Dissertation

Investigating the role of regulatory genes in heterosis for superior growth and biomass production in *Arabidopsis thaliana*

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‘Everything is simpler than you think
and at the same time more complex than you imagine.’

(Johann Wolfgang von Goethe)

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COMMONLY USED ABBREVIATIONS

ANOVA	– Analysis of variance
bp	– Base pair
cDNA	– Complementary DNA
chromDB	– The Chromatin Database
C_T	– Cycle threshold (number of cycles required for fluorescent signal to cross the set threshold)
DAS	– Days after sowing
DATF	– A Database of *Arabidopsis* Transcription Factors
DEPC	– Diethyl pyrocarbonate
dNTP	– Deoxyribonucleotriphosphate
DW	– Dry weight
EtBr	– Etidium bromide
F1	– First filial generation, produced by crossing two parental lines
FA(s)	– Fatty acid(s)
FDR	– False discovery rate
gDNA	– Genomic DNA
HAS	– Hours after sowing
IL	– Introgression line
MIR(s), miRNA(s)	– microRNA(s)
MPH	– Mid-parent heterosis
NCBI	– National Centre for Biotechnology Information
NOR	– Nucleolar organiser region
PCA	– Principal component analysis
Q-PCR/qPCR	– Quantitative polymerase chain reaction (real time PCR)
Q-RT-PCR/qRT-PCR	– Quantitative reverse transcription polymerase chain reaction (also called a real time RT-PCR)
QTL	– Quantitative trait locus
rRNA/rDNA	– Ribosomal RNA/ ribosomal DNA
ΔR_n	– Fluorescence signal
RNAi	– RNA interference
self	– An inbred line propagated by selfing (e.g. Col-0 self = Col-0xCol-0)
TAIR	– The *Arabidopsis* Information Resource
TC(s)	– Test-cross(es)
TF(s)	– Transcription factor(s)
T_m	– Melting temperature

1. INTRODUCTION

1.1. Heterosis

1.1.1. Definitions and heterotic traits

The word ‘heterosis’ is derived from the Greek word ‘heteroiōsis’ meaning ‘different in kind’. It is more than hundred years since the first examination of heterosis was published (Darwin, 1876) although it might have been observed by humans long before then. The definition of heterosis is one of the most difficult and controversial in genetic terminology (Tsaftarsis, 1995). Hoecker *et al*., (2008) defined heterosis as superior performance of heterozygous F1 hybrid plants compared to the average of their homozygous parental inbred lines after Shull (1952), and Falconer and Mackay (1996). The term ‘heterosis’ was used first by Shull in 1908 to describe the superiority of hybrids over their inbred parents in terms of size, vigour, and yield (East, 1908; Shull, 1908). Later, this definition was extended by evolutionary biologists to include heterosis for survival i.e. adaptive, selective, and reproductive advantage (Dobzhansky, 1950) or superiority of quantitative traits such as yield (Griffin, 1953), leaf area (Titok *et al.,* 1994), biomass (Liu *et al.,* 2002) or growth rate (Rao *et al.*, 1992). Heterosis can occur for all known characteristics of cultivars and can be observed in mature plants (Figure 1.1 a; Hochholdinger and Hoecker*,* 2007), embryos (Meyer *et al*., 2007) or seedlings (Figure 1.1 b; Hochholdinger and Hoecker*,* 2007).

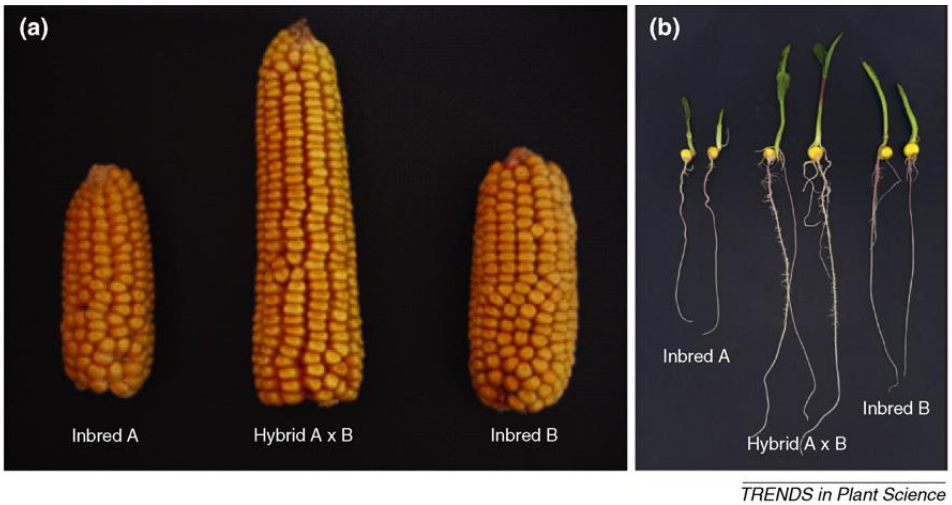


Figure 1.1. Phenotypic manifestation of heterosis in maize (Hochholdinger and Hoecker, 2007)

(a) Cob size and yield – examples of adult traits,
(b) Seedling development – example of ‘early’ traits.

Within a given hybrid the amount of heterosis can vary for different traits (Springer and Stupar, 2007) and its relative amount usually increases with the complexity of trait

(Becker, 1993). The degree of phenotypic difference of a trait in a hybrid compared to its parental inbred lines can be described as mid-parent heterosis (MPH) or best-parent heterosis (BPH). MPH exists when the average value of the trait for F1 hybrids is greater than the average value of the parents and is mostly of scientific interest. BPH exists when the trait level of the hybrids exceeds the best parent and is most important from an agronomical point of view. Maximum heterosis is observed in F1 hybrids and is lost in subsequent generations obtained through selfing (Meyer *et al.,* 2004) Heterosis was observed to be largest in allogamous (reproducing by cross-fertilization/cross-pollination) plants and smallest in autogamous (self-fertilising) species (Barth *et al.,* 2003).

1.1.2. Applications of heterosis

Heterosis or hybrid vigour has been utilised in plant and animal breeding programs for at least 90 years. Examples of plant hybrids used in agriculture include maize (corn), rice, sorghum, sunflower and alfalfa. Typically, hybrids grow faster and yield more biomass including seed. Application of heterosis in USA agricultural production is a multi-billion dollar undertaking, and nearly all field corn are hybrids (Swanson-Wagner *et al.,* 2006). By the end of the 21st century, 65% of maize production worldwide was hybrid-based with a similar proportion for other crops, such as sunflower or sorghum. Heterosis typically leads to increases in yield of up to 65% (Springer and Stupar, 2007) and 10-20% (Li *et al.,* 2008) in corn and rice, respectively, and it is therefore considered a major asset in meeting the world's food needs (Duvick, 1999). Many of traits of value to humans, such as plant disease resistance, tolerance to abiotic stress, and nutrient content may be subject to heterosis. Generally, it is believed that an understanding of the molecular basis of heterosis will enhance our ability to create superior new genotypes that may be used directly as F1 hybrids or form the basis for the future selection programmes (Tsaftarsis, 1995).

1.1.3. Hypotheses to explain heterosis

The genetic basis of heterosis has been discussed for nearly a century (Shull, 1908; Bruce, 1910; Jones, 1917). Multiple models have been proposed to explain heterosis (Birchler *et al.*, 2003). The most predominant quantitative genetic hypotheses to explain heterosis are 'dominance' (or complementation) and 'overdominance'. The dominance (Figure 1.2 a) hypothesis explains heterosis by the action of superior dominant alleles from both parents at multiple loci, which complement corresponding unfavourable alleles leading to improved vigour of hybrids. Such complementation may result in characteristics being equal to or better than the better of the two parents. Overdominance (Figure 1.2 b) postulates that diverse alleles

interact to create a superior function than that which could happen with homozygous alleles. It is a situation, in which heterozygosity for small regions of a genome produce a heterotic response. Other hypotheses to explain heterosis also exist like pseudo-overdominance (Figure 1.2 c), which is a genetic intermediate of dominance and overdominance. It can be considered as a simple case of dominance, in which the two recessive loci ('a' from P1 and 'b' from P2) are linked in repulsion (or '*in trans*' and there is a '*trans* hybrid'). This type of complementation in the hybrid resembles overdominance due to tight chromosomal linkage. Additionally, the multiple non-allelic genes can interact in ways that mask the action of each other in the process of epistasis (Powers, 1944). The relative contribution to heterosis of each of those mechanisms remains unclear (Birchler *et al.,* 2006; Lippman and Zamir, 2006; Hochholdinger and Hoecker, 2007).

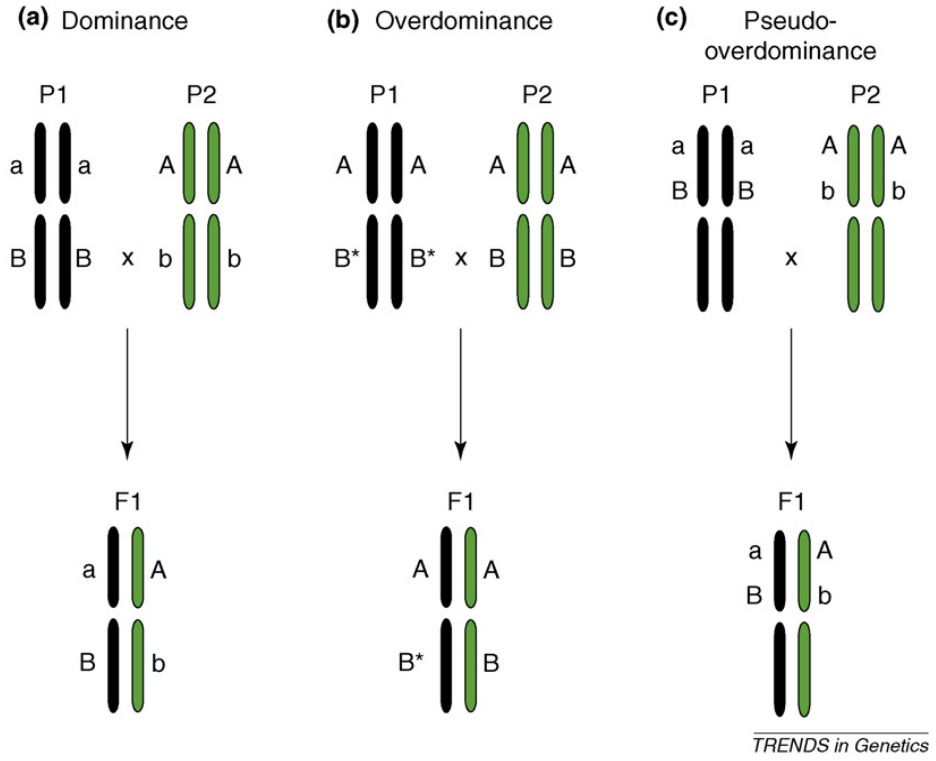


Figure 1.2. Scheme of main genetic models for heterosis (Lippman and Zamir, 2006)

There exist also molecular or physiological hypotheses to explain heterosis, including:

1. Heterosis may be a result of interactions of genetic and environmental stimuli (Jinks and Perkins, 1968; Parsons 1971; Griffing and Zsiros, 1971). Heterosis is 'multiplicative' or 'geometric' because it is based on complex interactions across development between various phenotypic components, each with their own inheritance, that are dynamically influenced by the environment (Williams, 1959; Griffing, 1990; Schnell and Cockerham, 1992; Lippman and Zamir, 2006).

2. Heterosis may be influenced by parental genetic distance.
   A positive correlation between genetic distance of parents and hybrid vigour has been reported (Melchinger, 1999; Barbosa *et al.,* 2003).
3. Heterosis may be dependent on epigenetic phenomena (Swanson-Wagner *et al.,* 2006).
4. Heterosis may be caused by differential accumulation of allele-specific transcripts in hybrids (Swanson-Wagner *et al.,* 2006).
5. In higher plants, at a functional level heterosis appears to be the result of a faster cell division rate rather than superior cell size or cell expansion (Srivastava, 1983). Ashby (1937) suggested that heterosis effects in tomato were associated with greater embryo weight and size due to larger cell number.

1.1.4. *Arabidopsis* as a model plant to study heterosis

Arabidopsis thaliana (common name: thale cress) was the first plant genome to be completely sequenced, in part because of its small genome size (Meinke *et al.,* 1998; AGI, 2002). There were collected over 750 natural ecotypes (accessions) of this plant from around the world (Passardi *et al.,* 2006). Among them Columbia (Col, and its different variations i.e. Col-0, Col-2), which was sequenced in the *Arabidopsis* Genome Initiative together with Cl-0, C24, Landsberg *erecta* (L*er*) and Niederzenz (Nd) are accepted as the standards for experimental analysis. Despite lack of agronomic importance, *Arabidopsis* has become the model system of choice for research on plant development, physiology, genetics, and biochemistry. Many tools, methods and technologies have been developed for *Arabidopsis* in these fields. Additionally, much data about *Arabidopsis* and analytical tools have been integrated in various international, publicly available, often interactive, databases such as The *Arabidopsis* Information Resource - TAIR (http://www.arabidopsis.org/index.jsp; Huala *et al.,* 2001). One of the original ideas behind using *Arabidopsis* as a model system was to facilitate the identification of related genes of importance in crop plants. *Arabidopsis* was proposed as a model species for investigating the molecular causes of heterosis by Somerville and Somerville, (1999) and The Multinational *Arabidopsis* Steering Committee (2002). Heterosis in *Arabidopsis* was reported for the traits of agronomic importance, including: rosette diameter (El Asmi, 1974 and 1975), stem length and biomass yield (Rédei, 1962; Griffing and Langridge, 1963; Li and Rédei, 1969; Corey and Matzinger, 1973; Corey *et al.,* 1976), seedling viability (Mitchell-Olds, 1995), seed number (Alonso-Blanco *et al.,* 1999) and phosphate acquisition efficiency (Narang and Altman, 2001). Additionally, heterosis between two divergent accessions of

Arabidopsis, Col-0 and C24 has been shown for biomass (Meyer *et al.,* 2004). In initial growth experiments under controlled, long day conditions, F1 reciprocal crosses between Col-0 and C24 displayed increased plant size and weight compared to parents. This was not due to differences in seed size, which was comparable in the hand-pollinated parent controls and the F1 hybrids. Detailed analysis revealed mid-parent biomass heterosis (MPH; the increase of a hybrid for a given character above the mean of its parents) of 40-60% for 10 DAS (days after sowing) seedlings grown at 120 µE. Differences in relative growth rate were only observed in the early phases of growth at the lower light intensities. Significant differences in seedling biomass were detected as early as 8 DAS (Figure 1.3).

1.1.5. Molecular approaches, tools and technologies to study heterosis

In the last few years a variety of functional genomics and applied genetics tools and methods were developed to improve our understanding of basic plant processes, including those, like heterosis, that are important for crop improvement. Genetic mapping tools, gene expression profiling, and gene knock-out technology belong to this tool kit. The essential challenge in molecular plant breeding is to identify the genes underlying the trait/phenotype/phenomenon in question and define their interactions. A gene that is identified as related to a particular trait is considered as a candidate gene for that trait. There are two categories of such candidates: positional and functional. The first are genes that are associated with the trait via QTL or map-based cloning approaches. The second are genes whose function has something in common biologically with the trait under investigation and are identified through transcriptomics, for example the function of candidate genes and the relationship to the trait of interest can be verified by functional genomics methods.

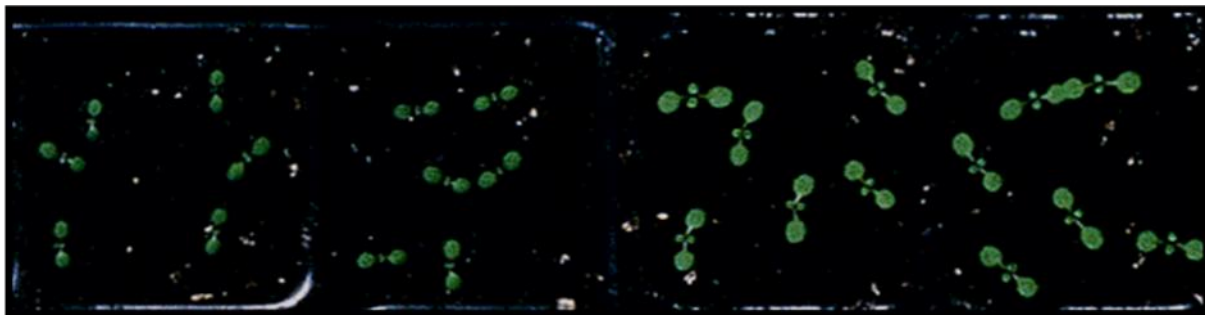


Figure 1.3. Differences in size between F1 hybrid and parental seedlings at 8 DAS (days after sowing) when grown in soil (Altmann T., Meyer R. – personal communication)

1.1.5.1. Mapping of quantitative trait loci (QTL mapping)

One of the most important tools to identify candidate genes (QTGs – quantitative trait genes), which might be involved in a certain quantitative trait/phenotype is QTL analysis or

QTL mapping (Doerge, 2002). A QTL or quantitative trait locus is a genomic region associated with a particular trait/phenotype that contains gene(s) affecting that trait. Complex phenotypic traits are usually determined by many genes (almost always interacting with environmental influences) generally located at multiple QTLs, which can be located on different chromosomes. To identify QTL, individuals in mapping population are first phenotyped for a trait(s) (i.e. each trait is measured or assayed) and genotyped at markers across the genome. Next, a genome-wide scan is performed, looking for statistical association between marker type and trait(s) values. QTL are identified as intervals across chromosomes, with high probability of association or linkage between markers and the trait of interest used in the mapping experiment. When mechanistically related traits map to similar map positions, this might suggest that variation for these traits at this locus is controlled by the same gene (El-Lithy *et al.,* 2004). QTL mapping has been broadly used in attempts to identify genes underlying heterotic loci (Xiao *et al.,* 1995; Li *et al.,* 2001; Luo *et al.,* 2001; Hua *et al.,* 2003). An achievement of gust work in crops was discrimination between classical genetic models for heterosis based on various modes of inheritance of gene expression i.e. dominance, overdominance, and epistatic interactions. However, further progress in identification of genes underlying heterosis has been hampered by the complexity of the genetic and environmental interactions that define the trait (Lippman and Zamir, 2006).

Different RIL (recombinant inbred line) populations have been developed for QTL mapping in plants. In *Arabidopsis*, several well characterised RIL populations now exist, including lines derived from Col-0/C24 crosses (Törjék *et al*., 2006). Such lines are generated by self-pollinating an F2 population for at least six generations to obtain lines that differ from each other but that are homozygous at 99% of loci. The variation among lines is immortalised and RIL lines can be propagated and multiplied. A set of probes that cover the genome and distinguish alleles from the two parents provide map positions for the genes controlling the traits of interest (Burr *et al.,* 1988; Maloof, 2003; Hake and Rocheford, 2004).

1.1.5.2. Introgression lines

QTL mapping with a segregating population provides only approximate positions of QTL (Kearsey, 2002), which is why a new set of lines to characterise and confirm the contributing individual loci is required (Koornneef *et al.,* 1997). Introgression lines (ILs) are of great use for this purpose. Such plant lines carry single homozygous, marker-defined chromosome segments introduced from a donor parent in the genetic background of a recurrent parent and result from successive backcrossing of initial F1 hybrids to the recurrent parent. Lines

of an introgression population have a common recurrent genotype, but different, short, donor segments from another line giving the ability to focus precisely on every desired region of the genome (Eshed and Zamir, 1995). Like RILs, ILs are homozygous and immortal because they can be maintained by self-pollination. The use of ILs is straightforward because of the fact that any phenotypic difference between an IL and the recurrent parent is attributed to the introgressed chromosomal segment. This eliminates most of the whole-genome epistatic interactions and the resulting need for complicated statistical analyses (Lippman and Zamir, 2006). ILs are highly effective for identifying QTL contributing to heterosis, particularly those showing overdominant effects (Semel *et al.,* 2006). However, a limitation is that epistatic interactions, which are important in heterosis, cannot be directly estimated (Li *et al.,* 2001; Luo *et al.,* 2001). IL lines were developed for different crop species and *Arabidopsis.* A new large set of reciprocal ILs covering the entire genome and a population of sub-ILs (smaller introgression regions) between the *Arabidopsis* accessions Col-0 and C24 were developed recently by Törjék and Meyer *et al.,* (2008).

1.1.5.3. Gene expression profiling

Gene expression profiling was one of the earliest technologies developed for functional genomics and allows measurements of transcript levels for thousands of genes from a single sample. Microarray technologies are based mostly on oligonucleotide chips (short oligos from 20-25 bases or longer from 50-70), which are used mainly for expression profiling studies of known sequences. Microarray data from various organisms and conditions is being integrated in different databases, which additionally provide a range of bioinformatic tools to interrogate the data. For example, GENEVESTIGATOR (Zimmermann *et al.,* 2004) helps to find a shared biological role (similar pathways and biosynthetic cycles) for multiple genes/proteins based on correlations of transcript levels. Much microarray research was focused on *Arabidopsis*. The Affymetrix ATH1 GeneChip 22K probe array contains approximately 22,750 probe sets for *Arabidopsis*. Certain genes like those encoded transcription factors, which are generally expressed at low levels in plants or in a cell-type or tissue-specific manner and only transiently during development, require more sensitive methods than microarrays to be detected. Q-RT-PCR is estimated to be at least 100-fold more sensitive than DNA arrays in detecting transcripts (Horak and Snyder, 2002). In 2004 Czechowski *et al.* showed that qRT-PCR provides extraordinary sensitivity, great dynamic range and high robustness (detection limit: 1 transcript molecule in 1000 cells), and has higher precision than the Affymetrix technology (ATH1 22K), especially for low-abundance transcripts.

1.1.5.4. RNA interference technology as a reverse-genetics approach

Reverse genetics is the process of determining the function of selected target genes by inferring with gene expression using mutants or other approaches. RNA interference (RNAi) technology is one such approach, which activates a naturally occurring post-transcriptional gene silencing (PTGS) mechanism that degrades target RNA via a double stranded RNA 'trigger' (Tijsterman *et al.,* 2002). In RNAi technology, dsRNA can be delivered e.g. by stably transforming plants with transgenes that encode hpRNA (hairpin RNA) (Helliwell and Waterhouse, 2003). One of the high-throughput applications of this technology was the AGRIKOLA project, which used recombinational cloning of gene-specific tags (GSTs) into the RNAi expression vector, pAGRIKOLA, to generate more than 20,000 plasmids. Each of these can be used to reduce or eliminate expression of a single *Arabidopsis* gene by PTGS (Hilson *et al.*, 2004).

1.1.6. Summary

Despite a hundred years or more of exploiting heterosis in breeding and agricultural programs, and the diversity of hypotheses about heterosis, and a variety of research initiatives aimed explaining the genetic/molecular basis of heterosis, the phenomenon remained a mystery at the beginning of this project.

1.2. Genes involved in this study on heterosis

This PhD project focused on an investigation of the role of transcription factors, which play a role in orchestrating gene expression, microRNAs involved in gene regulation, selected genes encoding proteins involved in the epigenetic control of gene expression and/or chromatin modification processes (called 'chromatin-related' in this work) and a group of genes with potential roles in growth because of our expectation that they might play key roles in heterosis.

1.2.1. Review of transcription factors (TFs) in *Arabidopsis*

Transcription factors are master control proteins that regulate gene expression levels by binding to specific DNA sequences in the promoters of target genes, thereby enhancing or repressing their transcriptional rates. There are two types of TFs, so called general, and regulatory or specific. General TFs are a small set of proteins required for the initiation of transcription, e.g. the TATA-box binding protein. Together with RNA polymerase they form the basal transcription complex. Specific TFs contain one or more DNA binding domains that attach to specific sequences of DNA adjacent to the genes that they regulate. These TFs bind proximal or distal (up- or downstream) of the basal transcription complex and act either as

constitutive or inducible factors. These proteins influence initiation of transcription by contacting members of the basal complex. They may also interact with chromatin remodelling proteins and other transcription factors. There are also TF hierarchies where relatively few 'master' transcription factors control expression of other TF genes (Ratcliffe and Riechmann, 2000; Riechmann *et al.,* 2002; Riaño-Pachón *et al.,* 2007). TFs contain several functional domains: (1) an activation domain that interacts with other parts of the transcription machinery (RNA polymerase or other TFs); (2) a DNA binding domain, which recognises specific bases near the start of transcription; (3) a nuclear localisation domain that targets the protein to the nucleus after being synthesised in the cytoplasm; and sometimes (4) a dimerisation domain, which enables formation of functional dimmers from inactive monomers. Regulatory TFs are far more numerous than general TFs and account for approx. 2000 proteins in *Arabidopsi*s.

The Database of *Arabidopsis* Transcription Factors (DATF at http://datf.cbi.pku.edu.cn/; Guo *et al.*, 2005) was the main point of reference on TFs for this thesis. It collects 1922 loci for transcription factors (~7% of all *Arabidopsis* genes) and classifies them into 64 families. Although regulatory type transcription factors can be classified according to mechanism of action or their regulatory function (Brivanlou and Darnell, 2002), family classification is based on their structure and mainly on their DNA binding domains (Luscombe *et al.,* 2000). Figure 1.4 shows transcription factor classification and connections between families.

The five largest transcription factors families present in *Arabidopsis* are the MADS (MADS-box), AP2-EREBP (APETALA2/Ethylene Responsive Element Binding Protein), MYB, bHLH (Basic Helix-Loop-Helix), and C2H2 families. The largest transcription factor families in *Arabidopsis* also appear to be the most prevalent ones in monocotyledonous plants such as rice or maize. TFs that belong to the same family often regulate similar physiological processes even among very different plant species. Overall regulation of most biological processes in the plant cell can be linked to one or more TF families (Century *et al.,* 2008). At least 45% of all TFs are plant-specific. Among the families found only in plants are: AP2-EREBP, NAC, WRKY, Trihelix, ARFs (Auxin Response Factors), the Aux-IAA (Auxin/Indole-3-Acetic Acid) proteins, which do not bind to DNA themselves but interact with the ARF proteins, and other small families (Ratcliffe and Riechmann, 2000).

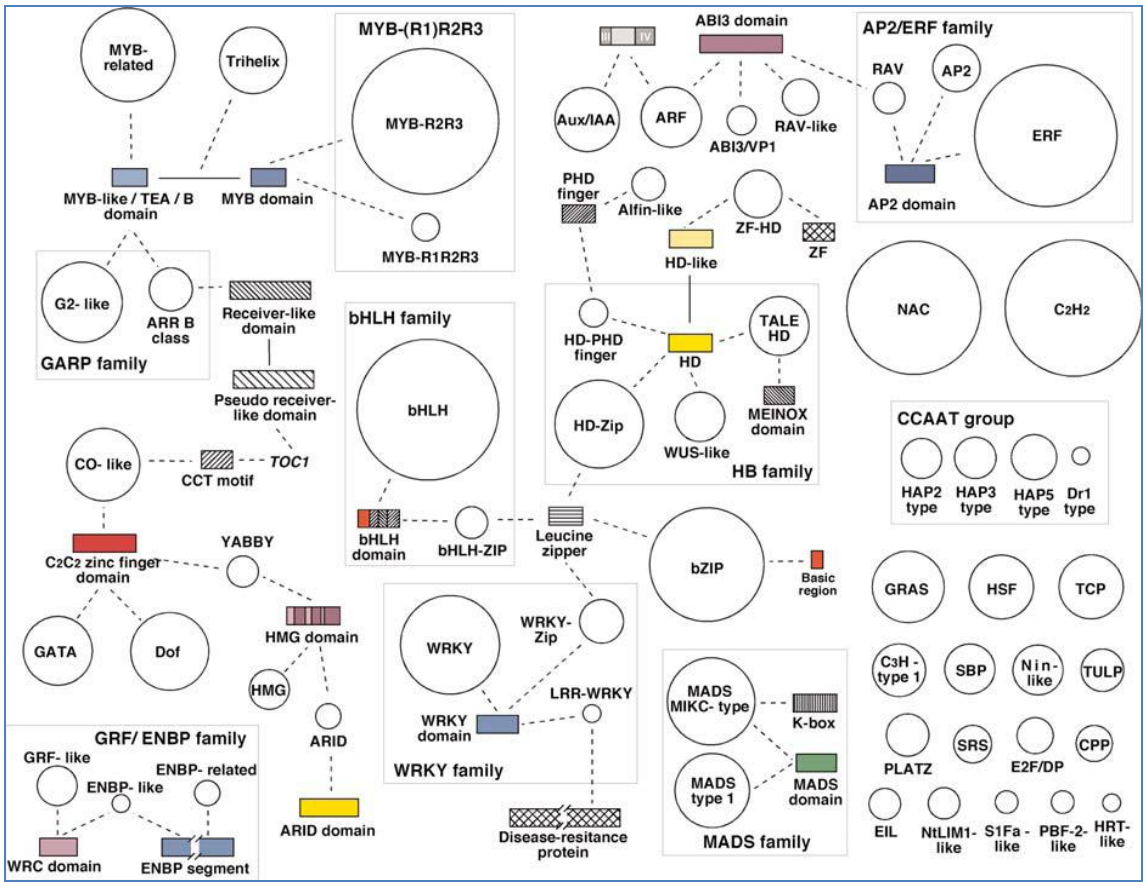


Figure 1.4. The *Arabidopsis* complement of transcription factors (Riechmann, 2002)

> Gene families are represented by circles, whose size is proportional to the number of members in the family. Domains that have been shuffled, and that therefore 'connect' different groups of transcription factors are indicated with rectangles, whose size is proportional to the length of the domain. DNA binding domains are coloured; other domains (usually protein-protein interaction domains) are shown with hatched patterns. Dashed lines indicate that a given domain is a characteristic of the family or subfamily to which it is connected. Gene names are written in italics (Riechmann, 2002).

TFs play fundamental roles in the life cycle of higher plants controlling or influencing almost all biological processes, including cell cycle progression, metabolism, growth and development, and responses to the environment (Riechmann and Ratcliffe, 2000). It is assumed that they have immensely important functions in the evolution of species. Development is based on the cellular capacity for differential gene expression, which is often controlled by TFs acting as switches of regulatory cascades. TF genes are generally expressed at low levels in plants, often in a cell-type or tissue-specific manner, and often only transiently during development. Many, if not most TF genes are themselves regulated at the level of transcription, so determining where and when TFs are transcribed, and how such transcription is affected by internal or external stimuli is valuable in elucidating the specific roles of cognate proteins. Since the identification of the first TF, *TF1*, from a bacteriophage SPO1 in the 1970s (Wilhelm *et al.,* 1972), knowledge about these proteins has increased rapidly resulting in

thousands of reports. About 10% of *Arabidopsis* TFs have been characterised functionally (Qu and Zhu, 2006). The importance of TFs is underlined by an increasing number of databases focused on this class of genes, for example: AGRIS with AtTFDB and AtcisDB (http://arabidopsis.med.ohio-state.edu; Davuluri *et al.,* 2003; Palaniswamy *et al.,* 2006), TrSDB (http://bioinf.uab.es/trsdb; Hermoso *et al.,* 2004), Athena (http://www.bioinformatics2.wsu.edu/cgi-bin/Athena/cgi/home.pl; O'Connor *et al.,* 2005), DATF (http://datf.cbi.pku.edu.cn/; Guo *et al.,* 2005), RARTF (http://rarge.gsc.riken.jp/rartf/; Iida *et al.,* 2005), TRANSFAC (http://www.gene-regulation.com/pub/databases.html; Matys *et al.,* 2005) AthaMap (http://www.athamap.de/index.php; Steffens *et al.,* 2004 and 2005; Bülow *et al.,* 2006; Galuschka *et al.,* 2007), PlnTFDB (http://plntfdb.bio.uni-potsdam.de/v2.0/; Riaño-Pachón *et al.,* 2007), PlantTFDB (http://planttfdb.cbi.pku.edu.cn/; Guo *et al.,* 2008). This allows exchange of knowledge and access to updated information and acceleration of worldwide TF research. Numerous reports have shown that change in activity of a single TF can have a profound effect on plant biology, causing phenotypic changes. Identification and functional characterisation of TFs is essential for the reconstruction or modelling of transcriptional regulatory networks. Diversity of transcription factors and the *cis*- acting elements that they bind are the source for an enormous combinatorial complexity, which allows fine-tuning of gene expression and gives rise to a huge spectrum of developmental and physiological phenotypes (Riaño-Pachón *et al.,* 2007). Identification of regulatory genes and networks that control agronomically important traits like biomass, growth rate, yield, and stress resistance may allow the modification of complex traits to improve crop plants. TFs have already played major roles in crop improvement via domestication and breeding, generally by way of increasing intrinsic yield through modification of plant architecture (Doebley *et al.,* 2006; Kovach *et al.,* 2007; Pourkheirandish and Komatsuda, 2007). TFs have also been identified in QTL analysis of some traits of agricultural importance in rice (Konishi *et al.,* 2006). Jiang (2004) showed that HERCULES1 (*HRC1*), an AT-hook family TF, increases plant organ size and yield when overexpressed in *Arabidopsis*, with associated increases in cell size and number. Oh *et al.,* (2005) reported enhanced drought tolerance in rice plants that constitutively overexpressed either *CBF3* or *ABF3* (*Arabidopsis* TF of bZIP family), with no obvious negative side effects. There is also some evidence that altering expression patterns of the E2F TF genes from *Arabidopsis* can benefit cell division and cell size, potentially increasing biomass and yield (Beemster *et al.,* 2005; Van Camp, 2005). In parallel with the work presented in this thesis, the latest studies on heterosis identified TFs among differentially expressed genes in hybrids (Wu *et al.,* 2003; Swanson-Wagner *et al.,* 2006; Meyer *et al.,* 2007).

Taking this wide background into account, an obvious question related to hybrid vigour is what is the role of TFs in heterosis? This is one of the questions addressed in this thesis.

1.2.1.1. A qRT-PCR platform for TFs

The Udvardi & Scheible groups at the MPI-MP designed and tested around 2000 pairs of gene-specific primers for qRT-PCR of all TF and putative TF genes of *Arabidopsis*. A subset of these (1465 primer pairs) was used to demonstrate the high sensitivity and specificity of qRT-PCR, and to identify root- and shoot-specific TF genes (Czechowski *et al.*, 2004). This was a key enabling technology for the project proposed here and can provide valuable information about transcription factors in a rapid, systematic, and comprehensive manner.

1.2.2. Epigenetic control of gene expression

Epigenetic phenomena are heritable changes in gene expression that occur without a change in DNA sequence, and gene expression level can be regulated by epigenetic modification via covalent modification of DNA or histones (Habu *et al.,* 2001). Chromatin structure is an important element of the mechanisms that determine gene expression patterns in eukaryotes, because nucleosome assembly eliminates the accessibility of promoter sequences to the basal transcription machinery. Gene expression requires unfolding of packed chromatin and, conversely, repression requires the formation and maintenance of condensed chromatin structures (Riechmann, 2002). The Chromatin Database (Chrom.DB at http://www.chromdb.org; Gendler *et al.,* 2008) contains lists of chromatin proteins in plants and classifies them into nine groups. DNA methyltransferases (METs, CMTs, DRMs) are enzymes that methylate DNA in various patterns, Methylcytosine Binding Domain Proteins (MBDs) are thought to bind to methylated DNA to mediate other chromatin modifying events, Histone Acetyltransferases (HACs) are enzymes that add acetyl groups to histones, Histone Deacetylases (HDAs) are enzymes that remove acetyl groups from histones, Chromatin Remodelling Activities (CHR, CHB, CHC etc.) are large multi-protein complexes that use energy derived from the hydrolysis of ATP to alter the positioning of nucleosomes on DNA, SET Domain Containing Proteins (SDGs) are proteins that methylate histones, Chromodomain Containing Proteins are histone-binding repressor proteins, Bromodomain Containing Proteins are proteins that bind acetylated lysines, and High Mobility Group (HMG) Proteins are abundant non-histone chromosomal proteins that bind and bend DNA, serving ‘architectural’ roles. Because of their role in transcription regulation, genes encoding these proteins (called ‘chromatin-related’ in this work) are interesting targets for heterosis research.

1.2.3. RNA silencing in plants

RNA silencing, which in plants is has also been called post-transcriptional gene silencing (PTGS), was mentioned above in the context of RNAi technology. RNA silencing refers to diverse RNA-based processes that all result in sequence-specific inhibition of gene expression, either at the transcriptional, mRNA-stability, or translational levels. A couple of different RNA silencing pathways have been characterised (for review see Brodersen and Voinnet, 2006). Nonetheless, these processes are still not fully discovered.

1.2.3.1. Chromatin-targeted RNA silencing

Chromatin-targeted RNA silencing is a gene silencing pathway in which chromatin structure and histone modifications play a role. Typically, short interfering RNA (a type of small RNA) guides the formation of heterochromatin, which is transcriptionally silent. Some proteins involved in this process belong to the putative chromatin proteins compiled by ChromDB. The two schemes of this silencing are shown in Figure 1.5.

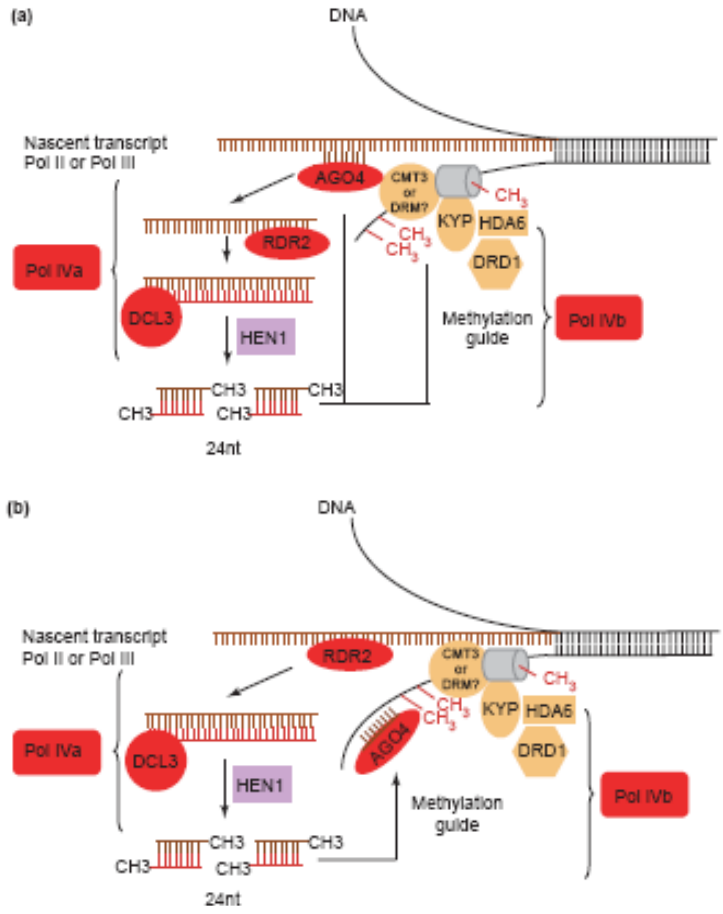


Figure 1.5. Chromatin-targeted RNA silencing schemes (Brodersen and Voinnet, 2006)

(a) A nascent Pol II or Pol III transcript is cleaved through the action of siRNA-programmed *AGO4*, resulting in a truncated RNA that is converted into dsRNA by the action of *RDR2*. The dsRNA is then processed by *DCL3* into 24-nt siRNAs that direct further cleavage of nascent transcripts and might possibly guide sequential activities of histone deacetylases (e.g. *HDA6*),

histone methyl-transferases (e.g. *KYP* or *SUVH2*) and/or DNA methyl-transferases (*CMT3* or a *DRM*). It is unclear whether histone modification precedes DNA methylation or not. The process might also involve siRNA-directed chromatin remodelling factors, such as *DRD1*. The positions of Pol IVa and Pol IVb in those reactions are currently ill defined.

(b) The same effectors are involved but, in this scenario, *RDR2* uses nascent transcripts as templates and siRNA-loaded *AGO4* is recruited to guide chromatin modifications rather than RNA cleavage.

1.2.3.2. MicroRNAs

Plant microRNAs (miRNAs) are another type of small RNAs, 70% of which were predicted to have TFs as targets (Steimer *et al.,* 2004). They are single-stranded 20-24 nt molecules, excised from endogenous non-coding transcripts with extensive fold-back structure. They cause gene silencing by acting *in trans* on cellular target transcripts to induce their degradation via cleavage, or by attenuating translation and protein production (Brodersen Voinnet, 2006). Most identified plant miRNAs have near-perfect complementarity to their targets. Approximately 100 miRNA genes have been identified in *Arabidopsis* and classified into 22 families (Xie *et al.,* 2005). MicroRNAs have important roles in plant development: they control key regulatory elements of plant response to auxin, take part in P-regulation (Bari *et al.,* 2006; Pant *et al.,* 2008), regulate accumulation of TFs involved in defining the identity or number of floral organs, leaf shape, and lateral root formation, and they are involved in primary and secondary metabolism. It is also predicted that miRNAs play roles in environmental adaptation (Brodersen Voinnet, 2006).

Insight into the importance of miRNA in gene regulation, plant physiology and development has increased rapidly (Hunter and Poethig, 2003; Dugas and Bartel, 2004; Steimer *et al.,* 2004; Kidner and Martienssen, 2005). This background makes miRNA an interesting target in study heterosis in plants. In 2007, the research group of Wolf-Ruediger Scheible at the MPI-MP designed and tested primer pairs for all 118 known miRNA genes of *Arabidopsis* for qRT-PCR (Datt Pant and Musialak-Lange *et al.,* 2009). This platform was used in this work.

1.2.4. Ribosomal RNA/DNA in relation to increased growth rate

There is substantial evidence that the rate in ribosome synthesis in meristem has a strong impact on growth (Kojima *et al.,* 2007). Elser *et al.,* (2000) shown that the growth rate of organisms is correlated with cellular ribosomal RNA (rRNA) content, with higher levels enabling faster protein synthesis and growth. Genetic mechanisms that may account for increased cellular rRNA levels include changes in rDNA structure/organisation, e.g. expansion of rDNA amount, and an increase in the transcription rate per gene of rDNA (Elser *et al.*, 2000). Endoreduplication may be one of possible ways that plant cells achieve this (Rogers and Bendich, 1987). Additionally, Kondorosi *et al.*, (2000) showed that increased DNA con-

tent or ploidy level correlates with increase in cell size, which may contribute to elevated hybrid growth. The bigger nuclei of polyploid cells meet the requirements of a higher metabolic activity, rRNA synthesis and transcriptional activity in larger cells (Weiss *et al.,* 2005). Another mechanism that may influence cellular rRNA levels is epigenetic regulation of transcription via covalent modification of DNA or histones (Habu *et al.*, 2001; Meyer, 2001). Results from genetic mapping indicated a biomass QTL (Lisec *et al.,* 2008) located in the top region of chromosome IV, which contains one of the two nucleolar organiser regions (NORs) with rDNA/rRNA genes (Figure 1.6). The nucleolus, which is created around those genes, is a key cellular structure that coordinates the synthesis and assembly of ribosomal subunits, plays a role in cell cycle regulation, and its function is tightly linked to cell growth and proliferation (Andersen, 2005). Total nucleolar size is an indicator of rRNA gene activity as shown in the study of Delany *et al.,* (1994). Thus, rRNA genes are an interesting target for research on heterosis for enhanced seedling growth rates in *Arabidopsis*.

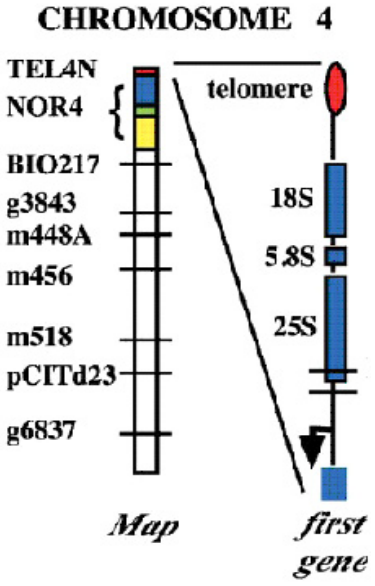


Figure 1.6. Organisation of the NORs at the top of *A. thaliana* chromosomes II and IV (Copenhaver and Pikaard, 1996b)

NOR2 and *NOR4* are each ~ 4 Mbp in size, including ~350-400 rRNA genes at each locus

1.2.5. Role of *FRIGIDA* and *FLOWERING LOCUS C*

FRIGIDA (*FRI*, AT4G00650) and *FLOWERING LOCUS C* (*FLC*, AT5G1014) are known to control flowering time variation in *Arabidopsis thaliana*. *FLC* is a MADS-box transcription factor that blocks the transition from vegetative to reproductive development (He *et al.,* 2003). *FRI* is a gene of unknown biochemical function (Veley and Michaels, 2008) and the FRI protein is predicted to contain coiled-coil domains in two positions (Johanson *et al.,* 2000). *FRI* (AT4G00650) acts epistatically to *FLC* causing its up-regulation (Shindo *et al.,*

2005; Michaels and Amasino, 1999). Up-regulation of *FLC* by *FRI* differs depending on the activity of both genes and is different for various genotypes. *FRI* was found to be functional in C24 but not in Col-0, whereas *FLC* is strong in Col-0 but weak in C24 (Gazzani *et al.,* 2003).

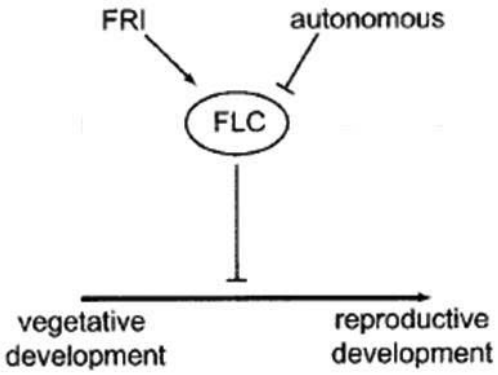


Figure 1.7. Scheme of *FRI-FLC* interactions (modified from Poduska *et al.*, 2003)

Studying *FRI* in relation to biomass heterosis seemed to be interesting since it is located in the biomass QTL 'hot-spot' at the top of chromosome IV (Lisec *et al.,* 2008). Moreover, Caroline Dean and co-workers have demonstrated the up-regulation of *FLC* expression by *FRI* in the shoot and root meristematic regions of L*er* seedling (data publicly available at the Caroline Dean's website www.jic.bbsrc.ac.uk/staff/caroline-dean/index.htm, 2007), which could contribute to biomass vigour of hybrid seedlings. Finally, Korves *et al.,* (2007) showed via association studies that the *FRI FLC* genotype (functional *FRI* in various *FLC* backgrounds) is associated with rosette growth. Boss *et al.,* (2004) emphasised that *FLC* integrates signals from the autonomous pathway (Figure 1.7), which stimulates flowering partially based on plant size. Although the *FRI* was not discussed in literature in the context of rosette growth, *FRI FLC* genotypes may differ in interactions with the autonomous pathway so the effects of *FLC* variation on rosette growth might be worth studying (Korves *et al.,* 2007). Furthermore, they also suggested that the specific *FRI FLC* genotype may be associated with very high water use efficiency, which may have contributed to slower plant growth in the studied conditions. All these considerations seemed to be a good basis for *FRI FLC* studies in the context of biomass heterosis.

1.3. Objectives of the study

I. The major goal of this PhD study was to provide an input into studies on molecular mechanisms underlying heterosis via determination of the role that regulatory (i.e. TFs and microRNAs), and additional genes of interest (i.e. 'chromatin-related' genes, *FRIGIDA*, and ribosomal genes) play in heterosis for biomass/growth in *Arabidopsis thaliana*.

II. The specific goals were:
    1. To determine the time in development at which the F1 hybrids and parents diverge phenotypically,
    2. To identify differentially expressed regulatory genes and additional genes of interest in F1 hybrids when compared to parents, before the point of divergence determined in point 1 (selection of candidate genes),
    3. Determine expression patterns (expression phenotypes) of selected candidate genes,
    4. To profile the expression of candidate genes at different time points of early development and compare their expression patterns,
    5. To determine, whether any of the candidate genes identified in point 2 map to the same chromosomal location as QTLs for growth and/or biomass heterosis, and for biomass *per se* (determined by others, Melchinger and Altmann),
    6. To validate the expression of a subset of candidate genes (from point 2) in crosses of other *Arabidopsis* accessions that exhibit biomass heterosis,
    7. To modify the expression of selected candidate gene(s) (from point 2) or additional genes of interest, using RNAi constructs in transgenic Col-0 or C24, to determine whether individual gene(s) contribute significantly to heterosis of biomass or growth,
    8. To analyse introgression lines (ILs) with an introgression containing selected candidate gene(s) or additional genes of interest in relation to biomass and biomass heterosis,
    9. To evaluate the possible role of rRNA/rDNA in growth/biomass heterosis using additional approaches.

2. MATERIALS AND METHODS

2.1. Plant material

- *Arabidopsis* accessions:
  - *Arabidopsis thaliana* (L.) ecotype Col-0
  - *Arabidopsis thaliana* (L.) ecotype C24
  - *Arabidopsis thaliana* (L.) ecotype Cl-0
  - *Arabidopsis thaliana* (L.) ecotype L*er*
  - *Arabidopsis thaliana* (L.) ecotype Nd
- Parental inbred lines:

  Col-0xCol-0, C24xC24, Cl-0xCl-0, L*er*xL*er*, NdxNd, N88/2/1/10 from generation BC5F3
- Reciprocal F1 hybrids:

  Col-0xC24, C24xCol-0, NdxCl-0, Cl-0xNd, L*er*xC24, C24xL*er*
- Test crosses (TCs) of introgression line (IL):

  Col-0xN88/2/1/10 and C24xN88/2/1/10

2.1.1. Plant growth conditions

- Early stages of development - until 10 DAS (days after sowing):

Plantlets were grown in Petri dishes and fine soil mixture (content as given below) was covered with nylon net. Plates were divided into four equal parts so that parental and hybrid seeds could be sown on the same plate. Up to 5 mg of seeds per genotype were sown on one plate to avoid tight growing.

- 15 DAS:

Plants were grown in a Latin square plot design with six replicates. Six plants of the same line were grown per well in 96-well-trays or per separate small pot.

Plants were grown in a 1:1 mixture of GS 90 soil and vermiculite. All seeds were germinated in a growth chamber at 4°C for two days and were then transferred to a long-day regime (16 h fluorescent light 120 mol/(m^2s) at 20°C and 60% relative humidity/8 h dark at 18°C and 75% relative humidity). To avoid position effects, trays/plates were rotated around the growth chamber every two days.

2.1.2. Technique of crossing

All the crosses were made according to Meyer *et al.,* (2004) to obtain seeds of all genotypes identical in size and mass: parental lines were inbred lines obtained by self pollina-

tion; five to six flowers per plant were left. Reciprocal hybrids and test crosses of ILs were produced by hand-pollinating emasculated flowers of the respective mother plant.

2.1.3. Methods of biomass difference determination

Prior to each experiment, hybrids of all crossing batches were tested to confirm biomass heterosis. Plants were grown until 15 DAS and then rosettes were harvested, and kept to dry in an oven at 80°C for 4 days for DW (dry weight) determination. Subsequent weighing was performed on a very sensitive balance (AX 205-balance, Metter Toledo) and statistical determination of DW differences via Student's t-test was performed in Excel (MS Office). Mid-Parent Heterosis (MPH) was used as a measure of a biomass increase level in hybrids vs. parents, and it was calculated according to the following formula:

MPH (%) = (mean DW of F1s – mean DW of Ps) / (mean DW of Ps)*100,

F1s - reciprocal F1 hybrids, Ps – parental inbred lines.

Only F1 hybrids of biomass levels that were significantly higher than those of parental plants i.e. showing at least 40% MPH were used for experiments.

2.2. Commonly used equipment and various consumables

2.2.1. Equipment

- Agilent Technologies, Santa Clara, CA USA: Agilent 6890 GC gas chromatograph with 7683 Autosampler, Agilent GC 6890N coupled with mass spectrometer,
- Applied Biosystems (& Perkin Elmer), Foster City, USA: ABI Prism 2X ABI Prism 7900HT Sequence Detection System and 7300 real-time PCR,
- Beckman Instruments Inc., Fullerton, USA: Avanti J30I centrifuge,
- Biometra, Göttingen, Germany: TGradient Thermal Cycler,
- Bio-Rad Laboratories, Hercules, USA: electrophoresis chambers, Power Pac 300,
- Becton-Dikinson, San Jose, USA: FACStar^{PLUS} flow cytometer,
- Dumont, Montignez, Switzerland: sharpened and unsharpened microscopic tweezers of different sizes,
- Eppendorf, Hamburg, Germany: pipettes, microcentrifuges: 5417, 5417C, 5417R, thermomixer, centrifuge tubes, PCR tips with filters,
- Gilson, France: pipettes,
- Hamamatsu Photonics, Herrsching Ammersee, Germany: ultra sensitive CCD camera,
- Leco, St. Joseph, MI, USA: Leco Pegasus III TOF MS mass spectrometer,

- Leica, Heidelberg, Germany: binoculars, stereomicroscopes supplied with cameras, microtome,
- Metter Toledo, Singapore, China: AX 205-balance,
- NanoDrop, Wilmington, USA: NanoDrop™ ND-1000 spectrophotometer,
- Retsch, Haan, Germany: MM200 homogeniser,
- Sorvall, Langenselbold, Germany: centrifuge RC5B Plus,
- Stratagene, Heidelberg, Germany: UV- crosslinker.

2.2.2. Consumables

- Applied Biosystems (& Perkin Elmer), Foster City, USA: 384-well Clear Optical Reaction Plates with Barcode PCR compatible DNA/RNA/RNase free with optical adhesive foil covers,
- Biozym Diagnostik, Hess. Olendorf, Germany: agarose,
- Eppendorf, Hamburg, Germany: centrifuge tubes, special PCR tips with filters,
- Eurogentec, Seraing, Belgium: 96 well PCR Plates with caps, oligonucleotides,
- Fermentas, St. Leon-Rot, Germany: O'RangeRuler DNA Ladder™ – various sizes of DNA ladders, 6X Orange DNA Loading Dye™,
- Invitrogen, Karlsruhe, Germany: 10 mM dNTPs Mix, 0.24 - 9.4 kb RNA-ladder,
- Merck, Darmstadt, Germany: other chemicals,
- Invitrogen/Molecular Probes, Karlsruhe, Germany: DAPI (4‘-6-diamidino-2-phenylindole) stain,
- Qiagen, Hilden, Germany: Oligo $(dT)_{16}$ primer™,
- Pharmacia, Freiburg, Germany: EtBr, other chemicals,
- Roth, Karlsruhe, Germany: phenol/chloroform/isoamyl alcohol 25:24:1 for DNA/RNA isolation, pH ~8.00, and other chemicals,
- Sefar, Heiden, Schwizerland: nylon net,
- Sigma-Aldrich, Taufkirchen, Germany: highly positively charged Nytran® SuPerCharge Nylon Membrane, diethylpirocarbonate (DEPC), basic chemicals,
- Shott Scientific Glass, Parkersburg, USA: glass tubes with caps and teflon discs, GC tubes.

2.2.2.1. Enzymes and kits

- Ambion, Huntingdon, Cambridgeshire, UK: TURBO™ DNase,

- Applied Biosystems (& Perkin Elmer), Foster City, USA: 2X SYBR Green® PCR Master Mix reagent and Power SYBR Green® PCR Master Mix reagent,
- Machery-Nagel, Düren, Germany: Nucleobond AX plasmid purification kit™,
- Invitrogen, Karlsruhe, Germany: Superscript III H⁻ Reverse Transcriptase™ (supplied with components: 5X First-Strand Buffer and 0.1 M DTT), Random Primers,
- Promega, Mannheim, Germany: RNasin® Ribonuclease Inhibitor™,
- Roboklon, Berlin, Germany: OptiTaq DNA Polymerase™ with corresponding buffers,
- Roche (Applied Science), Hague Road, USA: DIG-labelling system™, antibiotics.

2.3. RNA methods

2.3.1. RNA extraction protocol

The following protocol was a modification of Weber and Weschke RNA extraction method (IPK-Gatersleben, Germany). All solutions used for RNA extraction were prepared using autoclaved DEPC-water and were pre-chilled on ice before use; EtOH was kept at -20°C. Frozen tissue (50-150 mg FW or 40 mg DW) was ground using a retch-mill and quenched in liquid N_2. 700 µL of extraction buffer (1 M Tris - pH 9.0, 1% SDS, 10 mM EDTA, β-ME – 5 µL/mL added prior to RNA isolation) were added, the sample was thoroughly mixed by vortexing, 700 µL of phenol/chloroform/isoamyl alcohol (PCI, 25:24:1, pH ~8.00; Roth) were added and the sample were again vortex-mixed. Samples were centrifuged at 4°C for 15 min at 13,000 rpm. The aqueous phase was transferred into a new tube, 700 µL of PCI was added and vortexed, and the mixture was centrifuged at 13,000 rpm for 15 min at 4°C. The aqueous phase was transferred to a new tube, centrifuged at 13,000 rpm for 5 min at 4°C. The supernatant was transferred to a fresh tube and 1/10 of 3 M Na-Ac, pH 5.2 and 2.5 volume of the absolute EtOH added. The tube was three times mixed by inversions, incubated at -80°C for 30-45 min, and then centrifuged at 13,000 rpm for 10 min at 4°C. The supernatant was carefully removed. The precipitate was dissolved in 200 µL of DEPC-water and spun down at 13,000 rpm for 1 min at 4°C. 200 µL of 4 M LiCl were then added and thoroughly mixed by gentle pipetting. Samples were left to stand on ice overnight in a refrigerated room at 4°C. On the next day, all samples were centrifuged at 13,000 rpm for 15 min at 4°C. The liquid was removed very carefully, 1 mL of 2 M LiCl was added, the tube inverted once and centrifuged at 13,000 rpm for 15 min at 4°C. Liquid was removed very slowly and carefully. The pellet was washed twice with 70% EtOH, centrifuged at 10,000 rpm for 5 min at 4°C and the residual solution was removed. The resulting RNA pellet was stored in 500 µL of

70% EtOH at -80°C. Before use, the pellet was re-centrifuged, air-dried and dissolved in 40 µL of DEPC-water (approximately 1 µg/µL of RNA).

2.3.2. Assays of RNA amount and quality

RNA concentration and purity was determined by photometric measurements at 230, 260 and 280 nm using a very sensitive NanoDrop™ spectrophotometer. RNA quality was judged by two ratios: A260/280 (values in the range of 1.8-2.0 indicate low protein contamination) and A260/230 (ratios ≥ 2.0 indicate low polysaccharide contamination). RNA integrity was ascertained on a 1.5% (w/v) agarose gel stained with EtBr.

2.3.3. Removal of genomic DNA contamination from RNA samples

To remove all traces of DNA contamination, up to 10 µg of total RNA was digested with TURBO™ DNAse (Ambion), according to the manufacturer's instructions. RNAse inhibitor (Promega) was added to the sample in the proportion of 1 U/µL of RNA. Afterwards, RNA integrity was ascertained on a 1.5% (w/v) agarose gel stained with EtBr after DNAse digestion. The absence of genomic DNA contamination was subsequently confirmed by qPCR, using primers designed on an intron sequence of a control gene *AGL68*, (AT5G65080; primer sequences in Annex A); 1 µL aliquot of RNA solution per 10 µL reaction volume was applied in each of four technical replicates. For all reactions set up, a negative control (using 1 µL of H_2O instead of RNA solution), and a positive control (1 µL of 5 ng/µL of genomic DNA instead of RNA solution) were included into additional plate wells. If the amplification was detected earliest after 38 cycles, the RNA sample was considered as genomic DNA-free.

In case of total RNA samples used further to prepare a cDNA to measure the rRNA expression level the above mentioned procedure was modified. 1 µg of total RNA was digested with TURBO™ DNAse (Ambion). The absence of genomic DNA contamination was confirmed by qPCR, using primer pairs designed on sequences of 25S rDNA, 18S rDNA and 5.8S rDNA (primers sequences in Annex A) and applying a 1 µL of 100 times diluted RNA solution per 10 µL reaction volume. This was a crucial step because in higher plants ribosomal genes are present in multiple copies (Saini *et al.*, 2000). If the amplification was detected earliest after 38 cycles, the RNA sample was considered as genomic DNA-free.

2.3.4. Northern blotting with a DIG-system (based on Roche manual)

2.3.4.1. RNA electrophoresis and transfer to a membrane

6 µg of total RNA isolated via RNA extraction protocol (described in section 2.3.1), were separated by gel electrophoresis under denaturing conditions: 1.5% (w/v) agarose gel

contained 2% (w/v) formaldehyde (Lehrach *et al.,* 1977). RNA was later transferred directly from the gel to a highly positively charged Nytran® SuPerCharge Nylon Membrane (Sigma-Aldrich) and was fixed using a UV transluminator for 4 min at the wavelength of 302 nm.

2.3.4.2. Labelling of probes with dioxygenin-11-dUTP

Probes were labelled during PCR amplification of 10 ng of gDNA using gene specific primers. The PCR mixture contained all nucleotides at a concentration of 100 μM plus 17.5 μM dioxygenin-11-2'-deoxy-uridine-5'-triphosphate alkaline labile (dioxygenin-11-dUTP, Roche), and 82.5 μM dTTP. The following PCR program was used: 1 cycle of 95°C for 1 min; 30 cycles of {95°C for 1 min, 55°C for 1 min, 72°C for 1 min}, and 1 cycle of 72°C for 5 min. T_m of the primers was calculated from the following formula:

$T_m = 2*GC + 4*AT$.

Labelling efficiency was verified via agarose gel electrophoresis by monitoring the shift to the larger DIG-labelled DNA band, compared to the control PCR reactions (without dioxygenin-11-dUTP). Probes were used for hybridisation at a concentration 2 μL/mL of DIG Easy Hyb™ solution.

2.3.4.3. Pre-hybridisation and hybridisation conditions

Filters were pre-hybridised for 30 min at 50°C in pre-warmed DIG Easy Hyb™ solution in hybridisation tubes. The PCR DIG-labelled probe (see above) was diluted in 50 μL of ddH_2O and denatured at 95°C for 5 min. The probe was then immediately chilled on ice and added to fresh pre-warmed (50^0C) DIG Easy Hyb™ solution. The pre-hybridization solution was then replaced with 3.5 mL hybridization solution/100 cm^2 membrane, containing the probe, and hybridization was performed overnight at 50°C. Afterwards, the hybridization solution was decanted and stored at -20°C. The blot was washed twice in low stringency buffer (2X SSC, 0.1% SDS) for 5 min at room temperature, and then twice in pre-warmed, high stringency buffer (0.5% SSC, 0.1% SDS) for 15 min at 50°C.

2.3.4.4. Detection

Blots were washed in 250 mL of maleic acid buffer (0.1 M maleic acid, 0.15 M NaCl, pH 7.5, 0.3% Tween 20) for 2 min at room temperature, than blocked in 250 mL of Blocking Solution (Roche) for 30 min at room temperature. 20 mL of antibody solution (diluted 1:15,000 in Blocking Solution) was then added and the membrane incubated at room temperature for 30 min. The membrane was washed twice for 15 min in maleic acid buffer and equilibrated with 20 mL of detection buffer (0.1 M Tris-HCl pH 9.5, 0.15 M NaCl) for 3 min and

then briefly dried. The membrane was placed (facing up a DNA/RNA side) in a plastic bag and 500 µL/100 cm^2 drops of CDP-Star™ (Roche) were evenly applied to the surface of the blot. The plastic bag was laid for 5 min, any excess liquid was squeezed out, the bag was sealed and the membrane was incubated in room temperature for 1 h. A chemiluminescent signal was detected using an ultra sensitive CCD camera (Hamamatsu Photonics) with an acquisition time in the 'dynamic' mode for photon acquisition ranging between 10 min up to 2 h. The camera sensitivity was set to level equal to 255 and the threshold for background subtraction was 30. Images were analysed using HPD-LIS™ luminescence imaging software (Hamamatsu Photonics).

2.4. DNA methods

2.4.1. cDNA synthesis and quality check

Reverse transcription (RT) reactions were performed using 5 µg of total RNA with SuperScript™ III Reverse Transcriptase (Invitrogen) according to the manufacturer's instructions. The efficiency of cDNA synthesis was assessed by qPCR amplification of control genes encoding AT1G13440 or *GAPC2* (primer sequences in Annex A). Each 10 µL-reaction contained 1 µL of a 10-fold diluted cDNA sample. Negative (1 µL of H_2O instead of cDNA solution), and positive (1 µL of 5 ng/µL of genomic DNA instead of cDNA solution) controls were included into additional plate wells. Only cDNA preparations that yielded similar values of cycle threshold (C_T) for the control genes were used for subsequent comparison. Additionally, a quality of each cDNA sample was tested in technical triplicates based on the *GAPC2* amplification with two other primer pairs: designed on the 3' and on the 5' end of this gene sequence. The following ratio was the measure of the fold difference between both ends present in a given cDNA pool:

$$C_T\ GAPC2\_3' / C_T\ GAPC2\_5' = 2^{(CT\ GAPC2\_5' - CT\ GAPC2\_3')}.$$

A ratio of 1-3 was the accepted threshold. Samples showing higher values meant that 5'cDNA ends were underrepresented in the pool and were not considered for further analyses. Appropriate cDNA samples could be further diluted to the range of C_T ~ 18-19. cDNA samples were stored in -80°C prior to further analyses.

For a synthesis of cDNAs used to measure expression levels of genes transcribed without subsequent polyA tail addition (e.g. ribosomal genes), Random Primers™ (Invitrogen) were used. For these RT reactions, 1 ng of RNA was used and reactions were performed according to the manufacturer's (Invitrogen) instructions. Only cDNA preparations that

yielded similar C_T values for the control gene (see above) were used for subsequent comparison.

2.4.2. DNA isolation and quantity/quality assays

Genomic DNA was isolated from the youngest rosette leaf of 4-week old plants (for RNAi plants analysis) and from seedling at 6 DAS of different *Arabidopsis* ecotypes (for use as positive controls in qPCR) according to standard CTAB method (Sambrook *et al.,* 2000). The alkaline lysis method (Sambrook *et al.,* 2000) was used to extract plasmid from transformed *Agrobacterium tumefaciens* and *Escherichia coli*. The concentration and purity of DNA samples were determined by photometric measurements at 230, 260 and 280 nm using a NanoDrop™ spectrophotometer. Plasmid DNA integrity was tested on the 1.5% (w/v) agarose gel stained with EtBr. The Nucleobond™ AX plasmid purification kit with a protocol according to manufacturer's instructions was used to purify the plasmid DNA from *E. coli* prior to sequencing.

2.4.3. pAGRIKOLA clones validation via DNA sequencing and PCR amplification

pAGRIKOLA clones of *Agrobacterium* were validated according to AGRIKOLA (http://www.agrikola.org/) protocol 'Validating the pAGRIKOLA_clones' given at http://www.agrikola.org/index.php?o=/agrikola/html/pAGRIKOLA_validation. PCR conditions were modified and the touch-down PCR protocol was as follows: 1 cycle of 94°C for 5 min, 29 x [1 cycle of {94°C for 30 sec, 59°C for 30 sec, 72°C for 1 min}, 1 cycle of {94°C for 30 sec, 57°C for 30 sec, 72°C for 1 min}, 1 cycle of {94°C for 30 sec, 55°C for 30 sec, 72°C for 1 min}, 1 cycle of {94°C for 30 sec, 51°C for 30 sec, 72°C for 1 min}], 1 cycle of 72°C for 5 min. The reaction mix with a final volume 50 μL consisted of 5 μL of 10X Buffer B (Roboklon), 41 μL of ddH_2O, and a mix of equal volumes of four AGRIKOLA primers (Agri 51, Agri 56, Agri 64, Agri 69; primer sequences in Annex A) at a final concentration of 0.1 μM each, 1 μL of plasmid DNA template, 2.5 U of polymerase OptiTaq (Roboklon), and 0.1 μM of dNTPs (final concentration).

The sequencing of pAGRIKOLA clone plasmid was performed by AGOWA GmbH (Berlin, Germany) using Big Dye™ chemistry on a Perkin Elmer ABI Prism 377 DNA sequencer. As previously suggested by AGRIKOLA (http://www.agrikola.org/), there was used a sequencing method protocol developed by Esposito *et al.,* (2003) to prevent a hairpin creation during sequencing through inverted repeats. Sequencing chromatograms were analysed using Chromas v. 1.45 software. The obtained sequence was further compared with the one

deposited in the TAIR database. Plasmid clones with sequence identical to the one in TAIR and validated by PCR amplification were used to transform plants.

2.4.4. Primer design for qPCR

All qPCR primers were designed using the Primer Express 2.0 software (Applied Biosystems) with the following parameters: melting temperatures $T_m = 60\pm2°C$, primer lengths of 20-24 nucleotides, guanine-cytosine (GC) content of 45-55%, and PCR amplicon lengths of 60-150 base pairs. In addition, when possible at least one primer of a pair was designed to cover an exon-exon junction, according to the gene structure models at TAIR (http://www.arabidopsis.org) or NCBI (http://www.ncbi.nlm.nih.gov/) for ribosomal genes. If possible, primers were designed close (no more than 500 bp) from the 3' end of longest gene transcript annotated in TAIR. Primer sequences were further blasted against the *Arabidopsis* genome sequence using BLAST a tool of TAIR with standard parameters to check their specificity. For the experiments concerning the *FRIGIDA* (AT4G00650) expression study, the primers were designed on the part of gene sequence which was identical in Col-0 and C24. Sequence for the gene in C24 was provided to a laboratory of Thomas Altmann from laboratory of Caroline Dean (John Innes Centre Norwich, UK). The sequences of all primers used in this work were collected in Annex A.

2.4.5. PCR protocols

2.4.5.1. Q-PCR analysis condition and settings

PCR reactions were performed in an optical 384-well plate with an ABI PRISM® 7900 HT Sequence Detection System (Applied Biosystems), using SYBR® Green to monitor dsDNA synthesis. Reactions contained 5 µL of Power or 2X SYBR® Green Master Mix reagent (Applied Biosystems), 1.0 ng cDNA and 200 nM of each gene-specific primer in a final volume of 10 µL. Typically, 9 µL of a 'master-mix' consisted of 4 µL of 0.5 µM gene-specific primers (forward and reverse primers were mixed) and 5 µL of Power or 2X SYBR® Green Master Mix reagent, which was first dispensed into individual wells. Afterwards, 1 µL of cDNA template was pipetted in. The 'master-mix' was prepared to reduce pipetting errors and ensure that each reaction contained an equal amount of cDNA. Precise pipettes (Multi-Pro™ Pipettes, Eppendorf) with sterile tips with filters were used (Eppendorf) to aliquot the reagents and template to reduce possible air/pipette contamination. The following standard thermal profile was used for all PCR reactions: 50°C for 2 min; 95°C for 10 min; 40 cycles of {95°C for 15 sec, and 60°C for 1 min}. To generate a baseline-subtracted plot of the logarithmic increase in fluorescence signal (ΔR_n) versus cycle number, baseline data were col-

lected between cycles 3 and 15 (3-10 for rRNA genes). To obtain cycle threshold (C_T) values, all amplification plots were analysed with a set threshold of fluorescence signal $R_n = 0.1$. During qPCR analysis, melting curves were automatically created for each reaction by plotting fluorescence as a function of temperature as the thermal cycler heats through the dissociation temperature of the product. All data were generated and analysed using the SDS v. 2.1 software (Applied Biosystems).

2.4.5.2. pAGRIKOLA clones validation via PCR amplification and PCR-based screening for AGRIKOLA RNAi plant lines

Validation of pAGRIKOLA construct in of BASTA-selected RNAi plants (plant transformants, T1 generation) was performed via PCR amplification according to AGRIKOLA (http://www.agrikola.org/) protocol 'Validating AGRIKOLA RNAi lines' given at http://www.agrikola.org/index.php?o=/agrikola/html/seeds_validation. The AGRIKOLA primers (Agri 51, Agri 56, Agri 64, Agri 69; primer sequences are given in Annex A) and modified PCR protocol were the same as given in section 2.4.3; genomic DNA was a PCR template.

2.4.5.3. Semi-qPCR analysis

The reaction mix (total volume 50 μL) consisted of: 5 μL of 10X Buffer B (Roboklon), 41 μL of ddH_2O, forward and reverse primer mix for *FRIGIDA* (AT4G00650; primer sequences in Annex A) each at a final concentration 0.1 μM, 1 μL of cDNA template (undiluted sample obtained in a reverse transcription of 1 μg of total RNA), 2.5 U of OptiTaq polymerase (Roboklon), 0.1 μM of dNTPs (final concentration). PCR conditions were as follows: 1 cycle of 94°C for 2 min, 35 cycles of {94°C for 30 sec, 55°C for 30 sec, 72°C for 1 min}, and 1 cycle of 72°C for 5 min.

2.5. Transformations

2.5.1. Transformation of bacteria

For the purpose of sequencing the plasmid extracted from pAGRIKOLA clones of *Agrobacterium* were transformed ('back-transformed') into *Escherichia coli* strain DH5α using the heat shock method (Hanahan, 1983).

E.coli transformants ('back-transformants') and pAGRIKOLA clones of *Agrobacterium* (obtained from Magdalena Weingartner from AGRIKOLA project) were grown in LB media (Sambrook *et al.,* 2000) with the appropriate antibiotics (for growth on solid media, 1.5% agar was added). Filter-sterilised antibiotics were added at the following concentrations:

kanamycin 50 μg/mL gentamycin 25 μg/mL, rifampicin 50 μg/mL and tetracycline 5 μg/mL (for *E. coli* only the first antibiotic was used).

2.5.2. Plant transformation and selection of plant transformants

Transformation of wild type Col-0xCol-0 and C24xC24 (inbred lines) *Arabidopsis thaliana* plants with *Agrobacterium tumefaciens* was performed using the floral-dip method (Clough and Bent, 1998).

Transformants (T1 generation) were selected in soil-grown plants by spraying with herbicide BASTA (40 mg/L) according to a protocol 'Selection of transformants' given at http://www.agrikola.org/index.php?o=/agrikola/html/transformation of AGRKOLA project (http://www.agrikola.org/).

2.6. Metabolite analysis

2.6.1. Fatty acids

2.6.1.1. Extraction and derivatisation protocol

Lipids were extracted and fatty acids derivatised to form the corresponding methyl esters using a 'FAME (Fatty Acid Methyl Esters) procedure' (Browse *et al*., 1986). Typically, 5 seeds or seedlings were collected from a Petri dish for each sample and put directly in glass tube filled with 1 mL of 1 N HCl in MetOH, 0.9% NaCl and 100 μL of internal standard (50 μg/mL in MetOH of pentadecanoic acid, 15:0). Glass tubes were incubated at 80°C for 1.5 h. After cooling to a room temperature, equal volumes of 0.9% (w/v) NaCl and hexane were added to each sample. The samples were shaken vigorously by hand for 1-2 min and centrifuged at 1,000 rpm for 3 min. The FAME, which always partitions to the upper hexane phase, was transferred directly into fresh glass tube. The FAME was later slightly concentrated in a N_2 stream. The resulting solution (about 200 μL), was transferred directly into GC vials and the samples were further analysed by GC chromatograph (Agilent Technologies).

2.6.1.2. Data analysis

Individual fatty acids were identified and quantified on the basis of chromatogram analysis according to Browse *et al*., (1986). The differences in fatty acids level between parents and F1 hybrids were determined via the Student's t-test in Excel (MS Office). Each hybrid was compared to each parent at a significance threshold of P-value < 0.05.

2.6.2. Extraction of metabolites from the polar phase and GC-MS data analysis

Plant material was harvested and immediately frozen and stored at -80°C until further processing. For each sample preparation, 40 seeds or seedlings were used. The frozen tissue

was ground in liquid nitrogen and 1 mL of pre-chilled extraction solvent mixture (H_2O: MetOH: CH_3Cl mixed (v/v) in the proportions 1:2.5:1, pH 7.0) was added to each sample. Care was taken to prevent thawing (even partial). The mixture was vortexed for 10 sec, shaken for 4-6 min at 4°C and centrifuged at 14,000 rpm for 2 min. Afterwards, 500 µL of the supernatant were transferred into 1.5 mL conical Eppendorf tubes under argon gas. 200 µL of ultrapure water was added, the new mixture was vortexed for 10 sec and then centrifuged at 14,000 rpm for 2 min. The upper layer (polar phase) was collected and transferred into GC-MS vials. In parallel, several negative blank controls applying the total procedure without a biological sample were prepared. All samples were run on the GC-MS (Leco and Agilent). Raw data was normalised according to Fiehn *et al.,* (2000). The final data was presented as relative amount of metabolites in a time course as ratio of mean values of hybrids to mean values of parental lines, derived from peak areas detected in GC-MS. Annex C presents the summarised GC-MS data for 103 (26% of all detected compounds) metabolites which could be classified into chemical groups when using representative masses.

2.7. Preparation, flow cytometric analysis and sorting of nuclear suspensions

Parental and reciprocal F1 hybrid seedlings grown from three independent seed lots (as three biological replicates) were cultivated under standard conditions. 5-10 seedlings per genotype were harvested at different developmental stages namely at 4, 6, 10 and 15 DAS to prepare fresh extracts. First leaves and cotyledons at 15 DAS were harvested separately. Initially, the plant material was treated with fixative solution (10 mM Tris, 10 mM Na_2EDTA, 100 mM NaCl, 4% formaldehyde, 0.1% Triton X-100, pH 7.5) for 5 min in cold vacuum then for 10 min in a shaker, and was followed by two washing steps of 10 min each in washing solution (10 mM Tris, 10 mM Na_2EDTA, 100 mM NaCl, 0.1% Triton X-100, pH 7.5) at 4°C. Afterwards, the plant material was applied onto pre-cooled Petri dish and 700 µL of separation buffer (15 mM Tris, 2 mM Na_2EDTA, 0.5 mM Spermin, 80 mM KCl, 20 mM NaCl, 15 mM β-ME, 0.1% Triton X-100, pH = 7.5) were added. The plant material in this solution was chopped into very thin pieces with a sharp razor blade. The resulting suspension was filtered through fine-mesh filter tubes. 7 µL of DAPI stain was added and the whole mixture was applied into flow cytometer (Becton-Dikinson) to sort the nuclei according to different ploidy level. Peak heights in histograms obtained were directly proportional to the number of nuclei of the corresponding ploidy level. The differences in ploidy level between parents and hybrids were determined with the Student's t-test in Excel (MS Office). Each hybrid was compared to each parent at a threshold of significance of P-value < 0.05. Additionally, nuclei

of 2C and 4C DNA content were flow sorted from 6 DAS seedlings and applied directly onto microscopic slides containing one droplet of sucrose buffer (100 mM Tris, 50 mM KCl, 2 mM MgCl, 0.05% Tween-20, 5% sucrose). Three slides of 2C and 4C ploidy level were prepared per genotype. Approximately 1,200 nuclei were collected on every slide, each of which was previously supplied with a droplet of a of sucrose buffer. All slides were kept at room temperature for drying.

2.8. Silver staining

Air-dried microscopic slides were quenched in 4°C borate buffer (0.01 M boric acid, pH 9.2) for 10 min, and 100 µL of silver nitrate (50% of silver nitrate solution in ddH_2O whose pH was equilibrated to 4-5 using formic acid) was applied onto the sucrose buffer droplet and covered with a nylon mesh. Slides were incubated for 2 h at 65°C to dry. Later, they were washed and anti faint glycerol was applied before covering the slides prior to microscopic analysis. Slides were placed under the microscope and searched for good nuclei to measure their area using AnalySIS^{TM} software (Olympus). The areas of 100-150 nuclei were measured per genotype.

2.9. Microscopy methods and analysis

The plant material was fixed with 4% paraformaldehyde and 0.2% of glutaraldehyde in PBS, pH 7.2 at room temperature for 4 h. After dehydration with ethanol and xylol at room temperature, the material was embedded in paraffin. The plant material was sectioned using a microtome and five to ten individuals per genotype and developmental time point were subjected into microscopic analysis. The prepared sections were later stained with mixture of 1% aqueous toluidine blue 0 (for visualisation of cell wall, membranes, and nuclei) and 1% aqueous acid fuchsin for the detection of proteins. Lipids were determined by cytochemical staining with mixture of Sudan III and Sudan IV. Qualitative analysis of the spatial distribution and pattern of mobilisation of reserves was performed on the basis of microscopic studies of cells during reserve mobilisation in *Arabidopsis* (Mansfield and Briarty, 1996).

2.10. Transcript data analysis

2.10.1. Data normalisation

In order to compare data from different qPCR runs or cDNA samples, a C_T value of each of analysed gene was normalised to a C_T value of a reference gene. Four different reference genes were always included into each qPCR run. In the 1st transcript profiling experiment (refer to section Results), the most constant (AT3G01150 or *PTB*) of the four house-

keeping genes (AT4G05320 or *UBQ10*; AT1G13440 or *GAPC2*/*GAPDH*[1]; AT2G28390 or *'SAND family'*; and AT3G01150 or *PTB*) was selected for the normalisation. In the 2nd transcript profiling experiment and all other qPCR analyses, a geometric mean of C_T values obtained for all four house-keeping genes (AT2G28390 or *'SAND family'*; AT1G13320 or *PDF2/PP2A/PP2AA3*[2]; AT3G01150 or *PTB*; and AT1G13440 or *GAPC2*/*GAPDH*) was used. Gene expression was normalised by subtracting the C_T value of the reference gene (or geometric mean of C_T of four reference genes) from the C_T value of a gene of interest and is represented by ΔC_T. The average C_T value for *UBQ10* (AT4G05320) or *GAPC2*/*GAPDH* (AT1G13440) was 19.00 (+/-1) for all plates/templates measured in all experiments.

A PCR efficiency estimation method was based on data obtained from the exponential phase of each individual amplification plot and the equation (Czechowski *et al.,* 2004):

$(1+ E) = 10^{slope}$, E- amplification efficiency

E value was derived from the log slope of the fluorescence versus cycle number curve for a particular primer pair (Ramakers *et al.,* 2003). E = 1 meant 100% primer efficiency and the amount of cDNA was doubled in each reaction cycle.

Expression ratios of sample A to sample B (fold-change in expression) were obtained from the equation:

$A/B = (1+ E)^{-\Delta\Delta CT}$, where $\Delta\Delta C_T = \Delta C_{T\_A} - \Delta C_{T\_B}$

The relative expression value of an individual gene and genotype was calculated from the formula:

Relative expression $= (1+E)^{-\Delta CT}$

The qPCR efficiency values were calculated and loaded via the LinRegPCR software (Ramakers *et al.,* 2003). Regardless from cDNA genotype origin, genes for which the primer efficiency was below 70% were not considered in overall data analysis.

2.10.2. Melting curve analysis

For all qPCR reactions the melting curves of PCR products generated by SDS v. 2.1 software were analysed. This analysis was performed on the basis of work of Ririe *et al.*, (1997) showing that shape and position of this DNA melting curve are typically functions of the GC/AT ratio, length, and sequence, and can be used to differentiate amplification products separated by less than 2°C in melting temperature. Desired products can be distinguished from undesired products, in many cases eliminating the need for gel electrophoresis. Genes,

[1] AT1G13440 was annotated as *GAPDH* by Czechowski *et al.,* (2005), and as *GAPC2* by TAIR (February 2009)
[2] AT1G13320 was annotated as *PDF2* or *PP2A* by Czechowski *et al.,* (2005), and as *PP2AA3* by TAIR (February 2009)

for which a shape and position of dissociation curve together with specific melting temperature of PCR product varied between genotypes, were discarded from further analysis.

