,72373614
AT5G46160	-0,924979062	-0,830581775	-0,887714188	-0,860956835
AT5G46180	1,320438346	1,124281387	0,998594786	0,963172897
AT5G46710	-1,640210564	-1,258644821	-1,310071382	-1,587441606
AT5G46780	-1,126319752	-0,935055427	-0,779894333	-0,947546102
AT5G47020	0,726753853	0,654315801	1,06775585	0,945033965
AT5G47040	1,315493757	1,280905864	1,787690413	1,641357549
AT5G47180	0,732853379	0,693307333	0,85151613	0,78255583
AT5G47230	-0,98869358	-0,91294398	-1,536395202	-1,528195217
AT5G47560	1,590231489	1,200117864	1,320595674	1,25094795
AT5G47600	-1,001420459	-0,658693101	-1,024704658	-1,143984246
AT5G47650	1,328013798	0,76728989	0,633324349	0,748005644
AT5G47710	-0,917544039	-0,705356829	-0,890127767	-0,879477688
AT5G48030	-0,988647873	-0,815224634	-0,992072443	-1,035343186
AT5G48070	-1,532439513	-1,257297264	-1,141978444	-1,435497298
AT5G48180	1,543452401	1,647034412	2,816380059	2,746473586
AT5G48380	-1,06438003	-0,671633375	-0,767144441	-0,956060664
AT5G48657	-0,817163839	-0,851629484	-1,118269364	-1,259085499
AT5G48850	0,860848154	0,814258418	0,745821257	0,657382111
AT5G49360	1,674554737	1,533859238	1,133418308	1,087797178
AT5G49450	1,484601416	1,654580953	2,366786675	2,363629165
AT5G49510	-0,762712439	-0,724165779	-0,73844486	-0,760550003
AT5G50100	2,021083694	2,074675425	2,375311163	2,470271069
AT5G50210	-1,161710796	-0,913595511	-1,180859613	-1,168419898
AT5G50360	1,33258845	0,786111315	1,505916372	1,305804714
AT5G50370	0,653198607	0,656273383	1,021737923	1,068699537
AT5G50810	-1,530442429	-1,119943268	-0,927604873	-1,122327667
AT5G51540	-1,245152124	-0,946142523	-0,910255617	-1,011184817
AT5G52040	-0,942121864	-0,736794236	-0,646589496	-0,632222265
AT5G52310	3,371227155	2,722791175	2,214467621	2,312177339
AT5G52470	-1,482120102	-1,353187919	-1,495404867	-1,508546679
AT5G52490	-0,656687305	-0,896177457	-1,053075311	-1,199213014
AT5G52650	-0,750280986	-0,669299011	-0,628287026	-0,669368433
AT5G52820	-1,012029925	-0,714822018	-0,62979405	-0,689249738
AT5G52880	1,248926998	1,00042684	1,054837717	0,953052351
AT5G53120	2,584007764	2,553497449	2,120848404	2,005636794
AT5G53160	-0,741759789	-0,696235235	-0,753114381	-0,910725994
AT5G53590	2,053138824	1,284318137	1,990164864	2,067133496
AT5G53730	0,954514698	1,008836463	1,253236499	1,119040905
AT5G53830	1,183479578	0,876949238	1,381449683	1,339953031
AT5G53870	1,636688502	1,50118059	3,100208477	2,703792597
AT5G54080	1,362346141	1,440628251	1,637655609	1,570223915
AT5G54580	-1,104609137	-0,966368865	-0,928546739	-1,077262472
AT5G54910	-1,406185118	-1,23546502	-1,411513232	-1,401610729
AT5G55050	-0,671725064	-0,602409763	-1,702850421	-1,600972659
AT5G55620	-0,820739784	-0,759352823	-1,53164209	-1,777666597
AT5G55640	0,794249831	0,802594432	0,846087158	0,911055995
AT5G55930	-1,790180809	-1,610808714	-1,689333083	-1,673530467
AT5G56030	-0,812344147	-0,695346976	-1,079919851	-0,955461138

AT5G56350	-0,940293808	-0,620482598	-0,757033529	-0,867808267
AT5G56710	-0,946001003	-0,761347963	-0,804040449	-0,80318419
AT5G56940	-0,903281611	-0,679450509	-0,718892655	-0,642259762
AT5G57035	1,860375861	1,859818002	1,402645555	1,4467395
AT5G57035	2,883327809	2,881199527	2,306682716	2,23299969
AT5G57220	-1,094740594	-0,78308049	-1,096913278	-0,776966103
AT5G57350	1,307750793	1,522918611	1,879259023	1,82945097
AT5G57500	-0,770046176	-0,596024611	-0,6842785	-0,658265143
AT5G57785	1,689383465	1,228812414	1,941108284	1,81422435
AT5G57800	1,557992416	1,628565641	0,996488094	0,912509788
AT5G57815	0,818542121	0,913117437	0,760233242	0,725221145
AT5G57920	-1,049356209	-0,827132228	-0,945813329	-0,998493124
AT5G57990	-1,123489991	-0,87136764	-0,866588351	-0,849020666
AT5G58130	-1,362569973	-1,087857136	-0,932864516	-0,921293466
AT5G58140	1,236103249	1,340661964	1,387128653	1,202945465
AT5G58330	1,963804166	1,188682448	0,671985836	0,788189288
AT5G58420	-1,060758373	-0,949703923	-0,749731701	-0,878687487
AT5G58590	-0,875162977	-0,681340612	-1,079846249	-1,119556221
AT5G58800	0,74237964	0,755000415	0,771408008	0,682475036
AT5G59090	-1,446173014	-1,278205026	-1,840683822	-1,75916831
AT5G59220	4,171218453	3,798736119	4,189286489	4,069064256
AT5G59440	-1,120790998	-0,955803123	-1,016266696	-0,963331535
AT5G59450	-0,742652821	-0,660598112	-0,89007256	-0,818514773
AT5G59960	1,935338947	1,860956864	2,119869467	2,024699673
AT5G60030	-0,644732565	-0,799935406	-1,345022193	-1,204814863
AT5G60040	-0,947700872	-0,720184424	-0,787093255	-0,787276044
AT5G60360	2,516749253	2,352160655	2,078926415	2,112527342
AT5G60670	-0,83104938	-0,765164992	-0,846911684	-0,917715463
AT5G60680	1,386379472	1,015811917	0,814552373	0,639274272
AT5G60730	-1,081669472	-0,959056417	-0,92522492	-0,958057813
AT5G60890	1,142649857	1,490830616	2,061546561	1,884565275
AT5G60990	-1,53423324	-1,179109824	-1,070500103	-1,323345961
AT5G61130	-1,178159937	-1,102705771	-1,109595114	-1,149884661
AT5G61160	-1,458475105	-1,242805842	-1,412139976	-1,420520443
AT5G61330	-1,701554669	-1,410555331	-1,427528382	-1,57352975
AT5G61770	-1,329697194	-0,888125277	-0,652843245	-0,881724297
AT5G61880	-1,092668348	-0,746100675	-0,83574311	-0,937213404
AT5G62020	0,756139782	0,854217199	1,212074929	1,233742364
AT5G62130	1,454724527	0,940657778	0,83566519	0,803991371
AT5G62140	1,310149558	1,080045764	1,26496222	1,14912729
AT5G62270	-0,906712274	-0,708497068	-0,689446389	-0,741844443
AT5G62460	0,64508813	0,661386429	1,014489665	0,894786248
AT5G62470	1,703982562	1,461807787	0,968887122	0,836792639
AT5G62520	0,981031821	1,14380194	1,541594618	1,49789351
AT5G62740	-0,859805566	-0,756890534	-0,694194029	-0,767305751
AT5G63020	-0,950897209	-0,852306822	-0,703025718	-0,712159724
AT5G63180	-1,866274803	-1,590643888	-1,709310299	-1,698967479
AT5G63280	-1,024747132	-0,827729928	-0,6490238	-0,811213546
AT5G63600	-1,702866594	-1,137684889	-0,933190028	-1,010802676
AT5G63680	-0,73336338	-0,595925692	-0,664239804	-0,681455279
AT5G63690	-0,669072403	-0,716221682	-0,808058545	-0,817249849
AT5G63840	2,263688578	2,117057931	1,653204085	1,522232156
AT5G63920	-1,270297214	-0,946125548	-1,251319414	-1,226022382
AT5G64240	-1,220672899	-0,990569751	-0,687362325	-0,774536635
AT5G64500	-1,189287884	-1,25118604	-1,159451592	-1,23924585
AT5G64640	-0,978845543	-0,834499252	-0,72508069	-0,805997392

