Analysis of allelic gene effects in *Arabidopsis thaliana* biomass heterosis and metabolism

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Abbreviations

AGRIS	International System for Agricultural Science and Technology
BLAST	Basic local alignment search tool
bp	Base pair
cDNA	Complementary deoxyribonucleic acid
Chr	Chromosome
Col-0	Columbia-0 (*Arabidopsis* accession)
CTAB	Cetyltrimethyl ammonium bromide
DAS	Day after sowing
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
GC-MS	Gas chromatography mass spectrometry
GUS	ß-glucuronidase
IL	Introgression line
InDels	Insertions and deletions
Kb	Kilo base pair
LA	Leaf area
LB medium	Luria-Bertani medium
LI	Inserts to the left border of T-DNA
mQTL	Metabolite quantitative trait loci
mRNA	Messenger ribonucleic acid
MS medium	Murashige and Skoog medium
NAD(P)	Nicotine amide dinucleotide (phosphate)
NIL	Near isogenic line
OD	Optical density
ORF	Open reading frame
PCR	Polymerase chain reaction
qPCR	Quantitative real-time PCR
QTL	Quantitative trait locus
RH	Relative humidity
RI	Inserts to the right border of T-DNA
RIL	Recombinant inbred line
rpm	Revolutions per minute
SD	Standard deviation
SDS	Sodium dodecyl sulfate
SE	Standard error
siRNA	Small interfering RNA
SNP	Single nucleotide polymorphism
TCA	Tricarboxylic acid cycle
Tris	Tris(hydroxymethyl)aminomethane
TSS	Transcription start sequence
UTR	Untranslated region
v/v	Mass concentration (volume/volume)
w/v	Mass concentration (mass/volume)
YEP medium	Yeast extract peptone medium

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1 Introduction

1.1 General introduction

1.1.1 Allelic variation

An allele is an alternative form of a gene at a particular locus, and different alleles produce variation in the traits. Alleles can differ in expression, in property of the protein encoded or in patterns of inheritance. Differences can arise where sequence or epistatic polymorphisms are present. If the polymorphisms are in regulatory sequences, the alleles might express at different times and at different levels under the same stimulus or each allele might respond to a different stimulus. If polymorphic sequences are in the protein coding sequence, the properties of the protein encoded might differ. Allele-specific expression in plants closely correlates with polymorphisms between alleles in regulatory sequences (Swanson-Wagner et al. 2006, Zhang et al. 2008, Zhang and Borevitz 2009). Alleles of stress responding genes in a maize hybrid were differently regulated to various environmental conditions and at various developmental stages. The authors speculated that broad adaptability in adverse conditions and consequently enhanced qualities of hybrids might be caused by combinations of alleles which are differently regulated under certain stimuli (Guo et al. 2004). Various alleles of many genes and the variability of resulting phenotypic characteristics were identified in plants. Some important examples are *FRIGIDA* (*FRI*) and *FLOWERING LOCUS C* (*FLC*) for flowering time in *Arabidopsis* (Gazzani et al. 2003), *ACCELERATED CELL DEATH 6* (*ACD6*) for hybrid necrosis in *Arabidopsis* (Todesco et al. 2014), epistatic alleles of *AGAMOUS-LIKE6* for axillary bud formation in *Arabidopsis* (Huang et al. 2012), *Ghd7* for yield related traits in rice (Xue et al. 2008), *SINGLE FLOWER TRUSS* (*SFT*) for yield in tomato (Krieger et al. 2010), and *GIGANTEA* (*GI*) for circadian period in *Brassica rapa* (Xie et al. 2015). Naturally occurring allelic diversity in plants has been regarded as an important genetic source for phenotypic variation (Xue et al. 2008, Huang et al. 2012) and evolutionary fitness (Gazzani et al. 2003, Todesco et al. 2014, Xie et al. 2015). Therefore, identification and characterization of alleles has an informative value for knowledge on the evolutionary history of plants. Genetic information on the characterized alleles has practical value as a useful genetic marker in plant breeding practice.

1.1.2 Heterosis and hybrid plants applied in agriculture

The term 'heterosis' was introduced by Shull (1914) to simplify the phrase 'stimulation of heterozygosis'. Heterosis describes the phenomenon that F1 hybrids have better biological qualities than those of inbred parents, such as greater vegetative growth, higher fertility, increased yield, faster development and enhanced stress tolerance. Hybrid plants are used extensively in agriculture worldwide because of their beneficial traits. Growing hybrid crops is considered the most efficient way to increase yield production without increasing area, and hybrid maize and rice are the best examples. Since the commercial application of hybrid maize in agriculture in the 1930s, maize production had steadily increased six-fold by 1997 (reviewed by Crow 1998). Several studies showed that 50% of the increase was due to improvements of maize genotype (reviewed by Crow 1998). Currently, hybrid maize is planted in 60% of the world's maize fields and in 98% of US maize fields (Duvick 2005). Rice feeds half of the world population and is one of the most consumed staple crops in the world. Heterosis in rice had been noticed long time ago, but due to the self-pollination character of rice, application of hybrid rice was restricted. After introducing a male-sterile gene found in wild rice into cultivated rice, hybrid rice has been planted widely in China (reviewed by Cheng et al. 2007). Hybrid rice is cultivated in about 57% of rice fields in China, and resulted in a 20 % increase in yield (7500 kg/hm^2) compared to conventional rice (6150 kg/hm^2)(Yuan 2014). Growing hybrid plants is also preferred to inbred lines for their better adaptability against adverse abiotic and biotic environments. Hybrid maize is more resistant against drought stress than open-pollinated varieties (Crabb 1947). Hybrid rice is more resistant against pathogens and more tolerant to drought stress (Wang et al. 2008, Villa et al. 2012). Hybrid sorghum can survive in a wider spectrum of temperature by sustaining a higher carbon exchange rate (Blum et al. 1990, Yu et al. 2004).

1.1.3 Genetic models for heterosis and the supporting examples

1.1.3.1 Dominance

The dominance hypothesis was proposed by Davenport (1908), Bruce (1910), and Keeble and Pellew (1910), and conceptually established by Jones (1917). According to the dominance model, heterosis is caused by the masking of one parent's deleterious recessive allele(s) by the other parent's favorable dominant allele(s) in a heterozygous state. In numerous QTL studies on heterosis traits of several crops, plentiful dominant loci were reported (reviewed by Kaeppler 2012). Dominance was identified at the single gene level in elite rice: Substitution

of the fully functional Minghui 63 (M63, *Oryza sativa*) Ghd7 allele for the non-functional Zhenshan 97 (Z97, *Oryza sativa*) Ghd7 allele in a Z97 genomic background lead to increased yield, and RILs with a homozygous M63 Ghd7 locus in Z97 genomic background produced more yield than lines with heterozygous Ghd7 locus (M63 Ghd7/Z97 Ghd7) in a Z97 genomic background (Xue et al. 2008, Zhou et al. 2012). Increased yield in crosses of Z97 and M63 is caused by the presence of a dominant M63 Ghd7 allele, but not by an interaction between the M63 Ghd7 and Z97 Ghd7 alleles (Zhou et al. 2012). Dominance in hybrid maize, however, seems to be ascribed to more dynamic polymorphisms in genetic contents between parental lines, not only by allelic variation but also by presence/absence variation (PAV) and gene copy number variation (CNV). Fu and Dooner (2002) showed the intraspecific violation of genetic colinearity in the *bz* genomic region between B73 and Mo17 whose combination displays strong heterosis: the number of genes and intergenic retrotransposon clusters varied. The strong structural diversity between B73 and Mo17 was demonstrated by whole genome microarray-based comparative genomic hybridization: Several hundred CNV sequences and several thousand presence/absence variations (PAV) were present between the two genotypes. PAVs contained hundreds of expressed genes which may contribute to heterosis (Springer et al. 2009)

1.1.3.2 Overdominance

The overdominance hypothesis was postulated by Shull (1908), East (1908) and Crow (1948), and explains that intra-allelic interaction leads to superior performance of hybrids compared to either of the homozygous allelic states of their inbred parents. The overdomince model takes into account that heterosis is still displayed in an equally high magnitude in crosses of inbred lines which had been purified toward omitting deleterious alleles. Even though overdominance has been reported in many heterosis QTL studies, very few cases support true overdominance. The first molecular example was reported for tomato yield (Krieger et al. 2010). Heterozygosity of the functional *SFT* allele with the nonfunctional allele (*sft*) in hybrid tomato leads to a dramatic increase in fruit yield. *SFT* is orthologous to *Arabidopsis Flowering locus T* (*FT*) which functions in flowering hormone 'florigen' production (Shalit et al. 2009). The increased yield of the hybrid tomato was caused by a combination of an increased flower number from the wild type (*SFT*/*SFT*) and a shifted reproductive stage from the mutant (*sft*/*sft*) (Krieger et al. 2010).