2.10.3. Statistical methods

Comparison statistics was applied to identify differentially expressed genes. Comparison tests required replicates and used variability within the replicates to assign a confidence level as to whether the gene is differentially expressed. As a fold change does not address the reproducibility of the observed difference, it could not be used to determine the statistical significance (Draghici, 2002). Thus, 1-factorial ANOVA (Zar, 1999) was calculated using generalised linear model (GLM) function with the following models:

Relative expression ≈ Genotype x Time point x Replica,

Relative expression ≈ Genotype x Replica.

Analyses were conducted with the R (v. 2.1), SAS v. 9.2, and GeneStat v. 2.0 software. The differences in expression that could be explained by the effect of genotype were considered as 'significant samples'. Further, expression data was subjected into a false discovery rate (FDR) test of Benjamini-Hochberg (B-H), (Benjamini and Hochberg, 1995), and significance level of P-value < 0.05 was chosen to select candidate genes. FDR is the adjustment of the P-values obtained by ANOVA, which reduces the amount of false positives in the list (it is a correction for multiple testing performed in ANOVA). The *post-hoc* tests were performed to determine, which of the six possible comparisons between both parents and hybrids processed by ANOVA showed a significant difference. For this purpose, least significant difference test (LSD test; NIST/SEMATECH e-Handbook of Statistical Methods, 2003-2006) in GeneStat v. 2.0 software was performed. A significance level of P-value < 0.05 was chosen to select candidate genes.

In addition to ANOVA analysis, Student's t-tests were performed in R (v.2.1) software for data obtained in the experiments (chapters 3.3.2, Results section) to select genes differentially expressed in hybrid(s) when compared to parent(s). A significance level of P-value < 0.05 was chosen to select candidate genes.

Principal component analysis (PCA) analysis was performed in R (v. 2.1) software and was used to investigate trends in the data by suggesting the sources of highest data variation. In the cases where only two biological replicas were acceptable, the missing replica in the graph was represented by the mean of two present ones.

2.10.4. Expression patterns

The assignment of expression patterns was performed with the use of TIGR_MeV v. 3.0 software (The Institute of Genomic Research Multiple Experiment Viewer; Saeed *et al*., 2003). Candidate genes were analysed for patterns of gene expression. The median of three expression values per genotype was calculated and relative expression values were calculated by subtracting the median expression value across the four genotypes from the individual median expression value of each genotype. Only the median of three arrays per genotype and the function Pavlidis Template Matching (PTM; Pavlidis and Noble, 2001) was used to assign expression values to predefined patterns (P-values < 0.05). The defined patterns and PTM inputs are given in Table 2.1. If several expression patterns were significant for a gene, the one with the highest significance was retained.

Table 2.1. PTM function inputs to TIGR_Mev software

Template Number	Expression Pattern	Col-0xCol-0	ColxC24	C24xCol-0	C24xC24
1.	Intermediate_C24xC24_high	0	0.5	0.5	1
2.	Intermediate_Col-0xCol-0_high	1	0.5	0.5	0
3.	Overdominant (F1 high)	0	1	1	0
4.	Underdominant (F1 low)	1	0	0	1
5.	Dominant_C24xC24_high	0	1	1	1
6.	Dominant_Col-0xCol-0_low	0	0	0	1
7.	Dominant_C24xC24_low	1	0	0	0
8.	Dominant_Col-0xCol-0_high	1	1	1	0
9.	Maternal_C24xC24_high	0	0.2	0.8	1
10.	Maternal_Col-0xCol-0_high	1	0.8	0.2	0
11.	Paternal_C24xC24_high	0	0.8	0.2	1
12.	Paternal_Col-0xCol-0_high	1	0.2	0.8	0

3. RESULTS

At the start of this project, it was not clear from published data whether there were significant differences between the F1 hybrids (Col-0xC24 and C24xCol-0), and Col-0 or C24 prior to and during germination or at the early growth stages. As pointed out in Materials and Methods, seed size and mass were comparable for the four genotypes: Col-0xCol-0, C24xC24, Col-0xC24 and C24xCol-0. However, there may have been differences in embryo development or in the nature of seed reserves, the rate of germination, the rate of seed reserve mobilisation and utilisation, or a combination of these and other factors in the F1 hybrid seed, which could explain elevated growth of hybrid seedlings. Differences in the rate of cell division, cell sizes, specific metabolite composition, or gene expression pattern could conceivably lead to increased growth heterosis in F1 hybrids.

3.1. Determination of the developmental time point at which differences between F1 and parents are first manifested

3.1.1. Comparison of germination time and early seedling development in parents and hybrids

Germination rate and post-germinative growth of parents and hybrids were measured to identify any differences in parents and hybrids at the early stages. Prior to sowing, a seed material was stored for two months to break dormancy in C24xC24. Additionally, stratification allowed for synchronisation of seed germination. Typical early developmental behaviour of parents and hybrids is shown in Figures 3.1 A-D and the resulting growth heterosis, which was clearly visible in seedlings at 8 DAS, is shown in Figure 3.2.

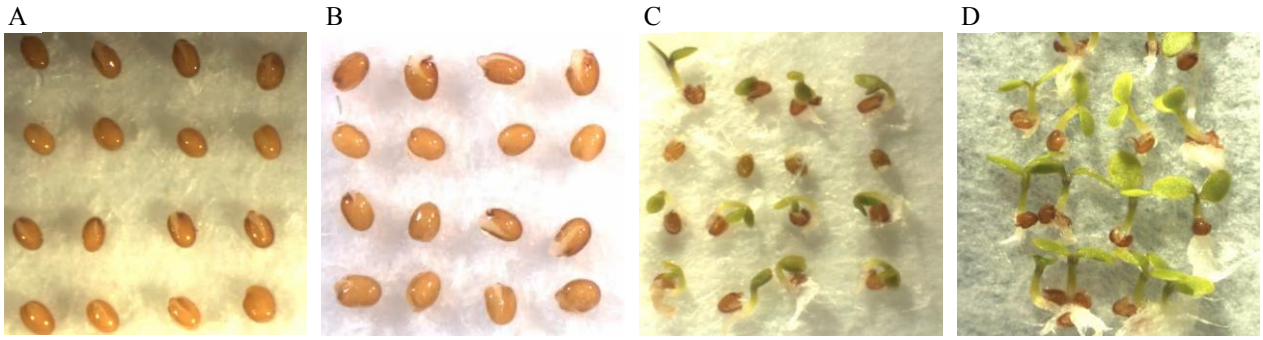


Figure 3.1. Photographs of a typical germination course and post-germinative growth in parents and their F1 hybrids

A – 36 DAS (1.5 DAS)
B – 48 HAS (2 DAS)
C – 72 HAS (3 DAS)
D – 96 HAS (4 DAS)

Seed or seedling layout:
row 1: Col-0xCol-0
row 2: C24xC24
row 3: Col-0xC24
row 4: C24xCol-0

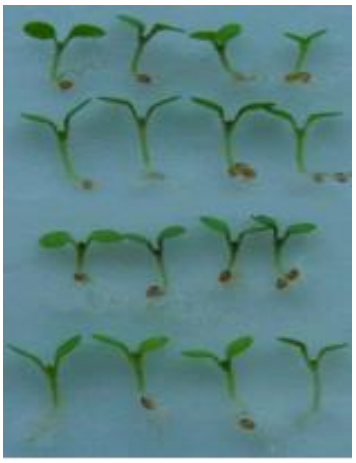


Figure 3.2. Photograph of parental and hybrid seedlings at 8 DAS. F1 hybrids outperformed parents in growth (heterosis)

Seedling layout:
row 1: C24xC24
row 2: C24xCol-0
row 3: Col-0xCol-0
row 4: Col-0xC24

The first observations revealed that C24xC24 seed required more time (over 48 hours, Figure 3.1 B) to germinate than Col-0xCol-0 or the reciprocal F1 hybrids (around 36 hours, Figure 3.1 A). The end of this phase is defined as radical protrusion through the seed coat according to Bewley and Black (1994) marks the onset of seedling growth. Heterotrophic growth (Eastmond and Graham, 2001) of C24xC24 continued until 72 HAS (germinated seed with root hair grown on hypocotyl), whereas the F1 hybrids and Col-0xCol-0 parent had proceeded to autotrophic growth by this time (Figure 3.1 C). Although all genotypes had progressed to photosynthesising seedlings by around 96 HAS (Figure 3.1 D), the delay of

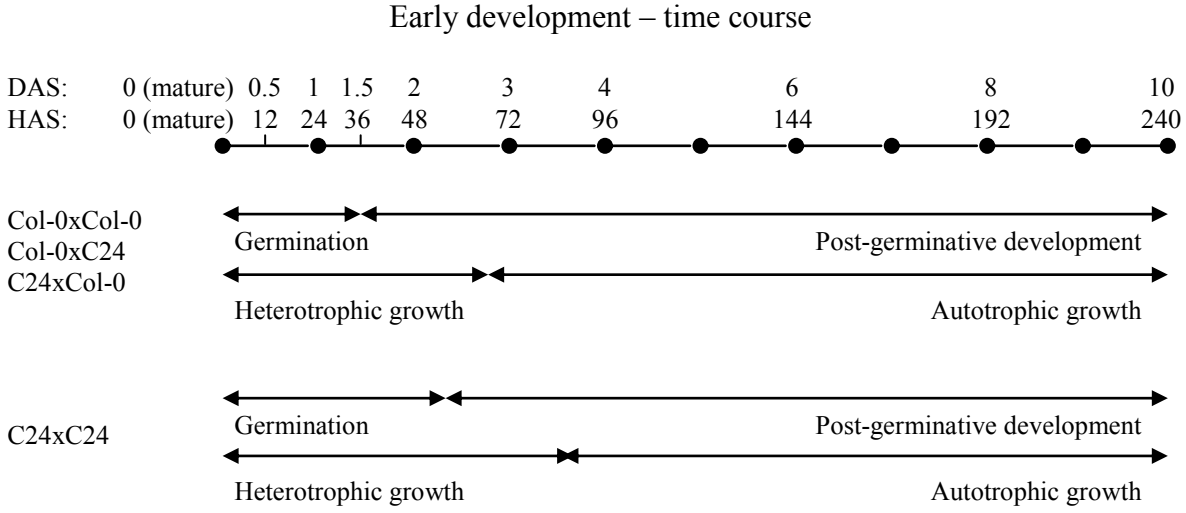


Figure 3.3. Schematic representation of differences observed in germination and post-germinative growth between F1 hybrids and their parents under typical experimental conditions

C24xC24 in germination should be taken into account when considering further data. To conclude, the C24xC24 was delayed in comparison to Col-0xCol-0 parent and both F1 hybrids (Figure 3.3), which were all three similar in germination rate. This difference disappeared by 96 HAS, at which point all genotypes had greened and were autotrophic (Figure 3.1 D).

3.1.2. Comparison of seed storage reserve mobilisation in parents and hybrids via microscopic analysis

Mobilisation of the main seed storage reserves in F1 hybrids and parents was investigated via microscopic imaging. The major seed storage reserves in *Arabidopsis* are lipids. They are stored in cytosolic organelles known as liposomes or oil bodies. Cells are packed full of oil bodies which occupy about 60% of the cell volume in the cotyledons of mature embryos (Mansfield and Briarty, 1992; Penfield *et al.*, 2005). *Arabidopsis* seeds also contain storage proteins, which are stored in membrane-bound compartments called protein bodies in cells of hypocotyls and cotyledons (Müntz *et al.*, 2007; Bentsink and Koornneef, 2002). Seeds and seedlings were sectioned and five to ten individuals per genotype and developmental time

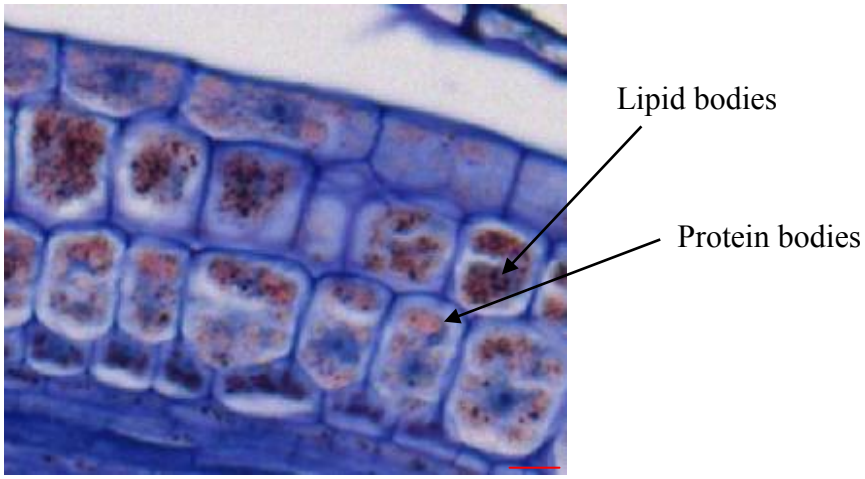


Figure 3.4. A fragment of microscopic cross-section from Col-0xCol-0 hypocotyl at 24 HAS

Scale bar = 10 μm

point were subjected to microscopical analysis. It was possible to detect the lipid bodies (small brownish dots) and separate protein bodies (bigger vacuolar structures containing pinkish protein globules) using appropriate dyes (Figure 3.4). F1 hybrids were compared to parents at the control stage of mature seed prior to imbibition (0 HAS), and 24, 36, 48, 72, and 96 HAS. Representative pictures collected in Annex B show one section per genotype at each time point. Qualitative analysis of the spatial distribution and pattern of mobilisation of reserves was performed in all genotypes at each developmental stage. The comparative analysis did not reveal any differences in storage mobilisation between parents and hybrids.

3.1.3. Comparison of metabolite levels in hybrids and parents during germination and early growth

3.1.3.1. Measurement of global metabolites via GC-MS

GC-MS analysis of plant metabolites (Fiehn *et al.,* 2000) was performed on mature seeds and on seedlings at 12, 24, 36, 48, 72 and 96 HAS. Data for each time point combined

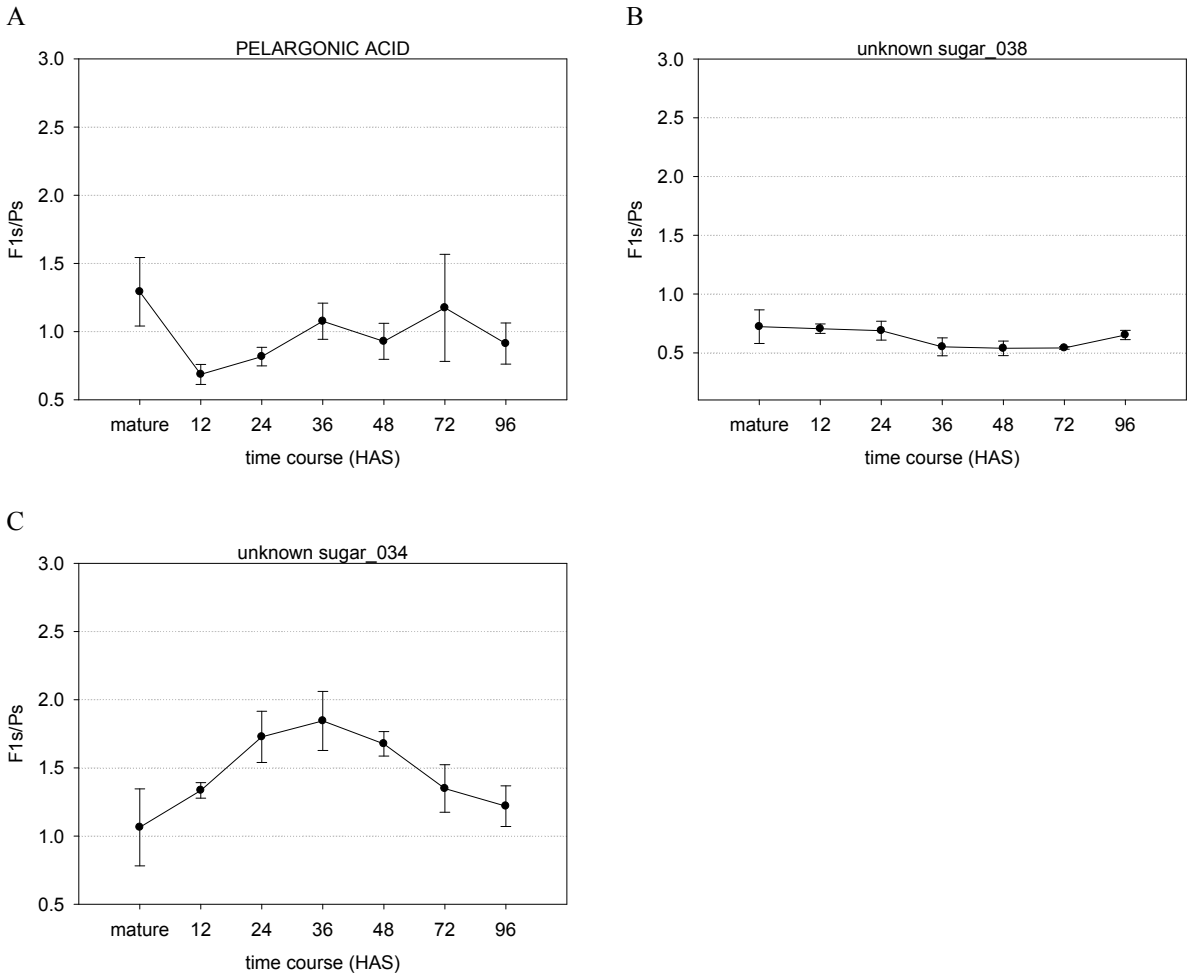


Figure 3.5. Differences between hybrids and parents in the level of three metabolites

F1s/Ps – the relative amount of metabolites in a time course shown as ratio of mean values of hybrids to mean values of parental lines from the peak area detected in GC-MS
mature – ungerminated seed

four biological replicates obtained in four independent experiments. 75 (around 20%) compounds were assigned a chemical structure by comparison with a METAB LIBRARY (Fiehn*.,* 2000), and 103 (26%) compounds were classified into chemical groups by using representative masses. These metabolites mostly represented amino acids, sugars, amines and organic acids. Significant differences were found in levels of some metabolites between F1 hybrids and parents within the time course (Annex C. Summarised GC-MS data). Some compounds differed in amount in mature seeds prior to germination, for example pelargonic acid (Fig-

ure 3.5 A) and an unknown sugar (Figure 3.5 B). Other metabolites had similar levels in the seed of hybrid and parent but changed in relative level during germination (e.g. unknown sugar_034, Figure 3.5 C).

3.1.3.2. Analysis of fatty acid content via GC

Arabidopsis seed accumulates lipids in the form of triacylglycerols (TAGs), esters of glycerol and fatty acids (FAs). TAGs are broken down during germination, providing both carbon skeletons and energy resources for the developing seedling (Mansfield and Briarty, 1992). GC measurements (Browse *et al.*, 1986) were performed to compare FA levels in F1 hybrids and parents at different developmental stages between 0-8 DAS. Three biological

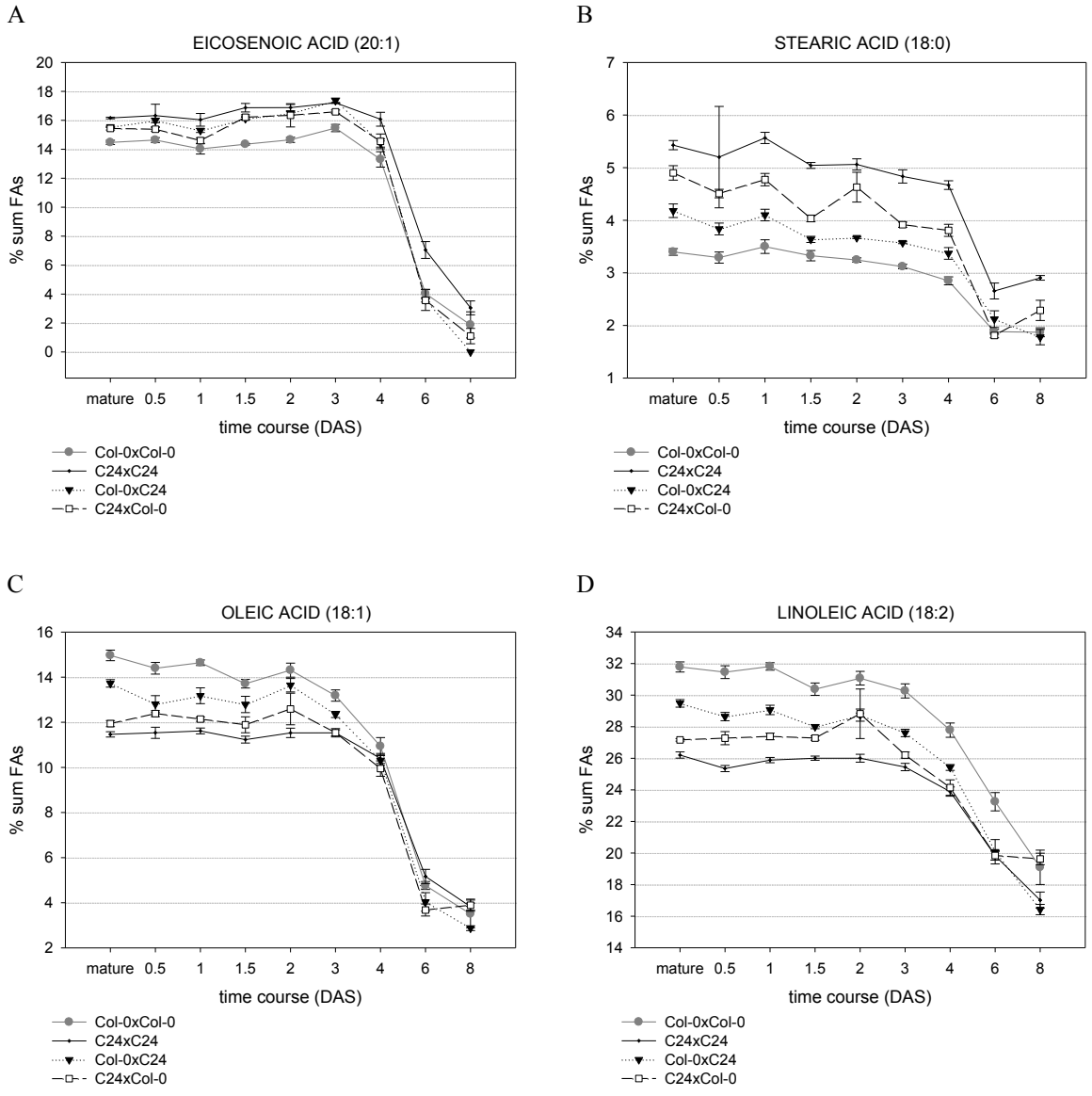


Figure 3.6. The content of fatty acids (FAs) in parents and F1 hybrids shown as % of the sum of total FAs measured by GC in the time course of 0 (mature seeds), 0.5, 1, 1.5, 2, 3, 4, 5, 6, and 8 DAS

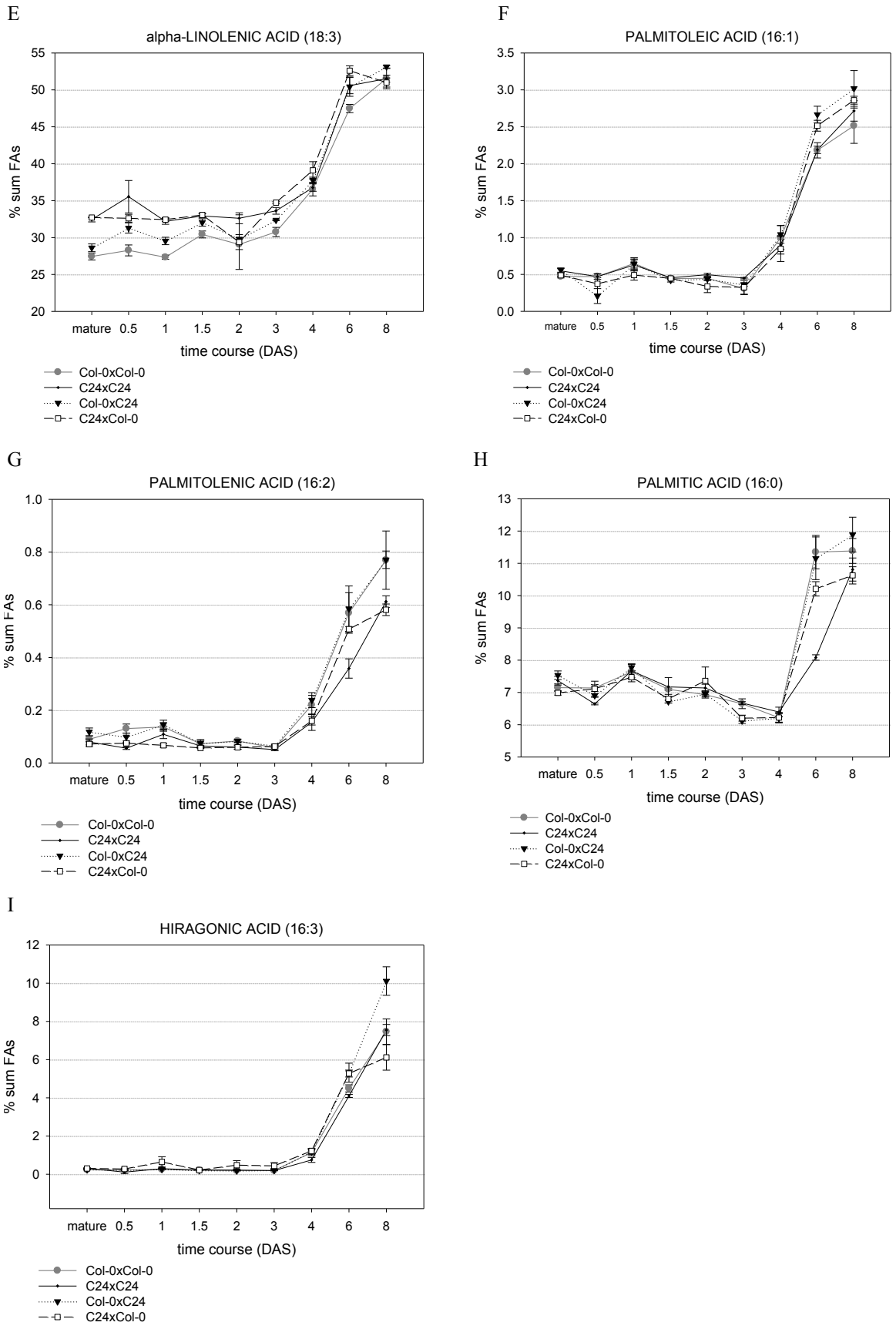


Figure 3.6. The content of fatty acids (FAs) in parents and F1 hybrids shown as % of the sum of total FAs measured by GC in the time course of 0 (mature seeds), 0.5, 1, 1.5, 2, 3, 4, 5, 6, and 8 DAS

replicates from three independent experiments were analysed. Reduced levels of 20:1, 18:0, 18:1 and 18:2 (Figures 3.6 A, B, C, and D, respectively) were observed for all genotypes as early as 4 DAS. Levels of 18:3, 16:1 and 16:2 FAs increased by 4 DAS (Figure 3.6 E, F and G, respectively), while 16:0 FA increased by 6 DAS in both parents and F1 hybrids (Figure 3.6 H). Levels of 16:3 FA, which is synthesised *de novo* and present only in leaves (Browse *et al.,* 1986) increased by day 4 and continued to increase through 6 and 8 DAS in all genotypes, although levels were significantly higher in hybrids than in parents at 6 and 8 DAS (Figure 3.6 I). Eicosenoic acid (Figure 3.6 A) is a marker for storage lipids and is present only in seeds (Lemieux *et al.,* 1990). Levels of 20:1 FA were lower in hybrids than in parents at 6 DAS, consistent with the greater increase in 16:3 FA in hybrids (Figure 3.6 I). To summarise: the switch to photoautotrophic growth marked by increases in chloroplast lipid such as 16:3 FA occurred in both parents and hybrids around 3-4 DAS. Differences in the synthesis or utilisation of FAs between hybrids and parents were apparent around 4-6 DAS.

3.2. Identification of heterosis candidate genes / reverse genetic approach

The results of metabolite level comparisons between F1 hybrids and parents at early development guided the choice of time point for transcript profiling. Differences in expression of transcription factors and other gene of interest between F1 hybrids and their parents were further studied at 4 DAS. Two independent experiments with two different gene platforms for each were preformed.

3.2.1. Efficacy test of qPCR primers of novel reference genes in the four genotypes

The efficacy of primer pairs of the novel reference genes used to normalise the expression of genes in a qRT-PCR approach (Czechowski *et al.*, 2005) was tested in the four genotypes. cDNAs were synthesised from comparable amounts of RNA extracted from seedlings at 4 DAS. Each sample was measured in three technical replicates. The majority of genes showed similar performance in both parents and F1 hybrids i.e. a comparable C_T value, PCR efficiency (Figures 3.7 and 3.8, respectively) and melting temperature (data not shown). Five genes were chosen for further normalisation of gene expression. These were: *UBQ10* (AT4G05320), *'SAND family'* (AT2G28390), *PTB* (AT3G01150), *GAPC2/GAPDH* (AT1G13440), and *PP2AA3* (AT1G13320*).*

Additionally, for *GAPC2* three different primer pairs were tested. They were marked in the figures as AT1G13440*, AT1G13440**, and AT1G13440***. The last two primer pairs were also used to estimate the quality of each newly synthesised cDNA prior its use in

expression profiling (see details about AT1G13440**/AT1G13440* ratio in Materials and Methods section). To test the efficacy of *UBQ10* two different primer pairs were used. The

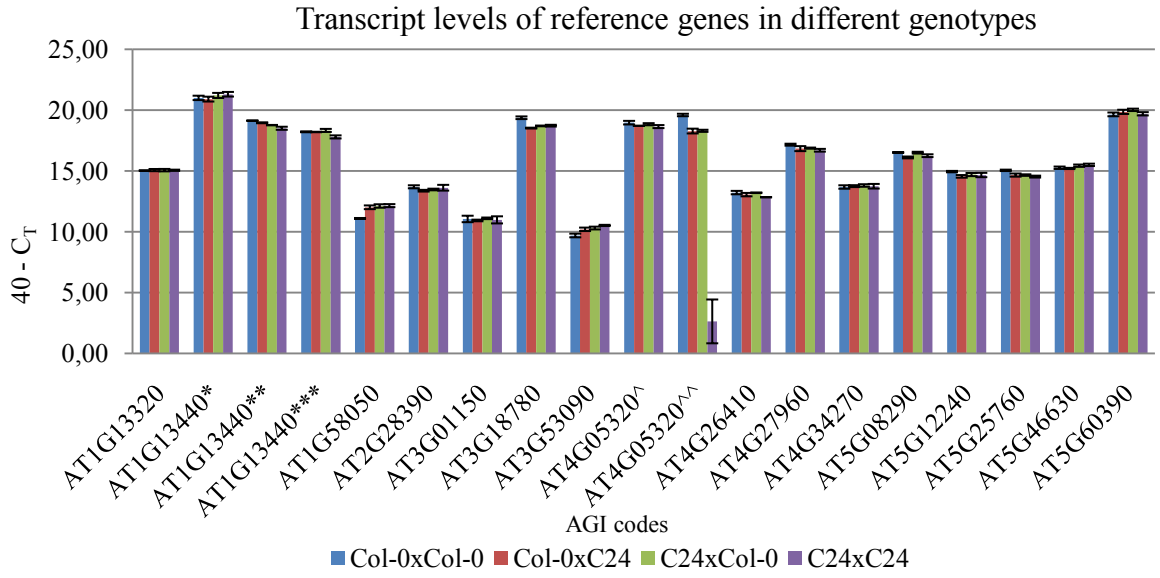


Figure 3.7. Comparison of transcript levels of reference genes in parents and hybrids

Error bars show SD

first, labelled AT4G05320^ was designed on sequences of three out of five polyubiquitin genes present in *A. thaliana* and worked well in all four genotypes. The second, labelled AT4G05320^^ was designed on five polyubiquitin genes and did not amplify the cDNA from C24 ecotype, as indicated by the low transcript level (Figure 3.7).

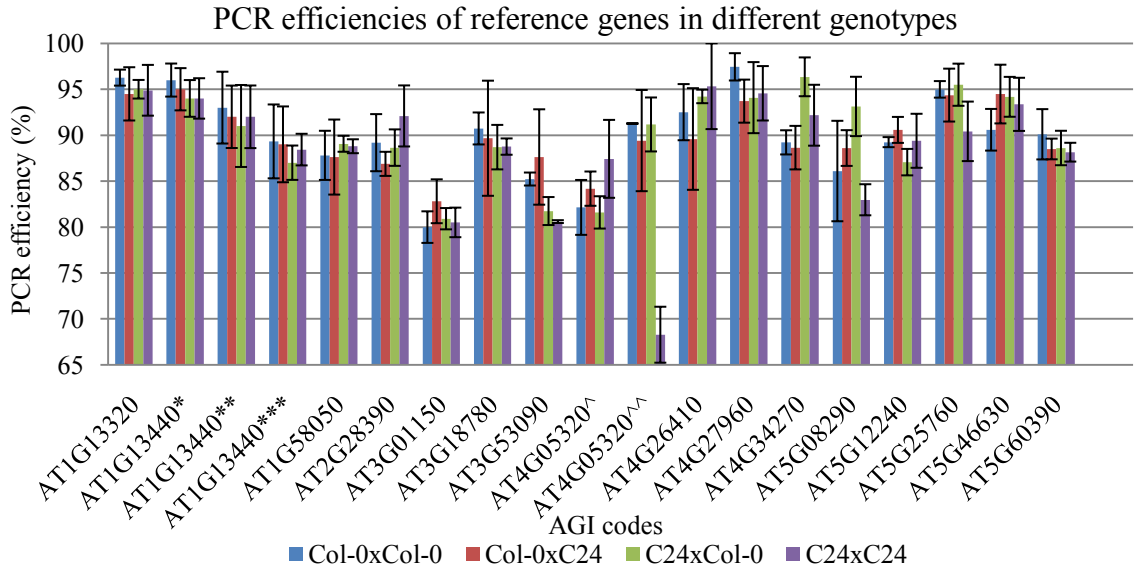


Figure 3.8. Comparison of PCR efficiencies of reference genes in parents and hybrids

Error bars show SD

3.2.2. Identification of candidate genes in experiment 1

The expression levels of 1198 TF and putative TF genes detected at 4 DAS were compared in F1 hybrids vs. parents.

3.2.2.1. Determination of the most stable reference gene(s) for transcript data normalisation

The most stable reference gene among 12 cDNA samples (3 biological replicas x 4 genotypes) was chosen on the basis of an algorithm developed by Vandesompele *et al.*, (2002). The gene expression stability measure, which was represented by M value generated by gNORM software, was calculated based on the assumption that the expression ratio of two ideal internal reference genes was identical in all samples, regardless of the experimental condition or the cell type. The most stable reference genes within this experiment were *PTB* (AT3G01150) and *'SAND family'* (AT2G28390), (Figure 3.9). Eventually, the *PTB* transcript levels were used for all data normalisations.

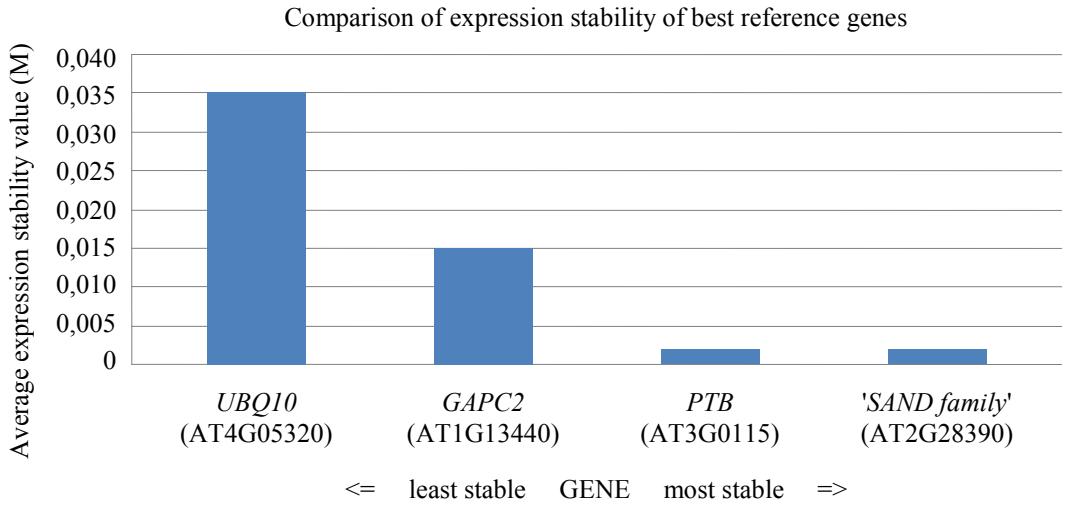


Figure 3.9. The outcome of gNORM calculation

The '*SAND family*' and *PTB* genes were the most stable across all of the replicates in the gene profiling experiment 1

3.2.2.2. Candidate gene selection criteria

ANOVA was performed on the normalised data for 1198 TF and putative TF genes. 188 genes were selected as a differentially expressed in parents and hybrids on the basis of a significance threshold of uncorrected (for multiple testing) P-values < 0.05 (79 genes) and/or of 3-fold difference in expression between parents and hybrids (109 genes). This group was further re-analysed via qPCR using a more sensitive reaction kit: Power SYBR Green. From this gene group, only those of uncorrected P-values < 0.05 and of 4-fold difference in expression between F1 hybrids and parents were selected. In total, 22 candidate genes were targeted

for further study (genes marked in bold in Tables 3.2 and 3.4). The annotations of two of them were later re-annotated in TAIR to SET-domain genes.

3.2.3. Identification of candidate genes in experiment 2

A second gene profiling experiment to identify heterosis candidates was performed using an updated set of primers for all known and putative TF genes as well as primers for microRNAs, and a subset of genes involved in the epigenetic control of gene expression and/or chromatin modification processes (called further 'chromatin-related'), especially from chromatin-targeted RNA silencing pathways (Annex A. List of primer sequences).

3.2.3.1. Identification of TF candidates and selection criteria

The expression levels of 1469 detected TF and putative TF genes were compared between F1 hybrids and parents. Gene profiling covered major protein families and around 80% of all known or putative TFs. ANOVA analysis of normalised expression data yielded 43 differentially expressed genes (Tables 3.2 and 3.4) with P-values < 0.05 significance threshold after Benjamini-Hochberg (BH) correction for multiple testing (Benjamini and Hochberg, 1995). These genes were targeted for further study.

3.2.3.2. Identification of heterosis candidates from a group of 'chromatin-related' genes via qRT-PCR

A set of 58 genes encoding proteins involved in the epigenetic control of gene expression and/or chromatin-modification processes (Brodersen and Voinnet, 2006; ChromDB at www.chromdb.org, Gendler *et al.,* 2008) including DNA methyltransferases (METs, CMTs, DRMs), histone deacetylases (HDAs), SET domain (containing) proteins (SDGs), and proteins of chromatin remodelling activities (CHR, CHB, CHC) were targeted for qRT-PCR analysis (Table 3.1 and Annex A. List of primer sequences). According to the comprehensive ChromDB database classification, these genes encoded the following protein groups: DNA modifying, histone modifications, nucleosome organisation: assembly and displacement, and RNAi components. Protein categories omitted in this study were: histones and histone linker proteins, histone modification-associated proteins and complexes, modified-histone binding proteins, non-histone DNA binding proteins, and proteins involved in chromosome dynamics. Expression data analysis was performed together with TF data analysis. The expression levels of 54 detected genes (93%) were compared between F1 hybrids and parents. Only one gene (AT5G43990 [52] or *SUVR2*; Table 3.4) was found to be significantly differentially expressed in hybrids when compared to parents.

Table 3.1. Set of 'chromatin-related' genes selected for expression profiling to identify heterosis candidate genes

No.	AGI code	Name(s)	ChromDB Protein Group	Additional Description of ChromDB or TAIR
1.	AT1G01920	*SDG42, SET42*	SET Domain Protein Superclass B	SET domain protein
2.	AT1G04050	*SUVR1, SDG13, SET13*	ARATH_SUVR4	Su(var)3-9 group of confirmed and predicted histone H3 lysine 9 methyltransferases
3.	AT1G14030	*SDG43, SET43*	SET Domain Protein Superclass B	SET domain protein
4.	AT1G17770	*SUVH7, SDG17, SET17*	SUVH1/SUVH3	Su(var)3-9 group; plant specific sub-group with YDG_SRA, Pre-SET, and SET domains
5.	AT1G24610	X	NOT PRESENT	(TAIR: SET domain-containing protein; similar to ribulose-1,5 bisphosphate carboxylase oxygenase large subunit N-methyltransferase)
6.	AT1G48410	*AGO1*	AGO1 (Dicots and Monocots)	A PIWI/PAZ domain containing member of the *Argonaute* gene family involved in RNA silencing
7.	AT1G63020	*NRPD1A, NRPDA1, SDE4*	ARATH_NRPD1A	One of two large subunits of a plant-specific RNA polymerase IV required for posttranscriptional gene silencing
8.	AT1G69770	*CMT3, DMT6*	DNA methyltransferases	Class II DNA methyltransferase; a DNA methyltransferase containing a chromodomain (chromomethylase)
9.	AT1G73100	*SUVH3, SDG19, SET19*	SUVH1/SUVH3	Su(var)3-9 group; plant specific sub-group with YDG_SRA, Pre-SET, and SET domains
10.	AT1G76710	*ASHH1, SDG26, SET26*	ARATH_ASHH1	predicted histone H3 lysine 36 histone methyltransferase; ASH1 group
11.	AT1G77300	*ASHH2, SDG8, EFS, SET8*	ARATH_EFS	*Arabidopsis* EARLY FLOWERING IN SHORT DAYS protein, a histone H3 lysine 36 histone methyltransferase
12.	AT1G80740	*CMT1, DMT4*	DNA methyltransferases	Class II DNA methyltransferase - a putative DNA methyltransferase containing a chromodomain (chromomethylase)
13.	AT2G05900	*SUVH10, SDG11, SET11*	ARATH_SUVH	Su(var)3-9 group; plant specific sub-group with YDG_SRA, Pre-SET, and SET domains
14.	AT2G16390	*DRD1, CHR35, CHA35*	SNF2 super family (Snf2, Ris1, Rad26 superclasses)	SNF2 Superfamily; RAD26 Superclass; DRD1 class
15.	AT2G17900	*ASHR1, SDG37, SET37*	S-ET interrupted and unclassified	S-ET protein containing an interrupted SET domain
16.	AT2G18850	X	NOT PRESENT	(TAIR: similar to SET domain-containing protein)
17.	AT2G19640	*ASHR2, SDG39, SET39*	S-ET interrupted and unclassified	SET domain proteins; Homology Subgroup S-ET; protein containing an interrupted SET domain
18.	AT2G22740	*SUVH6, SDG23, SET23*	ARATH_SUVH5/SUVH6	SUVH6; Su(var)3-9 group; plant specific sub-group with YDG_SRA, Pre-SET, and SET domains

No.	AGI code	Name(s)	ChromDB Protein Group	Additional Description of ChromDB or TAIR
19.	AT2G23380	*CLF, SDG1, SET1*	ARATH_CLF	CURLY LEAF; a member of the enhancer of zeste family of predicted histone H3 lysine 27 histone methyltransferases, works in concert with ATX1 (AT2G31650) to control the expression of the flower homeotic gene AGAMOUS
20.	AT2G24740	*SUVH8, SDG21, SET21*	SUVH1/SUVH3	Su(var)3-9 group; plant specific sub-group with YDG_SRA, Pre-SET, and SET domains
21.	AT2G27040	*AGO4*	Plant AGO4/6/9 class	A PIWI/PAZ domain containing member of the Argonaute gene family
22.	AT2G33290	*SUVH2, SDG3, SET3*	ARATH_SUVH2/SUVH9	Su(var)3-9 group; plant specific sub-group with YDG_SRA, Pre-SET, and SET domains
23.	AT2G35160	*SUVH5, SDG9*	ARATH_SUVH5/SUVH6	u(var)3-9 group; plant specific sub-group with YDG_SRA, Pre-SET, and SET domains
24.	AT2G36490	*ROS1, DNG1*	Superfamily of DNA glycosylases	DEMETER (DME) family of bifunctional DNA glycosylases; ROS1 is a 5-methylcytosine DNA glycosylase/lyase important for active DNA demethylation in *Arabidopsis*
25.	AT2G40030	*NRPD1B, NRPDA2*	ARATH_NRPD1B	One of two large subunits of a plant-specific RNA polymerase IV required for posttranscriptional gene silencing
26.	AT2G44150	*ASHH3, SDG7, SET7*	ASH1	Predicted histone H3 lysine 36 histone methyltransferase; ASH1 group
27.	AT3G03750	*SUVR3, SDG20, SET20*	ARATH_SUVR3	Su(var)3-9 group of confirmed and predicted histone H3 lysine 9 methyltransferases
28.	AT3G04380	*SUVR4, SDG31, SET31*	ARATH_SUVR4	Su(var)3-9 group of confirmed and predicted histone H3 lysine 9 methyltransferases
29.	AT3G07670	*X*	NOT PRESENT	(TAIR: SET domain-containing protein)
30.	AT3G21820	*ATXR2, SDG36, SET36*	S-ET interrupted and unclassified	S-ET protein containing an interrupted SET domain
31.	AT3G23780	*NRPD2a, NRPDB1*	RNA Polymerase IV Small Subunit	RNA Polymerase IV Small Subunit
32.	AT3G43920	*DCL3*	ARATH_DCL3	Dicer-like; similar to the *Drosophila melanogaster* gene Dicer, a multidomain ribonuclease essential for RNA silencing
33.	AT3G44530	*HIRA1*	HIRA protein	HIRA proteins are histone-interacting proteins, which function in the nucleosome assembly pathway to facilitate the deposition of histones onto the DNA
34.	AT3G55080	X	NOT PRESENT	(TAIR: SET domain-containing protein)
35.	AT3G56570	X	NOT PRESENT	(TAIR: SET domain-containing protein; similar to ribulose-1,5 bisphosphate carboxylase oxygenase large subunit N-methyltransferase)

No.	AGI code	Name(s)	ChromDB Protein Group	Additional Description of ChromDB or TAIR
36.	AT3G59960	*ASHH4, SDG24, SET24*	ASH1	Predicted histone H3 lysine 36 histone methyltransferase; ASH1 group
37.	AT4G08990	*METIIb, DMT8*	DNA methyltransferases	Putative DNA methyltransferase related to the mammalian DNMT1 methyltransferases
38.	AT4G11130	*RDR2*	RNA-dependent RNA polymerases	RNA-dependent RNA polymerase
39.	AT4G13610	*MET3, DMT3*	DNA methyltransferases	Putative DNA methyltransferase related to the mammalian DNMT1 methyltransferases
40.	AT4G13940	*HOG1, MEE58, SAHH1*	NOT PRESENT	(TAIR: Encodes a S-adenosyl-L-homocysteine hydrolase required for DNA methylation-dependent gene silencing)
41.	AT4G15180	*ATXR3, SDG2, SET2*	TRR_TRX	Member of the TRR (Trithorax-related protein) family of predicted histone H3 lysine 4 methyltransferases
42.	AT4G19020	*CMT2, DMT5*	DNA methyltransferases	Class II DNA methyltransferase; a putative DNA methyltransferase containing a chromodomain (chromomethylase)
43.	AT4G20910	*HEN1, CRM2*	HUA enhancer	HUA enhancer; acts to specify reproductive organ identities and to repress A gene in *Arabidopsis* and may function in microRNA biogenesis
44.	AT5G04560	*DME, DNG3, SET32*	Superfamily of DNA glycosylases	DEMETER (DME) family of bifunctional DNA glycosylases, required for maternal allele expression of the imprinted MEDEA (MEA) polycomb gene in the central cell and endosperm
45.	AT5G04940	*SUVH1, SDG32*	SUVH1/SUVH3	Su(var)3-9 group; plant specific sub-group with YDG_SRA, Pre-SET, and SET domains
46.	AT5G06620	*ATXR4, SDG38,SET38*	S-ET protein containing an interrupted SET domain	S-ET interrupted and unclassified
47.	AT5G09230	*SRT2, HDA16*	Histone deacetylases (SIR2 family)	SIR2 homology group; probable ortholog of yeast SIR2, an NADH dependent histone deacetylase
48.	AT5G13960	*SUVH4, SDG33, KYP, SET33*	ARATH_Kryptonite	Su(var)3-9 group; plant specific sub-group with YDG_SRA, Pre-SET, and SET domains
49.	AT5G14260	X	NOT PRESENT	(TAIR: SET domain-containing protein)
50.	AT5G14620	*DRM2, DMT7*	DNA methyltransferases	Class III DNA methyltransferase: a putative DNA methyltransferase with rearranged catalytic domains; similar to mammalian DNMT3 methyltransferases
51.	AT5G15380	*DRM1, DMT9*	DNA methyltransferases	putative DNA methyltransferase with rearranged catalytic domains; similar to mammalian DNMT3 methyltransferases
52.	AT5G17240	*SDG40*	SET Domain Protein Superclass B	SET domain protein
53.	AT5G42400	*ATXR7, SDG25, SET25*	ARATH_ATXR7	SET domain protein

No.	AGI code	Name(s)	ChromDB Protein Group	Additional Description of ChromDB or TAIR
54.	AT5G43990	*SUVR2, SDG18, SET18*	ARATH_SUVR4	Su(var)3-9 group of confirmed and predicted histone H3 lysine 9 methyltransferases
55.	AT5G49160	*MET1, DDM2, DMT1*	DNA methyltransferases	DNA methyltransferase related to the mammalian DNMT1 methyl-transferases
56.	AT5G55760	*SRT1, HDA12*	Histone deacetylases (SIR2 family)	SIR2 Homology Group; probable ortholog of yeast SIR2, an NADH dependent Histone Deacetylase
57.	AT5G63110	*HDA6*	Histone deacetylases (Rpd3/HDA1 superfamily)	Class I RPD3 type histone deacetylase protein
58.	AT5G66750	*DDM1, CHR1, CHA1*	SNF2 super family (Snf2, Ris1, Rad26 superclasses)	SWI2/SNF2 chromatin remodelling protein involved in the maintenance of DNA methylation

Legend:

X – does not exist

3.2.4. Selection of a final list of candidate genes for possible involvement in heterosis

A final list of heterosis candidate regulatory genes was compiled from both independent qRT-PCR profiling experiments, described in sections 3.2.2 and 3.2.3. The final list consisted of 61 genes including 57 putative or known TFs, three SET-domain genes (AT1G26760 [4], AT4G13460 [29], and AT5G43990 [52]), and one microRNA (AT5G08712 [36]); (Tables 3.3 and 3.4).

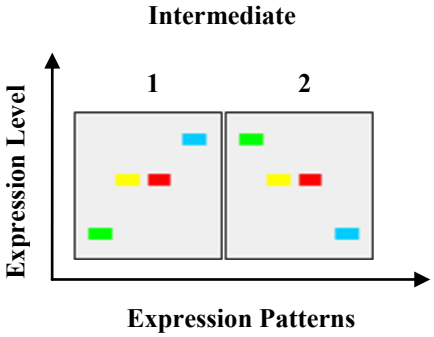
3.3. Characterisation of selected candidate genes

3.3.1. Analysis of expression patterns of candidate genes

The PTM (Pavlidis Template Matching) function within TIGR_MeV v. 3.0 software package was used to visualise expression patterns (or expression phenotypes) of candidate genes based on specific expression level relations in parents and hybrids (details are present Materials and Methods, section 2.10.4). Expression patterns could be classified into two general groups: additive and non-additive (Hoecker *et al.,* 2008). The additive pattern, synonymous to 'intermediate' pattern in this work, was defined for (or assigned to) these candidate genes, in which gene expression levels in hybrids fell in a range of average expression value of the two parental inbred lines (mid-parent expression value or MP). The non-additive patterns were defined for (or assigned to) these candidate genes, in which gene expression levels in hybrids were significantly different than the MP. The group of non-additive effects included 'dominant' (transcript levels in hybrids were on the level of one of the parents), 'overdominant' and 'underdominant' (transcript levels in hybrids were higher or lower than in parents, respectively), 'maternal' (hybrid transcript level was on the level of corresponding mother parent), and 'paternal' (hybrid transcript level was on the level of corresponding father parent) patterns. All the patterns were further discriminated in relation to expression level of a parent e.g. a 'dominant_C24xC24_low' pattern meant that the hybrid expression levels were on a level of C24xC24, and these three levels were lower than of Col-0xCol-0. Description of specific patterns is portrayed in Figure 3.10 and Table 3.2.

Most candidate genes (75%) fell into specific expression categories (or displayed expression patterns) that were defined in this thesis work (Figure 3.10). The most representative pattern was an intermediate (around 30%), following by a dominant (23%). Among candidate genes were also found maternal (19%) and paternal patterns (3%); (Table 3.2).

Additive expression patterns:



Non-additive expression patterns:

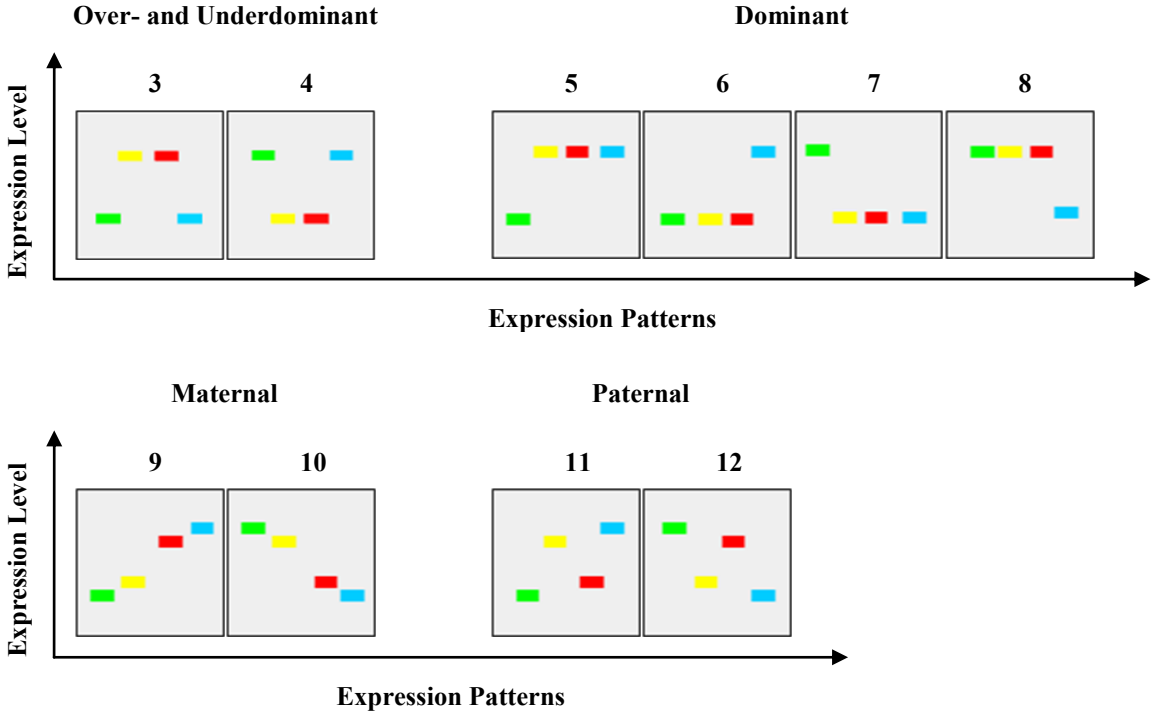


Figure 3.10. Templates scheme for expression patterns assigned to candidate genes

Colour bars represent the genotypes and their position indicates a transcript level

Col-0xCol-0 Col-0xC24 C24xCol-0 C24xC24

Table 3.2. Representation of expression patterns among candidate genes at 4 DAS

Template Number	Gene Expression Patterns	Candidate Genes [AGI order number]	% Candidates (rounded)	Sum % per Expression Pattern
1.	Intermediate_C24xC24_high	[3], [18], **[19]**, [45], **[46]**, **[49]**, **[56]**	11	29
2.	Intermediate_Col-0xCol-0_high	**[6]**, [10], **[11]**, **[16]**, [22], **[28]**, **[38]**, **[47]**, [50], **[52]***, **[59]**	18	
3.	Overdominant (F1 high)	x	x	0
4.	Underdominant (F1 low)	x	x	
5.	Dominant_C24xC24_high	[37], [58]	3	23
6.	Dominant_Col-0xCol-0_low	**[43]**	2	
7.	Dominant_C24xC24_low	**[20]**, **[36]**,** [40], **[61]**	7	
8.	Dominant_Col-0xCol-0_high	**[1]**, [4]*, [24], [26], [29]*, [44], [53]	11	
9.	Maternal_C24xC24_high	[41], [54]	3	19
10.	Maternal_Col-0xCol-0_high	[5], [13], [14], [23], [27], **[31]**, **[33]**, [39], [55], [57]	16	
11.	Paternal_C24xC24_high	[51]	2	3
12.	Paternal_Col-0xCol-0_high	[8]	2	

Legend:

* candidate SET-domain gene (a member of the 'chromatin-related' group of genes)
** candidate micro-RNA
[AGI order numbers] in bold – candidate genes with the highest ranking group number (I) in the group of 'statistical category' (refer to section 3.3.2)
x – not present

3.3.2. Rank of statistical significance of candidate genes ('statistical categories')

As described in previous chapters, pairwise comparisons of gene expression levels in different genotypes were performed using ANOVA to identify differences between parents and hybrids and select heterosis candidate genes. Note that although ANOVA analysis can identify difference between the mean of two or more groups, it cannot identify what means there is a significant difference between. For this reason, to validate expression patterns visualised by TIGR_Mev v. 3.0 some additional statistical analyses were required. A type of *post-hoc* analysis, a least significant difference (LSD) test was used to make pairwise comparisons among means of different genotypes. The results were shown as LSD P-values in Table 3.3 (columns 3-8). An LSD significance threshold of a P-value < 0.05 was applied (Table 3.3 fields in grey colour). There was also performed an additional ANOVA test to find significant differences between expression level of each of the hybrids and a mid-parent expression value (an average of the parental expression level or MP) to validate non-additive effects statistically (columns 9 and 10 of Table 3.3). These results were also shown as P-values and the same threshold was applied to determine a significant difference. Based on the results obtained from both of the above mentioned statistical analyses, it was possible to estimate a sig-

nificance level of the selected heterosis candidate genes and create their rank of significance (Table 3.3, the last column). The slower rank number of a group that candidate gene belongs, the more significant candidate gene is. The criteria of the rank classification were defined as follows:

- Ranking group I ('statistical category' I) – included 20 candidate genes (~33%), for which the assigned expression pattern could be validated in all of the performed statistical tests. (All the candidate genes of ranking group I were marked in bold in Table 3.2). The example may be given by AT4G29190 [31] (row no. 19 of Table 3.3), which was assigned by TIGR_Mev v. 3.0 tools to a non-additive, maternal_Col-0xCol-0_high pattern. To validate the maternal expression patterns defined in this work (template 10 of Figure 3.10), the significant difference between expression levels should hold true in the same time when the parents are compared between themselves, the hybrids are compared between themselves, Col-0xC24 is compared to C24xC24, and C24xCol-0 to Col-0xCol-0 (columns 3, 4, 7 and 8). In parallel a significant difference between expression level of each of the two hybrids and MP should be found (columns 9 and 10).
- Ranking group II ('statistical category' II) – included 26 candidate genes (~42%), for which the assigned expression pattern could not be validated in all of the performed statistical tests. The example may be given by AT1G72650 [13] (row no. 37 of Table 3.3), which was assigned by TIGR_Mev v. 3.0 tools to a non-additive, maternal_Col-0xCol-0_high pattern. To validate the maternal expression patterns defined in this work (template 10 of Figure 3.10), the same conditions as in the Ranking group I should be fulfilled however in this case, when one of the hybrids was compared to MP (column 9) the significant difference could not be found in the performed statistical tests. Thus, in contrast to above mentioned AT4G29190 [31], the AT1G72650 [13] could not be 'fully validated'.
- Ranking group III ('statistical category' III) – included 15 candidate genes (~25%) that do not exhibited any of defined expression patterns discussed above (Figure 3.10 and Table 3.3) and the results of required statistical comparisons represented unclear picture.

Table 3.3. 'Statistical categories' (ranking groups of significance) represented among heterosis candidate genes

No.	AGI code	P-values Col-0xCol-0 vs. C24xC24	P-values Col-0xC24 vs. C24xC24	P-values C24xCol-0 vs. C24xC24	P-values Col-0xC24 vs. Col-0xCol-0	P-values C24xCol-0 vs. Col-0xCol-0	P-values Col-0xC24 vs. C24xCol-0	P-values Col-0xC24 vs. MP	P-values C24xCol-0 vs. MP	Expression Pattern Type		Ranking Group of Candidate Gene Significance
1	2	3	4	5	6	7	8	9	10	11	12	13
1.	AT5G25810 [43]	0.000	0.001	0.001	0.156	0.324	0.604	0.047	0.020	Dominant_Col-0xCol-0_low	Non-add.	I
2.	AT2G45660 [20]	0.000	0.696	0.532	0.000	0.000	0.808	0.004	0.003	Dominant_C24xC24_low	Non-add.	I
3.	AT5G08712 [36]*	0.000	0.496	0.558	0.000	0.000	0.918	0.001	0.001	Dominant_C24xC24_low	Non-add.	I
4.	AT5G67480 [61]	0.000	0.469	0.374	0.000	0.000	0.858	0.011	0.014	Dominant_C24xC24_low	Non-add.	I
5.	AT1G12800 [1]	0.000	0.000	0.000	0.435	0.415	0.138	0.011	0.001	Dominant_Col-0xCol-0_high	Non-add.	I
6.	AT4G13460 [29]**	0.000	0.001	0.000	0.268	0.836	0.354	0.031	0.008	Dominant_Col-0xCol-0_high	Non-add.	I
7.	AT2G39250 [19]	0.000	0.003	0.023	0.006	0.001	0.149	0.785	0.155	Intermediate_C24xC24_high	Add.	I
8.	AT5G32460 [46]	0.000	0.020	0.017	0.001	0.001	0.913	0.062	0.072	Intermediate_C24xC24_high	Add.	I
9.	AT5G39760 [49]	0.000	0.002	0.001	0.004	0.008	0.589	0.636	0.291	Intermediate_C24xC24_high	Add.	I
10.	AT5G57390 [56]	0.000	0.001	0.000	0.000	0.001	0.446	0.925	0.431	Intermediate_C24xC24_high	Add.	I
11.	AT1G32870 [6]	0.000	0.018	0.017	0.001	0.001	0.965	0.119	0.128	Intermediate_Col-0xCol-0_high	Add.	I
12.	AT1G58220 [11]	0.000	0.005	0.004	0.003	0.004	0.777	0.794	0.947	Intermediate_Col-0xCol-0_high	Add.	I
13.	AT1G77080 [16]	0.000	0.006	0.003	0.004	0.007	0.664	0.868	0.735	Intermediate_Col-0xCol-0_high	Add.	I
14.	AT4G12020 [28]	0.000	0.005	0.009	0.002	0.001	0.630	0.692	0.355	Intermediate_Col-0xCol-0_high	Add.	I

No.	AGI code	P-values Col-0xCol-0 vs. C24xC24	P-values Col-0xC24 vs. C24xC24	P-values C24xCol-0 vs. C24xC24	P-values Col-0xC24 vs. Col-0xCol-0	P-values C24xCol-0 vs. Col-0xCol-0	P-values Col-0xC24 vs. C24xCol-0	P-values Col-0xC24 vs. MP	P-values C24xCol-0 vs. MP	Expression Pattern Type		Ranking Group of Candidate Gene Significance
1	2	3	4	5	6	7	8	9	10	11	12	13
15.	AT5G11270 [38]	0.000	0.002	0.001	0.013	0.047	0.360	0.291	0.061	Intermediate_Col-0xCol-0_high	Add.	I
16.	AT5G38860 [47]	0.000	0.006	0.015	0.002	0.001	0.502	0.523	0.184	Intermediate_Col-0xCol-0_high	Add.	I
17.	AT5G43990 [52]**	0.000	0.001	0.001	0.019	0.027	0.797	0.184	0.120	Intermediate_Col-0xCol-0_high	Add.	I
18.	AT5G63080 [59]	0.000	0.004	0.022	0.005	0.001	0.220	0.904	0.195	Intermediate_Col-0xCol-0_high	Add.	I
19.	AT4G29190 [31]	0.000	0.000	0.751	0.775	0.000	0.000	0.004	0.009	Maternal_Col-0xCol-0_high	Non-add.	I
20.	AT5G01160 [33]	0.004	0.001	0.152	0.376	0.001	0.000	0.009	0.004	Maternal_Col-0xCol-0_high	Non-add.	I
21.	AT1G26760 [4]**	0.002	0.006	0.001	0.307	0.583	0.141	0.124	0.010	Dominant_Col-0xCol-0_high	Non-add.	II
22.	AT3G50890 [24]	0.004	0.005	0.001	0.750	0.389	0.253	0.065	0.010	Dominant_Col-0xCol-0_high	Non-add.	II
23.	AT4G04880 [26]	0.015	0.031	0.007	0.592	0.538	0.269	0.244	0.036	Dominant_Col-0xCol-0_high	Non-add.	II
24.	AT1G73830 [14]	0.000	0.002	0.351	0.130	0.001	0.005	0.091	0.028	Maternal_Col-0xCol-0_high	Non-add.	II
25.	AT1G47760 [8]	0.001	0.119	0.000	0.006	0.055	0.001	0.229	0.001	Paternal_Col-0xCol-0_high	Non-add.	II
26.	AT5G10140 [37]	0.000	0.043	0.244	0.000	0.000	0.252	0.000	0.000	Dominant_C24xC24_high	Non-add.	II
27.	AT5G17300 [40]	0.000	0.009	0.137	0.000	0.000	0.086	0.009	0.001	Dominant_C24xC24_low	Non-add.	II
28.	AT2G28160 [18]	0.000	0.002	0.002	0.054	0.075	0.824	0.152	0.105	Intermediate_C24xC24_high	Add.	II
29.	AT5G41920 [50]	0.000	0.010	0.057	0.005	0.001	0.230	0.707	0.101	Intermediate_Col-0xCol-0_high	Add.	II
30.	AT5G27580 [45]	0.015	0.130	0.667	0.155	0.026	0.241	0.943	0.203	Intermediate_C24xC24_high	Add.	II

No.	AGI code	P-values Col-0xCol-0 vs. C24xC24	P-values Col-0xC24 vs. C24xC24	P-values C24xCol-0 vs. C24xC24	P-values Col-0xC24 vs. Col-0xCol-0	P-values C24xCol-0 vs. Col-0xCol-0	P-values Col-0xC24 vs. C24xCol-0	P-values Col-0xC24 vs. MP	P-values C24xCol-0 vs. MP	Expression Pattern Type		Ranking Group of Candidate Gene Significance
1	2	3	4	5	6	7	8	9	10	11	12	13
31.	AT1G53160 [10]	0.021	0.071	0.029	0.392	0.801	0.535	0.491	0.186	Intermediate_Col-0xCol-0_high	Add.	II
32.	AT3G46090 [22]	0.329	0.037	0.738	0.966	0.230	0.354	0.323	0.766	Intermediate_Col-0xCol-0_high	Add.	II
33.	AT5G61420 [58]	0.000	0.039	0.986	0.001	0.000	0.040	0.113	0.003	Dominant_C24xC24_high	Non-add.	II
34.	AT1G20696 [3]	0.000	0.003	0.001	0.001	0.010	0.035	0.669	0.036	Intermediate_C24xC24_high	Add.	II
35.	AT5G17320 [41]	0.001	0.015	0.828	0.048	0.001	0.020	0.622	0.021	Maternal_C24xC24_high	Non-add.	II
36.	AT1G28370 [5]	0.000	0.016	0.929	0.010	0.000	0.018	0.822	0.008	Maternal_Col-0xCol-0_high	Non-add.	II
37.	AT1G72650 [13]	0.000	0.000	0.012	0.010	0.000	0.006	0.061	0.046	Maternal_Col-0xCol-0_high	Non-add.	II
38.	AT3G49530 [23]	0.000	0.012	0.429	0.017	0.000	0.005	0.888	0.003	Maternal_Col-0xCol-0_high	Non-add.	II
39.	AT5G13790 [39]	0.000	0.001	0.056	0.003	0.000	0.010	0.522	0.011	Maternal_Col-0xCol-0_high	Non-add.	II
40.	AT5G47370 [55]	0.000	0.004	0.156	0.022	0.001	0.027	0.428	0.045	Maternal_Col-0xCol-0_high	Non-add.	II
41.	AT5G59820 [57]	0.000	0.012	0.438	0.014	0.000	0.005	0.954	0.003	Maternal_Col-0xCol-0_high	Non-add.	II
42.	AT5G25830 [44]	0.039	0.185	0.032	0.302	0.891	0.250	0.838	0.143	Dominant_Col-0xCol-0_high	Non-add.	II
43.	AT5G44080 [53]	0.000	0.001	0.004	0.001	0.000	0.138	0.993	0.097	Dominant_Col-0xCol-0_high	Non-add.	II
44.	AT5G46690 [54]	0.000	0.001	0.015	0.024	0.001	0.044	0.143	0.257	Maternal_C24xC24_high	Non-add.	II
45.	AT4G08250 [27]	0.000	0.001	0.012	0.004	0.000	0.030	0.306	0.077	Maternal_Col-0xCol-0_high	Non-add.	II
46.	AT5G43170 [51]	0.000	0.041	0.001	0.001	0.016	0.026	0.077	0.250	Paternal_C24xC24_high	Non-add.	II

No.	AGI code	P-values Col-0xCol-0 vs. C24xC24	P-values Col-0xC24 vs. C24xC24	P-values C24xCol-0 vs. C24xC24	P-values Col-0xC24 vs. Col-0xCol-0	P-values C24xCol-0 vs. Col-0xCol-0	P-values Col-0xC24 vs. C24xCol-0	P-values Col-0xC24 vs. MP	P-values C24xCol-0 vs. MP	Expression Pattern Type		Ranking Group of Candidate Gene Significance
1	2	3	4	5	6	7	8	9	10	11	12	13
47.	AT1G42990 [7]	0.000	0.007	0.940	0.012	0.000	0.006	0.809	0.004	No match		III
48.	AT1G76590 [15]	0.000	0.021	0.185	0.001	0.000	0.093	0.103	0.007	No match		III
49.	AT3G25990 [21]	0.015	0.446	0.059	0.043	0.001	0.020	0.354	0.004	No match		III
50.	AT4G14560 [30]	0.000	0.005	0.114	0.002	0.000	0.048	0.636	0.015	No match		III
51.	AT5G04760 [34]	0.000	0.012	0.182	0.001	0.000	0.002	0.128	0.000	No match		III
52.	AT1G16530 [2]	0.053	0.036	0.007	0.783	0.160	0.237	0.137	0.018	No match		III
53.	AT2G15580 [17]	0.299	0.091	0.018	0.415	0.082	0.273	0.147	0.022	No match		III
54.	AT5G07690 [35]	0.096	0.532	0.184	0.238	0.013	0.073	0.721	0.028	No match		III
55.	AT5G17810 [42]	0.016	0.144	0.645	0.154	0.009	0.074	0.978	0.048	No match		III
56.	AT1G51070 [9]	0.000	0.002	0.001	0.045	0.073	0.736	0.157	0.089	No match		III
57.	AT3G53370 [25]	0.000	0.001	0.000	0.001	0.005	0.097	0.699	0.112	No match		III
58.	AT4G37610 [32]	0.000	0.018	0.043	0.002	0.001	0.520	0.252	0.086	No match		III
59.	AT5G63160 [60]	0.027	0.034	0.034	0.878	0.875	0.991	0.187	0.188	No match		III
60.	AT1G69490 [12]	0.022	0.072	0.475	0.405	0.060	0.205	0.486	0.404	No match		III
61.	AT5G39610 [48]	0.036	0.030	0.343	0.816	0.420	0.342	0.217	0.991	No match		III

Legend:

Numbers in columns 3-8 are P-values from LSD analysis, whereas in columns 9-10 from ANOVA
The grey fields mark P-values < 0.05 (threshold of significance)
Non-add. – Non-additive; Add. – Additive
* candidate microRNA
** candidate SET-domain gene (a member of the 'chromatin-related' group of genes)

3.3.3. Biological significance of candidate genes

Annotations of 61 heterosis candidate genes were collected in Table 3.4, which also provided a specific reference for each known gene, where possible. For uncharacterised genes (30) a description of the gene family was given. According to the most comprehensive information source for TF genes which is a DATF database (Guo *et al.,* 2005), the candidate genes included 51 TF or putative TF genes. Figure 3.11 unveils a variety of TF families represented among the identified heterosis candidate genes. MADS, bHLH, AP2-EREBP and NAM families were highly represented.

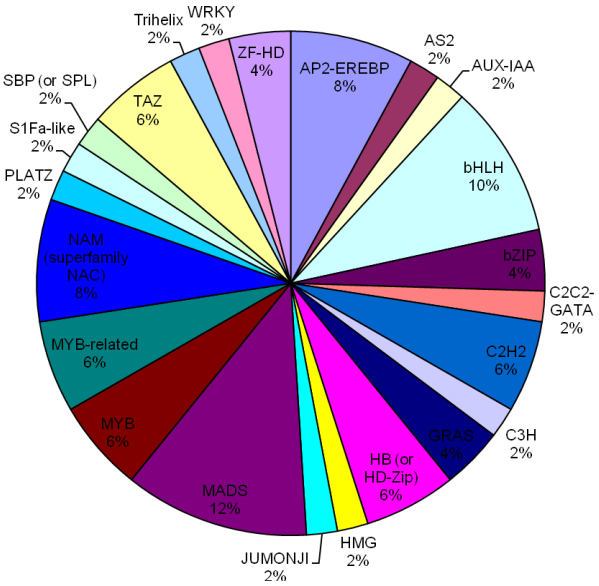


Figure 3.11. TF families represented by the identified candidate genes

The classification of AT4G13460 [29] and AT5G43990 [52] was questionable due to discrepancies that occur between databases referred in this work, a DATF (PcG TF family) and ChromDB (SET-domain family), respectively. There were also cases, in which a certain gene could not be found neither in DATF (the TF database of main reference for this work) nor in ChromDB (the reference database for 'chromatin related' genes), thus the TAIR database information was considered. All these candidate genes for which database information was not consistent or it was limited to a one source were collected in a Table 3.5. A final classification that have been approved for this work resulted in 57 TF or putative TF genes, the one microRNA, and the three SET-domain genes.