AT5G64680	-1,125008164	-0,825475055	-0,686136316	-1,063257387
AT5G64820	1,226005266	1,162358661	1,299725824	1,317394034
AT5G64960	1,14017516	1,173327214	1,658267496	1,598036612
AT5G64980	1,011325054	0,93232677	1,253866871	1,119075017
AT5G65880	-1,021870819	-0,776063747	-0,737023156	-0,76305033
AT5G65990	1,316051932	0,686564419	1,454462224	1,30799722
AT5G66040	1,156360927	1,033707201	0,86078031	0,663321223
AT5G67230	-1,373977666	-0,866978555	-0,634336992	-0,802027088
AT5G67300	1,565508905	1,159743756	0,868724344	0,86495306
AT5G67360	1,70628068	1,398096622	1,321710966	1,476016312
AT5G67370	1,959276508	1,893470737	1,889916502	1,746998183
AT5G67480	1,158030797	1,081486441	1,519058151	1,453837551
AT5G67590	1,610255822	0,802677952	0,759548046	0,688765459
AT5G67630	-1,614253856	-1,246107869	-0,863373383	-0,911831566
Nogene	1,879748188	1,844139889	0,95345368	0,959763014
Nogene	-2,876307926	-2,576883374	-2,305042181	-2,280124578

Table S4.1. ChIP/chip results

About 770 SAP promoter fragments were identified to be enriched after chromatin immunoprecipitation with the anti LEC1-antibody in at least three out of four biological replicates. The mean log ratios of three/four replicates are given. LEC1 target promoters were classified according to the genomic constellation of the corresponding gene, the size of the intergenic region (from stop to start) in case of head-to-head (hh) constellation and the representation of neighbour genes on the SAP macroarray (which contains only fragments of 8471 Arabidopsis promoters). In case of hh-gene pairs with an intergenic region of <3kB, the neighbour gene IDs and the size of the intergenic regions are given.

ATG	**Log ratio**	**classification**	**ATG neighbour Gene in hh <3kB**	**intergenic region (bp)**
AT1G07820	1,18	partner on SAP but not enriched	AT1G07830	297
AT1G16850	1,58	partner on SAP but not enriched	AT1G16860	1897
AT1G17120	1,17	partner on SAP but not enriched	AT1G17110	2074
AT1G51510	1,75	partner on SAP but not enriched	AT1G51520	2035
AT1G62600	1,61	partner on SAP but not enriched	AT1G62590	343
AT1G63880	2,13	partner on SAP but not enriched	AT1G63900	1008
AT1G67880	0,9	partner on SAP but not enriched	AT1G67890	1891
AT1G69295	1,12	partner on SAP but not enriched	AT1G69310	2679
AT1G74310	0,9	partner on SAP but not enriched	AT1G74320	884
AT1G74370	1,27	partner on SAP but not enriched	AT1G74380	661
AT1G75190	1,44	partner on SAP but not enriched	AT1G75180	1686
AT1G76405	1,2	partner on SAP but not enriched	AT1G76400	321
AT1G76680	1,23	partner on SAP but not enriched	AT1G76670	2412
AT1G78920	0,94	partner on SAP but not enriched	AT1G78915	1258
AT1G79910	1,46	partner on SAP but not enriched	AT1G79900	1450
AT2G29550	1,51	partner on SAP but not enriched	AT2G29560	702
AT2G33570	1,18	partner on SAP but not enriched	AT2G33560	1442
AT2G35680	1,99	partner on SAP but not enriched	AT2G35690	1371
AT2G39920	1,28	partner on SAP but not enriched	AT2G39930	1772
AT2G47200	1,18	partner on SAP but not enriched	AT2G47210	888
AT2G47770	1,27	partner on SAP but not enriched	AT2G47760	479
AT3G02570	1,5	partner on SAP but not enriched	AT3G02580	1569
AT3G07480	1,31	partner on SAP but not enriched	AT3G07470	682
AT3G08550	1,66	partner on SAP but not enriched	AT3G08530	1101
AT3G11330	1,15	partner on SAP but not enriched	AT3G11340	2032
AT3G12480	1,36	partner on SAP but not enriched	AT3G12470	1279
AT3G13930	2,42	partner on SAP but not enriched	AT3G13920	2111
AT3G15356	1,88	partner on SAP but not enriched	AT3G15351	1307
AT3G15640	1,49	partner on SAP but not enriched	AT3G15630	1761
AT3G17810	1,07	partner on SAP but not enriched	AT3G17800	1405
AT3G19180	1,16	partner on SAP but not enriched	AT3G19170	935
AT3G20240	1,21	partner on SAP but not enriched	AT3G20230	513
AT3G52070	0,93	partner on SAP but not enriched	AT3G52060	725
AT3G53470	0,9	partner on SAP but not enriched	AT3G53460	1389
AT3G54050	1,49	partner on SAP but not enriched	AT3G54040	1651
AT3G55440	1,37	partner on SAP but not enriched	AT3G55430	1789
AT3G57190	1,38	partner on SAP but not enriched	AT3G57180	461
AT3G61860	1,15	partner on SAP but not enriched	AT3G61870	572
AT3G62130	1,07	partner on SAP but not enriched	AT3G62120	1144
AT3G63120	1,01	partner on SAP but not enriched	AT3G63130	1214
AT4G21020	1,27	partner on SAP but not enriched	AT4G21010	899
AT4G26090	2,14	partner on SAP but not enriched	AT4G26080	2767
AT4G33440	1,29	partner on SAP but not enriched	AT4G33430	1731