1.1.3.3 Epistasis

Epistasis explains that hybrid vigor is caused by newly formed interactions between non-allelic genes which were originated independently from parents (Powers 1944). Therefore the sum of single locus variations cannot satisfy the whole magnitude of the trait of interest (Phillips 1998). The importance of epistasis for hybrid vigor has been highly emphasized in several QTL studies (Monforte and Tanksley 2000, Luo et al. 2001, Carlborg and Haley 2004, Zhou et al. 2012). Yu et al (1997) stated that digenic interactions play a major role in heterosis in rice hybrid: A QTL analysis was carried out on yield and yield-related components in elite hybrid rice in two ways, single-locus level and two-locus level, with an identical data set. Most of QTLs which were detected from two-locus level analysis were not detectable in a single-locus basis. The most representative molecular example of epistasis for biomass heterosis in *Arabidopsis* is an interaction between two flowering time related genes, *FRI* and *FLC* (Burn et al. 1993, Lee et al. 1993, 1994, Koornneef et al. 1994, Sanda and Amasino 1996). *FLC* encodes a repressor of flowering time whose expression is activated by functional *FRI* alleles (Lee et al. 1994, Koornneef et al. 1994). Therefore, the presence of the functional C24 *FRI* allele and the functional Col-0 *FLC* allele in crosses of Col-0 and C24 resulted in delayed flowering (Henderson et al. 2003), and consequently the hybrids grew larger by prolonged vegetative growth than Col-0 and C24 (Moore and Lukens 2011).

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1.1.3.4 Epigenetic modification

In general, the degree and frequency of heterosis positively correlates with genetic distance between parent lines (reviewed by Birchler et al. 2010; Chen 2010). This is because hybrids between two divergent plants have more enriched genetic materials in gene number and types of alleles than their parents and have more chances to build up new genetic networks or to change the flux in metabolic pathways by inflow of new types of alleles or genes. However, crosses between intraspecies plants which have fewer genetic differences also exhibit substantial heterosis. There are reports that the genetic distance between parental lines does not correlate with the degree of heterosis (Meyer et al. 2004, Zang et al. 2010).

Allelic variations can also be provided by epi-alleles in hybrid whose states of gene expression are altered by epigenetic modification, such as DNA methylation, histone modification and RNA-directed DNA methylation (RdDM) (Henderson and Jacobsen 2007, Ni et al. 2009, Ha et al. 2009, Groszmann et al. 2011, Shen et al. 2012). Research on

epigenetic modifications in heterosis in *Arabidopsis* found hybrid specific methylation patterns in regions that are differentially methylated between the two parents and covered with small RNA (Groszmann et al. 2011, Shen et al. 2012, Fujimoto et al. 2012). An important role of epigenetic modification in biomass heterosis of an interspecific *Arabidopsis* hybrid was demonstrated by Ni et al. (2009): Epigenetic repression of two circadian oscillators *CIRCADIAN CLOCK ASSOCIATED 1* (*CCA1*) and *LATE ELONGATED HYPOCOTYL* (*LHY*) in allopolyploids triggered the activation of their reciprocal regulators *TIMING OF CAB EXPRESSION 1* (*TOC1*), *GI* and evening element-containing downstream genes which were involved in chlorophyll and starch metabolic pathways. Consequently chlorophyll and starch synthesis increased in the allopolyploid (Ni et al. 2009).

1.1.4 Information on mechanisms of biomass heterosis in *Arabidopsis*

Like in other plant species, heterosis was also observed in crosses of *Arabidopsis* in several traits: Rosette diameter (Barth et al. 2003), stem length and biomass (Rédei 1962, Corey et al. 1976, Sharma et al. 1979, Barth et al. 2003, Meyer et al. 2004), photosynthetic efficiency (Sharma et al. 1979), seed number (Alonso-Blanco et al. 1999), and phosphate efficiency (Narang and Altmann 2001) have been reported. Many studies focused on biomass heterosis in *Arabidopsis* (Meyer et al. 2004, 2012, Ni et al. 2009, Groszmann et al. 2011, Shen et al. 2012, Fujimoto et al. 2012). Increased shoot biomass in *Arabidopsis* hybrids has been attributed to an extended vegetative growth period by interacting functional *FLC* and *FRI* alleles in a hybrid (described in 1.1.2.3) (Moore and Lukens 2011). However, biomass heterosis is also noticeable at early vegetative stages and the early growth vigor in hybrid is triggered independently from the flowering pathway: Hybrids which flower earlier by vernalization treatment or by introduced non-functional *FLC* allele show still a substantial degree of heterosis on biomass (Fujimoto et al. 2012). Rather, the incident of biomass heterosis in *Arabidopsis* at early developmental stages may be caused by consecutive cascades of multigene activity responding to underlying regulatory controls such as photosynthesis, cell growth and circadian clock. Biomass heterosis is associated with increased photosynthesis ability in hybrids: light intensity positively correlates with the degree of biomass heterosis (Meyer et al. 2004), and hybrid plants synthesize more starch and chlorophyll than parents in the same environmental conditions (Ni et al. 2009, Fujimoto et al. 2012). In transcription analyses in *Arabidopsis* hybrids 3-7 days after sowing, genes encoding chloroplast-located proteins were significantly overrepresented among the genes with greater

than mid-parent expression values in the hybrid, and many of these genes are involved in chlorophyll biosynthesis and photosynthesis (Fujimoto et al. 2012). However, the increased photosynthetic ability in hybrid plant is not caused by enhanced photosynthetic capacity per leaf area, but by an increase in the number of chloroplasts by increase in leaf area (Fujimoto et al. 2012). Increases in cell size and number were observed in *Arabidopsis* hybrids (Fujimoto et al. 2012, Meyer et al. 2012), which suggests that hybrid plants are superior in cell divisions and expansion for leaf growth. Auxin signaling pathway, which is fundamental for growth, development, cell division and cell enlargement, was also proposed to be involved in biomass heterosis in *Arabidopsis* (Shen et al. 2012): Indole acetic acid (IAA) was transported considerably faster in F1 hybrids than in either of the parents. An auxin transporter regulator *vacuolar H^+-pyrophosphatase* (*AVP1*) and a downstream gene in the auxin signaling pathway *ARGOS*, whose overexpression resulted in increased organ size, were expressed higher in the hybrid than in the parents at 15 DAS (Lohmann and Weigel 2002, Li et al. 2005, Pasapula et al. 2011).

1.1.5 Occurrence and roles of fumarase isoforms in plants

Fumarase (fumarate hydratase) is an enzyme that catalyzes the reversible hydration/dehydration of fumarate to malate (Bock and Alberty 1953). Fumarase occurs in a mitochondrial and a cytosolic form. Synthesis and localization of the two forms are achieved by different mechanisms in different organisms (Akiba et al. 1984, Tuboi et al. 1990, Stein et al. 1994, Knox et al. 1998, Karniely et al. 2006, Yogev et al. 2007). The mitochondrial fumarase participates in the TCA cycle which is a series of chemical reactions generating energy through oxidation of carbohydrates. Mitochondrial fumarases are found in all aerobic organisms and their protein structures and amino acid sequences are well conserved (reviewed by Yogev et al. 2011). On the other hand, cytosolic fumarases have different roles among organisms: Cytosolic fumarase in yeast was suggested to work as a scavenger of fumarate from the urea cycle and catabolism of amino acids (Ratner et al. 1953). Cytosolic fumarase in human and yeast plays a key role in the protection of cells from DNA double-strand breaks (Yogev et al. 2010). In most plant species whose sequences are available, such as tomato, rice, and poplar, only mitochondrial fumarase was found. The *A.thaliana* genome encodes two fumarases, a mitochondrial fumarase (*FUM1*: *At2g47510*, Behal & Oliver, 1997; Heazlewood & Millar, 2005) and a cytosolic fumarase (*FUM2*: *At5g50950,* Pracharoenwattana et al., 2010, Brotman et al. 2011). The two genes have the same exon structure and share 95% similarity in

protein coding sequences with the exception of the N-terminal region where *FUM1* encodes a mitochondrial targeting sequence (Pracharoenwattana et al. 2010). The two fumarases are thought to have arisen recently by gene duplication, but not by duplication of chromosome segments since they share high sequence similarity, while the direct neighboring genes of *FUM1* and *FUM2* are distinct (Pracharoenwattana et al. 2010).