Table 3.4. Annotations and published information about heterosis candidate genes

No.	AGI code [AGI order number]	Ranking Group of Candidate Gene Significance	DATF Gene Name(s)	DATF Gene Family	Gene Function/Process Involvement in *Arabidopsis*	Literature Gene-Specific	Literature Family-Specific
1.	**AT1G12800 [1]**	I	N/P	N/P	UNKNOWN	N/A	N/A
2.	AT1G32870 [6]	I	*ANAC13*	NAM/NAC	ultraviolet-B regulated	Safrany *et al.*, 2008	
3.	**AT1G58220 [11]**	I	x	MYB-related	UNKNOWN	N/A	Martin and Paz-Ares, 1997; Jin and Martin, 1999; Stracke *et al.*, 2001
4.	AT1G77080 [16]	I	*AGL27, FLM, MAF1*	MADS	inhibitor of flowering	Scortecci *et al.*, 2003	
5.	AT2G39250 [19]	I	*SNZ*	AP2-EREBP	regulation of flowering	Schmid *et al.*, 2003	
6.	AT2G45660 [20]	I	*AGL20, SOC1*	MADS	flowering control, floral pathway integrator; affects determinacy of all meristems; prevention of secondary growth and longevity in annual life forms	Lee *et al.*, 2000; Moon *et al.*, 2003; Simpson and Dean 2002; Mouradov *et al.*, 2002; Melzer *et al.*, 2008	
7.	AT4G12020 [28]	I	*MAPKKK11, WRKY19*	WRKY	UNKNOWN	N/A	Eulgem *et al.*, 2000
8.	**AT4G13460 [29]**	I	*SUVH9*	PcG	epigenetic control of gene expression	Baumbusch *et al.*, 2001; Ng *et al.*, 2007	
9.	AT4G29190 [31]	I	x	C3H	UNKNOWN	N/A	Wang *et al.*, 2008
10.	AT5G01160 [33]	I	N/P	N/P	UNKNOWN	N/A	Chrispeels *et al.*, 2000

No.	AGI code [AGI order number]	Ranking Group of Candidate Gene Significance	DATF Gene Name(s)	DATF Gene Family	Gene Function/Process Involvement in *Arabidopsis*	Literature Gene-Specific	Literature Family-Specific
11.	AT5G08712 [36]	I	N/P	N/P	targets genes that regulate diverse aspects of plant development, including apical and lateral meristem formation, leaf polarity, and vascular development; regulation of shoot apical meristem and floral development in *Arabidopsis*	Jung and Park, 2007	
12.	AT5G11270 [38]	I	N/P	N/P	mediates resistance to infection by necrotrophic pathogens	Coego *et al.,* 2005	
13.	AT5G25810 [43]	I	*TNY, TINY*	AP2-EREBP	might play a role in the cross-talk between abiotic- and biotic-stress-responsive gene expressions	Sun *et al.,* 2008	
14.	AT5G32460 [46]	I	N/P	N/P	UNKNOWN	N/A	Franco-Zorrilla *et al.,*2002
15.	AT5G38860 [47]	I	*BIM3*	bHLH	UNKNOWN	N/A	Toledo-Ortiz *et al.,* 2003
16.	AT5G39760 [49]	I	*ATHB23*	ZF-HD	establishing polarity during leaf development	Kim *et al.,* 2007	
17.	AT5G43990 [52]	I	*SUVR2*	PcG	epigenetic control of gene expression and possible involvement in regulation of rRNA expression	Baumbusch *et al.,* 2001; Thorstensen *et al.,* 2006	
18.	AT5G57390 [56]	I	*AIL5*	AP2-EREBP	roles in specification of meristematic or division-competent states especially in young tissues	Nole-Wilson *et al.,* 2005	
19.	AT5G63080 [59]	I	x	JUMONJI	UNKNOWN	N/A	Noh *et al.,* 2004
20.	AT5G67480 [61]	I	*BT4*	TAZ	Ca^{2+}/Calmodulin-binding	Du and Poovaiah, 2004	
21.	**AT1G26760 [4]**	II	N/P	N/P	UNKNOWN	N/A	

No.	AGI code [AGI order number]	Ranking Group of Candidate Gene Significance	DATF Gene Name(s)	DATF Gene Family	Gene Function/Process Involvement in *Arabidopsis*	Literature Gene-Specific	Literature Family-Specific
22.	AT1G47760 [8]	II	x	MADS	UNKNOWN	N/A	Parenicova *et al.,* 2003; Becker and Theissen, 2003; Messenguy and Dubois, 2003; Kaufmann *et al.,* 2005
23.	AT1G73830 [14]	II	*BEE3*	bHLH	brassinosteroid signalling, required for normal growth	Friedrichsen *et al.,* 2002	
24.	**AT3G50890 [24]**	II	*ATHB28*	ZF-HD	UNKNOWN	N/A	Windhovel *et al.,* 2001
25.	**AT4G04880 [26]**	II	N/P	N/P	UNKNOWN	N/A	x
26.	AT2G28160 [18]	II	*BHLH029, FIT1, FRU*	bHLH	required for the iron deficiency response	Colangelo and Guerinot, 2004	
27.	**AT5G10140 [37]**	II	*AGL25, FLC, FLF*	MADS	flowering control	Michels and Amasino, 1999; Sheldon *et al.,* 1999	
28.	AT5G17300 [40]	II	x	MYB-related	UNKNOWN	N/A	Martin and Paz-Ares, 1997; Jin and Martin, 1999; Stracke *et al.,* 2001;
29.	**AT5G41920 [50]**	II	x	GRAS	UNKNOWN	N/A	Pysh *et al.,* 1999
30.	**AT1G53160 [10]**	II	*SPL4*	SBP/SPL	UNKNOWN	Yamasaki, 2004	
31.	**AT3G46090 [22]**	II	x	C2H2	key role in the defence response of *Arabidopsis* to salinity stress	Ciftci-Yilmaz *et al.,* 2007	
32.	**AT5G27580 [45]**	II	x	MADS	UNKNOWN	N/A	Parenicova *et al.,* 2003; Becker and Theissen, 2003; Messenguy and Dubois, 2003; Kaufmann *et al.,* 2005
33.	AT1G20696 [3]	II	*HMGB3, NFD3*	HMG	UNKNOWN	N/A	Gupta *et al.,* 1997; Yamaguchi-Shinozaki and Shinozaki, 1992
34.	AT1G28370 [5]	II	*ERF11*	AP2-EREBP	possible involvement in ABA and glucose responses	De Luna *et al.,* 2007	

No.	AGI code [AGI order number]	Ranking Group of Candidate Gene Significance	DATF Gene Name(s)	DATF Gene Family	Gene Function/Process Involvement in *Arabidopsis*	Literature Gene-Specific	Literature Family-Specific
35.	AT1G72650 [13]	II	*TRFL6*	MYB-related	UNKNOWN	N/A	Martin and Paz-Ares, 1997; Jin and Martin, 1999; Stracke *et al.,* 2001;
36.	AT3G49530 [23]	II	*ANAC062*	NAM/NAC	UNKNOWN	N/A	Duval *et al.,* 2002
37.	AT5G13790 [39]	II	*AGL15*	MADS	recruitment of histone deacetylase complex components, promotes somatic embryo development	Hill *et al.,* 2008; Harding *et al.,* 2003	
38.	**AT5G17320 [41]**	II	x	HB/HD-Zip	UNKNOWN	N/A	Sessa *et al.,* 1997; Kim *et al.,* 2008
39.	AT5G47370 [55]	II	*HAT2*	HB/HD-Zip	regulation of auxin-mediated morphogenesis in shoot and root	Sawa *et al.,* 2002	
40.	AT5G59820 [57]	II	*RHL41, ZAT12*	C2H2	plays a central role in reactive oxygen and abiotic stress signalling, influences freezing tolerance, important component of the oxidative stress response signal transduction network	Davletova *et al.,* 2005; Vogel *et al.,* 2005; Rizhsky *et al.,* 2004	
41.	AT5G61420 [58]	II	*MYB28*	MYB	regulator of methionine-derived glucosinolate biosynthesis	Gigolashvili *et al.,* 2007b	
42.	AT4G08250 [27]	II	x	GRAS	UNKNOWN	N/A	Pysh *et al.,* 1999
43.	**AT5G25830 [44]**	II	x	C2C2-GATA	N/A	N/A	Teakle *et al.,* 2002
44.	AT5G43170 [51]	II	*AZF3*	C2H2	water-stress response in an ABA-dependent or -independent pathway		
45.	**AT5G44080 [53]**	II	x	bZIP	UNKNOWN	N/A	Jakoby *et al.,* 2002
46.	AT5G46690 [54]	II	x	bHLH	UNKNOWN	N/A	Toledo-Ortiz *et al.,* 2003
47.	AT1G42990 [7]	III	*ATBZIP60*	bZIP	endoplasmic reticulum stress response	Iwata and Koizumi, 2005	
48.	AT1G76590 [15]	III	x	PLATZ	UNKNOWN	N/A	Nagano *et al.,* 1991

No.	AGI code [AGI order number]	Ranking Group of Candidate Gene Significance	DATF Gene Name(s)	DATF Gene Family	Gene Function/Process Involvement in *Arabidopsis*	Literature Gene-Specific	Literature Family-Specific
49.	**AT3G25990 [21]**	III	x	Trihelix	UNKNOWN	N/A	Smalle *et al.,* 1998
50.	AT4G14560 [30]	III	*AXR5, IAA1*	AUX-IAA	required for auxin response	Yang *et al.,* 2004	
51.	AT5G04760 [34]	III	x	MYB	UNKNOWN	N/A	Martin and Paz-Ares, 1997; Jin and Martin, 1999; Stracke *et al.,* 2001
52.	**AT1G16530 [2]**	III	*LBD, ASL9*	AS2	exclusively regulated by cytokinin	Naito *et al.,* 2007	
53.	**AT2G15580 [17]**	III	N/P	N/P	UNKNOWN	N/A	Riechmann *et al.,* 2000
54.	**AT5G07690 [35]**	III	*MYB29*	MYB	regulator of aliphatic glucosinolate biosynthesis	Gigolashvili *et al.,* 2007	
55.	**AT5G17810 [42]**	III	*WOX12*	HB/HD-Zip	possible involvement in embryonic pattern formation	Haecker *et al.,* 2004	
56.	AT1G51070 [9]	III	x	bHLH	UNKNOWN	N/A	Toledo-Ortiz *et al.,* 2003
57.	AT3G53370 [25]	III	x	S1Fa-like	UNKNOWN	N/A	Zhou *et al.,* 1995
58.	AT4G37610 [32]	III	*BT5*	TAZ	Ca^{2+}/Calmodulin-binding	Du and Poovaiah, 2004	
59.	AT5G63160 [60]	III	*BT1*	TAZ	Ca^{2+}/Calmodulin-binding	Du and Poovaiah, 2004	
60.	**AT1G69490 [12]**	III	*ANAC029, NAP, AtNAP*	NAM/NAC	expression is associated with leaf senescence	Guo and Gan, 2006	
61.	**AT5G39610 [48]**	III	*ANAC092, AT-NAC2, ATNAC6*	NAM/NAC	salt stress response and lateral root development	He *et al.,* 2005	

Legend:

The genes in bold were selected from the 1st experiment on identification of heterosis candidate genes
N/P – not present in DATF database (refer to Table 3.5)
N/A – not available
x – does not exist

Table 3.5. Candidate genes for which the annotations varied depending on database source

No.	AGI code [AGI order number]	Ranking Group of Candidate Gene Significance	DATF Gene Name(s)	DATF Gene Family	TAIR Gene Name(s)	TAIR Gene Family/ Gene Prediction	ChromDB Gene Name(s)	ChromDB Protein Group
1.	**AT1G12800 [1]**	I	N/P	N/P	x	S1 RNA-binding do-main-containing protein	N/P	N/P
2.	**AT1G58220 [11]**	I	x	MYB-related	x	MYB	N/P	N/P
3.	**AT4G13460 [29]**	I	*SUVH9*	PcG	*SUVH9, SDG22, SET22*	SET domain, SU(VAR)3-9 protein subgroup	*SUVH9, SDG22, SET22*	ARATH_SUV H2, SUVH9
4.	AT5G01160 [33]	I	N/P	N/P	x	C2H2-type, RING-type Zinc Finger	N/P	N/P
5.	AT5G08712 [36]	I	N/P	N/P	*MIR166, MIR166C*	x	N/P	N/P
6.	AT5G11270 [38]	I	N/P	N/P	*OCP3*	Homeodomain	N/P	N/P
7.	AT5G32460 [46]	I	N/P	N/P	x	pseudogene, possible B3	N/P	N/P
8.	AT5G43990 [52]	I	*SUVR2*	PcG	*SUVR2*	SET domain, SU(VAR)3-9 protein subgroup	*SUVR2, SDG18,*	ARATH_SUV R4
9.	**AT1G26760 [4]**	II	N/P	N/P	x	SET domain	*SDG35, SET35, ATXR1*	unclassified
10.	**AT4G04880 [26]**	II	N/P	N/P	x	adenosine/AMP deami-nase	N/P	N/P
11.	AT5G17300 [40]	II	x	MYB-related	x	MYB	N/P	N/P
12.	AT1G72650 [13]	II	*TRFL6*	MYB-related	*TRFL6*	MYB	N/P	N/P
13.	**AT2G15580 [17]**	III	N/P	N/P	x	C3H (C3HC4-type RING finger)	N/P	N/P

Legend:

The genes in bold were selected from the 1st experiment on identification of heterosis TF candidate genes
N/P – genes not present in database
x – does not exist

3.3.4. Review of publicly available expression data for heterosis associated candidate genes

'Meta-profiles' tool of web-based application, a GenevestigatorV3 (Zimmermann *et al.*, 2004 and 2008; https://www.genevestigator.com/gv/index.jsp) was used to obtain the specific expression profiles for each candidate gene. Data for 54 out of 61 candidates was available in AtGenExpress, a target database to create the gene expression profiles, and the high quality data set of 1122 arrays was selected. Categories of different organs or anatomy parts (Figure 3.12), stages of development (Figure 3.13) and stimuli (Figure 3.14) were considered. The darkest blue colour (Figures 3.12 and 3.13) corresponds to expression values

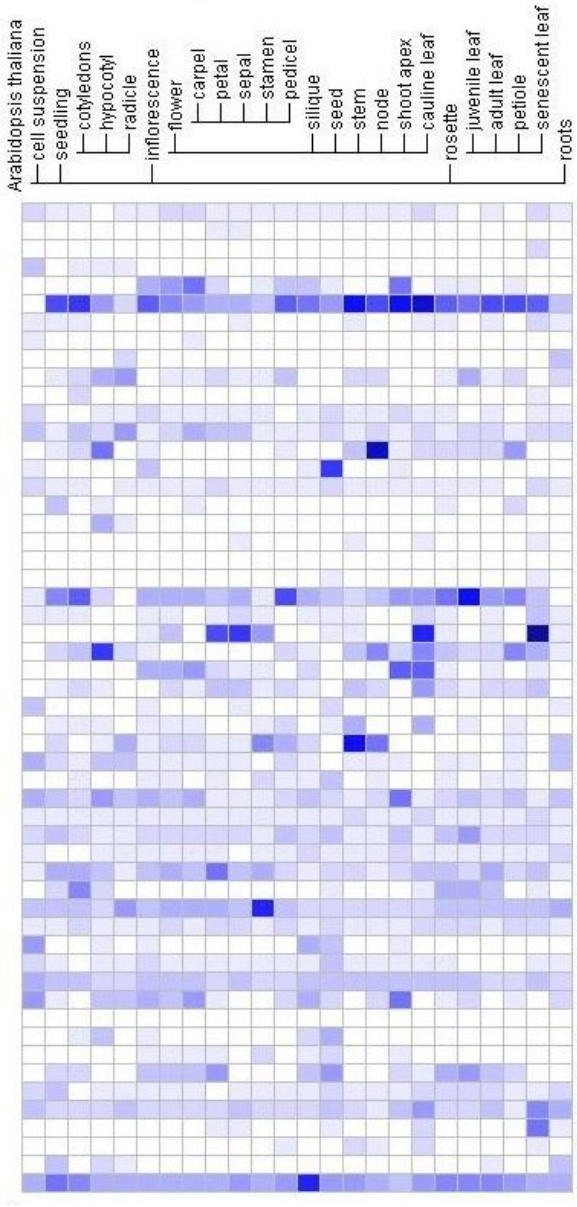


Figure 3.12. Candidate gene expression in different organs or anatomical parts

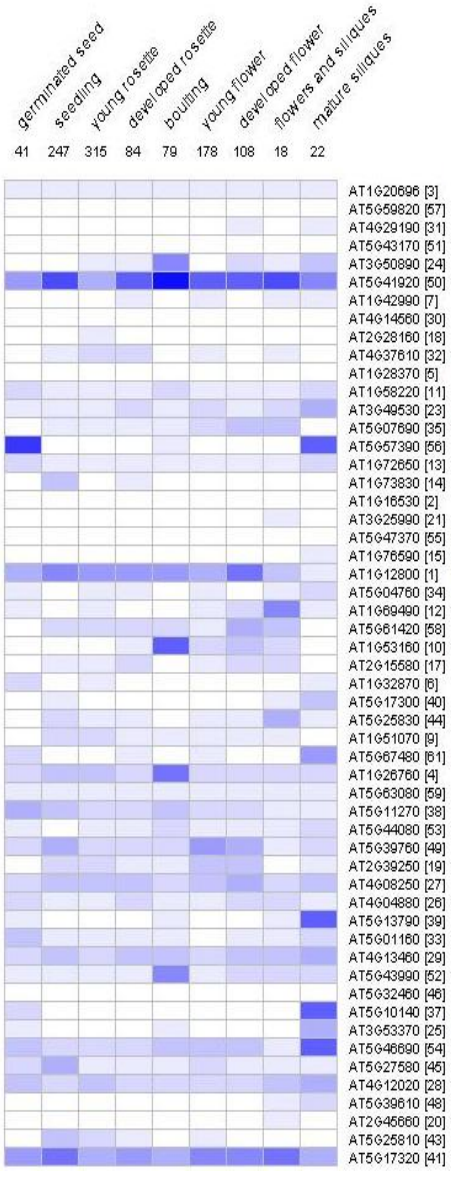


Figure 3.13. Candidate gene expression at different stages of development

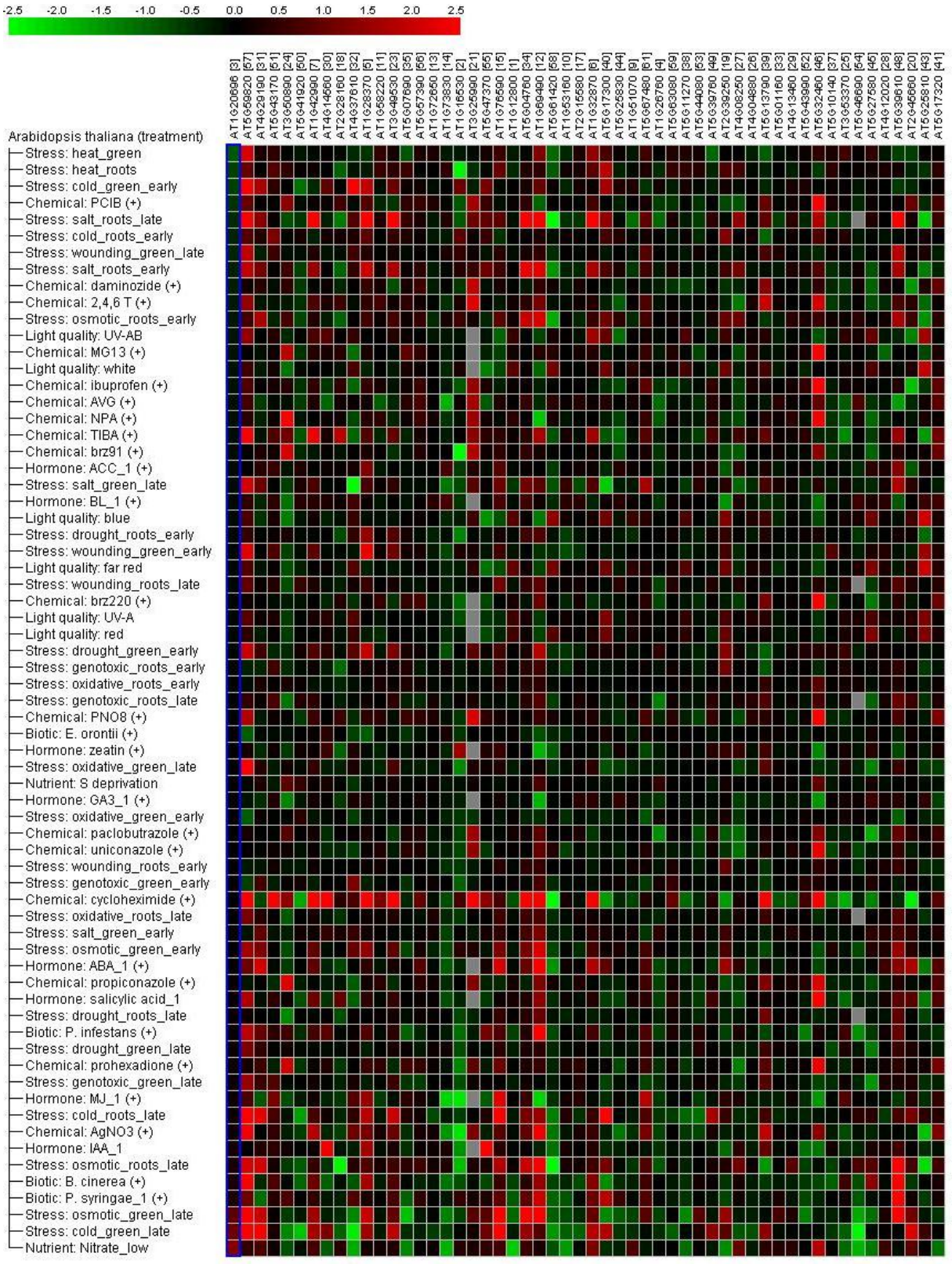


Figure 3.14. Candidate gene expression in response to different stimuli

(EPs), within the top 1% of signal intensities of probe sets/genes across all arrays in the database. EPs and hybridisation efficiencies are different between genes, which is why colour

Table 3.6. The summary of an array expression data for the previously uncharacterised candidate genes

No.	AGI code [AGI order number]	Ranking Group of Candidate Gene Significance	DATF Gene name(s)	DATF Gene family	Tissue Expression Pattern	Developmental Expression Pattern	Greater than 2.0 fold up- or down-regulation of gene expression in response to a given stimulus
1.	AT1G12800 [1]	I	N/P	N/P	most tissues, highly in cotyledons, pedicel, juvenile leaf	all stages, most at seedling, developed flower	low nutrient
2.	AT1G58220 [11]	I	x	MYB-related	most tissues, a bit more in cell suspension, shoot apex and seed	most stages, bit more at germinated seed, bolting, mature siliques	N/A
3.	AT4G12020 [28]	I	*MAPKKK11, WRKY19*	WRKY	all tissues in similar way, a bit more in cauline and senescent leaf	all stages in similar way, a bit more at mature siliques	N/A
4.	AT4G29190 [31]	I	x	C3H	senescent leaf	developed flower, mature siliques	cold and osmotic stress, ABA treatment
5.	AT5G01160 [33]	I	N/P	N/P	all tissues in similar way, a bit more in seed	all stages in similar way, a bit more at germinated seed	N/A
6.	AT5G32460 [46]	I	N/P	N/P	N/A	N/A	N/A
7.	AT5G38860 [47]	I	*BIM3*	bHLH	N/P	N/P	N/P
8.	AT5G63080 [59]	I	x	JUMONJI	in most tissues in similar way	at most stages in similar way	N/A
9.	AT1G20696 [3]	II	*HMGB3, NFD3*	HMG	most tissues, similarly	all stages similarly	N/A
10.	AT1G26760 [4]	II	N/P	N/P	most tissues, cell suspension, highly in hypocotyl, carpel, shoot apex	most stages, highly at bolting	N/A
11.	AT1G47760 [8]	II	x	MADS	N/P	N/P	N/P
12.	AT1G53160 [10]	II	*SPL4*	SBP, SPL	most (apart from in seedling parts) higher in shoot apex, cauline leaf, carpel	at bolting and flower stages	N/A
13.	AT1G72650 [13]	II	*TRFL6*	MYB-related	most tissues, similarly	all stages, slightly more at germinated seed and mature siliques	N/A

No.	AGI code [AGI order number]	Ranking Group of Candidate Gene Significance	DATF Gene name(s)	DATF Gene family	Tissue Expression Pattern	Developmental Expression Pattern	Greater than 2.0 fold up- or down-regulation of gene expression in response to a given stimulus
14.	AT3G49530 [23]	II	*ANAC062*	NAM, NAC	in most tissues, the strongest in carpel and radicle	at most stages, more at mature siliques	salt/roots, cycloheximide
15.	AT3G50890 [24]	II	*ATHB28*	ZF-HD	shoot apex, flower, silique	bolting, mature siliques, developed flower	chemical: NPA, brz91, propiconazole, prohexadione treatment
16.	AT4G04880 [26]	II	N/P	N/P	all tissues in similar way	all stages in similar way	N/A
17.	AT4G08250 [27]	II	x	GRAS	in all tissues in similar way, stamen and radicle much stronger	all stages in similar way, most at flower stage	N/A
18.	AT5G17300 [40]	II	x	MYB-related	all tissues in similar way, a bit more in cauline leaf and stem	seedlings, flowers and siliques, mature siliques	salt stress
19.	AT5G17320 [41]	II	x	HB, HD-Zip	all tissues in similar way, highly in silique	all stages in similar way, highly at seedling and flowers and siliques	N/A
20.	AT5G25830 [44]	II	x	C2C2-GATA	most tissues in similar way apart from rosette were lower, highly in stem, node, stamen	all stages in similar way, higher a bit in flowers and siliques	N/A
21.	AT5G27580 [45]	II	x	MADS	all tissues in similar way, more in seedling	all stages in similar way, higher at seedlings	genotoxic stress, chemical: paclobutrazole
22.	AT5G41920 [50]	II	x	GRAS	all tissues in similar way, more in seedling, stem, cauline leaf, shoot apex	all stages in similar way, higher at seedlings, bolting and flowers and siliques	chemical: NPA, ibuprofen, MG13, PCIB, brz220, PNO8, uniconazole, cycloheximide, propiconazole, prohexadione treatment, hormone treatment: salicylic acid
23.	AT5G44080 [53]	II	x	bZIP	in most tissues in similar way	at most stages in similar way	N/A
24.	AT5G46690 [54]	II	x	bHLH	in most tissues in similar way, a bit higher in petal, seed juvenile leaf	all stages in similar way, higher at mature siliques	cold stress

No.	AGI code [AGI order number]	Ranking Group of Candidate Gene Significance	DATF Gene name(s)	DATF Gene family	Tissue Expression Pattern	Developmental Expression Pattern	Greater than 2.0 fold up- or down-regulation of gene expression in response to a given stimulus
25.	AT1G51070 [9]	III	x	bHLH	most tissues, a bit stronger in cell suspension, radicle, hypocotyl, roots	most stages, slightly stronger at seedling and young rosette	N/A
26.	AT1G76590 [15]	III	x	PLATZ	seed, senescent leaf	mature siliques	cold and osmotic stress, hormone treatment: MJ, ABA treatment
27.	AT2G15580 [17]	III	N/P	N/P	in most tissues, a bit stronger in petiole, cauline leaf, stem, sepal and petal	at most stages, bit more at developed rosette and flower stages	N/A
28.	AT3G25990 [21]	III	x	Trihelix	senescent and cauline leaf, stem, sepal	flowers and siliques	cycloheximide
29.	AT3G53370 [25]	III	x	S1Fa-like	silique, seed, shoot apex, flower	mature siliques, germinated seed, bolting	cycloheximide
30.	AT5G04760 [34]	III	x	MYB	all tissues in similar way	most (not at silique and bolting) in similar way	salt and osmotic stress, cycloheximide treatment

Legend:

N/P – not present in DATF database (refer to Tables 3.4 and 3.5)
N/A – not available
x – does not exist

intensities can be only compared between values of the same probe set/gene (Zimmermann *et al.*, 2004 and 2008). The red-green colour system (Figure 3.14) corresponds to a fold change of gene expression in plants treated vs. controls. Various modes of gene expression were observed among candidate genes: tissue-specific; expression at certain times of development, or constitutive activity across the whole of development and in all tissues. Table 3.6 sums up the expression profiles of previously uncharacterised candidate genes which are supplemental to general information given previously in Tables 3.4 and 3.5.

3.4. Validation of selected candidate genes

3.4.1. Co-localisation of candidate genes with QTLs for heterosis of biomass and growth, and biomass QTL *per se*

Chromosomal locations of all the candidate genes were compared with map locations of QTLs for biomass heterosis and for leaf area/relative growth rate (RGR) (research group of

Table 3.7. List of candidate genes that co-localised with QTLs for biomass and leaf area/RGR heterosis, and biomass QTL *per se*

No.	AGI code [AGI order number]	Ranking Group of Candidate Gene Significance	QTL for leaf area/RGR (growth) heterosis	QTL for biomass heterosis	QTL for biomass *per se*
1.	AT1G12800 [1]	I	-	+	-
2.	AT1G58220 [11]	I	-	-	+
3.	AT4G12020 [28]	I	+	-	-
4.	AT4G13460 [29]*	I	+	-	-
5.	AT5G25810 [43]	I	+	-	-
6.	AT5G32460 [46]	I	+	-	-
7.	AT5G57390 [56]	I	-	+	-
8.	AT5G63080 [59]	I	-	-	+
9.	AT1G53160 [10]	II	-	+	+
10.	AT1G72650 [13]	II	-	+	+
11.	AT1G73830 [14]	II	-	+	-
12.	AT2G28160 [18]	II	-	+	-
13.	AT3G46090 [22]	II	-	+	+
14.	AT3G49530 [23]	II	-	+	+
15.	AT4G08250 [27]	II	+	-	-
16.	AT5G17300 [40]	II	+	-	-
17.	AT5G17320 [41]	II	+	-	-
18.	AT5G25830 [44]	II	+	-	-
19.	AT5G27580 [45]	II	+	-	-
20.	AT5G61420 [58]	II	-	-	+
21.	AT4G14560 [30]	III	+	-	-
22.	AT5G17810 [42]	III	+	-	-
23.	AT5G63160 [60]	III	-	-	+
24.	AT1G69490 [12]	III	-	+	+

Legend:

* – one of the three SET domain candidate genes (the ‘chromatin-related’ gene group)

T. Altmann – unpublished data) to obtain evidence for their relevance to heterosis. QTL data was obtained from IL and RIL populations derived from Col-0x Col-0 and C24xC24 reciprocal crosses. Nine out of 61 candidates co-localised with QTL for biomass heterosis and a further 11 co-localised with QTL for increased growth. Additionally, candidate gene locations were compared with QTL for biomass, a trait in and of itself (Lisec *et al.*, 2008). Five out of the nine genes that mapped near biomass heterosis QTL also co-localised with biomass QTL. Four of the 61 candidate genes co-localised exclusively with biomass QTL (Table 3.7). None of candidate genes belonged to an overlapping region of both heterotic QTLs. One third of the candidate genes that co-localised with above mentioned QTLs belonged to the 'significance category' I, a half to a category II, and the remaining four genes to category III.

3.4.2. Expression analysis of candidate genes at early stages of heterosis establishment

Expression of candidate genes in F1 hybrids vs. parents was further studied at various developmental time points: at 3, 6, 8, and 10 DAS. 3 DAS was a time point of developmental delay in C24xC24 (refer to section 3.1.1), accompanied with earliest changes in some FAs level in F1 hybrids when compared to parents (refer to section 3.1.3.2). The early onset of biomass hybrid vigour was associated with the 6 DAS stage (small, albeit insignificant changes in hybrid size were visible/become apparent) and at 8 DAS, where the difference in biomass between F1 hybrids and parents was statistically significant. 10 DAS was a time point where heterosis was established (Meyer *et al.,* 2004) and it is also the stage where first two rosette leaves > 1 mm are present (Boyes *et al.,* 2001).

At first, primarily trends present in the obtained expression data were investigated via PCA analysis since such data variation could be attributed to a given data component such as replicate, genotype or time point. The analysis yielded a good separation between parental and hybrid genotypes across all time points (Figure 3.15), which was a promising starting point for further statistical analyses. Significant difference in candidate gene expression between hybrids and parents at different time points was determined in ANOVA analysis followed by Student's t-tests (the most significant data was marked in green fields in a Table 3.8). Additionally, genes with less stringent significance criteria (Student's t-tests P value < 0.05 was a significance threshold) were considered as differentially expressed in hybrids (the orange fields in a Table 3.8). Comparisons were limited to differentially expressed genes at individual time points because transcript level differences were generally not maintained over multiple stages. Eighteen candidate genes were differentially expressed in hybrids across the whole time serious, while the remaining 33 occurred only transiently. Simi-

larly, when considering only the candidate genes that were ranked to 'significance category' I (i.e. group in which candidate genes were statistically the most significant) and genes that co-localised with QTL of interest (refer to section 3.4.1) the most abundant were genes which occurred only transiently (~59% and 45%, respectively). As many as 80% of candidate genes that were identified at 4 DAS were also significant at 3 DAS, 57% at 6 DAS, 55% at 8 DAS, and 72% at 10 DAS. There were only five candidates that were differentially expressed in hybrids only at 4 DAS (AT1G58220 [11], AT5G57390 [56], AT1G26760 [4], AT3G49530 [23], AT5G44080 [53]).

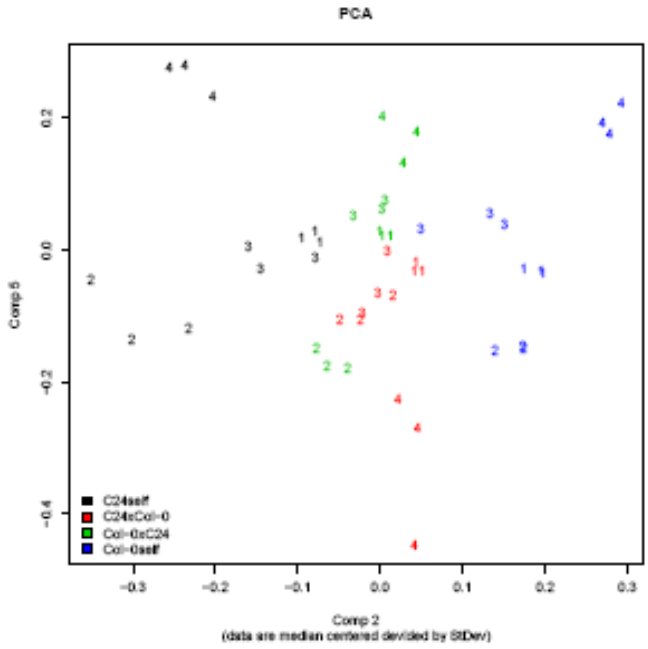


Figure 3.15. PCA analysis of gene expression differences in parental and hybrid genotypes from developmental time series 3-10 DAS

Numbers 1-4 represent the time points: 1 - 3 DAS, 2 - 6 DAS, 3 - 8 DAS, and 4 - 10 DAS
Different colours discriminate the genotypes (marked on the plot area)
Duplications or triplications of each of numbers represent two or three biological replicates, respectively

Gene expression patterns determined among candidate genes that were significantly expressed in hybrids at different time points were different than these defined at 4 DAS in almost all cases (95%, Table 3.8). Examples for changeable expression pattern across different time points are represented by Figures 3.16 A-C, and the maintained throughout time points by Figure 3.16 D. The analyses also revealed that among all the identified candidate genes, a predominant expression patterns at 3, 4, 8 and 10 DAS were the non-additive, whereas 6 DAS the additive and non-additive were almost equal. These results looked different for genes of 'statistical category' I (additive effects prevalent in all of the time points) and

the genes co-localising with QTLs of interest (additive and non-additive were almost equally frequent in all of the time points).

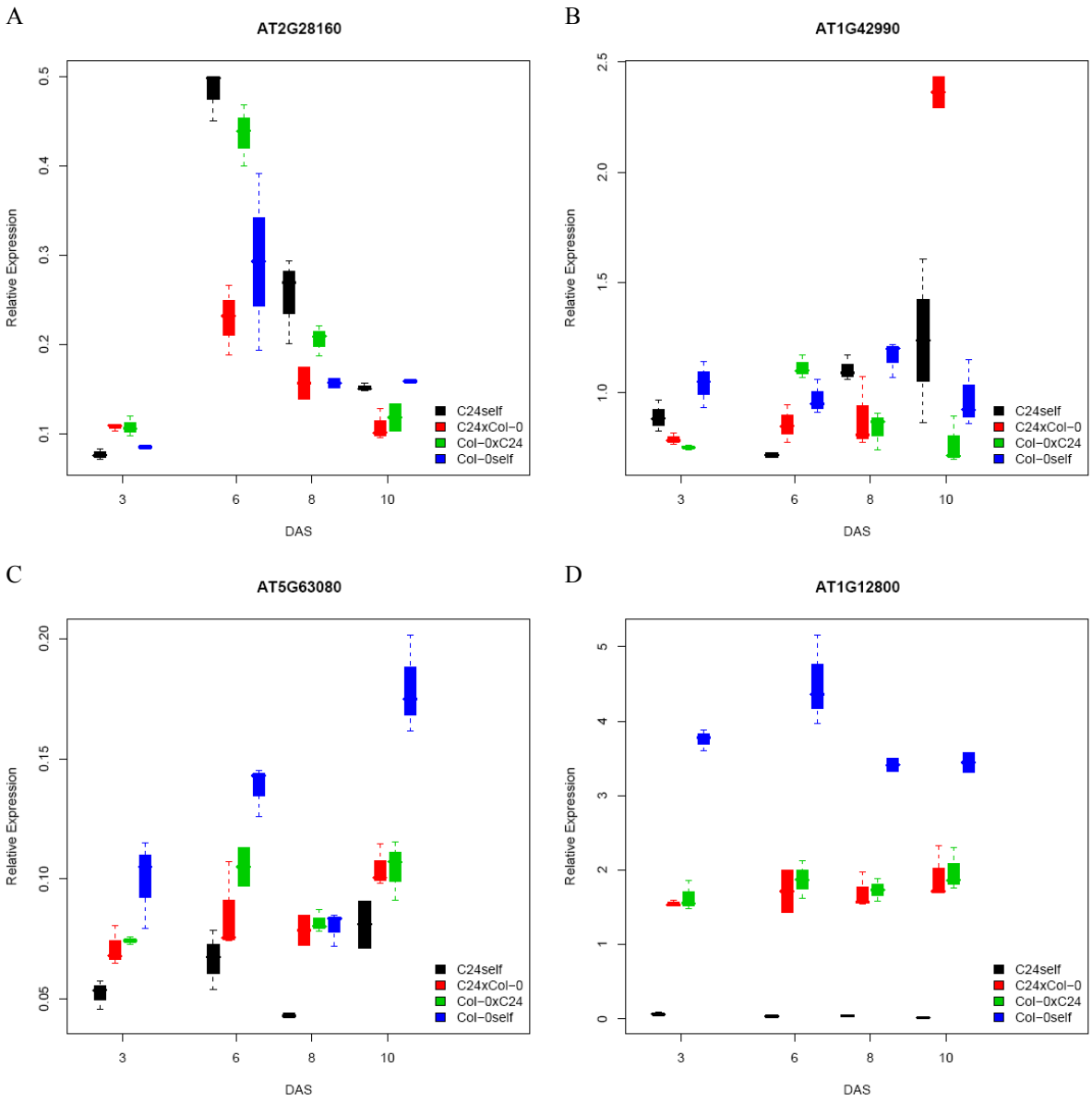


Figure 3.16. Gene expression levels of selected candidates at different developmental stages

A – Overdominant expression pattern at 3 DAS, and underdominant at 10 DAS i

B – Underdominant expression pattern at 8 DAS in AT1G42990 [7]

C – Intermediate_Col-0xCol-0_high pattern at 3 and 6 DAS, a dominant_Col-0xCol-0_high at 8 DAS, and a dominant_C24xC24_low at 10 DAS in AT5G63080 [59]

D – Intermediate_Col-0xCol-0_high expression pattern maintained across all time points in AT1G12800 [1]

Different colours discriminate the genotypes (marked on the plot area)
The box plot consists of median expression values from replicas (horizontal line marked on the box), box area where falls the middle 50% of the data (the upper edge of the box indicates the 75th percentile, and the lower hinge indicates the 25th percentile of the data set, respectively), and the whiskers, which ends mark the lowest and highest expression value.

Table 3.8. Summary of data obtained from candidate gene expression analysis across the developmental time serious (columns 5-9) and in crosses of different *Arabidopsis* accessions at 4 DAS (in columns 10 and 11)

No.	AGI code [AGI order number]	Ranking Group of Candidate Gene Significance at 4 DAS	Gene co-localisation with QTL	3 DAS Col-0/C24	4 DAS Col-0/C24	6 DAS Col-0/C24	8 DAS Col-0/C24	10 DAS Col-0/C24	4 DAS Cl-0/Nd	4 DAS C24/L*er*
1	2	3	4	5	6	7	8	9	10	11
1.	AT1G12800 [1]	I	biomass heterosis	2	8	2	2	2	1	1
2.	AT1G32870 [6]	I		12	2	2	2	2		1
3.	AT1G58220 [11]	I	biomass		2				2	
4.	AT1G77080 [16]	I		2	2	2	2	2	2	1
5.	AT2G39250 [19]	I		any	1			any		7
6.	AT2G45660 [20]	I		any	7	7		7	any	6
7.	AT4G12020 [28]	I	growth heterosis	2	2	N/A	any	N/A	2	1
8.	AT4G13460 [29]**	I	growth heterosis	any	8	6				2
9.	AT4G29190 [31]	I		10	10		any	any	10	any
10.	AT5G01160 [33]	I			10			7		
11.	AT5G08712 [36]*	I		any	7			any		any
12.	AT5G11270 [38]	I		2	2	2				any
13.	AT5G25810 [43]	I	growth heterosis	10	6	6		6		any
14.	AT5G38860 [47]	I		2	2	2	2	7		1
15.	AT5G43990 [52]**	I		any	2			8		
16.	AT5G57390 [56]	I	biomass heterosis		1				2	any
17.	AT5G63080 [59]	I	biomass	2	2	2	8	7	any	any
18.	AT5G67480 [61]	I		5	7		any	6	any	any
19.	AT1G20696 [3]	II		6	1	6		any		any
20.	AT1G26760 [4]**	II			8				12	
21.	AT1G28370 [5]	II		any	10	any		any	2	
22.	AT1G47760 [8]	II		any	12	any	any	7	1	1
23.	AT1G53160 [10]	II	biomass heterosis; biomass	6	2	any	any	7		any
24.	AT1G72650 [13]	II	biomass heterosis; biomass		10			7		
25.	AT1G73830 [14]	II	biomass heterosis	2	10		**4**	any	any	any
26.	AT2G28160 [18]	II	biomass heterosis	**3**	1	any	any	**4**		1
27.	AT3G46090 [22]	II	biomass heterosis; biomass	any	2					11
28.	AT3G49530 [23]	II	biomass heterosis; biomass		10					

No.	AGI code [AGI order number]	Ranking Group of Candidate Gene Significance at 4 DAS	Gene co-localisation with QTL	3 DAS Col-0/C24	4 DAS Col-0/C24	6 DAS Col-0/C24	8 DAS Col-0/C24	10 DAS Col-0/C24	4 DAS Cl-0/Nd	4 DAS C24/L*er*
1	2	3	4	5	6	7	8	9	10	11
29.	AT3G50890 [24]	II		any	8	6		any	any	7
30.	AT4G08250 [27]	II	growth heterosis	any	10		9			5
31.	AT5G10140 [37]	II		5	5	5	5	5	2	8
32.	AT5G13790 [39]	II			10	2	2	2		1
33.	AT5G17300 [40]	II	growth heterosis	10	7	10	any	7	2	7
34.	AT5G17320 [41]	II	growth heterosis	any	9	6	6	1	1	2
35.	AT5G25830 [44]	II	growth heterosis	6	8	1	any	any		12
36.	AT5G27580 [45]	II	growth heterosis	any	1	1	5	1		any
37.	AT5G41920 [50]	II		any	2	any	7	2	8	5
38.	AT5G43170 [51]	II		any	11	6	any	6	2	2
39.	AT5G44080 [53]	II			8					
40.	AT5G46690 [54]	II		12	9	any	any	any	7	2
41.	AT5G47370 [55]	II		6	10			any	any	2
42.	AT5G59820 [57]	II			10	any	any		2	9
43.	AT1G16530 [2]	III		any	any					
44.	AT1G42990 [7]	III		any	any	any	**4**	any		any
45.	AT1G51070 [9]	III		2	any					8
46.	AT1G69490 [12]	III	biomass heterosis; biomass	any	any		7		any	7
47.	AT1G76590 [15]	III		any	any			any	8	
48.	AT2G15580 [17]	III		2	any	2	**4**	7		1
49.	AT3G25990 [21]	III		7	any			7		
50.	AT4G14560 [30]	III	growth heterosis	1	any	any			2	
51.	AT4G37610 [32]	III		10	any		**4**	any	8	10
52.	AT5G04760 [34]	III			any		any	any	any	
53.	AT5G07690 [35]	III			any	any	any	8	12	
54.	AT5G17810 [42]	III	growth heterosis	6	any	6	any	N/A	2	
55.	AT5G39610 [48]	III		any	any	9		any	any	
56.	AT5G63160 [60]	III	biomass	any	any		any		any	

Legend is given on the following page

Legend:

Field colours:
grey – gene co-localisation with a given QTL,
dark or light green fields – candidate gene differentially expressed in the hybrid(s) when compared to parents confirmed via ANOVA and Student's t-test,
orange fields – candidate confirmed only via Student's t-test,
yellow fields – candidate confirmed only via ANOVA analysis,
white fields – no differential expression of candidate gene between hybrid(s) and parent(s).

N/A – data not available
* – microRNA
** – SET domain genes (a member of the 'chromatin-related' group of genes)

Expression patterns were following:
any – gene expression did not match any of specific patterns defined in this work,
1-12 – template numbers (for details refer to section 3.3.1).

Template Number	Col-0/C24	Cl-0/Nd	C24/Ler
	Gene Expression Patterns	**Gene Expression Patterns**	**Gene Expression Patterns**
1.	Intermediate_C24xC24_high	Intermediate_Nd_high	Intermediate_L*er*_high
2.	Intermediate_Col-0xCol-0_high	Intermediate_Cl-0_high	Intermediate_C24_high
3.	Overdominant (F1 high)	Overdominant (F1 high)	Overdominant (F1 high)
4.	Underdominant (F1 low)	Underdominant (F1 low)	Underdominant (F1 low)
5.	Dominant_C24xC24_high	Dominant_Nd high	Dominant_L*er* high
6.	Dominant_Col-0xCol-0_low	Dominant_Cl-0_low	Dominant_C24_low
7.	Dominant_C24xC24_low	Dominant_Nd_low	Dominant_L*er*_low
8.	Dominant_Col-0xCol-0_high	Dominant_Cl-0_high	Dominant_C24_high
9.	Maternal_C24xC24_high	Maternal_Nd_high	Maternal_L*er*_high
10.	Maternal_Col-0xCol-0_high	Maternal_Cl-0_high	Maternal_C24_high
11.	Paternal_C24xC24_high	Paternal_Nd_high	Paternal_L*er*_high
12.	Paternal_Col-0xCol-0_high	Paternal_Cl-0_high	Paternal_C24_high

3.4.3. Expression analysis of candidate genes of different *Arabidopsis* accessions

Candidate genes that were previously selected in Col-0/C24 crosses at 4 DAS were further validated in 4 DAS hybrids of different *Arabidopsis* accessions showing negative (Cl-0 crossed to Nd) and positive (L*er* crossed to C24) heterosis of biomass (Figure 3.17).

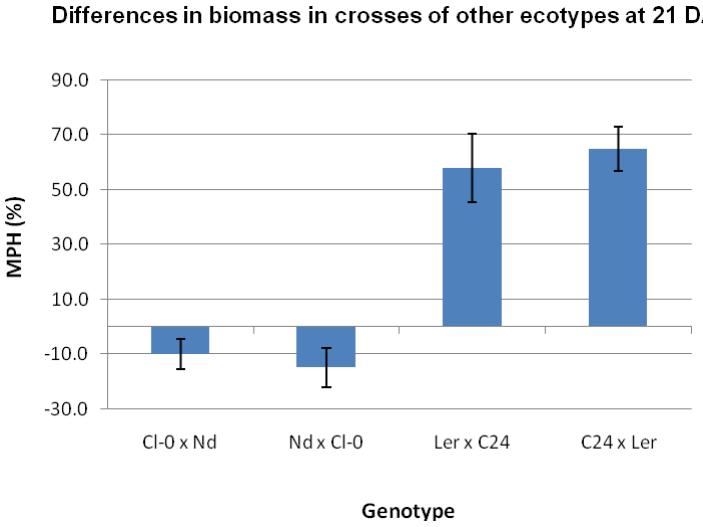


Figure 3.17. Biomass heterosis in L*er*/C24 and Nd/Cl-0 crosses determined at 21 DAS

The expression data obtained for parental and hybrid seedlings at 4 DAS were first subjected into PCA analysis. It yielded a good separation between parental and hybrid genotypes in both sets of crosses (Figures 3.18 A and B), which was a promising starting point for further statistical analyses.

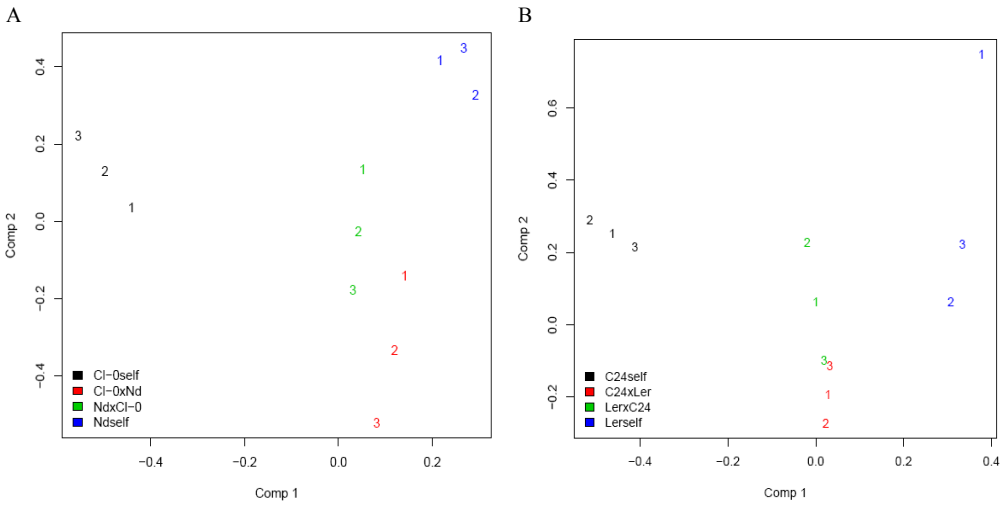


Figure 3.18. PCA separation of different genotypes resulting from crosses of other *Arabidopsis* accessions

A – Cl-0 with Nd, B – L*er* with C24

Numbers 1-3 represent biological replicates
Different colours discriminate the genotypes (marked on the plot area)

The candidate genes of a main focus in a validation analysis were exhibiting dominant expression pattern when hybrid expression levels were compared to parental in Col-0/C24 crosses at 4 DAS (refer to section 3.3.1). The scheme of the analysis performed to validate these candidate genes is given in Figure 3.19. The candidate gene was considered to be validated if it exhibited the same dominant pattern in the crosses of positive biomass heterosis (L*er*/C24 and Col-0/C24, Figure 3.19) and 'the opposite dominant pattern' in the crosses of negative biomass heterosis (Cl-0/Nd, Figure 3.19). The analysis was based on the assumption that if a candidate gene in question influences a biomass increase in the heterotic hybrids, a correlation between hybrid expression level in the range of a 'higher parent' and a biomass increase should be followed by a correlation between hybrid expression level in the range of a 'lower parent' and a decrease in hybrid biomass. The analysis resulted in confirmation of dominant expression patterns in only three from 12 candidate genes profiled in the L*er*/C24 crosses (AT2G45660 [20] – a 'significance category' I, AT5G10140 [37] – a 'significance category' II, AT5G17300 [40] – a significance category' II and co-localisation with growth heterosis). Unfortunately, the opposite effects in the Cl-0/Nd crosses could not be found (Table 3.8, columns 10 and 11).

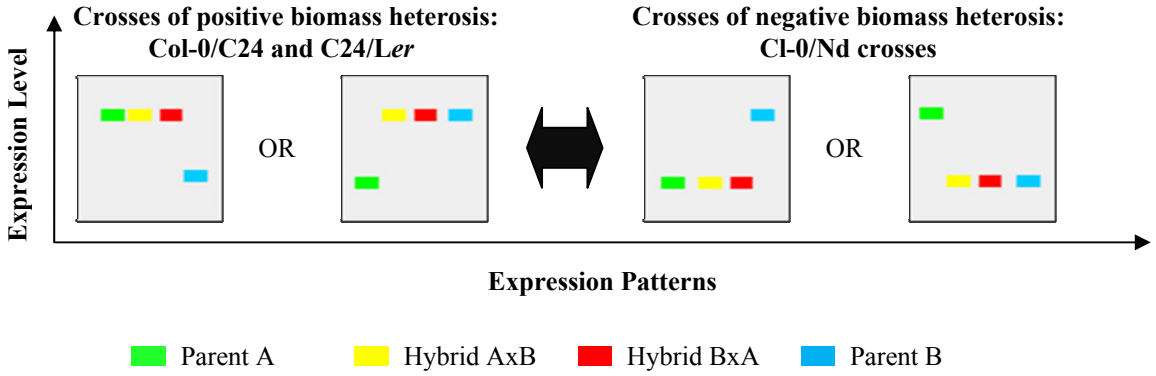


Figure 3.19. The analysis scheme for a validation of candidate genes with dominant expression patterns

Additionally, the remaining 44 candidates were expression-profiled in the same set of crosses of positive and negative biomass heterosis. Thirty nine candidate genes were differentially expressed in hybrids when compared to parents in L*er*/C24 crosses (green and orange fields, Table 3.8). In this group nine genes (AT2G28160 [18] – a 'significance category' II/co-localisation with biomass heterosis, AT5G17300 [40] – a 'significance category' II/co-localisation with growth heterosis, AT1G32870 [6] – a 'significance category' I, AT1G77080 [16] – a 'significance category' I, AT2G45660 [20] – a 'significance category' I,

AT4G12020 [28] – a 'significance category' I/co-localisation with growth heterosis, AT5G10140 [37] – a 'significance category' II, AT5G38860 [47] – a 'significance category' I, AT5G59820 [57] – a 'significance category' II) exhibited the same expression pattern as in C24/Col-0 crosses (3 dominant, 5 intermediate and one maternal; Table 3.8). Eighteen out of 39 candidate genes that were differentially expressed in the hybrids of L*er*/C24 crosses (green and orange fields) were not differentially expressed in hybrids of Cl-0/Nd crosses. Surprisingly, there were also found 10 candidate genes differentially expressed in hybrids of negative heterosis crosses but not of positive crosses.

3.5. Exploring of the possible role of rDNA genes in heterosis

Different approaches were taken to test if the increased growth of the F1 hybrids is correlated with the rDNA activity. The following parameters were measured and compared in hybrids and parents: expression level of rRNA genes (section 3.5.1), nucleolus area (section 3.5.2), and ploidy level (section 3.5.3).

3.5.1. Expression analysis of rRNA genes

To test whether F1 hybrids have enhanced levels of rRNA per cell which could account for increased early growth rates the expression of 25S, 18S, and 5.8S subunits were measured via qRT-PCR in parental and hybrid seedlings at 4 DAS (Figure 3.20). No significant difference in the expression level of any of these genes was found between hybrids and parental lines.

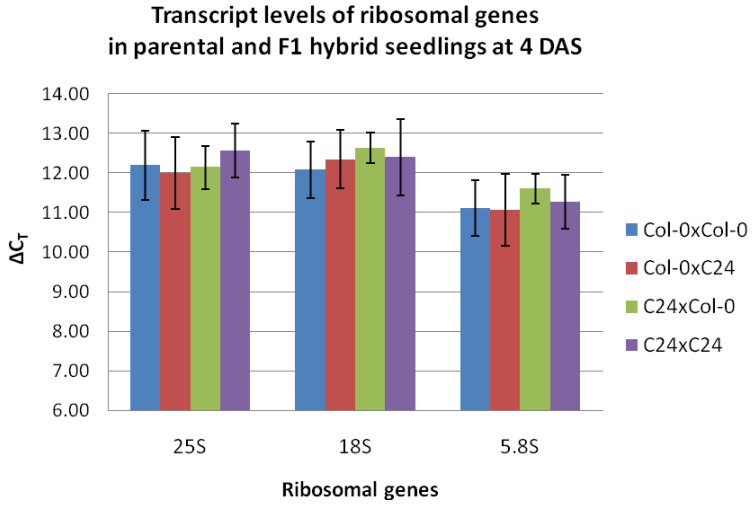


Figure 3.20. Comparison of transcript levels of ribosomal genes in parents and hybrids at 4 DAS

One unit corresponds to 2-fold difference in gene expression level. Here, a ΔC_T was given as $\Delta C_T = C_{T\ gene} - C_{T\ reference\ gene}$ due to high ribosomal gene expression, which is higher than that of reference gene.

3.5.2. Comparison of nucleolus area

Measurement of nucleolus area in F1 hybrids and parents was performed as a proxy for rDNA activity. Increased rDNA transcription leads to increased nucleolus area (Delany *et al.*, 1994). The suspended nuclei of 6 DAS seedlings were stained with fluorescent DAPI and subjected into flow cytometry to sort nuclei according to ploidy level. A ploidy level was determined on the basis of obtained histograms where relative fluorescence intensity represented a relative DNA content. At least 100 nucleoli of 2C (i.e., one unreplicated copy of the nuclear DNA) and 4C DNA content were flow sorted onto microscopic slides and silver stained (Figure 3.21) to perform measurements of nucleolus area. Unfortunately, the variability in nucleolus area within genotypes was greater than any difference between genotypes (Figure 3.22). Therefore, no significant difference was found in nucleolus size between hybrids and parents.

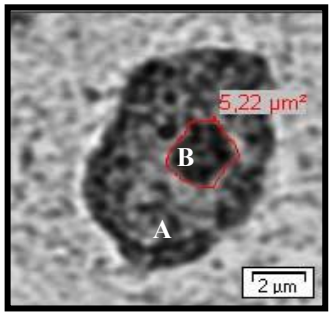


Figure 3.21. Col-0xC24 at 4C ploidy level

Nucleolus area was measured after the silver staining.
A – nucleus area
B – nucleolus area (5.22 µm^2)

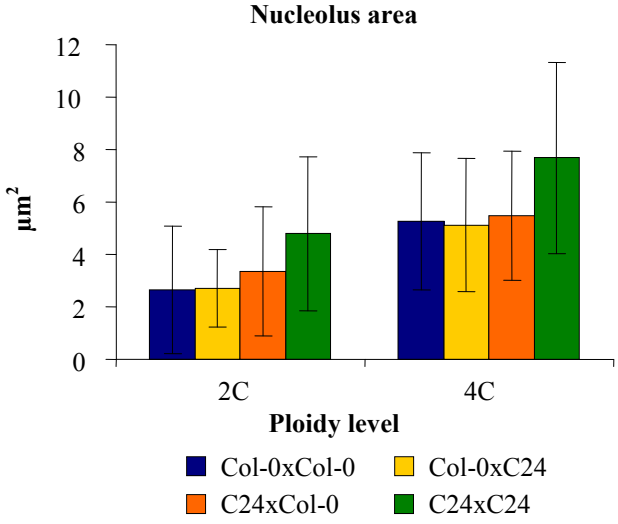


Figure 3.22. Comparison of nucleolus area at 2C and 4C ploidy level

Error bars show SD

3.5.3. Analysis of endoreduplication (endoreplication)

Changes in endoreduplication level across a developmental time serious were followed by measuring ploidy level in F1 hybrids and parents (the ploidy level was determined in the

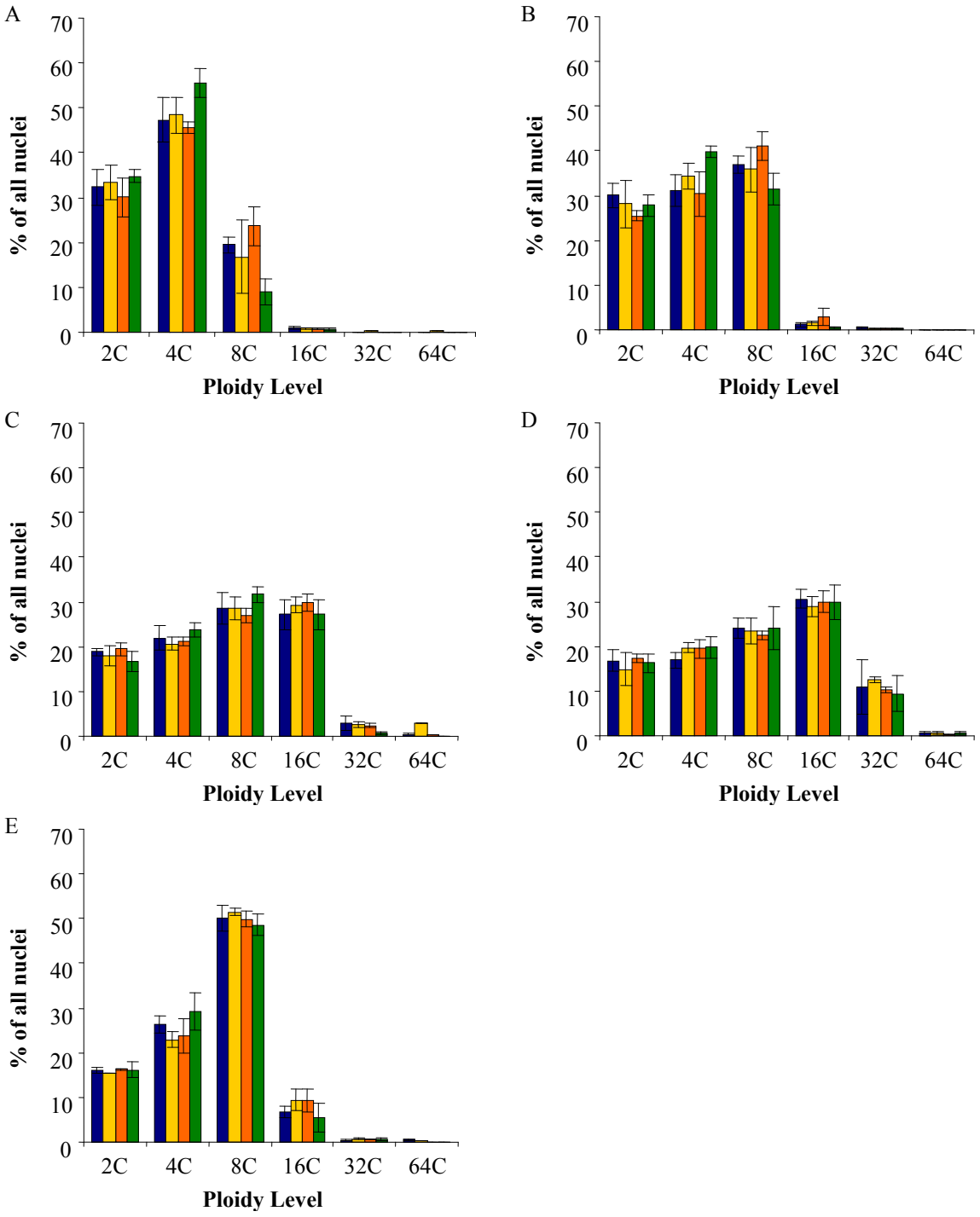


Figure 3.23. Ploidy level in the cells of parents and hybrids determined by flow cytometry

Col-0xCol-0 Col-0xC24 C24xCol-0 C24xC24

A – whole seedlings at 4 DAS
B – whole seedlings at 6 DAS
C – cotyledons of seedlings at 10 DAS
D – cotyledons of plantlets at 15 DAS
E – secondary leaves of plantlets at 15 DAS

same way as described in the previous chapter). An increase in ploidy level was observed for all genotypes from 4 DAS till 6 DAS (the whole seedlings; Figures 3.23 A-D) and from 10 to 15 DAS (cotyledons; Figures 3.23 C and D). Ploidy levels were higher in cotyledons at 15 DAS than in young leaves of the same plantlet for all genotypes (Figures 3.23 D and E). However, no significant differences in ploidy level were observed between hybrids and parents at any developmental stage (Figure 3.23).

3.6. Characterisation of *FRIGIDA* (AT4G00650) in relation to heterosis

The direction of PhD study, which was investigation of a role that selected regulatory genes play in heterosis, was further driven to an analysis of the possible involvement of the *FRIGIDA* gene (*FRI*) in biomass heterosis.

3.6.1. Analysis of an IL line carrying a segment containing *FRIGIDA*

The influence on plant dry weight (DW) of a C24 donor segment carrying *FRIGIDA* in both homozygous and heterozygous backgrounds, and a possible interaction with the *FLC* gene was examined. For this purpose introgressions line (IL) N88/2/1/10 BC5F3 and its test

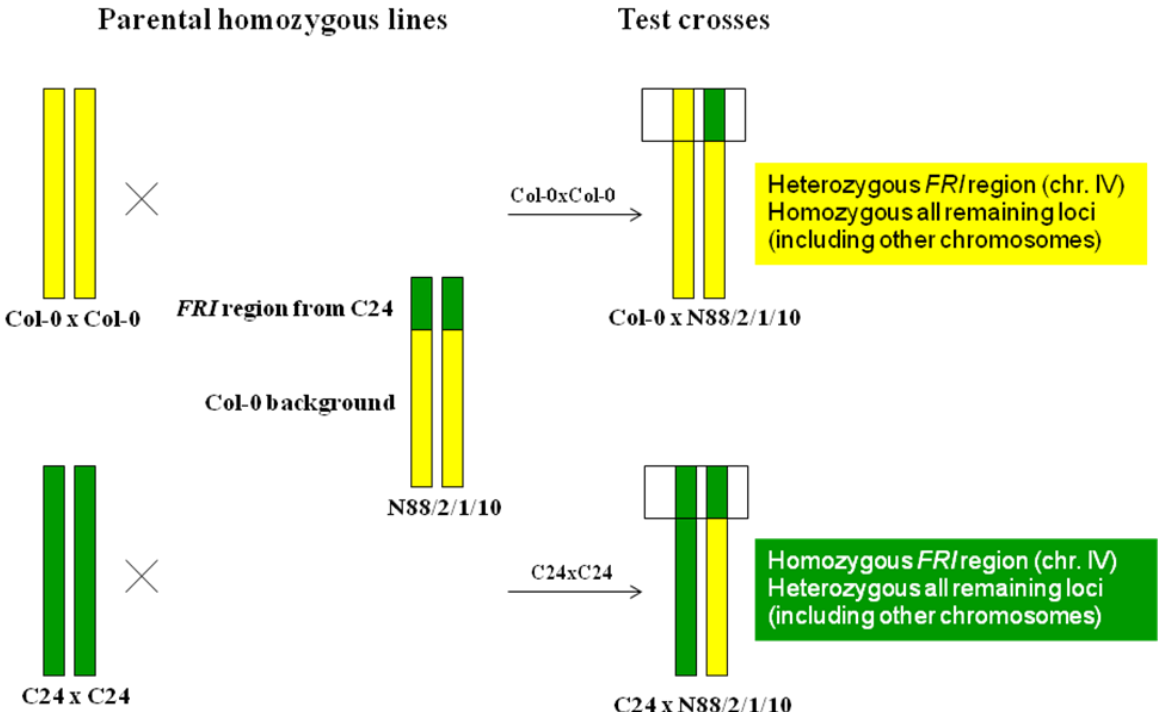


Figure 3.24. Schematic representation of chromosome IV in the IL line N88/2/1/10 and test crosses

crosses (IL crossed to Col-0xCol-0 and to C24xC24) were studied, (Figure 3.24). The substitutions were introgressed close to the top of chromosome (chr) IV where a QTL for biomass heterosis was identified (research group of Altmann T. - unpublished data). IL N88/2/1/10

BC5F3 was a homozygous line (Figure 3.24), in which the C24 segment of 340 loci was introgressed into Col-0xCol-0 background. It is known, that up-regulation of *FLC* by *FRI* differs depending on the activity of both genes and is different for various genotypes; *FRI* was found to be functional in C24 but not in Col-0 ecotype, whereas *FLC* is strong in Col-0 but weak in C24 (Gazzani *et al.,* 2003). This IL line carried functional *FRI* alleles from chr IV of C24 (FRI_{C24}/FRI_{C24}) and strong *FLC* alleles from chromosome V of C24 (FLC_{C24}/FLC_{C24}) and its DW was significantly greater than that of either Col-0xCol-0 or C24xC24 (Student's t-test P-values < 0.05 were used as a significance threshold in this experiment). There was a significant difference in DW between N88/2/1/10 progeny of test crosses and the Col-0xCol-0 and C24xC24 controls (Figure 3.25). Additionally, the biomasses of Col-0xN88/2/1/10, C24xN88/2/1/10 and N88/2/1/10 IL line were similar. In the both test crosses at least one of the two loci from *FRI* or *FLC* was represented by strong dominant allele (heterozygosity), whereas in both parents *FLC* and *FRI* loci came from the same ecotype (homozygosity). These results suggest that an introgressed region, and possibly the interaction of the strong dominant *FRI* allele from C24xC24 (FRI_{C24}) with the strong dominant *FLC* allele from Col-0xCol-0 ($FLC_{Col\text{-}0}$) might be involved in the observed biomass increase.

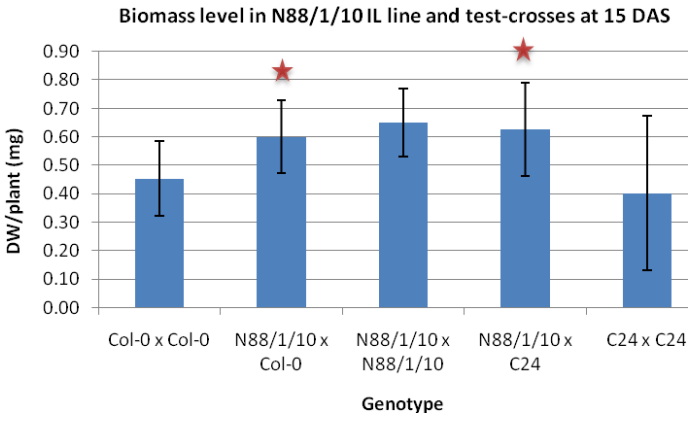


Figure 3.25. Comparison of biomasses in N88/2/1/10 IL, Col-0xCol-0 and C24xC24 homozygous lines and their test crosses

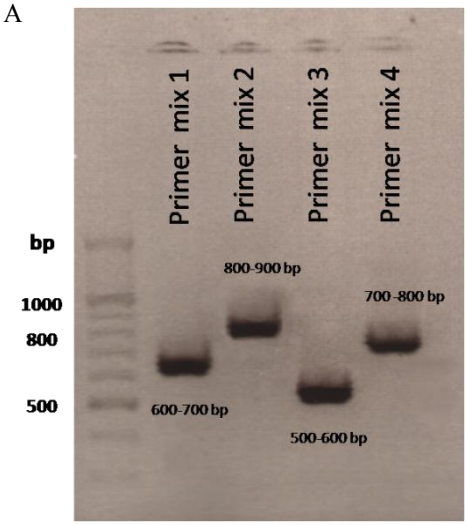
3.6.2. Creation and analysis of RNAi lines suppressing *FRIGIDA*

A reverse genetics approach was used to investigate the parental contribution of *FRIGIDA* to biomass and growth heterosis. The approach involved RNAi suppression of *FRI* expression in both Col-0xCol-0 and C24xC24 backgrounds. For creation and analysis of RNAi lines resources of AGRIKOLA project (www.agrikola.org; Hilson *et al.,* 2004) such as *Agrobacterium tumefaciens* colonies carrying hpRNA plasmid and required protocols set up and were implemented.

3.6.2.1. Validation of pAGRIKOLA clones via sequencing analysis

Agrobacterium tumefaciens strain GV3101 carrying hpRNA expression plasmid (Hilson *et al.,* 2004) for suppression of *FRIGIDA* was obtained from the AGRIKOLA project (http://www.agrikola.org). The hairpin (hp) construct of AGRIKOLA plasmid contained a GST (gene specific tag) with the length of 400 bp (CATMA ID: 4a00720). The GST was cloned on both sides of the intron spacer in inverted orientation to express hairpin (Figure 3.26 B). The clone validation (Figure 3.26 A) was based on PCR analysis according to AGRIKOLA validation protocols (Selection and validation of individual *Agrobacterium* clones; http://www.agrikola.org/index.php?o=/agrikola/html/transformation) and a sequencing analysis. Bacterial colonies verified for a desired hpRNA construct (Figure 3.26) were used to transform seeds from Col-0xCol-0 and C24xC24 plants.

A



B

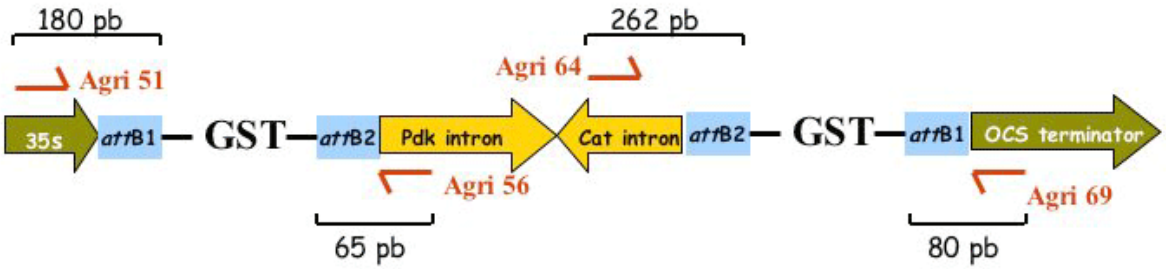


Figure 3.26. PCR verification of *A. tumefaciens* transformants (www.agrikola.org; Hilson *et al.*, 2004)

A. PCR products of expected length obtained from amplification of hpRNA expression plasmid with a set of AGRIKOLA primers (Agri 51/56/64/69). The four primers were positioned in such a way that the GST subunits present in hairpin cassette of the hpRNA expression plasmid (panel B) were easily distinguished by size in agarose gel electrophoresis.
B. The structure of the recombined hairpin cassette with the inverted GST repeats.

3.6.2.2. PCR screen and selection of *FRI*/RNAi lines of Col-0 and C24 background

Transgenic *FRI* RNAi T1 lines in Col-0xCol-0 background were produced and screened according to AGRIKOLA protocols (PCR on genomic DNA using primers Agri 51/56/64/69; www.agrikola.org) to validate the hpRNA construct in the same way as for bacterial plasmids. From these, 20 lines with desired construct were selected for further analysis.

Attempts to obtain *FRI* RNAi T1 lines in C24xC24 background failed. The subsequent trials and transformation protocol improvements allowed obtaining not more than 20 plants; however, in any of them construct could be validated. These plants did not survive even to reach the stage of fully developed rosette (Boyes *et al.,* 2001).

3.6.2.3. Analysis of the expression of *FRI* in selected RNAi lines

It was not possible to prove the silencing effect on *FRI* expression in RNAi T1 lines (Col-0xCol-0 background) at the developmental stage described by Shindo *et al.,* (2005) using the following methods: Northern blotting, semi-qPCR, and qRT-PCR expression analysis (Figure 3.27).

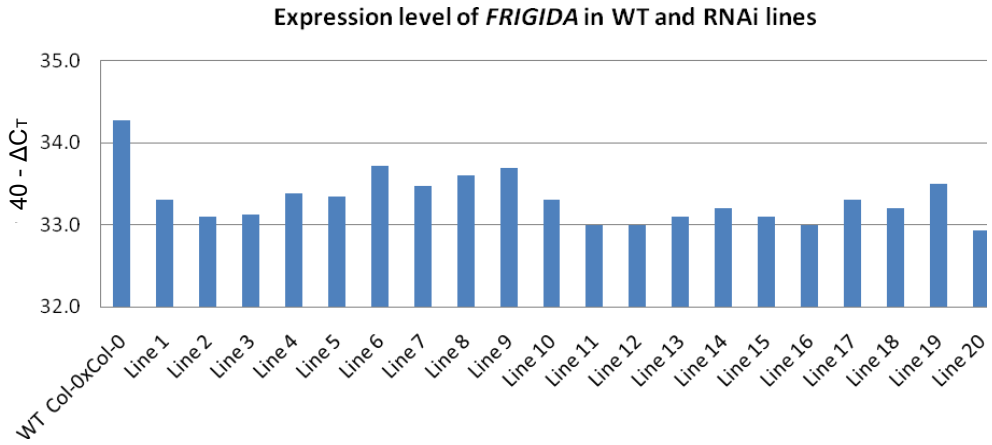


Figure 3.27. Levels of *FRIGIDA* suppression in RNAi lines

One unit on y-axis represents a two-fold difference of expression level.

No potential phenotypic differences between *FRI* RNAi and wild type plants were observed in the first three to four weeks following germination in plants grown in a greenhouse.

4. DISCUSSION

4.1. Determination of a divergence point between hybrids and parents

The first objective of this work was the identification of time point of the earliest divergence between F1 hybrids and parental inbred lines based on microscopic and biochemical studies of early development. The increased relative growth rate and biomass of young hybrid seedlings observed by Meyer *et al.*, (2004) could be explained, for example, by differences in the nature of seed reserves in the F1 seed, but not size and mass because these were identical in the four genotypes, as pointed out in the Materials and Methods. Additional observations and analyses on early development could provide insights into whether differences in seedling establishment resulted from faster germination, more rapid mobilisation and utilisation of seed reserves, or some physiological and developmental processes that follow the transition to photo-autotrophic growth. Because the earliest differences between hybrids and parents could potentially be driven by differential expression of regulatory genes such as TFs, which play master roles in the control of development, the identification of a time point of divergence was essential to select an appropriate time point for gene expression profiling to identify regulatory genes potentially involved in heterosis.

Observations performed in this study on germination and early seedling development suggested that the germination rate does not explain the differences in growth rate between parents and hybrids reported at 8 DAS. The markedly slower growth of C24xC24 manifested at 3 DAS was not apparent at 4 DAS (Figure 3.1), where development of all four hybrid and parental genotypes was homogenous.

While light microscopy revealed mobilisation of reserve material during seed germination (Microscopic pictures of mature seeds followed by stages 24-96 HAS in Annex B), resolution was insufficient to identify significant differences in the rate of mobilisation in the different genotypes. More sophisticated microscopic methods, including transmission electron microscopy (TEM) and the use of dyes of higher sensitivity (Mansfield and Briarty, 1996) could be used in the future to quantify any differences in sizes, numbers or structure of storage organelles of hybrid and parental seed during germination. Substantial increases in the volume of cells or vacuoles, plastids and other cell compartments are correlated with the mobilisation of storage reserves (Mansfield and Briarty, 1996).

Significant differences in metabolite levels between F1 hybrids and parents were detected at all time points (including mature seeds) via GC-MS and GC (Tables 1-2 in Annex C, and Figures 3.5 and 3.6). However, the most informative data concerning the point of diver-

gence between genotypes was obtained from fatty acid analysis, which revealed that the switch to photoautotrophic growth occurred in both parents and hybrids around 3-4 DAS and that differences in the synthesis or utilisation of FAs between F1 hybrids and parents were found at 4-6 DAS.

Taken together, the cellular and metabolite data prompted us to choose the stage 3-4 DAS as the most promising for gene expression profiling to reveal underlying genetic differences between genotypes. To avoid potential effects of non-homogenous development of C24xC24 on gene expression, the 4 DAS time point was selected in preference to 3 DAS. Another rational for this choice was the need for sufficient material for qRT-PCR, which required microgram quantities of high quality of RNA for cDNA synthesis.

4.2. Reliability of expression data

High-throughput qRT-PCR developed by Czechowski *et al.,* (2004) was used to study the expression of transcription factors and other genes selected in this work. Sample preparation and testing procedures including testing of gene-specific primers, were carried out previously for the Columbia genotype (Czechowski *et al.,* 2004). However, the methods and especially the gene-specific primers had to be tested on the C24 genotype used in this work. Based on sequence data, it was estimated that Columbia and C24 differ on average by 1 in 430 bp in coding regions (Thomas Altmann, personal communication). The most pessimistic estimates indicated that less than 10% of PCR reactions using C24 cDNA would be affected by single nucleotide polymorphisms between Columbia and C24. In fact, experimental testing showed that 98% of 380 randomly-chosen TF primer pairs designed on the Columbia sequence also worked on C24 (data not shown). Differences in the efficiency of cDNA amplification between reference cDNA obtained from Columbia and cDNA from other genotypes were determined from the kinetics of target amplification. In addition, amplification products of C24 were compared to those of Columbia, using dissociation/melting curve data (Ririe *et al.*, 1997). Every gene for which a significant difference in primer efficiency or amplification product was found between Columbia and C24 was excluded from overall data analysis. Among the genes excluded were those expressed at very low levels ($C_T > 35$). This 'filter' reduced technical variation in the data generated. Excluded genes constituted 22% of TFs, 25% of microRNAs, and 7% of 'chromatin-related' genes covered by gene-specific primers. As reference genes for transcript normalisation, four primer pairs from a set of reference gene primers available for qRT-PCR analysis (Czechowski *et al.,* 2005) were selected based on

experimental tests of cDNA from both Columbia and C24. These primers performed very well on cDNA from other genotypes used in this work.

Apart from the technical resources discussed above, appropriate experimental design and selection of the most suitable statistical methods for high throughput experiments (refer to sections of Materials and Methods, and Results) increased the reliability of expression data presented in this work. Additionally, analyses of three biological replicates (in almost all cases) allowed for more accurate estimation of expression values, as demonstrated by Lee *et al.,* (2000).

4.3. Significance of candidate genes

The complex phenomenon of heterosis was expected to involve global changes in gene expression. Several early studies in maize have shown that heterosis is accompanied by changes in gene expression, both at the RNA and protein levels (Leonardi *et al.*, 1987, 1988, and 1991; Romagnoli *et al.*, 1990; Tsaftaris and Polidoros, 1993; Tsaftaris 1995; Xiong *et al.,* 1998; Wu *et al.,* 2001). In this study, transcript abundances between parental inbred lines and their reciprocal hybrids were compared in order to identify regulatory genes potentially involved in the heterosis of relative growth rate and consequently biomass. Regulatory genes that were subjected to the analysis included known as well as putative transcription factors (expression of 1469 genes in total was detected via qRT-PCR in 4 DAS seedlings) and microRNAs (87). In addition, expression profiling of genes encoding proteins involved in the epigenetic control of gene expression and/or chromatin modification processes (58 'chromatin-related' genes, Table 3.1) was performed. A set of 61 heterosis-related gene candidates were identified including 57 TF or putative TF genes, three SET-domain genes, and one candidate microRNA gene. The TF group included six genes of uncertain or updated annotation, where AT4G04880 [26] was re-annotated as an adenosine/AMP deaminase (Table 3.5). As the last gene fell beyond category of regulatory genes, it was not considered further in the discussion. This section of Discussion focuses on the 57 candidate regulatory genes consisting of 56 TFs (or putative TFs) and one microRNA, whereas 'chromatin related' genes are focused on in chapter 4.4.

To date, no reports have dealt specifically with the role of TFs or microRNAs in heterosis although some reports have identified TFs among differentially expressed genes in the maize hybrids (Swanson-Wagner *et al.,* 2006; Meyer *et al.,* 2007; Użarowska *et al.,* 2007; Stupar *et al.,* 2008; Pea *at al.,* 2008), hybrids of *Arabidopsis* (Vuylsteke *et al.,* 2005) and of wheat (Wu *et al.,* 2003). Interestingly, most comparisons of gene expression in parents versus

hybrids were centred on stages after the manifestation of heterosis (Hoecker *et al.,* 2008), whereas this work is based on a stage (4 DAS) before any of the outward signs of heterosis are manifested.

Our focus on regulatory genes and a time point prior to the manifestation of growth heterosis reflected an expectation that such genes probably play key roles in genetic regulatory networks underpinning heterosis. It is generally agreed that heterosis effect on quantitative traits is determined by multiple genes or loci (Lippmann and Zamir, 2006; Pea *et al.,* 2008; Frascaroli *et al.,* 2007; Kusterer *et al.,* 2007; Melchinger *et al.,* 2007). Therefore, it was checked whether any of the differentially expressed regulatory genes were located in the QTL regions of biomass heterosis, heterosis of growth and of biomass *per se,* determined by the group of Thomas Altmann (personal communication). It was found that 40% of these regulatory genes (23) co-localised with QTLs: nine with the heterotic QTL for biomass, ten with the heterotic QTL for growth, and nine with the QTL for biomass *per se*. Of these 23, five genes were linked in the same time to heterotic QTL of biomass and QTL for biomass *per se* (Table 3.7). It was surprising that none of candidate genes fell in the overlapping region between the heterotic QTLs for biomass and growth. Among all the QTL co-localising candidate regulatory genes (the 'QTL group'), only TF or putative TF genes were found. The prediction of Salvi and Tuberosa (2005) that QTLs of quantitative traits will be found at microRNA loci seemed promising to investigate the role of microRNAs in biomass over performance of hybrids. However, among the whole set of microRNAs taken to expression analysis just one was found to be differentially expressed in hybrids and at the same time it does not co-localise with any of the three QTLs. Therefore, it is likely that heterosis of biomass does not operate on regulatory mechanisms driven by microRNAs.

In the absence of direct functional data, it was considered useful to determine whether information in the literature might shed light on the roles of some of identified candidate genes. Collected data revealed that the specific function of as many as half of TF or putative TF genes that co-localise with the three QTLs of interest is still unknown (Table 3.4). Among the remaining characterised genes, the following three that co-localise with biomass heterosis or biomass *per se* (Table 3.7) were proven to directly influence growth: AT5G63160 [60] (*BT1* of TAZ family) which belongs to calmodulin (CaM)-binding proteins known to be critical for brassinosteroid biosynthesis and plant growth (Du and Poovaiah, 2004 and 2005); AT1G73830 [14] (*BEE3* of bHLH family) involved in brassinosteroid signalling, required for normal growth (Friedrichsen *et al.,* 2002); and AT5G57390 [56] (*AIL5* of AP2-EREBP family) involved in specification of meristematic or division-competent states especially in young

tissues, and expressed primarily in young actively dividing tissues (Nole-Wilson *et al.,* 2005). It would be interesting to further investigate whether advantageous changes occur in the control of the brassinosteroid pathway leading to growth vigour and increased biomass in the hybrids.

All the candidate genes were also characterised based on the expression patterns they exhibited. Two main expression patterns non-additive and additive were recognised depending on whether hybrid gene expression level deviated from, or was the same as, the average of the two parents (mid-parent expression level), as described by Springer and Stupar (2007), and Hoecker *et al.,* (2008). All regulatory candidate genes displayed hybrid expression levels that did not exceed the range of parents (consistent with data from maize hybrids; Guo *et al.,* 2006), thus the two main effects could be simply evaluated further by defining more specific expression patterns. Among the genes with a non-additive pattern of expression, hybrid transcript levels were compared to those of the parents and classified as dominant (specified as dominant_C24xC24_high, dominant_Col-0xCol-0_low, dominant_C24xC24_low, dominant_Col-0xCol-0_high), over- and underdominant as well as maternal (specified as maternal_C24xC24_high, maternal_Col-0xCol-0_high) and paternal (specified as paternal_C24xC24_high, paternal_Col-0xCol-0_high) (Figure 3.10). Candidate genes with an additive gene expression pattern included two types of expression level relationships between hybrids and parents: intermediate_C24xC24_high and intermediate_Col-0xCol-0_high (Figure 3.10). Among all 23 TF candidates that co-localised with the QTLs of interest, non-additive effects were the most representative (6 genes of dominant expression phenotype and 5 of maternal), followed by additive (9 genes of intermediate expression phenotype). Expression phenotypes of the three remaining genes did not match any of defined patterns. The previously mentioned genes proven to directly influence growth varied in expression phenotypes: AT1G73830 [14] displayed maternal_Col-0xCol-0_high pattern, AT5G57390 [56] intermediate_C24xC24_high, whereas AT5G63160 [60] did not match any of defined patterns. When talking about expression patterns, it is important to remember that although these terms describe the relationship between parental and hybrid gene expression levels (expression phenotype of a gene), they do not imply quantitative genetics models like dominance or overdominance. In other words, they cannot be interpreted as an indication of correlation between genetic hypotheses and molecular events leading to heterosis (Hochholdinger and Hoecker 2007). Differential gene expression in hybrids could result from downstream regulation by other genes responding to heterotic growth effects. Therefore, investigations to relate the observed expression phenotypes to gene actions require subsequent analyses including expres-

sion QTL mapping (Lippmann and Zamir 2006; Hochholdinger and Hoecker 2007; Holland, 2007), which is focused on later in Discussion (chapter 4.7). For these reasons, improper usage of these terms can lead to confusion as noted by Lippman and Zachary (2006), and Springer and Stupar (2007).

The approach for selecting the most relevant candidate genes based on they are differentially expressed in contrasting QTL genotypes, are functionally related to and at the same time co-localise with QTL is not yet validated due to low number of QTLs cloned to date in plants (Salvi and Tuberosa, 2005). This means, alternative approaches to select candidate genes at the first screening stage may appear equally useful. As a result, all candidate genes identified in this work were ranked according to their statistical significance (Table 3.3; refer to chapter 3.3.2 of Results section). Significance ranking of candidate genes was as follows: category I included genes for which the expression phenotype matched one of the defined patterns (e.g. dominance, intermediate, maternal, paternal etc.) and the differential expression in hybrid(s) when compared to parent(s) could be proven in all performed statistical tests (i.e. results of tests passed a set significance threshold). This group contained 18 genes (including one microRNA), or constituted 32% of regulatory candidate genes. Category II included genes for which the expression phenotype matched one of defined patterns (24 genes or 42%) but the set significance threshold was not reached in all comparisons performed within *post-hoc* tests (i.e. expression levels differed in hybrid(s) when compared to parent(s), but not statistically). The lack of statistical significance could simply reflect insufficient biological replication (three replicates were performed). Therefore, genes of category II were considered further in this work. Category III group consisted of the remaining 15 genes (or 26%) for which the significance threshold was not reached in all comparisons performed within *post-hoc* tests but their expression phenotype did not match any of expression patterns defined in this work (Table 3.3). Nonetheless, genes from all the three categories of statistical significance (or 'statistical categories') were found among the group that co-localised with QTL for growth and/or biomass heterosis (seven of category I, twelve of category II, and four of category III). For this reason, genes from all the three categories were considered in subsequent analyses.