AT4G34860	1,32	partner on SAP but not enriched	AT4G34870	2431
AT4G36780	1,95	partner on SAP but not enriched	AT4G36790	2147
AT4G36800	1,25	partner on SAP but not enriched	AT4G36810	1364
AT5G04130	1,61	partner on SAP but not enriched	AT5G04140	1999
AT5G04480	0,88	partner on SAP but not enriched	AT5G04490	2073
AT5G06980	1,9	partner on SAP but not enriched	AT5G06970	1749
AT5G08680	1,35	partner on SAP but not enriched	AT5G08670	842
AT5G13100	1,65	partner on SAP but not enriched	AT5G13090	2221
AT5G18910	1,51	partner on SAP but not enriched	AT5G18920	698
AT5G23680	1,22	partner on SAP but not enriched	AT5G23690	1203
AT5G52300	1,21	partner on SAP but not enriched	AT5G52310	1524
AT5G63790	1,53	partner on SAP but not enriched	AT5G63800	2309
AT5G65430	1,16	partner on SAP but not enriched	AT5G65440	1759
AT5G66270	0,96	partner on SAP but not enriched	AT5G66280	867
AT1G01470	1,18	partner not on SAP	AT1G01471	424
AT1G02660	1,82	partner not on SAP	AT1G02670	1299
AT1G04530	1,68	partner not on SAP	AT1G04540	1360
AT1G04690	1,18	partner not on SAP	AT1G04684	3000
AT1G07510	1,78	partner not on SAP	AT1G07500	364
AT1G07980	0,72	partner not on SAP	AT1G07985	477
AT1G08880	1,31	partner not on SAP	AT1G08890	547
AT1G09080	1,04	partner not on SAP	AT1G09090	897
AT1G09730	2,15	partner not on SAP	AT1G09740	295
AT1G09870	1,42	partner not on SAP	AT1G09880	229
AT1G10590	0,95	partner not on SAP	AT1G10600	308
AT1G11400	1,19	partner not on SAP	AT1G11410	1078
AT1G13000	0,89	partner not on SAP	AT1G13010	749
AT1G14010	0,96	partner not on SAP	AT1G14020	1139
AT1G15580	1,57	partner not on SAP	AT1G15590	876
AT1G15980	1,27	partner not on SAP	AT1G15970	429
AT1G16300	2,11	partner not on SAP	AT1G16290	265
AT1G18320	1,84	partner not on SAP	AT1G18310	403
AT1G19310	1,26	partner not on SAP	AT1G19320	2222
AT1G20450	1,28	partner not on SAP	AT1G20460	2397
AT1G21350	0,86	partner not on SAP	AT1G21340	72
AT1G22850	1,11	partner not on SAP	AT1G22860	524
AT1G24625	0,91	partner not on SAP	AT1G24640	2067
AT1G25550	1,58	partner not on SAP	AT1G25540	2179
AT1G30380	1,26	partner not on SAP	AT1G30370	1566
AT1G33980	1,27	partner not on SAP	AT1G33970	389
AT1G48840	2,16	partner not on SAP	AT1G48830	218
AT1G50260	1,08	partner not on SAP	AT1G50270	400
AT1G51090	1,44	partner not on SAP	AT1G51085	1245
AT1G54340	1,07	partner not on SAP	AT1G54330	2527
AT1G54690	1,07	partner not on SAP	AT1G54700	610
AT1G55150	1,63	partner not on SAP	AT1G55140	131
AT1G63290	1,2	partner not on SAP	AT1G63280	389
AT1G64110	1,39	partner not on SAP	AT1G64120	1303
AT1G64620	1,45	partner not on SAP	AT1G64618	894
AT1G67360	1,64	partner not on SAP	AT1G67365	414
AT1G67785	1,28	partner not on SAP	AT1G67792	556
AT1G67840	1,32	partner not on SAP	AT1G67830	1183
AT1G68300	1,77	partner not on SAP	AT1G68310	269
AT1G68990	1,18	partner not on SAP	AT1G69000	408
AT1G70210	0,93	partner not on SAP	AT1G70209	219
AT1G74450	1,33	partner not on SAP	AT1G74448	456

AT1G75770	1,06	partner not on SAP	AT1G75760	1600
AT1G77690	1,03	partner not on SAP	AT1G77700	1429
AT1G80080	1,03	partner not on SAP	AT1G80090	540
AT2G03620	1,36	partner not on SAP	AT2G03630	1036
AT2G22430	1,7	partner not on SAP	AT2G22440	1297
AT2G23430	1,62	partner not on SAP	AT2G23440	1560
AT2G24420	1,63	partner not on SAP	AT2G24410	739
AT2G25880	1,6	partner not on SAP	AT2G25890	607
AT2G26150	1,06	partner not on SAP	AT2G26140	729
AT2G27810	1,14	partner not on SAP	AT2G27800	986
AT2G29180	1,22	partner not on SAP	AT2G29170	800
AT2G30110	1,94	partner not on SAP	AT2G30115	1299
AT2G33590	1,9	partner not on SAP	AT2G33585	454
AT2G34930	1,01	partner not on SAP	AT2G34940	610
AT2G35260	0,73	partner not on SAP	AT2G35250	979
AT2G35605	1,65	partner not on SAP	AT2G35600	2879
AT2G36530	1,34	partner not on SAP	AT2G36540	1450
AT2G37710	1,09	partner not on SAP	AT2G37720	1120
AT2G39270	1,95	partner not on SAP	AT2G39260	394
AT2G39290	0,7	partner not on SAP	AT2G39280	623
AT2G39730	2,06	partner not on SAP	AT2G39740	2904
AT2G41280	1,47	partner not on SAP	AT2G41270	360
AT2G41770	0,75	partner not on SAP	AT2G41780	1114
AT2G42540	1,16	partner not on SAP	AT2G42550	1229
AT2G43330	0,98	partner not on SAP	AT2G43320	1976
AT2G44310	1,03	partner not on SAP	AT2G44300	998
AT2G45870	1,35	partner not on SAP	AT2G45860	359
AT3G01590	1,79	partner not on SAP	AT3G01580	1192
AT3G02310	2,18	partner not on SAP	AT3G02315	2972
AT3G04240	1,28	partner not on SAP	AT3G04250	1227
AT3G05830	1,82	partner not on SAP	AT3G05820	1193
AT3G06750	1,33	partner not on SAP	AT3G06760	2762
AT3G08890	2,01	partner not on SAP	AT3G08885	422
AT3G10330	1,05	partner not on SAP	AT3G10320	2795
AT3G11880	0,93	partner not on SAP	AT3G11870	2603
AT3G11910	0,99	partner not on SAP	AT3G11920	2020
AT3G12110	1,11	partner not on SAP	AT3G12100	845
AT3G12300	1,22	partner not on SAP	AT3G12320	941
AT3G14190	0,75	partner not on SAP	AT3G14185	1092
AT3G17040	0,88	partner not on SAP	AT3G17050	2142
AT3G17300	1,11	partner not on SAP	AT3G17290	290
AT3G17680	1,18	partner not on SAP	AT3G17690	1114
AT3G20670	1,79	partner not on SAP	AT3G20660	961
AT3G22840	2,26	partner not on SAP	AT3G22845	1925
AT3G43120	1,57	partner not on SAP	AT3G43110	2358
AT3G46030	1,55	partner not on SAP	AT3G46040	839
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AT3G46830	1,27	partner not on SAP	AT3G46840	2648
AT3G47340	1,39	partner not on SAP	AT3G47341	377
AT3G49470	0,97	partner not on SAP	AT3G49460	455
AT3G49580	1,22	partner not on SAP	AT3G49590	2172
AT3G50830	1,28	partner not on SAP	AT3G50835	1515
AT3G51240	2,15	partner not on SAP	AT3G51238	145
AT3G51800	1,2	partner not on SAP	AT3G51810	1249
AT3G51820	1,97	partner not on SAP	AT3G51830	1302
AT3G52340	1,15	partner not on SAP	AT3G52330	1204