FUM1 is a life supporting enzyme involved in the TCA cycle, thus the elimination of *FUM1* leads to death (Pracharoenwattana et al. 2010). Likewise, suppression of mitochondrial fumarase causes a decrease in biomass and yield by restricting CO_2 uptake during the day and respiration during the night (Nunes-Nesi et al. 2007). FUM2 appears necessary for rapid vegetative growth under high nitrogen conditions: biomass decreases in both wild-type plants and *fum2* mutants grown in nitrogen poor soil, with no differences in biomass, fumarate and malate contents between the two. On the other hand, growing in nitrogen rich soil, wild type plants were twice as large as *fum2,* and the fumarate level significantly increased in the wild-type (Pracharoenwattana et al. 2010). The authors explain that accumulation of fumarate may be more advantageous to plants than accumulation of malate, because fumarate is involved in fewer metabolic processes. In addition to that, fumarate is more acidic (has lower pKa values) than malate (Pracharoenwattana et al. 2010), therefore using fumarate may be more beneficial than using malate for maintaining cellular pH by balancing the consumption of protons during the reduction of nitrate to ammonium (Zioni et al. 1971, Tschoep et al. 2009, Pracharoenwattana et al. 2010).

1.1.6 Fumarate in plants

Fumarate is usually found in high concentrations in photosynthetic organs such as leaves, stems, flowers, and immature siliques, but they are lacking in roots and mature seeds, and the level in leaves tends to increase with light intensity and plant age (Chia et al. 2000). The fumarate in *A.thaliana* is accumulated in high concentrations in rosette leaves, and sometimes the content exceeds the sum of soluble sugars, sucrose, glucose and fructose (Chia et al. 2000). The level of fumarate in rosette leaves shows diurnal regulation similar to the accumulation patterns of starch and sucrose: the concentration steadily increases during photosynthesis, reaching its maximum at the end of the day, and gradually decreases during the night, reaching its minimum at the end of the night (Chia et al. 2000, Fahnenstich et al. 2007). Accumulation of fumarate in light has been linked to nitrogen assimilation (Chia et al. 2000). Considering the gradually decreased level of fumarate during the darkness and the substantial

amount of fumarate in phloem exudates, fumarate may function in the transport of fixed carbon and nitrate from one part of the plant to another in the dark (Chia et al. 2000) similar to the putative function of malate (Zioni et al. 1971, Hamilton and Davies 1988, Touraine et al. 1988, 1992). Fumarate is supposed to serve as a metabolically accessible transient storage form of fixed carbon like starch and soluble sugar (Chia et al. 2000, Fahnenstich et al. 2007, Pracharoenwattana et al. 2010): Starchless mutants (*pgml*) have higher fumarate content than the wild-type (Chia et al. 2000), and *fum2* mutants accumulate more starch than wild-type (Pracharoenwattana et al. 2010). The two results imply that photosynthates which would normally be used to produce starch can instead be used to make fumarate in *pgm1*, and fumarate might serve as an alternative carbon sink for photosynthates (Chia et al. 2000, Pracharoenwattana et al. 2010).

1.2 Key background research toward this study

1.2.1 Forward genetic analysis on biomass heterosis in *A.thaliana*

Previously, a systematic survey on heterosis of biomass in *Arabidopsis* with a large number of *A.thaliana* crosses was carried out in our group: 63 *Arabidopsis* accessions were crossed with three reference accessions Col-0, C24 and Nd (Meyer et al. 2004). Among the 169 crosses, 29 exhibited significant mid-parent-heterosis (MPH) for shoot biomass, and the combination Col-0 / C24 was chosen for further in-depth studies on biomass heterosis in *A.thaliana* (Meyer et al. 2004). The reciprocal hybrids, Col-0×C24 and C24×Col-0, displayed 51% to 66% of mid-parent heterosis (MPH) for shoot biomass (Meyer et al. 2004). The degree of hybrid vigor positively correlated with light intensity: Hybrids growing under high light intensity (240 µmol m^{-2} s^{-1}) showed superior relative growth rates (RGR) throughout the entire vegetative growth (until 25 DAS) compared to the RGR of parents. In contrast, when the hybrid grew under low and intermediate light intensities (60 and 120 µmol m^{-2} s^{-1}), higher RGRs of the hybrids were only detected during 0-15 DAS (Meyer et al. 2004). From this observation it can be inferred that manifestation of hybrid vigor on shoot growth is influenced by environmental factors, in this case light intensities, but enhanced hybrid growth during 0-15 DAS occurs mainly through genetic factors independent from light intensity. In a following in-depth analysis on early vegetative growth of hybrids, a critical enhancement in RGR of the hybrids was observed during 3-4 DAS (Meyer et al. 2012).

To identify loci addressing heterotic biomass and biomass *per se*, QTL analyses were done with RILs (Törjék et al. 2006), ILs (Törjék et al. 2008) and their testcrosses (TC) with parents

Col-0 and C24 (422 RIL/C24, 416 RIL/Col-0, 20 IL/C24 and 22 IL/Col-0; Meyer et al. 2010). In the QTL mapping on heterosis biomass, the observation of all genomic actions - dominance, overdominance and epistasis - suggests that a combination of genetic actions contributed to biomass heterosis (Meyer et al. 2010). Ten QTLs which contribute contributing to biomass heterosis at an early developmental stage were identified, individually explaining between 2.4% and 15.7% of phenotypic variation (Meyer et al. 2010). Among the 10 QTLs, a QTL on top of chr.IV (position: 2-6 cM) was responsible for the largest percentage of phenotypic variation (15.7%) (Meyer et al. 2010). The locus on top of chr.IV was associated not only with biomass heterosis but also with biomass *per se* (Meyer et al. 2010). Interestingly, the Col-0 allele contributed to both, biomass *per se* and heterotic biomass to a greater extent than the C24 allele (position: 4/2-8 (chromosome/cM), effect [R^2]: 5.25%, standardized effect of the QTL (Std.Eff): -0.22, the negative value signified an increasing effect of the Col-0 allele), and heterotic biomass (position: 4/2-6, effect [R^2]: 15.7%, Std.Eff: -0.3) (Meyer et al. 2010), which means that polymorphism(s) between the Col-0 and the C24 genomic fragments of the locus might be the genetic basis for the phenotypic variation in biomass heterosis.

In addition, the locus on the top of chr.IV was also involved in additive to additive epistasis with a QTL at the bottom of chr.I, another major QTL for biomass heterosis (Meyer et al. 2010). In a parallel study on metabolite heterosis with the same population of RILs, ILs and their TC lines, a cluster of 23 metabolic heterotic QTL was located in the same genomic region of the top of chr.IV (position 4/4 : Lisec et al., 2009). Taken together, the results indicate the presence of (a) major growth/metabolism regulator(s) in this region. The corresponding QTL was fine mapped using additional markers and ILs. Fourteen candidate genes, from *At4g00238* to *At4g00335*, lay between the two markers MASC04036 and MASC07015.

1.2.2 Putative role of *Fumarase 2* allelic variation on different fumarate content in *A.thaliana*

In a metabolic QTL (mQTL) study using the same Col-0/C24 RIL population, a QTL for fumarate on chr. V (position: 71 cM, effect [R^2]: 3.6%) was identified (Lisec et al. 2008). The Col-0 allele of this mQTL was found to lead to higher fumarate levels than the C24 allele. A tentative fumarase (*FUM2*, *At5g50950*) was located within the confidence interval of the

mQTL, and the gene was identified as *FUM2* (Pracharoenwattana et al. 2010). In the RNA expression analysis on *FUM2* in Col-0 and C24, the expression level of *FUM2* was 17-fold higher in Col-0 than in C24 (Brotman et al. 2011).

1.3 Objectives

The encounter of different allelic variants in a genome is considered a prerequisite for the occurrence of heterosis. However, the genetic basis of heterosis was and still is largely unknown. To identify causative genes and the molecular basis for biomass heterosis in *Arabidopsis* at an early vegetative stage, a series of works was made by our group. In a heterosis QTL analysis with the Col-0/C24 RIL population, a QTL on top of chr.IV was responsible for the largest percentage of phenotypic variation not only for biomass *per se* but also for biomass heterosis. The Col-0 allele contributed to both the phenotypic variations to a greater extent than the C24 allele. The locus was fine-mapped to 14 genes (from *At4g00238* to *At4g00335*).

In a number of studies aimed at identifying a causative gene for biomass heterosis, several genes, such as *CCA1, TOC1*, *LHY*, *FLC* and *FRI* were identified (described in detail in 1.1.4) However, the genes can explain only the mid (2 weeks old) or the late (before flowering) vegetative growth vigor but not the early vegetative growth vigor in hybrids. The 14 candidate genes are linked to neither of the known genes, which inferred that an uncharacterized gene influencing biomass may be present at the QTL, and that the potential allelic variation may be the genetic basis for the phenotypic variation in biomass heterosis.