The genes of the most relevant (promising) 'statistical category' (i.e. category I) included three regulators whose function might be related to growth. Among them, the previously described AT5G57390 [56] as well as AT5G67480 [61], a Ca^{2+}/calmodulin-binding protein and the only candidate microRNA AT5G08712 [36] (*MIR166C* of miR165/166 family) were found. The last candidate regulatory gene is known to target class III Homeodomain leucine-zipper genes whose regulation is essential for normal meristem development (Zhou

et al., 2007). Additionally, it was demonstrated that overexpression of *miR166* causes an enlargement of shoot apical meristems and enhancement in vascular development (Kim *et al.,* 2005; Williams *et al.,* 2005; Zhou *et al.,* 2007). The mir165/166 family may regulate its target genes in a time- and tissue-specific manner and recently it was reported that it may also regulate floral development (Jung and Park, 2007). The first of the above mentioned genes (AT5G57390 [56]) exhibited the intermediate_C24xC24_high expression pattern, whereas the last two (AT5G67480 [61] and AT5G08712 [36]) the dominant_C24xC24_low. The category I gene group also includes a small subset of genes involved in flowering control: AT2G45660 [20] or *AGL20/SOC1* (MADS-box family; additionally it affects the determinacy of all meristems and is involved in the prevention of secondary growth and longevity in annual life forms; Melzer *et al.,* 2008), AT1G77080 [16] or *AGL27/FLM/MAF1* (MADS-box family; inhibitor of flowering, Scortecci *et al.,* 2003) and AT2G39250 [19] or *SNZ* (AP2-EREBP family; repressor of flowering, Schmid *et al.,* 2003). The three genes varied in expression phenotypes and exhibited the following patterns: dominant_C24xC24_low, intermediate_Col-0xCol-0_high, and intermediate_C24xC24_high (respectively). When considering all of the genes of 'statistical category' I (18 genes), the most represented effects were additive (11 genes of intermediate pattern, five of dominant and two of maternal), which was in contrast to the 'QTL group', where the predominant effects were non-additive.

There is an overlap of seven genes that co-localised with QTLs and belonged to the most relevant 'statistical category' I: AT5G25810 [43] (*TNY/TINY* of AP2-EREBP family), AT5G57390 [56] (*AIL5* of AP2-EREBP family), AT5G63080 [59] (JUMONJI family), AT1G58220 [11] (MYB-related family), AT1G12800 [1] (S1 RNA-binding domain-containing protein), AT5G32460 [46] (pseudogene, possible B3 family), and AT4G12020 [28] (*WRKY19* of WRKY family). From these, as many as five remain uncharacterised (Tables 3.4-6). The most representative expression phenotype, as seen independently in both candidate gene groups was the intermediate (5 genes), followed by dominant (2 genes). Apart from the role of AT5G57390 [56], which was previously described, the role of the Jumonji family to which AT5G63080 [59] belongs (intermediate_Col-0xCol-0_high expression pattern) seems worth mentioning. This TF family is involved in epigenetic regulation (Shirato *et al.,* 2009; Lu *et al.,* 2008) by antagonising the activity of the large number of putative SET domain-containing histone methyltransferases. Jumonji genes were shown to control flowering and flower development regulatory genes (Noh *et al.,* 2004; Sun *et al.*, 2008) as well as cell cycle genes (Shirato *et al.*, 2009). The lack of Jumonji N/C domain-containing proteins

results in impaired cell elongation and reduced expression of brassinosteroid target genes which are very important for plant growth and development (Yu *et al*., 2008).

It was also considered useful to determine whether any TF family was overrepresented amongst the genes when taking into account all the 56 candidate regulatory genes. Among as many as 24 distinct families, the candidate regulatory genes which were the most representative ones were at the same time the most representative among all known TF families. This indicated that it was not possible to find any prevalent family controlling specific process(es) or pathway(s), which could potentially contribute to biomass and/or growth heterosis. In parallel, review of the literature for all of the identified candidate regulatory genes revealed that they were involved in a wide range of processes. Apart from specific functions for some candidate genes that were already described above, the candidate regulatory genes influenced hormonal regulation, signalling and stress responses together with development of different organs or tissues. Additional information for candidate genes that have not been previously characterised (approximately half of candidate genes from at least 18 distinct TF families) was supplied by publicly available microarray expression data (https://www.genevestigator.ethz.ch/gv/index.jsp) and was summarised in Table 3.6. The majority of these genes were expressed in most tissues (~81%), throughout most developmental stages (~74%), and almost half (~44%) responded to a variety of treatments (hormone, chemical), stress, or affected nutrient conditions (Table 3.6 and Figures 3.12-14). Although it is sometimes possible to infer possible roles of genes based on when and where they are expressed and how they respond to stimuli, the expression information collected here provided little insight into the role, if any, of these candidate genes with respect to heterosis of biomass and growth. This result could favour previous conclusions from studies on heterosis in different species, namely that there may not be a predominant functional category to which differentially expressed genes belong and that no specific function is required during heterosis manifestation but rather an interplay of genes related to diverse functions (review of Hochholdinger and Hoecker (2007), Hoecker *et al.,* 2008).

Another interesting question was to determine expression phenotypes that are the most represented among regulatory candidate genes identified at 4 DAS (irrespective of the 'statistical category' or QTL co-localisation). Analysis revealed that among 74% of genes that matched expression patterns defined in this thesis, non-additive effects were prevalent (60%) with dominant pattern being overrepresentative (40% of all non-additive effects). This result is similar to the one for only QTL co-localising genes and it could be explained by the fact that the 'QTL group' consists of genes of all statistical categories. Still, the fact that the pre-

dominant expression phenotype of the regulatory candidate genes differs depending on which group of candidate genes is considered ('QTL' or 'statistical') should be kept in mind. On the other hand, among the seven candidate genes that are common for genes that co-localise with QTLs of interest and at the same time belong to the most relevant 'significance category' I, the additive effects prevail. Thus, the additive expression pattern appears more likely when discussing expression phenotypes of regulatory genes that associate heterosis for biomass and growth.

The expression phenotypes exhibited by candidate regulatory genes identified in this work fit to commonly observed trends in global gene expression studies of heterosis (Hochholdinger and Hoecker 2007), where more significant expression differences were found between parental inbred lines than between reciprocal hybrids. However, the maternal (12 genes) and paternal effects (2 genes) displayed by candidate regulatory genes both constituted as many as 25% of all the candidates (11% of all effects were found among the 18 genes of category I and an even higher percentage of 22% was observed within the 'QTL group'). Taking into account that reciprocal hybrids are genetically identical, differences in gene expression between reciprocal hybrids are mainly due to epigenetic effects (Hochholdinger and Hoecker, 2007). Hence, it would be worthwhile to check whether epigenetic control of regulatory genes is involved in heterotic performance.

The expression of candidate regulatory genes was analysed further at different developmental stages (3, 6, 8, and 10 DAS). Irrespective of gene group they represented (category of statistical significance or co-localising with QTLs), on average 30% of the regulatory candidate genes were significantly differentially expressed in hybrids compared to parents at all developmental stages (e.g. previously mentioned AT5G63080 [59]), 10% were significantly different only at 4 DAS (e.g. previously mentioned AT5G57390 [56]), and the remaining ~60% of genes were significantly different at 2-4 different time points (e.g. previously mentioned AT2G45660 [20]); (Table 3.8). These comparisons were limited to differentially expressed genes at individual time points because transcript level differences were generally not maintained over multiple stages. The expression patterns determined for all candidate regulatory genes at different developmental stages varied from this at 4 DAS and in many cases they were different when compared between different time points. Moreover, whereas at 4 DAS any over- or underdominant patterns were present, they were found at other developmental stages (Table 3.8). This hold true irrespective of group candidate genes represented (category of statistical significance or co-localising with QTLs). However, among the candidate genes that were differentially expressed at a certain time point, the ratio additive/non-additive was

similar for genes that co-localised with QTLs of interest. Genes of category I displayed different behaviour: additive patterns prevailed at 3-6 DAS and non-additive at 8-10 DAS. Taken together, these results seem to be enigmatic and require further studies. While it is certainly feasible that heterosis for superior growth could result from a transient boost resulting from changes in gene expression at a single growth stage, it is not possible to conclude weather one or more of the TF genes differentially expressed at 4 DAS conferred any growth advantage on the hybrids. Likewise, in earlier studies, it was not possible to identify any key genes or set of genes involved in heterosis (Hochholdinger and Hoecker, 2007). On the other hand, the growth heterosis could require sustained changes in gene expression involving enhanced expression of regulatory genes over many growth stages. Again, it remains to be seen whether any of the TF genes with this pattern of change in hybrids is involved in superior growth.

The candidate genes that displayed dominant expression pattern at 4 DAS were further validated in 4 DAS seedlings of two distinct sets of crosses: L*er*/C24 and Cl-0/Nd. The reciprocal hybrids of the first crosses showed positive biomass heterosis (Meyer *et al.,* 2004), and the second exhibited negative biomass heterosis (the hybrid biomass was significantly decreased in comparison to parents), Figure 3.17. One might assume that if a gene with a dominant expression pattern contributes to biomass heterosis then the pattern should be observed in the heterotic positive cross, and at the same time the opposite effect should be observed in the heterosis negative cross (Figure 3.19). Unfortunately, it was not possible to demonstrate this relationship for any of 14 candidate genes that exhibited a dominant expression pattern in Col-0/C24 crosses (Table 3.8). Although four genes displayed a dominant expression pattern in L*er/*C24 crosses, the opposite effect was not found in the Cl-0/Nd crosses (AT3G50890 [24] or *ATHB28*; AT2G45660 [20] or *SOC1,* AT5G10140 [37] or *FLC*, and AT5G17300 [40]; Table 3.8). Furthermore, the first two candidates did not match any of expression patterns defined in this work and for the last two candidates the intermediate patterns were detected in the Cl-0/Nd crosses. Such a result could be due to insufficient statistical power or it may be caused by the fact that various ecotypes possess different alleles of the same gene that may be regulated differently in different tissues and under different environmental stresses as shown by Guo *et al.*, (2004). Thus, even the smallest differences in the development of various ecotypes or the specific response to environmental conditions may affect the expression in hybrids. As a result, it is difficult or maybe even impossible to validate the pattern in the way presented in this project. Keeping this in mind, it became interesting to see what fraction of all the candidate regulatory genes would be differentially expressed in heterotic hybrids of

L*er*/C24 and what expression patterns they would exhibit. Expression profiling of all candidate genes on 4 DAS seedlings of L*er*/C24 crosses revealed that 38 genes (72%) were significantly differentially expressed in L*er*/C24 hybrids but only nine genes displayed patterns that were consistent with those found in crosses of Col-0/C24 (5 intermediate patterns together with 4 dominant effects that were already described above, Table 3.8). Although among these nine only three co-localised with QTL of growth or biomass, all belonged to the most relevant first or second category (I, II) of statistical significance. In addition, all candidate regulatory genes were expression profiled in Cl-0/Nd crosses (negative biomass heterosis) at the same time to exclude those which may be potentially involved in processes not leading to or associating specifically with biomass vigour in hybrids. In this way, as many as 25 (47%) genes were found to be differentially expressed in Cl-0/Nd hybrids. Comparison of data from candidate gene expression profiling in both sets of crosses resulted in 17 genes that were exclusively differentially expressed in L*er*/C24. Among them, only three genes exhibited the same patterns in L*er*/C24 and Col-0/C24 (additive expression levels), seven co-localised with QTLs of interest and six belonged to the most relevant 'statistical category' I. The last group included AT5G13790 [39], a TF involved in recruitment of histone deacetylase complex components (Hill *et al.,* 2008; Harding *et al.,* 2003; maternal_Col-0xCol-0_high expression pattern) as well as the only previously described candidate microRNA (mir165/166 family), and AT2G39250 [19] or *SNZ* (AP2-EREBP family; repressor of flowering). Surprisingly, the candidate genes involved in growth, which were described above (AT1G73830 [14] or *BEE3*, AT5G57390 [56] or *AIL5*, and AT5G67480 [61]) were differentially expressed in hybrids of in both L*er*/C24 and Cl-0/Nd crosses (Table 3.8). Once again, the results obtained appeared to be puzzling and require further studies. Therefore, the identification of regulatory genes that may contribute to heterosis under defined conditions in a limited number of *Arabidopsis* genotypes as presented here represents only the first step towards understanding the molecular basis of heterosis.

No simple model based on the classical genetic hypotheses to explain heterosis can account for the complex set of data generated here or elsewhere (Hochholdinger and Hoecker, 2007). This may be due, in part, to the difficulty of separating genes that cause heterosis, (which may conform to one predominant genetic model), from those affected by heterosis, (which confound data interpretation). Another impediment to understanding the molecular basis of heterosis, at least for a trait as complex as superior growth, is the complexity of gene interactions that lead to the trait, which is probably grossly underestimated by the number of QTLs that contribute significantly to the trait. It is certainly conceivable that heterotic traits

result from interactions of genes that are either up- or down-regulated in hybrids compared to parents. Lippman and Zamir (2006) suggested that there is no obvious link between expression changes caused by heterozygosity and hybrid vigour, which is an idea that will retain currency at least as long as we are unable to identify the genes responsible for heterosis. Another important issue that arises is whether heterosis, at the molecular level, is a general phenomenon or there exist different heteroses. Indeed, one of the latest studies on heterosis reported that heterosis is not an organism-wide phenomenon but rather a trait-specific, and probably is not a consequence of higher levels of additive or non-additive expression but likely is controlled by partially non-redundant sets of genes for different traits (Stupar *et al.,* 2008). Furthermore, Guo *et al.,* (2004 and 2006) suggested that changes in transcript abundance may not correlate with the biological process in question but may be achieved by the differential expression of genes involved in tissue- or cell-specific expression patterns or may be due to the fact that phenotypic value could result from additional regulation than only transcriptional controls. Such dynamic changes of gene expression in hybrids occurring in a response to genotype and environment may result from differential regulation of the two parental alleles. Moreover, allelic differences in expression were shown to be important genetic factors contributing to quantitative trait variation in various organisms. Thus, as they suggested, differential allele regulation may play a role in heterosis providing a new insight into understanding of molecular basis of heterosis. Additionally, Guo *et al.,* (2008) showed that *cis*-regulatory polymorphisms play a more predominant role in hybrid gene regulation than *trans*-acting regulation. However, as they claimed, since gene regulation is the result of *cis*- and *trans*-interaction, the roles of *trans*-acting effects may be through co-selection with *cis*-regulatory changes for optimised gene regulation, contributing to expression of heterosis. This simply indicates the need for the use of novel approaches that may shed additional light on rules driving heterosis. Further directions in the heterosis study will be discussed later in chapter 4.7.

4.4. Investigation of a role of 'chromatin-related' genes in growth heterosis

In an attempt to assess the possible role of epigenetic control mechanisms in growth and biomass heterosis, expression levels of 58 genes involved in the epigenetic control of gene expression and/or chromatin modification processes (Table 3.1, Results section) were compared in parents and hybrids at 4 DAS. The analysis resulted in one differentially expressed gene of the SET-domain group (AT5G43990 [52]). However, this result should be

extended by adding two additional genes which were re-annotated from putative TF candidate genes into SET-domain gene group (AT1G26760 [4] and AT4G13460 [29]).

The Su(var)3-9 group, to which AT4G13460 [29] and AT5G43990 [52] belong, was shown to be involved in the epigenetic control of gene expression (Thorstensen *et al.,* 2006; Baumbusch *et al.,* 2001; Ng *et al.,* 2007). AT4G13460 [29] or *SUVH9*, encodes one of the SUVH2/SUVH9 proteins known to control heterochromatic silencing, exhibiting histone methyltransferase activity and directing DNA methylation. In contrast, AT5G43990 [52] or *SUVR2* encodes a protein from the SUVR4 group which function as repressors of rDNA gene clusters in a decondensed part of the nucleolus (Thorstensen *et al.,* 2006). The function and group affiliation of the protein encoded by AT1G26760 [4] or *ATXR1/SDG35* is currently unknown.

Among the three identified candidate genes, only AT4G13460 [29] co-localised with a QTL for growth heterosis (Table 3.7). This gene, together with AT1G26760 [4], exhibited a dominant expression pattern (dominant_Col-0xCol-0_high) in contrast to AT5G43990 [52], which exhibited an intermediate pattern (intermediate_Col-0xCol-0_high), (Table 3.2). Only two genes, AT5G43990 [52] and AT4G13460 [29] were differentially expressed in hybrids at developmental stages other than 4 DAS. The expression level of AT5G43990 [52] was different in hybrids at 3 DAS (no match with any of defined patterns) and 10 DAS (dominant_Col-0xCol-0_high), whereas AT4G13460 [29] at 3 (no match with any of defined patterns) and 6 DAS (dominant_Col-0xCol-0_low), (Table 3.8). AT4G13460 [29] was differentially expressed also in the 4 DAS hybrids of L*er*/C24 (intermediate_Col-0xCol-0_high) but not of Cl-0/Nd; interestingly AT1G26760 [4] was expressed in Cl-0/Nd hybrids (paternal_Col-0xCol-0_high) but not in L*er*/C24 (Table 3.8). Taken together, similar conclusions as those previously discussed for other regulatory genes could be made: at this stage, these data alone are difficult to interpret and require additional studies. Taking into consideration that Salvi and Tuberosa (2005) predicted that the regions controlling chromatin methylation and/or organisation (e.g. folding) supposedly co-localise with QTLs of complex quantitative traits, the small number of identified candidate genes seems to be a little surprising. In addition, the recent study of Zhao *et al.,* (2008) revealed that there are differences in methylation status between parental and hybrid chromosomal regions, suggesting that epigenetic mechanisms might play a role in the performance of heterosis. Also Ni *et al.,* (2009) proposed a general mechanism for growth and biomass heterosis demonstrating that epigenetic modifications mediate expression changes in downstream genes of circadian clock genes and in the physiological pathway which has an impact on growth and development. Two genes involved

in epigenetic regulation were also found among candidate regulatory genes identified in this work. These included AT5G13790 [39] or *AGL15*, and previously mentioned AT5G63080 [59], a TF gene of the JUMONJI family (chapter 4.3). The first one was shown to be involved in recruitment of histone deacetylase complex components (Hill *et al.*, 2008), whereas the second antagonises the activity of the large number of putative SET domain-containing histone methyltransferases (Shirato *et al.,* 2009; Lu *et al.,* 2008) and it was found among genes co-localising with QTL of biomass *per se*. These data altogether could support the idea to further validate candidate genes identified in this project to determine whether epigenetic control of regulatory genes is involved in heterotic performance. Given that epigenetic silencing mechanisms are widely used by plants to control development and parent-of-origin imprinted gene expression (Henderson and Jacobsen, 2007), special consideration could be given to candidate genes that exhibited paternal or maternal expression phenotypes (refer to chapter 4.3, Discussion section). One approach to study this would be the creation of RNAi lines (Ni *et al.*, 2009) or overexpressing lines. Here, the possible effects of this genetic manipulation on the expression of downstream genes (e.g. ribosomal genes for the AT5G43990 [52]) regulated by candidate genes in relation to growth vigour should also be studied in detail. At the same time, comparative analyses of methylation status of the candidate genes in parents and hybrids would also be appropriate.

4.5. Investigation of a role of ribosomal genes in growth heterosis

In parallel with the investigation of the role of regulatory genes in biomass and growth heterosis, a study on the possible role of ribosomal genes in heterosis was initiated. Elser *et al.,* (2000) reported that the growth rate of organisms is correlated with increased levels of cellular ribosomal RNA (rRNA) content, which may result from an increase in the transcription rate per gene of rDNA or from changes in rDNA structure/organisation (e.g. expansion of the rDNA amount, which can result from endoreduplication; Rogers and Bendich, 1987) and/or epigenetic regulation of transcription (Habu *et al.,* 2001; Meyer *et al.,* 2001). Transcript analysis of ribosomal genes at 4 DAS in F1 reciprocal hybrids of Col-0 and C24 inbred parents showed similar levels of rRNA in all four genotypes (Figure 3.20). Measurement of nucleolar size (Figure 3.21), an indicator of rRNA gene activity (Delany *et al.,* 1994), yielded data with very high variation (Figure 3.22) which made it impossible to find significant differences between genotypes. Also, measurements of the ploidy level, which could reveal endoreduplication and, therefore, gene copy number differences between genotypes, were made

across developmental time (Figure 3.23). However, these measurements did not reveal any differences between parents and hybrids.

Recent studies on nucleolar organising regions (NORs), where several copies of rDNA/rRNA are located, revealed that *NOR2* and *NOR4* are the main determinants for differential NOR DNA methylation (Riddle and Richards, 2002; Lewis *et al.,* 2004). It was also shown that the NOR DNA methylation pattern can be inherited by inter-ecotype hybrids and that epigenetic regulation, reconfiguration of parental NOR DNA methylation or *trans*-acting modifiers can be involved in this process (Woo and Richards, 2008). Taken into account the biomass QTL was found at *NOR4* (Thomas Altmann, personal communication), this information may be a hint that further investigating the role of ribosomal genes in growth and biomass heterosis could involve epigenetic regulation. One approach to study this would be to compare the methylation status of rDNA in hybrids and parents and to correlate it with differences in cellular rRNA/rDNA transcript levels (differential transcript levels of cellular rRNA/rDNA are likely to reflect changes in transcription rate per gene when considering no differences in ploidy level between hybrids and parents). Since the result of qRT-PCR expression analysis was negative and there was a concern that it might be affected by its normalisation procedure (a reference gene is not a high copy gene as rRNA), it would be worthwhile to use another method such as S1 nuclease protection assay (Gaudino and Pikaard, 1999) which allows for the determination of steady-state levels of nascent rRNA transcripts. If there were differences in parental and hybrid expression levels, it would make sense to determine the methylation status of chromosomal regions in hybrids and parents. The differential methylation status of rRNA/rDNA genes would suggest that epigenetic mechanisms may play a role in heterosis. Such results obtained for rRNA/rDNA genes would allow further investigation for their role in heterosis in the context of epigenetic regulation in parallel to candidate genes identified in this work (refer to chapter 4.4).

4.6. Investigation of a possible role of *FRIGIDA* and *FRI-FLC* interaction in heterosis

Further investigation of the possible role of regulatory genes in heterosis included the study of the *FRI-FLC* interaction in relation to biomass vigour. The main objectives of this study were already presented in the Introduction. Generally, the most promising points included the co-localisation of *FRI* with the 'hot-spot' for the biomass QTL (Lisec *et al.,* 2008), expression activity of *FRI* and *FLC* in meristematic regions (Caroline Dean's lab website in 2007: http://www.jic.ac.uk/staff/caroline-dean/FRIGIDA.htm), and a *FRI-FLC* genotype association with rosette growth (Korves *et al*., 2007). Interestingly, *FLC* was identified here

among the candidate TF genes that may contribute to biomass/growth heterosis (AT5G10140 [37]).

In the first set of experiments the introgression lines (ILs) N88/2/1/10 BC5F3 that contained a C24 introgression in the region of *FRIGIDA* (with a functional *FRI* allele, FRI_{C24}) in a Col-0xCol-0 background (with a strong *FLC* allele, $FLC_{Col\text{-}0}$) were used. Comparison of biomass (DW) between introgression line N88/2/1/10 BC5F3 (N), IL test crosses (C24xN and Col-0xN), and both parents (C24xC24, Col-0xCol-0, Figures 3.24 and 3.25) suggested that the increased biomass observed in hybrids and the homozygous IL line may have been related to the interaction of the dominant FRI_{C24} allele with the dominant $FLC_{Col\text{-}0}$. Unfortunately, the second part of this study, which involved another IL line, M63/9/3 that had the Col-0 introgression in the region of *FRIGIDA* (non-functional *FRI* allele, $FRI_{Col\text{-}0}$), in a C24xC24 background (with the weak *FLC* allele, FLC_{C24}), was not completed due to unexpected problems with Col-0xCol-0 growth, and lack of sufficient seed for proper replication of experiments. Although introgression of the region containing FRI_{C24} into the Col-0xCol-0 background yielded increased biomass, it is not possible to conclude that FRI_{C24}, rather than another introgressed gene from C24 near FRI_{C24}, was responsible for the growth phenotype. One way to confirm this result for FRI_{C24} would be to overexpress the FRI_{C24} gene in Col-0xCol-0.

In parallel to the introgression study, RNAi lines suppressing *FRIGIDA* (*FRI* RNAi) in Col-0xCol-0 and C24xC24 backgrounds ($FRI_{Col\text{-}0}$RNAi and FRI_{C24}RNAi, respectively) were created to investigate the role of *FRI* with respect to growth and biomass heterosis. The effect of suppressed *FRI* was also to be studied in complex crosses of RNAi lines (validated for *FRI* suppression effect) with the opposite genotype (Col-0xFRI_{C24}RNAi and C24x$FRI_{Col\text{-}0}$RNAi). Unfortunately, this work met two problems that blocked progress. The first was that none of the RNAi lines appeared to have reduced transcript levels, as determined by qRT-PCR (Figure 3.27), semi-qRT-PCR, non-radioactive and radioactive Northern blot (data not shown). Typically, the tissue or developmental stage of highest expression of the gene of interest is selected to test for silencing in RNAi lines. Publicly available microarray data did not point out any suitable time point or tissue for *FRI* transcript detection. However, the *FRI* transcript signal was detected in 4-week-old young rosette leaves by radioactive Northern blot by Shindo *et al.,* (2005); therefore, similar material was used to measure *FRI* levels in WT and RNAi lines in this study. Unfortunately, *FRI* transcript levels were too low to measure differences between WT and RNAi lines. Thus, the solution to this problem requires finding a time point and tissue or cell type of strong *FRI* expression and using qRT-PCR to measure transcript levels. It is possible that the shoot meristems would be a proper choice. Unfortunately,

this work could not be performed within the remaining time of this PhD. The second problem that was encountered was an inability to recover transformants containing the *FRI* RNAi construct in C24xC24, a possible reflection of a poor seed lot use during transformation. Transformation using the same construct and methods worked well in the Col-0xCol-0 background indicating that the methods were effective and also that presumed loss of *FRI* activity in Col-0 was not lethal. However, bearing in mind the difficulties faced in measuring *FRI* transcript levels in WT plants mentioned above, it is possible that *FRIGIDA* expression was unaltered in *FRI* RNAi lines of Col-0xCol-0 and that loss of *FRI* expression in *FRI* RNAi lines of C24xC24 was lethal. Perhaps, overexpression of the FRI_{C24} allele in Col-0xCol-0 would help to resolve the role of this gene in heterosis.

4.7. Further directions in heterosis study

Studying heterosis at the level of gene expression is an important approach to unravel the genetic nature and links between genotype and phenotype; however, to assign the role of identified candidate genes in this process, further validation steps are required. Some of previously discussed approaches involved functional testing of the selected candidate gene by applying reverse genetics. These tools are based on the analysis of transgenic plants in which the activity of specific genes of interest (e.g. TFs or TF target genes) was altered by genetic engineering. Apart from genetic complementation of a known mutant (Doebley *et al.,* 1997) or RNAi lines (Helliwell and Waterhouse 2003), further studies may also include either the analysis of knock-out lines e.g. from publicly available T-DNA and transposon-insertion mutant collections (e.g. http://signal.salk.edu/cgi-bin/tdnaexpress) or overexpressors (OEs). Possible limitations, such as difficulties in interpretation of phenotype obtained from constitutive expression of cDNA or RNAi constructs, can be omitted by making use of alternative (i.e. nonconstitutive) promoter systems. They include tissue-specific, developmental or inducible promoters which allow the introduced gene to be expressed in a specific tissue or can be turned on/off at any time of development. Such flexibility may be helpful in discriminating primary from secondary, pleiotropic effects of ectopic gene expression. Reverse genetic tools are important constituents of TF-based technologies that are currently undergoing substantive development despite potential problems related to the complexity of transcriptional networks and TF engineering (Century *et al.,* 2008). The rationale behind is that TFs are considered excellent candidates for modifying complex traits in crop plants of third generation biotechnology. This would certainly accelerate attempts taken to discover the roles of TF and other regulatory genes as well as pathways they regulate in heterosis. A most recent and excellent

example of applying this approach to study heterosis is the (previously cited) work of Ni and his colleagues (2009). They used OE and RNAi lines to manipulate an expression of *CCA1*, a circadian clock gene, demonstrating its effects on circadian-mediated physiological and metabolic pathways that contribute to growth vigour and increased biomass.

Ongoing improvements of genomic tools, together with development of novel approaches (including different experimental designs with parallel analysis of more genotypes, developmental stages, organs etc.) as well as more advanced methods for data analysis and validation may bring more insight into the potential contribution of differentially expressed genes to heterosis. These new experiments may be helpful for heterosis predictions based on expression profiles as reviewed by Hochholdinger and Hoecker, (2007). At the same time, making use of novel expression profiling platforms of increased detection sensitivity may be helpful to reconfirm differential expression of selected candidate genes and/or allow identification of a higher number of candidate genes. For this purpose, apart from commonly used microarray analysis, serial analysis of gene expression (SAGE) and its improvements such as Robust Long SAGE (Gowda and Wang, 2008) or SuperSAGE (Matsumura *et al.,* 2008) could be used. SAGE was shown to be extremely powerful and efficient global approach that provides digital analysis of overall gene expression patterns, which allows for direct comparison with data generated by other laboratories. In this method, transcription profiles are created by isolation of specific SAGE tags representing individual transcripts, their further sequencing and quantitative analysis. Whereas SAGE has been primarily used to collect data for various cancerous cell lines (Zhang *et al.,* 2007; Aldaz, 2003), recently it was successfully applied in plants to study the molecular mechanisms involved in transcriptional regulation in response to abiotic stress (Byun *et al*., 2009; Robinson and Parkin, 2008). The resolution of transcript profiling can be increased even more to reach a specific cell type level when combined with laser-capture microscopy (LCM; Kerk *et al.,* 2003, and Schnable *et al.,* 2004). This approach allows for rapid and precise isolation of variety individual plant cell types from heterogeneous tissues using laser beam and microscope. The LCM-harvested cell samples are next subjected to expression analysis. LCM combined with cDNA microarrays was successively used for the identification of phloem-specific genes in rice (Asano *et al.,* 2002) as well as genes preferentially expressed genes in the epidermis or vascular tissues of maize coleoptiles (Nakazono *et al.,* 2003). This method could be used to compare the expression activity of candidate genes (including previously mentioned *FRI* and *FLC*) in meristematic regions of parental and hybrid plantlets. Another approach that may shed more light into understanding the molecular basis of heterosis is allele-specific expression analysis (Knight 2004, Guo *et al.,* 2004 and 2006;

Hochholdinger and Hoecker, 2007) especially when combined with MPSS technology (Guo *et al.,* 2008). It outranks all gene expression studies that have been focused on total level of gene expression by taking into account the allelic contribution to gene expression in the hybrid. This enables discrimination of *cis*- and *trans*- regulation of gene expression. This novel technology was used to study heterosis in maize giving an indication that *cis*-regulatory polymorphisms may play a more predominant role in hybrid gene regulation than in *trans*-acting regulation.

In parallel with approaches presented above, trends in the study of heterosis focus on making use of novel derivatives of classical QTL mapping called an expression QTL (eQTL) mapping, the previously mentioned method which nowadays is considered to accelerate analysis of the molecular basis of quantitative traits. Here, since the expression level of a gene is considered as a quantitative trait (expression trait) it is used to identify loci controlling the expression variation of genes/gene networks associated with diverse biological functions. Mapping results may also suggest *cis*- *or trans*-acting mechanisms for eQTLs. This means, in the first case, that sequence variations around the gene region of the expression trait may directly influence the transcript abundance of the gene; in the second example, the variation in the expression level of one gene may be affected by sequence polymorphisms in other genes (Kliebenstein *et al.,* 2006; Zou *et al.,* 2007; Kliebenstein 2009). eQTL analysis has been applied to identify *cis*- and *trans*-acting regulatory regions in various organisms, also revealing the presence of eQTL 'hot spots' or chromosomal regions that possibly control the simultaneous expression of many genes (Brem *et al.,* 2002 and Schadt *et al.,* 2003). eQTL studies in plants focused to unravel the genetic control of gene expression during shoot development (de Cook *et al.,* 2006) and of complex traits like cell wall degradability (Shi *et al.,* 2007). The usefulness of eQTL was also demonstrated for the prediction of transcription factor binding sites (von Rohr *et al.,* 2007). Once the QTLs that associate specific traits are identified, they are further subjected in diverse cloning methods. These mapping and cloning strategies together allow for the simultaneous handling of multiple, key, genetically-unrelated genes and may lead to discovering their relevance in heterosis (Hohcholdinger and Hoecker, 2007).

Achievements in extensively developing bioinformatics may also bring new insights to the functional characterisation of identified candidate regulatory genes. Bioinformatic tools are used to find connections between candidate TFs and their target genes by reconstructing transcriptional regulatory networks which control a pathway (Qu and Zhu, 2006; Riaño-Pachón *et al.,* 2007) that may contribute to heterosis. Once again, the work of Ni *et al.,* (2009) may serve as good example of how regulation of expression of a few circadian clock pathway

genes (proven by Michael *et al.,* 2003, and Dodd *et al.,* 2005 to control metabolic pathways and increase plant fitness) mediate expression changes in downstream genes that control the chlorophyll and starch metabolic pathways, which ultimately induces increase of hybrid growth and development. Bioinformatic analyses and tools may also become helpful to determine whether the evolutionary divergence of genes contributed to heterosis. The combined evolution of transcription factors and their targets (including changes that occurred within a regulatory regions close to e.g. promoters or far e.g. enhancers or silencers) in different parental species may influence the regulation of certain pathways in hybrids causing phenotypic difference variation in quantitative phenotypes. The completion of genome sequencing projects in various ecotypes will allow data collection from various sources. This information when reprocessed by bioinformatic systems may clarify the relevance of genome organisation in heterosis in determining whether the heterosis is a general phenomenon or a very specific one.

Taken together, the integration of data obtained from studies performed at many levels and with different sources seems to be promising in explaining the phenomenon after hundred years of heterosis studies. This 'systems biology' approach, in which genetic, expression and interaction data are combined to assemble all genes into transcription or protein interaction networks underpinning major biological processes, is a current trend in biological sciences (Century *et al.,* 2008). Because of the specific roles that TFs and other regulatory genes play, data obtained in this work in combination with future discoveries on heterosis could be directly applied to increase significantly the effectiveness of crop improvement.

5. SUMMARY

Heterosis or hybrid vigour has been utilised in plant and animal breeding programs for at least 90 years. An understanding of the molecular basis of heterosis will allow the creation of new superior genotypes to be used directly as F1 hybrids or form the basis for the future breeding programmes.

This PhD project investigated the role of transcription factors, microRNAs, selected genes encoding proteins involved in the epigenetic control of gene expression and/or chromatin modification processes (called here 'chromatin-related') and a group of genes with potential roles in growth (*FRIGIDA*, ribosomal genes) because of our expectation that they might play key roles in heterosis.

The heterotic F1 hybrids of two divergent *Arabidopsis* inbred lines (Col-0xCol-0 and C24xC24) were used. Significant differences in seedling biomass were detected as early as 8 days after sowing (DAS), whereas differences in relative growth rate were only observed in the early phases of growth at lower light intensities. The self-created reciprocal hybrids (Col-0xC24 and C24xCol-0) used for all experiments exhibited a mid-parent heterosis of 40-60% at 15 DAS when seedlings were grown at a light intensity of 120 μE.

Because the earliest differences between hybrids and parents could potentially be driven by differential expression of regulatory genes such as TFs, in the first part of the PhD project it was essential to identify an appropriate time point for gene expression profiling to later identify regulatory genes potentially involved in heterosis. Based on microscopic and biochemical studies of early development the 4 DAS stage was selected.

High-throughput qRT-PCR developed by Czechowski *et al.,* (2004) was used to study the expression of transcription factors and other genes selected for this work. The technical resources developed for the ecotype Columbia were tested (and modified when needed) in other ecotypes used in this study to obtain a reliable data. Additionally, careful experimental set-up and selection of the most suitable statistical methods for high throughput experiments increased the reliability of the expression data generated in this work.

The transcript abundances between parental inbred lines and their reciprocal hybrids were compared at 4 DAS. The subsequent several-step data processing and analyses allowed for the identification of 57 candidate regulatory genes (56 TF/putative TF genes and one microRNA), and three candidate 'chromatin-related' genes.

The most relevant regulatory candidate genes in this work included those which co-localised with QTLs for biomass/growth heterosis and QTL for biomass *per se*, or those

which belonged to the highest statistical significance and matching with predefined expression patterns. In the first group 23 candidate regulatory genes were found, whereas the group of the most significant 'statistical category' I included 18 genes (seven genes overlapped in these two groups). The most represented expression phenotypes found among these candidates were non-additive or additive, respectively. The literature and publicly available microarray expression data searches indicated there was no prevalent family controlling specific process(es) or pathway(s), which could potentially contribute to biomass and/or growth heterosis because of the wide range of processes the candidate genes are involved in. However, some specific functions related to growth or development were found among candidate genes, suggesting the potential contribution of brassinosteroid signalling and meristem development to heterosis, for example. Moreover, the identification of candidate genes exhibiting paternal and maternal effects suggested the epigenetic control of regulatory genes could be involved in heterotic performance.

The expression of candidate regulatory genes was analysed further at different developmental stages (3, 6, 8, and 10 DAS). The analysis revealed that on average 30% of the regulatory gene candidates were significantly differentially expressed in hybrids compared to parents at all developmental stages; the gene expression patterns varied at 4 DAS and also when compared between different time points. These results did not clearly determine whether one (or more) of the TF genes differentially expressed at 4 DAS conferred any growth advantage on the hybrids.

The candidate genes that displayed dominant expression pattern at 4 DAS were further validated in 4 DAS seedlings of two distinct sets of crosses: L*er*/C24 (positive heterosis biomass) and Cl-0/Nd (negative heterosis biomass). Unfortunately, it was not possible to demonstrate any relationship between the dominance effect of candidate gene and the hybrid biomass differences observed in the crosses of L*er*/C24 and Cl-0/Nd. This might be caused by the fact that various ecotypes possess different alleles of the same gene that may be regulated differently in different tissues and under different environmental stresses. All the identified candidate regulatory genes were further expression profiled in both sets of crosses resulting in a fraction of 17 genes that were exclusively differentially expressed in L*er*/C24 but not in Cl-0/Nd, potentially revealing another relevant group of candidate genes for heterosis whose involvement requires further validation.

To assess a possible role of epigenetic control mechanisms in growth and biomass heterosis, the 'chromatin related' genes were expression profiled and resulted in surprisingly small number of candidate genes (three). However, this number reflected findings in the re-

cent literature highlighting that a fraction of the candidate regulatory genes is generally involved in epigenetic regulation. Therefore, these genes still should be considered as targets for future studies on heterosis.

In parallel, a study on the possible role of ribosomal genes in heterosis was initiated based on reports that the growth rate of organisms is correlated to increased levels of cellular ribosomal RNA (rRNA) content. Transcript analysis of ribosomal genes at 4 DAS in F1 reciprocal hybrids of Col-0 and C24 inbred parents showed similar levels of rRNA in all four genotypes. Measurement of nucleolar size, an indicator of rRNA gene activity (Delany *et al.,* 1994), yielded data with very high variation, which made it impossible to find significant differences between genotypes. Also, measurements of the ploidy level across developmental time, which could reveal gene copy number differences between genotypes, resulted in negative results. Still, because of the potential role of ribosomal genes for growth they should be a subject of further studies on heterosis.

Further investigation of this work included the study of the *FRI-FLC* interaction in relation to biomass vigour due to co-localisation of *FRI* with the biomass QTL 'hot-spot' for the biomass QTL, expression activity of *FRI* and *FLC* in meristematic regions, a *FRI-FLC* genotype association with rosette growth, and identification of *FLC* (AT5G10140) among the candidate genes. The study using introgression lines indicated that introgression of the region containing *FRI* from C24xC24 (FRI_{C24}) into the Col-0xCol-0 background was correlated with increased biomass, however, it could not be concluded that *FRI* from C24 is responsible for this growth phenotype without further analyses. At the same time, the RNAi lines created to suppress *FRIGIDA* (*FRI* RNAi) in Col-0xCol-0 and C24xC24 backgrounds were problematic hindering further progress.

Taken together, this PhD study provided an input into studies on molecular mechanisms underlying heterosis allowing these findings to be further exploited when investigating the phenomenon. Alone, the overall analyses performed in this work to discover a role the selected regulatory genes play in heterosis do not provide the final answer. However, when integrated with some future results of other research groups, they together will allow to discover the phenomenon after hundred years of studies. This could be directly applied to increase significantly the effectiveness of crop improvement.

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Annex A. List of primer sequences

Table 1. Primer sequences and references

AGI codes of genes	Remarks	Sequence of forward primer 5' - 3'	Sequence of reverse` primer 5' - 3'
AT5G65080		TTTTTTGCCCCCTTCGAATC	ATCTTCCGCCACCACATTGTAC
AT4G05320	for intron sequence	TTTTTTGCCCCCTTCGAATC	ATCTTCCGCCACCACATTGTAC
AT4G05320	for 5 polyubiquitin genes	GGCCTTGTATAATCCCTGATGAATAAG	AAAGAGATAACAGGAACGGAAACATAGT
AT4G05320	for 3 polyubiquitin genes	CACACTCCACTTGGTCTTGCGT	TGGTCTTTCCGGTGAGAGTCTTCA
AT1G13440		AGGTGGAAGAGCTGCTTCCTTC	GCAACACTTTCCCAACAGCCT
AT1G13440	for 5' cDNA end	TCTCGATCTCAATTTCGCAAAA	CGAAACCGTTGATTCCGATTC
AT1G13440	for 3' cDNA end	TTGGTGACAACAGGTCAAGCA	AAACTTGT CGCTCAATGCAATC
another reference gene primers		Czechowski *et al.*, 2005	
5.8S rRNA		GAAGAACGTAGCGAAATGCGA	GACTCGATGGTTCACGGGA
25S rRNA		CGGGCTTTTGATACGCTTGT	TTAGGCGCGTGCTGCAG
18S rRNA		ATTGTGTTGGCTTCGGGATC	AAATACGAATGCCCCCGACT
AT4G00650 for semi-quantitative PCR		CAATTATCCACCGACGGTGG	TCCATTTTCTCAGCCGCAG
AT4G00650 for DIG-system		CAAGTATGGACATTACGATCGGTC	TTCGACGTCTCCGGTACAATC
AT4G00650 for qPCR		GTCATTTATTTAACTCCCAACAGTCTCA	GCATTCTTAAGCCCCAAACATTA
pAGRIKOLA validation primers Agri 51/56/64/69		http://www.agrikola.org/seeds_validation.html	
Set of 'chromatin-related' genes			
AT1G01920		GCATGCCCTTCACTTAGCTCTCT	AATAAAACACCGATGAGATATCAGTCA
AT1G04050		CACGTTCTTTTTTGTAGTAAACCATAAATT	TCAGGGATCCGTTGGCC
AT1G14030		GAAAATCTTTACTGGTTTGGTCTCAGA	TCTGATGTCTTGAGGTTGATTCAAGT
AT1G17770		AGAGCCACGACATTTGACTGAACC	TGTGTGGAGAGGAGCGAAGAAGAT
AT1G24610		CCAAGGTTAGTATGAGAAAGTGGACA	CAACCTAACTTCCCTTCTACTTCATCA
AT1G48410		CCTGGAGAGGATTCAAGCCC	TTTCAGGCCAATCCTGAGATG
AT1G63020		CAGTTGCACCCCAACAGTGT	TAGCGACCCGGATTCCTTT
AT1G69770		TCTGCCTGAAGGTTTTGCATT	CAGTGTTCATGCAAGCTCGG
AT1G73100		ATTCGTGAAGGAAACGGTGAA	GGATATGACGCATGGCAAAGA
AT1G76710		CCGGCCTTATCTCGTCGTACAATG	ACGAGGAGGTGAAGAAGGAAGCA
AT1G77300		GTCCCATCTGCTTTTGCGCTTG	GGCTGCAACTCCTGAAACAGCATC
AT1G80740		CGAGTCAAGGACTGACGGATG	CATTCGGGATACTTGAATGGC
AT2G05900		TCTTCCGAAAAGCGGCTTCTAGTG	CGGGTTTGGTGGTACGGACATGTA

AGI codes of genes	Remarks	Sequence of forward primer 5' - 3'	Sequence of reverse` primer 5' - 3'
AT2G16390		CGCTTGTTGGAGCATCTCG	CGGGTCACAGAAGGGTTTAGG
AT2G17900		CATGGCTGATCCGCAGAATGTCAA	ACTCGAATGGTTGCTGGGGGAA
AT2G18850		CTCTGCCGCTCTTTGAACTCCAT	TCGACAGAGAAAACGCGGATTGG
AT2G19640		CCATTGCTGCTGCCACTACCTT	TCCAAGCTCGTTTCCTCCTCTCTG
AT2G22740		CACCTCACCATTGCTCTGAGAC	CACCGATTGTGAATGGAAATGGGA
AT2G23380		AACCATTCTCCTGAACCTAACTGTTAC	CACCCTGTGATCTCCAGCAA
AT2G24740		CGATGAAGCACATTCCTCCA	TCCACACAAGAAATTCCATAGTCATAT
AT2G27040		ATTTCTGTTGTTGCGCCGAT	TGAACGTCCCAAGCTGAGC
AT2G33290		GTGTGATGCTTTTTGCGCTG	CCGTAATCCAGGCTTAGCTCG
AT2G35160		ATAAACGCAGCACAGAAAGGG	AGATTCGGCGAGCAGCTATG
AT2G36490		GATTGCACTTCCCGGCG	CCAACGGATTAGGCGAGGT
AT2G40030		GAAGAAATACCCTGACCGTGCT	AGGCCGAGGTTTCGTAAAGTACT
AT2G44150		ACCATCCTCGAACATCACCGAGTG	GCCCCACGAGTGATAGGTGTTTTG
AT3G03750		GTGGCCGGAGAGAACAGAGAC	CCCAAACAGCAGGAACTACCA
AT3G04380		TCATGTTTGCATCCTCGCATCTGT	AGATGAAGAAGCTCTCTGCCTGGA
AT3G07670		AACTGCAATGCGTCTTCCTCGAT	TTGAAGAGATGGCGAAAGCTGCT
AT3G21820		TGCTTGTCCGTCTCTGTCCTCTTC	AAGGAACGGCCTTCTTCCCTCT
AT3G23780		GTAAGGGTTATCCGAACCGAAGA	CAGTCATCAGTGGCTCGGTTT
AT3G43920		GGGCCTCGTGGAACTCTACA	CGAAAGTAGGCATTGGCCA
AT3G44530		AAAACGGGTTCTGAGGACCG	TTCAAGAGCCCGAGTCTCTTG
AT3G55080		TTATTGCACAACCTTGCTTTCAGA	TTGCTATCGTAGAGACTGTAAAATCGAT
AT3G56570		TGGAAGATGACATTGTTAGGGTGA	TGATATAGTCTCCTGTCTCTCGGAAGA
AT3G59960		GCTTGTCCTGACGCATCCAAATTG	ACCTGGAAAACGCCCAAGCTTC
AT4G08990		ATAATTTGGGCAGCTTCACCA	AGACATGCATCGGCTCAGG
AT4G11130		TACAATCCAAACCATCGCGA	TCACCTACGATCCATGGGAAA
AT4G13610		GGCAATTTACCCACTTGCATC	ATGGAAGCACATTCCCACCA
AT4G13940		CTCTTCAAACAAATGAGAAACCAAAA	AACACTGGGATGCCGAAAAC
AT4G15180		GCATCGAACATCGGGCTTCCAAA	TGTCTAAGCAACCTCAAAGACCGT
AT4G19020		CAGTCTCGGTTTCTCGTGCTT	GCGGGCTAGACCACGAAAA
AT4G20910		CAACACAATTCTCCAGCGGTC	CTGTGGCTCCGAGTTGTTTTC
AT5G04560		ATCCTCCTGTAGCCATCCCG	CTTGCTAGGGATTTCTCCAACG
AT5G04940		GATCAGCGTATTGCCGTGG	TGTTCACCAGAGATCGCCTTT
AT5G06620		TGTTTCGACATCCAAAAACCCCTT	GCCCTTCTCTTTTAAAGCTCGACT
AT5G09230		TTGTTCCATTGAAAATCAATGCTAG	CCCACGTCAAGAACTCTGTGC
AT5G13960		TTTCCCCAATGCAGGAGCT	GGTCCATGAACGCTATCAAGC

AGI codes of genes	Remarks	Sequence of forward primer 5' - 3'	Sequence of reverse` primer 5' - 3'
AT5G14260		AGATCCAGCCATGAACCAGACAGT	GGTAGTCCTACCAAGGCCGAAGTT
AT5G14620		TTCCCACATGGAATCAATGTTC	GTGCCACTTCCCCACCAC
AT5G15380		CCGTGTAACAATCTTGCCGG	AGTGCTCTCCTCCAAGACCG
AT5G17240		TCAGGGAAGTCGGAGACGGAAAGA	GATGGGCAGCAGAGATTGGCATT
AT5G42400		TCCGACGTGAAGGTACATTGGAGA	TCACAGAAGGGCCGTAAGTCGT
AT5G43990		TCTCTCCAGCAAGCTCACAGACAA	TGCAGGTGTTTTTCACGCCCAAT
AT5G49160		GAGTACTCTGCCACTGCCTGG	AAATCCCTGACATGGAGGTCC
AT5G55760		CCCGTAAACGCTCTAGAACCG	GCCTTGGTTTCTTCTGCCAG
AT5G63110		GGACAGGGACTCTACCGGTG	ATTCACGTCTGGCTCTGGGT
AT5G66750		TTCCAGTGAAGGGTCCAGGT	GAAGACAGCATTCCTCCCGA
The old TF platform (experiment 1)		MPI-MP Golm / *Arabidopsis* TF platform	
The new TF platform (experiment 2)		Table 2 of Annex A (Czechowski *et al.*, unpublished results)	
The microRNA platform		Datt Pant and Musialak-Lange *et al.*, 2009	

Table 2. Primer sequences of 'The new TF platform (experiment 2)'

No.	Gene group	AGI	Sequence of forward primer	Sequence of reverse primer
1.	TF	AT1G01030	TCTTTCCAACGAGGCATCGGAGAT	AACGAGGCTCATGTCGGGTCTATG
2.	TF	AT3G16280	GTCATCATCGCCGCATGATATCCA	CCGGAGCCTCATCCTTCTCTAACA
3.	TF	AT1G28300	ACGAGGACGAAAGCAAGAATCTCT	GTGGTTCCTTTCCCTCGACTCATT
4.	TF	AT3G16770	CAAACTCCATCCCACCAACCAAGT	TCTGTTGCCTGCTCCTTCTTCACT
5.	TF	AT2G30470	ATGCTGGAGACATGCAGGGTTGTG	ACACCAGACGATGATGTGTCCTCA
6.	TF	AT3G20310	TCTCCAACCTCTCACCTACCTCCA	CCGCCGTATAACCGATGGTCCATA
7.	TF	AT2G36080	TTATCCTCATGCAGGGGCTCAAGC	TGGCACTCCATGTTCACTCCGAA
8.	TF	AT3G20840	AGCACTGAGGAAGAAGCAGCAGA	CCGGTTGATCTCGAAGTTGGTCAC
9.	TF	AT2G46870	TGCACGGGAGAGCTAATCAGGAAC	TCTCGCCGCATTCCATATCAACTC
10.	TF	AT3G23220	GGGACGTTTAACACAGCGGAAGAC	CTCATGCCGAAAGCTGCTCTATCA
11.	TF	AT3G11580	AGTATTCACACTATGGCGCCG	CCTGCGACCACCGGTG
12.	TF	AT3G23230	GGAGCAGCAACAACCAATCACAAG	TTCTTCGAACTCCCCGGAAACGA
13.	TF	AT3G24650	GGCAGGGATGGAAACCAGAAAAGA	GGCAAAACGATCCTTCCGAGGTTA
14.	TF	AT3G23240	TTGATCACCGCTCCGTGAAGTTAG	TCAGAAGACCCCAAAAGCTCCTCA
15.	TF	AT3G26790	ATGATACTCCCGAAGAAAGCCGC	TCCCTTCCTTGCATTCAAGTGCC
16.	TF	AT3G25730	TGCCATGAGTAGCGTAGACGAGAG	AGTAAACTCGCCGGAGACGATGAC
17.	TF	AT3G61970	TGGTGCTGGTTCTCTCCTCCAGTT	TCAAGACTTGCAGCTTCCATTGCT
18.	TF	AT3G25890	CCACTTCTGCTGCTTCCTCTGTTT	TCAATGCTTGACCCTGAGGCTGAG
19.	TF	AT4G01500	ACGTGGCGAAATGGGAGTAGCA	ACGTGGCGACCTCCAAAATGATT
20.	TF	AT3G50260	TACCTCAGAGGACCAACTGCTACG	CCTGATCGTTGCCGCTGACATATC
21.	TF	AT4G21550	GGGAAGCCTACTGATGTTGCAGGA	TGTCGTGGGTGCTTTGTGGTTG
22.	TF	AT3G54320	TTCCGGCAGAGACGTACACAAAGG	GCGGCGGAGAGAAGCCAAATATTC
23.	TF	AT5G60450	TTGTTGGTGCGATGGGATGAGTC	TGTGGGAGAGAAACCGAGGGATCA
24.	TF	AT3G18990	CAAGAAAAACGTCTGAGGGTCCCA	TCAGGTACTGTGAGTGCAACAGC
25.	TF	AT5G62000	TTGGACCCTGAGGCTGCTCTTTAC	TCGTCTTGTCTAGGAACCGTCACA
26.	TF	AT3G46770	CAAAATAGAACGCGCATACCGTCT	TGTCACGCATGATAACCATGTTGG
27.	TF	AT1G04880	TCTAACCTTTACCCCACAGCCCAA	TCCAGCAAGTGATCCTCCGAGGAA
28.	TF	AT3G53310	TTGAAGGGAACTTGGACGTGGAAG	CACCACATTGGCTGGGATCAACA
29.	TF	AT1G20910	TGGGCTGCAAAAACACAAACAACA	CGGGTCCAACATCAACAACCTCAG
30.	TF	AT4G00260	ACTCTGCGGTTCGAGGACG	AATCCTTGCCATCCTCCTACC
31.	TF	AT1G55650	TCTGCAAACCGTGACAAAGACTCT	GCTTTTCTGAGCCTACCTTTGTGC
32.	TF	AT4G01580	TGATGAGGATTCCGCCAAGATTCG	ACGCTTATATCCCGCAGGAGTCAC
33.	TF	AT1G76110	ATCTCTTCACTGCTCGTGGTCC	GAGGGATTTGCATGAAATGTAGC
34.	TF	AT4G31610	CGTGAGACTACCGGGCAACTATCA	TGGTTCAGCAGAATTACCTCCCCA
35.	TF	AT1G76510	AAGGTTGTGAACTTTCCCGCAAGA	GCTCAAATGGGACTCGCACAAACA
36.	TF	AT4G31620	TCCGAGGCCAAGATAGAGCAAGA	TGCGACGAGAGTGTCCTACTTCT
37.	TF	AT2G46040	CTTGGCAGAAAGTCCAGAAGATGC	GCCTATATCCGAGCCATTCCCAGT
38.	TF	AT4G31630	TGTTCCAAGGTGAAACAAGCAGCA	TTGATCCGTCATTCGGGGTTTCC
39.	TF	AT3G13350	GGGTGGTGAAGGATCGGAAATGGA	CGATGCACTTGTTATCGTTGTCGG
40.	TF	AT4G31640	CCCGGTTTCAAGAGCCACATCAAA	CTTCAGTGAGTTTATGGCCCTCCA
41.	TF	AT3G43240	TTCTAGTGCTCCAGCACCTCCTCT	CGAGACATCACAACGCATGGTCAC
42.	TF	AT4G31650	CCCGGTTTCAAGAGCCACATCAAC	GGTCTTTCCTTCGTGTTTCCCCTT
43.	TF	AT1G06280	TGGTGTTGGTGTGAATCGGGAGAT	CCCAACCACCTTGCACATTCCTTG
44.	TF	AT4G31660	TTCCCGGCTTCGACTCTTACCTTA	GTCGGTTCTTAGCTCCACTGTCCT
45.	TF	AT4G32010	GCGACACAGATGTTCAAGGGAAGC	TGTCACCACATCCCGGATTCAAGC
46.	TF	AT3G54990	TGGGCGGGTTTGATACTGCTTACG	TGTCTGCATCGAGACCACGGAATT
47.	TF	AT5G06250	CACTAACTCCGCCGTGAATACGA	GCGGCTCCATAGTGGGAATACTCT
48.	TF	AT3G57600	TCAGTACCGTGGAGTCAGGCAAAG	TGTAGCGAAAGAGCCAAGCCAAAG
49.	TF	AT1G14510	TGGCAAGCCTCGTCATTCTGAATC	TCTTGGTGGAGGGGACATCTTTGA
50.	TF	AT3G60490	TGTCTACCGCTCAGTCTTCGACTT	CACCGTCTCTTCGTTGCTTTCCTT
51.	TF	AT2G02470	TGGCAAGAATGAGAGGAAGAGGCT	TTGCCGCTCACAACTTCGAAAATG
52.	TF	AT3G61630	CGTCAACGAGGAAACAGAGCATGA	CAAACCCGCCGACGATTCTTCT
53.	TF	AT3G11200	CCCGTCTTAATCGCAATGAGAGGA	AGTGCCATTTCTGGATTTGCTTCC
54.	TF	AT4G06746	GGCCTACGATACCGCTGTGTTTTA	TCCTAAGCCTTCACCGCCGTTT
55.	TF	AT3G42790	CGCTTGGTTACTGTCTGTCTCGTT	ACGCTTCCTCTCTTCCTTGTGGA
56.	TF	AT4G11140	TTAACTTCCCTCCTCCTCCGGTGA	GAAGACATTCTCCACCGCCAATGA
57.	TF	AT5G05610	TCCAGAAACGGCGTAAAGAGATCA	TCCTCCACAACTTCCACAGAGTG
58.	TF	AT4G13040	AGCCGAAGAACTCGGTCAAAACAT	GCGGCTGGTATTTTGGTGAGATGG
59.	TF	AT5G20510	GGCTTTCTCTCGTTGCTGTCCA	GCGCTTCCTGTCAGCTCTATCGAA
60.	TF	AT4G13620	TCGCAGAGATTAGGCTTCCAAGGA	GCTTGCTCAGCGGTTTCAAAAGTG
61.	TF	AT5G26210	TCCGACTTTGTGATCCCGAAAAGG	GGCAAATTCACTTCCCAGTGCTCA
62.	TF	AT4G16750	GGTGGCTCTGCCCACCTTAATTTC	GCGGCGGCTTCTTGAATGTCTTT

No.	Gene group	AGI	Sequence of forward primer	Sequence of reverse primer
63.	TF	AT1G01250	TTGCGGCGGAGGAGGTAAAGAA	CCCATTTTCCCCATCGGCGTTT
64.	TF	AT4G17490	GAGTTTCTGAGCATGCCGCT	GTGGATAACCAAACGGTGGG
65.	TF	AT1G03800	TGGAAATGCCAACCTCCCTCTCGT	TTCACCGGAGAAGCCAAAGCATTC
66.	TF	AT4G17500	GGTGTACGGACGAAACCCTAGCTT	AAATCTCCCCAGCTCTCGGTGAAG
67.	TF	AT1G04370	TGACCGAGCCGCCTATTCAATGAG	AGAATTAGCCGCAGTGGATGAGGA
68.	TF	AT4G18450	TGGGCGAATTACATCGGAACACCA	AAGGGTAGGCAACGCTTCCCAAGT
69.	TF	AT1G07900	GCAGGAACTTCCAGAATCACAAAG	AATGGTATATGGCACCTGCACAT
70.	TF	AT4G31690	TTAGCCAATCCGTGACGCA	AAATCTCTCGGCACACTCACTG
71.	TF	AT1G16530	AGATGTTGCAGGAGCTGTCGGAGA	TTGTATCCTCGCGTTGGCCTCGTA
72.	TF	AT4G33280	AGCTGCTGGAAGCAATAAGAAAGC	GCATGTTCTTCACGCACCATTTGT
73.	TF	AT1G31320	TCAGGAATTACCAATCCACCAGCG	TGGAGGGACGAAATTGCCCCAA
74.	TF	AT4G34400	ACGAGCTGTTGGTTCATGCACAA	GCACTTAGTTTCCCGAACCTGTCG
75.	TF	AT1G36000	CAAAAGCTCATGTGGAAGATTATGTT	TCTTCTCCTGGAGTTCACGGA
76.	TF	AT5G09780	CCTGGCGATCCCGAAACCTTTT	ACAACCTTCCATGTCTTCTCCCCA
77.	TF	AT1G65620	TACTCGCAGGGAGAGGGAAAAGAG	AAGCGGCGCATGGTGAGTTT
78.	TF	AT5G18000	CGGTCGGAACAAGAGAGAAGAGAG	AGTTAAGCTCCTGTTTCTGCGTCA
79.	TF	AT1G72980	TGCAGCAGCAGCAACAGAATCC	GCTGGTTTGTTCAGGCATCTCCAA
80.	TF	AT5G18090	CGGTCGGAACAAGAGAGAACAAAG	TGTGACCAGGATAGCTCGATTCAG
81.	TF	AT2G19510	GGTGGATTTGGTATGGTGCAA	GAAGGTAGGCTTTGTGTAACATAATCTTC
82.	TF	AT5G32460	TTTAGAATCACACGCGGCTG	TACATCCAGGTTTTTGACCGTTC
83.	TF	AT2G19820	GCACCATATTTTCCTGCCGAAAGA	TGGACGATGCCAACATGTCTCT
84.	TF	AT5G57720	AAGTGGAACGAAATGTTTACGAGG	TCTCACATATGATTACGTCTCCTTGC
85.	TF	AT2G23660	CGGTTACAGCCACGGGTTT	AACGAACCACCAGCACCTTG
86.	TF	AT5G58280	ACATAGCTCTACATGGGCGACCT	TGCAGCCTTGAATGCTCCAACC
87.	TF	AT2G28500	TGCAGGAACTTCCAGAATCGCAAA	CGCACATCCGTAAACCGGATCTCT
88.	TF	AT5G60130	TCCGAAGAAGAAAAGCCAACTGCT	GATCTGCTTCTCCAATGTCCCGAA
89.	TF	AT2G30130	AGCAAAATGTTGCAGGAGCTACCA	AGCCGTAGACCGGATCTCTTACTC
90.	TF	AT5G60140	GCCAATTGCACATACCGGCTCAT	CTCCAATGCTCCCATTTCGTCCA
91.	TF	AT2G30340	CGTGAATGTCCCGAGAAAGGGAAG	ACGGCATGATCCGTCGTTGTCT
92.	TF	AT5G66980	CGGGCTGGAGATACTTGTGACATA	TCCTAACAAAATGCGGATGCTTGG
93.	TF	AT1G06160	GGGATAAGAGTGTGGCTTGGGACA	TGAGTACTGCGAGGCTGCCTTT
94.	TF	AT4G23750	TTGTCTCTGCTCTCCGGTGTCTGT	GCCGCGACTGGTGATGAAGAAATG
95.	TF	AT1G12610	TAAGAAGCGTGCGGGAAGGAGAGT	TTGTCACCGTTCCTCCGCCTTATG
96.	TF	AT4G25470	CGGAATCAACCTGTGCCAAGGAAA	AGACCATGAGCATCCGTCGTCATA
97.	TF	AT1G12630	TAGTTCGAAGTCGAGAGCGCGTGA	TCTGTCCCACACCACGACGAAGAT
98.	TF	AT4G25480	GGATCATGGCTTCGACATGG	GCTCTGTTCCGCCGTGTAAA
99.	TF	AT1G12890	CAGCCGAAGAAGCTGCTTTTGC	TGTTGTAGCTAGAGAGCCGCTGAT
100.	TF	AT4G25490	CCGCCGTCTGTTCAATGGAATCAT	TCCAAAGCGACACGTCACCATCTC
101.	TF	AT1G12980	CAGACGGTGGTTTATCGTTGGGAT	TCGGCAAGTACAGCCTAACTGAGT
102.	TF	AT4G27950	TGTTTCCGGCCAAAACCAGAAGAA	GCGTTGCTCAGGATCACGAATCTC
103.	TF	AT1G13260	TCTACGTGCTGGTGACGTGGTTAG	CGGATCTCGACTTCCACCCAATGT
104.	TF	AT4G28140	TGCAAGGCCAGAAGCAAATGATCT	GGCCATATACTGCTGCTGCTGTTG
105.	TF	AT1G15360	AGAGGTGTCAGGCAACGCCATT	TCCCTAGCCAAATCCTCCGTTTCA
106.	TF	AT4G31060	AGGCAGCTAGAGCCTATGATGC	TTGTAAACTCCCTTCTCACCCC
107.	TF	AT1G16060	CCTTGGAACTTACGCGACGCAA	CCACGGTACTCGATAGCTGCGATA
108.	TF	AT4G32800	TCTCATCGTCGTCTTGGTCCTCTG	GGCTCGTTCCTAGACTCGGTAACT
109.	TF	AT1G19210	TTTCGACGCGGCTCTTTATTGTCT	CCGGAGATCACCGGAGGATTATCA
110.	TF	AT4G34410	GTCAGGGTTTTTCCAGTGACAGCA	GTGTCTGAATCCAACCGAGGCATT
111.	TF	AT1G21910	GCTGCTCAAGCTGCCAACTCATTT	TGACGAGACGGCTGATGAAGTAGG
112.	TF	AT4G36900	GAGGAGTGAACGGTGGTGGAGATA	GCTTCTAACGCATCCACTTGTGCT
113.	TF	AT1G22190	CCTTCCAACGATTCATCCGCGTTT	AATGAGTGGAGATCCGACCCGTAT
114.	TF	AT4G36920	TGCCGAGTCATCAGGGAATCCTAC	TCCCAAGCTCAAATCGAGGTTGTG
115.	TF	AT1G22810	GCGTGAACTCTGGATGCGGAGATA	TGGCCGCCCAGATAATCATACACT
116.	TF	AT4G37750	GAGGAGTCACAAGACATCACCAGC	GTTTCCAGCGACTCTACCAATCCG
117.	TF	AT2G31310	TCCCTGATCATGACCGATGTGAC	AACAACCTGTTGTTGGAGGGAGAA
118.	TF	AT1G01260	TGAAGGAAGTTCCAAGCACGGATG	TCTGCGATGATTGCTGCAAAGGTA
119.	TF	AT2G40470	TGGAAGTACCTGAGAGCCAGAGAG	AGCTCGGCTTGTAAAGCTTGGA
120.	TF	AT1G02340	GATGCGTAAGCTACAGCAACTCGT	AGAACCGAAACCTTGTCCGTCTTG
121.	TF	AT2G42430	TCACCATCGCCTACGAAGCTCA	TTGCAAGAAAGCCACCTGTTGTTG
122.	TF	AT1G03040	TAAGGTCCTGAGCATGAGCCGTCT	TTCAGTGACTAGTGGTGCGACAGC
123.	TF	AT2G42440	TTCCCTCCAACAACAAGTTGTGAA	AATAGCCATCATGCTTTGTGTTGC
124.	TF	AT1G05805	GGTTTACCGTGACTAGGCCCAGTA	ACTGCGAAAAGAGACCAGAGTCCA
125.	TF	AT2G45410	TCTTCTCTCTTCAACACCAGGTGA	TGTTGTGGCGACTGTAGAGGAAA
126.	TF	AT1G06150	AACAGTCCATCGGATCGGCCAAAA	ATCAGGGGATGCCTCATGTAAGCA
127.	TF	AT2G45420	GCAACAGGTGGTGAATCTACAGGC	TGCGGTTGAGGTAGCTCTAGTGAT

No.	Gene group	AGI	Sequence of forward primer	Sequence of reverse primer
128.	TF	AT1G06170	TCAACGCCAAGATTAGTGAAGGA	GCCGAAAGAGCTTCCATGTATTGT
129.	TF	AT3G03760	TCCTGTCTATGGCTGCGTCTC	CTGTAGCGAGGCCACCTGTT
130.	TF	AT1G09250	TGTTCCCGAGCATCGAAAACACAG	TCGTTGACACGTCGTGCCTCTATC
131.	TF	AT3G11090	TCGCACTCGACTACTTGCCCAT	TCAAGAAAAGCAGCAAGCTCAGGA
132.	TF	AT1G09530	GCTCAAGACAGGAACCCTTCTCCA	ATTTTCCCACACCAGCTCCACAAC
133.	TF	AT3G13850	GGTTTCTAGCGAACGACACGAGTT	TGATGTATTGTGCGGAAGGCAAGT
134.	TF	AT1G10120	TCTTGCAGAGCGGGTTAGAAGAGA	TCTTGTTGCATCCGGGAACAAGTT
135.	TF	AT3G26620	TAGCCAAAACTCAAGCTGAG	GAAATATGGGTTTGGCTAAG
136.	TF	AT1G10610	TGGAAATGTTCAGCACTCGTGTTC	CACCATGCTTTCATCAAACGGTCT
137.	TF	AT3G26660	TCAAGCTGAGATTGCTGTT	CATAAAATCAGAATTTTGGG
138.	TF	AT1G12540	GCTTCTGAGGTCGAGGAGGG	TGATTATCTTTTCTTGAAGCTCTGAGA
139.	TF	AT3G27650	TTGTTGCGATCCATTTAAAGGGGA	GGCGGAAAATAGGGTGCGAATAC
140.	TF	AT1G12860	AAGTTTGACATGAACAGCGACGGT	TTCTGACCCAAGACGCAGCTTCAC
141.	TF	AT1G22985	ATTGGACCTGATGCGCCGACTA	TCTTCAGAAGCACCACCACTAGCA
142.	TF	AT4G39780	ACCCGACTCTGGCTTGGAACTTT	CGCGAACTCGCCTCTTAGCTTGTA
143.	TF	AT1G24590	TGCCAGAGAGCGGTTTTCAGACAG	TGACCGAATCCGTTTCCTTCGACA
144.	TF	AT5G05410	TGTCTGGAGAATGGTGCGGAAGAG	TCAAACTCGCTCAGCCAATGCTTA
145.	TF	AT1G25470	TTCTGGTGTTGAAGTATTTGGGGA	AGGTTTAGGTAACCATTCATCGCT
146.	TF	AT5G07310	AACAAGATCCTAACCCACCGGCTC	AGTGCCTCTTCCTCAATAGCCCTT
147.	TF	AT1G25560	TCGGCTTTTCTTGACGCTCATTCT	CTCTGCTCAAACTCATCGGCGTAA
148.	TF	AT5G07580	TGCTGCAAGAGCCTATGACTGTG	GCTTCATATTTCCCGGCGTCAAGA
149.	TF	AT1G28160	TGCAAGCATAGACACGTTTGCCTT	ACCCTTCTGTTCCACTCTGACCAA
150.	TF	AT5G10510	ATTGCCTCCCTCAGGAGGAAGAGT	CCTTGCTTGCCAACGTCCTTGTT
151.	TF	AT1G28360	TCTTCTACAGAAGCGCCGCTTTAC	CCAACCACTTGATAACCAGGCGAT
152.	TF	AT5G11190	TGAAGAGAAGAGTGTGGCTTGGAA	GCGTTTTGGCCGTTCATTAGAAGA
153.	TF	AT1G28370	TGAAGGACGACGTGTGGTTTTGGA	CAGTTCTCAGGTGGAGGAGGGAAA
154.	TF	AT5G11590	TGACTCATTCCCTCGACCCGTTTC	TGAGCTGCTTTAAGAGCTGCTGTC
155.	TF	AT1G33760	GGCCAACGAGATTACCGCTTCAAC	TACCGGGTTCTCGGATCTCCGATA
156.	TF	AT5G13330	ACATCAACCAGACCAAGATCAACC	GCTTTCTTTGGATCGCGGATTTCT
157.	TF	AT1G36060	TTCCCAGCTCTCCGATACCAAACC	TTTAGCGTCTACGGCAGCTTGAAT
158.	TF	AT5G13910	AGCTGCCTTGGCCTACGATAGA	TGACGGATGAGGAAGGAGGCAT
159.	TF	AT1G43160	GCTGTGACTAAAGAATGTGAAAGC	CCTTGTGTGGGTCTCGAATCTC
160.	TF	AT5G17430	ACTCGCAAAGGAAGACAAGGAGGT	GTGCGGCTAAATCGTAAGCCCTA
161.	TF	AT1G44830	TGGCTTGGTTCTTACTCAACTGCT	AGACATAGGAGTGCTGCGTCGT
162.	TF	AT5G18450	TGGGGCAAGAAAAGAAGACGGAAC	TCCTTGTTGTCCGACGATCCACAC
163.	TF	AT1G46768	TAAGGGAAAAAGCGGCGGAGGT	TCGGGTTTTATTACCGGCGGAGTG
164.	TF	AT5G18560	TGTCCCAAACCACTGCCTTAAACC	TTGCTTGCTACCGGAGTTGTGAAA
165.	TF	AT3G27940	CAACAACTGCATGTTTGCTCTGCT	CGAGTCCAAAGATGCGATTGACGA
166.	TF	AT1G18400	GGGTTCGGCGAGGGAAAATAAACG	AGCCTTATAACATCCGGGCACCAT
167.	TF	AT3G47870	GCCTTACTTCCCAGCCGAACAA	ACTTCTTACGCCGAATAGCCTGTG
168.	TF	AT1G22490	TGTCACCACTCTCCACAACTCCAT	GCTTCCTTCTTCAACCCTGACGCT
169.	TF	AT3G50510	CAACGTTTTGCATCCACACAA	TCCTCATCGTGCTGCATCTG
170.	TF	AT1G25310	CATTACAAGGGTTCGCAGGG	CGGCACCAGCTTCAGTAAACA
171.	TF	AT3G58190	TTTGCTCTCCAACAACAGGTTGTG	GCAAAAATCATGCTTTGTGCTGCT
172.	TF	AT1G25330	TGGCTGAAAGGGTACGAAGAGAGA	CCATTGCCTTGTAGCATCCTGGAA
173.	TF	AT4G00210	GCCCTTCAACATCAGGCGGAGTTA	TTGTTTTGAGAATTCGGCGGAGGA
174.	TF	AT1G26260	TTGCGACAAGGTGACTGGTAAGG	GCACAGGATTCACAGCCGAAAGTT
175.	TF	AT4G00220	CGTCTCCAAACTCCTCCACCAT	TGCAAACTTACCACCTGTTGCTGA
176.	TF	AT1G27660	TTCCTACAGAGCCAGATCGAGACT	ACTAGACATAGCCCACGGCTTCT
177.	TF	AT4G22700	ATGCAACTCCCAACTGCTGCT	TGCACCATCAGCTCTATGCCCATC
178.	TF	AT1G27740	GCCTTTATGCTCGGAAACGAAGAG	TGTCCCGTTTGGCACAAGGTT
179.	TF	AT5G06080	GCTATCACCATCTCCTACGAGGCT	GTCACAACCTGTTGATGAAGAGCG
180.	TF	AT1G29950	TGGCGTTGGACATTTCTCAAACCA	GCGCTAGACCGATGATTTACCTCC
181.	TF	AT5G35900	ATGCCAGCTGATTTTTTGACTAATAA	CCTCCATCTTTTCAATATAAGGATGTAA
182.	TF	AT1G30670	AGCAGACCAAAATGCAGACTCTGG	CCCTTGGAACCACACACACTTCTT
183.	TF	AT5G63090	GGTTTTTCAGCCTCAGCCGCTT	TGGCATCTGATTCATCACCGGACT
184.	TF	AT1G31050	TCACAACAAAAGGGGACCGAAGC	TCGCGGTATGCGATTGGGGTAT
185.	TF	AT5G66870	ATTGAGGCTCTCAAGTCTGAAAAG	TGGTGGGAAATATGGAGCGAATA
186.	TF	AT1G32640	GATGAGGAGGTGACGGATACGGAA	CGCTTTACCAGCTAATCCCGCA
187.	TF	AT1G67100	ACCACCTCCGTCCTGCGATATTTA	CGGATTCACAATCCTCCCGCAT
188.	TF	AT1G35460	CGTAGCATTGCTGAACGGGTGA	AGGAACAAGCTCTTGCAGCCTC
189.	TF	AT1G49120	ACAGGGAATAAAGCAGCCGGAAAC	CGGATTTCTTATCTCCGCCGCAAA
190.	TF	AT5G19790	TTCCTTATTCGTGGCCCCTTGTCT	CTGCCTGAGCCAATGGAATCTGAT
191.	TF	AT1G50640	TCTGTAATCGACGACGACGACGA	GATCGAATTGAAACGGCGGATTCC
192.	TF	AT5G21960	TGGCGACCATAATACCGAGGAAGA	CTTCCCCAACTTGCCCTAACGTAG

No.	Gene group	AGI	Sequence of forward primer	Sequence of reverse primer
193.	TF	AT1G50680	TTGTGGCTTCTGGCAATGTC	CCCCAATGACCGTTCTGTTG
194.	TF	AT5G25190	ATGGCACGACCACAACAACG	TCAAGAGAGGGTGACGAATTTCGG
195.	TF	AT1G51120	TGCCTTGTCCAACACGACGAAATT	CGATGGTCTGCGTAAATCTGAGCA
196.	TF	AT5G25390	TCTCTTGAAGAGAAGAGTGTGGCT	TCCAAGGAATTTGAACCGTTCGAT
197.	TF	AT1G51190	GGCCGAGTTGCTGGAAACAAAGAT	TGCTTCTTCCTCCGTGCTGAATG
198.	TF	AT5G25810	TCGCTAGAGTCTCTCGTGTCTTCC	TACAATCTCCCCTAGCTCCTCGGA
199.	TF	AT1G53170	TGTGGTGTCCAGAGCGAGTCTGAA	TCCCAGCTCCACCTTCGAAATCAA
200.	TF	AT5G43410	TGGCTGGATCTTCCTCCG	CAAATTCAAAAACTTGCCTAGAAGAA
201.	TF	AT1G53910	GCTGCGGAAGGTTCAGTTTTTGGT	TGCAGATTTCTCAGCGTCCCCATC
202.	TF	AT5G44210	TCGATCCGTCAAGAGCTGCTTCGT	ACCGAACCGGACAAACCCGAGAAA
203.	TF	AT1G63030	AGGAGACACGTCACCCAATCTACA	AGCCAGACTCGACGCTGATGAATC
204.	TF	AT5G47220	TGTTTCACAGAGAGTTGGGGAGGT	GAGGAGTCCGTACACCAACATGTC
205.	TF	AT1G63040	TTTAATGGCCACTGACACCG	AATTCCCCGATAAACCGGAT
206.	TF	AT5G47230	CGCTTCTGTCGCCGTTATCT	CAAACAACGGTCAACTGGGAA
207.	TF	AT1G64380	TGGGAGTGGACACTGATGGGTTTT	CCCAAATCAGCTCTGGATCGAACG
208.	TF	AT5G50080	GATCGAGGAAGCAAGGGAGAAAAA	CCAAACCCTAGCGGCTCTATGT
209.	TF	AT1G68550	CTGCATCAGCTCTCACTTGTGTCA	CCTTGTTTCCACCAGCAGGAACAT
210.	TF	AT5G51190	AAGCTCAGGTTCAGGCTGATG	TCCCAAAACCCCTTCCAACT
211.	TF	AT1G68840	TCCTACGACATCGCAGCTTGTAGA	GCTAAATCGCCGTCTTCCAGAACG
212.	TF	AT5G51990	TGGTCGCTCTGCTTGTCTCAATTT	GTCTCAGGAATACGAAGCCGCCAA
213.	TF	AT1G68510	CCTGATCACCTTCGTCCCGCAATA	TGACCACATCAAACCAACCGAACC
214.	TF	AT1G43770	CCGATATGGAGGGGATTGATGTCT	GTATTTCAGCAGAGAGCCGACC
215.	TF	AT3G02550	CGTGCTGGACTCATGAACCTCATC	GATCGGAAAATCCCAGGACGAAGG
216.	TF	AT1G49770	CGTGATCCAAGCACAGGTAAATCC	ACTCAAACCGAAGCAGCCAGTTAT
217.	TF	AT3G49940	GCCCTGCTTTGTTTCAGTCTTTGC	ACATTCCAATTCCCCGTCCACAAC
218.	TF	AT1G51070	AGCAAGAGATGAAGCGCAGAAACT	CGCAGCTCGTTCTTCTCATCCTT
219.	TF	AT4G37540	ACGTCCTGCTTTGTTTCAGTCGTT	TGCCAGTTCCTGGTCCACAACATA
220.	TF	AT1G51140	GCATAGCCGAGAGGGTGAGAAGAA	GCGTGTCCATGTTTGGAACAAGG
221.	TF	AT5G67420	GCCACCGTCTTCGTCGCTAAATT	ACAAAGCAGGACGTTGAGAATCCG
222.	TF	AT1G59640	AAAGAGTTTGGTCAGCAAGCG	TCGTAGACTGCGACCCGAAC
223.	TF	AT1G04100	CGCAACCAGACAAGTTGCTGTAGG	AGCCGCCTTCCGTAGCTAAAGACT
224.	TF	AT1G61660	CTGACAGTGGAGGAAGTACCGTGA	GTCCTAGACCCATCATCTGCAACG
225.	TF	AT1G04240	AACATCCCCTCCTCGAAAGGCT	TCCTTGACCCTCATGCTCAGATTC
226.	TF	AT1G62975	TTGCCTCTCAGCTAGACGGCAACA	TTTGCCATCCTCCACCTGCGAAAC
227.	TF	AT1G04250	GCCAAGGCACAAGTTGTGGGAT	TTTGGCAGGAAACCATCACGTTCT
228.	TF	AT1G63650	AAGGAATCCCCGGAGGAGCGTTAT	GCGGTTTCAGCGTTACAAAGCCAT
229.	TF	AT1G04550	TGGGTCTAAACGCTCTGCTGAATC	ACCACTTGACTTGAACGAGGAGGA
230.	TF	AT1G66470	TTCTCACACGGGAGAGAGCACTCA	TTTCCGGTCACACCGCTACTCA
231.	TF	AT1G15050	TGGTAGGTCGCAAGGTCTGTGTTC	ATCCCGACACACTCTGCATCCCAA
232.	TF	AT1G68240	TGATGCCTCAGCAAAACATGACCT	TGGCGGCACTGTTCCATATACTCT
233.	TF	AT1G15580	TTCCGCTCTGCAAATTCTGTTCG	CGATCCAAGGAACATTTCCCAAGG
234.	TF	AT1G68810	ACCCAACACCACCAAAACGGATAA	CTCCTCCGTGAAAGCTACCGTTAA
235.	TF	AT1G51950	CAGAACCAAAGAGACAAGGAGGCA	TTTGAGCTGCAAGAAGACCTCTGA
236.	TF	AT1G68920	GCAGAAGAACTCTGAAGCAGCTCA	TGGACTTTGCTCATCATTGCGCT
237.	TF	AT1G71130	TGGCGGAGATCAGATGTGGAAGAG	GAGCAGCTTCCTCAGCAGTGTTAA
238.	TF	AT5G52020	TCAGGGGGATTCGACTACGTAACG	ACCGGATAAGTCCCGAGCCAAATT
239.	TF	AT1G71450	ACCGAAGGAACAGAGTCAGCCAT	GCTTGAATTTCCCTGGGCGAGAGT
240.	TF	AT5G53290	TGAGCCTAGCTGTAACAACGTCGT	CGACGCCGGAGATTGAGTTTCATC
241.	TF	AT1G71520	TTACAACCTCCCTCGCCTCCAATA	GCGTCGGAAGCAGCTTTTTGGAT
242.	TF	AT5G57390	TTCTCCAGTCGAACGGCAAGATGG	AAGTTTAGGACCGCCGGGGTATA
243.	TF	AT1G72360	ATGGGCGGCTGAGATACGT	GTTGAAAGTCCCGAGCCAAA
244.	TF	AT5G60120	GGCAGCTAATGTTAAGCTCGACCT	TGCTTCGGACCATCTCCTAGTGAA
245.	TF	AT1G72570	TGAGGAGGAATAGCAGCGGGTTTT	CAATTCTGGCTTGCCACCTTCCAT
246.	TF	AT5G61590	CGGAAAGTATGATGCTCCGGTCAA	TGTGGTACATCGGTTCTCCTCCTT
247.	TF	AT1G74930	AATCCACCGTCGATCTCCGTAGAA	AGCGAATCTAGCAGCAGCTTCC
248.	TF	AT5G61600	TAACCAGCGTAAACCGCCCTTACC	TCCCCTGTAGTGCCTCTCTTCTTC
249.	TF	AT1G75490	TGAGGCTCATCTCAACCTCCCTGA	GGTGTTGCTGCTTGGTGTAGTCTG
250.	TF	AT5G61890	AAGACCAAGGGGACTTGAGGAGGA	CCGCAGATTCAGCGGTTTCAAAT
251.	TF	AT1G77200	TGACTCGGCGTATCCACCTAGTTC	TGCCATTTTCGTCGCTGTGGTAAT
252.	TF	AT5G64750	AAAGGAGGAGAGAGGTGGAGGA	ACTTTAGAAGTAGAGCTACCACCG
253.	TF	AT1G77640	GTTCTTACTCCACCGCAGAAGCA	TAAGACACAAGAGAGCAGCGTCGT
254.	TF	AT5G65130	TGGGCTAAACCAGCTCACTCCAAC	TGCCGGAGATGTAACTCTGTCTGA
255.	TF	AT1G78080	TTCGCCCGGCTTAACTTCCCTAAC	TTCACCGAAATCGCCTCCGATGTG
256.	TF	AT5G65510	TCGTGGAGTCACCCGACATAGATG	TTTCTGGCTTGACCTTCCCTCCT
257.	TF	AT1G79700	TCAAGGGGCTTACGACGAAGAAGA	GTGTGTCTCGTCCCCAGTACTTCA