AT3G53180	1,42	partner not on SAP	AT3G53170	909
AT3G55260	0,85	partner not on SAP	AT3G55258	847
AT3G56270	1,57	partner not on SAP	AT3G56260	1446
AT3G57560	2,07	partner not on SAP	AT3G57570	390
AT3G58990	1,72	partner not on SAP	AT3G59000	1095
AT3G62600	1,21	partner not on SAP	AT3G62610	1411
AT3G63060	1,92	partner not on SAP	AT3G63070	1210
AT3G63510	1,15	partner not on SAP	AT3G63500	550
AT4G00850	1,4	partner not on SAP	AT4G00840	569
AT4G01120	1,29	partner not on SAP	AT4G01130	1897
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AT4G14540	2,11	partner not on SAP	AT4G14530	779
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AT4G23590	1,19	partner not on SAP	AT4G23580	1797
AT4G23930	0,76	partner not on SAP	AT4G23940	413
AT4G27020	1,02	partner not on SAP	AT4G27030	570
AT4G27700	1,76	partner not on SAP	AT4G27710	846
AT4G27940	1,31	partner not on SAP	AT4G27930	1766
AT4G29410	0,77	partner not on SAP	AT4G29415	313
AT4G29890	1,76	partner not on SAP	AT4G29880	488
AT4G33530	1,32	partner not on SAP	AT4G33540	528
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AT5G04885	1,01	partner not on SAP	AT5G04880	786
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AT5G19430	1,63	partner not on SAP	AT5G19420	1131
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AT5G52570	1,26	partner not on SAP	AT5G52580	1164
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AT5G55470	1,41	partner not on SAP	AT5G55480	1691
AT5G58330	2,14	partner not on SAP	AT5G58340	319
AT5G58575	1,65	partner not on SAP	AT5G58570	1267
AT5G59910	2,11	partner not on SAP	AT5G59900	499
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AT5G61820	1,5	partner not on SAP	AT5G61830	1111
AT5G62000	1,42	partner not on SAP	AT5G61997	2474

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AT1G15340	1,73	not head head
AT1G15870	1,26	not head head
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AT1G19110	1,24	not head head
AT1G19350	1,7	not head head
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AT1G19490	1,44	not head head
AT1G19530	1,2	not head head
AT1G19650	2,71	not head head
AT1G19660	2,6	not head head
AT1G19860	1,41	not head head
AT1G19960	1,52	not head head
AT1G20693	1,48	not head head
AT1G21010	1,12	not head head
AT1G21400	1,35	not head head
AT1G21590	1,89	not head head
AT1G21640	1,58	not head head
AT1G21790	1,56	not head head
AT1G22370	1,43	not head head
AT1G22750	1,18	not head head
AT1G25275	1,43	not head head
AT1G25490	1,44	not head head
AT1G26670	1,53	not head head
AT1G27290	1,01	not head head
AT1G27300	1,42	not head head
AT1G27930	1,3	not head head
AT1G28395	1,3	not head head
AT1G29070	1,45	not head head
AT1G29390	2,26	not head head
AT1G29395	1,24	not head head
AT1G30720	1,18	not head head
AT1G31230	1,73	not head head
AT1G32380	1,27	not head head
AT1G32640	1,18	not head head
AT1G49200	1,42	not head head
AT1G49780	1,19	not head head
AT1G52890	1,61	not head head
AT1G53320	0,84	not head head
AT1G53400	1,14	not head head
AT1G54130	1,65	not head head
AT1G54210	2,18	not head head
AT1G56200	1,57	not head head
AT1G56330	1,68	not head head
AT1G56340	1,22	not head head

AT1G59990	1,29	not head head
AT1G60430	1,37	not head head
AT1G60790	2,47	not head head
AT1G63000	1,31	not head head
AT1G64630	1,17	not head head
AT1G65230	1,36	not head head
AT1G68190	1,75	not head head
AT1G68520	1,06	not head head
AT1G69010	1,51	not head head
AT1G69800	1,56	not head head
AT1G70590	1,05	not head head
AT1G70650	1,96	not head head
AT1G71100	1,34	not head head
AT1G72790	1,27	not head head
AT1G73430	1,48	not head head
AT1G73480	2,22	not head head
AT1G73880	1,66	not head head
AT1G73920	0,97	not head head
AT1G74010	2,03	not head head
AT1G74840	1,62	not head head
AT1G75400	1,16	not head head
AT1G77450	2,13	not head head
AT1G77460	0,49	not head head
AT1G77500	0,52	not head head
AT1G78510	1,43	not head head
AT1G78680	2,13	not head head
AT1G78700	1,81	not head head
AT1G79110	1,31	not head head
AT1G79460	2,37	not head head
AT1G79530	1,5	not head head
AT1G80130	1,32	not head head
AT1G80380	1,44	not head head
AT1G80440	1,34	not head head
AT1G80530	2,27	not head head
AT1G80570	1,19	not head head
AT2G02160	1,31	not head head
AT2G05710	0,9	not head head
AT2G17280	1,79	not head head
AT2G18876	0,98	not head head
AT2G20580	1,52	not head head
AT2G20750	1,08	not head head
AT2G20835	1,86	not head head
AT2G20890	0,98	not head head
AT2G21780	0,88	not head head
AT2G21970	1,04	not head head
AT2G23790	2,21	not head head
AT2G24100	1,69	not head head
AT2G24150	1,35	not head head
AT2G24360	0,6	not head head
AT2G25900	1,67	not head head
AT2G27830	1,69	not head head
AT2G28420	0,92	not head head
AT2G28550	0,78	not head head
AT2G28950	0,86	not head head
AT2G29390	2,1	not head head
AT2G31970	1,29	not head head

AT2G33700	2,4	not head head
AT2G34250	1,87	not head head
AT2G34510	1,16	not head head
AT2G35290	1,03	not head head
AT2G35650	1,02	not head head
AT2G36620	1,01	not head head
AT2G36630	1,21	not head head
AT2G36770	1,02	not head head
AT2G36800	1,94	not head head
AT2G36840	1,64	not head head
AT2G36870	0,9	not head head
AT2G36895	1,86	not head head
AT2G38090	1,43	not head head
AT2G38280	1,33	not head head
AT2G39800	1,29	not head head
AT2G41100	1,11	not head head
AT2G42010	0,84	not head head
AT2G42530	1,06	not head head
AT2G42590	1,58	not head head
AT2G42750	1,33	not head head
AT2G42790	1,09	not head head
AT2G43710	1,03	not head head
AT2G45490	1,41	not head head
AT2G45660	0,72	not head head
AT2G46150	1,5	not head head
AT2G46735	1,27	not head head
AT2G47490	1,15	not head head
AT3G01510	2,21	not head head
AT3G02690	1,69	not head head
AT3G02910	1,14	not head head
AT3G03550	1,03	not head head
AT3G04120	2,63	not head head
AT3G04730	1,03	not head head
AT3G05200	1,37	not head head
AT3G05510	1,12	not head head
AT3G05810	1,21	not head head
AT3G08720	0,85	not head head
AT3G09370	1,15	not head head
AT3G09390	2,19	not head head
AT3G09980	1,06	not head head
AT3G10410	1,38	not head head
AT3G11700	1,36	not head head
AT3G11770	0,97	not head head
AT3G11900	2,01	not head head
AT3G14180	1,82	not head head
AT3G14230	1,76	not head head
AT3G14690	1,93	not head head
AT3G15351	0,47	not head head
AT3G16530	0,92	not head head
AT3G17020	1,58	not head head
AT3G17310	1,03	not head head
AT3G17365	0,98	not head head
AT3G17790	1,35	not head head
AT3G18000	1,33	not head head
AT3G19130	1,49	not head head
AT3G19500	1,6	not head head