To identify causative gene(s) among the 14 candidate genes for biomass heterosis, and their potential allelic variations, the following steps were taken:

1. To gain potential insight into the biological functions of the 14 candidate genes related to biomass heterosis, previous research work on the genes was compiled.
2. Sequence polymorphisms between Col-0 and C24 on genomic fragments covering the 14 candidate genes were analyzed.
3. Transcription analyses of the 14 candidate genes in reciprocal hybrids, Col-0xC24, C24xCol-0 and in parents, Col-0 and C24, were carried out.
4. The Col-0 and C24 alleles of the 14 candidate genes were transferred into Col-0 and C24 by two complementation approaches:
   (i) To assess presumable combinational effect among the candidate genes on the target trait ‘biomass’, a vulnerable quantitative trait (Elwell et al. 2011), several

neighboring candidate alleles were introduced together into plants using Col-0 and C24 genomic DNA libraries which were constructed in cosmids.

(ii) To monitor the effect of individual alleles of the candidate genes, every single allele of the candidate genes was introduced into plants.

To dissect allele-specific effects from overdose of genes (dosage-effect), the alleles were transformed into their native plant genomic background as well.

5. Leaf areas of obtained transgenics were determined at an early vegetative stage.

Fumarate in *A.thaliana* is accumulated in high concentrations in rosette leaves. Fumarate functions in nitrogen assimilation and can serve as a metabolically accessible transient storage form of fixed carbon like starch and soluble sugar. Fumarate can be synthesized from malate by fumarase and also from succinate by the reversible reaction of the fumarate reductase/succinate dehydrogenase complex. In the *Arabidopsis fum2* mutant, the fumarate level was 10% of the wild-type Col-0 level. FUM2 is responsible for 85% of fumarate activity in rosette leaves (Brotman et al., 2011; Pracharoenwattana et al., 2010). In a metabolite QTL study using Col/C24 RILs, *FUM 2* (*At5g50950*) was found within the confidence interval of a fumarate QTL. Lines with the Col-0 alleles showed higher fumarate levels compared to the lines with C24 alleles. Taken together, we hypothesized that *FUM2* allelic variation might be responsible for fumarate content variation. To identify a potential allelic variant of *FUM2* influencing fumarate content variation, the following steps were taken:

1. Sequence polymorphisms between $FUM2_{Col}$ (*FUM2* Col-0 allele) and $FUM2_{C24}$ (*FUM2* C24 allele) were analyzed.
2. $FUM2_{Col}$ and $FUM2_{C24}$ were separately introduced into three genomic backgrounds, Col-0, C24 and the T-DNA insertional knock-out mutant *fum2*.

   To study the genetic inheritance pattern of the two *FUM2* alleles, such as dominance, recessiveness or co-dominance, each of the *FUM2* alleles was transformed in its reciprocal genomic background. In addition, the alleles were transformed into their native plant genomic background to distinguish allele-specific effects from dosage effects.
3. Metabolite abundances in all obtained transgenic lines were analyzed.

2 Materials and Methods

2.1 Materials

2.1.1 Bacterial strains

***E coli* strains**

DB3.1: used for propagation of pMDC123 harboring *ccdB*, genotype: *F⁻ gyrA462 endA1 glnV44* Δ(*sr1-recA*) *mcrB mrr hsdS20*(r_B^-, m_B^-) *ara14 galK2 lacY1 proA2 rpsL20*(*Sm*^{r}) *xyl5* Δ*leu mtl1*

DH10B: used for propagation of pCR8/GW/TOPO, genotype: *F⁻ mcrA Δ(mrr-hsdRMS-mcrBC) Φ80dlacX74 deoR recA1 endA1 araD139* Δ(*ara,leu*)*7607 galU galK* λ^- *rspl nupG*

HB101: the helper strain which was used for tri-parental mating, genotype: F⁻ *mcrB mrr hsdS20*(r_B^- m_B^-) *recA13 leuB6 ara-14 proA2 lacY1 galK2 xyl-5 mtl-1 rpsL20*(Sm^R) *glnV44* λ^-

SURE Tet^S: used as a host cell to carry Col-0 cosmid (pCLD04541) library, genotype*: endA1 glnV44 thi-1 gyrA96 relA1 lac recB recJ sbcC umuC::Tn5 uvrC e14* Δ(*mcrCB-hsdSMR-mrr*)*171 F'*(*proAB*$^+$ *lacI*^{q} *lacZΔM15 Tn10*)

EPI100-T1: used as a host cell to carry C24 cosmid (pCLD04541) library, genotype: *F⁻ mcrA Δ*(*mrr-hsdRMS-mcrBC*) *ϕ80dlacZΔM15 ΔlacX74 recA1 endA1 araD139 Δ(ara leu)7697 galU galK* λ^- *rpsL nupG*

DH5α: used as a donor strain of C24 cosmids for tri-parental mating, and used for propagation of pMDC123, genotype: *F⁻ endA1 glnV44 thi-1 recA1 relA1 gyrA96 deoR nupG Φ80dlacZΔM15* Δ(*lacZYA-argF*)*U169 hsdR17*(r_K^- m_K^+), λ^-

***Agrobacterium tumefaciens* strain**

C58C1 (pGV2260): C58C1 contains helper Ti plasmid pGV2260 (Deblaere et al. 1985). C58C1 is resistant to rifampicin; pGV2260 is resistant to carbenicillin.

2.1.2 Col-0 and C24 cosmid libraries

The Col-0 cosmid library was obtained from GeTCID (BC library from Ian Bancroft, JIC, Norwich, UK), and the C24 cosmid library was custom-made by Eurofins (Eurofins MWG GmbH, Ebersberg). Both Col-0 and C24 genomic libraries were constructed in the same binary cosmid pCLD04541 (Jones et al. 1992) (Fig. 1) but in different *E.coli* strains, Sure Tet^S (Col-0) and EPI100-T1 (C24). The Col-0 cosmid library was composed of 73728 clones, with

an average insert size of 17kb, at 17x genome coverage. The C24 cosmid library was composed of 61440 clones, with an average insert size of 15kb, at 8x genome coverage.

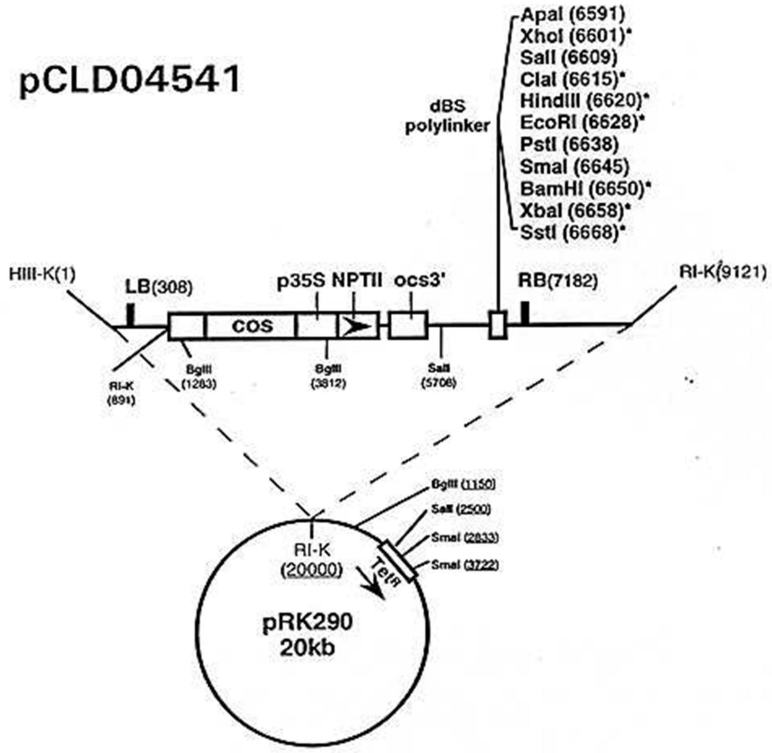

Figure 1: Map of pCLD04541 (Source: http://weedsworld.arabidopsis.org.uk/Vol4ii/fig2.html)

2.1.3 Vectors

2.1.3.1 Cosmids

Four Col-0 cosmids (Cos_013_{Col}, Cos_062_{Col}, Cos_125_{Col} and Cos_170_{Col}), five C24 cosmids (Cos_395_{C24}, Cos_401_{C24}, Cos_411_{C24}, Cos_507_{C24} and Cos_523_{C24}) and one empty cosmid (Cos_EV) were used for biomass heterosis study. A Col-0 cosmid harboring the Col-0 *FUM2* allele (Cos_FUM2_{Col}), a C24 cosmid harboring the C24 *FUM2* allele (Cos_FUM2_{C24}) and a Cos_EV were used for *FUMARASE 2* study.

2.1.3.2 Genomic clone vectors

Individually cloned Col-0 and C24 alleles of the 14 candidate genes and the ORF_{C24} were ligated in a binary vector pMDC123 cassette C1 (Curtis and Grossniklaus 2003). A total of 30 cloned vectors were used for biomass heterosis study. The nomenclature of cloned plasmid vectors is as follows: $pXXX_{Col}$ and $pXXX_{C24}$; the three digits (indicated 'XXX' in the

examples above) represent the last three digits of the corresponding AGI code taken from 'At4g00XXX'. The subscript Col and C24 ($_{Col}$ and $_{C24}$) refer to Col-0 and C24 alleles of the genes, respectively. pEV is a pMDC123 cassette C1 without *ccdB* in the insert. The *ccdB* was eliminated by LR reaction with pCR8/GW/TOPO. All nucleotide positions of the vector inserts were blasted against the *Arabidopsis* reference sequence Col-0 (TAIR 10).