No.	Gene group	AGI	Sequence of forward primer	Sequence of reverse primer
258.	TF	AT5G67000	TTGTTGCACCACACCCTTCTTCTT	TCGCAGAGCAGAGACCATGATCT
259.	TF	AT1G80580	GCGAGGAGGCTTAGAGGGAC	TGGGAAAAAGCGGAGGAATC
260.	TF	AT5G67010	CATCAGGAAAATGGTCGGCGGAA	CCAAGCCACCTCCTTGTTCTTGT
261.	TF	AT1G52830	CATAGGAGTGGCGAAGGAGGGTAA	TGCCAAGGTACATCTCCGACGA
262.	TF	AT1G69010	CTCTAGTGCCTACTCGCATGAGTT	TGCTTGCGACAGATCAATGCCT
263.	TF	AT1G80390	GGAGAGGAAGGGAGAGTTTGTTGC	CCACAAACATCATCCACGGCACAT
264.	TF	AT1G71200	ACGCTAAGGAGCGTTTAAGGAGGA	TGGTCAGGAAGAAGAGTGCCAAGA
265.	TF	AT2G01200	TGTTTGACCGTGTCGGGATTGAG	CCCCAACATTCCTCCAAATGCCTT
266.	TF	AT1G72210	GCGAAAAAGAGACCGAGACAGCTT	TTGGCTCCCTTCTTCAACCTTGAC
267.	TF	AT2G22670	GCCAAGGCACAGGTTGTTGGTT	TCCATGCTCACCTTCACAAACAGA
268.	TF	AT1G73830	TGTGGAATCCATGCAGAAGGCAAA	ACAGAACTCCCATCCCTCCCTTGA
269.	TF	AT2G33310	GCTAATGGACTCGCTGCACGAAAT	TAAACCGGCTGCTTTCGCTGTCTC
270.	TF	AT1G74500	GTCGTTCCGACAAGGTTTCAGCA	GCAGCTTGTGCAGTGTCTGAGTTT
271.	TF	AT2G46990	CGCATCCATTCTCTGGGCTGAAGA	TCTCCAACCATCATCCAGTCACCT
272.	TF	AT2G14760	GCGTGCCACAACAACTGATAA	GCATTTCTGGCTCCTTCGTG
273.	TF	AT3G04730	AAACCACCAGCCAAGGCACAA	CGGACATGACGTTCTTGCGGAAAG
274.	TF	AT2G16910	GGACAAGAAATGGAGCCACAGGTG	TGAAGCCTCCTGGTTTGTATTCGC
275.	TF	AT3G15540	TCGGTGTGGCCTTGAAAGATGG	TGCATGACTCTAGAAACATCCCCC
276.	TF	AT2G18300	GGGCGAGAAGAGAAAAGATCAGCA	GCATACCAGCTTTTCCTGTGACCT
277.	TF	AT3G16500	CAGAAAAGAACTGCTCCTGGTCCA	TTGAAGAGCTTGTGCTCGCTAGA
278.	TF	AT2G20100	TACAAGTTGGCAGCGACAATG	TGGAGAGTGGTTCCGAACG
279.	TF	AT3G17600	TGCGGTAATCGAGATCGAAAACAT	TCCGACCATCATCCAATCTCCATC
280.	TF	AT2G20180	TCACCCTGCTCGAACTTGGATACG	GGATCACGGAAACCACACAACAGA
281.	TF	AT3G23030	ACCTCCTACCAAAACTCAAATCGT	GCTCGGGGTAGTTTTTGTATGTCT
282.	TF	AT2G22750	GCTCAATCCTTGACTCGAAGCCAA	GCCTTGTCCATCTTCTTTAGGCCA
283.	TF	AT3G23050	TCTGCTGTTCCCAAGGAGAAGACT	GCCATCCCACCACTTGTGCTTTAG
284.	TF	AT2G22760	TGTTTTAGCCGAGAGAAAGCGCC	TACCTTGTCCGCCTTCTTAAGCCC
285.	TF	AT2G20350	CCCACGACACGAGGCGATAATTAA	GCCGCCCATTTACCTGATGGTTTT
286.	TF	AT5G67180	TGGGACTGTGGGAAGCAAGTGTA	TAATCGCCGCTCGATCATAGGCTC
287.	TF	AT2G20880	TTATGCTACTCCTCAGCCGCCAAC	TCGCTGGAGGGAGGTAAAAGGGAA
288.	TF	AT5G67190	TTAGAGGACCTACGGCGAGGCTTA	TTCCTGATGGTCGCAGCCGACATA
289.	TF	AT2G22200	GGGACATTCGAAACCGCCGAAAAA	GCGATATCTCCACGGAGCTGAAAA
290.	TF	AT1G19220	TCCAACGAAGGAGAGAAGAAGCCA	TGAAACTAAAGGCCCTGCACAAGC
291.	TF	AT2G23340	TTGTGAATACCGGCGGCGAGAATC	TCGACCCGTTCCAATGACCCGTTA
292.	TF	AT1G19850	CGGGAAACGAAGGTACATGGGAAC	TACGCCACTTAGAACCAGGCCATC
293.	TF	AT2G25820	AGTGGTCATCCTCATCGTCCTCAG	TCTCCTAGCTCCCCCGAAAGCATA
294.	TF	AT1G30330	TTCTATAACCCGAGGGCGAGTCCA	CCAACAGAGACGCGAGTGTGATAA
295.	TF	AT2G28550	GCCGAGGGAAGAGCAACAGAAAAG	TCTGCCATCCCCAGTTACTCATCA
296.	TF	AT1G34170	GGCGAAGCCTAAAAGTGCAATGG	AGATGCTCGATGTCCCAAGGTGA
297.	TF	AT2G31230	TTTCCCGGTGGAAGTGGTTAGAGA	TCAAGGCCATAACCGGAGATCCT
298.	TF	AT1G34310	TGGCGCAGCTTAGAAGTGCAGT	ATGTTCGATGTCCCAGGGTGACAC
299.	TF	AT2G33710	TCTTCGTCGCTTACTCTTCAAGAA	TCACGCCTCTGTAGTTTCGTTG
300.	TF	AT1G34390	TGGCGCAACTTAGAAGTGCAGTG	TGTTCGATCTCCCAGGGTGACA
301.	TF	AT2G35700	TCGCCATCTCCCACAGTTACGGAA	GCGTCGTCGGAGAGTGCTATCATA
302.	TF	AT1G34410	GCAGCTTAGAAGTGCAGTGGGATG	ACGCAGGCACTAAATGTTCGATCT
303.	TF	AT2G36450	TTTCCTCTTTGCCTGCCCCAACCT	TCCCTAGCAGCACCAAAAGCAGCA
304.	TF	AT1G35240	TGGCGCAGCTTAGAAGTTCAGTG	TGTTCGATCTCCCAGGGTGACA
305.	TF	AT2G38340	TTTGGGTGGGGGAAGGAAGAAGGA	TCCAACCAATAGCCTCCCGAACTT
306.	TF	AT1G35520	TTTCACAGGTAGTGACGAGGATGA	GCAGAATTCAGGCCATGGAT
307.	TF	AT2G39250	TGGGTGGTTTCGACACAGCCTACA	GGAATCTGATAGCAGCTCGGTCGT
308.	TF	AT1G35540	TGGCGCAGCTTAGAAGTGCAGT	TGGTGAAACTTGGTTTGGTCTCGG
309.	TF	AT3G62100	TGCTTCAATCCTTTGGGCTGAAGA	TCTCCAACCATCATCCAGTCACCT
310.	TF	AT2G22770	TGGCCTCAAAAAGACGGACAAGG	TCCTCCAGCTTCTTTACCCGCTCT
311.	TF	AT4G14550	ACGAGGACAAAGATGGTGACTGGA	ATGACTCGACAAACATCGGCCAGG
312.	TF	AT2G24260	TCGCCGAAAGGTTACGAAGAGAGA	TGCCTTGTCTGTCTTATTGCCGTT
313.	TF	AT4G14560	ATCTGCTCCTCCTCCTGCAAAAAC	CGGTTAGATCTCACTGGAGGCCAT
314.	TF	AT2G27230	GAAGCAAACCGGGGAATCCAAGAT	TGACCCTACTTCGAAAGCCCATGT
315.	TF	AT4G28640	CAACTAGTGGGCAAGTTGTGGGAT	TCAGTGGCTGAAGCCTTAGCTTGG
316.	TF	AT2G28160	CGGTATCAATCCTCCTGCTTCCAA	TGGAGCAACACCTTCTCCTTTGT
317.	TF	AT4G29080	TGGTCGGAGATGTCCCTTGGGAAA	TCACCCTTGGAGCTAAGCCGATAG
318.	TF	AT2G31210	CAGAACGTGAGCGAAGATGTCACT	TCTCCCTTACTCGGGCTAGGAATG
319.	TF	AT4G32280	GAGGGTGACTGGCTACTTCGA	GATGAACAGATTCCGCAAAGATCT
320.	TF	AT2G31215	GCACAACAACATTGAAGTAGACAATAAAA	ATGCTCTATCTTGCTAGTCCCATAAAT
321.	TF	AT5G25890	GCTCCTCCTTGTCACCAATTCACT	ACTGGAGCTACCTCAACCCTGTTA
322.	TF	AT2G31220	ACCTCATTCCAAATCCCACAAAGA	TGCTCCTGAATCTCCCACATCT

No.	Gene group	AGI	Sequence of forward primer	Sequence of reverse primer
323.	TF	AT5G43700	GCTGAGATTGGGATTACCAGGGAC	GGCCATCCAACAATCTGAGCCTTT
324.	TF	AT2G31280	GCAGGGGATGGTGCTTATCGAGAT	TCCATGTTTTCTCGCCCTGAGTC
325.	TF	AT5G57420	TGGAAGGACTTTGTTCGTGTAGCG	TGTGTTTCCCTTGACCGGCAAGAT
326.	TF	AT2G34820	ACTTAACACCGCCGAGATGTTCC	ACCCTTCTTTGTGGTCTGCATCAG
327.	TF	AT5G65670	CAGCCAAGGCACAAATTGTCGG	GCCTCCCATCAACTTCGTCACTGT
328.	TF	AT2G40200	GCTGATGATTGTGTCGGTGGGAT	AGCTGCTTTGTCTAACTTGTCGGA
329.	TF	AT1G16640	CTTCCCTCAAGTCGCAGTAGAGGT	GTTTGTCTTGATCGTCGCCGTCT
330.	TF	AT2G41130	GGAGAAACGATGGCTCAAGACAGA	AGCAGTGTGGCTTTATCGGTCTTA
331.	TF	AT1G26680	ATCTCGCACTCACCTCAACATTCC	CGCGTCTGATCTTAGCGTCACA
332.	TF	AT2G41240	GTCTTCCTCCCACCAATCAAACGA	AACTTGCTCTTGCAGCTCTGGTA
333.	TF	AT2G40220	TCAATAACTCATCCACCGCCGTTG	AGGCCAAATGGTCGAAGATCCATC
334.	TF	AT1G43950	TGAATGTCTCCCTCCGCTGGAT	TGCATTCCAACCAGTTGTGTCTCT
335.	TF	AT2G40340	TGTGGCTCGGTACTTTCTCCAGTT	CATATATAGCTTTGGCCGCCTCGT
336.	TF	AT1G59750	GGTCCATCTGGTCCTGTTACTCCA	ATGGCACTGAGGAAGGAGTGTCTG
337.	TF	AT2G40350	TGGTCAGATCTCTAACTTCTCGCA	AGTAACGCCCCAACTTAACCAATG
338.	TF	AT1G77850	TGGAAGCAGCTTCAGATCACATGG	TGTGCAGCAATTTCCACTTGCCA
339.	TF	AT2G41710	TTCAAAGCCGATGGGACGCATCAG	ACGATCATCACCTGCCCCGTAATG
340.	TF	AT2G28350	TCTCCTTCAGGTAGCTTGGGACGA	ACTAACCACGGACTAACCCGCTTA
341.	TF	AT2G44840	TTCCGTAACCCGAGTTTCAGCAAC	TAACGGCAAGTCGCTCCAGTTATC
342.	TF	AT2G33860	TTGGTAAGGTGGGACGACATTGTG	GCTGCCTGAATTGGAGATGGAACC
343.	TF	AT2G44940	TGGCAAGATTCGGTCAACGATGTG	TCGGCTCGGCTTCAACTATTTCAG
344.	TF	AT2G46530	TTGAGGGGTGAAACCGGGGATTT	TCGAAATAACGGATGCGGGCATCG
345.	TF	AT2G46310	TGCGCTACTGATTCTTCCAGCGAT	ACGTTTCACCCTCGGAGCAACAGA
346.	TF	AT3G61830	GCAGGTACAATGGGATGAGCCAA	GGGAAGTTGCCAAGAAAGGCTCT
347.	TF	AT2G47520	GCGTAAACCCGTCTCAGTGAGTGA	TGGCCTCTGCCTTATCCCTCTGTA
348.	TF	AT4G23980	AAGAAGCCGTACCAAGGTGCAGA	TTTAAATCCACAGCCCTGCCCACA
349.	TF	AT3G11020	TGAATGAACCTGGTCCCCATCAGA	TCGAGATGAAGCGGATGCAAATCA
350.	TF	AT4G30080	GTCTCTGATCCTATCCGTTGGCCT	TCATCCCACGCCACCTGTAGAA
351.	TF	AT3G14230	TCGTCTCCACTGTAGGTTCAGCAT	TTTCTCAGCTTGCTCAGCGGACTC
352.	TF	AT5G20730	TTTCTACAACCCGAGGGCTGCT	ACCGCATACCGAGGGAAACTTGA
353.	TF	AT3G15210	TTTTGGACCTGATGGGGATCGGTA	GCGATCTAAACGCCGATGTCACAG
354.	TF	AT5G37020	TATCTTTCGGGGACAGCCCAAACG	TGGCACTGACAAAGACACTCCATC
355.	TF	AT1G49480	TGTACTTGCCATCTGGGTTTGCTG	CCATTGTTTCTCACCGAGCTGGAG
356.	TF	AT2G42280	GTGGTTGCGCTACACATCCTCGAA	CGCTTATCCGCGTTCTTCTTACCC
357.	TF	AT2G16210	ACAAGAGAGCACTTCCACATGACT	CCCAGCCAGACTTCTCCATGAA
358.	TF	AT2G42300	GAACGCGAGAAGAAGGTCAAAAGC	TGGCAACTTGTCTGACTCCACG
359.	TF	AT2G24650	TGCGTCTCCCAAAGGTATTCACGA	AACAGAGTAATCCTCCCCGGCTTG
360.	TF	AT2G43010	CGACTCAGCCGATGGAGATGTT	GTTGTTGACTTTGCTGTCCCGC
361.	TF	AT2G24680	AGCTCATAAGACCTCCCAGTGTGT	ACAGCGGCTAGGTCCTAAATCTGA
362.	TF	AT2G43140	ACGCAGCATTGCTGAAAGGGAGAG	GCGTAGCTCGTTTGCTTGTCCATA
363.	TF	AT2G24690	AGACTGCGTCTTCCACTGCAATTC	ACTTACCAGCCACTTCGCACCA
364.	TF	AT2G46510	AGCAGCGTTTTACCCCGAG	CTGTGAACTAGAAGAAGGCAATGG
365.	TF	AT2G24700	GGTTTCACCAACCACCTGGACATT	AGCTCAGCCGTTTTTCCTACGTT
366.	TF	AT2G46810	CAGAGGGGAGACCAAGCGTCAATA	TGTGCTTCAAGGGATTGCAACTGT
367.	TF	AT2G35310	TCATGCAAGATGGCAAGGAGATGC	AGGTGGCCTAGAAGAAGCCATGAA
368.	TF	AT2G46970	AATGCGTGCTTTGCAGGAC	CAACAATGAAGCCTTATCATCCTTG
369.	TF	AT3G06160	CAGATCAACGGGTTTGCATAAA	TCTGTGAACTGGTTTCTCTCGC
370.	TF	AT2G47270	TCATGATCCGACCAAGGAAGAGTG	TCTGCCGTTTGTCTAAAGAGTCCA
371.	TF	AT3G06220	CGAGCAGTCGTTCATGTTGAA	CAAGAGAATCGTTGTCACTTTCTGAC
372.	TF	AT3G05800	TTGGAAATGCAAGTCCGAGCCAT	CGGCTATAAAGCCGAGCCGAGATT
373.	TF	AT3G17010	ACGAGCAGAATGGCTTAGAGATGC	TGATCCCACTAGAAGGACCCATCG
374.	TF	AT3G06120	TCGAAGAAGGCAAATGAACGAGCA	CGATGATCGAAGCTTGATCTCCCC
375.	TF	AT3G18960	CCCAAAACACGAAGGCCAGAGT	TCACGAATCTTGGCGGAATCTTCA
376.	TF	AT3G06590	TGACTACGGTGGTTAGTAGCAGCA	CTCAACACCGACACTCTCCGTTT
377.	TF	AT3G07340	ATCATTGCAACGACAAGTTGAGTT	CAGCCTGGTGTTCACTGACG
378.	TF	AT1G03970	ACTCCTCCACCCATTGATCCTTCA	TTTTCCGATGCAGCGACACAGTTC
379.	TF	AT3G17100	TCTCCGCCGTTAGTTCTTCTCCT	CATGTGTGTTTGTCCCCCTTCGT
380.	TF	AT1G06070	ACAGGTCCACCTACAGGATGCTTT	TGCCCCGTCAATACCTTAAGATGC
381.	TF	AT3G19500	GATTGCAGGAACTCAAACGACGC	TACCCGTTCCGGTTCTCTTCTTGG
382.	TF	AT1G06850	CCCAAACGCGCCAAAAGGATT	TGAGCAGAGAGAGTGGTAGCTTCG
383.	TF	AT3G19860	GACCCAAGAATGACAAAGCCACGA	CGTTTTTCTCCTGTGTCAACTCGC
384.	TF	AT1G08320	CCCACGAGAGACACTGATGAGTGA	TGGAGAAGTGGTTCCGAGATGACT
385.	TF	AT3G20640	CGTCTCCGTCACCAGCTTTCAAGA	TTGTTGGAGCGCAGCGATTCTGTC
386.	TF	AT1G13600	TCACACAACGAAGACGACAACAGT	ACTCAAAATCTCTGCCGCTTTGGT
387.	TF	AT3G21330	TTGCAGCGAGACAGAGAAGGGAGA	TCGTCCCACCTGGAACCAATGT

No.	Gene group	AGI	Sequence of forward primer	Sequence of reverse primer
388.	TF	AT1G19490	CAATTCGGCGAAGACAGGCAATG	GCCCAATCCTTTTCCCTCCTCAAA
389.	TF	AT3G22100	TGCCGTGGGAGAGAAAGATGAACT	GGCATCCAACGGAGTGAAGCAATC
390.	TF	AT1G22070	GAGAGTTTTGTGAACCAGGCGGAT	GAGCAGCCTGTCTTGTCGTCAATA
391.	TF	AT3G23210	GGGAAGAGGAGACGAACTGGATCA	GGAGTCCTGCCAGGCTCTAAAACA
392.	TF	AT1G32150	GACACAACGGGAAGGATGGTGAAA	TTCCATTACGGGGTGGTCCATGAG
393.	TF	AT3G23690	CACAGAGATGATTCAACCGGGGGA	TGCTCTGAGCAAGCCATTGCGT
394.	TF	AT1G35490	GACCATCCAAGTGCTACAAGTTGA	GCATGAGTAACTGCTGATCCAAGT
395.	TF	AT3G24140	GGTCGAAAGAAACCGTAGGAAGCA	TCCCCTTTGAACGTAGGAGCCA
396.	TF	AT1G42990	TGGCTAAAAAACGAAGAAGGAGAG	TCAAGCATACGTCCTAGTCTCAAG
397.	TF	AT3G25710	CCAAAACGGACAAAGCTTCTTTGC	TCTACGGTCAGATCATCGCACTC
398.	TF	AT1G43700	GCAACAAGCTGAACTTAGGGATGC	TCATTGCCGATTGCTGAGATGAGA
399.	TF	AT2G24790	CCAAACTGTTTCGACGAGAACGAT	CCTCAAAACCCTTGCTTCCCTCT
400.	TF	AT1G65110	AATGGGTGAAGAATGAAACTGAAAA	TCCCAGTCCAATGCCTTTGT
401.	TF	AT2G31380	TCCCTGCGACATCTGCCTTGAGAA	ATGGGTCGCCTCATCGCAATCTCT
402.	TF	AT3G62850	CATGTTGTGTCTTTGTGATTGCA	CTTCACTTGAGAGATGCGTCCTC
403.	TF	AT2G33500	AACGATACCCGAACCACCAA	CGGAATCCTCCTGCTGGTAAC
404.	TF	AT1G01930	TCCACAGAGCCAAGGCTGGTAAAA	AAGTGAGGCTCCGGCAGAGTGTAT
405.	TF	AT2G47890	TGCCTAAGAATCGTCACGCCACCT	TAGTCCACGGAGCTGTCGGATCAT
406.	TF	AT1G02030	TGTCGAGAGACAAGTGGGAGAAGG	TTCTCGCAAGTCTCGCACTCGAAC
407.	TF	AT3G02380	GCGCATCCGTTTCATCAATGGACA	AGCTGTTGCACCACCTGAGTAGGA
408.	TF	AT1G02040	TTCAGTCGTGGACAACACGATGAG	TGGACATCGATCTCTCCCTCTTCC
409.	TF	AT3G07650	TAAGCACCAAACAGCTCCAGAGGG	GAATCATTACTGCCTGCTGGCTGC
410.	TF	AT1G03840	CCCTGGAAATCCTGATCCAGAAGC	CGCAGAGGAAACGATTTGTGGC
411.	TF	AT3G21150	TGTGTCTCAAGCTCCGAGCTATCG	TTTCCCTCCCTCGCGCTCTGTTTA
412.	TF	AT1G04445	GAACCCCAAGAATGTGCGGTTTG	AGGTGGAATACTGGCGATTGGAGT
413.	TF	AT3G21880	GCGCTCTTTTGGGAAACAGATTCG	TCTTTTCCTCGTGTCAGCTCTTGC
414.	TF	AT1G04990	ACCTGGACAACCAGCTTGTGGTAA	ATGGCAGCATAGGGTGGTCGAA
415.	TF	AT3G21890	GCGTGCTATGCACTTCTTGTCAGA	CGTCGTCACCGACGGTAAAACAAC
416.	TF	AT1G08290	TGTCGGTCCAATGCAATTTGCTTG	CCATGTCCCCACATATGCATCTGC
417.	TF	AT4G10240	TCACCGAGTCGCCTTACAAAAAGA	AGTACCCTTTTCTCTCCTGGCAGA
418.	TF	AT1G10480	TCGTCGAGGTCGTCGCAGATTAAC	TCGAACACCGTACAGCTTACCCAT
419.	TF	AT4G15250	GGGAACGTTGTACCGAACATGTCA	AGAAACCCTGGTGAAATTCCGCAA
420.	TF	AT1G11490	TTCCCGGTCGTTGTTCAGAG	ACTCCGAAACCGCAGATGTC
421.	TF	AT3G26744	CGCTGAGCAATGCCAAGAAGGA	ACCAGCATACCCTGCTGTATCGAA
422.	TF	AT1G45249	GCAGGCAAGGATCATGGAAATGC	CCGACTCTGTCCTCCTCAGCTTTT
423.	TF	AT3G47640	AGCCGATACTCTTGAACTGAATCA	CTCAATTTGACCAAACACGTCCT
424.	TF	AT1G49720	GGCCTGGAGAAGGTTGTTGAGAGA	GCCTGTTTTCGAGCCCTTGATCTA
425.	TF	AT3G47710	TTGTCCTCCAGCTTCATCGGCTTC	GCTGAAACCTTTCCAGAGCGTCT
426.	TF	AT1G58110	CGAGCCAAACAGCAATTTGCACAA	GCCTTCTGCCTGCAATGTCTGTA
427.	TF	AT3G50330	TGCGGGTACTGTTGGTGGAGGATA	TGATCAGACCGCATAATGCCACAC
428.	TF	AT1G59530	CGGACAATCCAAGCGGTATAAACG	TGGCCGATTCCCGGTTTGATAT
429.	TF	AT3G56220	ACTGATTCTCATGCTGAGAGCGAA	CGGAAAGATTGCTCAGACGTGGT
430.	TF	AT1G68640	ACGGTTATGGATTGTGGTGGTGGT	TGCCCTGTGTCCAAACACTCCTCT
431.	TF	AT3G56770	ACTCCAAGACAGACAAATCCACAC	TTTCGTCAGTCTCCGACGGTAT
432.	TF	AT1G68880	TGGAAGAACTGTGGTCCATGCTTG	TTCCCTGGCTTGGCTTAGCTCATC
433.	TF	AT3G56970	TTCCAGCTTCTGATCAATCGAAGA	TTGACCCGATACTCGTACCAAAAT
434.	TF	AT1G75390	TTCGACGGCGTGATGAATCCTATG	CAGCAGTAGAAGCAGAAGCCATGA
435.	TF	AT3G56980	CGTGACCGTCGCAGGAAAATTAAC	TCGCAGGAATGCTTAGCTTCTTCG
436.	TF	AT1G77920	AAGCAGCAGGGCCATTTAGGACCA	GCGCTGTTCGTAGTTCGCTAACTC
437.	TF	AT3G57800	GCCATAGCTTAGCAGAACGAGCA	GGGACCAGTTCCTGTAACAGCTTC
438.	TF	AT2G04038	TAAGCTGAACCGCGTATCGGAGAC	AACAAGCTGTCGGAGATCAGAAGC
439.	TF	AT3G59060	GCGGGAAATCAGACCGTGCAACAA	CGCCGGAGATCCAAATCCCAACAT
440.	TF	AT2G12900	AGCTTTTCTCCGTCAGCTCCTGTG	CCATCAACCAAAGGGGGCAATGAA
441.	TF	AT3G61950	CAAGCAGATGGAAGATGAGTGCGA	AAAATCCGATGAACAGCCGCCG
442.	TF	AT2G12940	GATACAGGCACAACTTCGGGATGT	AGCCTTTCACTTTCTCCGTGCAAA
443.	TF	AT3G62090	GAAGGATCGATGTATCTAAGCAGTAGTC	CTTGTGGCCTCGCATCATCT
444.	TF	AT2G13150	TTGAATCATTGGAGCAACACGCC	CTACGTGCAAATGTTCGGTCAAGG
445.	TF	AT4G27310	AGCGGAGGTTCAGTGACGAAGA	CGATCTCATCATCGGAGCAGAGAA
446.	TF	AT1G13290	CCGCTTCAACAACATGCAGATGC	CGAAGATGACGACTTCGTCCCTCT
447.	TF	AT4G38960	TCGCCCCTGCGATGAAAAAGTTC	ACCAACACGTACATGCCGACTAGC
448.	TF	AT1G14580	GGAACCAACCCGGAAACCCAAATC	GGAACCTGTTCGTCGCCATTATCG
449.	TF	AT4G39070	TCGCCAATAAACTAGCCGGGAAAC	ACGCCTCTCCCCGCAAATATCA
450.	TF	AT1G24625	TCACCAGAACGCACACAAGC	TCTTCCCATGTGCATTGCTC
451.	TF	AT5G15840	TGGCTCCTCAGGGACTCACTACAA	TTGACTCCGGCACAACACCAGT
452.	TF	AT1G24625	TTTGGTAACACCGTGCCGTTGTTC	CCAGAAGAAGTCGCTACCACCATC

No.	Gene group	AGI	Sequence of forward primer	Sequence of reverse primer
453.	TF	AT5G15850	GGCATGTACCAAGAGCAACAGAAC	TTCACCATATGACTGAGGGAACCA
454.	TF	AT1G25250	GGGACACCAGCTAACAGATCCAGA	TGCAGATCTCACACACGTACCGAT
455.	TF	AT5G24930	CAGTCACAGCGTGTCATCATCGT	ACTGGTTGCAGGACCACCGTAA
456.	TF	AT1G26590	TGAGTGCCCAATTTGCTTCAGGAT	GCGTGGTTTGCAATGGAATGTGAT
457.	TF	AT5G48250	TCATGAGGGCTCAATGCAGCA	TGGCTCGGTTCTACAAGTCATGAA
458.	TF	AT1G26610	GCAGCAGCAGCAACAACTTCAAG	GAGTGACAAGCCATGTGACCACAA
459.	TF	AT5G54470	AAAGGACGAGACTTGATTTGGATC	AATGGTCTAGATTGGTTCTCCTCATC
460.	TF	AT1G27730	TCGAGCACTGGACAAAGGGTAAGC	CCTCAGTGAGGTTTTGGTGGTGGA
461.	TF	AT5G57660	AAAACAACGCCGGAGGAGAT	AACGGGAGAGGCTCTGTTTTC
462.	TF	AT1G29570	CAGATCCGACCCACTGTTTCA	TCAGCAACACTACGGCTCGA
463.	TF	AT1G07640	TTTGAGCCCCTTTGCTCTCTTCG	CGTTGTTCCCGATATTACCGTCGT
464.	TF	AT1G29600	AAAGAAGTGGGCCAGTTTCGTGAT	GCCTCTTCCGCATCTCAACATCTC
465.	TF	AT1G21340	ACGAGAATCGGCCAGATGGTCTTA	TGTGCGAAAACCGCAGCCAA
466.	TF	AT1G30970	GCACGATGAAACCAGCCAGATGAA	CCCAGCAAGCCTACTCTCTGAGAT
467.	TF	AT1G26790	TGGCTTGTGGCTCTATTGGTATGT	CCGGTGTAATTGTCCAGCCAAACA
468.	TF	AT1G34370	TTCTCGCACCGCATACTCACTTCT	TCGCGTCTCTCTTGAAACCCTTGC
469.	TF	AT4G00050	CATACTACGAGGTAGCCGAGCTGA	ACCTAAGCCGTGCAAGCCTAGTTG
470.	TF	AT2G16770	AGCAAGGGCTAAATGGTTGTG	TCGAGGCTCACCTTTTCTCTTATT
471.	TF	AT4G00120	TCCACCTCCCGAAACCCTAATTCA	CTGCATCTCCTTCATCGCATCCAT
472.	TF	AT2G17770	TCCTGCCACTGTCCTCAGCTTAAA	AGATTCCTCAAAGGAGCGAGGGTT
473.	TF	AT4G00480	GAGCTGGGCGTCACTGAATTGA	GTCATCGTTGTCTTGGTGTGCAGA
474.	TF	AT2G18160	TCACCGCTCAGATGGAGGAGCTTA	TCCTGCACCGTTGGATTGAACAAG
475.	TF	AT4G00870	GTGGAAACAAGAACAACAATTCCC	GCTCACACTCGATGCTGTTTG
476.	TF	AT2G21230	AACTCCGCGATGCTCTGTCAGAGA	GTTCGGCTCCCCTATCACCAGTTT
477.	TF	AT4G01460	TCTCTCCGATCTCTCATGCCTCCT	TACAATCGAAGCTTGGTCACCCCG
478.	TF	AT2G21235	ACAGGCCCCTCTTCTTACTGCTTT	TCGACACTCCCTCGTTCATTTGC
479.	TF	AT4G02590	TGTTTTCCACGGGCAGCCTATG	TCGAACCCTGGGACGGATTGAA
480.	TF	AT2G22850	CGGTTTTGCAGGTGACTGACGA	TTTACGCATCCTCGACCGCTTC
481.	TF	AT4G05170	TAGAATCACTGCGCTTCAGCAACT	ACAGAAGCTGTATCTGTCTTGCCA
482.	TF	AT2G31370	GCCCAGTTGACCCTCTTACAGAGA	TGTAACCGCAGCTTCAGCTCATTG
483.	TF	AT4G09180	ATTGCCGAGAGGGTACGAAGGA	TGCAGTGTTGGTTTGCTTGTCCAT
484.	TF	AT2G34600	TCAAAAACTGCGACAAGCCTT	TCGCATTTTGTTTGCATCTCC
485.	TF	AT4G09820	TCCTCCATCTGGGATGCCAGGAAA	CCACTTAGCCATACGTGCTTCCTC
486.	TF	AT2G35530	AATGCCTCCGGGTTCTTATCCCT	ACCATCAGTGCCGCCTGTAGTAT
487.	TF	AT4G14410	ATTGCTCCAGAAAGCGGGCAAG	AGCTTCTCCCTCCTCAACCTTTCA
488.	TF	AT2G36270	TGGAGAGGAAGAGGAAGCAACAGT	CATCAATGTCCGCAATCTCCCGTT
489.	TF	AT4G16430	TTGTAAGGCTAAGCTGTCCGTTGG	GCCACGTTGGAATCATGAGGCATA
490.	TF	AT2G40620	TGCGAGATGCACTGAACGAGCAAC	GTGAGACTTCTCCCGTGGCGAATT
491.	TF	AT4G17880	CTCCAGCAACGTCTCCAAGCTTTA	TGTTGTCTTCTCCGGCGAAACC
492.	TF	AT2G40950	TGAAGGTGTTGCAGGTCCCATGTT	TGCAGGAATGATGGCTCCAGAGGT
493.	TF	AT1G28310	TGGCTTCATCTCATCAGCAGCAAC	GGAAGAATCGCAACGAGGGCATTT
494.	TF	AT1G34790	CGCTACAACAATCTTCAGATGCAC	CACCCTTCAACGCAGCAGTA
495.	TF	AT1G29160	TCAGGTCGAGCTAGATGCTTTGCT	ACGGAGCCTCTTCACCGGAAAATC
496.	TF	AT1G43850	ACACGAACAGGACCAATCGAGAGT	AGAAGGACCAGGCAGTGCAGAT
497.	TF	AT1G47650	GATTGTCACACGGTTAGGGAGAA	TTCGCACATGAAGCAACTTCA
498.	TF	AT1G43860	TTCCAAGAAGCAGGCACTTGATGT	AAGACGCAGTCTCATTGGAGAACG
499.	TF	AT1G47655	GGATTACGGGTTTGGGTACGGGTA	CCACAACCATCAACCACCGGAAT
500.	TF	AT1G47860	TGCTTGTTTGCCGTGTGATC	TCTTCCGGCACATCACTCTG
501.	TF	AT1G51700	TCTCAAGAACCAAACCGTCGCTGA	TTTTCTTCTGACCCGGACCCATGA
502.	TF	AT1G51220	GATACAACAACATGCAGATGCACA	GCACAGCAGAAACATGGTAGTCTT
503.	TF	AT1G64620	AACAACTACAGCCTGACGCAGC	ATCTCTTGTTCTTGCGGACGCC
504.	TF	AT1G55110	GGAAATCCAGACCCAGAAGCAGAA	GTCCTCTCTTGTGAAGTTGCAAGT
505.	TF	AT1G69570	TGTCCCCTGTTCACGATGTTTCTT	CGGAACCCTGATTGGTCCAATAGT
506.	TF	AT1G65120	CCTGCTGAAACGATATCAACGGGA	TCGGATAAATGTTCGGCTTGTGCT
507.	TF	AT2G28510	AAGGCGGTACTCTTCGTAACGTTC	AGGATCGTTTGTTTCGACGGCAAC
508.	TF	AT1G65130	CACGAAGAGAACTTCAGCGAGCAT	CTTTTGCCTGTTCGACATCCTGGT
509.	TF	AT2G28810	GGCCACAGTCTGCGAATCCAAATA	TTAGGGTTTTGTGGGAAGTGGTGA
510.	TF	AT1G66140	TTCACGGAAGCGGAAACGGGAACA	GGAATGTGCCCGGATTCCCAAAGT
511.	TF	AT2G34140	TGTCGTGGTTGGTATGCTTGGAGA	CACTCCTCAACGAGCAAGCCATTT
512.	TF	AT1G67030	TTCGCCGGAGATAGTGATCGGAGT	TGGAGGTCCAGCCCAATACCATTC
513.	TF	AT2G37590	GCAACAGGCTCCTCCGAGCAATTA	TGAGGACGAAGAACATGACCTCCA
514.	TF	AT1G68130	GGCCGAGTTTTTTCCAGAGTGG	CGGTTTGTGTAGCGTTTGTGGTAT
515.	TF	AT2G46590	TCATCAGCAGCAGCCTTCATCATC	TGAAACAGGAGACGACGAGGAACC
516.	TF	AT1G68360	TGTCTCATGGCTGCGTCATCCAAG	ACCCTGAATCACGAGCCCCATACT
517.	TF	AT4G20970	AGGAGCTGAGATCACTCATGCTGG	CGTGTTCTTCCACCTTGCAGTGAA

No.	Gene group	AGI	Sequence of forward primer	Sequence of reverse primer
518.	TF	AT2G41070	GGAGGCTAAAGGAGGTGGAGAAGA	AGAGAAGCAGAGTTTGTTCGCCG
519.	TF	AT4G21330	TGAGGATGAGCCGTAGGAAACAAG	TGTTGGTGACAATGGGGACATGAG
520.	TF	AT2G42380	AGCTCGAACTGTTATCGCCAAGAG	GCGCTGTTGTCAACGTTAAGAAGC
521.	TF	AT4G21340	GGAGATCGAATTACCGCGCTTCAA	CAGAAGCAGTGTCTGTCTTGCCAA
522.	TF	AT2G46270	TGGGATCACTGCCTCAAGGTCAAA	TCAAAAGCGTCCCCGGAGTTGTTA
523.	TF	AT4G25400	TCCAGGGAAAACGTTCAACGTCA	TTCGTCCAGACAATAACACGAGGT
524.	TF	AT3G10800	GGCTCAGGGCCACTAATGGATTAC	TGCTGGAGACTGATGACGGGACTA
525.	TF	AT4G25410	AAGCTTCTCCACCGCGACAT	TCCGACACAGCTCTTTTTCCCTT
526.	TF	AT3G12250	TGGCAATGGGGCTTTGGCATTT	TTCAGGGCAGATCTCAGCTCGTTC
527.	TF	AT4G28790	GGCCTCCTGCATTCATACCTT	AACAGGACCTACACCTTCCGC
528.	TF	AT3G17609	TAAACGCCGCCGTGGAAGAAAC	CTCTCTCTTGCTTGTTGCGCTGA
529.	TF	AT4G28800	TCGCCGAAAGGAGAAGGAGAGAA	GCGAGGAATGAGTTGTTGCAGAGT
530.	TF	AT3G19290	TGAGCTGAAAGAAACGTCGAAGC	TCCGGTTAATGTCCTTCTCAAGCA
531.	TF	AT4G28810	GGTAATTCTTTCTCAGTATCTATTGCCG	CGTGGCTGAAACTAGGCTGTG
532.	TF	AT3G30530	CTCCTCTACGGATTCCAAAGCCCT	GTCTGCTGCTCTTCTGCTTCATCT
533.	TF	AT4G29100	TGGTCAGCTCGTGAACGATCAGTG	ATTGTCCGTAGATGACGACGAAGC
534.	TF	AT3G44460	GCCCGGAGACAAGCTTATACTGTG	TTCGTGTTTTCTTCCGTGAGGTTG
535.	TF	AT4G29930	TCCGAGAGAAACAGACGGCAAAAG	ACAGATGCCTTGTCCAACTTGCTT
536.	TF	AT3G49760	CGAAGCTCATCAAACTGTGCCACT	ACATGATCGACGGATCTTGCTGGA
537.	TF	AT4G30180	GCCACTTGGTTCCTGCACTCAAGA	TGAGCAGACAAAGCCAAAGCCAT
538.	TF	AT3G51960	TCTCATCATCTCTCAGGATCAGCA	TTCCTCACTGCCTCTCTATTACCA
539.	TF	AT4G30980	TCCCTCAAGGATCGGGAGGTCAAA	TTGTCTGAGGTTGCGCCGTT
540.	TF	AT3G54620	AGGAGGATGCTCTCAAACCGAGAA	CGGCTCTTAATTGGCCTACCTGTG
541.	TF	AT3G21270	TTGGAGGAGGAGGGATCATGCTTG	TCAAACCCAAACCCATACCCGGAT
542.	TF	AT1G68480	GACAAGAGAGGGAGACCGAGACAT	AGCCGAAAGGAGAAATTCCAGGTG
543.	TF	AT3G45610	ATGCATCAGAATGTGATGGGAGT	ACAGCGAGGGCATCTTAATGATT
544.	TF	AT1G72050	GAAACAAGCACGAGAATTCCGGGT	CAGTTTCGACAAAATCACCGCAGG
545.	TF	AT3G47500	AAATCACCAGAGAAGGTAACTCCA	AAGGTTTCGGGTTTCGAATTGT
546.	TF	AT1G75710	TGATCGTTGACCCGAGCAGGTAT	CCACATTGGGAACAAGCACAAACC
547.	TF	AT3G50410	GTTCCGTTACAAACGACGCCTGTT	AGCACTTCCCTTTCCGTCGCTTTC
548.	TF	AT1G80730	GCTCATAAACGTGAGCGCACCTTA	GCTGAAGAAGGCAAGGAGGAGAGA
549.	TF	AT3G52440	TTTATATGGCGGCCAAACGCAAAC	ACATCTTGGACAACTCGGCGTGAT
550.	TF	AT2G01940	CCAGATCCAGATGCAGAAGTTGTG	TCTGGTCTCTTTGAAACCCTTGGT
551.	TF	AT3G55370	CATTACTAGAAGGCGGGGTTAGCG	ATTCACTCCATCCCCATCCCTACC
552.	TF	AT2G02070	ACTGTGGTACACTCTTCTCTCGGC	TGGGTGTCTCGCACTCTCTTGA
553.	TF	AT3G61850	ACGAAGTGGACTCAGGGTTTTCAA	GTTGGTGGCGATGAATGTTGGTT
554.	TF	AT2G02080	TGTTCTAAGCGTTACGCTGTTCAA	CAATGCATCGCAGAAAGCCCTAT
555.	TF	AT4G00940	TCTCCTCCCCTATGCAAGACAAGC	TGGAAAAGACAGTTCTGCCCTCCT
556.	TF	AT2G15740	AATGGTGAGCCTCAAAAGACCAAA	ACGACCACAAGATGTTGGAAAGTT
557.	TF	AT4G21030	CGCGAGTGTGTCCAAGGTGTTATT	GCATTTGTAGCGCGGTTGAGACTT
558.	TF	AT2G17180	TGAGGAACGGTTTGAGTGTGATGG	TGTGTTGCTCTATGTCCGCCTAAC
559.	TF	AT4G21040	TTCAACCGCGCTACTTCTGCAAG	TTAAAGCCCCACCATGAGTCCAGT
560.	TF	AT2G18490	AATGTTTTCCATGAAGGCCGATCT	CGACCATCATATTGAGCATCGTCA
561.	TF	AT4G21050	CACGAGTGTGTCCAAGGTGTGATT	TGCAAAAGTAGCGCGGTTGAGAT
562.	TF	AT2G23740	GCTTCACCAGAGGCTGATAGTGTC	GCAATGTCATGCTTCCACGTTTTC
563.	TF	AT4G21080	TGCCAAACGGGCAAGGGTAAATC	CCTCGTTGGGTCTCAACAGAAACC
564.	TF	AT2G24500	CAGCAGCAGCTATGTCAATGGAGA	TTCACTGACTCACCGGACACAACC
565.	TF	AT4G33880	CTCGTCCCCAATGGAACAAAGGTC	GCAATCGGCGCATACATCCATAGA
566.	TF	AT3G56660	TGATGCTTCGAACGACCAACTCAA	GAACTGAACATTGGCCCTGCAAC
567.	TF	AT4G34530	TGTGAATCCAAGGCCGGATTTTGA	GAGTTGAGGCAACCTCTTTGGCA
568.	TF	AT3G56850	TCCCGAGCTAGGAAACAGGCTTAC	TTTCCACCTCCTTTTGCTTCCTGA
569.	TF	AT4G36060	GGGACAAAATCTCAGAGGAGCTG	GGGAACAAACTGCTTCCTTCTTCA
570.	TF	AT3G58120	GGAGCGTTACTTCATTGCAGACTG	CGCTGATGATCCAAAAACGCAAC
571.	TF	AT4G36540	ACACCACATCTCTCGGCGCTAAAG	GACACTGTGAGTGAGTTTCCCAGC
572.	TF	AT3G62420	TGGGGTCGTTGCAAATGCAAACAA	CCGTGGCGTACCTCGGATCATTAT
573.	TF	AT4G36930	TGTGAAAGCGAGGAAGGAGGAGAA	GAAGGACCTGACTTGGAAGAGGGA
574.	TF	AT4G01120	TCGCCAATGATGGCTCCTTATGGA	GTGGTTGTGAGCCCATTTGAACAC
575.	TF	AT4G37850	GGCTCAACCGTTTTCAAGAAACCA	GCCTTGTCCATCTTTTTAAGGCCA
576.	TF	AT4G02640	TTACTCCAAGCGCCAACCCGTA	TTTTTCGGCCATGCTGAATCGTTC
577.	TF	AT4G38070	ACAGAGAAGGTGCTTCGCAGAACC	GAGGAGTTTACGGCACGTTTCGAT
578.	TF	AT4G34000	AAATCAGCTTCTGGAGCCTCTGC	CAAGCATTGCCTTTTGCATCCCAT
579.	TF	AT5G01310	TGGTCTCGTATCTGCCAGGACATT	ACACTGAGCCTTTGTTCCAGCTTT
580.	TF	AT4G34590	GGAGCAGAGGAAACGTAAACGGAT	CCTGAGCCGTTAGATCGTCTAGGA
581.	TF	AT5G04150	TGCCTCTTTCTGATCAAAAGAGG	TTTCACTACTCTCGCTACCGTCAT
582.	TF	AT4G35040	GAAACGGATCATTCAGCAACGTCA	GCACGATGCCCACCTTTTCTCTTA

No.	Gene group	AGI	Sequence of forward primer	Sequence of reverse primer
583.	TF	AT5G08130	TGGGAAGAAGGGACAGACAAGGAA	AGTTGCTGCAACATCAGCTCTCAT
584.	TF	AT4G35900	CGCGCTAGGAAACAGGAATGCT	GCATTTTCTGCCTGCAAGTGAGC
585.	TF	AT5G09460	TCTGGTTTGAGCGACGAGCAGT	TCTTTTCCCTTTGCCCCTGGAAC
586.	TF	AT4G36730	GCCAATCAACAGGAACAGGGTTCA	GCGTCAGCAAGCATCTGTCCAAAG
587.	TF	AT5G09750	GGCTAGACATCGCCGTGAGAGAAT	ACCTGGCACGAGTCTCTGAAGAA
588.	TF	AT4G37730	TTGTGACGACGCCGACATATCAAA	GAGTCCGGTTCATCTGCTTGAGAG
589.	TF	AT4G24060	TCAGAAGCGAGGAAGCGGTAGTGA	CATCCCAGTCCAATACCCACCAGA
590.	TF	AT2G26940	TGAGTCTCCTCAGCCTCAGAGGAA	ACGTGACCACCATAAGCCTTACCA
591.	TF	AT4G38000	ACGGTGACGACGGATTGGGTTTTC	GATTGCCACTTGTTGCACCTCCT
592.	TF	AT2G27100	TGGGCCACTGGGACCATTTGTA	CCGCTACCTTCAAAAGGAGGGTTG
593.	TF	AT5G02460	ACATCAACCAATCACAACCACCGT	AGGCGTTTGTTGCTGTTGCG
594.	TF	AT2G27630	TCTGACAGATGCGAAGAGGGAGTC	TCCGATGCGCCTTCGAGAACAT
595.	TF	AT5G39660	TTCCTGTTCGGTTATCAGATTCGT	CCCTTTTTATCAGTCTCGCTCTCT
596.	TF	AT2G28200	TGGAAGTGGAGGAGAAAGAGCTGT	TCAGTAGTCACCGGAGACGAAACC
597.	TF	AT5G60200	TGCCGGAAAAACAAACGATCCACA	AACCTGCCGCCGAGAAGACTTT
598.	TF	AT2G28710	CGATGAAGAAATCAGGCGGTGGTA	AGGCGTCAAGTTCAAGTCCAAACA
599.	TF	AT5G60850	TGGAGATCGAACGGCTCAGGTTGA	CCACTTCCCCAATCCAACGGTTCA
600.	TF	AT2G29660	TTGGGCAGAGTGGGAACAAGAGTG	CAAGGAAGCACAGCCCTTGGATTA
601.	TF	AT5G62430	GCCGTACACGTGGAATCCTG	CAACACTGGCATGCTCCAAT
602.	TF	AT2G32930	TCAAGGCTGGGCCACTTACATGAC	ACCGATGCGCTAGAACTGGAGT
603.	TF	AT5G62940	TTGGCTCCAGGAATCCGAGTCATC	GCTGCTGACGGAGAATTCGTTGAA
604.	TF	AT2G36480	TTCAGCAGTCGGGACGGACAAAAG	AATTGGGTGCGGTCTCTGGGACAT
605.	TF	AT5G65590	TTCAGCAACAGAGGATGGCGATGC	TTGTGGCCTCTCGACGACAACAGT
606.	TF	AT2G36930	ACTCTGCCATCGGAACTTCTCCAA	GCGCTGGTCGCTCTATCTTCTTCA
607.	TF	AT5G66940	TTTTCCCTCAGTCGTCTTCCAACG	TGATCAAAGAGCTGAAACCGCCAT
608.	TF	AT2G37430	TTTTGGGACCGGACAGGCTTTAGG	GGCATGGATGGAATCATCGGAGAG
609.	TF	AT1G08000	GAACGAACCTGCAATCCCTAAAC	ACAGAGAAAAGGACGACGAATTTG
610.	TF	AT2G37740	TGTGCCAAAAATCGGGCATAAAGC	CACCGAGAGATCAGTGGTGTTTCC
611.	TF	AT1G08010	CATTCCTGCTTTGAAACAGTCC	AAGATTGGTGGAGAGTGCTGG
612.	TF	AT2G41940	TGGACGAAACCAACGGACGAAGAG	TTTGGGAGGAGTACGGCGGATGAA
613.	TF	AT5G10570	GCAGGCTTCTTGTTTTGAGGTTGG	GACATCTTCCTCCATAACCTGCGT
614.	TF	AT4G38900	TGCTCTGAACGAAGCACTGAATGG	TTCTGACTGCTCTCACCGATTGC
615.	TF	AT5G15160	GAACCGTCGTTCCAACACGGTAT	TCACTGAGGTCATCGGCTTCCT
616.	TF	AT5G04840	TAGGCATGTTGCAGACGGTGGAAG	TGGCTATTCTCCAGGGACAGTGTT
617.	TF	AT5G37800	AAACGGCCTTTCACGGGAGAGAAC	TCCATTCGTACCGCTACTCGGCTT
618.	TF	AT5G06839	GCTTCGTTCTCCAGGCGGATAATT	AACGGCTAGAAGACACCGTGCT
619.	TF	AT5G38860	CCACTGTCTGCTCACAAAGGGT	CCTCAGAAGCAAACGCAGCAAC
620.	TF	AT5G06950	ATCATCCTGATCTTGGGTCGGAGG	GTCACTCGAATCAGAAGCAGCAGT
621.	TF	AT5G39860	CGTTCTGATAAGGCATCAGCCTCG	ACAAACGCTCGCTCAGATTGTCAA
622.	TF	AT5G06960	TACAAAGAGCACGGCAACAGGG	ATCTCCAGCGGTAGAATGGGCT
623.	TF	AT5G41315	ACATTGGTGAAGGAATGCCTGGAC	TTACTATCCGCCGTATGAGCGTTG
624.	TF	AT5G07160	TGCGTCAGAATATTGATCCCAC	CGGTTCGAAATTATCCGTTTGA
625.	TF	AT5G43175	TCAGAGCCTATACGCTAGGAAACG	GCAGGAACTTCACGTAATGGACAG
626.	TF	AT5G08141	TGGCGTCTAATCGAGAATCAGCA	ACTTGCATTTGCAACCCCTCTTTC
627.	TF	AT5G43650	TGCCAATTTCTCTCCTCAAGAGTT	ACCTCCTCTCCTCTAATCTGAGTC
628.	TF	AT5G10030	TGTGGAAAACTTCAGCAGAGCGG	TGCGGTAACAGAACCTTGAGAAGC
629.	TF	AT5G46690	TGCCGTCGAAAGAAATCGAAGAAG	TGATCACCCTTGTGAGCAAAAGGT
630.	TF	AT5G11260	GGCGACTGTCGGAGAAAGTCAAAG	TCAACAACCTCTTCAGCCGCTTG
631.	TF	AT5G46760	TGGCAGATCTCACACGACTTCGAT	CCCAGCCGAGGATCACTGTGTTAT
632.	TF	AT5G15830	TCTTCGTCATCAACGAGAGGAAGC	GCAACCTGTGAGAGAAGCTCATCT
633.	TF	AT5G46830	TGACGTGGAAGTGACGGATATGGA	AGCAAACGCCTTACCCGCTAAC
634.	TF	AT5G24800	TCGACCTCATGAACCGGGATTACA	CGAAAAGGTCCAGCCGGAAACAAT
635.	TF	AT5G48560	TCCCGGAAGAGAAAATCTGTGCCT	CCGCCGTCTTTGAGAAACTAGGAG
636.	TF	AT5G28770	CGAGCAAAGGTGAAAATGGCTGAA	GGCTGCTTGTAGTGTCTGGAGAAT
637.	TF	AT1G51600	TCCGCAAGGAGGTAGCTTTGAGGA	AGCTAGATCCAGCAGATGCAGCTT
638.	TF	AT2G42410	TGTGAGATAAGCCGTGGGGATCTG	TCAAACTCATCCCAAGCTCCAACC
639.	TF	AT2G18380	CGGACCAAAAGGACCTAAGTCG	CACGCCTCTCCTCTTTCTTGAA
640.	TF	AT2G45120	ACGTTGAAAGTGAAACCGAGTCGT	TTCGTTTAGATCGTCGCCGAGTTG
641.	TF	AT2G28340	GCATGTGCCAATTTTGCTTG	TACCACATCCCAGTTGCCAA
642.	TF	AT3G01030	TCCAGAGAAGAAACTTGTGGGGAT	TTCTTGTTTGCCAAGTAACCCGAA
643.	TF	AT2G45050	TCCACGACATTTGCGTTCCCAGTG	CGTCCACGAATTGCGAAAGCCATT
644.	TF	AT3G01460	TGCTGAGGAGAGAATCTTGCTGCT	GCACACTGCTCAAGGTGTTGATGG
645.	TF	AT3G06740	TGGTACCAGCAAAACCCCTCT	GCATGCGTTACAAAGCGACTT
646.	TF	AT3G02790	GACTAATCGAGGCGGAGGAAAAGC	ACTTAGCGTGGCCTCCTTTCTCTT
647.	TF	AT3G16870	GACTTGCGTTGATTGCGGAA	ACAATGACTTTGGTCCGGCAG

No.	Gene group	AGI	Sequence of forward primer	Sequence of reverse primer
648.	TF	AT3G02830	CGCTCGAATGAGGTTGATTGTGCT	TTGGTTGAGGCTGAGGGTGGTT
649.	TF	AT3G20750	GGTTTGCCAAATTCGACTGTTG	ACGACGTTGGTTCCCTGATCA
650.	TF	AT3G05760	TGGGCAAGACACAGGTCGTTACAC	CGACAGAAATAACCAGCCTGCTGA
651.	TF	AT3G21175	GTGTCTCGCCTGAGAAGGTTCAAG	AGGGTTGTTGGGAGTGTATGTGGT
652.	TF	AT3G09290	GCGTTGATGATTCCCTGAACCAGT	TCGAGAAGCCCCTTATGCAGAAAC
653.	TF	AT3G24050	CTGGATTACGGCCAACAGACT	CTTCAACCACTCCAAGGTCGT
654.	TF	AT3G10470	TTCCGGCTACTGCAAACACGGCTT	AGCCTGAATTGGACCCTCCGACAT
655.	TF	AT3G45170	CTACTTGAGGAATCTCGAGAGTTTGATAC	CGGTTGGCAGATTTGAGAAGTT
656.	TF	AT3G13810	CCAGGGAATCCAGACCCAGAATCA	ACACACGAATCTGTTTGTTGCCAT
657.	TF	AT3G50870	AGAGGCCCTAAGTCCCTATGCA	GGTGTTTCCTGTAGCCGCAGTA
658.	TF	AT3G14740	GGCGATGAAACCTACGAATGA	CGGAACAAACAACGCACAAG
659.	TF	AT3G51080	CCGATGGATGATATAGCGGAAC	CGGCGTGAAAGAAGAATCATCT
660.	TF	AT3G19580	TCTCAACAACCGCCGATGATTCAA	GGAGGCAGCAATCGGATGTTTTTC
661.	TF	AT5G50010	CAGGCCGTGAGGACCAAAAAACA	TCCCGCATACTCTCATTCGAGGT
662.	TF	AT5G38800	GGTTGTCCACCTTCGTACCTTTCC	AGGGTTTTGTCCGCAAAATGGTGA
663.	TF	AT5G50915	TTGCTGAGAGGGTGAGAAGGGAA	CCTTATCACAGCCCGGAACAAGGT
664.	TF	AT5G42910	GGCTCGAAAGCAAGCTCAAACTAT	CTGGTTCCATTTGTCTTTTCCGCA
665.	TF	AT5G51780	CGCGGTTTTCGAGTGTTCTTCAAG	TGACCTCGGCCTGTATGGTGTAAA
666.	TF	AT5G44080	TCTCGTCGCATCTCTTCTGCTTCT	TTCTTCCGCCGTAACCGACACTTG
667.	TF	AT5G51790	CATCAAGGCTCTTTCTTCGCAGG	CTAGTAGGGTCACCAATCTCTCGC
668.	TF	AT5G49450	AACGCGGGTCTTAGATCGGAGAAG	TCAGCGTTAAACTCGTCGTAGCAA
669.	TF	AT5G53210	CGCCTCTTAGCCCGCTTAGTAGTA	TCCACATCAGCCAAAGCCGATTTT
670.	TF	AT5G60830	TGGAGTCCCACTACACGACGATCA	TGCGGGTCGATACGTTTGACTG
671.	TF	AT5G54680	AAGACTGAGAAAAACGAGCTGCGA	AGCTGCTGCTCCAGCTTTTCTTT
672.	TF	AT5G65210	TCTTCGTCATGTCAGGGATGTGGA	AACCTTGAGAAGATCGGAGGGTCG
673.	TF	AT5G56960	TGAGGGTGAATGGGACGAATCAGC	TCAGTGAGCCAAGTCAGCAACAAC
674.	TF	AT1G19350	TTGTTCTGAAGCTGGTTGGGTTGT	AGAGGCTTGTGTCCCTTGCGAT
675.	TF	AT5G57150	GACAGAAGCTGGCCTTTGGAAGAA	ATCCGGCGAACTCGAATCATACGA
676.	TF	AT1G75080	TGTGTTGAAGCTGGTTGGGTTGTT	AAGGCTTGCATCCCTTGCGAT
677.	TF	AT5G58010	TGAGAGGCTTAGAAGGGAACGCAT	TGGTGTTGGGAACCAGTTCTTGGA
678.	TF	AT1G78700	CCGCAAGGGATGTAGTAGACCTGT	AGGAAGAGCAAGGACTAGCGGT
679.	TF	AT5G61270	CAGCAACAGTTTCAGATGTCGTT	CACCACCTCCCATTCCCAT
680.	TF	AT3G50750	TCGAAAGGGTTCTCGACCAACAGA	GAATTGGGCTTTGAAAGGCCGATG
681.	TF	AT5G62610	GGGTGCAAGTACTGGTCCGACAAT	GGTAAAGTCCCGAGATCACCGGAA
682.	TF	AT4G18890	CGCAAGGGATGCAAACCAATGGAT	TGTAGGAAGCACGAGGGCTATGTT
683.	TF	AT5G64340	ACCGATGGATAATTGCCAGGCTGA	AAATGCAGCGAAATGTCACCAACC
684.	TF	AT4G36780	TCGAAGCTGGTTGGATCGTC	TGGTGGCTAAACCCCTTGC
685.	TF	AT3G54810	TGTCGGTTTCGGCATAGGTGA	ATGTCCTCAAACGGAACATAGAGC
686.	TF	AT3G20880	GGTCCAACACAGTTCTCTTGTCCT	TCTCAGCGACTCCGGTCCTTTT
687.	TF	AT3G60530	TCCTACTCTCCTCACCGACTTCACT	ATGAGCTGCGTCGTCACTGG
688.	TF	AT3G23130	TGGCAAACTCTCCTCCTCCTCATC	AGGAACGGATCAAACCTGCCCTAT
689.	TF	AT4G17570	TTGTAACCATTAAGAGGCGCTATG	AGCCCCCTTGACTCTGACTTTA
690.	TF	AT3G23140	TCCCTCCTCCTCCACAAACTTCAC	TGGCTGAGACCCTTGGTGTAGGTA
691.	TF	AT4G24470	CACAGATCAAGATTCTGCCCAA	TGCCGCAATGAGTACACGAT
692.	TF	AT3G29340	CAAGACTTCGGTGAACTGCTTGC	CCGACAAAAACGCCAAACAGTGAA
693.	TF	AT4G26150	GCTCCGATTGTAACACAACCAA	CCACAAGCGTTACAAAGAGACTTG
694.	TF	AT3G44750	TGAGCCACAAGGCTATTCTGAGGA	CAGCATTCCCAGCAGGAACTTCT
695.	TF	AT4G32890	GTTCCAACTCCTCCTCGCTTTT	CGTCATTCGGAATATATAGGTCGG
696.	TF	AT3G45260	ACCGTTCTTGGCCGTTATTG	GAGGCCGGAGGAGACGAG
697.	TF	AT4G34680	CTTCCTTCTTTGCCGGATGAA	CATCCACAACACGAGATACCCA
698.	TF	AT3G46070	AGTGGGAAGAGAGTGGCGTGTTT	GTTGACAAAGCTCTCCACCGAAGT
699.	TF	AT4G36240	TTCACCGCCAGAGGATCTCTT	AGTCCTCCAAATCACCAACCG
700.	TF	AT3G46080	TGAGAAAGCCTCACCAGGCACGTT	CACCGTCGTCGTCTCCGGTAAAAA
701.	TF	AT4G36620	CCCAAGTCGTTGTGTAATGCG	GGTCGATGCACGTCTCTCTTCT
702.	TF	AT3G46090	CTCAGGCTCGTTGGTTACACGTT	CAAACAAGCCACTCTCTTCCCACT
703.	TF	AT5G25830	TCTCCGGTGACCTTTGTATACCTTC	CCACAATGTTCGAAAGCCACTC
704.	TF	AT3G47890	CACAGCCTCCTTTCGGTTGCAT	GCTTCAGAAACAACGTCAGCCTCT
705.	TF	AT5G26930	AGTCACTTTGCAATGCATGTGG	AGGCTAAGCTTTTGTGGCTGC
706.	TF	AT3G49930	CCACCGTCGGATCATCACTCTCTT	GATTTGCCACAGACGGAACACTTG
707.	TF	AT5G47140	TCGTCCTGATAGCCTCAGAAGC	CTGCCACAAGTTGCTGAAACAA
708.	TF	AT3G50700	CATGCCTGATCCAGAGTCAGAAGT	TGTGTCCACGACGATGAAGTTGAA
709.	TF	AT5G65320	AGCGTCAAGGTAGAGGCAGATTGT	TGCACCGTATTTGCGACCTCATTA
710.	TF	AT1G06040	TGCCAAGAGAAGGCAGCTTTCATT	GGATTCATCGCAGTCCCTGCAAA
711.	TF	AT5G65640	CGCCAAAACCGGGATTGCTACTAT	TGCTCAGCTCCCTCAGAACAAGAA
712.	TF	AT1G25440	TGGTGGAGAATGGAGGAGAAAGTA	TCCAAACCCACTTGATGGTAAACA

No.	Gene group	AGI	Sequence of forward primer	Sequence of reverse primer
713.	TF	AT5G67060	GGGGGAGTGGTTATGAAAGGGTGT	TGCATTGCCCACCATCTGATGAGT
714.	TF	AT1G28050	AAGGAAAAGAGGAAGACACGGAGA	GCTTTCACAAATCTGCCTCTGACA
715.	TF	AT5G67110	GCAGCTTCAACTTCAAGTCCAGAC	GGTGGAACCTGTGGTAATCGCAT
716.	TF	AT1G49130	TTCCTCCAGGGATAGAGGAGAAGA	GTCGGCGTTAACCTTTCTAACCT
717.	TF	AT1G14685	GTACTAAACGCCGTGGAGCG	TTCTTAAACGCGCCTTGACTC
718.	TF	AT1G60250	TGCGACCGGATGATTCACAGTCAC	TGAGAAAACGGCGAGAAGGTCTCT
719.	TF	AT1G68120	TGTACCGGAATGCCTCAACA	AACAAGCAGACTGCCATCCC
720.	TF	AT1G68190	CCTAAGGTGCAAAGAGAAGGTGGT	GGAAGCTCCAATGGAGATGGATCA
721.	TF	AT2G21240	ACCGTCGAGTTCCTGCTCCT	CCGACATCTTGACTGTCCCA
722.	TF	AT1G68520	GGGTGCCATCTGAAATAGACCTCG	CCTCTGCTCCACTTTCACCCAT
723.	TF	AT2G35550	AAAAATCCCAACAGTCGTGGA	TGCATGACCGAAGTTACCCAA
724.	TF	AT1G73870	AGTGCTTGGGATAATCACGGTTCG	CCAACATGCACTCCGGTTTGATTC
725.	TF	AT4G01930	CCATATGCTTCAAATGCGCA	GGATGGTCGTCAACTCTATGCTT
726.	TF	AT1G75540	CCTCTCTGCGACATCTGTCAGGAT	GCGTGGATCGATGAATCGCAATCT
727.	TF	AT4G38910	TGCCGAGAAGAGTGGGAATAA	CACAATCGCCAAACTCTCACA
728.	TF	AT1G78600	AGACATCATGGGCGGTTCC	GGCCAGTTTAGACCAGACGCT
729.	TF	AT5G42520	CAACAAAAGGCATGCTCGAG	AAGCTTGTTGAACGCGCTTC
730.	TF	AT2G21320	TGCTCCTGCGACGAAAAAGTTCA	TCGGATCAGCTAAGCCTACACGAA
731.	TF	AT5G49300	TTGTGCTGATTGTGGAACCAGT	CAACGACTTTGGACCAACAGG
732.	TF	AT3G53600	TCGTTGACCAAGAACAGGTGAAGC	CCGGTCCCAAACATTTGATCGCAA
733.	TF	AT5G56860	GGCCAAGATGTTTGTGGCTAAC	GTCTCCTTCTTTGGCACCATGT
734.	TF	AT3G53820	GGGCAAAACTTCGACAAGCGAACC	TGGTGCAAATGGCATCTTCACTGT
735.	TF	AT5G66320	CTACAAGCGAACTCTCTCTTCCG	CCACGAAATGAGAGAGCCACT
736.	TF	AT3G57480	GATCTGGGTAAACACTGCTCCGTC	GACAATACACCTGAAGGCAGCGAT
737.	TF	AT1G08465	TTGGATTAAAGCTGGATGGCA	TGGCCTGCAACTGACTGGT
738.	TF	AT3G57670	TGGCCCTACTCAGTTCTCATGTCC	CCATGTCCCCACATATGCATCTGC
739.	TF	AT1G23420	GCTCAGAATCCAAGCATGGCTCAC	GGCCCAATTTTTGGCAGCTAAGC
740.	TF	AT3G58070	TGGTCCTAAGCCGTCGGGGTATTA	GCGGTAGACGCCATAGTCCTAACT
741.	TF	AT1G69180	GCTGCTGCCAAAAATTGGGCTAAG	TCTTCTCACCGAATCCCAAGCCAT
742.	TF	AT3G60580	GCGAACTCGGAAACTCGATTCGTT	GCTGAGCTATGAGGAGGCTCTTGA
743.	TF	AT2G26580	TCTTGCGGTGAATGTCCCATGC	AGCTGCCATGTTTACAGACCACAG
744.	TF	AT3G62240	TCAGCGAAGTGCTGCATTACAGAT	GGGGTCTTCCTCGACGATTCTCTT
745.	TF	AT2G45190	GCCAAACCATCCTTGCGGTTAATG	TGGTACAGCAACCACATCGGACAG
746.	TF	AT4G02670	TCCTGGAAATCCTGATCCAGATGC	CATGGAAGATTGTGGCCTCTCCT
747.	TF	AT4G00180	TGACCCCAGAGAAGCGACAAAGA	GGTTGCCTGCCTTTATACGTTGGA
748.	TF	AT4G12240	TTGCGGTGTTTGGGATTACGAGTT	TCTCCACAGGTTCCACCTCTGTTC
749.	TF	AT1G13400	CACCAACATAGGCAACAACGG	ACCACCGCAAACGCTATTG
750.	TF	AT4G15420	TGTCCTCTTCGCCTAATCGC	CAGAGTTACCCGCTTCCACC
751.	TF	AT1G49900	AAAGCAGAGCCGAAAGGGA	GATCATTAAACTCCGGCGATG
752.	TF	AT4G16610	GGTTACGAGAGAACCCGAGGAAGA	TTCAGGTTCGGTCTCTGTCTCACT
753.	TF	AT4G17810	CCGTTGCCTACAACGACACTTATA	AAGCCGAGGAGGAACTTCTACAT
754.	TF	AT3G14020	CAAGCTCATCAAAGTCCGCAAACC	TTTGTGTTGAGGAAACGTCCACCA
755.	TF	AT4G25610	GCCGTCAAAGAAAGCCGGAAAAGT	GCGCATCCCTCTCTTTGCCAAAAT
756.	TF	AT3G20910	CAGTTGGATGGGCATCCTCAAATC	TTCTTGAATGAGGCATCCCACCA
757.	TF	AT4G26030	TCGTGTCAACCGTTTCATGACTTC	TGACGACATGCATAGTCTTGTTGC
758.	TF	AT5G06510	TCCAAACTCGAAGCAAGCTGATTG	CTCTTCACTTTCTCACCTGGTGGT
759.	TF	AT4G27240	TCTAACCAAACACGCCGTGACTG	TCCACTATTCTCCTCGACGAGTCT
760.	TF	AT5G12840	GGAAAGTCATCCGGGACAGAAAGC	TTTCTTCGCAAACCGGCCTCCA
761.	TF	AT4G31420	TCTCAAGACAGCAACCAGATAATTG	TCAGACCCAGGCTCTTGTACCT
762.	TF	AT1G09030	CCACAGGGAGAATCGGAAGA	GAGAGTGCTGAGAGCCCACC
763.	TF	AT4G35280	TTGGGAAAAGGAGGAGGAGCCGAT	ACAACACCCTGATGTGTCCGAAGT
764.	TF	AT1G21970	TTTCACGGCCCATCTCATGGCCTA	AGTACCGACCACCTCCCATAACCA
765.	TF	AT4G35610	TGGTGATGCGCCAGGATCTGAA	AGTCGCTTCCTCTACTTGCTCCA
766.	TF	AT2G13570	GCTGAAACAAATCCAGGAAGCCCT	TTCCGACATTCGCAATGGGAAGAA
767.	TF	AT4G35700	GGCTTGTTTCAAGTATCGGAAGG	TCCTCCTCGACTGGATCGG
768.	TF	AT2G27470	CGCCACGGCAAATGATTTTTGTA	TGCCTTAAACACATCATCAGCCTT
769.	TF	AT5G01160	TTTTGCCTTGAATGTGCTCG	TCTGAATCCGCTCATCACATAGA
770.	TF	AT2G37060	AGAGGGTGACACAAAGGGATCAGC	GGCCATTTTGGCTTGATTGCCCA
771.	TF	AT5G01860	TTTCCCTCCCATGTCTCACCTCTC	GGTCGTGAATGCTGCCCTCTAGAT
772.	TF	AT2G38880	TAGCGAGGTACAGGGAGGGTGATA	CCGCCACCAGCATCTCTATTTGAT
773.	TF	AT5G03150	GGCACGTTGTTCTCCAGGAAAGAT	GAACTCATCCTCGCTCCTTCCTCA
774.	TF	AT2G47810	TTACAACTTCGGGAGCAGCTCATC	ACCACCACCACTAACCCATCATGT
775.	TF	AT3G25790	GTGACTCAAGCGATTGAGGCGTAT	TGAGCACTCCGACTGTCCGTATAA
776.	TF	AT1G52150	TTTGGAGGCTTGTAGCGTGCCTGA	GTGCCGCCATTGTTGTCTTCTGTG
777.	TF	AT3G46640	TGGCGTTTAGTATCACACCGGAGA	AAGATCCACCACGTAGCGACGAGA

No.	Gene group	AGI	Sequence of forward primer	Sequence of reverse primer
778.	TF	AT1G62360	TCTCCGGTTATGGAGAGACAGCAA	TCGACTTCTTCCTCGGATGACCCA
779.	TF	AT4G04580	TGCACTGGACCGACGATCTTGATA	CACTCTCTTCGCCACCGAGTTTTT
780.	TF	AT1G62990	GCAAATGGCCTTACCCTACGGAA	TGCAATCCTGTCTCCTCCACCAAT
781.	TF	AT4G13640	ACACGAGCAACTAGAGGTGCAA	GTACTTCCCTTGTGCCTCGATC
782.	TF	AT1G69780	CATGGATTTGCTTCGTTTCTAGGT	CTCTTCTTCTCTCCCATTTGTGAC
783.	TF	AT4G17695	ACCCTCCACGCCCATTTTGTACAC	GGTTGCTCTTTCATGACCACCCAA
784.	TF	AT1G70510	GCTCTTTCAGATGATGGTGCGGTT	CGCGGTCATTGCTTCTTTGTTGG
785.	TF	AT4G18020	CGAAAGAAGGTGGATTGGACACCA	TGATCAACGCCGAGTTGCTCAA
786.	TF	AT1G70920	AACCATACCTTAACCCCTAAGCA	TCAACTTGCCTTTGACTAAGCTTC
787.	TF	AT4G28610	ACAGCAATAACGGAACGGGCAAG	GCTCTTTCACTACCGCCAAGACTG
788.	TF	AT1G73360	AGAACCCGTCCTCAGTGGGATGTT	GTGAGCAACTTCTTGCACTGCGTT
789.	TF	AT4G37180	TTGGAGGGCCACAAGTTGCTACAC	TTGTCGGATGCAGCGGATGCTTAC
790.	TF	AT1G75410	AGGACTATCGAAAAACCAGGTTGC	TTCCATAGTCGAACTCTCGCGTTA
791.	TF	AT5G05090	TACCTCAAACCACACCGCCACAAC	TTGCTGGTTCGTCTCCGGTAGAGT
792.	TF	AT1G75430	CCGTCAGTTTTCTTGTTGCTCAAT	TTGGTTCTTCGAAAGTCCTGTTTG
793.	TF	AT5G06800	ATTTCACGTTAAAAGCCACTTACAGA	TTGAGACTCAGGCATGTATTTCG
794.	TF	AT1G79840	GGCATGAGTGGGATGCTTTGTCAA	TGGATTGCCACTGAGTTGCCTCTG
795.	TF	AT5G16560	CCAAGGATGCGTTGGACTAGTAGC	TGCTCTTTCATGGCCGCCTAGAAG
796.	TF	AT2G01430	GCAAGGAGCAAATTGAAGCAAACC	GAGGCAGAGTTCACCGTTGTTG
797.	TF	AT5G03510	AGCAAAGAGCAGCGACGATCATTT	ACCGTCTAGCCCTAACCCTAGAGA
798.	TF	AT3G53340	CTCAGTTCTCGCAGGTTCCTCAAC	AAGATGCTCGATGCTATTGCCGAA
799.	TF	AT5G03740	GAGGAAACCCCAAAGAAGCCTGAA	GGTTCTTGGAGGAGTTGGGTTCTG
800.	TF	AT4G14540	GGGAGACAAGGCGATAAGGAAGGT	TAGTCACCATGCCACCACCGTACA
801.	TF	AT5G04340	TGTGAAGTCGCACGTTTGCTCTAT	GCCTCCGTTCTTTCCTTCGTAGTG
802.	TF	AT5G47640	CGCCACCTGTGGCTCCATACATAT	TGGTGGGATGCAGTATCACCAACA
803.	TF	AT5G04390	TGCCAGCGAAAAAGAAAGCTAGGA	CAAAGCTGAATCCGTTGACCCGAT
804.	TF	AT5G47670	GTCATACTAACGGACCCAGCACTG	TTGTACCTCCACCGCTACAGAGAG
805.	TF	AT5G05120	TGCACACAGAAAGGAGAGAGAGGT	GGTCGGTTCAAATGCGCCAAAAAT
806.	TF	AT1G07980	TCCTTGCAGATAGTGTTCCCGAGA	TGCCTCTTTCCCATTCCTCCAACG
807.	TF	AT5G06070	TTCGTCATGGAAGAGGGCAAAGAC	GGCCGTTGATCTCGGAAATTCCAA
808.	TF	AT1G08970	TGTTCATCCTGGAGCTGACACTCA	AGTCACAGCAGCAGCAATATCGTT
809.	TF	AT5G06650	GCTTTCTTCCACCGCCAACAAAAC	CGCGTCGTTGATTTGAACAGCTTC
810.	TF	AT1G54830	CAACGCCATGACCACTACACCAAC	TGGTGGATCTGATGGTAAGCTGGA
811.	TF	AT5G09740	GCTTGAAGATGACACGTCACCAGA	GCATCCAGCTCTTCATGACCCTCT
812.	TF	AT1G56170	TTTCCAGGACCGACGTGTTTGATT	CTTTCAGCTCGTCCCTCGGGATTA
813.	TF	AT5G10970	CGGGTCAACTTCCACTGAACAGAA	CAAGCGCCTGTGAGCTGTAGAAT
814.	TF	AT3G12480	AGAGAAGGAAGCCCATCAGCGAT	TGCCACTGGTCTTAGCACTCCCTA
815.	TF	AT5G14010	CTGGAAACGAACCGCTTCAGGAA	CGGATGAAACGGATCGTAGCCAT
816.	TF	AT3G48590	TTATCCGCCGATGGGACAACCA	ACGGAGGCTGGACATAAACACCA
817.	TF	AT5G14140	TTGTGAGACCGATGGCTACGGGTA	TGGGTTCCAGTACGGAAAGCCTAA
818.	TF	AT5G27910	TGGAATCCACCAACCACAACCACA	TTTTCCCACGCGCTTCTTCCTCCT
819.	TF	AT5G15480	TGCATTTATCGCCGAACGAGAAGG	TTCACAAACCCAAGGCTACGAGAC
820.	TF	AT5G38140	GTCTCTTCGTTGGACACAGCTCTG	TGAGTTTGGCCTGCAAAGTTTCCT
821.	TF	AT5G18240	GACCACAGCCGAGCATGAACTTA	GTGTAGCCGTCTCTGGACCTCTAT
822.	TF	AT2G01500	CGACCACAGCCACAGCATGAATTA	TTTACCAACCTCTGATGCCCTCTG
823.	TF	AT5G29000	ACTTCACGAGGCATTCGTTGAAGC	TTAGGGGTGGCTCGTTCACTACCA
824.	TF	AT2G02540	TGGTAATAGTGGAGGTGGGCA	TGGTGCAGAATTGGCATGAT
825.	TF	AT5G42630	ACGAAGCATTCGTGCTCCAAGAA	TCTTTCATGGCCGCCAAGAAGTT
826.	TF	AT2G16400	TGAGCATTTCCTCCACCCTTATCC	TCGGCTCAACCCCGTTTGTCTA
827.	TF	AT5G44190	TCCCATCGACATTCATCCCTCGAA	GGCGGTTTCAGTCCCAAAGGAA
828.	TF	AT2G17950	TCCCAGCTTCAATAACGGGAAT	GCCATTAGAAGCATTAACAACACC
829.	TF	AT5G45580	GCTCAGATGGACAGCCGATCTTCA	TTTGTCAGCGCCACCGAGCTTA
830.	TF	AT2G18550	GTACCACAACAAGGAGGAGAAGCA	TCTAAACCACCCATTGCCTTCGTT
831.	TF	AT5G59570	TTTCAAGACGGCGGAGGAAGTAAC	TGGAATAGGCACCATCGACGGAAC
832.	TF	AT2G22430	ACAACTTCCACAGATGAGCAGAGT	CCTCGTATCCTTCAAGCATCGACT
833.	TF	AT1G11510	AGTGGGTCTTGGCATGGATGAAC	GCATCGCTTTCCACTGCTCGTAAA
834.	TF	AT2G22800	TCCCAAACAAAAGCAAGTTCTGGC	TTCAGCTTTGTCCTGGCTCTTCT
835.	TF	AT1G44810	GGAAGTGGTGGTTCTTGGAGGAGA	TCATCCACACCAAGCCTCGCAA
836.	TF	AT2G23760	TTGAAGAGGTGGACCGACGGTA	GCCGTAACCCATTACTTGGTCGAA
837.	TF	AT1G61730	TCCAACGTCGTCGTCAGCTACT	CGTTTCGTTGAAGTCGTAGCAGCA
838.	TF	AT2G27220	CTGGCCTTACTAAGAGTCAGGTGT	CCGGTCACTTCCTTTTCTGCTTTC
839.	TF	AT1G66420	TGAGAGTCTTGGTGTGGATTCGGT	TCAGCCTCCAACAACTCCAACCTC
840.	TF	AT2G27990	TTCATCCGTACCCCACTGATTCTG	ACCTGGTTACGAGAGAGACCAGTT
841.	TF	AT2G01370	AATTGCGAGGTTAGGGGTGAGTGA	ATCGGAACCATGCTCCAACTCTGT
842.	TF	AT2G28610	TCAGGGAACTGGAGTAGGAGAAGC	TCTTCAGCTCCACTTTTGGTGCAG