AT3G23940	0,87	not head head
AT3G26580	2,32	not head head
AT3G27170	1,92	not head head
AT3G27360	1,52	not head head
AT3G27570	0,99	not head head
AT3G27820	1,5	not head head
AT3G44880	2,18	not head head
AT3G45030	1,34	not head head
AT3G46010	1,45	not head head
AT3G46540	1,18	not head head
AT3G47070	1,44	not head head
AT3G47470	2,05	not head head
AT3G48090	1,86	not head head
AT3G48690	1,07	not head head
AT3G49680	1,26	not head head
AT3G50060	1,42	not head head
AT3G51260	1,25	not head head
AT3G51510	1,61	not head head
AT3G51750	0,83	not head head
AT3G51890	2,06	not head head
AT3G53670	2,05	not head head
AT3G53800	1,5	not head head
AT3G54390	2,16	not head head
AT3G54890	1,71	not head head
AT3G54960	1,41	not head head
AT3G55800	1,21	not head head
AT3G56090	2,34	not head head
AT3G56800	1,67	not head head
AT3G57400	2,06	not head head
AT3G58200	1,38	not head head
AT3G58840	1,38	not head head
AT3G59050	1,16	not head head
AT3G59060	1,81	not head head
AT3G60110	1,15	not head head
AT3G60410	1,31	not head head
AT3G61950	1,38	not head head
AT3G62260	1,03	not head head
AT4G01026	2,48	not head head
AT4G01060	0,82	not head head
AT4G01610	1,07	not head head
AT4G01860	1,27	not head head
AT4G02120	0,97	not head head
AT4G02130	0,97	not head head
AT4G03210	1,17	not head head
AT4G03560	1,59	not head head
AT4G10340	1,84	not head head
AT4G11220	1,73	not head head
AT4G13180	2,82	not head head
AT4G13530	2,07	not head head
AT4G14420	1,21	not head head
AT4G14600	1,88	not head head
AT4G14900	1,43	not head head
AT4G16390	0,59	not head head
AT4G16660	1,83	not head head
AT4G17420	1,61	not head head
AT4G18810	1,1	not head head

AT4G18920	1,72	not head head
AT4G21510	1,69	not head head
AT4G22240	2,16	not head head
AT4G22820	1,39	not head head
AT4G23650	1,7	not head head
AT4G23750	0,99	not head head
AT4G24000	0,91	not head head
AT4G24190	1,46	not head head
AT4G24350	1,33	not head head
AT4G24730	1,95	not head head
AT4G24740	2,83	not head head
AT4G25260	1,14	not head head
AT4G26740	2,15	not head head
AT4G27440	1,68	not head head
AT4G27720	1,75	not head head
AT4G28440	1,5	not head head
AT4G31750	1,78	not head head
AT4G33560	0,94	not head head
AT4G33905	1,08	not head head
AT4G34120	1,34	not head head
AT4G34131	1,48	not head head
AT4G34135	1,1	not head head
AT4G34490	1,44	not head head
AT4G35000	0,98	not head head
AT4G35140	1,48	not head head
AT4G35250	1,96	not head head
AT4G36490	1,34	not head head
AT4G37460	0,97	not head head
AT4G38740	1,56	not head head
AT5G01420	1,99	not head head
AT5G01530	1,53	not head head
AT5G01990	1,03	not head head
AT5G02500	0,9	not head head
AT5G03520	1,41	not head head
AT5G04590	1,9	not head head
AT5G05220	0,66	not head head
AT5G05250	1,08	not head head
AT5G07020	1,27	not head head
AT5G07340	1,49	not head head
AT5G07450	1,38	not head head
AT5G09600	1,44	not head head
AT5G10730	1,76	not head head
AT5G11260	1,42	not head head
AT5G11730	1,12	not head head
AT5G12050	1,31	not head head
AT5G13760	2,05	not head head
AT5G14620	0,97	not head head
AT5G15160	1,39	not head head
AT5G15850	1,65	not head head
AT5G15860	1,44	not head head
AT5G15950	1,98	not head head
AT5G15960	1,39	not head head
AT5G15970	1,53	not head head
AT5G16210	1,31	not head head
AT5G16710	1,43	not head head
AT5G17300	0,76	not head head

AT5G17460	1,6	not head head
AT5G17510	1,94	not head head
AT5G19690	2,03	not head head
AT5G19850	1,51	not head head
AT5G20030	1,51	not head head
AT5G23890	1,27	not head head
AT5G24430	1,38	not head head
AT5G24590	1,8	not head head
AT5G24930	1,36	not head head
AT5G27420	0,98	not head head
AT5G27670	2	not head head
AT5G35180	1,23	not head head
AT5G41100	1,02	not head head
AT5G42100	1,58	not head head
AT5G43940	1,34	not head head
AT5G44710	1,18	not head head
AT5G44785	1,07	not head head
AT5G46690	0,79	not head head
AT5G47180	1,27	not head head
AT5G47420	1,81	not head head
AT5G47590	0,94	not head head
AT5G47610	2,21	not head head
AT5G47670	1,47	not head head
AT5G51460	1,68	not head head
AT5G52420	1,19	not head head
AT5G53140	1,45	not head head
AT5G54510	1,03	not head head
AT5G54770	1,34	not head head
AT5G56100	1,68	not head head
AT5G57030	1,92	not head head
AT5G57100	1,35	not head head
AT5G57490	0,99	not head head
AT5G57580	0,97	not head head
AT5G58070	1,63	not head head
AT5G58380	2,34	not head head
AT5G59000	1,26	not head head
AT5G59690	1,99	not head head
AT5G59960	1,23	not head head
AT5G60360	1,37	not head head
AT5G61060	1,24	not head head
AT5G61790	1,97	not head head
AT5G61810	1,21	not head head
AT5G62130	1,77	not head head
AT5G63370	0,97	not head head
AT5G63380	1,94	not head head
AT5G63810	1,29	not head head
AT5G63860	0,86	not head head
AT5G64050	1,08	not head head
AT5G64400	1,26	not head head
AT5G64920	0,58	not head head
AT5G66460	1,6	not head head
AT5G66580	1,31	not head head
AT5G66780	1,68	not head head
AT5G67330	1,15	not head head
AT5G67370	1,25	not head head

Table 4.2. ChIP/chip candidate target genes of LEC1.

After initial data normalization and statistical analysis, a number of 449 LEC1 candidate target genes were identified by ChIP/chip according to the following criteria: (1) Genes with genomic constellation in head-to-tail or tail-to-head. (2) Genes with genomic constellation in head-to-head with an intergenic reguion >3kB. (3) Genes with genomic constellation in head-to-head with an intergenic reguion <3kB that were confirmed by qRT-PCR or micro/macroarray. The given log-ratios represent the mean value out of three/four biological replicates.

ATG	mean log-ratio
AT1G01070	1,81
AT1G01240	1,71
AT1G01370	0,86
AT1G01470	1,18
AT1G01720	1,89
AT1G02205	1,65
AT1G02340	1,87
AT1G02500	1,13
AT1G04920	1,26
AT1G04980	1,3
AT1G05340	1,73
AT1G06760	1,1
AT1G07080	1,46
AT1G07430	1,54
AT1G07470	1,53
AT1G07770	1,19
AT1G07980	0,72
AT1G09200	0,69
AT1G09210	1,4
AT1G09350	1,56
AT1G09780	1,79
AT1G10360	1,12
AT1G10760	1,96
AT1G10960	1,65
AT1G11210	1
AT1G12845	1,15
AT1G13640	1,98
AT1G13740	1,66
AT1G13950	1,18
AT1G14900	1,5
AT1G15340	1,73
AT1G15580	1,57
AT1G15870	1,26
AT1G18400	1,03
AT1G19110	1,24
AT1G19350	1,7
AT1G19400	1,47
AT1G19490	1,44
AT1G19530	1,2
AT1G19650	2,71
AT1G19660	2,6
AT1G19860	1,41
AT1G19960	1,52
AT1G20693	1,48
AT1G21010	1,12