Genomic clone	Insert
$p238_{Col}$ and $p238_{C24}$	chr.IV: 102091-108441
$p240_{Col}$ and $p240_{C24}$	chr.IV: 105146-113312
$p250_{Col}$ and $p250_{C24}$	chr.IV: 111042-115207
$p260_{Col}$ and $p260_{C24}$	chr.IV: 112970-118505
$p270_{Col}$ and $p270_{C24}$	chr.IV: 115437-121485
$p280_{Col}$ and $p280_{C24}$	chr.IV: 119641-125021
$p290_{Col}$ and $p290_{C24}$	chr.IV: 121271-127746
$p300_{Col}$ and $p300_{C24}$	chr.IV: 125649-131395
$p305_{Col}$ and $p305_{C24}$	chr.IV: 130211-133473
$p310_{Col}$ and $p310_{C24}$	chr.IV: 131762-137160
$p315_{Col}$ and $p315_{C24}$	chr.IV: 134414-141454
$p320_{Col}$ and $p320_{C24}$	chr.IV: 137855-143388
$p330_{Col}$ and $p330_{C24}$	chr.IV: 141687-146430
$p335_{Col}$ and $p335_{C24}$	chr.IV: 144300-150427
$pORF_{C24}$	chr.IV: 118575-124978
pEV	empty

2.1.4 Plant materials

Two divergent *Arabidopsis thaliana* accessions (Columbia (Col-0) and C24), the F1 hybrids (Col-0xC24, C24xCol-0) and the transgenic plants listed below were used.

2.1.4.1 Cosmid transgenic plants used for biomass heterosis study

The nomenclature of cosmid transgenic plants is as follows: *Col* (or *C24*)+$XXX_{Col\ (or\ C24)}$Y. 'Col' and 'C24' refer to the genomic background of the transgenic plants Col-0 and C24. The following three digits and the subscript Col and C24 (indicated '$XXX_{Col\ (or\ C24)}$' in the examples above) refer to transferred genomic fragments and are taken from cosmid 'Cos_XXX $_{Col\ (or\ C24)}$'. The following letter ('Y' in the examples above) signifies the transgenic lines originating from independent transformation events. The following 20 genotypes were used in this study: *Col+*013_{Col}*Y*, *C24+*013_{Col}*Y*, *Col+*062_{Col}*Y*, *C24+*062_{Col}*Y*, *Col+*125_{Col}*Y*, *C24+*125_{Col}*Y*, *Col+*170_{Col}*Y*, *C24+*170_{Col}*Y*, *Col+*395_{C24}*Y*, *C24+*395_{C24}*Y*, *Col+*401_{C24}*Y*, *C24+*401_{C24}*Y*, *Col+*411_{C24}*Y*, *C24+*411_{C24}*Y*, *Col+*507_{C24}*Y*, *C24+*507_{C24}*Y*, *Col+*523_{C24}*Y*, *C24+*523_{C24}*Y*, *Col+EV Y* and *C24+EV Y*.

2.1.4.2 Cosmid transgenic plants used for *FUMARASE 2* study

The nomenclature of cosmid transgenic plants is as follows: *Col (C24 or fum)+FUM2*$_{Col\ (or\ C24)}$Y. 'Col', 'C24' and '*fum2'* refer to the plant genomic backgrounds of transgenic plants. *fum2* is an *Arabidopsis* (Col-0) mutant line containing T-DNA insertion in the *FUM2* gene (*At5g50950*) (GABI_107E05; Pracharoenwattana et al. 2010, Brotman et al. 2011). The following *FUM2*$_{Col}$ and *FUM2*$_{C24}$ refer to the introduced Col-0 *FUM2* allele and the C24 *FUM2* allele which use the cosmid vectors, Cos_FUM2$_{Col}$ and Cos_FUM2$_{C24}$, respectively. The EV refers to a T-DNA of Cos_EV whose insert was eliminated. Cos_EV was transformed into the three genomic backgrounds for transgenic plant controls. A total of 9 transgenic genotypes were used in this study: *Col+FUM2*$_{Col}$, *Col+FUM2*$_{C24}$, *Col+EV, C24+FUM2*$_{Col}$, *C24+FUM2*$_{C24}$, *C24+EV, fum2+FUM2*$_{Col}$, *fum2+FUM2*$_{C24}$ and *fum2+EV*.

2.1.4.3 Transgenic plants harboring individual allele of the 14 candidate genes

The nomenclature of transgenic plants harboring a single candidate allele is as follows: *Col* (or *C24*)*+XXX*$_{Col\ (or\ C24)}$ *Y*. The initial Col or C24 refers the genetic background of the transgenic plants Col-0 or C24. The following three digits and the following subscript Col-0 and C24 (indicated 'XXX$_{Col\ (or\ C24)}$' in the examples above) represent the last three digits of the transferred genes taken from the genomic clones 'pXXX$_{Col\ (orC24)}$'. The following letter (indicated 'Y' in the examples above) signifies transgenic lines originating from independent transformation events. A total of 60 transgenic genotypes were used in this study.

2.1.5 Oligonucleotides

The primers listed below were synthesized by Biomers.net (Ulm, Germany).

2.1.5.1 Primers for probe preparation

The following primers were used for the probe synthesis for the screening of the cosmid libraries.

Name	Forward primer (5'-3')	Reverse primer (5'-3')
At4G00238	AGGGGCATGCTCAGAGAGTA	TCCTCAAACCACTCCGAATC
At4G00240	TATCAGTCGCTGGTGCTGTC	GAGTTGCCCCTGGTTTACAA
At4G00250	AGGCGGAAGAAGAAAGAAGG	ACCATCAGATCCCCAAATCA
At4G00260	TCATCAAACGAGGATGGAGA	TGAGATTTTCTCGTCGTGGA
At4G00270	TGATTGGGATGCGTTTTGTA	CATCACCTTGTCCCCAAATC
At4G00280	AGCTTCCCTCAAGGCAACAG	GGTTCAACCTCATTAACAAAACG
At4G00290	GAATGGGAATGATTGGGTTG	GCAGTTTAGCGGAACTTTGC
At4G00300	CCCTTCAGGATCGTCGATAA	CACATAAACGCCAACATTGC
At4G00305	GTTTGTTTGGTTCGGCATCT	GGGGTCCTACACAAAGGACA
At4g00310	GGATTCGGAAGAACACGAAA	ATGGAAGCGAAATCGAAATG

At4G00315	GGCCATTGAAAGCTTGAGTC	CACACGTCCAAGGAACAATG
At4G00320	GCCTTTGCTTGCTTTTATGC	GTTGGCACCATCCTGAAACT
At4G00330	CTTCCTTCCGTGTTCACCAT	GGGAGGAGTGAAGCAGTTGA
At4G00335	AAGAGCTTGTCCCTTCAGGA	AGCGTTTCAAAACTGCTTCC
FUM2	TGTAGACAATCAGTTCACAGGTCA	GGCCTTATCGACCAATTCAT

2.1.5.2 Primers used for cloning of the 14 candidate genes

The following primers were used for amplification of the Col-0 and C24 alleles of the 14 candidate genes of the top of chr. IV for cloning purposes.