No.	Gene group	AGI	Sequence of forward primer	Sequence of reverse primer
843.	TF	AT2G25650	CTCAAATAGCACAGCAGAGCGAGA	AGCCACTTCCTCATGCTCCTTCA
844.	TF	AT2G32370	TTTCAATCTGTCGATTCCGCGCTA	TTCCAGCAAAGAAGGTTCTCGCAA
845.	TF	AT5G16470	AGCTCCAAGCTAAAGCCGATGCAG	TGCGTGACCACCTTTCTCCTTACC
846.	TF	AT5G43250	GAATCGCCGTGAAAAGACACCAAC	GCAACGGAAGCGAGTCTAAGAGGA
847.	TF	AT5G16540	TGTGTCTTAAGCTCGGGAGAACCA	AGCTTGGACCAAACTTGCAGATCC
848.	TF	AT5G50470	TCGTTACCGATGATCCCGTGCTAC	CTATCACCGTTCCCGGAGGAAGTA
849.	TF	AT5G18550	ACCAGGCTTCTGTAAGTGCGATGC	AGCTGGTTCCCATTGACGGTTGAG
850.	TF	AT5G50480	CAACAACAACAACGGCGACAACAT	ACGTAACTGAGGGTTGGATGATGC
851.	TF	AT5G22890	TGTCCTCAACACGGGTGTAGATGG	TGGCGCAAATCACAGATTTCAACG
852.	TF	AT5G50490	TCGCTGCTGAGGCTCCAAATCTCT	AGTCGGTTGCTCTCTTGAGCATGG
853.	TF	AT5G22990	TCCCATCGAAATGAGTTCCAACCT	ACCATGATGATTCGTGTGGTCAGA
854.	TF	AT5G63470	AACCACCACCAACCTCCGTCTATC	TCCTCCGGTGACTATTGATGCAGA
855.	TF	AT5G25160	TCTCCACTTCCTTTCCACGGACAG	GCTTGTGGCTTATCGAATGCGCTT
856.	TF	AT2G21810	GGAAATGTGAGACCTGCCGCTT	ATGTCTTGATGGTCGCCCCAACTC
857.	TF	AT5G26610	AAGAGCCAACCCACAAGCAGCATC	TCTTCGTCGCTATCGTTCCCGAAA
858.	TF	AT3G27500	TTGTAACGATTGCGGGGGAGAAAA	TCCTCGGCTCCATTAGTACTCAGC
859.	TF	AT5G27880	TCAACCTCTCAAGCCTTAGGTGGT	CAGCTTCCATCTCCTTCCGCTTTT
860.	TF	AT4G01350	AGCACAAACGATGTGCAGCATGTA	TGAATGGACACGTTTCACAGCAGT
861.	TF	AT5G37890	ACCCCGCAAGAGAATTTCATG	TTTCCAACACATCACCACGC
862.	TF	AT5G02350	TGCGGTGCATGTCAACTATCGAAA	ACTCCACGCATTCTTTGTGGAACA
863.	TF	AT5G39550	GACCAGTGATGAGCATGGCGAT	TTTCTCACAAACAGGTCAGCAGCA
864.	TF	AT2G20110	CCAATTAGACATCCGAGGCCCGAA	TCCATCTCTAGTTTCCCCAGCAGG
865.	TF	AT5G40310	TGGGCCATTGCCGAAACAAGA	TGGGGCTTTCGAGGATCGTAAGAC
866.	TF	AT3G04850	TGAGCAATGTGCGGGAGATTCTGA	TTGAAGCATCGCCATGATCACCAT
867.	TF	AT5G40710	GCGAGTCATGGTAAATGTCTGTCG	GGACTTAAGCCGAGCTGTGTCTAC
868.	TF	AT3G16160	TCGCAGAGAGCATTCAGAAGCAAA	GGACTGCTTGCATCGACATCCTTT
869.	TF	AT2G36340	TTGGACCAAGCCAAGGATGTTCC	CTCTTGCTCCACACAAGGCACA
870.	TF	AT2G33880	TCAGGATGTGAAGTGGAGAGGAGT	GGAGGATTCACCATCCCGGAGTTA
871.	TF	AT3G04930	GCCGTTCAATTTCGGTTTTGGTGT	CTGTTGTTTCCTCCACCGCTCAT
872.	TF	AT2G34710	TGGATCTCCCGGAGTTACAGCCTT	GCAACGTGTCACCACTGGTTTGAG
873.	TF	AT4G00250	ATGTGAGTTGTTGTTGGCGG	TTGAGCAAGCACCGAAGTCA
874.	TF	AT2G35940	ACTCGTAGCCAGGTGTCGAACT	TGCCTGCTCCTTCATTTCCTCCA
875.	TF	AT4G00270	CCGTCGCAGAAGAAGAGACTAT	AGCTCTGTAATCAACTAACCCCTT
876.	TF	AT2G36610	TCAGGAAACCAAGACAACAGATGT	TTCTCCATAGCCACTCTCTACCAT
877.	TF	AT4G00270	CTCCAACGGTACCCTCTCCC	AGCACGCTTTGAGGAAGGAAT
878.	TF	AT2G44910	GTAGGGCAAGGACGAAGCTGAAA	CCGTCGATTCTCCTCGGTCAGATT
879.	TF	AT4G00390	GGTGTGTCGAAAAAGAGTGCGAGT	ACCAACAAACGCCAAGTCTTGCTT
880.	TF	AT2G46680	ACTTGGCTTCTCAGTTCGAGTCCT	GCCTCTTTTAGCCTCTGCAACTCA
881.	TF	AT4G00610	TGCTACTGCTCTTGTGAAGCCAAA	ACGCACCATCAACAAACGACCT
882.	TF	AT3G01220	GAGCTAGGTGGAAGACTAGACAGC	AAGCCATTACCTCAGCAAGGAGTT
883.	TF	AT4G01260	CTGCTGCCAAGAGATCGTTTGAGA	TCACATCCTCCTCACCCATTGAGT
884.	TF	AT3G01470	TCCGAGGTTACTTCCCTGACCGAA	GGCACTTGACCAGGTGGTTCATTA
885.	TF	AT4G25210	TGCGGATGGAGCTGAGAAGAGAGA	TCACGAGTCCAGACCTCTGCAAAC
886.	TF	AT3G03260	TCGACACTCGTCAACAGTGGGACA	TTTCACTTGACCCGGTGACAATGC
887.	TF	AT5G14280	GGAGAGGAGGTGTTTGAGGAGGAT	CCACTTTTCGCAGCCCCTTTGT
888.	TF	AT3G03660	TTCAAAACCGGCGGTCAAGGT	GCTAGAAGTGTTGGTGGTTGCGT
889.	TF	AT5G28040	GTGGGTTTCCTGGAGGTGGT	GACGGCATTAGCATCGGACT
890.	TF	AT3G11260	GGCAGAAACGTCGTAAAATCTCCA	TCCTCTTGACAATCTTCTTCGCTT
891.	TF	AT5G28040	CGGTGTGTTGAGTCCGATGCTAAT	ATCTGTTGTCTCCTCCACCGTTCG
892.	TF	AT3G18010	GCGACACGCAACCAGAGAAACCTT	AACGAGCATTGTGCTCCACCCGTA
893.	TF	AT5G42640	CAAACCATCGTGAATCGCTTCCC	TGGATCGAACCATTCGAGAGTTGT
894.	TF	AT3G22760	CCATTTAGACAACCACTGGCGCAA	TGGAGGTAGCAGTCTGTTGTTGGA
895.	TF	AT5G43170	TCAGCAGTAGTAGCCACCGTGGAT	TCGCCATCGGACTCATCACTTCGT
896.	TF	AT3G22780	GGCTGTGCTTCCTACAAACGAGT	TCTTCCGTTTCCCTGGCATCTCA
897.	TF	AT5G43540	TTGGAGGCTACGAGCAAGTAGACT	CCATCGAGCCGATTCGTAAGTGTT
898.	TF	AT4G14770	CGACACCAACGCCGATTTACAGAC	TGTGGAGGAGGCATCCTGTTCTTA
899.	TF	AT5G44160	GGCACCATTTTCTCAAGGCGAGAC	TTCCGCTAAGGCATCGCAGAAAGC
900.	TF	AT4G29000	AGGCTTAGCCACTTTCCACAGGTA	GCACGAGAAACAGGTGATGGAGAT
901.	TF	AT5G48890	TTTTCCGACGGTTCATCCCGACCA	AGCCAACACGACATGAGAACCACT
902.	TF	AT5G25790	GGGGATCTTAGAGCGTAACCCTGA	TGCTGCACATTCTCCTGCAAATCT
903.	TF	AT5G52010	TTGGAGTGGTGTTGAGAAGGGAGA	TCCCACCTCATCCTCCTCAAACCT
904.	TF	AT1G47870	GAAGGGTGCTGACAATCTTGGACA	TCCAACCTGCTTTCCTCAGATTGC
905.	TF	AT5G54340	TGGAGAGGCAACAGATCGAGTCAA	TGTGAAGACCCACCAAAGAGCAAG
906.	TF	AT2G36010	ACCAGGTTTCCAGAACCAGACTCT	GCACTTCCAAAGTTGTGCCATGAG
907.	TF	AT5G54360	GTTTTGCCCTAGGCGGTCA	CGCTGCTTCTCCAATTCTCG

No.	Gene group	AGI	Sequence of forward primer	Sequence of reverse primer
908.	TF	AT3G01330	TGGGGTTCTCGTCTCCAATTTCCT	AGCCCAATGAGATCCACATCGTCT
909.	TF	AT5G54630	GCTTGAAGCTGCTGAAGCTCATC	TCTCCTTCTACAAGCTCCGTTACG
910.	TF	AT3G48160	TCTCCCTTGATGACGCTGCAAAAT	GCCGCCTCACTTTAGTTCGCATT
911.	TF	AT5G56200	CAACAGGTCAAGCTCTTGGTGGT	CCACAGTAGAAACTGGTGCAGTCC
912.	TF	AT5G02470	GCGGGAACTCTCACCCAAAAGAAG	TCAACGCTCACTGAAGGATGTTGC
913.	TF	AT5G57520	AGGATTTCTCGAAAGTGGCGGCTC	TGATCAAGACCACTCTCCGGCA
914.	TF	AT5G03415	GCGACACTACTTTTCAACGCCTGA	ACACCAGAAGCTCCTTGAGAACCA
915.	TF	AT5G59820	GTGCGAGTCACAAGAAGCCTAACA	GCGACGACGTTTTCACCTTCTTCA
916.	TF	AT5G14960	GGGCTTGTTGCAAGAAGTGGGAA	AGCACGAGGAACAGCTCCAAAACC
917.	TF	AT1G05055	TCGTCATTGACTTCTCTCGGGCAG	TAGCCATCCGGCTTGGTCTGAAAT
918.	TF	AT3G19510	GGCTGGAAAGGTTCAAGCCTGGAA	TGTGGCTCGTTCAAGCTCTTTCTC
919.	TF	AT2G34210	TATAACCCTGGAGCTGGAGGGAGA	TGAAAGGCCCCAGACGAATTTTGA
920.	TF	AT3G27970	ATGCGCAGAACCGTAGCAAC	GGAGACATCCT CTCGGCCTC
921.	TF	AT4G26170	TGCGAGGATCACAAGGGAATGAGA	CGGCTTTATCACGTTCTGTTGGGT
922.	TF	AT3G49530	TCGATTCTCAAGAGCCCAGACGA	GCGCAATCTGATCTGGTGCTACAA
923.	TF	AT1G01160	TCATGGAAAACCAGAATCTCGG	TGGAGAAGAGCTTGGTACTGGG
924.	TF	AT3G55210	CTCTTCTCACAACAACGACAAGGA	TTCCACAGGTGCAACTGAAACAA
925.	TF	AT4G00850	ATCAAATGCACCACCATGAAAC	CTAGCATCGTTAGGACCCGC
926.	TF	AT3G56520	TCCGCATGATCTGCCTGGTTATC	ACTTGGTTGTCACGTTTTCTGCAA
927.	TF	AT5G28640	CTTAGCGAATGCGCCGAG	TTGCAGCTAGGTACATTAGGTTGC
928.	TF	AT3G56530	CAACAACGAGAAGGTGGATCATGC	TTTGCTGCCGCTGGAGTCAGATA
929.	TF	AT1G07520	TGTGTTCTTGTCTGCGTCTCCGTT	AACCGAAGCATCCTTGGCAGCATC
930.	TF	AT3G56560	TTTCAGGCAGAAACGTGGCGACAC	GCCAACGACACCATCTCCAGCTTT
931.	TF	AT1G07530	TTTCACTGCAAACGCCAACACGAT	TGCTGAGCGAGAGGCGATGAATCA
932.	TF	AT3G60390	TCCAAAACCGAAGGGCAAGGA	TGCAATCTCCGATTCTCATCCGTT
933.	TF	AT1G14920	GCGGTTAACTCTGTTTTCGAGC	ACCTTATCGATCGCACCAGGT
934.	TF	AT3G61150	CAGCGCGATAAATGCGAATCAGAG	TGGGATATCAACAGGCGCGTACAC
935.	TF	AT1G21450	CCCTTTTCCTCTAATCTCTCCTGT	CCATTCTCATTCAACGTGTAAAGC
936.	TF	AT3G61890	AGCAATCTCTGGTCTCTGAGCTGC	GCCAGTCCTTGATCACCACAACAC
937.	TF	AT1G50420	GATGTTGCAGGCTAGGAGATTGCT	GCACCCGCTCTCTTCCTTGATTCT
938.	TF	AT3G61910	GCTCTTGAAGTATTACCTCCGCAA	ATCTCTTGAATATCCCAAGGCTCG
939.	TF	AT1G50600	AGAGAAGAGAGGCACGAGCCACTA	AAATCCCGCCATGTGAAACCGAGA
940.	TF	AT4G00730	GCTCCAATGCAATGAACGCGAATC	TCGACAGGCGCGTATACTACAAGC
941.	TF	AT5G60470	TCCAGGAAGGACAGTTTCATCTCA	TGGTCGAATTGGCGGCTAATG
942.	TF	AT5G22220	GAGGTTCCAGATCCTGATGAGGCT	CACGTCTATTGGTCCCATTGTGCT
943.	TF	AT5G61470	TGGCTCGATCACTCCATCACTACG	GTGGACAAATCTCGCCAGAACTGT
944.	TF	AT1G73730	CGTAGCAGACATCAGGATGGAGAA	GTCTCTCAAGGTCGTCAGCATCAA
945.	TF	AT5G63280	TCTGCGAGAGCGTTGCCAATAG	TGAAGACGACTAGCTGAGGGACCT
946.	TF	AT2G27050	TGTGGAAGAGCGGAAACCAGAGAT	TGGCGACCTCCTCCTTTATGGGAA
947.	TF	AT5G64610	GAGGAAATGCGATTTGAAGCACCC	TGCCATCCACCTCAAACATTGACA
948.	TF	AT3G20770	TTGCAAGAGCTTCAAGACACGACT	ACGTCTCTGAGGAGGATCACAGTG
949.	TF	AT5G66730	CGTCTCATCGACCGGAAACCAAA	TTCAGCGTCTGGATCAGGCATTC
950.	TF	AT5G10120	TCGAAAGCCGCACGATTTGAGAAA	TTTAATCACAGCCGCGAGAACACT
951.	TF	AT5G67450	TCCGCCGTACAATCTCCTCCTCTT	GGACTTCCCACAGACCGTACACTT
952.	TF	AT5G21120	TGTGTTCTGATTCGCATACGGCTT	CGCTGCTTGTCTCTCCAGATCTTC
953.	TF	AT1G03790	CCGGGAGGAGATCTGACTTGAGAA	TTGACCAAACCGTTACGAACCGAT
954.	TF	AT5G65100	TCGCAAACCGCACGATCTAAGAAA	CGAGCCGTCTCACTCTCTCCAAAT
955.	TF	AT1G32360	TGCAACGAGCGTCTAGTGCGGTTA	ATCCGACTGATTTTCGCCGGTCCT
956.	TF	AT1G60700	TGGGAGATCATCTGGTGGCCTGAA	TAACCAATGCCTGACGCCGAGA
957.	TF	AT1G68200	ACCGTCACTAAGCCTGGGACTTGT	TCCTCGCACATACACCTTCTGCGT
958.	TF	AT1G75530	CCAGAGGTGTTACTTGGAAGAGCA	AGAGCCTGTCGTCGAGAGAATCT
959.	TF	AT2G19810	TGCATGGGTCGGATTGAACCGGAT	ACCCACCCGACATCAGGAGTATCA
960.	TF	AT3G07220	CCGGAGAGAAGAAGAGAGAGGGAA	TCCCGGACCACACAAATCGGAA
961.	TF	AT2G25900	TGGGAAAGAGCTGAGAGCGGAGAT	TCGAGTCGCCACGTCATCAAAGAC
962.	TF	AT3G07260	AGGAGGATATCAGAGGCAGTGGAA	TCTCTCTTCTTCTCTCCAGAGGCA
963.	TF	AT2G35430	TCCTTTTGGTTCTCACTGCCAT	TCCGCCAAATGTATGCAACTC
964.	TF	AT3G54350	TCAACAGAAGACCTCGCTGTGGA	CGTATGATCGCCTGTCGTCGAGAT
965.	TF	AT1G55580	TCCAATTCCAGTTTCACACGC	TGTAGCAAAAGTCCGGCGAG
966.	TF	AT4G01520	CGATTCGCATACCGTCATAGCGAT	TGGCTGCTCAACCTGAAGCTCA
967.	TF	AT1G63100	TTGGCAGGTTGTTCGAGTTCATCA	TGCTGCTACTGCTTCACTCCTCAA
968.	TF	AT4G01550	CCTTATCTATGACCGAGAACCGCA	TGTTCCTTGGTAACTCGTTGCAGA
969.	TF	AT1G66350	TCGGAGCAAGAGGACGAGAATTGA	TAGCGCGTGGACTAAACGCACT
970.	TF	AT4G02560	TGGCATGTACCACCAGGAATGGAA	TGCTATTACCACCAGCGGCTACTC
971.	TF	AT2G01570	TCCACTCATTACCACCTCCGCTTG	TTCACTCGACTCGACTCCACCA
972.	TF	AT4G03250	AAATCGGTCTGGTGTGGAACTGCC	AGATTCGTCAGTCTCATCGTCCCC

No.	Gene group	AGI	Sequence of forward primer	Sequence of reverse primer
973.	TF	AT2G04890	CGGTTGCTGAGGATGGTGAAGAGT	GAAGGAAAGGGGAAGTGTTCGTGT
974.	TF	AT4G04890	TCGGCTCCGCGAAGAGATTGATAG	GGCTTCCCAACGTATTTCGCAGCA
975.	TF	AT2G29060	AGTCTTCGCCTCTCGGTGAC	TCAAGCGCGTTAGCGAAAC
976.	TF	AT4G08150	TCCTAAAGAAGCACGGCAGAAGC	GCCAACGCTACCTTCTCTGACTCA
977.	TF	AT2G37650	TGCAAGGCTGGAAGGGAAGAACTG	TGGTCTCTCATATGCCAACGAGGA
978.	TF	AT4G10350	GCCAACCCTAGTGAAGATGGATGG	GAGCTTGAAGTGCATGGTTGTTGT
979.	TF	AT2G45160	GGATATTGGCGCGGCTCAATCA	AGTGAGAGGAGAGCTTCAGCGAT
980.	TF	AT4G16780	ACAGACGAGCAAGAACAAAGCTGA	TTTTGTAGCCGACGGTTCTCTTCC
981.	TF	AT3G03450	CAGACGCGACCACTCATCACAA	TTTTCCCGGTTTCAGGCGAGTC
982.	TF	AT4G17460	TCCGCAGTTCTCGAAGACACTTTC	CCAAAGCCAGCTTCTGTTTGGGAT
983.	TF	AT3G13840	TGCAGTTGATGGAGCCAAATC	CATTCTTATTTCCCAATTGTTGTCA
984.	TF	AT4G17710	GCCACAATCTCCGCCTCGAAAA	TGCTGCGTAAACGATCAAGCTCTT
985.	TF	AT3G46600	GAAGCTCTCTTCCATTGCTCGTCA	TAGTTCCCTCTCCACCAGTGTCCT
986.	TF	AT4G21750	CCGGCTTGTGATCTTTCCGTGATA	TCCGGTACAGAAGCTCATCACCAT
987.	TF	AT3G49950	TCGGGTGGGGAATGAAGAAGGAAG	AAATGGGAACCCAAACGGTAGCAA
988.	TF	AT4G25530	ACACAGGCAAATGGGTCAACGTG	AGTGAGCCACTTTTGGTTCCACC
989.	TF	AT2G40140	AGAGGGTTGGGAATACGGTGA	CACGACGGCAGCTTAAACTTG
990.	TF	AT1G49190	GGCAAGTCATCTTCAGAAACACCG	TTGGAGAGAGGGAGCTAGTGTACC
991.	TF	AT2G41900	CCGGTAATGGATCATGCTGGGCTA	TCTCAATTCTGCTGAGCCACAAGC
992.	TF	AT1G67710	TCGGATGCGATCACAAAGC	GAGCCATGGAACATTCATGAGA
993.	TF	AT3G06410	CACGAGATCGTGGAGCGGTTATT	ATGTCCCATCCTCTCCGGTAAAGC
994.	TF	AT2G01760	TCCTGGAAACTCGAAGAAGTCACG	GAATCCGCTTTGGTACAGCTTTGT
995.	TF	AT3G12130	TCAGTACTTCTGGCTGTCCTTTCG	TGGTCCCATATTTGTCATCTGCGA
996.	TF	AT2G25180	GGCCAGTCATCTTCAGAAATTCCG	TGATTAGCCACACCACTGATCCTC
997.	TF	AT3G12680	CAAAACGGGAAGCTGCAAGTATGG	GGGGAATGAATGCAGTCCTCTCAG
998.	TF	AT3G16857	TTGAAGAAACCGCGTGTCGTCT	CCTTCTCAACGCCGAGCTGATTAA
999.	TF	AT3G19360	CGCCGCCATCATTGATCCAGTTAT	GATTGCACGAACATGGGACGAGA
1000.	TF	AT3G62670	GCCAGTCATCTCCAGAAGTACCGT	TCTTCTTGAGGCTCCTGAGGAGTG
1001.	TF	AT3G48440	GACCGGGAGAAGTAGAATGCCCTT	CAGCTCCATATTTGCAGGAGCCAT
1002.	TF	AT4G16110	ACGCAACAGTTGTGGGTGAG	TGATACAGATTCCGGCTCGG
1003.	TF	AT3G51120	TCAATGAGGCTCTGGAAGCTGAGA	TTTTTGCCCGATCACGGAGATGGT
1004.	TF	AT4G31920	TCAGAAATTCCGCGTTGCTC	TAGCCGCCCTGTTAGCTTGTT
1005.	TF	AT3G55980	TCCTCTCCAAGAAACGGCGGATCA	TTGAGCTGCAAAGCCGGTGGAGTA
1006.	TF	AT5G07210	CAAGCATCAACAAGGAGAAGCGGA	AGGTCCAGTCACCATCGTCTTCAT
1007.	TF	AT4G00305	CCGGTGTTTGAGGAATTGCGTACA	GGGTCCTACACAAAGGACACGTCA
1008.	TF	AT5G49240	TCTTGGACTCGAAAGAGCTGTTCC	TGACTGGCTACGTTTTCTCGACTG
1009.	TF	AT4G01020	AAGCTGATCGCCTAAAGGTGCAAT	ATCTCGTGGCAGAGATGCCCATTC
1010.	TF	AT5G58080	CCTCGACAAGGCTGTTCCCAAAAA	GCAAATGACTGGCTACGTTTTCCC
1011.	TF	AT4G29190	TCCTGGGTTTCAGTCTCTGCCTAC	ACACGCTCCATTACGGGTTCTTCC
1012.	TF	AT1G13300	TCAACTCTGGAATCAACCCGACCA	TCTCCTGCTGCAACCTTTCCTCTT
1013.	TF	AT3G50650	TCGGAGCCAGGTTTCATCTCCTT	TCGGACCAAGTAGTCAACGCCAAG
1014.	TF	AT4G29940	TGTGGACAGCAACTGGCAGGATAT	CCGAAGGCCAATCTGCTTCATTGT
1015.	TF	AT3G54220	CACTCTCTGGTTACTCCAAAGATTAGCT	CGTGGCTCAAATCTTGTTCCA
1016.	TF	AT4G32040	TGGAACAGCAACTCTTCCACGTCA	CCCGGTCCGTTTACGTTTGTTCTT
1017.	TF	AT3G60630	GACGAATCGTTACCGTTGGATGGA	GTCTGACTCAGCGTCACAGGAGTA
1018.	TF	AT4G32880	TGGAAGACGGGAGCCTTGTGATAT	CGAAATGAGGAGACGGAGGCATAC
1019.	TF	AT4G00150	ATGTTGGCAAAGGACAGAACTCGT	AACTCCGGTGGAAATCAGGAGGA
1020.	TF	AT4G32980	CTTCCTTCACCCTTACCCGAAAGA	CCATAGCCTAACCCGCGCATTTAT
1021.	TF	AT4G08250	AGCTTTACCAACCGTTGTCAAGCA	AGCCCGTTTTGGCCCAATTCTT
1022.	TF	AT4G34610	TGTTTTGATTCTCCGTGCTTGGC	GAACCAGTTTGAAACCTGACCGC
1023.	TF	AT4G17230	CAGCTTCTCTCCTTCAAAAGGTTT	ACCCCAGCAATACACTACACA
1024.	TF	AT4G35550	ACGGCACCATTTGTGAGCGTCT	CTCCCATTCTCCCTCCTGCAAGAT
1025.	TF	AT4G36710	ACGTGGCGAAACGACAAGGAGAAT	AAACCGCCAAGCTGATGTGGCAAC
1026.	TF	AT4G36740	ACCAGTAGGAGAAGTGAAGCAGCC	AAACAAACCGTTGCCTCCATCTG
1027.	TF	AT4G37650	TTTGCACTCAATGGCCGACT	GTGAGGCGTGTCGTCTGATCTT
1028.	TF	AT4G36870	TTGAAGAGGTGGACCGACGGTACA	ACCGTGGCCCATTACTATGTCGAA
1029.	TF	AT5G17490	CAGTTGGTTCGTTGGTCGTGGA	TGAAAACACTTGCTCGTGCTGTTG
1030.	TF	AT4G37790	TGGTTCCAAAACAGGAGAGCTAGA	TCTCATCCGTTAAAGTCTCGCAAC
1031.	TF	AT5G41920	CGGCGACACAAGCGGGTTTATT	AGCCAAGACGGAGGGTTCCATT
1032.	TF	AT4G40060	TCTCTCCGCCACAATTTCGATTCT	TCCTCTTCCTTAACACCCTCCGTA
1033.	TF	AT5G48150	CTCCGAAAAAGTCAGTTATTGTGC	ACCGTTTACGAGAGTTGTTAACC
1034.	TF	AT5G02030	GCGGCGGAGGAGATAATGGAAAGA	TGTAAACCTCGTCGAGCATGGAGA
1035.	TF	AT5G52510	AGCGGAGTCGATGAAGAGTCGT	CGTCCCATCCAACCAAAGCACA
1036.	TF	AT5G03790	CGATGAGGTGAAGAAGCTGAGAGC	TTGATGGTCCCGGCAGAGATTTG
1037.	TF	AT5G06420	TGATGGTTCTGCGATTAACGCTTC	GTGCAAACTTGTTGAGACTCCTGA

No.	Gene group	AGI	Sequence of forward primer	Sequence of reverse primer
1038.	TF	AT1G14600	TGCTCGGCGGTCAATATAAAGCAA	TGGAGATGGCTCTTGACGTGTGA
1039.	TF	AT5G06770	CACAACTCCAATGGCGGATTCAA	CAAATGGACATCCAGAAGTGCTGA
1040.	TF	AT1G25550	CCGGAGTTACACCGCAGATTCTTG	GCGTAGCAACATGTGATCCACCA
1041.	TF	AT5G07060	TACATGCGAATGACCCGAGCGGAT	TGGTCGCCACCTAAAAGCTGTGAA
1042.	TF	AT1G32240	GCAGCAGCTTCGTCAGGACAAT	TCGTCAATTCCTCGCTATCTCTCG
1043.	TF	AT5G07500	TGTAACGCCGGGAACTTGTGTCAG	ATGCGTACCTGCACCTGTGCTTTC
1044.	TF	AT1G49560	TGTTGGAATCCAGAGTTGCATCGC	TTTGTTTAGGAGTTGCCACTCCCG
1045.	TF	AT5G12850	TTGCGTCCCTTGTACCCTTCCACA	AGTAGAGGCGGAAACAGCAGACGA
1046.	TF	AT1G68670	TCGCAGTCGCATACGCATAGAAAA	ACATGAGATCCTCCAAGCTGCTGA
1047.	TF	AT5G44260	CGTCGAGCTTCTTTGGCGATGAAT	TGCAGCCACCGAGAAATCGCTTAG
1048.	TF	AT1G69580	CAACGGATGCTAAGCCAAGGTTGA	GGTGTTGCTTTGTTAGGTCCTCCA
1049.	TF	AT5G58620	TCAGAGTGAAATCAATAGAGCGGG	GCAGGCTGATGATTCTAGCAGAAG
1050.	TF	AT1G79430	CCTAAACCGCGTTTGCGTTGGA	AATCGTCTTTGGGGTCGCTTTGT
1051.	TF	AT4G16150	CGCTGCGTTTAGAGGTTTCCAAGT	TGCCTTCTCAAGAACTCCCACTGA
1052.	TF	AT2G01060	TTCGTTGATGCCGTTGCTCAACTT	AAGAACGCCTTTGGGTGTAGCTCT
1053.	TF	AT1G67310	GGCTGCACAATTTGGAAGTGA	AGCCGATGCCCCTGAAG
1054.	TF	AT2G02060	CCGGATCTTCACCGTTGTTTCGT	ACAAGTTTTGGTGTTGCTCGGTGT
1055.	TF	AT1G67910	TTCCGGTGTAGGATTCACCG	ATGCAGCCTCGATACTCTTGG
1056.	TF	AT2G03500	CCATCTCCAGAAGTATCGGCTTCA	GGTGTACTCCGGTGGAACCCATAT
1057.	TF	AT2G22300	GCGTTGATTTTCGCGATGTA	CTCCCTGCCAAAGAAAGCTG
1058.	TF	AT2G20400	GCTTCAAAGGTCCAGCAACCAAGT	GGGCGCAATTCCACACAAGAAGAA
1059.	TF	AT2G22900	TTCTCATTAGGAATTGTCAGTGGTCT	GGTCCCATACCTGTCCACGT
1060.	TF	AT2G20570	CATCCAATGCATAACGGGACGACT	TGGCGGTGCTCTAAATCTCGTAGC
1061.	TF	AT5G59450	TGATTTCCTGCGAGGGTGCAGA	CTGGCTTAAACCCGGCTCTCAAAA
1062.	TF	AT5G05770	TGCCGGAAAATCTCCACCGTCAAG	TCGGCGAGGCTTAGAAAGATCTGT
1063.	TF	AT5G66770	TGGCTTCATCTCTTTGGCCTGGA	GGTTTGGTTTGGTTATCGCCAGGA
1064.	TF	AT5G06710	AGCAGTTGAATCTTCGTCCTCGTC	GCTTTGTCCTGGCTCGTCTGTTTT
1065.	TF	AT2G06200	GAAGCAGCCGGATCGACAT	TGGCCTCTCACGTGTCCTC
1066.	TF	AT5G11060	AGCTTGACCACTTCATGACGCAT	ACAGCTTCCATTGCATGAACACGA
1067.	TF	AT2G22840	TGGCTAACAGAGTTCAAAATTCTCG	TGTAAGTTCATCGTGGCAGGAA
1068.	TF	AT5G11270	AAGCTGGGCGTCGTAAAACTAGTA	TGGCGGTTTTTCATCTGGTAGTGT
1069.	TF	AT2G36400	TCTTCACCATCCTCTTCAACACC	TGCCCTTCCCAAATACCAAG
1070.	TF	AT5G15150	ACATGCTGAGTTAGTGGCATTGAA	TGCTTCCATTGTTGCTCCATGAA
1071.	TF	AT2G45480	CCAGACACTGTTCTTCAAGAACGT	CATTGTCTGTTTCAACTCCTTGTAAAC
1072.	TF	AT5G17320	CGGAGAAATGGGCGAGGCTTTT	TTTCCTCGATGGTCAACGGAATCC
1073.	TF	AT3G13960	TCAAGACTCGACACTGGTAGCTAATT	TTGGTGCAGATCCATCATTGAT
1074.	TF	AT5G17810	CGAACAAGAAGGGTTTATGACGGT	TCAGTGGGAAGAGGAAGACCAGAG
1075.	TF	AT3G52910	GACGAGCGATTATCATCAAAGACC	CAGGATGCAAATTCAAGGGC
1076.	TF	AT5G19520	TCGGAGAATACTTGGTGGCAAACT	TCACCATCACCTGTGCTTCTGGAT
1077.	TF	AT4G24150	GCTTTCTACTCTTCCGTCTGCC	AAACCACCTCAGTCCTCTGTGG
1078.	TF	AT5G25220	TCTGTTCTCAAAGCTTGGTGGCAA	ACCAACCTCGCCTTATCTTCCTCA
1079.	TF	AT4G37740	CTCCTAGTTCCTTCGGATGGG	GGGTCCATGTTACCGCCTG
1080.	TF	AT5G46010	AGAATGGAAACCAAATCAACATCA	CGTACCGCCGATGAAAAGTT
1081.	TF	AT5G53660	GGAGATTTAGAGCCGGGAAGA	CTTTCGCGCATCTCCATTTC
1082.	TF	AT5G46880	TCCGTGAAGAGCTTGATCGTCTGT	GATCAATGGCTGAGATGGTGGCAT
1083.	TF	AT1G05230	TGGCGTGATAACCAACCAAGAAGG	TTGAAGCACTCACTCCTGCACAAA
1084.	TF	AT5G60690	CGCCAAGCTAATGCAACAGGGATT	TGTCTTCCCATCGTTGACACACAG
1085.	TF	AT3G16940	AGGCAGTTTTGAGGTGGAGACA	TCTGCTGCAACCTGGAGTCC
1086.	TF	AT2G38300	GATCTAAAGTCCCTCGACTCCGGT	TTGCTCTTTCTTGACCTCCAAGCC
1087.	TF	AT5G09410	GCGTTTAGTGGCAGGGAGG	TGCCCCAGCATCAGCAC
1088.	TF	AT2G40260	TTGGGTGGCCCAGATAGAGCAACA	TGGGCAATACTTAGCCCCTTGACG
1089.	TF	AT5G64220	CCGTAGACTATTGACTGTCGTTGAA	TGCCGAGCTAGAAGAAGCCTC
1090.	TF	AT2G40970	TTAGGAGAATGCAAGGCGGGAACG	GCCGATCAGTAGCCGAATCAGAGA
1091.	TF	AT5G08190	GCTTCCGAAAGCTACCATGACGAA	TCTCTAGCAACACGAACATCTGCG
1092.	TF	AT2G42660	CAGACAGAGCGACGCCGAAATT	AAGCTCCAAATCCAGGGCGAGAAG
1093.	TF	AT5G23090	GACGCTTCTCTTCCAAAAGCTACG	TGCAACACGAACATCTGGTGGTA
1094.	TF	AT3G04030	GCATTGGACCACAGCCAAACAAGA	AGCTCAAGTTGCTCATGAAGCCTT
1095.	TF	AT1G17590	AATTATGAGGAGGAGGCAACAGCG	GAAGATACGGCTTACGGGCTTTGA
1096.	TF	AT3G04450	TGGTAGTGAACGAGCCACCCCTAA	CGGTCAACCCAGGGCTATTGATGA
1097.	TF	AT1G30500	GTCACGTAAGCCGTATTTGCATGA	TTTAGAAACCGCCCGCCACATC
1098.	TF	AT3G10760	TCACGGAAACGGAAACTCAAACCA	GGAAGCTGAAGCTGAGACGGTGTA
1099.	TF	AT1G54160	GAATATGCATCAACACCAACGGCG	TGTGATGAGGCAATGGCACTCTTG
1100.	TF	AT3G12730	ACGAGATGCAAATGGAGGTGCAA	GCCGCTATCCTTTGATTCACCTGT
1101.	TF	AT1G72830	TACCTACCACAGGCACCAACATGC	GCTGGTAAAGGAACACGACCAGGA
1102.	TF	AT3G13040	CTGACGAGAAGAAGAAAGGGGCCA	CTTCCATCTGCATACGCAGTGCTT

No.	Gene group	AGI	Sequence of forward primer	Sequence of reverse primer
1103.	TF	AT2G34720	GAAGCGTCTTTGCACAGCAAGC	ACTGCAATTGGACCCCAGGATAGG
1104.	TF	AT3G19070	CAGGCCCAAGAACCAACAAT	GGCCTGGAGAGATCTGTGGTATT
1105.	TF	AT3G05690	TGAGTAGTAGATGCCGCAAGCCAT	AGTGTTCAAGAATCTCCCACCGGA
1106.	TF	AT3G24120	AAGATGGGGAGAGTCAGGACACAG	TGACTTGGTAACCCTCGTTCTGCT
1107.	TF	AT1G17920	GGAGGAGTGATTCCATCGCCAGAA	TGTGCCAACACTCAAGCAGAAGTT
1108.	TF	AT1G20693	CAAACCAAAGAGGCCAGCC	GAAAGTCTCACGGAAATCTTCCAT
1109.	TF	AT1G19700	GGATTGTCAAAGAACCAGGTTGCT	TTCGATCATCGGTTTCCACAGAC
1110.	TF	AT1G20696	TCCGTGTGACGTACAAGGAGG	GCTTTGCCAACAGCAGCA
1111.	TF	AT1G20700	TCTCATCAGGAGTGAGGCCAATGG	TCCACCGATGCCTAGTCGATATCC
1112.	TF	AT2G17560	CGCTACTGTTGGTAAGGCTGCT	TGGTTCCACTAGCCAGTTTCAAGT
1113.	TF	AT1G20710	GCAACCTCCGACAACGACAATTAC	TCCCTAAATCAGGACTCGGGAACA
1114.	TF	AT2G34450	GCGGAGGCTCTAGTCGGAAC	GACCTCCTTGCACCTTCACTTG
1115.	TF	AT1G23380	GTGGCCTTACCCTACTGAAGGAGA	TTTTGGTCTAACCCCGTTGCATCA
1116.	TF	AT3G28730	CCAAAGTCAAACCAACCACACACG	GGGGTACATTGTCTGACCTTTCCG
1117.	TF	AT1G26960	AGCTTCAAGCTCAGGTAATGGCAT	TATCGATCTCCGGTGGCCTGAT
1118.	TF	AT3G51880	AGCCAGTTGATGACAGAAAGGTGG	GTTTAGTAGGCTTCTCAGCCGGTG
1119.	TF	AT1G27050	AAGTTGGAGGTGGTTTGGGCGA	TCGACGAAAACGACGACGTTCT
1120.	TF	AT4G11080	GCAAACGAGAGGAGAGCTGCTTTA	TCCAGCCATCTTAGCGACCTCAAT
1121.	TF	AT1G28420	TGGTGCGTTTGGAACACCTATAGC	TGTCGCTCATACAGATCGCTCTCA
1122.	TF	AT4G23800	AGGAGAGCTGCTTTACGCGA	CTCCAGTGATCTTTGCGACCT
1123.	TF	AT1G30490	TGCAGCAGGGATATGCGAATCTTC	ACCGTCGCTTGCTCATACGAAAC
1124.	TF	AT4G35570	TCTTCGTCTTCCTGGACGATTTCC	GCTCTACCAACATTACCGACGGAT
1125.	TF	AT1G34650	TGACTTTTCTCCAGCCGTCAAGTG	GGAGCGTATACCACCATACCTCCT
1126.	TF	AT5G23420	AACAAGCCCAAGCGACCTCT	ACGTTTTACGGAAGTCACTCATGA
1127.	TF	AT1G46480	TGAGAGAACCAATGGTGGAGAAGG	TCAAATCCCCAGCTCCTACATGTC
1128.	TF	AT5G56770	GGACATATTCTCGGAAGGATACTCA	TGTTGCAGCAGCTTCTGGTG
1129.	TF	AT5G56780	ATACAGATGGGCTCCGATGG	CAAGAGCATCCCTTCTGTTGC
1130.	TF	AT3G04100	GCGACAATATGGCTCACTTACG	CGTCCCACACGAACAGAAGAT
1131.	TF	AT1G32330	TCGTTTGGAAACCACCGGAGT	CTGGGTCAACCTTCCTGAAACCAT
1132.	TF	AT3G18650	AATTCGGATGCGGGAAATTA	GCCCATTGATCCCAGTTCCT
1133.	TF	AT1G46264	GCAAAACGAGACTTGTGTTGGA	GTAGAAGCAGTCATGAGATTCAAAGC
1134.	TF	AT3G30260	GCTTCGCCGGACAATATCTC	CCTTTGTGATGTATCCCCCG
1135.	TF	AT1G67970	TCGTCAGCTCAATATCTACGGTT	TCCTCCTGATAACATTCTTCAGCA
1136.	TF	AT3G54340	TGGGCCACTCAATATGAGCGAATG	ACACTCACCTAGCCTCTGCTTGAT
1137.	TF	AT1G74250	TCCCAGGATTACAAGAAGGCTCCC	GCCCTTGGTTTTCATTCCTCTGGT
1138.	TF	AT3G57230	TGGCGTTCGAATGAAAAAGG	TGGTGAACGAGATTCCCCTCT
1139.	TF	AT1G77570	GCAATCATATCGTGGAGCGAAAGC	TGAACCCATGAGAACGAAGGTTGG
1140.	TF	AT3G57390	ACTTCCTTGCAGTTGGGGTTGTC	GCCACTTGACTCCCAGAGTTATCG
1141.	TF	AT2G26150	CTTGCCAAGGCGTTGAACAATCC	TCCGTTTCCTCCCCACATCCAAA
1142.	TF	AT3G58780	TGTACCTGCGAGCAAAGATAGCCG	ATCACACTCGATTCCTGCTGGTCC
1143.	TF	AT2G41690	GTTCGCCAGCTCAATACTTACGGT	TGCTCATAAGCTCTCTTTGCCCCT
1144.	TF	AT3G61120	TTCCGCAACCCGAAAACAAAAGAC	TCCCTCTCCTTTCTCCGAAGCTCT
1145.	TF	AT3G02990	TCCAGCTTCGTCAGACAGCTCAA	TTTGGCCTCTTAAAAAGCCCTCGT
1146.	TF	AT3G66656	GTGCAAGACACAAACACGAAGCAA	GCAAGCTCGCTCGCTTTCTTGAA
1147.	TF	AT3G22830	TCCGCCAGCTCAACACATATGG	TTTGCCCTCTAAGAAACCCTTCGT
1148.	TF	AT4G09960	TCAGGTCCAAGAAGCATGAGTTGC	TCAATCTCCCTTTTCTGCGCGTTT
1149.	TF	AT3G24520	TCTCGCAACGAATCTTACCTGCTT	ACCCATAGGTGTTGAGTTGACGAA
1150.	TF	AT4G11250	TTGGATCCTAGCAAGTTCTCGC	TGGATTGTGAAGCAGACAGTGG
1151.	TF	AT1G68320	GCCAAATGTGCTGGGCTAAAGAGA	TCCCTCGTCTTATGTCGGGTTTCA
1152.	TF	AT5G02320	GGACCTTGGACTCAAGAGGAGGAT	TGGTAAGGACTTGGCGATGACAGA
1153.	TF	AT1G69560	TCGCCGTCTACGGTCCACAAAACT	TCGAAGCCTACAGCTTTTCCCGGA
1154.	TF	AT5G03780	TCCCTCGATTCGCTTCTGGATGAT	CGCTTCTTGTGTGGCACATCTCTT
1155.	TF	AT1G73410	TCCTGGTCGCTCTGGTAAGA	TCCGATGAGCCGCTAAAAGTC
1156.	TF	AT5G04110	AGAAGTCGAAGCTTGTTGAGGACA	AGACAATGCAAGTCAGTCCTTCCA
1157.	TF	AT1G74080	TGGAGAGTTTAGCCAAGACGAGGA	TGGCCGACCATTTGTTGCCAT
1158.	TF	AT5G06100	TGGGCACGTATGGCTGCACATT	AACCAGCTCGTTGTCGCCTCTT
1159.	TF	AT1G74430	CCGGAAGCTCGTGGTTTACATCAA	TAGACCGGCTCTCTTAGGCAGTGA
1160.	TF	AT5G06110	AACCAAGAAACCCACAGCCTAACC	TGAGTCCACGCTCATCTCTGTCAA
1161.	TF	AT1G74650	ACCACCTTGTTGCGAGAAGATTGA	CTCTTGCTACACCTTAGCAAACCG
1162.	TF	AT5G07690	GCATGGACTGCCGAAGAAGACAAG	TCCAGCTTTTTGGGGAATGTCACG
1163.	TF	AT1G79180	TGGGCTATTGAGGTGTGGGAAGA	TCCTCCTCTGAAGTGAAGTTGCCA
1164.	TF	AT5G07700	CCAGAAAAAGCTGGGCTGAAACG	GCCACGAGATGCATGAAGCATGAT
1165.	TF	AT2G02820	TGGCCCAGAAAGTTAATGCAG	AGAACCTTCCAGGCATTCTCC
1166.	TF	AT5G10280	CAAGTGGTCAACGATTGCGAAT	GGTGAGTCATTGGATCAAAACCCA
1167.	TF	AT2G03470	GATGCAAAAGTGCAATGCTAAGC	TCTCACAGAGACCAATCAGATCCT

No.	Gene group	AGI	Sequence of forward primer	Sequence of reverse primer
1168.	TF	AT5G11050	TGGACAGCCGATGAAGACAGGAA	AGCTTCTCCGAGATTACTGCCCAT
1169.	TF	AT2G06020	TCTCGTGAAACCAACTTATCGTCT	ACTTATCCACTGGATCTGCTTCATC
1170.	TF	AT5G11510	TGCCTGGAAGGTCGGATAATGGAA	CTGATCCAAAAGGCCAGACGACAT
1171.	TF	AT2G13960	TTGGACTCCAGAGGAGGATGAGAC	TTCTGCCACCTGTGCAAGCA
1172.	TF	AT5G12870	AAGCGTGGCGCTTTCTCTCCTCAA	CAATCTGAGACCACCTGTTGCCGA
1173.	TF	AT3G51910	TCTTTCTCCACGATTCTCCTTCCT	GCAAATTCCCATCTCTCTGCTTCT
1174.	TF	AT4G11880	TGCTGATGGAGAAGTGTGAGATGC	TGTCGAGTCTCAGGAGGTCCAATG
1175.	TF	AT3G63350	TCCACATTCCTTCTCGGCCACT	TCTGCCTCGATCTTTCTGAATCCA
1176.	TF	AT4G18960	GCCAAATTGCGTCAACAAATAA	TCTCACCCATCAATTGCCTGT
1177.	TF	AT4G11660	TGATCTCGTGGAACGAAGATGGAA	CCGTAAGTATTGAGCTGACGAACA
1178.	TF	AT4G22950	TCAGCAAGCGAGAGACGAAACATC	TGCATCAATGCCTTCTCCAAGCAA
1179.	TF	AT4G13980	GAATTCAACAATGAACGGCGCAT	CGATACGATCTGGTCCGTTGATGA
1180.	TF	AT4G24540	CAGTGGAACTCCCCTTGAGGAT	TCCCAAGATGGAAGCCCAA
1181.	TF	AT4G17600	ACGACGAACCAGTGCTTTTCGAT	GCCGCACGACCATTTAAGAGTTCA
1182.	TF	AT4G36590	TCTCCAACTTGTTGAAACCCGTCC	TTTCCTGGTTTGCCAGCACCTC
1183.	TF	AT4G17750	TCTCATGGAGTCCGACGAACAATA	ACCATAGGTGTTTAACTGGCGAAC
1184.	TF	AT4G37940	CGAGGTCGGTCTCATCATCTTC	AACCGACTTCATGCTGGAGCT
1185.	TF	AT4G18870	CAGAGCGGCAAGAGTTTCATCATT	CGCGAATTCCCATTTTCCAGAGTC
1186.	TF	AT5G04640	TCATAGCCACTCCGGTCTCTTCCA	TTCGTGAGGAAAGCGGCTACAACG
1187.	TF	AT4G18880	TCCGCCAGCTTAACACATATGGTT	TCGCAAATTCCCATTGCTCAGGA
1188.	TF	AT5G06500	TGACGATAGGATGAGGAGGGCAAG	GCGCATATCGCACAGAATGGACAG
1189.	TF	AT4G19630	CGTGGAGCAAAAGCAACAATGGTT	ACTCTGAGAGTTTGCCGCAATTGA
1190.	TF	AT5G10140	TTCAACTGGAGGAACACCTTGA	CATGAGTTCGGTCTTCTTGGC
1191.	TF	AT4G36990	TCGTCAGCTCAACACTTACGGATT	CCGTCGTATGTCCGTCAACAGAT
1192.	TF	AT5G13790	GCAAAGCCTTGAGCAGCAA	CAGCAATCGTTCCTTTCGCT
1193.	TF	AT5G03720	GACAGCTTAACACTTATGGATTTCGA	TCGTTAGCGAATTCCCACTTGT
1194.	TF	AT5G15800	TGACCAGCTCTCGGATCTTCA	CATCCAGCTTCATTGCCAAAG
1195.	TF	AT5G16820	GCACAACAACTTCTCCAGCTTCGT	GCAAATTCCCATCGGTCAGGATCA
1196.	TF	AT5G20240	AAATCTGATGGCTGTCGAGCAC	TCTGGTGGTCTCGGACTTTGTC
1197.	TF	AT2G16720	GGCAACAAGTGGTCTTTGATTGCG	TGGCTGGATCAATCCCTTTGCTC
1198.	TF	AT5G14340	CAATGGCATCCACTGCTGGAGAAT	TTCCCGCATCTCAACAAACCTGC
1199.	TF	AT2G22710	CTCCAAACAATCATTATCTTCCCG	TGGCTTTCTTGTTGACTGTTGC
1200.	TF	AT5G14750	ATTTCACCGAGCAAGAAGAGGAT	CCACCTATTACCAAGCAACTTGTG
1201.	TF	AT2G23290	AAACCGTTAAAACGGAGGGCGAGT	ACTTAACGCCGTCACCACAACAAC
1202.	TF	AT5G15310	ACAAAACCACACGAAGATCAACA	CAGAGAATGACACTGTAGATGTCGG
1203.	TF	AT2G25230	ACCATGCTCGTCCTAACATTAAGA	GCGATCTCGACCCATTTGGTA
1204.	TF	AT5G16600	TGGGGAGGCAACCATGTTGTGA	CGCAATAGTCCAGAAAGCTTGGGA
1205.	TF	AT2G26950	TGTTCAGCCTAGCCGCAATC	TCCACCCGTGAAAGGAAATG
1206.	TF	AT5G16770	AGTGGTCGTCGATAGCCGGTAAT	ACCGGATCAATCCCCATTTGGAGA
1207.	TF	AT2G26960	ATCATGAAGAGCCTGGCGG	GACTCATGTTCCGGCATTGG
1208.	TF	AT5G17800	ACCACCTTCTTGGTAGATCAGGCA	TCCTCCGTGAAGGCTCTCTTGTTA
1209.	TF	AT2G31180	CGCAGGTTTACTTAGATGTGGGAA	TTGCCTAAGCTTTCATGCAGATTG
1210.	TF	AT5G18620	GAAGGGGTCGCCATTCTTCCAAAC	AACCGTGTGCCTCCAGATCCAACT
1211.	TF	AT2G32460	TCATCATCGACCTTCACGCTAAGC	GTAACTGAGAAGCCATACGAGCCC
1212.	TF	AT5G23000	CCTTGGTCGCCTGAAGAAGACTCT	TCCCACATCTCCTCAAACCGGCTT
1213.	TF	AT2G33610	TGGCTCAGGCTGCTTTTCTTTCAG	CGAGCTGCTGCTTCTGCAACATTT
1214.	TF	AT5G23650	CAATGGTTGGACCAATGCTCT	TGAGATGGAGCCAAGTGGTTC
1215.	TF	AT2G36890	TCCATTGGAAGCAGGTGGTCAGTA	TAAGTGGTGATGTGGAGGAGGAGC
1216.	TF	AT5G26660	CTCCATGCTGCATTAGGCAACAGA	TGTTCTTCCCGGTAACCGCGTT
1217.	TF	AT2G36960	GGATGTATACTGGCCCGACTCGTT	CCCGAGGCTCTCACTAAGGATCAA
1218.	TF	AT5G35550	ACTCTCCCTAACCAAGCTGGTCTC	CCCCGGTCTTAGGTAGTTCTTCCA
1219.	TF	AT2G37630	TGACAGAGGAAGAGCAGAGGCTTG	TCCACTTGTTGCCGTGTTTCTCC
1220.	TF	AT5G39700	TCAATCGAAGAACAGACCATGCTG	ACTACCAGGAAGAAAGTTGGAGGT
1221.	TF	AT5G43840	TGCCCTAATTTGCCTCCCTAGATG	TGCCTCTCTCCCTTCAGAAAATGC
1222.	TF	AT5G23260	CGAACAGAACAGGATGCCTCA	AAGTCGCAATCCGTTGGTATG
1223.	TF	AT5G45710	GCCTGCTCCAAAAACAGGCGTTAA	TCGGTTGATCCAGGGTTCTCTGTC
1224.	TF	AT5G26580	TGAATCCGATGGTGGAACAA	ATGAAAGGAATGCTCTCACGC
1225.	TF	AT5G54070	TTCGTCAGCTTAACTCTTACGGTT	GCAAATGTTTCTTCCCTCCTTGAA
1226.	TF	AT5G26630	CTTTGTGGCGTCCCGATCT	ACACCTCCGGGTTCAGCTC
1227.	TF	AT5G62020	TCGTCAGCTCAACACTTACGGAT	GACGTTGGATCTCACGGAGAAGA
1228.	TF	AT5G26650	TGAATCATGTTGGAGGGCGTGAAA	AACGGCTGGTAGTTGATTGGATGG
1229.	TF	AT1G08620	TCACCCGCAATCGTACAGGCTATC	GAACTGAACTTGTGGCCGGGAATA
1230.	TF	AT5G26870	CGAGTTAGGTCAGATCCCCG	ACACACCGATATTCACCAATGG
1231.	TF	AT1G30810	TCCACGTCATCGGAAGGTTGTTGC	GATGGAGTGAATACGGGAGCGTCA
1232.	TF	AT5G26950	CCTCCTCAGATGCAGACACAAACC	GCAAACGACGGTGCTTGATTCCAT

No.	Gene group	AGI	Sequence of forward primer	Sequence of reverse primer
1233.	TF	AT1G63490	GCCTGAATGCGCTGATTCTGATCC	CATGCAAAAACAGATGGCCTGCAA
1234.	TF	AT5G27050	CATGGCCGGAGGATGAATC	CCGTCGCTCTCTCTCGTTTAGTT
1235.	TF	AT2G34880	GTGCTGCTCAGAACAAAGAGGACA	GACAACGCCTACGAGGAGGTTCTA
1236.	TF	AT5G27070	TGGTTTTTCCTCCTCAGATGCA	CCATGCCGCAAACTGATCA
1237.	TF	AT2G38950	AGGTTGGAATCTGAACAGTACGGC	TCCAGAACTGGGAAGACAAGCAC
1238.	TF	AT5G27090	AGCTTCTGTTACTCCAACCCGAGA	GAATAAACCGCACAGCTCTGACCT
1239.	TF	AT3G20810	TTCCCGTGGAGGTGGGGAAAAA	ATTGGTCCGCATCCGTTCAAGG
1240.	TF	AT5G27130	TGTCAACGAGTATTGCAGCGATCA	GACGGAGACGAGAGGTTAGGGTTT
1241.	TF	AT3G48430	GCCGGAATACCGTGTTGCAGGTTA	AGCTCCCGGAAAGGTGACGACAAA
1242.	TF	AT5G27580	TTACTCCAACCCGAGAACCAGACC	GATTCGTTGTGGAAGAAACCGCAC
1243.	TF	AT4G20400	TTCTCTCCCCGCACGTAATTAAGC	GGATCAAGAGCCTCAACCACCTGT
1244.	TF	AT5G27810	GAAGTGTGGCCATCAAATTCTG	CCGGGAGTGTCCTAAATTCG
1245.	TF	AT2G39880	GGGCCGAGGAACTGGAATCTAATC	TGCTCCTCCTCATCAGAGAAAGGT
1246.	TF	AT5G40330	CACAAGCTCCTCGGCAACAGAT	CGGCTTTGACGGCAGTTGAATGAT
1247.	TF	AT2G42150	CCGACTCGAACGTCAGGAAACAAT	TACAAGCCTCCTTCGACTCGCTTC
1248.	TF	AT5G40350	TGCAAAATGGGGAAATAGGTGGT	GGAGCTTTGTGATCCAACGGT
1249.	TF	AT2G44430	TTGATCTGATTCGGTCCCATCCGC	TGTAATCCTTAGCCTCCTGGCTCC
1250.	TF	AT5G40360	ACGAGTTGTTGGTGAGAATGGTGA	GGTTATGCCACCTCTCTCGACAT
1251.	TF	AT2G47190	GGCAATAGGTGGTCGAAGATTGCG	GTGTTTGGCTTGCTTTTGGACTCG
1252.	TF	AT5G40430	AGGAGGCGAGCACAAGCAAAT	GGGTTCAAGTGCCACCATCTCTTT
1253.	TF	AT2G47210	AATGCCAGACACTCCAAAGGATCG	GGACCCTTGCGTTTTTGTTCCTTC
1254.	TF	AT5G41020	TTGCAGATGCAATGGGTAAGCACA	ATCTTCCTCTCCTCCAAGCGTCTT
1255.	TF	AT2G47460	TCTCTCCCCAAAAATGCCGGATTA	TGTTTCCACGCTTGAGGTCTGAT
1256.	TF	AT5G45420	AGGCAGCTATGAGATGGGAGAA	AATCCCGTACAGATGTCCTCTAGT
1257.	TF	AT2G47620	CACTTATGAAACAGGTCGCTGCCA	GGCTTCATCACAAAGAGCTGCAAG
1258.	TF	AT5G47290	GCAGAGATGTTGTGCCCGA	TCCCTTCTTCGCCACTATCG
1259.	TF	AT3G01140	GCAGCTCCTCAACAACATTGCTTC	GGTCTCCGTTTCCTTGGTTTGGTT
1260.	TF	AT5G49330	GCATTCCCTTCTCGGCAACAGAT	GGAAACGGCAGTGAAGGCATAGAT
1261.	TF	AT3G01530	TCGCCAAAGCCTCTGGTCTAAAAC	TATGTTCCCTCGCCGCACATCT
1262.	TF	AT5G49620	GCCGAACTCAAAAGGACCGGAAAA	TGTTTCCACGGCGCACATCT
1263.	TF	AT3G02940	CTTGGGAACAAGTGGTCGTCT	ATGGTCGGTTCTTGGCCTATG
1264.	TF	AT5G52260	AGCTGGATTGCAAAGGAATGGGAA	GACCCCCTCTTTAGTCCTGGTCTT
1265.	TF	AT3G05380	CTTGGGCGTTGAGTTGGTTATGGA	TTGCCTCCTTAGACCCTCTGGCAT
1266.	TF	AT5G52600	TCATGGTGAAGGAAACTGGGCAGA	TTTCCTCCTCTCTTCAACCCGGAT
1267.	TF	AT3G06490	TCATTCCCGTTGGGGAAATAGATG	TTCTTGATCTCGTTGTCCGTTCT
1268.	TF	AT5G54230	GGAAGACGAGAAGCTCGTAGGCTA	CGCATCTTTTTAACCCGGCGTTC
1269.	TF	AT5G04240	TTTCAAGGCAGTGCCACGATCATT	CGATCTCTTAGTCGGGAGCTACGA
1270.	TF	AT5G27960	ATGATGGATCAAGAAACCCA	AGCCAAATTCTTTAGTTGCTCTTT
1271.	TF	AT5G46910	TGCTGGTTTCAGCCACGGTTTTAA	CCAATCACCCATCGCAAAGTTCAC
1272.	TF	AT5G37415	TGGACCATCTTCAAGAAAGCTTCA	TTCTCTCGCTCCTTAGGCCA
1273.	TF	AT5G63080	TCTTGCTGCCAACACAGGAA	AGAGAACCGTGACATGAAGAGGA
1274.	TF	AT5G38620	AGAAGAGCGATGTTCACGGAA	ACTGCAGAAGCGGACTGAAAA
1275.	TF	AT1G01780	TCAGCAAAAACTTTCAACCAGG	TTGCTGGGAGTCCTAGTCAGCT
1276.	TF	AT5G38740	TGGTTGTCATCAAAACATGTCTA	CCAAGGATGTTGAAAGCTG
1277.	TF	AT1G10200	GAGAGACCTGCTGGAACCAAAGTT	ACTTGAAGCAGCTCTTGTGGTACA
1278.	TF	AT5G39750	CAAGATCACTCTGCTCCTGTGG	GCGGCATCAACCCATAGTTG
1279.	TF	AT2G39900	AGTCACCTGCAAAGCCATTGA	CGACTAGGTGTCCTATTCAGCTCC
1280.	TF	AT5G40120	TAAGAAGAACGTGAATGTTTTAAAGTA	AACTTGGTCGGGAGGGTAA
1281.	TF	AT2G45800	TCAGGCAGGAAAGACCGAGA	TGATAACTTGCTTGGAGTTCGAGTC
1282.	TF	AT5G40220	GCTTGAATTTCTTGAGTCGCG	TGCTAAACTGTGGTGATCCGG
1283.	TF	AT3G55770	AAGGTAACAGTCGAGAGCCAGACA	GTTGGAAGGTGAAATTGGGCAACC
1284.	TF	AT5G41200	TTCTCCACTGATTCAGTCCTTGG	AAATCGAAGCCTTTCTTGGAATT
1285.	TF	AT3G61230	GAGAAATCGAATGATGCGACG	GTGTGCCGCTAAAGAAGGATG
1286.	TF	AT5G48670	TCACCAGCCACAGGAGCAC	GGCATCAAGAGCGTTGGAAG
1287.	TF	AT4G32551	CTTACCCCTCGCTGCTCGTA	TCTGACATGTTCCATAGTTCTAAAGACTG
1288.	TF	AT5G49420	CGATCAAGACATTGGCTTCGACAC	TGGTCATGTTCTCATCGCATGAGT
1289.	TF	AT5G65070	CCGGAAAGTAGCTCTGACAACA	TGATGGTGGTTACTTGAGAAGCA
1290.	TF	AT5G49490	ATTTGTTGCGTCCCTGATGAA	TTGGTGAGATTCCAACAATCTCG
1291.	TF	AT1G01530	CTGAAAGCCGCACAAAGCTC	CAGCCATCACCTCAGTTAAAGACTC
1292.	TF	AT5G51860	TGCTCAAGTGGCAGCTATGATC	TCCTGATATCGGAGCTAGCGA
1293.	TF	AT3G08500	TCCATTCTTGGTAACAGGTGGTCT	TGTTGTTCTTAAGCCGCTTCTTCA
1294.	TF	AT5G55020	ACCTTAACAGTTCCGGTGCTCA	AAGACATCCTCTAAGAGGCTGCAG
1295.	TF	AT3G09230	TCTTCGTTGGTGTAATCAGCTCAA	AGCCTGATCCTCTACCTCAGTAAA
1296.	TF	AT5G56110	GCTGGGTTGCAAAGATGTGGGAAG	CGGACGCAGATAGTTTGTCCATCG
1297.	TF	AT3G09370	AAGGGACCTTGGACACATGAGGA	GACCATTTCGCAGGCCCGTATT

No.	Gene group	AGI	Sequence of forward primer	Sequence of reverse primer
1298.	TF	AT5G57620	GGTCACCGGAAGAAGATGTGAAGC	CCCAATTTTCTGAGGCAGTGCGAT
1299.	TF	AT3G10113	TCCAAGATGGCTCAGGAAGCTGAC	GAGGCGGGATCACAATCGCTTTAA
1300.	TF	AT5G58850	TCGCTAGAAGTGCAGCCAGAGATT	ACTTCCTGTCTTCTTCCGCCGT
1301.	TF	AT3G10595	TGGACCAAAGAAACACATGAATGG	GCCACTGGTAAAAGTTTTCTGCAT
1302.	TF	AT5G59780	GCATCCTGGTCTCAAACGTGGTAA	CCGGGCAATTTTTGACCACCTGT
1303.	TF	AT3G11440	TTGGGTCGCTCCGAGATTACAAGT	AGCTGATGAAAAACCACCTGCACT
1304.	TF	AT5G60890	CTTAAGGGTAACAAGTGGGCCGCA	TTTCGTTGTCAGTTCGTCCCGCCA
1305.	TF	AT3G11450	ACTCGCTAAAGCCAAGGAGGCAAA	AAGCAATCCGATGGGACCTCGTCA
1306.	TF	AT5G61420	TCCCCAAAAAGCTGGGTTGAAA	TTTAAGGTAGTTGGTCCATCGCA
1307.	TF	AT3G12560	TGAAGATCCTGCTGTGCCAAC	AGGAGACGCCGCAGACC
1308.	TF	AT5G62320	ATCTCCGGCCAGATCTCAAGAGAG	CCATCTATTGCCAAGGCGAGCAT
1309.	TF	AT3G12720	TGGTCTCCAGAGGAAGACGAGAAG	TCTGCAACCCGGCAAGCTTT
1310.	TF	AT5G62470	TCGACCCGGTATTAAGCGTGGAA	GCTGCCCATCTGTTGCCTAAAAGG
1311.	TF	AT3G12820	CGCTGGATTGATGAGATGCGGAA	TGAAGTTGCCTCGTTTGAGACCTG
1312.	TF	AT5G65230	TCATGCGGTTCTAGGCAACAAGTG	TCAGTTCGTCCAGGTAAGTGGCT
1313.	TF	AT3G13540	GACGAGGAAGATCTCATCCTCCGT	GATCAATGACCACCTGTTGCCGA
1314.	TF	AT5G65790	GCAGGTGGTCTATAATTGCTGCAC	TCTCTTGCTTCATTCGCGCTTC
1315.	TF	AT3G13890	CACAGCATTCTTGGTAACAGATGG	TGATGGTGAGACATGAGCTTCTTT
1316.	TF	AT5G67300	AGACGACGTTTCGATAGGAAGCGG	TTCAAGCCTGTGCCTAGACCTGGT
1317.	TF	AT1G17310	TTTGGCCATGTAGACGCATTG	CCTCCAACTTCACCGGA
1318.	TF	AT5G51870	TGCAGGAGCTGAAGATGGAAA	CCCAACAGCTTACGGTGATGA
1319.	TF	AT1G18750	CAACAACACAGTTTCACAGCGG	GCCAAGACATGGAGTGAGCTTC
1320.	TF	AT5G55690	TTCTCGAATGTGACAGAGCCGATG	CCGAACAACTCCCGTAAAACAAGC
1321.	TF	AT1G22130	GACATCGCTCTTCAAGTCACGA	CATGCTCAAGTTCCTCGACGT
1322.	TF	AT5G58890	GATGTGGTGATTTCCGAGCC	TGATGGCCCTGACTTTGGTC
1323.	TF	AT1G22590	CTTGTGCCATCATCTACAGCGA	CCTCGTTAAGGTTTGGCCAGA
1324.	TF	AT5G60440	TCATCTTACTCAGGTGTTGAGTCA	CGAGTTGAGATAACGCAAGTTCC
1325.	TF	AT1G24260	AGAGCTCTCAGGACACAGTTTATGCT	GCATGCGTTCCTTACTCTGAAGAT
1326.	TF	AT5G60910	TCTTCTTCAGTTCTTCTGCCTCAA	TCTCCACAAAGCCATCTCTGG
1327.	TF	AT1G26310	TCTCACGTTAATGCACAGACGA	TCAATCTTGGCCTTAAGCCTG
1328.	TF	AT5G62165	ACTCCTTGAGTTTCACAAGCGGAA	ATTGAGCCTTTCTTTCTCGGACCT
1329.	TF	AT1G28450	AGGCGAAGAAGATGATGGAGAA	AAGCAACTTCCTCACGCAGTTT
1330.	TF	AT5G65050	TGCTGATGAACTTGAAGCCTTAGA	TTTGAGTGGCAGATAATTCCGAG
1331.	TF	AT1G28460	ATGTGTGGTGGAAAGTGGATCC	CATCATCTTCTTCGCCTTTTCG
1332.	TF	AT5G65060	AGTTGCTGATAGAAGAGAACCAGA	GCGAAAGAGTCTCCGGTACTT
1333.	TF	AT1G29960	TGGCTTATGCTTATGGTCCGAG	CCGCTAATAACACATTTCCCGA
1334.	TF	AT5G65080	CGTCTCTTCCACCGGCAA	GATGATCTTGGCCATGCTGTC
1335.	TF	AT1G31140	TCTTTTGAGCTCCATCTCGAATCT	TGCATGAACTCAGACTTGCGA
1336.	TF	AT5G65330	ACGCAAGAAGAGCCGCAATCT	TCGTCTTCCCCTTGTCCTTGTCCT
1337.	TF	AT1G31630	CGTCAACCGAAGGTGTTCAAG	TTGGATTGTTCTGTCGCCG
1338.	TF	AT2G42680	CGAGACCGTCAGAAAATTCAATG	CAGAGATGTGCCGCTTGATG
1339.	TF	AT1G31640	TGAAGCATGCACACATCCCTT	TAAGACCAACGGTTGGTGGCT
1340.	TF	AT3G24500	AAAGAGCCAAGACCTACGCG	CGCGACACCGTTTCTCAGA
1341.	TF	AT3G15320	TGCAAGTTCCCAACCAATCCATGT	TTTGGCTGCCTTCACACCAGGA
1342.	TF	AT1G01060	ACGAAACAGGTAAGTGGCGACATT	TGGGAACATCTTGAACCGCGTT
1343.	TF	AT3G18100	TGAAGAAGAAACGCAAAGCCAAGC	CCGTCTCTTTGGTTGTCTCTCTGC
1344.	TF	AT1G01380	AGCAATGGCTCAGGAAGAAGAGGA	TCCCACCTTTCACCGACAAGCTTA
1345.	TF	AT3G23250	TCCATGCTGTGAGAAGATGGGGT	AAAGACCAGCTTGCTTAGGGAGGG
1346.	TF	AT1G01520	AGCCCTTCATCTATTTGACCGGGA	TGTGCGTGGCTTCGTATCTGTATC
1347.	TF	AT3G24310	TGCTAAGTGGGGCAATAGGTGGT	GGTTCTTTGTCTTCTCCGCACTGT
1348.	TF	AT1G09770	AAAGGGTCATGAGAGGAGGGCAGA	GCCTGCTTCAATGTCGCCTCTATT
1349.	TF	AT3G27220	AAGTGCTCACGTAGACGCGAGA	TTGGCATTGGTGGCAACATTTTCC
1350.	TF	AT1G15720	TTCGGATGGCTTCTTCGGTGATGC	TCACGAGTCACCAAGTCGCTCTCT
1351.	TF	AT3G27785	AGTTATTGGTGCAGCTAGTGGACC	TCCAACTCGTCCTTGAAGCATCTT
1352.	TF	AT1G17460	TGTGGATCTCAAGGACAAATGGCG	TCGACCCGTGTTTCTTCAGACCT
1353.	TF	AT3G27810	CAGGTGGTCGAAAATCGCCAA	TGATGAGATCCAACGGACGATG
1354.	TF	AT1G17520	GAGCAGCAACGACAAGAAGTGC	TTCAAGCTTTCCCTGAGCTGCAA
1355.	TF	AT3G27920	CGCATCGTCAGAAAAACTGGGCTA	CCGAGGAGCTTGTGGAGACGAATA
1356.	TF	AT1G18330	TCCAAGATGGCTCAGGAAGCTGAC	GGCGGGATCACAATCGCTTTAACC
1357.	TF	AT3G28470	ATTGGCAGCAGGTGGTCTTCCA	AGTCACCGGGTCTATCCCCATTTT
1358.	TF	AT1G19000	CAACCGTCGCCGAAGAAGATCTA	GGCCATTTCTGTAACCGTCTCAGT
1359.	TF	AT3G28910	GTTCCTACCAATACTGGGCTGCTT	TGCCTCTTTTGATTCCTGGCCTTA
1360.	TF	AT1G49950	TCCACCAGACTTCAAACGCTTGTT	AACCTTCACAAGTTTCCCGCAAGA
1361.	TF	AT3G29020	AGCTTGTTTCGGTTTACGGTCCT	TGCAGCTCTTTCCTGTTCTTCCTT
1362.	TF	AT1G70000	TCCTGATCCCAACCCAACCGAT	TCCTCGTTTGCGTTCACGGTTTC

No.	Gene group	AGI	Sequence of forward primer	Sequence of reverse primer
1363.	TF	AT3G30210	TTGAGGCCAGGGCTTAAGAGAGGA	AGACCACTTGTTACCCCAAAGGGA
1364.	TF	AT1G71030	CCTTCCGGTATGCAACACCGTAAG	AGTGCATGAAGCTTGAGGATGAGA
1365.	TF	AT1G33070	ATTTCAAGCATGCGCACATC	ACGGTTGGTGTTTGGTGGTAG
1366.	TF	AT3G58680	GACCGTTCGAAAATTCAATGC	GTTCAAGGAGGTGCCGCTT
1367.	TF	AT1G46408	TCATCAGCCGTGTTCTGCAAGG	TGCTCTTCCGTGTTAAACCCATCG
1368.	TF	AT1G01150	TGCGGAGAAGCTTATGCTGTTGT	TCTCCACTCCCTCCCTCAACATCT
1369.	TF	AT1G47760	GGAGGATTTGAGGAAGAAACAGC	CGCCTTGAAAGCAACTAAGTCC
1370.	TF	AT1G06180	CCTAAACTAGCCGGGCTACTTC	GACCATCTGTTGCCTAAGAGTTGA
1371.	TF	AT1G48150	CCGATGTTCACAGAGGAGGATC	CCAAACACGTCGTCGACATCTA
1372.	TF	AT1G06910	AGGGCGTGGGTAATCAGAACAGT	AGAGGCTCTCTTGGTCGTCTTCTT
1373.	TF	AT1G54760	GTAGATGAGGATGCGTGGTGG	TCGCCTCTTCGTGATCTTCC
1374.	TF	AT1G07540	CGAGCTGCCAGATCACCAAGAAAG	TGCAGCCAACAAATCGAAAGACGA
1375.	TF	AT1G59810	ATCTGTTACGGTCCCGACAGCGAT	TCAAGGCATTGAACTTGGCAACGA
1376.	TF	AT1G08810	ACACTGGGTTATTGAGATGCAGCA	TGGACGCCCATTTGTTACCCAA
1377.	TF	AT1G60040	TCAAAAGATGCAGCTTACACGG	CCTGAAGATAATTACCGCCGAA
1378.	TF	AT1G09540	TCCTTGGAAACAGATGGTCACAG	TGTGTTTGGGTCAATGCCTCTTT
1379.	TF	AT1G60880	GCGGCTCCTATGACTTCCAAA	CGACAGAGGAATGACCGAAAGA
1380.	TF	AT1G09710	TGGACGACGATAGTGACATGGAGT	TGTGCAATAGCCTCCACTGATGC
1381.	TF	AT1G60920	GCGGACTCTTCCACAAAGCTT	ATTGCGATTTGAGTCCCCG
1382.	TF	AT1G13880	GGAGAAGCCAGGTGTCGTAGGATA	AGAGAAAGGCGCATTGGTCTCAAA
1383.	TF	AT1G65300	TGCAGAAGCTACGTGATGAGAACC	TCGTCTCCCCTTTGAGACAACCAA
1384.	TF	AT1G14350	GGAGGTTGGTCCCCTGAAGAAGAT	TTCTGCCTGAAACCACCTTTGCT
1385.	TF	AT1G65330	CTGTCCCAATCGGTTTTGATG	CGGCTCTTGCTGATTTTGGT
1386.	TF	AT1G16490	TCCCAAAGCAAGCTGGATTGTTGA	TGCACTGAAATTGCCACGTTTCAC
1387.	TF	AT1G65360	TGAGGTGAAGGCGGAAGTAGAA	ACCACTCCTCAGCGTTTTCGT
1388.	TF	AT1G17950	GATGTGTAGTCGAGGCCATTGGA	CTCTTACCAGATCGACCAGAGAGC
1389.	TF	AT3G46130	TCACGCTAAGTGGGGAAACAGGTG	ATCCGTTCGTCCCGGCAATTT
1390.	TF	AT1G72650	TCCCTCAAACAGCGTGGGAAGTCT	GCAAGCTCCCTTACCCGTAACAGA
1391.	TF	AT3G47600	TGCCTACTCACACAGGTTTGAGGA	ACGCTTGATCCCAGGTCGAAGAT
1392.	TF	AT1G72740	TTTCATTGAGCCCAGGCATGAAGT	TGCAAGCCTCCTCAGTCTTGTACT
1393.	TF	AT3G47680	GCCACAAACCCATGAAGCAGAAGA	TGCTTTCGCCTTTGCAGCTTTAAC
1394.	TF	AT1G74840	GGCAAAATCGAGAGGAAACGAGGA	GCCCAAGCAAGAACAGCTTGTGT
1395.	TF	AT3G48920	ATTCATTGGACCATGTGGCC	TGGCACGAAATCAGGCAAA
1396.	TF	AT1G75250	TGGCAGTTTACGACAAGGACACAC	GTGGCGCTTCACTTCTTCTACAGT
1397.	TF	AT3G49690	GCAGGTGGTCTATAATCGCTGCTC	GCTTCTTGAAGCTCCTTGCGTTGT
1398.	TF	AT2G21650	ACGTGCCATTCCCTGACTACAAGA	GCAGCTTCATGCTTCTCATCCTCT
1399.	TF	AT3G50060	ATTCCGTCACCGTCGTCTCCTGTT	GTAAAACCGCCGGAAATCGGCA
1400.	TF	AT2G27070	ACCGATTGGTCGTTTTTGGA	CCGAAATACCCGATCCGAA
1401.	TF	AT3G52250	TCCCCAGAGATCAGTCACCCAAGA	GGTGAGTGAGAAGCCTTCACCTGT
1402.	TF	AT2G30420	CGATCCCGATATGACTCTGAAGA	CGGTCATACTGATAAACTCCCATTC
1403.	TF	AT3G53200	GTTTCCGGTTTGAAGAGGAGTGGT	TTCTTGACTCATCGGTCCACGC
1404.	TF	AT2G38090	TGGACAGAAGAAGAACACAGACAA	TGGTCACAAAGTTACGAGCTATGT
1405.	TF	AT3G55730	TCTCAAGCGTAAACCTTTCTCTGA	TGAACCGCGTGAGCAGAAAT
1406.	TF	AT2G46410	TTGGCGACAGGTGGGAGTTGAT	GCAAAAACGACGCCGTGTTTCATA
1407.	TF	AT3G57980	CGGCTCGAAACCCAGGAAACAT	ACCCTCTTCCACACGACTTCGAAT
1408.	TF	AT2G46830	TCTGTGTCTGACGAGGGTCGAATT	ACTTTGCGGCAATACCTCTCTGG
1409.	TF	AT3G60460	TCTTCTTCAACGCACCGGCAAAT	TCAGCCGAGAACTTGCATCCATTT
1410.	TF	AT3G09600	GCACTTCAACTGTTTGATCGTGAC	GCACATGAGCTAATGTCCCGTTTT
1411.	TF	AT3G61250	CTTGCTGGTTTACTTCGCTGTG	TGGCATGAAGCTGGATAACAAGTT
1412.	TF	AT3G10580	GGGAAAAAGAAGGGAATACCTTG	TTAGTCCATCCAAAAACAATCTGTGT
1413.	TF	AT1G69120	GCACCAAATCCAGCATCCTT	CAGACCACCCATGTTGAGAAAA
1414.	TF	AT1G18570	TCACGCCCTTCACGGCAACAAAT	TCGGTTCTTCCTGGTAGTCCACGA
1415.	TF	AT1G69540	ACCTTATGTTGCGGTCTTCGA	CGGAGCTACTTGTCAGTTTGGC
1416.	TF	AT1G18710	ATCAACGAGCATGGCGTTTGTGA	TCTCTGCAAACCAGCTCTTTTGGG
1417.	TF	AT1G71692	GAGGAACAAGGAAGGAGTCCTCAA	AGTTTGTCTCCATGACTGCGAAG
1418.	TF	AT1G18960	GCAATGGGAAGCAGATGGCAACTT	TGAGAAAAGGGACGGTGAGTGACA
1419.	TF	AT1G72350	ACGTTTTCCAAACGACGATCC	GCCGCAGAGAACGCTTAATTC
1420.	TF	AT1G19510	GTTCGAGAGGGCTTTAGCCGTTTA	ACTTCCAACTGCTTTAGCCACGTT
1421.	TF	AT1G77080	AACAACTTGAGACTGCTCTGTCCG	ACTCCATCATCAGTTCTGCCTTCC
1422.	TF	AT1G21700	TCAACAGCAAGATGGAGCACACAA	CGGCTCTAAATGCTGCCATAACCT
1423.	TF	AT1G77950	TGTGACATCGATCTTGCCCTC	CGATCCTTGTTTGACCGGAA
1424.	TF	AT1G22640	GCGCCGCTGGATTACAAAGATGTG	TTGTTACCGAGCAAGCTATGGAGC
1425.	TF	AT1G77980	GATCAAAGTCGACGCCCAGA	GTTGCTGCAAAGTCCGGAGT
1426.	TF	AT1G25340	ATGAAGAGGGTGCAGAGCAGAG	TCCAGAAGATTTAGCGAGCAGATT
1427.	TF	AT2G03060	CGCGAATTTATAGCCTCAAGGA	TCAGAAATCCGAGCCTGCA