AT1G21400	1,35
AT1G21590	1,89
AT1G21640	1,58
AT1G21790	1,56
AT1G22370	1,43
AT1G22750	1,18
AT1G23710	1,37
AT1G23750	1,18
AT1G25275	1,43
AT1G25490	1,44
AT1G26670	1,53
AT1G27290	1,01
AT1G27300	1,42
AT1G27930	1,3
AT1G28395	1,3
AT1G29070	1,45
AT1G29390	2,26
AT1G29395	1,24
AT1G30720	1,18
AT1G31230	1,73
AT1G32380	1,27
AT1G32640	1,18
AT1G42990	2,94
AT1G49200	1,42
AT1G49500	0,98
AT1G49780	1,19
AT1G52890	1,61
AT1G53320	0,84
AT1G53400	1,14
AT1G54130	1,65
AT1G54210	2,18
AT1G56200	1,57
AT1G56330	1,68
AT1G56340	1,22
AT1G59990	1,29
AT1G60430	1,37
AT1G60790	2,47
AT1G62390	1,55
AT1G63000	1,31
AT1G64630	1,17
AT1G65230	1,36
AT1G68190	1,75
AT1G68520	1,06
AT1G69010	1,51
AT1G69800	1,56
AT1G70420	1,39
AT1G70590	1,05
AT1G70650	1,96
AT1G70710	1,28
AT1G71100	1,34
AT1G72450	1,2
AT1G72790	1,27
AT1G72820	1,31
AT1G73430	1,48
AT1G73480	2,22
AT1G73880	1,66

AT1G73920	0,97
AT1G74010	2,03
AT1G74310	0,9
AT1G74320	0,9
AT1G74450	1,33
AT1G74840	1,62
AT1G75400	1,16
AT1G75410	0,78
AT1G75440	0,93
AT1G77450	2,13
AT1G77460	0,49
AT1G77500	0,52
AT1G77760	0,77
AT1G78230	2,61
AT1G78510	1,43
AT1G78680	2,13
AT1G78700	1,81
AT1G79110	1,31
AT1G79460	2,37
AT1G79530	1,5
AT1G80080	1,03
AT1G80130	1,32
AT1G80380	1,44
AT1G80440	1,34
AT1G80530	2,27
AT1G80570	1,19
AT2G01850	2,18
AT2G02160	1,31
AT2G05710	0,9
AT2G14890	1,29
AT2G17280	1,79
AT2G18876	0,98
AT2G20580	1,52
AT2G20670	0,95
AT2G20750	1,08
AT2G20835	1,86
AT2G20890	0,98
AT2G21780	0,88
AT2G21970	1,04
AT2G23790	2,21
AT2G24100	1,69
AT2G24150	1,35
AT2G24360	0,6
AT2G25890	1,6
AT2G25900	1,67
AT2G27250	1,09
AT2G27830	1,69
AT2G28330	0,83
AT2G28420	0,92
AT2G28550	0,78
AT2G28950	0,86
AT2G29390	2,1
AT2G31970	1,29
AT2G32980	1,68
AT2G33585	1,9
AT2G33700	2,4

AT2G34250	1,87
AT2G34510	1,16
AT2G35290	1,03
AT2G35650	1,02
AT2G36530	1,34
AT2G36620	1,01
AT2G36630	1,21
AT2G36770	1,02
AT2G36800	1,94
AT2G36840	1,64
AT2G36870	0,9
AT2G36895	1,86
AT2G38090	1,43
AT2G38280	1,33
AT2G38820	1,44
AT2G39270	1,95
AT2G39800	1,29
AT2G41100	1,11
AT2G41310	0,99
AT2G41900	1,6
AT2G42010	0,84
AT2G42530	1,06
AT2G42580	1,09
AT2G42590	1,58
AT2G42750	1,33
AT2G42790	1,09
AT2G43710	1,03
AT2G44920	1,27
AT2G45420	1,01
AT2G45490	1,41
AT2G45660	0,72
AT2G45960	1,26
AT2G46150	1,5
AT2G46735	1,27
AT2G47180	1,14
AT2G47490	1,15
AT3G01510	2,21
AT3G02230	1,02
AT3G02690	1,69
AT3G02910	1,14
AT3G03550	1,03
AT3G04120	2,63
AT3G04730	1,03
AT3G05200	1,37
AT3G05510	1,12
AT3G05810	1,21
AT3G06760	1,33
AT3G07350	1,19
AT3G08720	0,85
AT3G08890	2,01
AT3G09370	1,15
AT3G09390	2,19
AT3G09980	1,06
AT3G11410	1,48
AT3G11700	1,36
AT3G11770	0,97

AT3G11900	2,01
AT3G14180	1,82
AT3G14230	1,76
AT3G14690	1,93
AT3G14860	1,86
AT3G15290	2,21
AT3G15351	0,47
AT3G15540	1,95
AT3G15630	1,49
AT3G16060	1,51
AT3G16530	0,92
AT3G17020	1,58
AT3G17310	1,03
AT3G17365	0,98
AT3G17680	1,18
AT3G17790	1,35
AT3G18000	1,33
AT3G18710	1,26
AT3G19130	1,49
AT3G19500	1,6
AT3G19540	1,95
AT3G23820	1,22
AT3G23940	0,87
AT3G26580	2,32
AT3G27160	0,61
AT3G27170	1,92
AT3G27360	1,52
AT3G27570	0,99
AT3G27820	1,5
AT3G43720	1,18
AT3G44880	2,18
AT3G45030	1,34
AT3G46010	1,45
AT3G46540	1,18
AT3G47070	1,44
AT3G47470	2,05
AT3G48090	1,86
AT3G48690	1,07
AT3G49680	1,26
AT3G50060	1,42
AT3G50660	1,1
AT3G51260	1,25
AT3G51510	1,61
AT3G51750	0,83
AT3G51890	2,06
AT3G52070	0,93
AT3G53670	2,05
AT3G53800	1,5
AT3G54050	1,49
AT3G54390	2,16
AT3G54890	1,71
AT3G54960	1,41
AT3G55250	1,65
AT3G55800	1,21
AT3G56090	2,34
AT3G56710	1,09

AT3G56800	1,67
AT3G57400	2,06
AT3G57810	0,98
AT3G58200	1,38
AT3G58570	0,93
AT3G58840	1,38
AT3G58990	1,72
AT3G59050	1,16
AT3G59060	1,81
AT3G60110	1,15
AT3G60410	1,31
AT3G61460	1,65
AT3G61950	1,38
AT3G62260	1,03
AT3G62980	1,78
AT4G01026	2,48
AT4G01060	0,82
AT4G01120	1,29
AT4G01130	1,29
AT4G01610	1,07
AT4G01860	1,27
AT4G02120	0,97
AT4G02130	0,97
AT4G03210	1,17
AT4G03560	1,59
AT4G10340	1,84
AT4G11220	1,73
AT4G13180	2,82
AT4G13530	2,07
AT4G14420	1,21
AT4G14600	1,88
AT4G14900	1,43
AT4G16370	1,48
AT4G16390	0,59
AT4G16660	1,83
AT4G17420	1,61
AT4G18390	1,65
AT4G18700	1,66
AT4G18810	1,1
AT4G18920	1,72
AT4G21510	1,69
AT4G21960	1,01
AT4G22240	2,16
AT4G22820	1,39
AT4G23100	1,02
AT4G23650	1,7
AT4G23710	0,96
AT4G23750	0,99
AT4G23940	0,76
AT4G24000	0,91
AT4G24190	1,46
AT4G24350	1,33
AT4G24400	1,96
AT4G24730	1,95
AT4G24740	2,83
AT4G25260	1,14