Name	Forward primer (5'-3')	Reverse primer (5'-3')
$p238_{Col}$ and $p238_{C24}$	AGCGAAAGCACTCGACTTTG	AGGCTGCTAAGCCTCACAGA
$p240_{Col}$ and $p240_{C24}$	TGATGAAGAAGAGTGAGTGAGAGA	TGATTTGGGGATCTGATGGT
$p250_{Col}$ and $p250_{C24}$	GCCGGTCACATATTTCCACT	CTCTTTGACGCGTCTGATCT
$p260_{Col}$ and $p260_{C24}$	ACCGAAGTCACATCCTTCAA	CGCCCTAGAATCAGCAAGTC
$p270_{Col}$ and $p270_{C24}$	AGAAACGTGGTTTGCTTTTG	GCACTACAGAGACAACTCAAC
$p280_{Col}$ and $p280_{C24}$	GCCATTAGCATGATAGGAACTT	GTTACTGGCTCTGGTGATGG
$p290_{Col}$ and $p290_{C24}$	CATGCATGTAATCCTCCTATTACC	GAACTGTGTATCCCCACGAG
$p300_{Col}$ and $p300_{C24}$	CTTCAACTTCTGGGCCTTTT	GGCGGGATTTAGAGAAGAAG
$p305_{Col}$ and $p305_{C24}$	TCATAAGGGTGTCAATGTGTTG	AAGAATCGACCCACGGTAAC
$p310_{Col}$ and $p310_{C24}$	CCGGATATGCCAAGATGAGT	GGCAAACGAACAAGGAAATG
$p315_{Col}$ and $p315_{C24}$	TTGTAATGACACTGCTCAAAGG	TATCTGACCCGCTACATCTTCT
$p320_{Col}$ and $p320_{C24}$	AGGTATGTCTCCCCTCCTTG	AGAGCCACCTATCATCCACA
$p330_{Col}$ and $p330_{C24}$	TCAAGCTGCTACAAGTTTGCT	TCCCATCAATAGCTGAAACTTG
$p335_{Col}$ and $p335_{C24}$	GTTGTGTCGGTGGATGTACG	GTGTCCGACCTTATCCGAGA
$pORF_{C24}$	ATTCCCAATTTTGACGACCT	AGCACTACATGCACATGTCAAC

2.1.5.3 Primers used for genotyping of vectors and transgenic plants

The following primers were designed to amplify both ends of plasmid inserts or cosmid inserts and used to check structural changes of the inserts, such as truncation, rearrangement and chimeric fusion in *Agrobacterium* and in transgenic plants. In the present study, inserts to the left border of T-DNA are called 'LI' and inserts to the right border 'RI'.

Name	Forward primer (5'-3')	Reverse primer (5'-3')
Cos_013_{Col} LI	CACCAGAACCACCTGTAAAG	GGAAACAGCTATGACCATG
Cos_062_{Col} LI	AATCAAGAGCCATTAGATCG	GGAAACAGCTATGACCATG
Cos_125_{Col} LI	TGCAGAAAGCTGGAAGATTG	GGAAACAGCTATGACCATG
Cos_170_{Col} LI	TGGCTCCTTTGGAAAGTC	GGAAACAGCTATGACCATG
Cos_395_{C24} LI	CCGTCGCCAGCATTTATTC	GGAAACAGCTATGACCATG
Cos_401_{C24} LI	TCGCTTCCCTTACTCTATCC	GGAAACAGCTATGACCATG
Cos_411_{C24} LI	TCTGCCGGACCATTACAAC	GGAAACAGCTATGACCATG
Cos_507_{C24} LI	TAGTGCCAATGGACTAAGAG	GGAAACAGCTATGACCATG
Cos_523_{C24} LI	GATCTCAATGACGCTCTATTTC	GGAAACAGCTATGACCATG
Cos_FUM2_{Col} LI	CACTGCAAGCTGCTCTTCTC	GGAAACAGCTATGACCATG
Cos_FUM2_{C24} LI	CAGCATCAATGGCTCTCTGT	GGAAACAGCTATGACCATG
Cos_013_{Col} RI	CGCTTACGTACATGGTCGA	ATAACCCCATCCTGTTCATGT
Cos_062_{Col} RI	CGCTTACGTACATGGTCGA	CTTTGACCGATGGAGTATTG
Cos_125_{Col} RI	CGCTTACGTACATGGTCGA	TGGAAACCTATAACTCGTACTC
Cos_170_{Col} RI	CGCTTACGTACATGGTCGA	TGGGATGTGATTCCTGTTTC
Cos_395_{C24} RI	CGCTTACGTACATGGTCGA	GAGGGCGGGATTTAGAGAAG
Cos_401_{C24} RI	CGCTTACGTACATGGTCGA	CGGCCTTTCTCTATGGGTG
Cos_411_{C24} RI	CGCTTACGTACATGGTCGA	TCCCAAGCATAACGAAAGAC
Cos_507_{C24} RI	CGCTTACGTACATGGTCGA	GTGGCAGTATCCGATAAGAG
Cos_523C24 RI	CGCTTACGTACATGGTCGA	GACCGGCAATAGTAAGTTAAG
Cos_FUM2_{Col} RI	CGCTTACGTACATGGTCGA	CATGCGATTTTAAGCCAAGA
Cos_FUM2_{C24} RI	CGCTTACGTACATGGTCGA	ATCCGGCATCAGAAATTAGG

pCLD14541 NPTII	TACCGTAAAGCACGAGGAAG	GATCTCCTGTCATCTCACCTTG
Cos_EV	CGCTTACGTACATGGTCGAT	CACCCCAGGCTTTACACTTT
fum2	AGAAGTAAGTTGGCCGCAGT	GAGCTGCATGTGTCAGAGGT
$p238_{Col}$ LI	GTGGAATTGTGAGCGGATAA	TTGGTTGCATCAGTTTCACA
$p240_{Col}$ LI	GTGGAATTGTGAGCGGATAA	CAAAAGAGGGCATTACATCC
$p250_{Col}$ LI	GTGGAATTGTGAGCGGATAA	CCCATAAAACCGTCAGGTCT
$p260_{Col}$ LI	GTGGAATTGTGAGCGGATAA	TGATTTGGGGATCTGATGGT
$p270_{Col}$ LI	GTGGAATTGTGAGCGGATAA	TTCCATGATCTGCCCTTTTC
$p280_{Col}$ LI	GTGGAATTGTGAGCGGATAA	TGTTAGCAACCCCTGAAAAA
$p290_{Col}$ LI	GTGGAATTGTGAGCGGATAA	GGGAAAAGGCTTCGAGTATT
$p300_{Col}$ LI	GTGGAATTGTGAGCGGATAA	GAGTCAAAGCTGTTTTTGGAAC
$p305_{Col}$ LI	GTGGAATTGTGAGCGGATAA	GACGTTCGGTTATCCATTC
$p310_{Col}$ LI	GTGGAATTGTGAGCGGATAA	TGGCGTCTTTAGCATTTTCA
$p315_{Col}$ LI	GTGGAATTGTGAGCGGATAA	ACCTTGCTTTGTGAGTGTTTCT
$p320_{Col}$ LI	GTGGAATTGTGAGCGGATAA	ACGCCATCTCCAATACCTTC
$p330_{Col}$ LI	GTGGAATTGTGAGCGGATAA	CCAATGATTAGCCCGTATCC
$p335_{Col}$ LI	GTGGAATTGTGAGCGGATAA	GGAGTCTCTTCGATCCTTCG
$p238_{Col}$ RI	GGACAATATTTGTGGACCAAAG	GGCGATTAAGTTGGGTAACG
$p240_{Col}$ RI	TGTTAGCAACCCCTGAAGAA	GGCGATTAAGTTGGGTAACG
$p250_{Col}$ RI	GTCCTGAACCGAACCAGAGT	GGCGATTAAGTTGGGTAACG
$p260_{Col}$ RI	AACTATCATTAGCTGCCTCTGC	GGCGATTAAGTTGGGTAACG
$p270_{Col}$ RI	CATGCATGTAATCCTCCTATTACC	GGCGATTAAGTTGGGTAACG
$p280_{Col}$ RI	AGCACTACATGCACATGTCAAC	GGCGATTAAGTTGGGTAACG
$p290_{Col}$ RI	TGACGACGATACCGTGTTTT	GGCGATTAAGTTGGGTAACG
$p300_{Col}$ RI	TCATAAGGGTGTCAATGTGTTG	GGCGATTAAGTTGGGTAACG
$p305_{Col}$ RI	AGATTCGGAGGTGAGGATCA	GGCGATTAAGTTGGGTAACG
$p310_{Col}$ RI	AGTGAAACTGCCACGTGTGT	GGCGATTAAGTTGGGTAACG
$p315_{Col}$ RI	TCTGATCCATTGGGTCTTCA	GGCGATTAAGTTGGGTAACG
$p320_{Col}$ RI	CCTCAATGGAAGAAGACTTGG	GGCGATTAAGTTGGGTAACG
$p330_{Col}$ RI	CATTGCTTTAGTCGCAAGGA	GGCGATTAAGTTGGGTAACG
$p335_{Col}$ RI	GTTGTACAGCTGGCGTCAAT	GGCGATTAAGTTGGGTAACG
$p238_{C24}$ LI	GTGGAATTGTGAGCGGATAA	TTGGTTGCATCAGTTTCACA
$p240_{C24}$ LI	GTGGAATTGTGAGCGGATAA	CAAAAGAGGGCATTACATCC
$p250_{C24}$ LI	GTGGAATTGTGAGCGGATAA	GTCCTGAACCGAACCAGAGT
$p260_{C24}$ LI	GTGGAATTGTGAGCGGATAA	AAGCAAACCCAAAGATCCTG
$p270_{C24}$ LI	GTGGAATTGTGAGCGGATAA	CATGCATGTAATCCTCCTATTACC
$p280_{C24}$ LI	GTGGAATTGTGAGCGGATAA	TGTTAGCAACCCCTGAAAAA
$p290_{C24}$ LI	GTGGAATTGTGAGCGGATAA	GGGAAAAGGCTTCGAGTATT
$p300_{C24}$ LI	GTGGAATTGTGAGCGGATAA	GTGATGTCATGTCCTGTCCTG
$p305_{C24}$ LI	GTGGAATTGTGAGCGGATAA	AGATTCGGAGGTGAGGATCA
$p310_{C24}$ LI	GTGGAATTGTGAGCGGATAA	TGGCGTCTTTAGCATTTTCA
$p315_{C24}$ LI	GTGGAATTGTGAGCGGATAA	TCTGATCCATTGGGTCTTCA
$p320_{C24}$ LI	GTGGAATTGTGAGCGGATAA	ACGCCATCTCCAATACCTTC
$p330_{C24}$ LI	GTGGAATTGTGAGCGGATAA	CATTGCTTTAGTCGCAAGGA
$p335_{C24}$ LI	GTGGAATTGTGAGCGGATAA	GGAGTCTCTTCGATCCTTCG
$pORF_{C24}$ LI	GTGGAATTGTGAGCGGATAA	AGGGATCATCTCATCAGAGACA
$p238_{C24}$ RI	GGACAATATTTGTGGACCAAAG	GGCGATTAAGTTGGGTAACG
$p240_{C24}$ RI	AATCCGAATGTCCAAGCCTA	GGCGATTAAGTTGGGTAACG
$p250_{C24}$ RI	CCCATAAAACCGTCAGGTCT	GGCGATTAAGTTGGGTAACG
$p260_{C24}$ RI	TTGGGATCATTCCCTTGATT	GGCGATTAAGTTGGGTAACG
$p270_{C24}$ RI	TTCCATGATCTGCCCTTTTC	GGCGATTAAGTTGGGTAACG
$p280_{C24}$ RI	AGCACTACATGCACATGTCAAC	GGCGATTAAGTTGGGTAACG
$p290_{C24}$ RI	TGACGACGATACCGTGTTTT	GGCGATTAAGTTGGGTAACG
$p300_{C24}$ RI	GAGTCAAAGCTGTTTTTGGAAC	GGCGATTAAGTTGGGTAACG
$p305_{C24}$ RI	GAAGTCCGGTGTTTGAGGAA	GGCGATTAAGTTGGGTAACG
$p310_{C24}$ RI	AGTGAAACTGCCACGTGTGT	GGCGATTAAGTTGGGTAACG
$p315_{C24}$ RI	ACCTTGCTTTGTGAGTGTTTCT	GGCGATTAAGTTGGGTAACG
$p320_{C24}$ RI	CCTCAATGGAAGAAGACTTGG	GGCGATTAAGTTGGGTAACG
$p330_{C24}$ RI	TGATAGCGACAGAGGGTTTG	GGCGATTAAGTTGGGTAACG
$p335_{C24}$ RI	GTTGTACAGCTGGCGTCAAT	GGCGATTAAGTTGGGTAACG
$pORF_{C24}$ RI	CGATTTGGGACCGATAAAAC	GGCGATTAAGTTGGGTAACG