No.	Gene group	AGI	Sequence of forward primer	Sequence of reverse primer
1428.	TF	AT1G26580	GCCACGGTGAATGAGGGAAATCTT	ATCTCTTCCACCACAACCTTGTGT
1429.	TF	AT2G03710	CTTGAGCATCTCGAACGCCAAGTA	TCAAGCATAGACCGAGCCTTGGTA
1430.	TF	AT1G26780	TCTGGGAAGAGCTGTAGACTACGA	TCTTCGGTTTATCCTCGGGTCCAA
1431.	TF	AT2G14210	TTGGCAAAGAGAGGTTGCAAG	TCTCCCACTAGTTTCCTGTGGC
1432.	TF	AT1G34670	CAGCCGAAGAAGAGCAAACAATCC	GCGTCGCAATAGCTGACCATTTA
1433.	TF	AT2G15660	AAGCAGTAGCAACGGATGCC	AGTCATCGCTGGGACCCTAA
1434.	TF	AT1G35515	AGAGCAAATCATTGTCAAACTTCATAG	TCCCAGCAATCAAAGACCATT
1435.	TF	AT2G22540	TGGTTTGACGCGTGTGATTG	CCCTTTTTCTGAAGTTCGCTGA
1436.	TF	AT1G42670	ACTCTGAACCTGTTGGCGTTG	TTTGCCGCCTTAACACCAAT
1437.	TF	AT3G62610	ACCAGGTGGTCAACAATTGCGAG	CGTGGAGTTTACGGCTGAGATGAG
1438.	TF	AT3G10590	CCTTGGACAGAAGAGGAACACAGA	GACACTTGCCGTGGAGTCTTTG
1439.	TF	AT4G00540	AGGATTGCTGAATGCCTTCCTGG	TCAAGAACCTTAAGCCAACGGTGC
1440.	TF	AT3G11280	CTTGGACAGAGGAAGAACACAGGA	TGGCGTCTTTGACACCACGAA
1441.	TF	AT4G01680	TGCCGTTCTTGGCAATAGATGGTC	AAGAGCTTGTGTGTAACCGGGTC
1442.	TF	AT3G16350	TGGTAACCGATTCATCGCCAACAC	TCCTTGCTTGGAGAGGAACCGTT
1443.	TF	AT4G01980	TCAAGCTTCCGGTTTTATGGA	GGTCTCATTGTTGTGGCTTTCTT
1444.	TF	AT3G24870	GCTTACAGAAGCAAGCCTCTTTTA	TGCTACTTCCAGACTTCTCACAA
1445.	TF	AT4G05100	TGGTAAGAGTTGTCGTCTCCGGT	ACCACTTGTTTCCCATGATGCTGT
1446.	TF	AT3G49850	ACCTCCAAGACCTTCTACAAGTGT	TCTTCAAGCTGGTAATAGCCTCCA
1447.	TF	AT4G09460	GGATTGTTGCGTTGTGGTAAAAG	TGACCATTTGTTACCGAGTAAGC
1448.	TF	AT4G01060	CCCAAGACCAACTCCATCGTTACT	CCAGCTATCAGTTCCCACCTGTCA
1449.	TF	AT4G12350	ACGCCGAGGAACAGCTTGTCAT	TCTTGGACCATCTGTTGCCGAGAA
1450.	TF	AT4G01280	TCAGATACGAAGCCACGCTCAGAA	GGTGGAAGATGTTCGTTAGCACCA
1451.	TF	AT4G13480	CGCAGGATTGAAAAGGAATGGCAA	TGACCACCTATTTCCCCACTTAGC
1452.	TF	AT4G09450	TGACGGAAGCAGGTGAGTCTAAGG	CAACCTCTGTTCCTCTTCTGACCA
1453.	TF	AT4G16420	TGTTGTAAGCGAGTACCGCATGG	CCTGCCACTTGAGCTTCCTTGAGA
1454.	TF	AT4G11400	GGTAACAAGTGTTGGCTCCAAGTG	TTTGGTGACCCCGAAATGGATGA
1455.	TF	AT4G17780	GGGCTTGGACAATACTAGCCTTGG	TTGGAAGGTTTCTTGCGTGACACT
1456.	TF	AT4G36570	GCGCTTCTTGGACACGTAAGGAGA	GGCTCTTGCAACGTTATGCCAAC
1457.	TF	AT4G17785	GGTGGATGAATTATCTCCGGCCTG	TTTGTTGCCAAGGAGGGCATGG
1458.	TF	AT4G39250	AACAAAGCCTTTGAGCAGGCTCT	CCTCTGTTGTTTTTCCACCCACGA
1459.	TF	AT4G18770	TGGACTGCTGAAGAAGACAGGGT	AGCGATATGCGACCATTTACGCAA
1460.	TF	AT5G01200	CCGAGGACGAACACCTACGATTTC	TCGGCGTTCGAGTCGTCACAAA
1461.	TF	AT2G22630	TGCCAGCTCCAGTGTGAAATC	TTGCTCCTCCATCTTAGCCGT
1462.	TF	AT1G48000	AACTCCCTCTCTCGTTCTGCTGGA	GCCACCGCAATCTGCAACTTTTC
1463.	TF	AT2G24840	TGCATTGGAGGAGTTGAGGAAG	CGTCTTTGGCCTCATTAAACGA
1464.	TF	AT1G49010	GCTCCTTATGCTGTACCGGC	ACGGAGCTGGATGTTGCTG
1465.	TF	AT2G26320	CTCGTTCTAGATCATTCCATACAATCC	CCCGTTCTTGTAATGAAAGCATG
1466.	TF	AT1G56160	GGACGTGAAGCGAGGCAACTTTAG	GAAGGACGCGATCTTTGACCACTT
1467.	TF	AT2G26880	CCATTCGAGGTGCAAGTCG	CACCCCCTAAAAAAGCGTCC
1468.	TF	AT1G56650	GCTGCGAAAAGGTGCTTGGACTAC	CCCAGCTCTTACAGGAACTTGGTG
1469.	TF	AT2G28700	GCGATGTTAATGCATGTGCG	GGCCACACATCTGGATTTGAGT
1470.	TF	AT1G57560	AACAGATGGTCGCAAATTGCTGC	CCAACCTCGGAGAGGGGTTTATGT
1471.	TF	AT2G34440	TGAGACGCTTACCCTCGATGAACT	GCCTGCAAGTGATTGACTTGACCT
1472.	TF	AT1G58220	TTGGAAGCATCCCCTGGAGTTAGT	GCACATAGGAAGCAGCCATCACTT
1473.	TF	AT2G40210	TGGTGTTCGCCAAGAACCAT	CGTCGCATCACCATTGTTTTC
1474.	TF	AT1G63910	TGCAACCAGCAAAAGGTGAAGAGA	TGAAGCCCTGCTTTTTCAGGGAC
1475.	TF	AT2G42830	TCCGATCCAAGAAGCACGA	TGCAGCTCGATTTCCCTTTT
1476.	TF	AT1G66230	TGGCAAGAGTTGCAGACTTCGTTG	TGACCACCTGTTTCCAAGCTGG
1477.	TF	AT2G45650	AAGTTTGAAACGGAAGGCCATGCT	ATGCCGCCGAGTTTGCCCATAA
1478.	TF	AT1G66370	AGAACTGGTCTCAATCGGTGC	TCGGAGCAGAGTTTTCCTCTCTTA
1479.	TF	AT2G45660	ACGAGAAGCTCTCTGAAAAGTGGG	TGGGCTACTCTCTTCATCACCTCT
1480.	TF	AT1G66380	TGGGCTAAATCGGTGCAGGA	CCAGCAATCAAGGACCACCT
1481.	TF	AT3G02310	GCTGGAAGATATGATCGGCGT	TGATCACCACCTTCCCATCCT
1482.	TF	AT1G66390	TGGGCTAAATCGATGCAGAAAGA	CCAGCAATCAAGGACCACCTAT
1483.	TF	AT4G21440	GGGCCTTGGACATCTGAAGAAGAC	GCAAACCGGCATTTTTGGGGAG
1484.	TF	AT5G02840	TCAGATCAGGAGCCATGCCCAAA	GGGGGTGGAACATGTGCTAAAGTC
1485.	TF	AT4G22680	ATGATTTTGGGTTTTGACTGTTCA	TGTGTTCATCTCCATAGAGTTGCC
1486.	TF	AT5G04760	CCTTGGACAGAGAACGAACACAAA	TCCTCGTCACCACAACGTTTC
1487.	TF	AT4G25560	CCCATCAAAGCTGGGTTACAAAGG	CGACCACTTGTTACCCAAGGAAGA
1488.	TF	AT5G05790	TGGACGGAGGAAGAACACAGGAGA	TGGGCATGACTTGCAACCTGAG
1489.	TF	AT4G26930	TGCTCAGTTACCAGGCAGAACAGA	GCGTTTCAACCTCGTGTTCCAGT
1490.	TF	AT5G08520	GTGGACAGAAGATGAGCACAGGTT	ACTTTTGAGCATGGCTCGCAAC
1491.	TF	AT4G28110	TACGCTCCCCAAAAATGCTGGACT	GTCCATCGAAGACGGCAGCTTTT
1492.	TF	AT5G17300	GAGCTTGAGACTCTGAAGCTGGA	AGACTTTGCTTCGTTGGTTCCTTG

No.	Gene group	AGI	Sequence of forward primer	Sequence of reverse primer
1493.	TF	AT4G32730	TGGACACCTGAAGAGGACGAAGTC	CTCTGGGTTCAAGACCTTTTGCCA
1494.	TF	AT5G37260	TGGCTATGCAGGAACGTTGTGA	TGCTCTGCTTCTGTCCATTTCTCT
1495.	TF	AT4G33450	GGAAGATCAGGGAAAAGCTGTAGA	TTGGTGATGTTTGGATCAAGTTGG
1496.	TF	AT5G47390	GCTGGTGATGGTTACGCTTCTGA	TCCTTTCTTTCTCTCGCGGCTAGA
1497.	TF	AT4G34990	ATCTCCGACCTGATCTCAAGAGGG	GAGACCACTTGTTACCGAGAAGGC
1498.	TF	AT5G52660	CCTCCCAAAAGGAGCAGGAGCTAA	TCTTGCGTCCCTGTTGGATCGT
1499.	TF	AT4G37260	ACCGTCTCGTCAGTCCAAGGAA	TCTGAGGTTACAGCGTCATCGTCT
1500.	TF	AT5G53200	TCGCCCTCCATGACTCTGAAGAAG	TCCCACCTATCACCGACAAGTCTG
1501.	TF	AT4G37780	TGGGATAAAGAGGTGCGGGAAGAG	CGGTGAAGCCTCCATGCTTTAAGT
1502.	TF	AT5G56840	AAAACACCTGACCGCAAAAAAGGG	TCCAATTAGAAACGTCCGGTGCTC
1503.	TF	AT4G38620	TCACCGAGGAAGAAGACGAACTCA	TCCCGGCAATAAGCGACCATTTG
1504.	TF	AT5G58900	GGACGGAGGAAGAACACAAGCTA	TCGCGTTATCACAAAGTTCCGAGA
1505.	TF	AT5G61620	TCATGAGAAGAAAAAGGGGAAGCC	ATGACTTGCGACTTGTGTTGGT
1506.	TF	AT5G47370	TCGATCCTACATCAGATCTTCGCA	TCTCACTTCTCTTCCCGCTAATGG
1507.	TF	AT5G67580	GTGGATCACCACGCACTTGT	CGGTGGAGTCTTGAAATTCTCCTC
1508.	TF	AT5G52170	ATGGACACGAACAAATGGGCAGAA	AAGGGTTGATGCAACCGCGACTAT
1509.	TF	AT1G01010	AGCAGCAGAGCAAAGAAAAGGTGA	ACCGTGTTGGTGGATGGAGGTAT
1510.	TF	AT5G53950	GTGCCATCGCAGAAGTTGATCTCA	TTCTCTCCCATCTTAGCTCTCCCG
1511.	TF	AT1G01720	TCAGGCTGGATGATTGGGTTCTCT	GCCTCTCGGTAGCTCCTTTTTTGT
1512.	TF	AT5G53980	TCGAACCAGCTTGGTCTACCTCAA	GAGTGCAGTGTTGGACCTCAAGAG
1513.	TF	AT1G02210	TCCAACACACCAATCATTGGCAAG	GGCAAAAACGTTGGTTGATACACG
1514.	TF	AT5G56620	AGATCCTTTGATGAGGGGACAGCC	TCCCATCATGTACTTCCTCGCACG
1515.	TF	AT1G02220	GGGAGTTACCTTGCCAGTCAAGGA	TCATCTGCTGATCACCTCTGCCA
1516.	TF	AT5G59340	GCTAGGCAACGCCAAAAGC	TGTGGAGGAGGCGATTGAAG
1517.	TF	AT1G02230	TCTCTTGGAGAGTCGCCAACCAT	TCGCCCAAAGAAGAGCTTCCATTT
1518.	TF	AT5G61430	CTTGCCGAAAACCGCAAAGAATGA	TTCCCTCCAGCACTCTTTTGGAAC
1519.	TF	AT1G02250	GAGGGATCGTTGTTTCACAGCCAA	AGTGCATCCACATCAAGAAAGCCA
1520.	TF	AT5G62380	GGGACATCCAAGAGTTATGTGGAA	CCCGTTGCTCTATTGGTTCGT
1521.	TF	AT1G03490	ACCATCAGGCTCATCAGACTCAGC	ATCAGCCTCTTCTTTCTGCTCACG
1522.	TF	AT5G63790	ACTTGATGATTGGGTTTTGTGTCG	GTACTCATCTTTTCCGTCGGTTTC
1523.	TF	AT1G12260	TCCTCAGGAAGAAGGATGGGTTGT	CGTACCAATGTGAAGGGGATGAGT
1524.	TF	AT5G64060	CGAACCTCCTGATTTACCCGACAA	GTCTCTCCCTGTGGTCTTCCAGTA
1525.	TF	AT1G19040	TCAGAACGTCCCACCAAGTGTCTT	TGGTGAATGGGCCTTTGGAGTGAG
1526.	TF	AT5G64530	TATTCCCTCCCGGATTCCTC	AAGCACGGCTTGATCTCTTAGAA
1527.	TF	AT1G19790	GTGGTGGGGATGACGATGAT	AGTCTCTAACCCTGAAGAACTAGC
1528.	TF	AT1G66550	CGACCAAGTTGTTCCTGAACCGAT	TGCGGAGTTTTCCTCCACGGTAA
1529.	TF	AT1G75520	AAGACCATAGTGGTGGTGGTGGAT	TGTTGACTCACCTCCAACCCTGAA
1530.	TF	AT1G66560	AACCTCACCGTACCAAAGGTGTT	GGATCATCTGCACCCGCTTTCTA
1531.	TF	AT2G18120	CACTTTTCCGATGCGTCAGA	TGGTATGCGTATTGGCCCTC
1532.	TF	AT1G66600	CCAAAGGTGTTACTATCGTTGTGC	TCTGTAAACTGGAGGACTGTCTTG
1533.	TF	AT2G21400	ACCACGGTGAACATTGGAGG	TGTGGAGTCCTTGATCGTGAAG
1534.	TF	AT1G68150	CCGCAACAATGAATGATGGATGCC	TGGATTCCCTTTCGCGGTTTTCTG
1535.	TF	AT3G51060	TCGCATACCTTCTCATTCAGGGCT	CACCTAACACCGCCGATGAACT
1536.	TF	AT1G69310	ACCGGAGACACCAGTGAAGGAGAA	TGAATGCGAATCTTGGTTGCCGAA
1537.	TF	AT3G54430	CGACAACCACTTCTCGTCAAGA	GCTTCAATTTTCCCCGGTAAC
1538.	TF	AT1G69810	TCAATAAACGACGGTTGCCAGTGG	TGGAGCAACGGTAATAGGCTCGTG
1539.	TF	AT4G36260	TCAGCAGGGTTAGAAATGGGGGAG	CACACCGAAAAAGCGCATCTGAG
1540.	TF	AT1G80590	AACCTCTCCGCACCAAAGGTGGTA	CGCTTGGTAGCGTCGCAGTTTT
1541.	TF	AT5G12330	ACAAGACGGAGGAGGGTCAA	TTAAAGACATGGCCGCCGAT
1542.	TF	AT1G80840	TGCGAGTTGAAGAAGATCCACCGA	TCCGAGAGCTTCTTGTTCTCAGCA
1543.	TF	AT5G33210	GCCTCAAACTCAGCTGCCTC	CGGTAAATTCTCACGGTGGC
1544.	TF	AT2G03340	AGGAGGTTGGGAATGCAGAGACT	TCTTCGCTTGGGATCAGGCTCATC
1545.	TF	AT5G66350	CACTTCAGGGTTGGAGGTTGGGAA	AACTCACACGCACGCACCGAAA
1546.	TF	AT2G04880	GTCCTACCGCAAAGCGAAGGAAGA	ACCTCTCCACTGGACTCAGCTCAA
1547.	TF	AT1G05690	ACCTTCGAGCTTAATGCGCCTT	TGCAGCCAGGACACTTGAATGG
1548.	TF	AT2G21900	ACGGGTAGTCCATTTCCAAGG	TCTTCACGTTGCAATCTGGG
1549.	TF	AT1G25580	GAGGTTTCTCTTGCCGAGACATCC	GCTCAGGCCCAGAACTTGGTCTTT
1550.	TF	AT5G65310	TCGCCATTCTACAACAGACAAACA	CGTCGTCTTCTAATGCGTCAAACA
1551.	TF	AT1G26870	ATGGCGGCTGATCCTTCGGAAATG	TCCGGGCAACAATACATCCTCCA
1552.	TF	AT5G66300	TCTCCTCCACAGGAAGAAGGATGG	TTGTCGAGAGGTTCCAAGAGAGTG
1553.	TF	AT1G28470	TGGAGTAAGCAAGGACGGGCAA	TTCGTTTTCGTGTTCCGGTCGTAT
1554.	TF	AT5G66700	GTACGCCAAACTCAAAAACCACCA	TTCAAGTCGTTCCGACAGTTTTCG
1555.	TF	AT1G32510	TTTGGTGGTAGAACCGAGTGGGTA	ACAGCGCGAAGTCTCCCTTGAA
1556.	TF	AT5G39690	GAGGATATTGGCACGCAACA	GCCAACGACACCATCTCCA
1557.	TF	AT1G32770	GTGCCTCCAGGTTTCAGATTCC	TCCTCTTGAATATCCCAAGGCTCA

No.	Gene group	AGI	Sequence of forward primer	Sequence of reverse primer
1558.	TF	AT5G50820	GGTGGAGGATGAGCACGAGT	GTCGTCACCGGCTTTGAATAA
1559.	TF	AT1G32870	TGCAGGAGCTAAACAATGACGGAC	CAGATGAATCACCTGTACCAGGCG
1560.	TF	AT1G18790	TTTGAAGAAGAGATGCCGCGAAT	CCCTTCCATCTTCTCTAGCTCCTT
1561.	TF	AT1G33060	TGCCATGTCCAAGGTCGTAGAGGA	TGAGCAGTCCCCTGAGCACTTAAA
1562.	TF	AT1G18790	TTCAAGGCTAACCATAAGAGGAAGAG	ATGATGATGTGATGGGCGTG
1563.	TF	AT1G33280	GGATCATGCACGAGTACCGTATCG	ACCCATCCATCTTCACAAGGGTCA
1564.	TF	AT1G20640	TTGCAGAGCACTTCAGGCAGTTGA	AGAGAAGGTGGAATCGGAAGCTCG
1565.	TF	AT1G34180	TCAATGAAATCCCAGATGCACCCG	CCCTGTGGAACACCAACAAACTCA
1566.	TF	AT1G64530	GCGTGCTTCTTGCAAAGTGAATCC	AATCGTCCAAGGCCACTTGTCTG
1567.	TF	AT1G34190	TTTGCTTCACCCTCATCAGGTGTG	TGGATTTCATGGCCTTGGGCATTC
1568.	TF	AT1G74480	TGATCAGTAACGTCAAGGAGCTGC	TCCTTCTCAAGCATCTCCAACGC
1569.	TF	AT1G52880	CTTCCCGCGAAAGCTTCGTTT	AATAACCGGAAGTCGCAGCTCG
1570.	TF	AT1G76350	ACCGCCTTCTCTAAAGGGACCAGA	TGCGTCTCGCAAGCACATCT
1571.	TF	AT1G52890	TCGTAGAAACGGAAGCACTAAGTT	CGTTGTTGCTGAATTCTCTAGCAT
1572.	TF	AT2G17150	GCTTCGACTGGTGGGGGAAATATG	AACACCAATGCTCTTGGCTGC
1573.	TF	AT4G37610	TGTCATCGGAATGGCTTCAGATGT	GAATGAAAACTCGAAGAGCGTGGT
1574.	TF	AT2G23320	AAGGATCTCCACATCCAAGAGGA	ATCAGCGGCGGAGAGAGAAT
1575.	TF	AT5G63160	CGTGTCCCTCTTTGCAGGCAATAT	ACGCTACTCTTCTCACCAGAACCT
1576.	TF	AT2G24570	TGCCATTGCAAGAAAAGCCGAAAA	TTTGCACTTACCGCCGGTACTCTC
1577.	TF	AT5G67480	ATCCTTGGAACTGCTTCCACTGTG	CATCATGAGGGACACCTCGGATTG
1578.	TF	AT2G25000	TTGGTGCAACAAATGGCTTC	TCGCAAGAGCTGCAGTGAAC
1579.	TF	AT1G30210	TCTCTCGCCAATCTTGGAGTGGAA	CATATACATGCTCAGGGCTGCACA
1580.	TF	AT2G30250	TGCTTCTCAGCTCCTCACACAGTT	AGCCTTGCAATGGAAACGTTCCT
1581.	TF	AT1G35560	GGTCGGTTCAGCTAGGGTCTATGT	CGCCGGAAAATAGCCCCAAATTGT
1582.	TF	AT2G30590	GCCACTGCGCTAAGAAGAGGAAAC	GCCCTTGATGGGCTTCTGACCATA
1583.	TF	AT1G53230	CTGCTTCATCCGATTCTCGCCATT	GCATTCGAAGCGCCCTGGAATATG
1584.	TF	AT2G34830	AGAGAAGGAAGAGCCAGGCAAAGA	TCCACTGGACCGGCTGTTCATA
1585.	TF	AT1G58100	TTCCCAGATCTACACCTCCCGAAG	CCGTTGCCGTAGATGAGGTTGTTG
1586.	TF	AT2G37260	TGCTTCAGGGGAATCGTCAACAAA	TTGGCTCACACTCTTCCCATAGGA
1587.	TF	AT1G67260	TTCAAACGGCACAAGGGATTA	TGGCGAGCAATCCCAATAG
1588.	TF	AT2G38470	AGCAAAGAGATGGAAAGGGGACAA	GCACTACGATTCTCGGCTCTCTCA
1589.	TF	AT1G68800	CAAGGCGAGCAAGACGATTGAATG	ATCCTTTCCTCCTCCTTCCGATGC
1590.	TF	AT2G40740	CGGAGAAGGAAGGACGAAGGAGAA	TGGTTATCGTCGGGTGGCAGAT
1591.	TF	AT1G69690	TTTCTGGACAGCCTTTGGCTTCTG	GCAGCATTCAACGCCGCTAAAACT
1592.	TF	AT2G40750	TGCTACACTAGAAAGACGAGATCA	TTCCTCCAAGCATATCTGTCTTCA
1593.	TF	AT1G72010	TGCACCAATGGGGTCAATGATGTT	TGTTGCCCTCTGCCACTCCTAATC
1594.	TF	AT2G44745	AAGAACAGCCTTCACCCCAGGA	TCTTCCGATAGTCGCTCCACTCTC
1595.	TF	AT2G31070	CAACACAGTCGTAGTTCCCGAGAC	TCCATCCAGAGATTCTCCCGAACG
1596.	TF	AT2G46130	TCAAGAACAGCTTGTATCCCAGGA	GTTGTCTCCACAATGCTCGTCTC
1597.	TF	AT1G54330	GGCTCCTCATGGCCTTCGTACTAA	GGCACAATGCATACGACTCCTTCA
1598.	TF	AT2G43500	CAACGCTGATCGTAAAGGCC	TGATGGCTCGAACTTGAAACG
1599.	TF	AT1G56010	AGCTCTCCAAAGGAAGACTGGGT	TGCAGAGGCTGTCTCATCAAAACA
1600.	TF	AT2G43500	GGAGCAGGATGTGAGCAAAGCAAG	CCCAGAGAAGTGTTGTTGGAGAGC
1601.	TF	AT1G60240	GGCCCAGCACATTCGAGGTTACTA	ACGGGTTCAATAACGTGACACCAA
1602.	TF	AT3G59580	GCAGGCCGTGAACTTACAAACATC	TGCATAGCACACTGCTCTGAGAAC
1603.	TF	AT1G60280	TCTCTCCACTAGATCACCGCTTCC	CCCTCCGCTTCTGAATCGAAGAAA
1604.	TF	AT4G24020	GAGTTTGCCCGACGACAATGAAG	GGCCTCCATCAGTACCTTGAACAG
1605.	TF	AT1G60300	CTGGACGCGATAATGACTTCGGAA	AGATCCACAAGCTCCAATGGATGC
1606.	TF	AT4G35270	TCCACCTCGGTTACCGGAGAATAG	GTTTGGCTTCACCAAATGTGGCTT
1607.	TF	AT1G60340	AAGGAAGAAACAGAGAGGTTAGGT	GACAATCTCCTCCGGTTGCT
1608.	TF	AT4G35590	CATCCACGATCTTCAGAGGGAAGC	CTTAGCTACCGCCATTGCTGCT
1609.	TF	AT1G60350	CGTATGGTCGTCAACGGAGACTCA	GCTCTCAGCATCGAACTCCACATT
1610.	TF	AT4G38340	ACCTCATCCCTACCCCCACT	TCATCCCGTCCTTTGCTTTC
1611.	TF	AT1G60380	CTTCGCTAACAGGACTTGTGGTGT	CATGATCAGCTTCTCACGGTGCTT
1612.	TF	AT5G53040	AAGTTGCCTCCGCTTGATTCTCTC	AGTGCAACCCTGCATTTGGTTTCA
1613.	TF	AT1G61110	CCAAGCAAGGCGAGTTTTGGAGAG	GGTTTGGCCTAACCCCATTTGGA
1614.	TF	AT5G53040	ACTCAGTGATGGAACCAAGAAGCTA	TCTTCTCTTATAATTGGCTTTGAAACAA
1615.	TF	AT1G62700	TGGAACCCCTCAGGAAGAAGGATG	TTTCCTCACTGTCGCTGCCAATT
1616.	TF	AT5G66990	TCCCGATTTGGAATTTGGG	TTTAGCCTTGAAGCAAGCCTGT
1617.	TF	AT1G64105	TGTTGTGGAATTGGCAGAGG	TGTTTGTCGAGGAAGCAGCC
1618.	TF	AT5G66990	TGACCTGGTTAGAGGCCAACAACT	TGAAAGAGCTGTGAGGATCGACGA
1619.	TF	AT1G65910	TGGGAAGTCCTTGCTGCCAAGTA	TGGTCGCCCGGTTTGTTCTTGA
1620.	TF	AT4G27330	CAAATCATACGGGACCAATGG	CCATTTCTAGGGTTTCCTTCCA
1621.	TF	AT2G37000	ACGGCGGAATCTGAGGTTGAGA	CTAATGGTGACGGCGTCTACGTTC
1622.	TF	AT2G46400	TAGAGCATATTACAGATGCACGCA	TGATTTCTGAACTTGCTTCACTGC

No.	Gene group	AGI	Sequence of forward primer	Sequence of reverse primer
1623.	TF	AT2G45680	GGTCCCCAAATGGTTAGAGCTACG	GTGGTGAGCCCCCACCAAAAAA
1624.	TF	AT2G47260	TTCCCAGGAGTTACTACCGTTGC	CAGTGGAAATAGGACGAGACGTGA
1625.	TF	AT3G02150	CCGAGGCAAATGTAGCAACAATGG	CGGTTGAATTGGACCGACGACATC
1626.	TF	AT3G01080	ACTCATCTTCTCACCTCGCCCAT	AAAGCCGGTATGAGCTGACGACT
1627.	TF	AT3G15030	TCAGGGCTTGTCTAGCTTCCATGC	GGCCATTGACTACACAAACCGAGA
1628.	TF	AT3G01970	GAACAATCCATTCCCCAGGAGCTA	GTATGTCGTCACCACCACTCCTT
1629.	TF	AT3G18550	GGCAGCAATACTAACACGACCGAA	TCTGGCCTCTTTCTCTTCCCTCTC
1630.	TF	AT3G04670	TTCACCGCATCCACGGGGATACTA	TTCCTTGCTGGACAACCTCTCACA
1631.	TF	AT3G27010	ACCTCTGCAAAAACACAAGGTGG	GCCTCTCTGCAACTTAAAGACTCC
1632.	TF	AT3G56400	GTTTGAAGATTCCGGCGATAGTC	ACACGTCTCCGATCTCTTTTTTCT
1633.	TF	AT3G45150	GCCGAGTGTTGCTCCCCAACTATT	CATTCTGGAGGAGCCAACTGACAG
1634.	TF	AT3G58710	CCAACTCCAAAGAAAAGTAGGAGG	CCATGAATCGGACGGTGGATATA
1635.	TF	AT3G47620	TTTCCGGCGACTCTCAAGCTAGTG	TCTTGCTGATCCTCCTCATCACCA
1636.	TF	AT3G62340	GCAGCATAAGACAAAGAAGCGGTT	CCTCACTCCTCGTGATGAATGACA
1637.	TF	AT4G18390	GGGGACCCTTCAGTCCAATTCAAC	AAGAACTGTGGACCTCCTCCACTT
1638.	TF	AT4G01250	AAAGGTTCACCATATCCAAGAGGA	TCTATTTCGCTCCACTTGTTTACG
1639.	TF	AT5G08070	GGCGGCGGAAACTATGAGTTCT	AGTTGAAGTTGACCACCACCGAGA
1640.	TF	AT4G01720	TGCCACTACGGTAAATGACGGATG	TGGTGCAACGATAATAAGCGCGA
1641.	TF	AT5G08330	GGCTTCAGCAGCTAGAGTTGGGAA	ACCCGTTAGCTCCACCAGACAAAG
1642.	TF	AT4G04450	TCACCTAGCTACGACCGAGAACAA	TCAGAATGCGGTCCCAAATCGATG
1643.	TF	AT5G23280	CAACAAGCTGCAATGGGTGAAGC	CCGGGAGATCCACCGGATAAAGAA
1644.	TF	AT4G11070	AGAGGAAGATGTTGCCAAAGTGGA	CCTTCTAAGCCTCTCTCTGGGCTA
1645.	TF	AT1G69490	CGAAACGTAACGGTTCCATGAGGT	TTCGTCCATGAAACCCTCTTGCT
1646.	TF	AT5G61850	AAATGCCCCACCAAGGTGACGAAC	ACTCGCTCCTGATTTCTTCGCGTA
1647.	TF	AT1G71930	TCCGACAGAGGAAGAGCTTGTG	ACCCTAGTTTACACCTCGCTTGT
1648.	TF	AT5G35770	GGAGAAGTTGACGCCATTGTTGC	ACTCTGAGCCGTTGATGAAGCTGA
1649.	TF	AT1G76420	TGGTCTCTCCGGCATTCACATTTC	TCTGGTAGCTCCCAAGGTTCACAG
1650.	TF	AT1G14410	CGAGAAGCAGAGGTTCGGTG	AGCAGGCAATCCTTCAGCAG
1651.	TF	AT1G77450	CCGACATGGCTTTGTACGGTGAAA	AGCTGCACGGTTGGGTCTTGAA
1652.	TF	AT1G71260	AGCCCCACGCAGATGGTAG	TTGAGGATGCTATTGTTAACGCTC
1653.	TF	AT1G79580	AAGAGAAATGCAGAATCGGGTCGG	TTCGTTCGGGTCCCAGTCGGATAT
1654.	TF	AT2G02740	TGCAGCTGGTGTTCGTCAAT	TGTAACCGACAACGAGAAGACCT
1655.	TF	AT2G02450	TCGCCGAAAAGTTGAAGGCAAAC	TATCGCCGCCATAGCAGGAAGTTC
1656.	TF	AT1G05380	TGCGATTGAATCGGCTATGCGTT	AGTCTGGAATGGCAGGGATAACCA
1657.	TF	AT2G17040	GCTCCTTACCAAAGGATGTTGTGC	TCATCTTAGCTTCCATCTCTGCCC
1658.	TF	AT2G36720	AAACTTGCACCTGAACAGCTCAGT	TGCAGCATGGATGCTCCTTTGAAC
1659.	TF	AT2G18060	CCCAGGAAGAAGGATGGGTGGTTT	TCTTGGCTTGCCCTGTAGCTCT
1660.	TF	AT3G14980	CGTGCAGCAAATATCGCCGACA	CATCTCTTCAATGGCAGCCACGA
1661.	TF	AT2G24430	ACCTCCAAGCAAGACGAGTGGGTA	TGATGACTTGTGCTGCTGCTACTG
1662.	TF	AT3G53680	TCAGGGAAGGGGCTACTTTCAAGG	CTGCTGGGAGAAGCAAGTTCTCAA
1663.	TF	AT2G27300	TGGGACTTGCCAGAGGAATCGAAA	CGCGCAGAAGTAAAACCACTCGTT
1664.	TF	AT4G14920	TGCCTAGACATACGGGGTCATCTG	TTGGGGCAATGCCAATCACCA
1665.	TF	AT2G33480	TTCTGTAATACCGGAAACCGATGT	TTCACAATCACCTGGTAAATCCCA
1666.	TF	AT5G12400	GTTGTGCCTTTGAGTGAAGCTGC	TGTACATGCCTTCCTTCTCTCCCC
1667.	TF	AT2G43000	TCTCCAGCTCAACAAGCAGAGGTA	CCGGTTTTCGGTTTGGTGGTAAGA
1668.	TF	AT5G22260	TCGTGGTGGAGTCAGTGGTG	AAGGCCATTTCATCGACCCT
1669.	TF	AT5G41030	TTCTCAACCTCCTCTCACGGCTGA	TCTCCACAACCCATTTGCCTCCA
1670.	TF	AT4G12020	GCAGACGGGGACTTCTTTGCTTT	CCCCCCTCGACTTGTTGTATCCAT
1671.	TF	AT5G51910	AATACACAGGGGTTTGTGCCGTT	GGAATAGCCCACATTTGGCCCATT
1672.	TF	AT4G18170	TCGTTCCAAGATCCAACGGT	GAATCGGGTGGTTGTGTTGAC
1673.	TF	AT5G60970	TTTTCGGTCCTACTCCTCCGGCAA	CGGCTCCATCGACGACATGATGAT
1674.	TF	AT4G22070	TTATTATCGTTGCACAATGGCC	TGCGCAACGCTGCACTT
1675.	TF	AT1G13450	GAGAGAGACTCCTGGAAACGGTGA	ATGACCCTACCACCAAAAGGCTGA
1676.	TF	AT4G23550	AAGGATCTCCATACCCAAGGAGT	TCGACTTGTTTTCTTGCCAAACAC
1677.	TF	AT1G21200	GTTGCATTTGCCTCATGACCTTGC	CATCTCTGCTTCTAAGCGCCAACT
1678.	TF	AT4G23810	TTTGCCGATGGAGGAGGTTCTAGC	GCCTCTCTCTGGGCTTATTCTCAC
1679.	TF	AT1G23540	AGTTTGGTTGAATGGGCGC	TCGCTTAAATCTCCGGTCTCA
1680.	TF	AT4G24240	CGGGGATATTACAAGTGCAGCAGT	ACGTCACGATTAGCATCATCGCA
1681.	TF	AT1G31310	TGCTCAGCCAATACTGCCCACAGA	TCTCCTTCTCTTCGCCGGAGATGT
1682.	TF	AT4G26440	CCTTTGCTATCTCCAACAACCGGG	GCGATGTTAGTAGGATCAAGGCCA
1683.	TF	AT1G33240	CCACAAGGAACAGAAAAGCCAGAA	TGCTGTTGTTGTTGAATCAGCTCT
1684.	TF	AT4G26640	GATCAGCCAGAACCTTCCCCTACT	TGGAACCCCCTGCCTGTATAAGAA
1685.	TF	AT1G54060	GGCGAGGGCGATACTTGGATTTAC	CCTCTCCTTTTCCAGTTCCGCCAT
1686.	TF	AT4G30935	GGGCAGAAAATGGTGAAAGG	CCGCTGAAGTGCATCGGTA
1687.	TF	AT1G76880	TCTAGGAAAATGGCGGAGCATGG	GACCTTCTTTGGTTCGTTTGTGGT

No.	Gene group	AGI	Sequence of forward primer	Sequence of reverse primer
1688.	TF	AT4G31550	AGTGCCATTGCAAGAAAAGCAGGA	TCCGGTGGAATATCGGCGATCT
1689.	TF	AT1G76890	TCCAGGAAAATGATGGAGCTTGGT	CACGTTCTCGAACTTCTCCTTGC
1690.	TF	AT4G31800	AGAAGGTACAACGCAGCGCAGA	TGCGTCCCTTCGTATGTCGCTACA
1691.	TF	AT2G33550	GCTTGTGTAGCAGATCAAGGTAGA	TTGTGATGTCGATCCACCTTCC
1692.	TF	AT4G39410	CATGGGCATGGAGTTGTCAAGC	ACCACCATCGCCAACCTCATCA
1693.	TF	AT2G46770	TCGAGCCTTGGGACATTCAAGAGA	ACCAGTCGTTTTGTGGCGTTGTT
1694.	TF	AT5G35210	ACGTACTAATCTGCCGGAACACCT	TCCTCCAAGTTCCAACCTCGGTCA
1695.	TF	AT3G01600	CCCTCAAAACCTTCCTGGTGCAAA	TTCTGCGTTTCCTCTGTCCTGTTC
1696.	TF	AT5G36670	TGTCTGGCAATTCTGCTGCTATCC	TGCGAAGCCAAAGCCTGAGAAAAA
1697.	TF	AT3G03200	TGGGACTTACCAGACCAAGAATGG	TCCAACTAACTCGGCGGTCTTTAC
1698.	TF	AT5G58610	GCAAATTGTCTTGGGCTGGAGGA	TGTCCACATGCACCACAACAACAT
1699.	TF	AT3G04060	ACCAAAGATTGCAAAGATGGGGGA	TCCAATAACCAGACACGGTCGC
1700.	TF	AT5G63900	ACTGGTCTTACCGGCTGCAAAAG	AGCCGCTCCGAACTGTTCATTAC
1701.	TF	AT3G04070	ACCAGCTAAGGCTCCATTTGGGGA	GCTCTGTTTGGTCTTGCTCCGTTT
1702.	TF	AT1G21000	GAAATGCCTTTTGCTCTTACTGTTT	CGTATCTGAACAACACGATGGTCT
1703.	TF	AT3G04410	GTTTCAGATTCCGTCCGACCAA	GATATTCCTATCGACATGGCTCGT
1704.	TF	AT1G31040	TGGACGGACATGATCAGCTC	ACCAGTATATCCTCCCCATCCTC
1705.	TF	AT3G04420	GAGGTGAGCCAAGCTCTGATAACC	ATTTTTGCCCTCCAGCCCCAGT
1706.	TF	AT1G32700	TCGAAGCCTCGTTGATTCCT	CGATATTCCAGAGATCTTGCAACC
1707.	TF	AT3G04430	TTTACCTGGGCTGGCGAAGATAGA	TCCACCGGCGATATGAAGTACCAA
1708.	TF	AT1G43000	TTTGTTTGGATTGTTCAGGCAA	TGGGTTCTGTGATGAGCGAG
1709.	TF	AT3G10480	TGGAAACAACCAGATGGACCAGGA	GAGTAGCATCTCTCGGTAGCTCGT
1710.	TF	AT1G76590	ATCGTGTCCTCCAGATACGGA	TGAATCTCGTTTACTCTCACAACGTT
1711.	TF	AT3G10490	ACGGAAACCTGGTGCAAGATGC	TTCCCACTTGGTGGCCCAATGT
1712.	TF	AT2G27930	CCTCCCATGAACCCTTCTTG	AAAACGAACCTTGCAACCCA
1713.	TF	AT3G10500	CCAGACAAGTCGAGGCTGAAAAGT	AGTCGCACGATTCGTCTTTGATCC
1714.	TF	AT3G60670	GTCTCTCCTGCAAGATTAGTGACG	CGGAGAAACCCTGAGAGTCCT
1715.	TF	AT3G15170	TCCTCCTCCGCTAAGGATGAATGG	TCGTCTCTCTACTAACTACGCCGC
1716.	TF	AT4G17900	GAGTTTCAGCCCATCAACACC	TCGCTTGACGATTCTGCAGT
1717.	TF	AT2G35640	ACCGCCCACAGTAGGTACATCATC	TCACAGACGTGCATCTAGACAACG
1718.	TF	AT5G01900	CCACTTCACAAGAGGGGAAGAAAG	TGGTAGATCGGGGTTGAAGATTCG
1719.	TF	AT2G38250	TGAACAGTGCAAGTGCAAGTGGAA	TGCCTCCATTGTCTCACATCCCTT
1720.	TF	AT5G07100	TTCACTATGCCTCCTGGCCTTACT	GGAGACGGCAAAATGTTGGAGGAA
1721.	TF	AT2G44730	AGCTAGTGCGATAAAGCTGCTTGG	TCGTCATCTCCATTCTCGTCTGCT
1722.	TF	AT5G13080	CCAAAAGGCCGTCAAGAACAACAA	TGCTTCTTCACATTGCATCCTCCA
1723.	TF	AT3G01560	CAATGTACGATGGAGCGGGT	TCAGACAAGTAGCCGGACGG
1724.	TF	AT5G15130	CAGGTCCAAAGATGTGCAGATGAC	ATGGTGGTGGCTGAGAGTGGAA
1725.	TF	AT3G10000	TCCCAAAATCCCCTTTCCTTGAAC	CGACCGGATTTGCCTTCTTTTGTT
1726.	TF	AT5G22570	GGGGAGCCCCTCCAAGAAAAGAAA	TCCGGTGAATCGTCCCTCCAATT
1727.	TF	AT3G10040	TTCCGATTCCGAGTCAGCAGCAGA	TCTCCGCAATTCTCGCCATTTTCC
1728.	TF	AT5G24110	TCGGAGCCAAATTTCCAAGAGGAT	CCTCGGTAACTGATCTCAAGGAGC
1729.	TF	AT3G11100	AACGAAGAGCTGCTTCAGGTAAGA	TCCAACCATTGCTCCTTGCTTCA
1730.	TF	AT5G26170	GCTTCTGCCGACAACCAAA	TGAACGCAACTCTCCCTTTAATTT
1731.	TF	AT3G14180	ACTGCAACAGGTGGTTGAGATGGA	GCATTCTCTGCAACTCAAGCTCCT
1732.	TF	AT5G28650	GGAGGAAATACGGACAAAAGCCGA	ACCGCGCACACTGCTACATTTAT
1733.	TF	AT3G19020	GACAACGACGACGGCGATA	GCGATGCATATTGGTGTCCA
1734.	TF	AT5G41570	GCACAATGCTCATCCCAGGAGCTA	CGACGACGTTTGGATCTTTTGCCA
1735.	TF	AT3G24490	GGTGACAGCGAAGATGAAACAGGA	CGCGTGACGCTTAGGTATTGGTCT
1736.	TF	AT5G43290	GCCCTAACCCAAGGAGTTATTACA	TGGAAGTGGAAACCTTCGTAGGTA
1737.	TF	AT3G24860	GAAACAACCACCCTCTCCTCCTCA	CGGAGGTTGATGCGGCTAAAGCTA
1738.	TF	AT5G45050	TCGTTCAAAGAGTCGCCGAAAGAA	CGATCCACTACGCAAACCACTCTC
1739.	TF	AT3G25990	TTGAAGCTAATGGCAGGCCAACGC	TGTGATGGGATCAGCAGCAATGGG
1740.	TF	AT5G45270	GGACTTGGCGAAAGTACGGTCAAA	CGCACCTGTAGTAACCCCTTGGAA
1741.	TF	AT3G15500	CGTCGAAATGGAAGCACCAAGC	GCTGTATTCACGACCACTCGTCAT
1742.	TF	AT5G46710	GCCTTGCCGAAAACTGCTT	CCAGATGTTCCTGCAACCTTG
1743.	TF	AT3G15510	TCCCGCTAAAGCATCGTTTGGAGA	AAGTCGCCGCTCTGTTTGGTCTTG
1744.	TF	AT1G02580	AGGCGAGTGGTTAATGGAGAAGGA	TAGTTCGGGTGGCAAACCCTCA
1745.	TF	AT3G17730	TGGGACCTCGCAGAGAAGTCGTTT	AACCCGTTCGGATACTTCCGGTCA
1746.	TF	AT2G35670	CGCCTGAGACTTGAACGTCTTGT	CATCCGCGATCACCCTTTGTTTTC
1747.	TF	AT3G18400	CCCCACGAATAAGGAGGAATGGGT	TTCTTGTGCTTTCTTTGCTGCCG
1748.	TF	AT4G02020	TAAACCCAATTGCTACGCTAAGGT	AAAAATCCCGACCCTGTGATC
1749.	TF	AT3G29035	TGGGACTTGCCTTGGAAGGCTAA	ACCAGTCGGGTATTTTCGGTCTCT
1750.	TF	AT4G16845	TTCGCTCTCTAGGCAACCCATCGT	ACTTTCTTTTGCGCTTTGCCCCAA
1751.	TF	AT3G44290	CGCCAAAAGGAGGAAGAACGGAAT	GTCGGCAAATAACCAGAGCATCCA
1752.	TF	AT5G51230	AGGAACCGAAGCCTCCTTCAGAA	TCTTCACTATCCCGGTCCGAAAGT

No.	Gene group	AGI	Sequence of forward primer	Sequence of reverse primer
1753.	TF	AT3G44350	CGTTGAGCCTACTCAGCTTCCAAA	TTCACGCTCTTGTCTTGGCACA
1754.	TF	AT3G27700	AGGTACTGTTGTCAAGAGGGAGGA	CCGAATCAGAGGCTGTGTCTAGTT
1755.	TF	AT4G01540	CGATTCGCATACCGTCATAGCGAT	TGGCTGCTCAACCTGAAGCTCA
1756.	TF	AT3G47120	GATCTTCTCGCCGTCTTCTCTCAA	TTTACCCGTTCCCTTGTCTCGAAT
1757.	TF	AT4G17980	TCCTTGGGAGTTGCCAGGTAAATC	GCCCTATTGGTTCGAGAACCGTTT
1758.	TF	AT3G51950	TCAAAGAGCTGCTGCTGCTTTGAT	GCTGGACAAGCCATAGCAGAAAGA
1759.	TF	AT4G27410	GCTCCAAGTTGGATGATTGGGTGT	TGACGACCCATTCGTGCTATGC
1760.	TF	AT2G37120	GTTCCGCCGGAAAGGC	AGCAGCACTATCAATCCAGGGTT
1761.	TF	AT4G28500	AATTGCCAGGAGTGAACAAGGACG	CCTTCGACGGTCGGTGGAAGAAAT
1762.	TF	AT3G53370	CTGCTGAAGCCAAGGGATTG	AGCGGACCTCCAACAACAAG
1763.	TF	AT4G28530	CTTGGATCATGCACGAGTTCCGT	TCTGCACAAGACCCAGTCTTCCTT
1764.	TF	AT1G02065	CCAGCAATGCAGCAGGTTCCATTT	ATGGTCAGCAAGTCGCTTACGG
1765.	TF	AT3G54390	GCTCATCCGCCGCAGATTTCTTAC	TCCGGCTTGAATCCGTCTTCCTTA
1766.	TF	AT5G46350	ACAGTCCTTATCCGAGGAGTTACT	TGTGTTGACTCTCGTAGGTTGTG
1767.	TF	AT3G58630	CAACTGTTCACGGAGATGCAGGT	TCCCATAATCAAGAGCGGCGGA
1768.	TF	AT5G49520	CCAGAAGCTATTACCGTTGCACCA	TGGGGAAAGGATGGGTATGCTGAC
1769.	TF	AT4G17050	TGGTATCCAGTTCAGGCTGGTGAT	GTCTTTCCGAGTGCAGCATACCAT
1770.	TF	AT5G52830	CAAAGGCTCTCCTTATCCAAGGAA	TGTTCTCCGGTGTAAGTAACGATG
1771.	TF	AT4G31270	ATCGAGCTTTTTGAAGCCATCA	GCTAATTCAGCAGAGAGATCAACA
1772.	TF	AT5G56270	AGCCCTGCAACACTCTTGGAATC	AGTTGGAGAAGGTTGAGCCAATGG
1773.	TF	AT5G01380	ACCTCGTCACTAGATACAAGGCGT	ACTGCTGCCTAATAGCATCTGGCT
1774.	TF	AT5G64810	CTGTCAAAAACAACATTAACAAGAGGA	ACCGAGCAACCTTCACTTGAG
1775.	TF	AT5G03680	GGCCGAGACAAGAAACCCTAACTC	CCTTTGGTATCCATGTTCCTCGGA
1776.	TF	AT1G14440	ATCGCTACCAAACCTGCCAT	TTCCGGCGTGAACTTTGTC
1777.	TF	AT5G05550	TGAGTTGGAGAAGCAGAGAATGGA	TGCTTACCTGAAGCACTTGCG
1778.	TF	AT1G14687	CGCAGCTACCACCGTCGTAT	GGAAGCGCGTGTGATTGATT
1779.	TF	AT5G14540	ATGATTTCCAACCTATGCGCCCTA	GCCATAACTTCTTGCGGAAGAAGC
1780.	TF	AT1G69600	GTCACCGGAGAAATGGGTGT	CGAGATTAGCCGCGTGGTT
1781.	TF	AT5G28300	TGGGAGCATACTTCAAGAAAGTTG	TGCATTCTTGTGGACTCCTCTTAA
1782.	TF	AT1G74660	GACGGTTGCCGTGAGTTCAT	CGCATCTCAAAGCATCAACG
1783.	TF	AT5G38560	TTGTTGAATGGGCAAGACCC	AGCTCGTCGAATTCTTCGTTCT
1784.	TF	AT1G75240	TCGAAGCTCTCAGATGCGCT	TCCATTTCTTTCCGGTGGAA
1785.	TF	AT5G47660	TGGTCTCTCTCTCAGCTCCTCTGT	TTTCCCGCTTTCTTTTACCCGTGA
1786.	TF	AT2G02540	AGGAAGAAGGCGGAGGAAGCTTGA	CTATACGGTGATGGTGGTGGTGGT
1787.	TF	AT5G63430	GCTGAAGGAATCAATCGAAGCCCG	TCTCTCATCAGCCTTGCTCTCCTC
1788.	TF	AT2G18350	GAAGGCACGGTGGAGTCTCTT	TTCTATGGAAGCTCCGGTGG
1789.	TF	AT4G35580	TGGGAACCTTGGGATCTTCCTGCT	TAGGGTATTTCCGATCACGAGGGC
1790.	TF	AT1G20980	AACTCTGATGCTCAGGACCGCACT	TCCCAGGGAGCTGACTTGGATCTT
1791.	TF	AT4G36160	CAAGAGAGCTGCCGAATCGGATAT	CGCTCTGTTTGTTCTTGTTCCTGT
1792.	TF	AT1G27360	ACTTCATGGCGAAGATGTGGGAGA	CAGAAGAGAGAGAGCACGGTGGAT
1793.	TF	AT5G04400	TTCCGCAAAACAATACAGGGAGCA	CGTCATCTGGAACATGGATCGCAA
1794.	TF	AT1G27370	TTCTGCCAACAGTGTAGCAGGTTC	TGCGGCAGCTTCGTTTCTTTTC
1795.	TF	AT5G04410	TGACCAGAAACCTGCCCCTAAAGA	CGCCACTTTCCTTTTCCTCCACTG
1796.	TF	AT1G53160	TCAGGACTTAACCAACGCTTTTG	CAAACTCTTGGAGGTCATGAAACC
1797.	TF	AT5G07680	ACAACTTGCCTAAAACCGCTAAGA	GGTAAACTAGACCCGGTTCCGTAA
1798.	TF	AT1G69170	ATTTCCTACCGGGTAAGCGC	TTGTTGCCTACTACATCTTGAGAGGT
1799.	TF	AT5G08790	AGCAGATCTCGGCTCCGGTTAT	AGACATCTCTGGAAGCTCCCAAGG
1800.	TF	AT2G33810	ACAATGCAGCAGGTTTCACG	TCTCCTGCAACTCCGCTTG
1801.	TF	AT5G09330	TGTGCAATTCTGAGGTTGTGGGAA	TCCTGAGGAACATTCATCTGCGTC
1802.	TF	AT2G42200	TGCAGCAGGTTTCATCAGCTTCCG	TGTGGCTTCCTTCGTCGCTCATTA
1803.	TF	AT5G13180	CGAGTTCTATGGGTCCCACTCAGA	CGTCGTTCTTGTTACCGGCTCTTT
1804.	TF	AT2G47070	GATCCTGCAATGGTGGGGATTGAA	TGTATGAGAAGTGACCGCGTAAGC
1805.	TF	AT5G14000	CGACCATTTTACGGTTGGGT	GGTAGGAGGAGAAGGCGGTG
1806.	TF	AT3G15270	AAGGCATCTGCTGCGACTGTT	GGTAGCTCATGAAACCTGCTGCAT
1807.	TF	AT5G14490	CACCCTCAAAACCTTCCAGGTGTG	TTCTCCGCTTTCTCTGCCCATTTT
1808.	TF	AT3G57920	TTTACGGAAACCCCAATGCTGCAA	ACCACGCAGTAGGATCTCCCAAAA
1809.	TF	AT5G17260	GAACTTTATGGCGGATCTCGGACC	AGTTGTCGTTGTCTTGTGCTTGGT
1810.	TF	AT3G60030	TGGAGATGCACAGAGCCGTACTGA	TCTGTCCTCGTAAGGCAACTGGAA
1811.	TF	AT5G18270	ATCGTCTTGAAGGCAAATATTCG	ACCCATTCGTCCCTTGCA
1812.	TF	AT5G18830	TCCCACGGAGACTACGTCATCAAA	CGGATATAGCCCTCCAGCTCAACA
1813.	TF	AT1G16070	TACACATGAAGGTCGGGGGCGTAA	ACGTCGGCTATGGTAAGCAACTGC
1814.	TF	AT3G28920	CGGTGGTGGTGGGAGATTT	GTTTACGTTTCCGCCACCAT
1815.	TF	AT1G25280	CTTCGGTTTGTCGGTCTTGGAGAG	GGCTGTTTGAGGGAAACAGGGAAA
1816.	TF	AT3G50890	TCCGGCGTTTTACAGCAGTA	CTCACCCGTTGGATGCATTAC
1817.	TF	AT1G43640	TGGTGTCTGCAAGACATGGAGACT	CCGGGCTGTTTCAACGAAACTG

No.	Gene group	AGI	Sequence of forward primer	Sequence of reverse primer
1818.	TF	AT4G24660	CTTAAGTGTGCAGCTTGCGG	CGATGCTTTCGGTTTCCTTG
1819.	TF	AT1G47270	TAAGCCCTGCTCTTTCCGGTGACA	GATACAACAAACTCCGCACCCGTC
1820.	TF	AT5G15210	GGAGGATGCCGAAAGCTGA	TCAACTCCAATCTCCCGACAA
1821.	TF	AT1G53320	GCCTCAAATTGCCAGGTCCTAGAG	GCCGCCAGAAGAAACTTTCCCTTA
1822.	TF	AT5G39760	GCTGCCACCGTAATTTCCAC	GGGATTTGGGAAGAGTCGTTG
1823.	TF	AT1G61940	TTCCAGTTTGCGAAAGTCGG	TCCATTCAGCCTCATATAAACTGAAG
1824.	TF	AT5G42780	CGGCGTTACTGAGACGGTTC	GATCCGACGAAACTGACACGA
1825.	TF	AT1G76900	TCAGTCCTGCTTTGTTGGTTGAGA	GGCGTGCATAGAGATCACGTACTC
1826.	TF	AT5G60480	GACCCTCCGTCCCTTAGGTG	GACTACGACGGTGGAAGTTGC
1827.	TF	AT2G18280	TTCGGAGACTGAGAACGACAAACT	AACATAAGTACTGCTGCTCCGTGA
1828.	TF	AT5G65410	ACGACGCCGTTTACGACTCT	CTTTCTGTCGAGGCTTTGGG
1829.	TF	AT2G47900	TCGGGTTAAACCAAGCAGCTTCAA	GAAACCTCTTGGCAGCAAGAAGGA
1830.	TF	AT1G17380	TCCACCAGGGAAACAAAATGCGAT	TGCCTCCTGATGAGGTAGAGGGTT
1831.	TF	AT3G06380	CGTTTGCTATCTGCCTGAGCAGTT	ACAGACCACAACAACGAGCACAC
1832.	TF	AT1G19180	GCCTAGCTTCTCACAGACGTGTAG	TGACGTGAGTTGCCTAAAGTTCCA
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1834.	TF	AT1G30135	CGATCGCAAGCAGAGAAATG	ACTTGTTTGGAGGATCCGACC
1835.	TF	AT2G20825	GGAGAACTTCAAATCACCTGCC	CAGGCGTCAACTTGTCTTCG
1836.	TF	AT1G48500	ACATAGCTCCTGAAAAGGCCC	TTAGCATGAGGTCCATTTCCG
1837.	TF	AT5G18300	TTGGCTTCCACCAACCAAACACGA	TGGCGTAGATGTTGTCCCTGTCTT
1838.	TF	AT5G43270	CCTAAAGTCGTTGTGAGTGGCGT	ACTCAGAGAGACAGTGGAACCTGC
1839.	TF	AT5G22290	TCGAGCCTTGGGATTTACCCGATA	TTTCCCACGCGCACAGAAGAAG
1840.	TF	AT5G50570	AATGCAGCAGGTTTCATGCTTTGG	GGCTTCCGTCGTCTTCGATTATGT
1841.	TF	AT5G22380	CTGGAAAAGCACCCACAGGAAGAA	GCTTAGGGATTGTGGAAGCGTTGA
1842.	TF	AT5G50670	TCAACAATGCAGCAGGTTTCA	CCTACAACTTCTCTTCCCTTCATCAA
1843.	TF	AT5G24590	TCCCCAGCGAATGTCGTAGTGGAT	TATCGTCAAGCCCGGTGGTTGT
1844.	TF	AT1G05830	CGGCGTTATTTCATGTCTGCACGA	AGAGGGGAAAGTTAGCCAGAACCA
1845.	TF	AT5G39610	TCTTCCCCAAACAGCTAAGAACGA	GGCTGGTTCCATTCGGTTAATGTG
1846.	TF	AT2G31650	GCTGTGCTCGGACAGAGCCTTATA	AAGAAGCAGCAGCAAGAGCTTCA
1847.	TF	AT5G39820	TCCCAACGATGGGGGAAAAAGAGT	TCGGTTAGGTCTTGTGCTGTTGC
1848.	TF	AT3G61740	CGGGCAGGTTACAACATGGAGGAA	GCCAAATACTCCTGAGGGTGTGTG
1849.	TF	AT5G41090	TGGCTATGGAAGCGAAGAGCATTG	TTCTCCCGTTGGTATCCAGAGGTT
1850.	TF	AT4G27910	GCTCGGCTCATCAATCATTCGTGT	CATTGGCCTTGGCTATCAGGACAA
1851.	TF	AT5G41410	TGCTCTCCATGCTCGAAGAGCTTA	CGCAACTCTCATTTGCTCTCGGTA
1852.	TF	AT4G30860	AGAAGCTGTGTTTGCAGGGTTCAA	TGCGGAATGGTCTGTTTCCACAAC
1853.	TF	AT5G44180	TTCCATGACTATGACCCACGGCTA	AGCGTTCTTACCACACCCTCGATA
1854.	TF	AT5G09790	GGGATGCAGGTGCTATGCAAAGAA	CAAATACCACAACGAGAGGAGGGC
1855.	TF	AT5G45980	TCCTTTTCCTCAGATCGGATACCA	TTTGAATCTCCTCTCTAGGTGGGT
1856.	TF	AT5G24330	AATGCCCGCCACTTATGGTCGTCT	TGTCCGCCTCTACTGTGAACCCTT
1857.	TF	AT5G46590	TCGATCCTTGGGAGTTACCAGACA	GTCCCTTGAGCAGAAGAAGTACCA
1858.	TF	AT5G53430	GAACGGCTTCACCACTTGCAGA	CGCAAAAAGGCCCCATCCATGT
1859.	TF	AT4G28190	TGATCCAAACTGGAAATGCTCA	TTCTTCCTCCTCACATGTTATCTTGT
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1861.	TF	AT1G28520	GAAGGAGCTGCAACTGCTAAATC	AGAACCGTGAGATCAAAGAGCTCT
1862.	TF	AT1G72450	GCTAAACGAAAAGACAGGGCTG	GACTACCGTGTTGGTTCACTTGAT
1863.	TF	AT2G42400	CATGGAACGCAGCAGAGCTA	CACTCTCTAATTGTTTCGCCTTCAA
1864.	TF	AT1G74950	GCAGCACAAGAGCCAATTCA	TTGCAATCGGGAGTTCGC
1865.	TF	AT1G13960	TCAAGCAAAACAGACCAACCGC	GTGACTTGAGCTAGAGCTTGCTGA
1866.	TF	AT2G34600	ATGCGACTTGGAACTTCGCCTT	AGAGCTGCTTGATTCGTCCAACG
1867.	TF	AT1G18860	AGACACCAACGATGAACGACGGAT	AGCTCGGGGACATGGATTGCCTTT
1868.	TF	AT3G17860	TAATGGCTCCAACAGTGGCA	TCTAAAAACCTAGCCAGGGATGC
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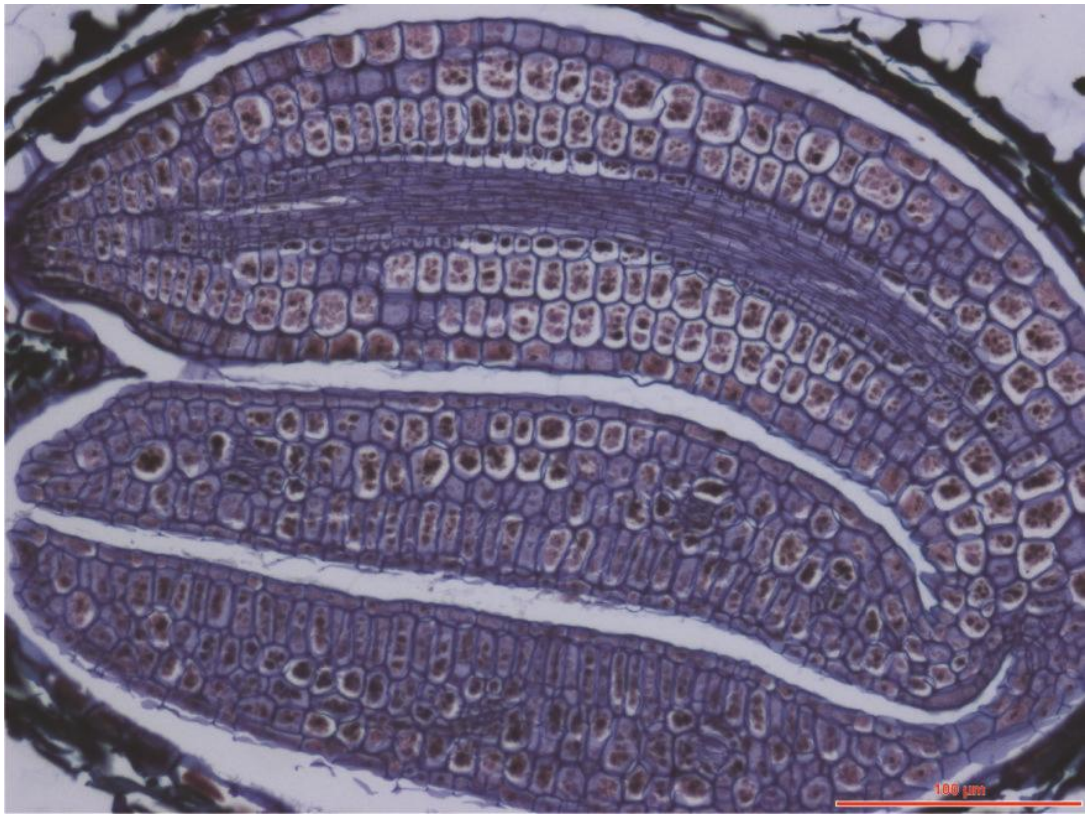
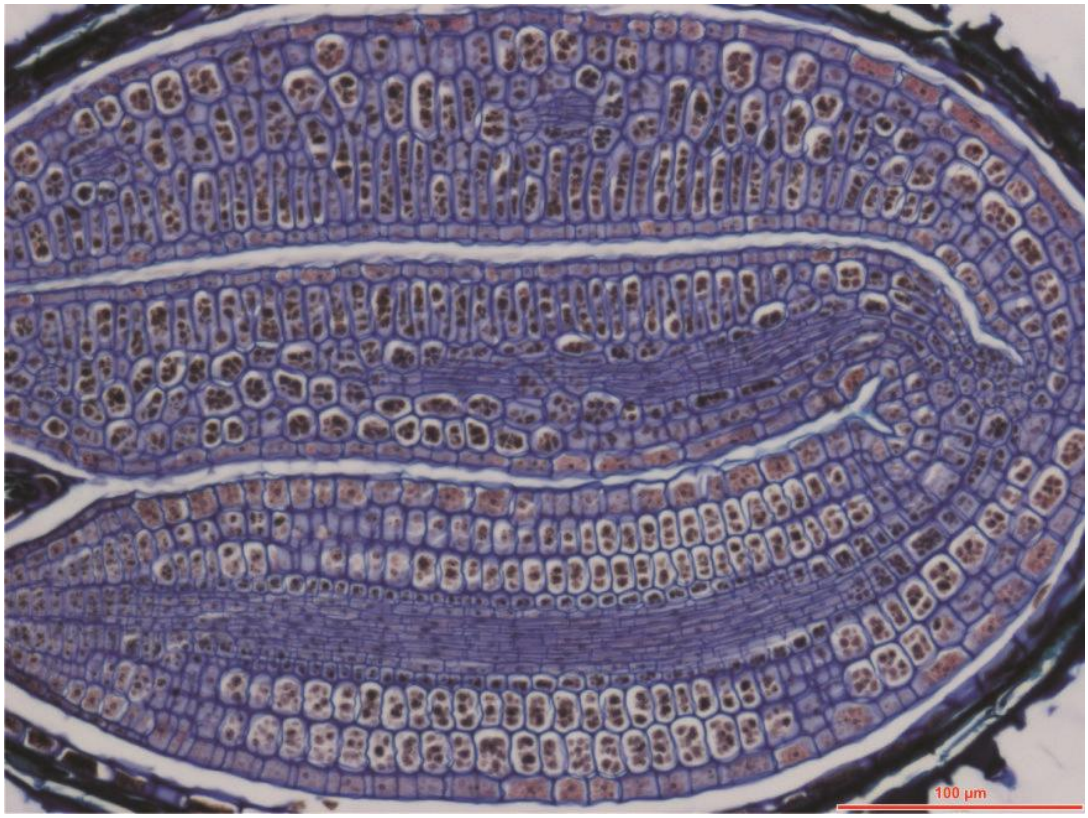
1870.	TF	AT3G43440	TTGAGAAAAGACGGCATCGA	TGCTTCCGAAGTCGTAGCAGA
1871.	TF	AT1G29860	AGGCGAGCCTAAAGAGAACACCA	GTCAGTTGCTTGGAGCTTTCACCA
1872.	TF	AT4G14713	GTATCCGAGAAAGATGGCCACA	CACTTGTTTCAGCTGGGCTTC
1873.	TF	AT1G30650	AGGCTCTCCTTTTCCAAGGGGTTA	GATCGGTTCGGCTTCTTTCGACTT
1874.	TF	AT4G14720	GAGCTTCCCCAATATGGACTTG	GACCCTCAACATCAGAATCACG
1875.	TF	AT1G55600	CCTCCACCTCCAAAGAGAAGGAGA	TGCTTGTTCTTGTGGCTCCAATCA
1876.	TF	AT4G32570	TGACGTTCACCCAAACAAGG	TGAGCCTCCGCTTGATCCT
1877.	TF	AT1G62300	GGAAGCTCCGATGATAAGCGATGG	ATCGTGCAGCGGTAATATGCCC
1878.	TF	AT5G13220	AGATCAGCCTCAGATCCCGAT	TTCGGTACTAGACCTGGCGAG
1879.	TF	AT1G64000	GTTCATAATCATCCTTGTGAGAAGCT	GAAGTTGCCTAAGGAGAGGGC
1880.	TF	AT5G20900	GCAGATCCACGGCTGATCTAC	TTCTCGAGGAATCGTTGAAGC

Annex B. Microscopic pictures

Mature seeds

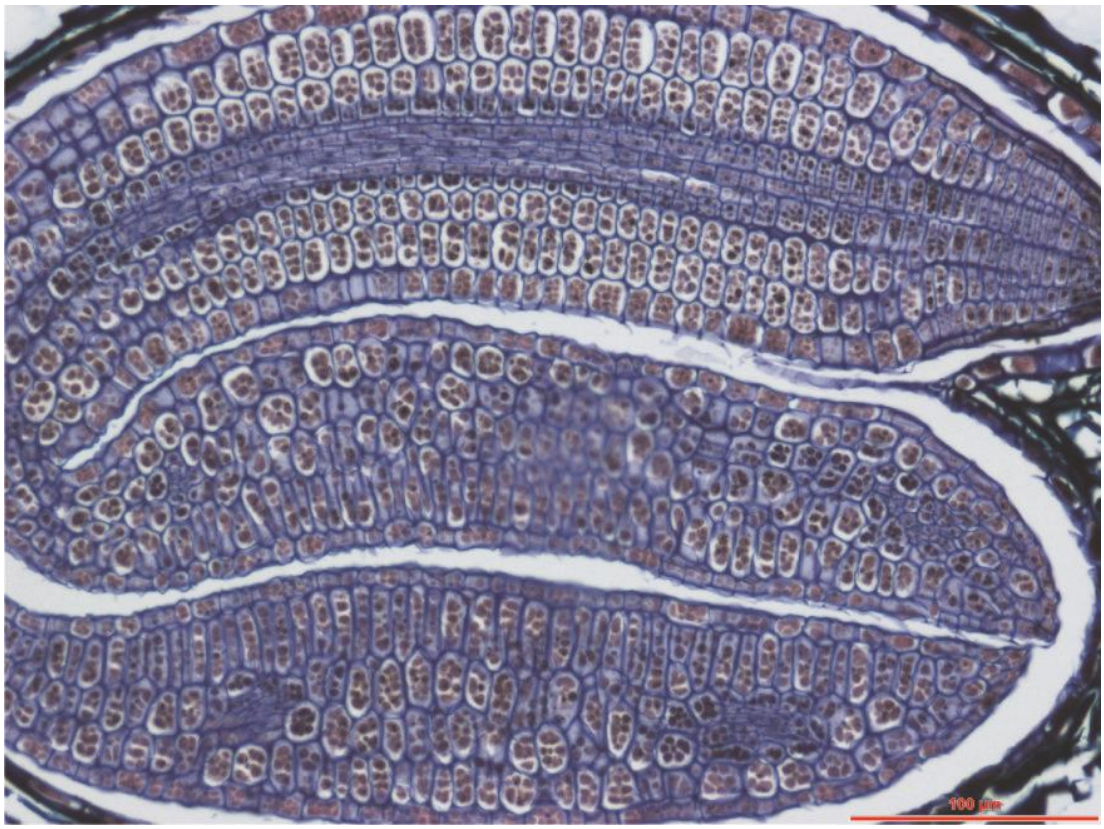


Col-0xCol-0 mature seed Magnification: 20x

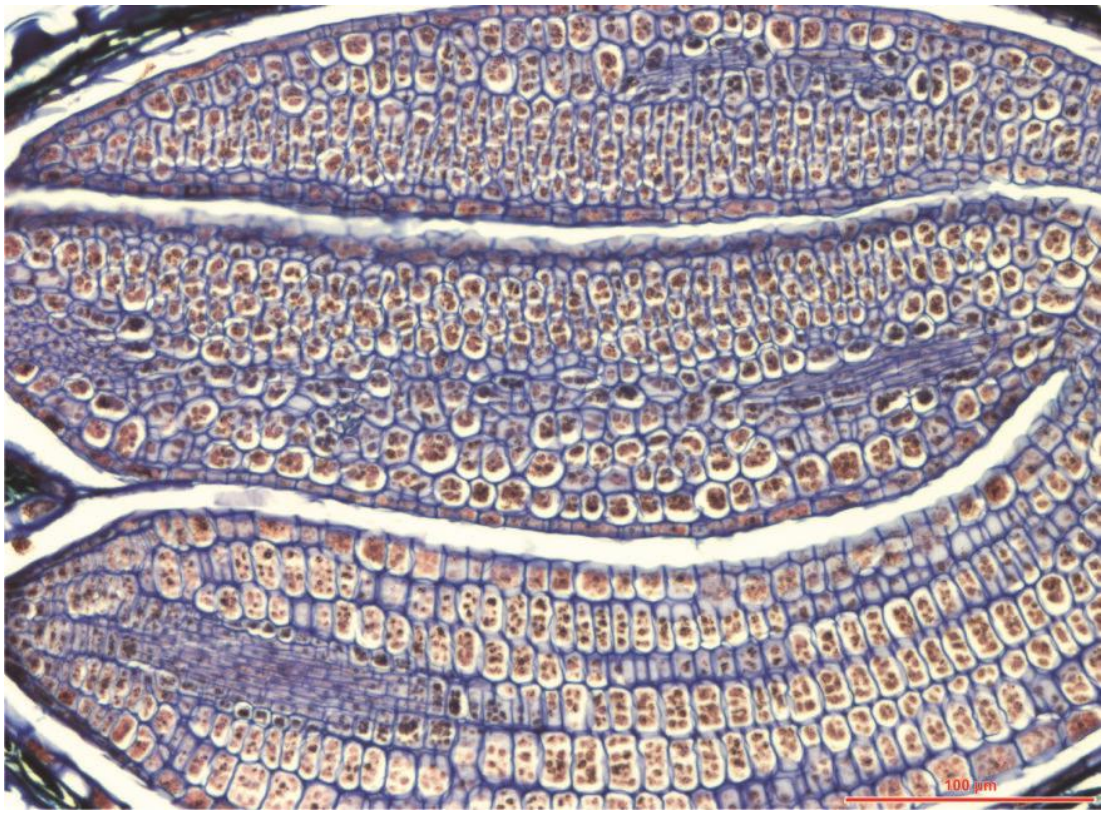


Col-0xC24 mature seed Magnification: 20x

Mature seeds

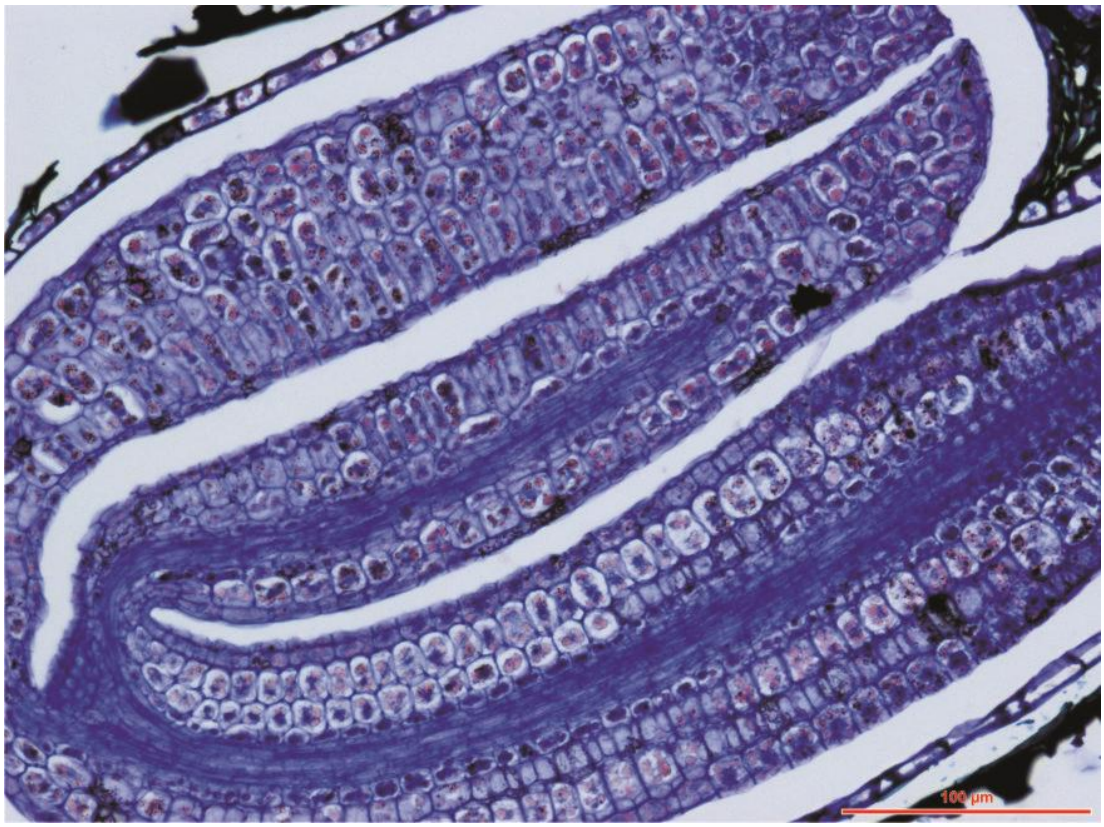


C24xC24 mature seed Magnification: 20x



C24xCol-0 mature seed Magnification: 20x

24 HAS



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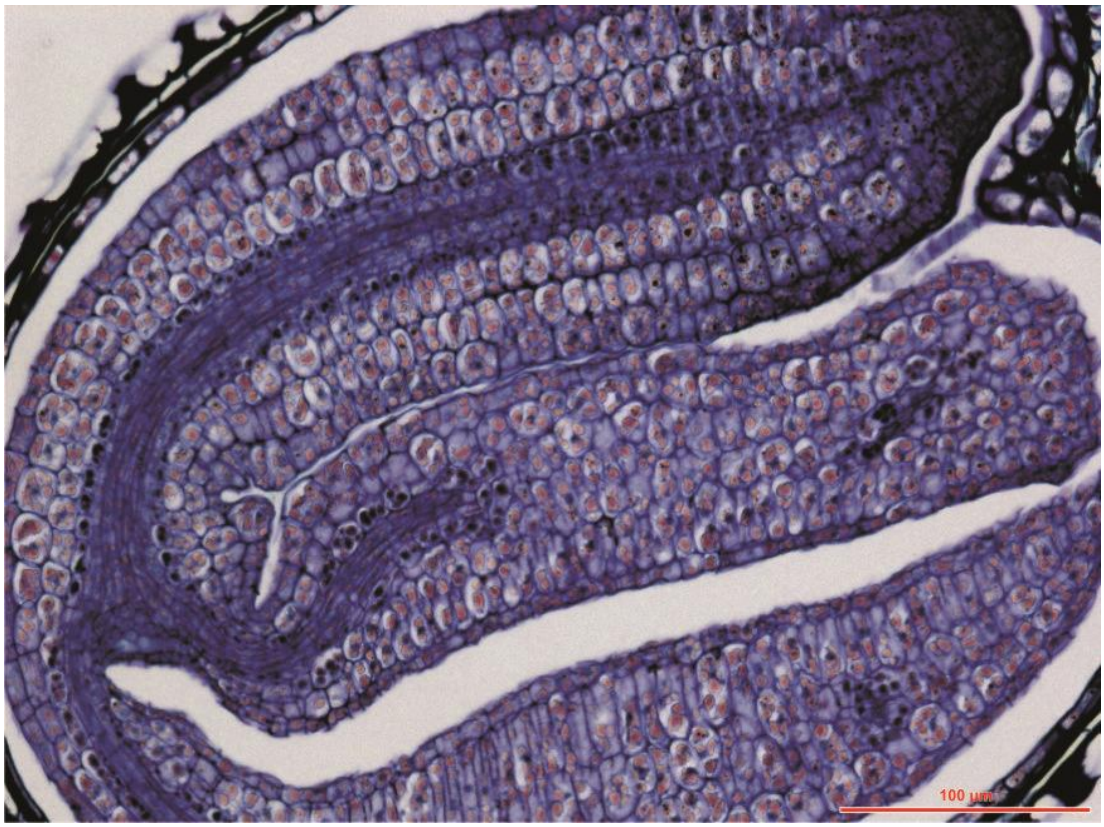
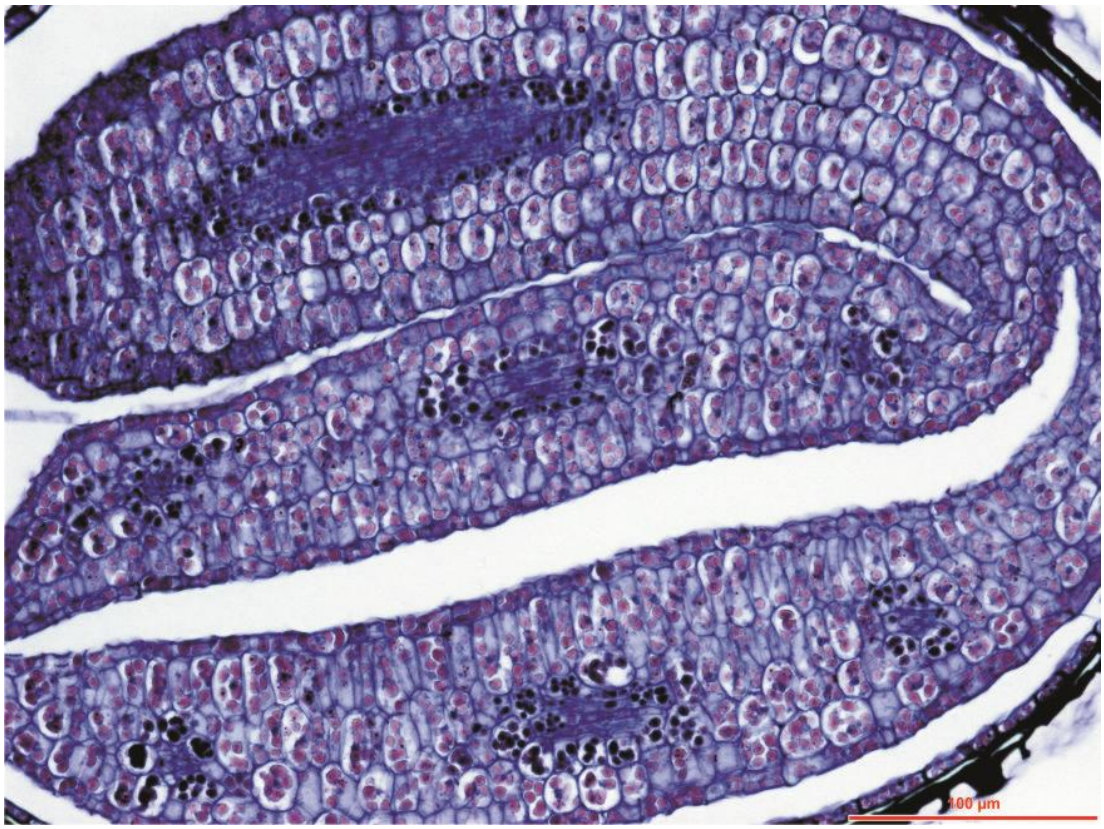