AT4G25620	1,48
AT4G26740	2,15
AT4G27440	1,68
AT4G27720	1,75
AT4G28440	1,5
AT4G29330	1,47
AT4G31750	1,78
AT4G33560	0,94
AT4G33905	1,08
AT4G34120	1,34
AT4G34131	1,48
AT4G34135	1,1
AT4G34490	1,44
AT4G35000	0,98
AT4G35090	0,85
AT4G35140	1,48
AT4G35250	1,96
AT4G35920	2,05
AT4G36490	1,34
AT4G36780	1,95
AT4G36800	1,25
AT4G37460	0,97
AT4G38740	1,56
AT5G01420	1,99
AT5G01530	1,53
AT5G01990	1,03
AT5G02500	0,9
AT5G02960	1,13
AT5G03520	1,41
AT5G04590	1,9
AT5G05220	0,66
AT5G05250	1,08
AT5G07020	1,27
AT5G07340	1,49
AT5G07450	1,38
AT5G08139	1,18
AT5G09600	1,44
AT5G10730	1,76
AT5G11260	1,42
AT5G11730	1,12
AT5G12050	1,31
AT5G12250	1,15
AT5G13760	2,05
AT5G14620	0,97
AT5G15160	1,39
AT5G15850	1,65
AT5G15860	1,44
AT5G15950	1,98
AT5G15960	1,39
AT5G15970	1,53
AT5G16210	1,31
AT5G16710	1,43
AT5G16760	2,69
AT5G17300	0,76
AT5G17460	1,6
AT5G17510	1,94

AT5G19690	2,03
AT5G19850	1,51
AT5G19940	1,89
AT5G20030	1,51
AT5G23890	1,27
AT5G24430	1,38
AT5G24590	1,8
AT5G24850	1,68
AT5G24930	1,36
AT5G27420	0,98
AT5G27670	2
AT5G35180	1,23
AT5G41100	1,02
AT5G42100	1,58
AT5G43940	1,34
AT5G44710	1,18
AT5G44785	1,07
AT5G46690	0,79
AT5G47040	1,7
AT5G47120	1,87
AT5G47180	1,27
AT5G47390	1,16
AT5G47420	1,81
AT5G47590	0,94
AT5G47610	2,21
AT5G47670	1,47
AT5G47770	1,83
AT5G49525	1,17
AT5G51460	1,68
AT5G52030	1,15
AT5G52420	1,19
AT5G52550	1,93
AT5G53140	1,45
AT5G53540	1,09
AT5G54430	1,62
AT5G54510	1,03
AT5G54770	1,34
AT5G56100	1,68
AT5G57030	1,92
AT5G57100	1,35
AT5G57490	0,99
AT5G57580	0,97
AT5G58070	1,63
AT5G58380	2,34
AT5G59000	1,26
AT5G59320	0,8
AT5G59690	1,99
AT5G59960	1,23
AT5G60360	1,37
AT5G61060	1,24
AT5G61790	1,97
AT5G61810	1,21
AT5G62000	1,42
AT5G62130	1,77
AT5G63370	0,97
AT5G63380	1,94

AT5G63810	1,29
AT5G63860	0,86
AT5G64050	1,08
AT5G64400	1,26
AT5G64920	0,58
AT5G65920	1,47
AT5G66460	1,6
AT5G66580	1,31
AT5G66650	0,88
AT5G66780	1,68
AT5G67330	1,15
AT5G67370	1,25

Table S5. Overview of the LEC1 target genes identified by different methods.

The current list of putative LEC1 target genes contains 114 genes which were confirmed by at least two different methodical approaches. A rough functional classification, the ATG gene identifier, a short gene name and the fold change values obtained by macro-, microarray, ChIP/chip and qRT-PCR are given for each gene. Dashes indicate missing confirmation and dots mean 'not tested'. The activation of several genes that are genomically located in head-to-head position was tested in order to identify further candidate targets. Corresponding hh-pairs are marked alternately in grey and white and non-activated, negative genes are written in grey. Noc-not represented on the REGIA or SAP macroarray.

	Gene ID	Name	macroarray	microarray		ChIP/Chip	qRT-PCR			
			DEXABA	DEX	DEXABA	DEX	DEX 4h	DEXABA 4h	DEX 24h	DEXABA 24h
REGIA										
misc	At3g21270	DOF2	6,76	-	-	-	1,32	3,50	1,10	6,98
misc	At1g43160	RAP2.6	13,73	-	-	-	0,59	20,31	0,80	30,72
light	At3g17609	HYH	8,38	-	-	-	•	•	1,14	0,19
Overlap REGIA/CATMA										
flowering	At3g54990	SMZ	5,91	-	2,65	noc	1,05	5,75	0,81	8,06
misc	At3g16870	GATA17	-3,88	-	-1,89	noc	0,54	0,11	0,69	1,03
misc	At1g28370	ERF11	-11,33	-	-1,87	-	•	•	•	•
CATMA										
lipid	At3g54320	WRI1	noc	-	2,65	noc	-	-	17,78	55,87
lipid	At2g34590	trans-ketolase	noc	-	2,27	-	-	-	1,44	4,65
lipid	At5g46290	KAS1	noc	-	2,09	-	-	-	1,20	2,92
lipid	At2g05990	MOD1	noc	-	2,12	noc	-	-	1,54	5,37
lipid	At5g51210	OLEO3	noc	-	4,38	-	1,54	18,19	•	•
hormone BR	At5g08130	BIM1	noc	-	1,54	noc	•	•	1,16	2,21
hormone BR	At5g02820	RHL2	noc	-	1,67	-	•	•	1,49	1,73
hormone BR	At2g07050	CAS1	noc	-	2,16	-	•	•	3,00	6,97
hormone AUX	At1g31340	RUB1	noc	-	-1,56	noc	•	•	3,20	1,20
hormone AUX	At2g22670	IAA8	-	-	1,83	-	•	•	3,17	4,69
hormone AUX	At2g46690		noc	-	1,90	noc	•	•	2,87	12,04

hormone AUX	At3g10540	put sim PDK1	noc	-	1,61	noc	•	•	2,82	2,92
hormone AUX	At5g19140		noc	-	1,86	-	•	•	4,31	11,81
hormone AUX	At5g25890	IAA28	-	-	-1,66	-	•	•	2,84	7,96
hormone AUX	At3g06810	IBR3	noc	-	1,80	-	•	•	2,34	5,05
hormone AUX	At4g18010	IP5PII	noc	-	-1,99	-	•	•	4,00	3,98
flowering	At5g57390	AIL5	noc	-	2,63	noc	0,77	1,68	1,11	5,23
misc	At2g30040	MAPKKK14	noc	-	4,11	-	0,52	9,02	•	•
misc	At3g58990	aconitase	noc	-	-4,96	-	0,87	0,27	•	•

overlap CATMA/ChIP

hormone AUX	At3g62980	TIR1	noc	-	1,81	3,43	-	-	0,95	1,96
misc	At3g46540	ENTH domain protein	noc	-	-1,97	2,27	•	•	1,20	1,14
misc	At5g52550	unknown	noc	-	1,61	3,81	•	•	•	•
misc	At2g25900	CTH	noc	-	1,61	3,18	•	•	0,53	3,27
misc	At2g39270	unknown	noc	-	1,64	3,86	•	•	2,46	3,42
misc	At3g08890	unknown	noc	-	1,61	4,03	•	•	•	•
CDPK	At4g23650	CDPK6	noc	-	1,79	3,25	•	•	2,73	2,98
misc	At5g47770	FPS1	noc	-	1,70	3,56	•	•	•	•
SnRK3	At5g58380	SnRK3.8/CIPK10	noc	-	1,94	5,06	•	•	1,19	2,63
misc	At4g01026	unknown	noc	-	1,83	5,58	•	•	•	•
misc	At1g23750	DNA-binding	noc	-	2,04	2,27	•	•	4,17	3,32
misc	At1g19650	SEC14	noc	-	2,24	6,54	•	•	0,67	3,07
misc	At5g16760	I3P-kinase	noc	-	2,16	6,45	•	•	0,75	1,39
misc	At2g38820	unknown	noc	-	2,65	2,71	•	•	0,87	5,60
misc	At1g69800	CBS domain protein	noc	-	2,34	2,95	•	•	1,97	4,80
misc	At2g02160	zinc finger (CCCH)	noc	-	0,64	2,48	•	•	4,89	6,38
misc	At2g36530	LOS2	noc	-	-1,67	2,53	•	•	2,09	1,75
misc	At2g41310	ATRR3	noc	-	1,74	1,99	•	•	2,67	6,38
misc	At2g42530	COR15B	noc	-	3,21	2,08	•	•	•	•
misc	At3g62260	putative PP2C	noc	-	2,13	2,04	•	•	2,59	20,18