2.1.6 Media

All media were sterilized by autoclaving at 121°C at 15psi for 20 min. Antibiotics and heat-labile solutions were sterilized using syringe filters with 0.22 µm pore size (GE Healthcare Life Sciences, Freiburg, Germany) and added to cooled-down media and solutions.

2.1.6.1 Media for bacterium culture

LB (Luria-Bertani) medium: 1% (w/v) bacto tryptone, 0.5% (w/v) yeast extract and 1% (w/v) NaCl were dissolved in ddH_2O and adjusted to pH 7.0. For agar plates, 1.5% (w/v) of bacto agar was added to the above broth.

YEP (Yeast Extract Peptone) medium: 1% (w/v) bacto peptone, 1% (w/v) yeast extract and 0.5% (w/v) NaCl were dissolved in ddH_2O and adjusted to pH 7.0. For agar plates, 1.5% (w/v) of bacto agar was added to the above broth.

SOC (Super Optimal broth with Catabolite expression) medium: 0.5% (w/v) yeast extract, 2% (w/v) tryptone, 10 mM NaCl, 2.5 mM KCl and 10 mM $MgCl_2$ were dissolved in ddH_2O and adjusted to pH 7.0. 20 mM filter-sterilized glucose was added to the solution and stored at -10°C.

AB (*Agrobacterium*) minimal medium: The salts (3 g K_2HPO_4, 1 g NaH_2PO_4, 1 g NH_4Cl, 0.3 g $MgSO_4 \cdot 7H_2O$, 0.15 g KCl, 5 mg $CaCl_2$ and 2.5 mg $FeSO_4 \cdot 7H_2O$) were dissolved in the order given in 1L ddH_2O; the pH was adjusted to 7.2. 2% (w/v) bacto agar was added to the broth. 20% (w/v) of filter sterilized glucose was added to the medium prior to plating (Matthysse 2006)**.**

2.1.6.2 Media for plant culture

½ MS (Murashige & Skoog) medium: The half required amount (2203 mg/l) of MS medium salt including modified vitamins (Duchafa, Haarlem, The Netherlands) and 1% (w/v) sucrose were dissolved in ddH_2O and adjusted to pH 5.8. For agar plates, 0.8% of bacto agar (w/v) was added to the above broth.

2.1.6.3 *Agrobacterium* infiltration buffer

The half required amount (2203 mg/l) of MS medium salt including modified vitamins (Duchafa, Haarlem, The Netherlands) and 5% (w/v) sucrose were dissolved in ddH_2O. 0.05% (v/v) Silwet-77 (Lehle Seeds, Texas, USA) was added. The infiltration buffer was prepared fresh before *Agrobacterium* inoculation and not autoclaved.

2.1.7 Buffers and solutions

2.1.7.1 Solutions for heat-shock competent *E.coli*

Solution I: 30 mM calcium acetate (pH 7.5), 100 mM KCl, 10 mM $CaCl_2$, 15% (v/v) glycerin, 25 mM $MnCl_2 \cdot 4H_2O$ were dissolved in ddH_2O. The solution was adjusted to pH 5.8 and filter-sterilized.

Solution II: 75 mM $CaCl_2$, 15% (v/v) glycerin and 2% (v/v) PIPES sesquisodium salt were dissolved in ddH_2O, filter-sterilized and stored at 4°C.

2.1.7.2 Buffers for colony blotting

Denaturing solution (1X): 1.5M NaCl and 0.5M NaOH were dissolved in ddH_2O and adjusted to pH 13.0.

SSPE (20X): 3M NaCl, 0.2M $NaH_2PO_4 \cdot H_2O$, 25mM EDTA and 0.2M NaOH were dissolved in ddH_2O and adjusted to pH 7.4.

Pre-hybridization buffer: 10X Denaturing Solution, 6X SSPE and 1% SDS were dissolved in ddH_2O and stored at -10°C.

Hybridization buffer: 6X SSPE and 1% SDS were dissolved in ddH_2O and stored at -10°C.

Washing buffer: 6X SSPE and 0.2% SDS were dissolved in ddH_2O.

2.1.7.3 *Arabidopsis* DNA isolation buffer

CTAB buffer: 20% (w/v) CTAB, 100 mM Tris (pH 8.0), 20mM EDTA (pH 8.0), 1.4M NaCl and 1% (w/v) PVP 40 (polyvinylpyrrolidone) were dissolved in ddH_2O and adjusted to pH 5.0.

2.1.7.4 DNA Gel loading buffer (6x)

0.25% (w/v) bromphenol blue, 0.25% (w/v) xylene cyanol FF and 30% (v/v) glycerol were dissolved in ddH_2O.

2.1.7.5 Hot borate RNA extraction buffer

200 mM Na-Borat (pH 9.0), 30 mM EGTA (ethylene glycol tetraacetic acid), 5 mM EDTA (ethylenediaminetetraacetic acid), 1% (v/v) SDS and 1% Na-Deoxycholate (w/v) were dissolved in ddH_2O.