Col-0xC24 24 HAS Magnification: 20x

24 HAS



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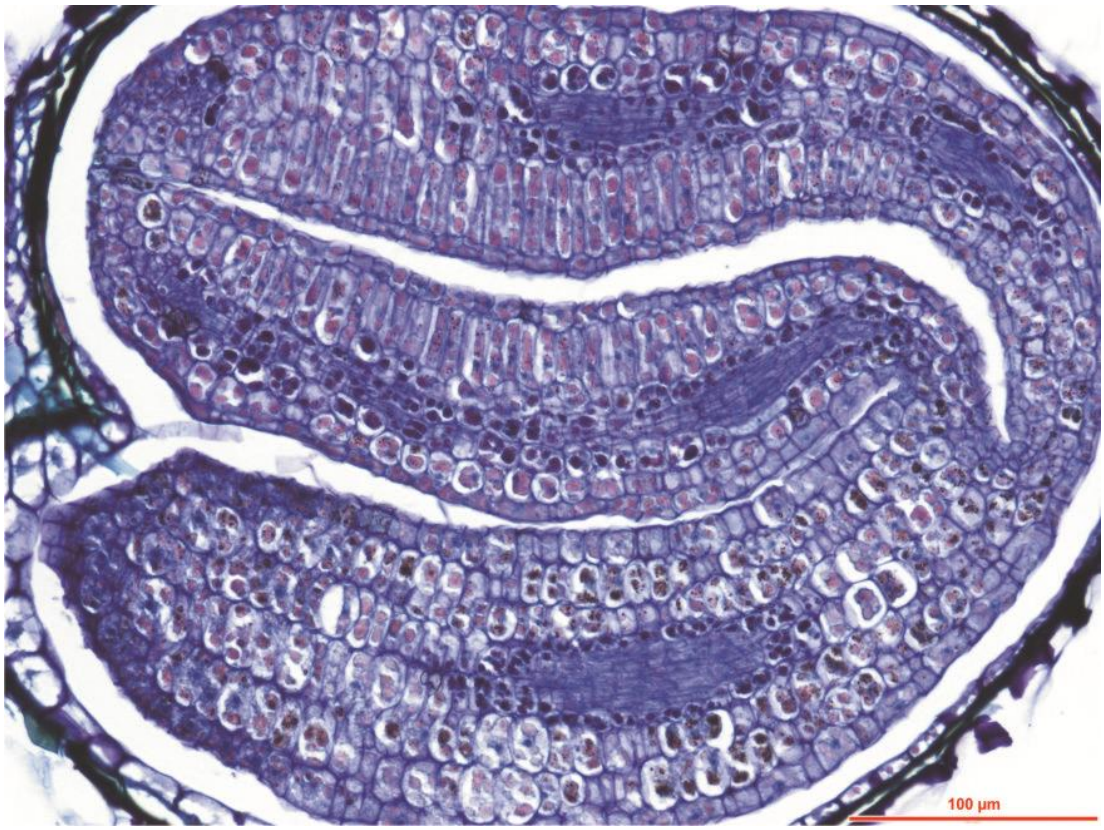
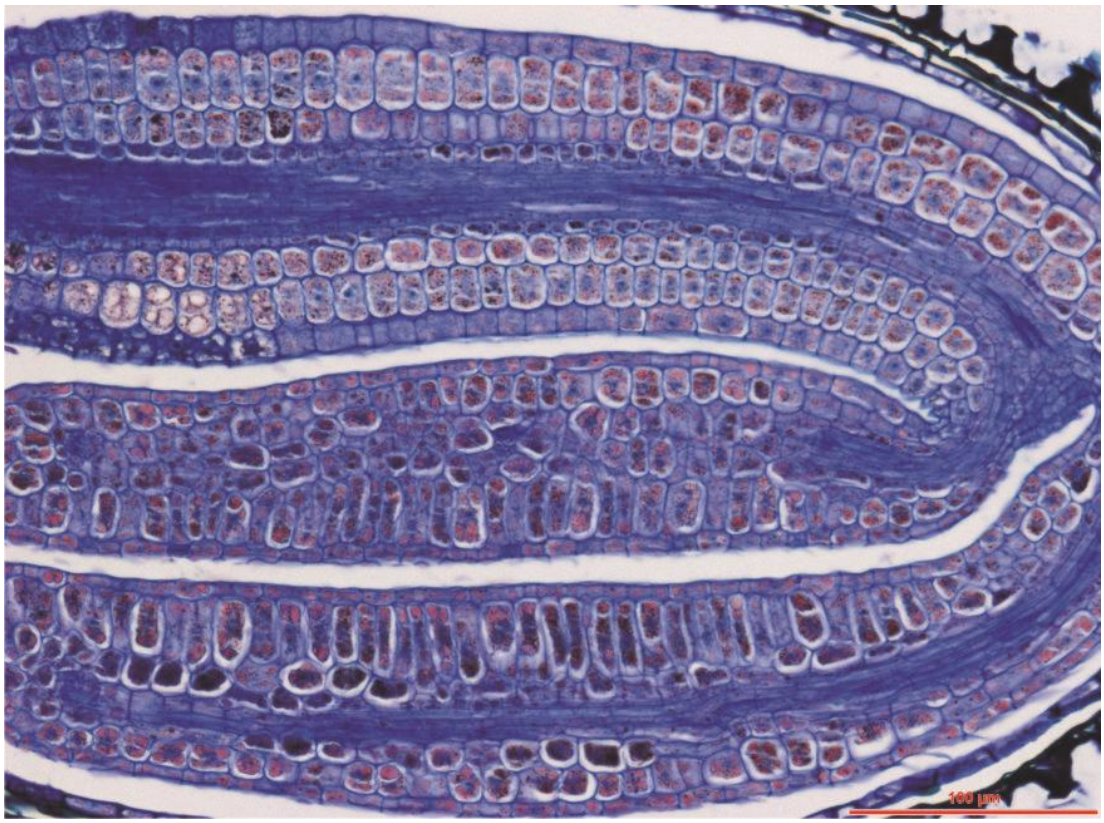


C24xCol-0 24 HAS Magnification: 20x

36 HAS



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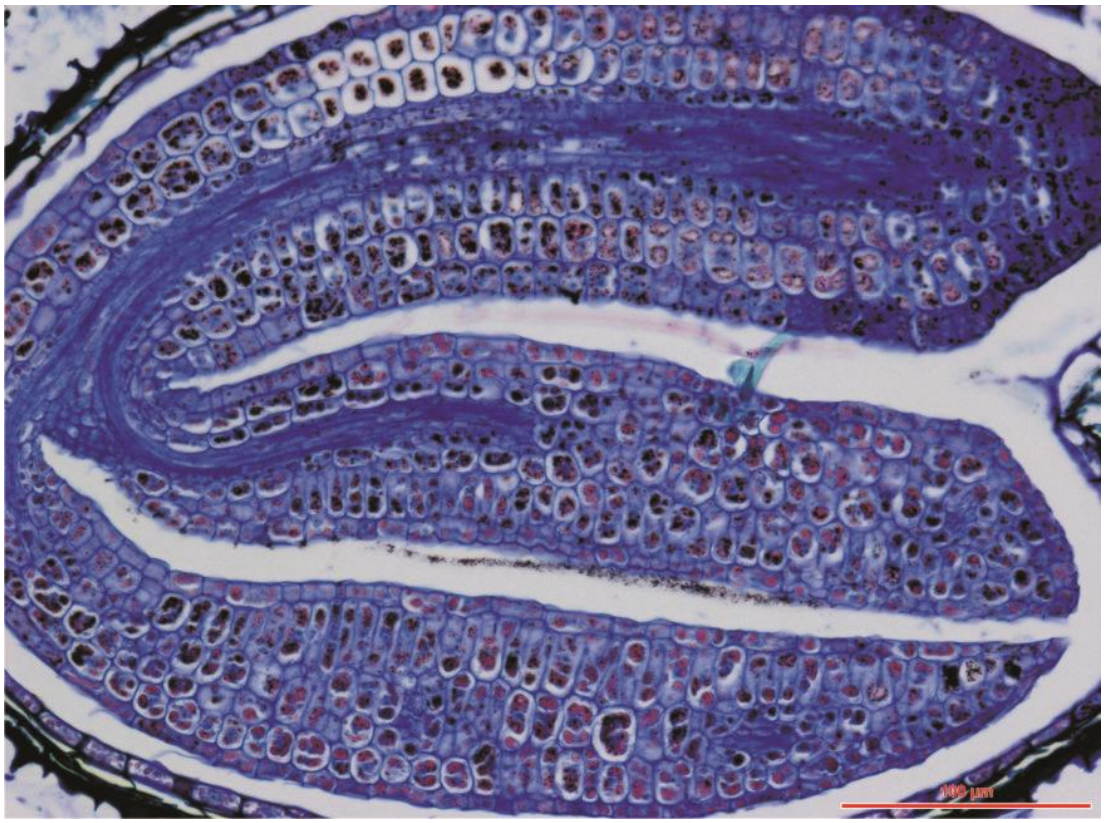
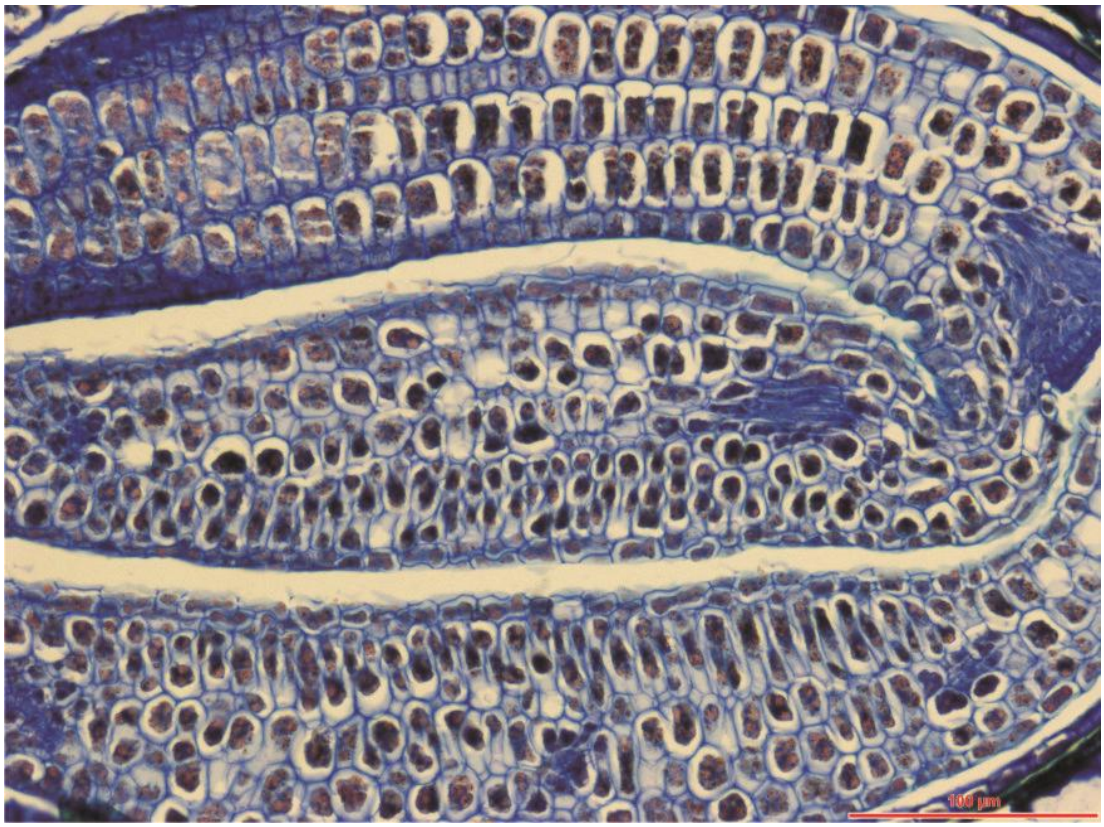


Col-0xC24 36 HAS Magnification: 20x

36 HAS



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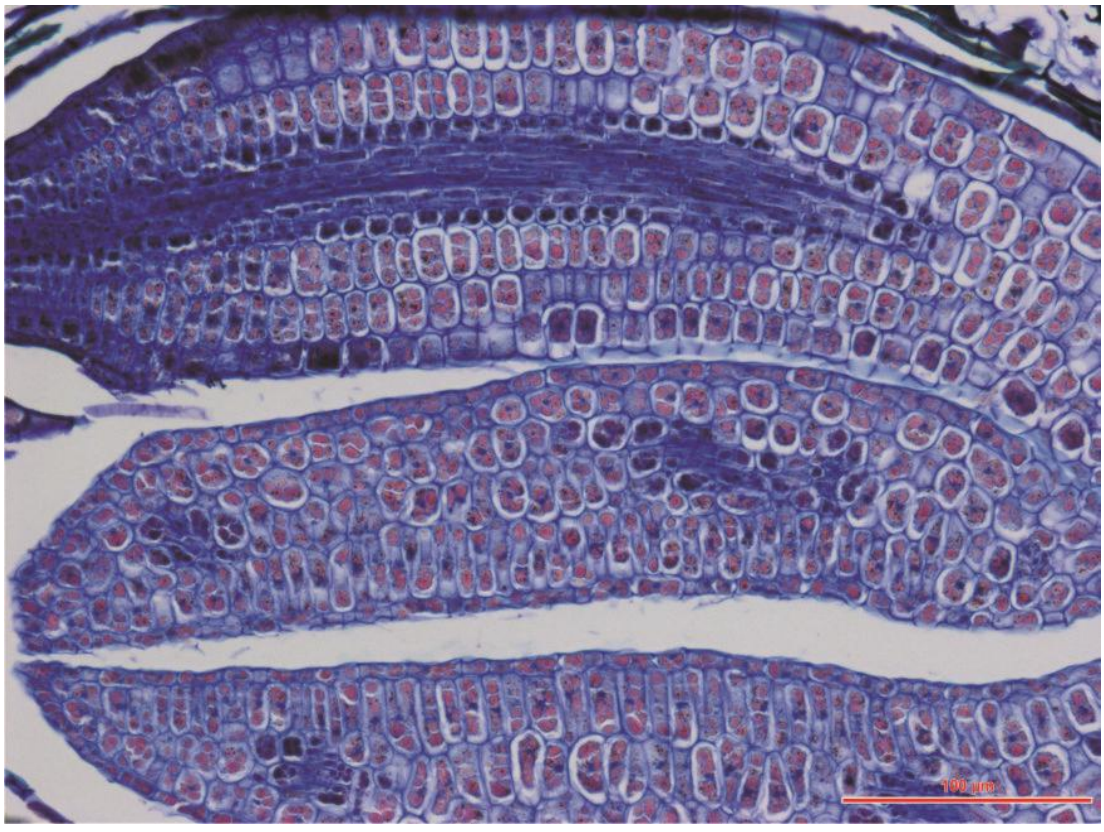

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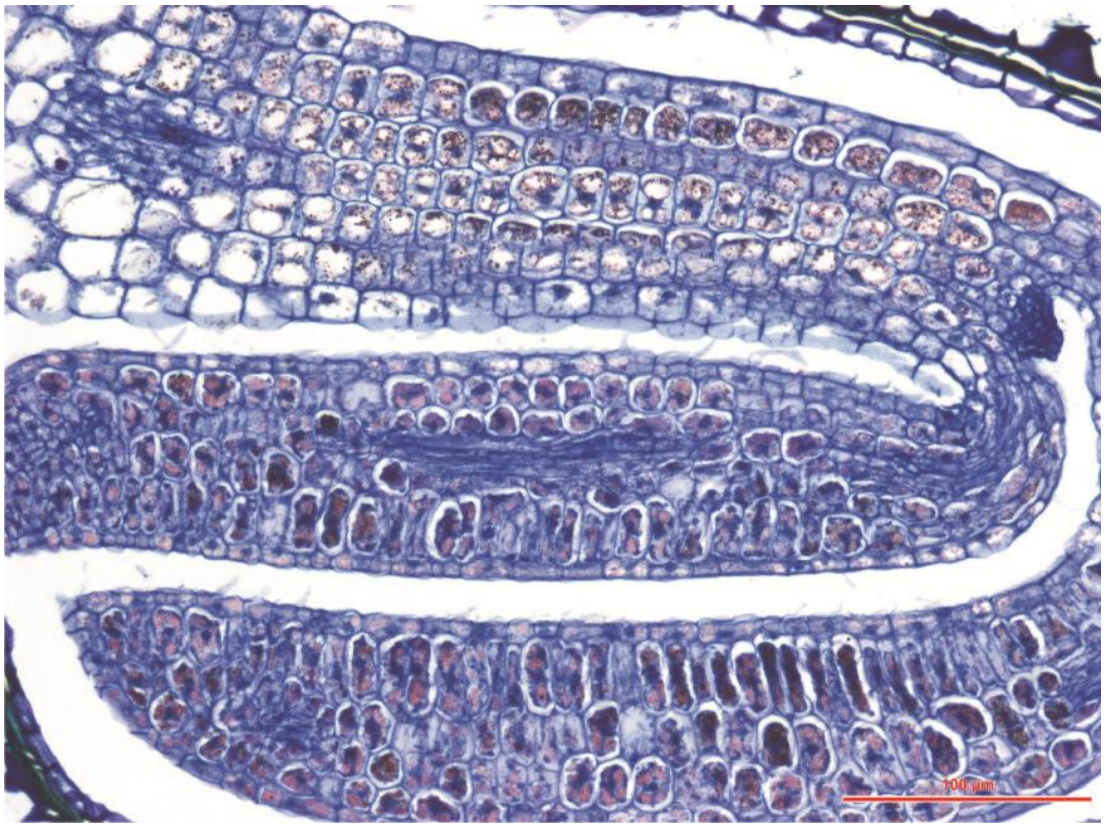

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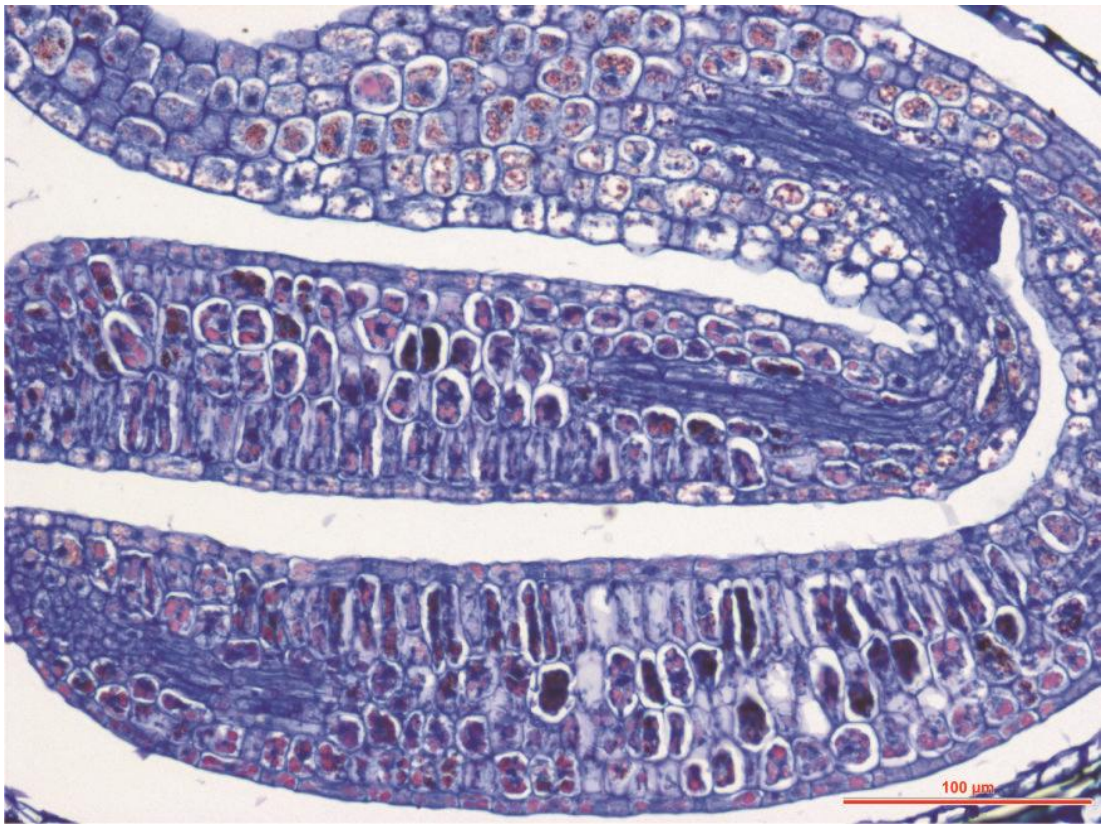

Col-0xC24 48 HAS Magnification: 20x

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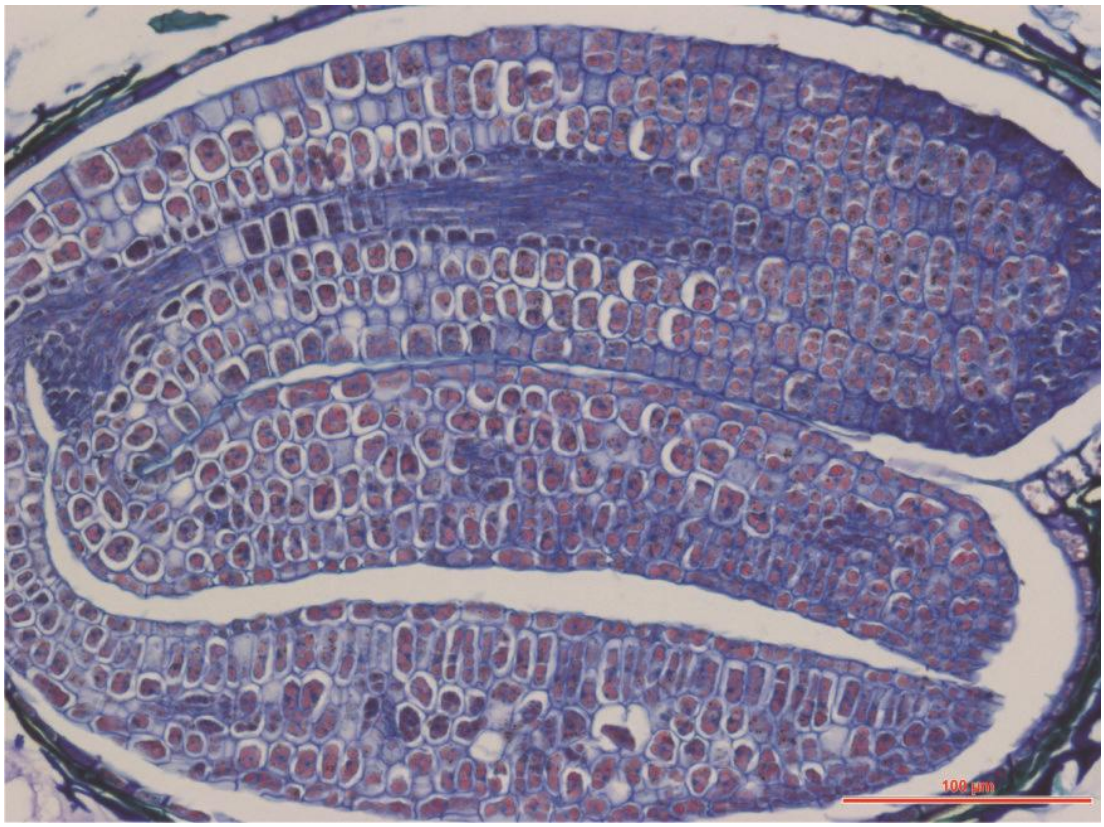

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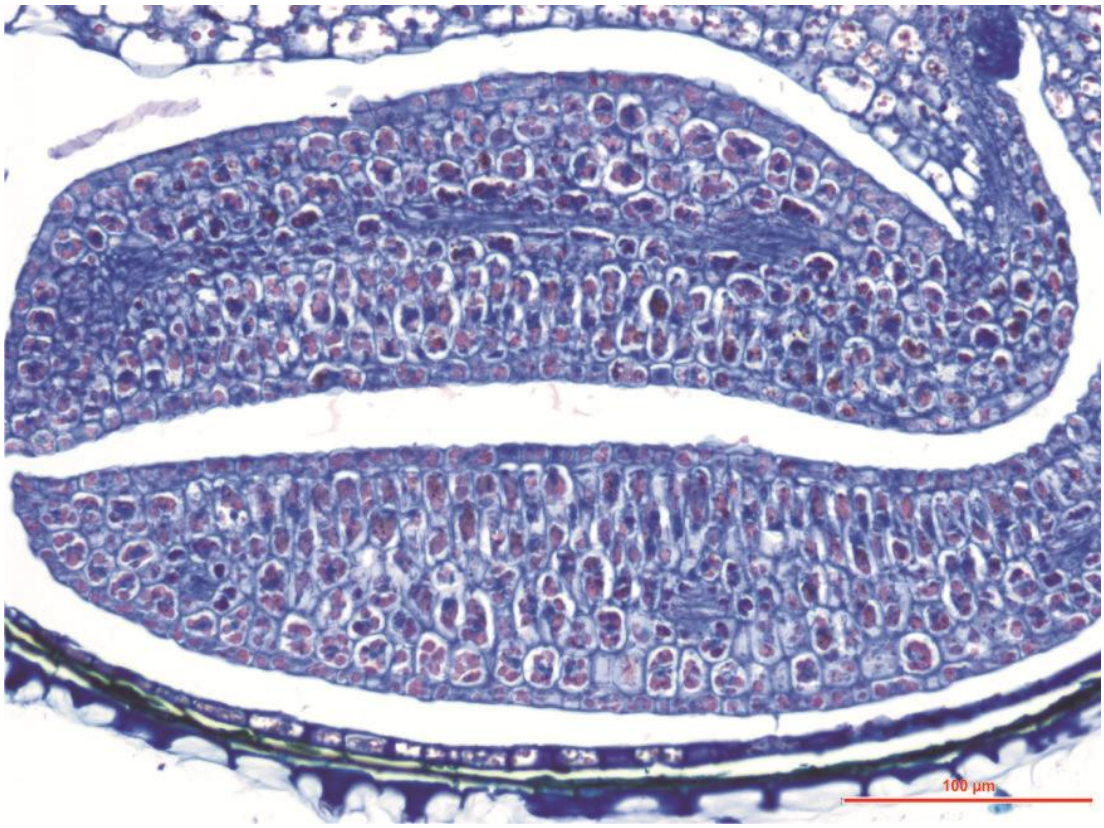
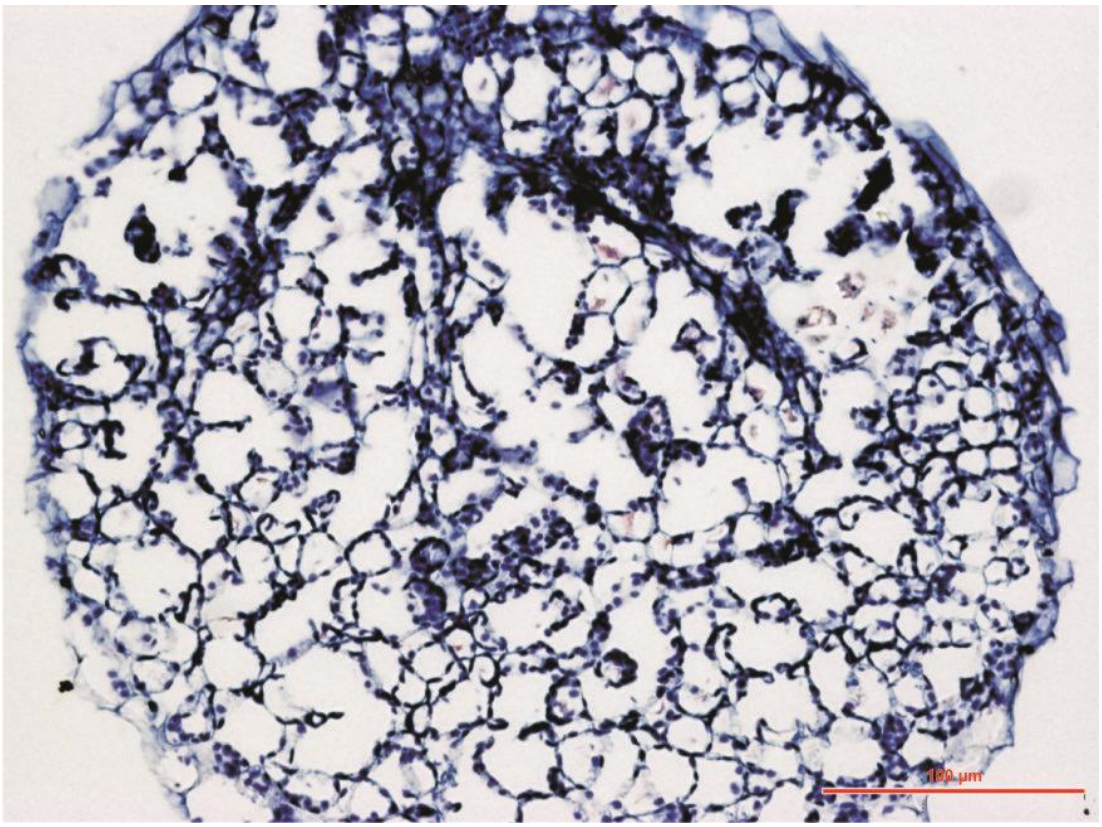


C24xCol-0 48 HAS Magnification: 20x

72 HAS



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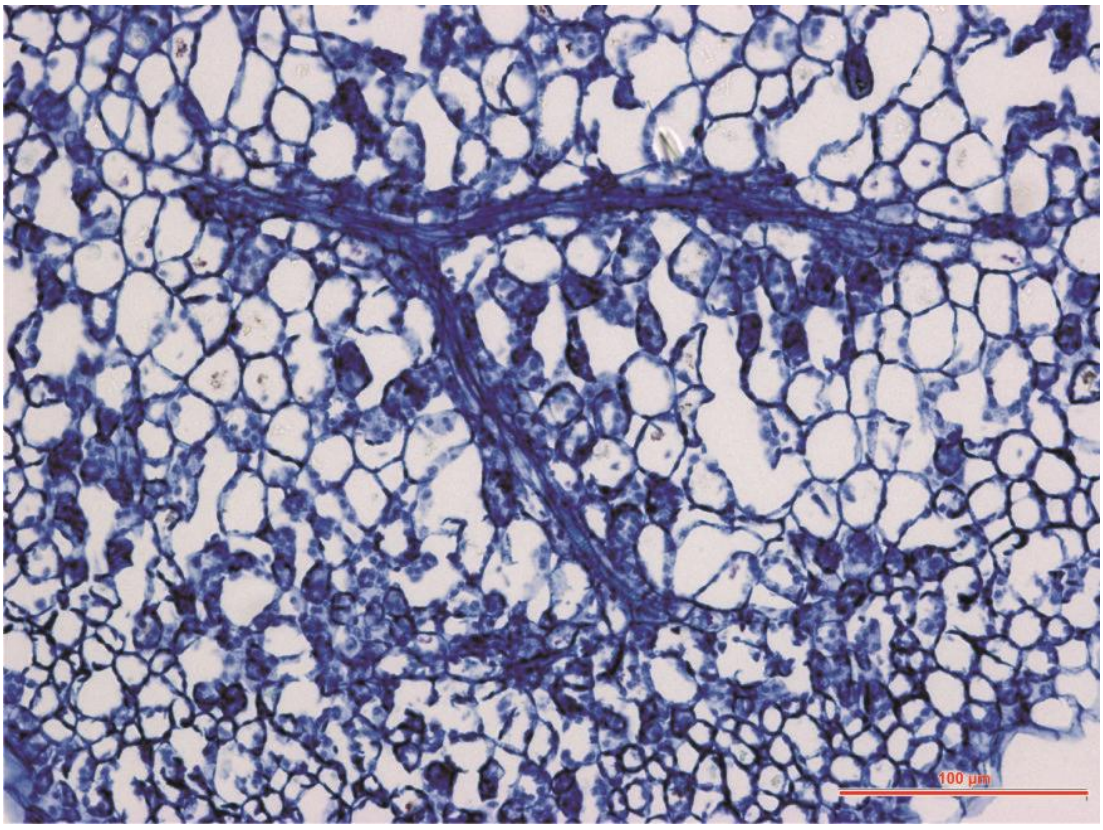
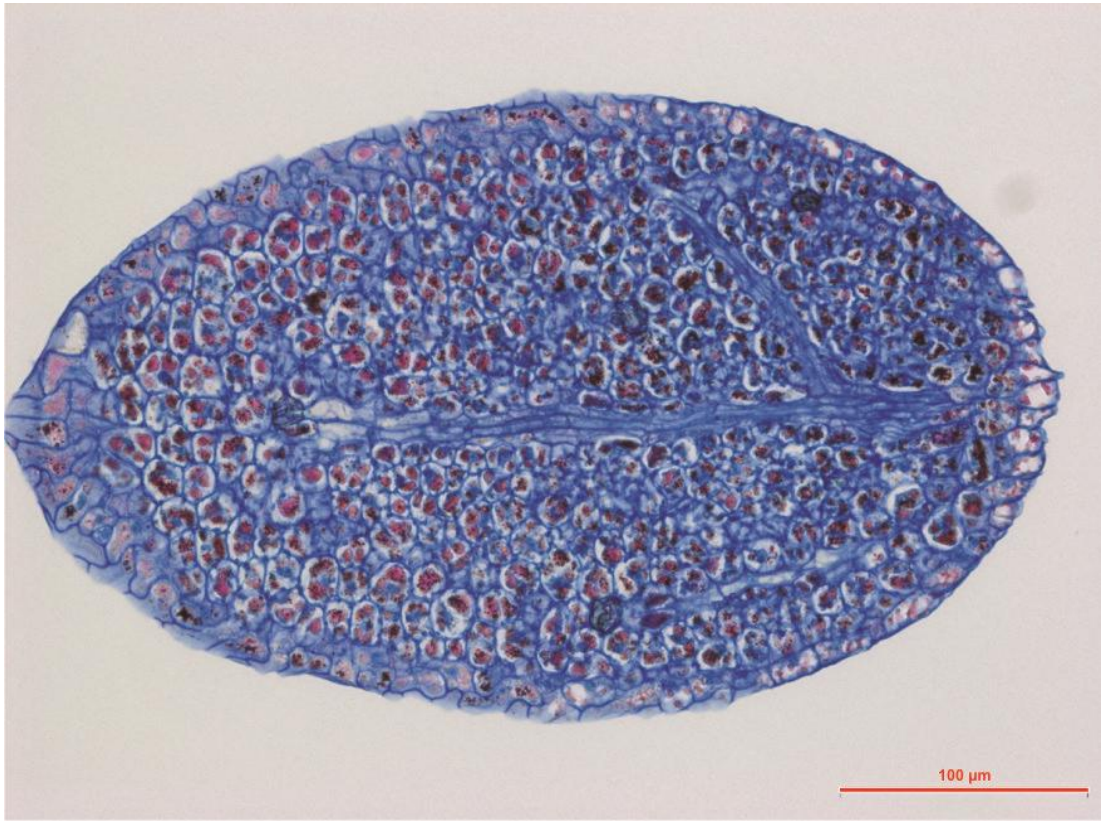


Col-0xC24 72 HAS Magnification: 20x

72 HAS



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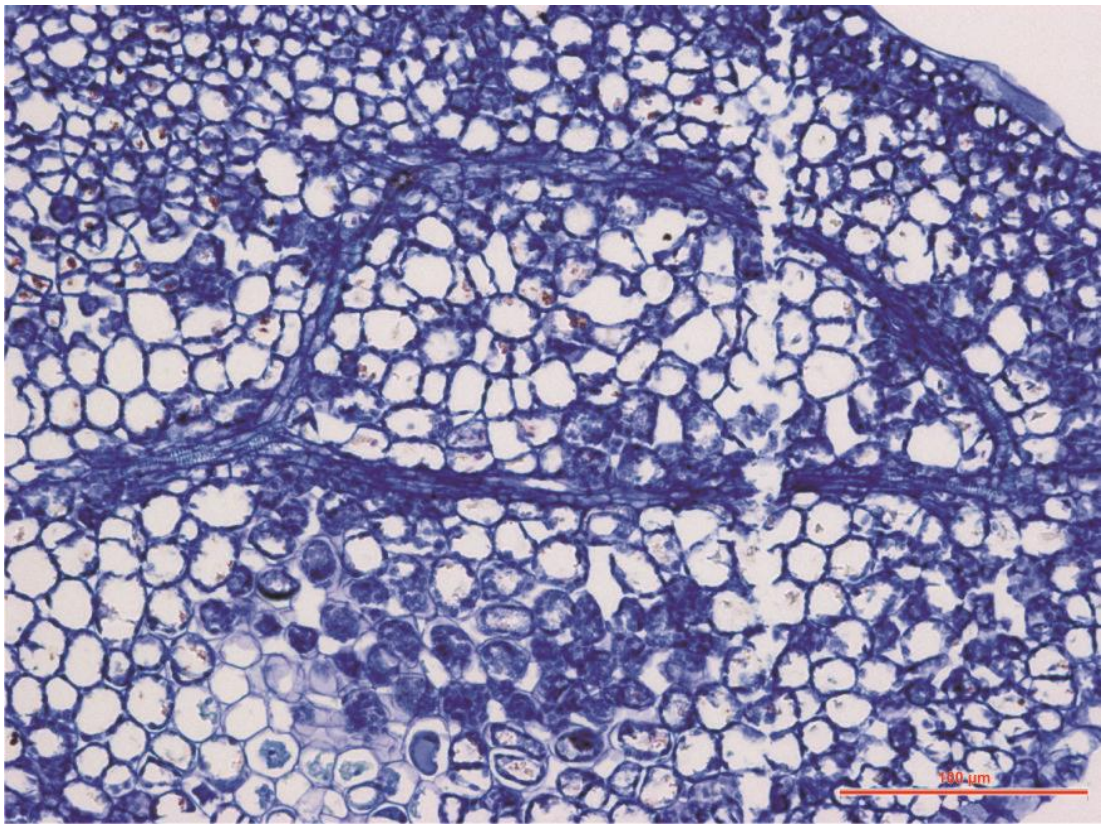
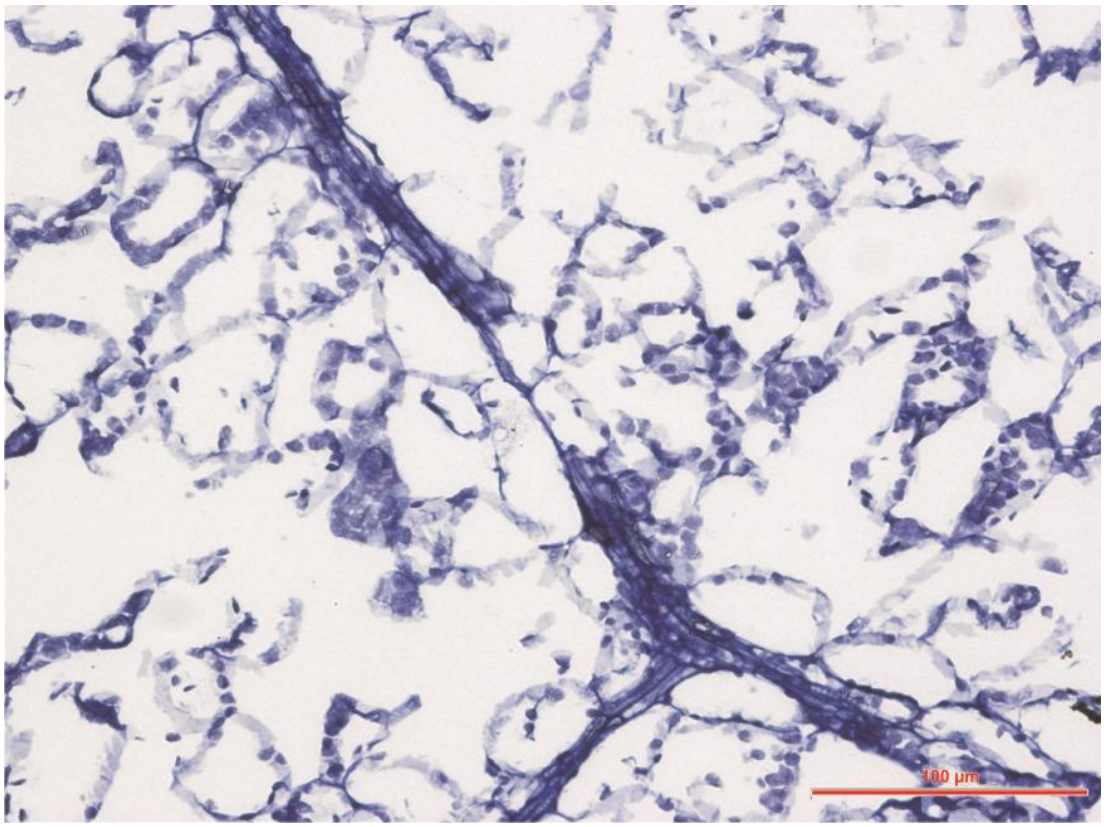
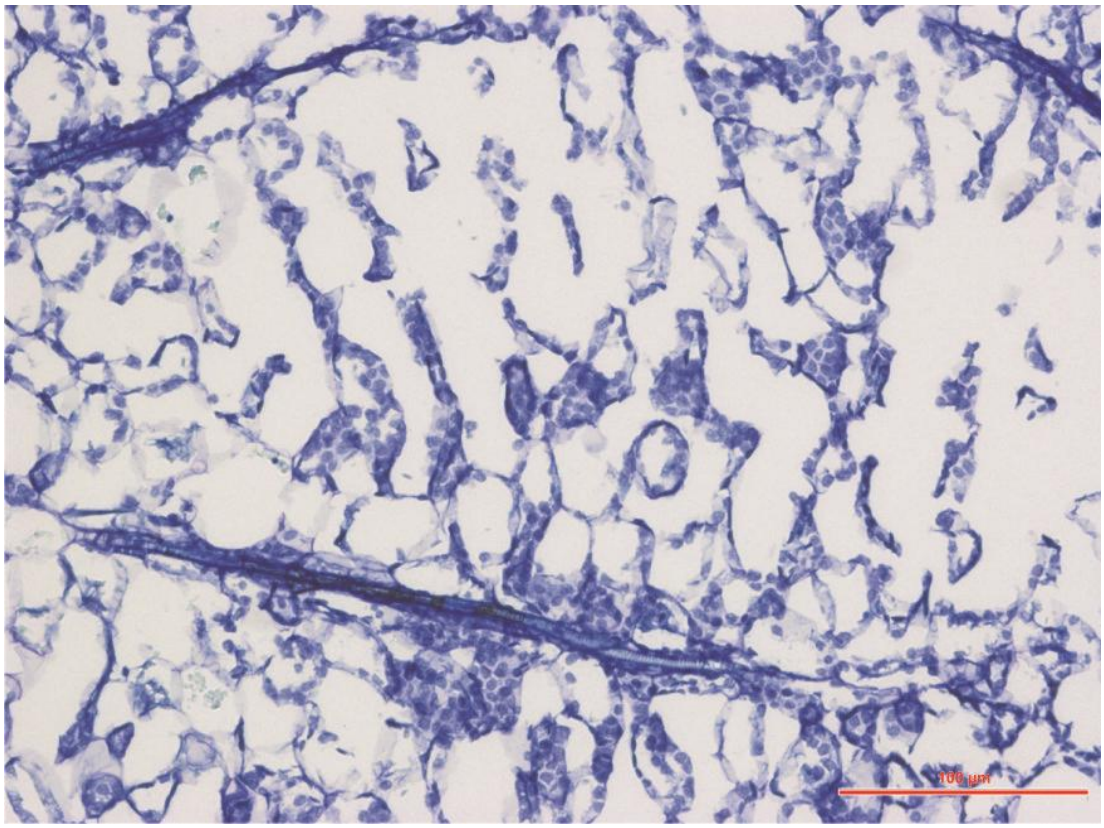


C24xCol-0 72 HAS Magnification: 20x

96 HAS

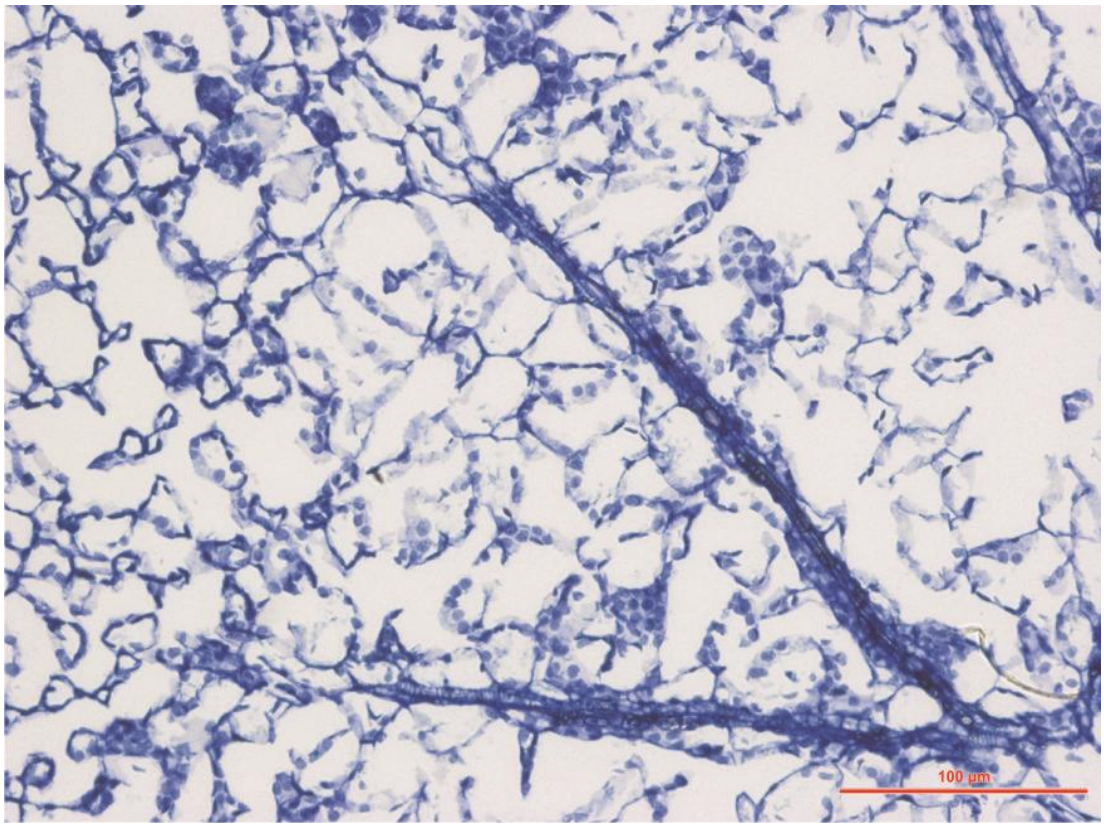


Col-0xCol-0 96 HAS Magnification: 20x



Col-0xC24 96 HAS Magnification: 20x

96 HAS



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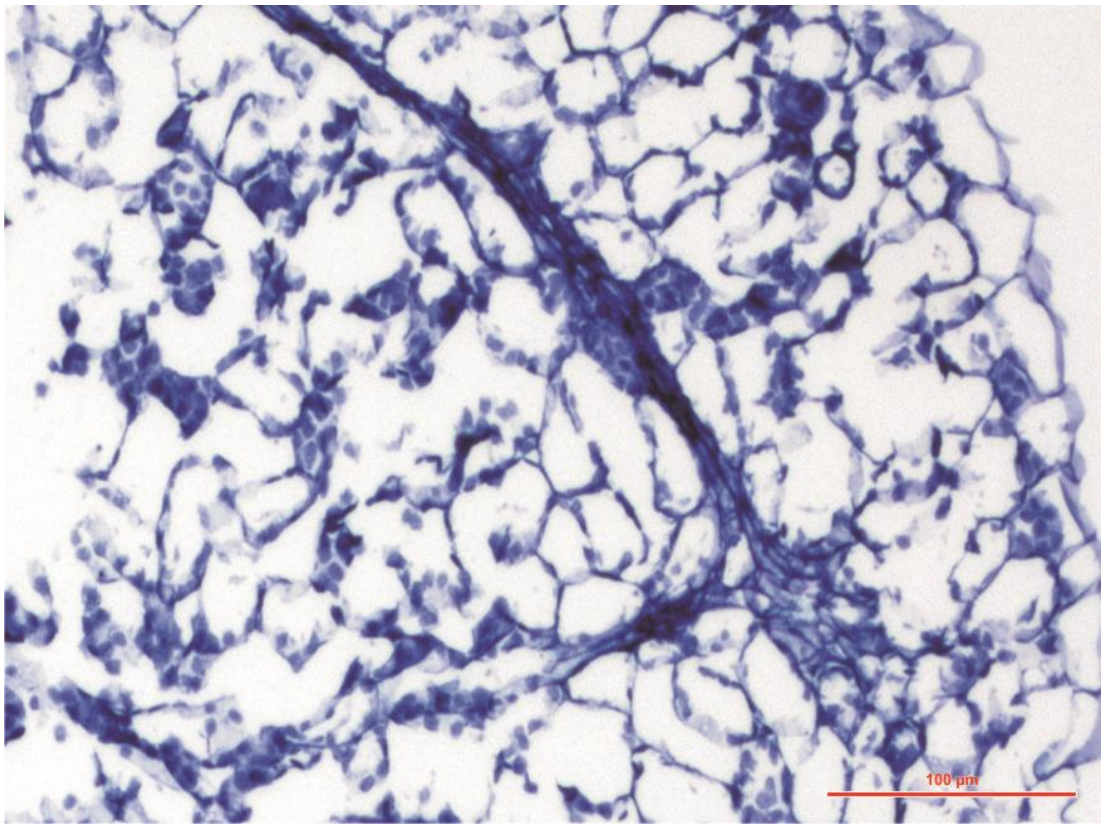

C24xCol-0 96 HAS Magnification: 20x

Annex C. Summarised GC-MS data

Table 1. The list of 75 identified compounds

No.	Identified compounds	MEAN F1s / Ps							SE						
		mature	12 HAS	24 HAS	36 HAS	48 HAS	72 HAS	96 HAS	mature	12 HAS	24 HAS	36 HAS	48 HAS	72 HAS	96 HAS
1.	adipic acid	**1,47**	**2,71**	**1,34**	0,96	**1,71**	1,01	0,80	0,72	1,23	0,45	0,14	0,61	0,15	0,08
2.	alanine	0,79	**0,64**	**1,53**	**1,91**	0,81	**1,56**	**4,98**	0,14	0,03	0,38	0,74	0,16	0,33	4,34
3A.	alpha-tocopherol	1,05	1,06	1,18	1,13	1,05	0,90	**0,61**	0,14	0,09	0,20	0,15	0,07	0,25	0,12
3B.	alpha-tocopherol minor	**1,49**	**0,51**	0,98	**1,41**	**1,48**	**1,42**	1,10	**NA**	**NA**	0,11	0,31	0,17	0,47	0,13
4.	aspartic acid	1,01	**1,85**	1,11	**1,66**	**1,52**	**1,32**	**1,32**	0,06	0,19	0,14	0,11	0,10	0,12	0,20
5.	behenic acid	0,98	0,93	0,98	0,95	1,12	1,03	1,01	0,03	0,03	0,04	0,06	0,10	0,16	0,14
6.	benzoate	0,98	1,19	1,16	**1,44**	**1,42**	0,80	**1,98**	0,11	0,08	0,04	0,37	0,11	0,09	1,09
7.	beta-glucopyranose 1,6-anhydro	1,03	1,15	1,12	1,08	1,04	0,90	1,01	0,12	0,07	0,18	0,18	0,20	0,12	0,07
8.	c15:0 fatty acid	1,10	0,76	0,87	1,10	1,08	1,03	1,20	0,20	0,08	0,33	0,44	0,10	0,14	0,41
9.	c20:0 fatty acid	1,01	0,84	0,90	0,82	1,05	1,08	1,09	0,09	0,16	0,10	0,13	0,11	0,22	0,54
10.	capric acid	0,93	0,73	0,80	0,88	0,92	0,98	1,05	0,07	0,08	0,06	0,04	0,14	0,24	0,25
11.	citramalic acid	0,88	0,88	0,87	0,89	0,92	0,88	1,00	0,13	0,11	0,14	0,04	0,07	0,13	0,17
12.	citrate	1,23	**1,40**	**1,69**	**1,50**	0,94	**1,56**	0,96	0,43	0,26	0,23	0,32	0,15	0,30	0,15
13.	citrulline	1,08	**1,54**	**1,46**	1,15	0,96	1,08	1,29	0,17	0,31	0,26	0,19	0,08	0,12	0,18
14.	cysteine	0,81	1,03	**0,67**	1,13	0,90	1,16	0,82	0,05	0,18	0,13	0,18	0,15	0,06	0,09
15.	dodecanoic acid	**1,52**	0,82	0,76	1,06	1,02	**1,76**	1,10	0,49	0,12	0,20	0,18	0,12	0,71	0,11
16.	ergosterol	0,99	1,08	0,89	0,97	1,02	1,06	0,98	0,09	0,10	0,08	0,10	0,04	0,14	0,08
17A.	fructose1	0,86	1,11	1,15	0,95	0,90	0,74	**1,34**	0,06	0,13	0,22	0,11	0,08	0,14	0,08
17B.	fructose2	0,88	1,11	1,18	0,93	0,91	0,74	**1,41**	0,06	0,14	0,21	0,11	0,07	0,14	0,13
18.	fumaric acid	0,72	0,81	1,00	1,02	0,87	1,18	**2,16**	0,04	0,10	0,15	0,14	0,03	0,07	0,50
19.	gluconic acid lactone	0,93	1,05	0,87	1,13	1,00	0,85	0,84	0,07	0,13	0,06	0,07	0,07	0,12	0,16
20A.	glucose1	1,03	**1,51**	**1,41**	0,77	1,03	0,99	**1,31**	0,17	0,38	0,28	0,17	0,22	0,43	0,20
20B.	glucose2	1,01	**1,56**	**1,48**	0,83	1,12	**0,64**	**1,54**	0,13	0,35	0,30	0,19	0,26	0,22	0,39
21.	glucose-1-phosphate (degradation product)	0,90	1,13	1,01	1,25	1,03	**1,33**	0,87	0,07	0,07	0,08	0,25	0,09	0,10	0,09
22.	glutamate	0,94	1,14	**1,33**	**1,67**	1,29	**1,48**	**1,69**	0,04	0,14	0,18	0,05	0,16	0,18	0,31
23.	glutamine	0,74	1,28	1,08	**1,36**	0,85	1,08	1,04	0,13	0,08	0,17	0,15	0,16	0,11	0,14
24.	glutaric acid	0,80	1,17	1,03	0,89	0,92	0,83	1,05	0,10	0,34	0,10	0,06	0,06	0,05	0,09
25.	glycerate	0,82	0,93	1,13	1,12	1,03	1,30	0,99	0,12	0,11	0,16	0,07	0,05	0,14	0,09
26.	glycerol	0,95	0,83	1,18	0,78	**1,44**	0,94	1,06	0,01	0,09	0,11	0,21	0,38	0,12	0,17

No.	Identified compounds	MEAN F1s / Ps							SE						
		mature	12 HAS	24 HAS	36 HAS	48 HAS	72 HAS	96 HAS	mature	12 HAS	24 HAS	36 HAS	48 HAS	72 HAS	96 HAS
27.	glycerolphosphate alpha	0,85	**1,33**	1,08	**1,71**	1,26	**1,38**	1,09	0,12	0,06	0,15	0,18	0,13	0,07	0,09
28A.	glycine	0,70	1,05	1,23	1,24	0,93	**1,38**	0,87	0,04	0,16	0,20	0,13	0,08	0,16	0,11
28B.	glycine minor	0,82	0,79	**1,56**	1,18	0,74	0,93	0,93	0,10	0,06	0,24	0,06	0,10	0,08	0,12
29.	glycolic acid	0,90	0,85	1,13	1,10	0,88	0,94	0,91	0,06	0,12	0,10	0,31	0,04	0,14	0,08
30.	heptadecanoic acid	1,06	0,77	0,82	0,85	1,06	0,85	1,21	0,12	0,05	0,24	0,23	0,08	0,11	0,30
31.	hexanoic acid	1,00	0,74	0,83	0,70	0,97	**0,61**	**0,69**	0,31	0,14	0,12	0,06	0,24	0,11	0,13
32.	hydroxybenzoate	0,79	0,79	0,95	0,70	0,78	0,89	0,92	0,07	0,07	0,13	0,02	0,07	0,08	0,14
33.	indole-3-acetonitrile	1,20	1,03	1,05	0,96	0,72	1,25	0,85	0,23	0,14	0,05	0,10	0,09	0,11	0,11
34.	inositol myo-	0,81	1,28	1,22	**1,40**	1,11	0,75	1,15	0,07	0,08	0,15	0,08	0,07	0,04	0,02
35.	inositol-2P	1,23	0,90	1,04	1,25	**1,40**	1,18	0,90	0,38	0,17	0,08	0,02	0,28	0,14	0,10
36.	isogalactinol	1,03	1,01	1,11	**1,33**	1,19	**1,32**	0,89	0,11	0,08	0,09	0,12	0,09	0,10	0,09
37.	isoleucine	0,90	0,94	1,16	**1,65**	0,95	**1,36**	**0,62**	0,10	0,02	0,24	0,04	0,14	0,17	0,07
38.	isopropyl beta-D-thiogalactopyranoside	1,01	0,92	**1,36**	1,19	0,93	1,06	0,78	0,30	0,18	0,25	0,17	0,14	0,22	0,29
39.	isoribonic acid (put.)	0,94	1,09	0,97	0,89	1,06	**1,37**	**1,40**	0,07	0,18	0,16	0,08	0,12	0,37	0,10
40.	isosinapinic acid	0,71	1,22	1,16	1,24	1,08	1,04	1,22	0,07	0,15	0,11	0,31	0,03	0,11	0,12
41.	itaconic acid	0,77	0,87	0,99	0,94	0,92	0,95	0,94	0,03	0,13	0,17	0,08	0,07	0,13	0,09
42.	lactic acid RI 192920	0,87	0,74	1,05	0,89	0,84	0,70	0,74	0,04	0,19	0,05	0,12	0,12	0,09	0,31
43.	leucine	0,97	0,90	1,23	**1,56**	0,92	1,29	0,80	0,11	0,05	0,16	0,08	0,13	0,12	0,08
44.	lignoceric acid	1,10	0,97	0,90	1,03	1,10	1,16	1,12	0,07	0,05	0,12	0,08	0,12	0,19	0,10
45.	lysine	0,76	0,80	0,96	**1,50**	1,04	1,08	0,76	0,12	0,20	0,31	0,31	0,15	0,17	0,19
46.	malic acid	0,89	0,99	**1,61**	**1,62**	**1,33**	**1,66**	1,26	0,06	0,12	0,09	0,07	0,14	0,08	0,14
47.	methionine	0,95	**1,36**	**1,34**	**1,55**	0,85	1,19	0,84	0,12	0,10	0,20	0,18	0,11	0,09	0,07
48.	myristic acid	0,94	0,85	0,88	0,87	1,03	0,98	1,08	0,13	0,05	0,13	0,23	0,11	0,07	0,23
49.	nicotinic acid	1,09	1,08	1,13	1,28	1,23	0,93	1,10	0,12	0,06	0,06	0,20	0,07	0,09	0,20
50A.	octadecanol	0,92	1,14	0,79	0,94	0,96	0,78	1,29	0,12	0,16	0,05	0,04	0,21	0,07	0,32
50B.	octadecenoic acid1	0,85	0,94	**0,61**	0,85	0,93	0,82	**1,59**	0,15	0,11	0,12	0,22	0,11	0,12	0,58
51.	octadecenoic acid2	0,91	0,95	0,81	0,76	0,96	0,77	1,14	0,08	0,08	0,05	0,08	0,04	0,23	0,20
52.	octanol	1,01	0,80	0,78	0,97	0,98	0,96	0,74	0,24	0,15	0,08	0,09	0,23	0,49	0,15
53.	oxalic acid	**0,54**	0,90	1,03	0,96	0,84	0,84	1,06	0,04	0,13	0,29	0,19	0,17	0,20	0,08
54.	oxamic acid	**0,57**	1,21	1,06	0,78	0,92	0,93	**0,62**	0,15	0,32	0,21	0,05	0,08	0,06	0,06
55.	oxoproline	0,96	0,91	0,95	0,98	1,04	1,02	1,29	0,05	0,09	0,07	0,22	0,06	0,09	0,26
56.	palmitic acid	1,03	0,86	0,94	0,89	1,00	0,95	1,12	0,08	0,03	0,07	0,15	0,11	0,14	0,16
57.	pelargonic acid	1,29	**0,69**	0,82	1,08	0,93	1,17	0,91	0,25	0,07	0,07	0,13	0,13	0,39	0,15

No.	Identified compounds	MEAN F1s / Ps							SE						
		mature	12 HAS	24 HAS	36 HAS	48 HAS	72 HAS	96 HAS	mature	12 HAS	24 HAS	36 HAS	48 HAS	72 HAS	96 HAS
58.	phenylalanine	0,76	1,08	1,07	**1,36**	0,85	0,84	0,90	0,06	0,07	0,10	0,13	0,04	0,05	0,05
59.	phosphate	0,92	1,02	1,13	1,23	0,99	1,26	1,03	0,13	0,13	0,08	0,25	0,07	0,08	0,11
60.	picolinic acid	0,71	1,15	**0,68**	0,86	0,96	0,86	1,00	0,10	0,19	0,15	0,09	0,10	0,11	0,14
61.	proline	0,85	1,07	**1,40**	**1,57**	1,08	**1,42**	**0,55**	0,05	0,12	0,24	0,08	0,14	0,19	0,11
62.	propanedioic acid 2-ethyl (put.)	0,73	0,95	1,03	1,13	1,02	0,88	0,86	0,11	0,21	0,18	0,16	0,09	0,16	0,13
63.	rhamnose	1,00	1,03	1,02	**1,44**	0,77	0,87	0,73	0,14	0,14	0,16	0,23	0,08	0,13	0,08
64.	serine	0,93	1,25	1,19	1,29	0,96	1,02	1,06	0,13	0,05	0,13	0,02	0,08	0,08	0,08
65.	sinapinic acid	1,04	1,20	1,13	1,24	1,14	0,85	0,96	0,11	0,14	0,07	0,16	0,03	0,05	0,07
66.	sitosterol	0,95	1,09	1,06	1,01	1,04	1,08	1,00	0,07	0,08	0,11	0,11	0,02	0,17	0,09
67.	sorbitol	0,88	1,16	0,71	0,95	0,99	1,19	0,99	0,06	0,36	0,15	0,18	0,09	0,23	0,15
68.	stearic acid	1,02	0,81	0,87	0,95	0,99	0,79	1,01	0,09	0,06	0,03	0,05	0,20	0,12	0,10
69.	succinic acid	**1,36**	1,07	1,05	1,07	0,87	1,23	**1,31**	0,55	0,21	0,12	0,16	0,05	0,19	0,07
70.	sucrose	0,88	1,17	1,06	1,13	1,05	**0,69**	**0,67**	0,11	0,13	0,14	0,13	0,09	0,09	0,21
71.	threonine	0,85	1,24	1,20	**1,48**	1,05	1,14	1,12	0,08	0,09	0,14	0,08	0,08	0,09	0,24
72.	tryptophane	0,84	0,89	0,93	1,09	1,02	**0,66**	**0,41**	0,08	0,11	0,05	0,03	0,12	0,03	0,11
73.	tyrosine	0,81	1,19	1,19	**1,67**	0,93	0,90	0,79	0,05	0,14	0,13	0,07	0,11	0,07	0,06
74.	urea	1,28	0,84	1,10	1,24	1,18	0,83	**1,57**	0,21	0,10	0,20	0,24	0,11	0,14	0,54
75.	valine	0,85	1,12	**1,31**	**1,50**	1,05	1,28	0,94	0,06	0,08	0,26	0,06	0,10	0,15	0,07

Legend:
MEAN F1s/Ps – ratio of mean values of hybrids to mean values of parental lines from the peak area detected in GC-MS
HAS – hours after sowing
N/A – only one or two biological replicates analysed
Red marks: over 30% increase of the compound level in hybrids
Blue marks: over 30% decrease of the compound level in hybrids

Table 2. The list of the 103 compounds classified according to their chemical group only

No.	Chemical Class	MEAN F1s / Ps							SE						
		mature	12 HAS	24 HAS	36 HAS	48 HAS	72 HAS	96 HAS	mature	12 HAS	24 HAS	36 HAS	48 HAS	72 HAS	96 HAS
1.	acid001	0,71	0,87	0,89	1,20	**1,49**	0,88	0,87	0,09	0,05	0,22	0,26	0,37	0,10	0,08
2.	acid002	0,84	1,08	0,81	1,03	0,98	0,85	1,12	0,11	0,04	0,02	0,05	0,09	0,04	0,12
3.	acid003	1,04	0,92	1,07	1,00	0,95	0,85	1,18	0,18	0,17	0,11	0,02	0,09	0,11	0,14
4.	acid004	0,99	0,77	0,94	0,86	1,04	1,07	0,88	0,06	0,10	0,10	0,05	0,11	0,24	0,11
5.	acid005	0,95	0,88	**1,81**	**1,67**	0,70	0,79	0,99	0,06	0,04	0,30	0,20	0,05	0,05	0,04
6.	acid006	0,99	0,84	0,93	1,06	1,01	0,77	1,01	0,08	0,03	0,22	0,31	0,03	0,04	0,08
7.	acid007	**0,65**	0,81	1,23	0,94	0,81	0,99	1,24	0,06	0,06	0,18	0,05	0,13	0,04	0,32
8.	acid008	1,02	0,80	0,73	0,82	0,89	0,81	0,89	0,07	0,04	0,11	0,15	0,09	0,12	0,14
9.	acid009	0,70	0,99	1,01	0,91	0,79	1,12	1,11	0,11	0,21	0,07	**NA**	0,06	0,05	0,12
10.	acid010	1,01	0,99	0,87	1,12	1,25	1,02	1,07	0,11	0,05	0,09	0,04	0,16	0,14	0,06
11.	acid014	**1,71**	**1,91**	1,06	**1,93**	**0,62**	0,85	1,01	0,67	0,69	0,21	0,15	0,23	0,28	0,39
12.	amino acid001	0,86	1,21	0,78	1,17	1,01	0,85	1,03	0,04	0,01	0,02	0,16	0,10	0,12	0,16
13.	amino acid002	0,85	1,17	0,79	1,14	0,99	0,82	1,01	0,01	0,00	0,01	0,14	0,06	0,10	0,16
14.	amine001	**0,64**	0,80	1,17	1,12	0,85	**0,53**	0,95	0,13	0,08	0,34	0,26	0,11	0,16	0,19
15.	amine002	1,07	0,80	1,15	1,09	0,89	**0,59**	1,09	0,03	0,08	0,23	0,24	0,12	0,11	0,20
16.	amine003	0,98	0,90	1,18	1,21	0,92	0,86	0,86	0,08	0,15	0,11	0,13	0,12	0,24	0,19
17.	amine004	1,01	0,90	1,05	0,93	1,11	0,78	**0,66**	0,18	0,09	0,08	0,09	0,21	0,01	0,16
18.	amine005	1,05	0,82	1,02	0,89	0,88	0,80	0,99	0,26	0,10	0,11	0,03	0,07	0,10	0,09
19.	amine006	0,78	0,81	1,01	0,77	1,02	1,14	**0,28**	0,19	0,12	0,16	0,07	0,19	0,30	0,08
20.	amine007	**0,63**	1,05	0,91	1,02	1,16	0,95	1,00	0,06	0,20	0,28	0,42	0,21	0,11	0,26
21.	amine008	1,06	1,00	1,08	**1,46**	1,24	0,70	**0,58**	0,36	0,02	0,07	0,42	0,04	0,13	0,20
22.	amine009	0,82	**1,77**	**1,57**	**1,54**	**1,54**	**2,09**	0,74	0,20	0,36	0,38	0,73	0,81	0,58	0,17
23.	amine010	**4,38**	0,80	**0,61**	0,93	**10,17**	0,79	0,97	2,90	0,08	0,14	0,03	9,46	0,15	0,15
24.	amine011	0,85	0,77	**1,36**	0,85	1,29	1,00	**0,66**	0,09	0,06	0,21	0,07	0,40	0,20	0,13
25.	amine013	0,92	**0,62**	1,00	0,98	1,02	0,90	0,90	0,15	0,14	0,21	0,20	0,06	0,11	0,19
26.	amine014	0,94	1,02	0,85	0,82	1,01	0,78	0,99	0,12	0,26	0,13	0,10	0,08	0,15	0,15
27.	amine015	0,73	1,03	0,96	1,00	1,16	0,98	0,72	0,10	0,33	0,22	0,12	0,24	0,22	0,11
28.	amine016	**0,44**	**1,32**	0,95	0,84	0,91	0,92	**0,68**	0,10	0,36	0,22	0,15	0,30	0,10	0,18
29.	amine017	0,81	0,82	0,75	0,78	1,09	**0,65**	1,25	0,08	0,01	**NA**	**NA**	0,11	0,06	0,37
30.	amine018	0,94	**1,39**	1,07	1,21	**1,48**	**1,89**	1,30	0,11	0,24	0,16	0,17	0,37	0,23	0,62
31.	amine019	0,93	**1,81**	**1,79**	**1,69**	1,27	1,19	1,10	0,13	0,43	0,20	0,09	0,20	0,18	0,18
32.	amine022	0,85	1,15	0,96	1,06	**1,38**	**1,41**	0,95	0,06	0,11	0,11	0,10	0,26	0,09	0,07

No.	Chemical Class	MEAN F1s / Ps							SE						
		mature	12 HAS	24 HAS	36 HAS	48 HAS	72 HAS	96 HAS	mature	12 HAS	24 HAS	36 HAS	48 HAS	72 HAS	96 HAS
33.	amine023	0,85	1,26	**1,55**	1,22	1,30	1,13	**1,46**	0,11	0,14	0,04	0,13	0,24	0,06	0,42
34	amine024	0,89	1,02	**1,51**	1,03	1,06	0,86	**1,32**	0,09	0,15	0,32	0,09	0,10	0,06	0,09
35.	amine025	1,02	1,06	1,10	0,94	1,19	**1,31**	1,17	0,13	0,16	0,12	0,09	0,13	0,28	0,25
36.	amine026	0,99	1,02	**1,34**	0,83	**2,40**	1,19	**0,44**	0,34	0,29	0,49	0,23	0,08	0,27	0,01
37.	amine027	0,74	0,98	0,92	0,86	0,97	1,00	**0,66**	0,18	0,19	0,13	0,05	0,19	0,23	0,13
38.	amine028	**1,35**	0,91	0,80	0,95	0,82	0,81	0,87	0,26	0,10	0,03	0,04	0,11	0,13	0,07
39.	amine029	1,26	0,87	0,86	0,93	0,78	0,82	0,87	0,21	0,10	0,04	0,03	0,11	0,15	0,04
40.	cho001	0,92	0,74	1,04	1,14	0,94	0,73	0,94	0,11	0,08	0,10	0,34	0,05	0,05	0,03
41.	cho002	0,95	1,04	1,13	1,23	1,02	1,24	**0,67**	0,28	0,21	0,09	0,29	0,17	0,30	0,17
42.	cho003	1,05	1,19	1,00	1,07	1,14	0,92	0,79	0,08	0,08	0,07	0,09	0,09	0,06	0,07
43.	cho005	0,93	1,15	0,87	1,26	0,93	0,84	0,95	0,15	0,07	0,06	0,26	0,03	0,04	0,09
44.	cho006	1,25	0,95	0,82	1,05	0,93	0,96	0,87	0,20	0,06	0,14	0,06	0,06	0,13	0,17
45.	cho007	1,11	0,88	1,00	**1,62**	0,87	1,09	0,75	0,32	0,03	0,21	0,61	0,07	0,23	0,07
46.	cho008	1,03	1,02	0,90	**1,40**	0,89	0,99	1,03	**NA**	0,13	0,06	0,23	0,10	0,12	0,23
47.	cho009	0,73	0,78	0,94	0,86	0,90	0,73	1,03	0,02	0,14	0,26	0,00	0,04	0,09	0,18
48.	cho010	**1,37**	1,12	1,05	0,87	0,82	**1,62**	0,88	0,52	0,07	0,13	0,08	0,05	0,38	0,40
49.	cho011	1,05	1,05	0,99	0,88	0,96	**1,58**	1,00	0,17	0,08	0,10	0,06	0,08	0,17	0,35
50.	cho012	**1,71**	1,00	1,06	0,93	0,88	**1,47**	0,94	0,74	0,07	0,08	0,10	0,09	0,23	0,37
51.	cho013	1,00	1,24	**1,57**	0,79	1,01	1,17	0,75	0,04	0,08	0,40	0,08	0,12	0,44	0,36
52.	cho014	0,89	**1,31**	1,24	0,86	0,99	1,29	1,10	0,08	0,18	0,14	0,11	0,13	0,67	0,37
53.	cho015	**0,51**	**1,53**	0,85	1,28	0,75	0,81	0,89	0,07	0,54	0,50	0,19	0,09	0,03	0,19
54.	cho016	0,90	1,09	1,09	1,03	1,16	1,05	0,71	0,20	0,11	0,20	0,07	0,16	0,17	0,29
55.	cho017	0,86	1,13	1,11	0,99	0,96	0,91	0,97	0,06	0,06	0,14	0,09	0,03	0,09	0,07
56.	cho018	0,94	1,10	1,10	0,78	0,88	**0,69**	1,05	0,12	0,10	0,26	0,16	0,13	0,07	0,21
57.	cho019	0,71	1,20	**1,32**	**1,50**	1,01	**1,45**	0,82	0,17	0,20	0,28	0,39	0,24	0,42	0,25
58.	cho020	0,95	1,09	1,11	0,81	0,86	0,75	1,04	0,11	0,11	0,26	0,17	0,12	0,08	0,25
59.	cho021	0,87	1,17	1,11	1,29	0,98	**1,47**	0,78	0,27	0,21	0,09	0,33	0,20	0,29	0,15
60.	cho022	**1,35**	**1,41**	1,25	0,85	1,04	1,28	0,85	0,44	0,18	0,13	0,12	0,15	0,64	0,42
61.	cho023	**1,31**	0,92	1,23	1,26	1,06	1,24	**0,61**	0,58	0,31	0,16	0,26	0,19	0,28	0,14
62.	cho024	0,93	1,12	1,21	1,07	0,95	0,81	0,74	0,22	0,07	0,14	0,11	0,05	0,03	0,06
63.	cho025	0,77	0,73	0,77	1,04	0,93	0,78	1,07	0,10	0,08	0,21	0,25	0,08	0,36	0,06
64.	cho026	0,88	1,02	1,10	1,29	0,99	1,16	0,74	0,29	0,27	0,19	0,42	0,18	0,29	0,20
65.	cho027	0,83	1,26	1,04	1,18	0,96	1,13	0,78	0,21	0,25	0,21	0,27	0,16	0,21	0,17

No.	Chemical Class	MEAN F1s / Ps							SE						
		mature	12 HAS	24 HAS	36 HAS	48 HAS	72 HAS	96 HAS	mature	12 HAS	24 HAS	36 HAS	48 HAS	72 HAS	96 HAS
66.	cho028	0,88	0,93	1,08	1,17	0,87	1,20	0,75	0,32	0,26	0,23	0,32	0,15	0,37	0,22
67.	cho029	0,91	**1,31**	**0,64**	1,04	0,80	**0,54**	**1,31**	0,14	0,26	0,05	0,17	0,05	0,04	0,51
68.	cho030	0,92	1,15	1,25	**1,41**	1,17	**1,31**	0,77	0,29	0,25	0,15	0,32	0,23	0,31	0,27
69.	cho031	0,86	0,88	1,04	1,00	0,78	0,79	1,02	0,21	0,09	0,13	0,10	0,05	0,09	0,29
70.	cho032	0,95	1,07	0,83	0,96	0,97	0,82	1,15	0,02	0,14	0,08	0,14	0,07	0,06	0,28
71.	cho033	1,00	0,78	0,74	1,19	1,13	**0,61**	**3,83**	0,13	0,08	0,07	0,33	0,43	0,15	2,35
72.	cho034	1,06	**1,33**	**1,73**	**1,84**	**1,68**	**1,35**	1,22	0,28	0,06	0,19	0,22	0,09	0,17	0,15
73.	cho035	0,83	1,23	1,30	**1,60**	1,19	0,98	0,76	0,03	0,49	0,31	0,38	0,23	0,29	0,09
74.	cho036	1,16	0,89	0,77	**1,45**	1,02	0,75	0,90	0,26	0,17	0,10	0,35	0,36	0,13	0,15
75.	cho037	1,02	1,26	0,96	1,06	0,96	0,70	0,98	0,02	0,11	0,05	0,12	0,18	0,11	0,18
76.	cho038	0,72	0,71	**0,69**	**0,55**	**0,54**	**0,54**	**0,65**	0,14	0,04	0,08	0,08	0,06	0,01	0,04
77.	cho039	0,89	1,09	0,87	**1,51**	**1,40**	**1,68**	0,86	0,13	0,06	0,19	0,47	0,24	0,40	0,23
78.	cho040	0,83	0,82	**2,89**	0,71	0,92	0,87	0,98	0,10	0,01	2,16	0,22	0,34	0,11	0,15
79.	cho041	**1,60**	0,93	**1,39**	**2,31**	0,97	0,79	**0,55**	0,67	0,22	0,56	1,19	0,29	0,32	0,17
80.	cho042	0,86	1,07	0,85	1,20	0,82	0,99	**3,06**	0,11	0,12	0,10	0,33	0,12	0,16	1,37
81.	cho043	1,27	**1,47**	1,13	1,25	**1,54**	1,16	1,01	0,20	0,41	0,19	0,31	0,30	0,18	0,27
82.	cho044	**1,46**	**2,07**	1,19	**1,80**	1,24	0,88	**0,64**	0,61	0,92	0,19	0,99	0,14	0,17	0,15
83.	cho045	0,99	0,94	0,88	1,13	0,89	**1,53**	0,86	0,18	0,20	0,16	0,41	0,13	0,15	0,09
84.	cho046	0,80	**1,70**	0,79	**2,57**	**2,24**	0,88	0,82	0,11	0,47	0,12	1,35	1,63	0,14	0,03
85.	cho047	1,09	1,16	**1,72**	1,19	**1,44**	**1,67**	0,87	0,23	0,40	0,70	0,07	0,32	0,54	0,07
86.	cho048	0,98	0,83	1,29	0,78	1,06	**1,32**	1,15	0,03	0,08	0,32	0,03	0,12	0,19	0,26
87.	cho051	1,28	1,05	0,96	**1,49**	**1,46**	0,75	0,76	0,50	0,28	0,38	0,22	0,58	0,05	0,00
88.	cho052	**3,55**	1,24	0,72	1,19	0,99	**0,59**	**0,62**	2,38	0,28	0,27	0,09	0,19	0,20	0,10
89.	cho054	1,07	0,94	0,98	**1,75**	**1,41**	**0,67**	**0,58**	0,23	0,30	0,26	0,66	0,39	0,11	0,22
90.	cho055	1,03	0,97	0,96	1,24	1,12	0,78	**0,58**	0,12	0,12	0,08	0,23	0,26	0,11	0,03
91.	cho056	1,08	0,81	1,00	**1,42**	**1,44**	0,74	**0,65**	0,24	0,20	0,22	0,42	0,48	0,23	0,10
92.	cho057	0,92	0,85	**1,53**	**1,68**	**1,69**	0,77	0,98	0,12	0,10	0,49	0,73	0,63	0,20	0,30
93.	cho058	0,98	0,94	**4,31**	**1,55**	**1,68**	0,95	0,98	0,11	0,14	2,16	0,82	**NA**	0,05	0,38
94.	cho060	0,86	0,81	0,88	1,08	0,98	**0,40**	0,92	0,07	0,12	0,09	0,23	0,27	0,07	0,24
95.	cho062	1,17	1,09	0,98	1,11	**1,39**	0,77	0,95	0,11	0,34	0,28	0,13	0,25	0,18	0,34
96.	cho063	1,15	1,12	1,28	0,97	**1,34**	0,75	**0,52**	0,22	0,12	0,30	0,11	0,41	0,14	0,08
97.	cho065	0,86	0,94	1,03	1,05	0,98	0,84	1,23	0,07	0,10	0,13	0,12	0,09	0,09	0,46
98.	cho066	0,97	1,07	0,98	**0,66**	**1,35**	0,85	**0,62**	0,14	0,08	0,23	0,18	0,63	0,23	0,05

No.	Chemical Class	MEAN F1s / Ps							SE						
		mature	12 HAS	24 HAS	36 HAS	48 HAS	72 HAS	96 HAS	mature	12 HAS	24 HAS	36 HAS	48 HAS	72 HAS	96 HAS
99.	cho067	0,81	1,01	0,91	**1,38**	**1,31**	0,84	**0,69**	0,04	0,14	0,15	0,34	0,35	0,07	0,11
100.	cho069	**2,23**	**1,95**	1,27	**1,82**	**2,80**	0,88	0,89	0,77	0,60	0,27	0,26	1,82	0,27	0,37
101.	indol deriv001	0,88	1,16	1,15	1,01	0,86	**1,48**	0,86	0,20	0,24	0,15	0,08	0,18	0,28	0,06
102.	indol deriv002	**0,33**	**1,62**	**1,49**	0,80	0,96	**1,68**	**0,47**	0,10	0,50	0,50	0,16	0,34	0,48	0,06
103.	indol deriv003	**1,64**	1,23	0,78	**1,38**	**1,68**	1,18	**1,66**	0,64	0,09	**NA**	0,35	0,08	0,01	**NA**

Legend:
MEAN F1s/Ps – ratio of mean values of hybrids to mean values of parental lines from the peak area detected in GC-MS
HAS – hours after sowing
N/A – only one or two biological replicates analysed
Red marks: over 30% increase of the compound level in hybrids
Blue marks: over 30% decrease of the compound level in hybrids

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Journal of Heredity 2008, 99 (4): 396-406
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