misc	At4g18390	TCP TF	-	-	1,68	3,14	•	•	1,98	2,33
misc	At4g35090	CAT2	noc	-	1,93	1,80	•	•	1,61	3,58
misc	At5g03520	AtRab8C, AtRABE1d	noc	-	1,68	2,66	•	•	3,69	3,42
misc	At5g07020	proline-rich protein	noc	-	2,00	2,41	•	•	1,47	3,42
misc	At5g17300	myb TF	-	-	2,12	1,69	•	•	19,88	206,26
misc	At5g47120	ATBI1	noc	-	1,58	3,66	•	•	2,28	5,95
	At5g47390	myb TF	noc	-	1,59	2,23	•	•	na	na
ChIP										
hormone AUX	At1g25490	RCN1	noc	-	-	2,71	•	•	3,32	4,76
hormone AUX	At1g71090	auxin efflux carrier	noc	-	-	3,66	•	•	1,85	3,11
hormone AUX	At2g41100	TCH3	noc	-	-	2,16	•	•	2,52	0,58
hormone AUX	At3g04730	IAA16	-	-	-	2,04	•	•	292,4	645,05
hormone AUX	At3g50060	MYB77	-	-	-	2,68	•	•	1,62	3,34
hormone AUX	At4g36800	RCE1	noc	-	-	2,38	•	•	3,29	5,33
hormone AUX	At5g01990	auxin efflux carrier	noc	-	-	2,04	•	•	3,12	14,63
hormone AUX	At5g54510	DFL1	noc	-	-	2,04	•	•	10,09	23,42
hormone AUX+BR	At1g15580	IAA5	noc	-	-	2,97	•	•	12,67	128,34
hormone AUX+BR	At3g15540	IAA19	-	-	-	3,86	•	•	5,69	8,40
hormone AUX+BR	At5g62000	ARF2	noc	-	-	2,68	•	•	3,53	5,12
hormone BR	At1g18400	BEE1	noc	-	-	2,04	•	•	4,89	0,88
hormone BR	At1g19350	BES1	noc	-	-	3,25	•	•	1,63	1,50
hormone BR	At1g69010	BIM2	noc	-	-	2,85	•	•	2,16	1,71
hormone BR	At2g36800	DOGT1	noc	-	-	3,84	•	•	1,96	9,18
hormone BR	At3g50660	DWF4	noc	-	-	2,14	•	•	4,36	4,24
hormone BR	At1g78700	BEH4	noc	-	-	3,51	•	•	4,03	2,44
hormone BR	At3g61460	BRH1	noc	-	-	3,14	•	•	5,07	2,73
hormone BR	At4g36780	BEH2	noc	-	-	3,86	•	•	3,35	4,08
misc	At5g16750	TOZ	noc	-	-	7,41	•	•	1,61	0,58
HAP3	At5g47670	L1L	-	-	-	2,77	•	•	3,34	4,06
misc	At2g38280	FAC1	noc	-	-	2,51	•	•	1,74	4,94

misc	At1g80080	TMM	noc	-	-	2,04	•	•	3,73	6,04
flowering	At2g28550	TOE1	-	-	-	1,72	•	•	2,43	1,84
HAP3	At1g07980	CBF/NF-Y	noc	-	-	1,65	•	•	3,73	4,92
misc	At4g24190	SHD	noc	1,80	-	2,75	•	•	5,05	14,71
hormone ethylene	At5g64050	ATERS/ERS/OVA3	noc	-	-	2,11	•	•	2,74	4,38
misc	At4g23100	RML1	noc	-	-	2,03	•	•	3,03	3,19
misc	At1g01470	LEA14	noc	-	-	2,27	•	•	4,29	6,76
misc	At1g13640	PI- 3- and 4-kinase	noc	-	-	3,94	•	•	3,46	1,82
misc	At2g39290	PGPS1	noc	-	-	1,62	•	•	2,49	2,02
misc	At4g36490	put SEC14	noc	-	-	2,53	•	•	5,22	0,61
misc	At1g42990	bZIP60	-	-	-	7,67	•	•	1,44	5,83
HAP3	At4g14540	HAP3	-	-	-	4,32	•	•	2,84	1,31

ChIP/chip hh-pairs

hh pairs	At1g23740	oxidoreductase	noc	-	-	4,17	•	•	4,19	2,46
hh pairs	At1g23750	DNA-binding	noc	-	2,04	2,27	•	•	4,17	3,32
hh pairs	At4g23940	FtsH protease	noc	-	-	-	•	•	3,68	2,31
hh pairs	At4g23930	proline-rich protein	noc	-	-	1,69	•	•	3,47	2,49
hh pairs	At2g25880	AUR2	noc	-	-	3,03	•	•	1,13	0,70
hh pairs	At2g25890	glycine-rich / oleosin	noc	-	-	-	•	•	11,96	1927,49
hh pairs	At1g74310		noc	-	-	1,87	•	•	2,06	5,48
hh pairs	At1g74320		noc	-	-	-	•	•	4,38	2,26
hh pairs	At3g15630		noc	-	-	-	•	•	5,89	3,71
hh pairs	At3g15640		noc	-	-	2,81	•	•	1,75	1,49
hh pairs	At4g01130		noc	-	2,63	-	•	•	110,6	18,80
hh pairs	At4g01120	GBF2	-	1,66	-	2,45	•	•	130,1	577,74
hh pairs	At3g02220		noc	-		2,13	•	•	1,52	1,27
hh pairs	At3g02230		noc	-	-2,38	2,03	•	•	1,03	1,11

hh pairs	At3g15280		noc	-	-	5,21	•	•	na	na
hh pairs	At3g15290		noc	-	1,80	4,63	•	•	1,68	3,31
hh pairs	At3g16050		noc	-	-	1,84	•	•	1,05	1,36
hh pairs	At3g16060	kinesin	noc	-	1,56	2,85	•	•	1,73	4,18
hh pairs	At4g24390		noc	-	-	1,49	•	•	1,34	6,09
hh pairs (SnRK3)	At4g24400	SnRK3.13/CIPK8	noc	-	1,66	3,89	•	•	1,64	5,45
hh pairs (BR)	At4g33430	SERK3/BAK1	noc	-	-	-	•	•	1,13	3,74
hh pairs	At4g33440		noc	-	-	2,45	•	•	0,87	6,99
hh pairs	At3g02580	STE1	noc	-	-	-	•	•	1,30	3,40
hh pairs	At3g02570	MEE31	noc	-	-	2,83	•	•	0,95	2,65
hh pairs	At3g51810	AtEM1	noc	-	-	-	•	•	1,72	20,11
hh pairs	At3g51800	ATG2	noc	-	-	2,30	•	•	1,12	1,59
hh pairs	At3g62610	MYB11	-	-	-	-	•	•	40,77	na
hh pairs	At3g62600		noc	-	-	2,31	•	•	6,80	22,74