2.2 Methods

2.2.1 Cosmid manipulation

2.2.1.1 Colony blot for screening of cosmids from Col-0 and C24 genomic libraries

Bacteria from the plates were transferred to nylon membranes and fixed (IPK internal service at PGRC). These membranes were stored in a cool dry place. For the screen, 50 µl of salmon sperm DNA in 50 µl ddH_2O was denatured at 95°C for 3 min and added to 100 ml hybridization buffer. Wet membranes with 40 ml of 65°C pre-warmed 2xSSPE buffer with denatured salmon sperm DNA were pre-hybridized at 65°C for 4 h in hybridization bottles (Thermo Fisher Science, Schwerte, Germany) in a hybridization oven (Bachofer, Germany). Probes were prepared using Col-0 or C24 genomic DNA templates with primers described above and the PCR reaction mix and thermo cycle condition as mentioned in section 2.2.4. The PCR product was purified using the PCR purification kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. For radioactive probe labeling, 50 ng of denatured probe was dissolved in 45 ml distilled water, and mixed with ready prime kit (RediPrime Kit, GE Healthcare, Germany) and 5 µl of [α-^{32}P]-dCTP (Amersham, Freiburg, Germany). The probe was then incubated at 37°C for 30 min. The labeled probes were purified using spin columns (illustra MicroSpin S-300 HR Columns, GE Heath Care Life Science, Germany) according to the manufacturer's protocol. The purified ^{32}P labeled probes were denatured at 95°C for 3 min, added to the pre-hybridized membrane and hybridized overnight at 65°C. The following day, the radioactive labeled membrane was washed twice with 50 ml of washing buffer in a hybridization bottle for 5 min and another 15 min and washed a third time with 1000 ml of a 65°C pre-warmed washing buffer in in a Tupperware container with gentle shaking for 15 min. Then the washed membrane was briefly dried using Whatman paper, sealed in a plastic bag and imaged in an X-ray film cassette (Fuji imaging plate BAS-MS, Fuji Photo Film, Japan) with X-ray films (Retina, Germany) overnight. The X-ray films were developed using a phosphorimager (FLA 5100 Fuji). The acquired image was analyzed using the TINA2.09 software (Raytest Isotopenmessgeräte GmbH, Germany).

2.2.1.2 Cosmid transformation into *E.coli*

Preparation of electro-competent *E.coli*

10 ml of fresh grown *E. coli* (DH5α) was added to 1L LB broth and cultured at 37°C at 120 rpm until OD_{600} 0.5-0.6 was reached. The cells were collected at 8000 rpm at 4°C for 10 min

and washed in 500 ml, 250 ml and 100 ml ice-cold autoclaved ddH_2O and 10 ml of 10% ice-cold sterile glycerol. The cells were pelleted at 8000 rpm at 4°C for 10 min again and the supernatant was discarded. The pelleted cells were suspended in 5 ml of 10% ice-cold glycerol, aliquoted into 50 µl in 1.5 ml microtubes, frozen in LN_2 and stored at -80°C.

Cosmid transformation into *E.coli* via electroporation

Col-0 cosmid library was transformed in a *E.coli* strain Sure Tet^S which lacks a conjugation factor (Beutin and Achtman 1979). To transfer Col-0 cosmids into *Agrobacterium* via tri-parental mating, the Col-0 cosmids were transferred into *E. coli* DH5α which has conjugation factors. 100-400 ng of salt-free cosmid DNA was added to 50µl of electro-competent DH5α, and the mixture was transferred into an electroporation cuvette with a 1 mm gap (Eppendorf, Hamburg, Germany). The cells were pulsed (Eletroporator 2510, Eppendorf, Hamburg, Germany) once at 1.8 kV for a few seconds, then 250 µl of SOC medium was added immediately. The cells were incubated at 37°C at 120 rpm overnight. A fraction (50-200µl) of the cell culture was plated out on LB medium containing 10 mg/l tetracycline and incubated at 37°C for 2 days.

2.2.1.3 Cosmid transformation into *Agrobacterium* using tri-parental mating

The recipient *Agrobacterium* strain C58C1 (pGV2260) was cultured in YEP medium containing 50 mg/l carbenicillin and 200 mg/l rifampicin at 28°C for 2 days before conjugation. The donor *E.coli* strains, Col-0 cosmids in DH5α and C24 cosmids in EPI100-T1, and the helper *E.coli* strain pRK2013 in HB101 were cultured in a LB medium containing antibiotics (10 mg/l tetracycline for the donor strains and 50 mg/l kanamycin for the helper strain) overnight at 37°C. 0.8 ml of the recipient, 0.2 ml of the helper and 0.2 ml of the donor were mixed in a 1.5 ml tube and the cell was collected by centrifugation at 13000 rpm for 1 min. The suspended cells in 500 µl of LB medium were spread onto antibiotic-free LB agar plates and incubated at 30°C overnight. On the next day, a layer of bacteria mixture growing on the plate was collected and suspended in 1 ml of 10 mM $MgSO_4$. This mixture was diluted at 1/100 with 10 mM $MgSO_4$. 20 µl of this diluted mixture was spread onto an antibiotic-free AB minimal medium and incubated for 3 nights at 27°C. A single colony growing on the medium was streaked on YEP medium containing 2 mg/l tetracycline and 50 mg/l carbenicillin, and incubated for 2 days at 27°C. The transformed colonies were analyzed using PCR.

2.2.2 Cloning of the individual candidate alleles

The Col-0 and C24 alleles of the 14 candidate genes and ORF_{C24} were cloned with the own promoter and terminator sequences. The putative promoter sequences of the alleles were obtained from the *Arabidopsis* Gene Regulatory Information Server (AGRIS; http://arabidopsis.med.ohio-state.edu/AtcisDB/: Palaniswamy et al. 2006) where all cis-transcription elements and trans-transcription factors identified in *A. thaliana* are integrated. The promoter sequences which were used for the allele cloning were larger than the putative promoter sequence of AGRIS (Appendix 5). Terminators of the genes were roughly estimated to range from 1 kb to 2.7 kb in length depending on the distance to the downstream gene (Appendix 5). Col-0 and C24 alleles of a gene were amplified with the same primer pairs whose sequences were present in both the Col-0 and C24 genomic regions (2.1.5.2). All the alleles and ORF_{C24} were amplified by PCR using proofreading DNA polymerase (Bioline, Germany) according to the manufacturer's protocol. 5 µl of the PCR products were incubated with 0.5 U *taq* DNA polymerase at 72°C for 10 min in order to add 3′ A-overhangs to the blunt ends of the PCR products. The A-overhanging PCR products were ligated into pCR8/GW/TOPO (Invitrogen, Darmstadt, Germany) and transformed into DH10B according to the manufacturer's protocol. The clones were determined by *EcoRI* (Thermo Fisher Science, Schwerte, Germany) restriction fragment-lengths. Constructs having the correct restriction fragments were sequenced by the IPK internal sequencing service using Sanger sequencing on a 96-capillary ABI 3730 instrument. The clones without PCR errors in the coding sequences, splicing donor and acceptor sites, and cis-regulatory element binding sites in the promoter were selected. The inserts of the cloning vectors were transferred into destination vector pMDC123 (Fig. 2) (Curtis and Grossniklaus 2003) by a LR reaction using the Gateway LR Clonase (Invitrogen, Darmstadt, Germany) according to the manufacturer's protocol. Positive colonies on the selection medium (LB agar plate containing 50 mg/l kanamycin) were analyzed based on the *EcoRI* restriction fragment-lengths (Appendix. 6), and used for further study.

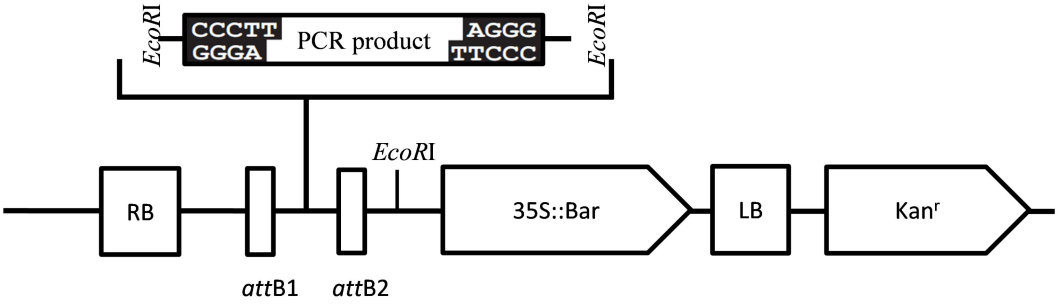

Figure 2: Schematic drawing of a partial map of pMDC123 after LR reaction with the entry vector pCR8/GW/TOPO.

2.2.2.1 Plasmid transformation into *E.coli*

Preparation of heat-shock competent *E.coli* cells

0.5 ml of overnight grown *E.coli* (DH5α and DH10B) suspension culture was cultured in 100 ml of LB medium at 37°C for about 2 h until the concentration reached OD_{600} of 0.2. The bacteria culture was placed on ice for 20 min and centrifuged for 10 min at 4°C at 3000 rpm. The pelleted cells were suspended in 25 ml solution I, and placed on ice for 1 h. The cells were collected for 10 min at 4°C at 3000 rpm and suspended in 2 ml solution II and kept on ice for 5 min. The suspended competent cells were dispended into 50 µl aliquots, deep frozen in LN_2 and stored -80°C.

Transformation of plasmids into *E.coli* via heat shock

50-100 ng of plasmid DNA was added to 50µl of heat-shock competent cells, and incubated on ice for 30 min. After a heat-shock treatment for 30 sec at 42°C, 250 ml of SOC medium were added. The cells were incubated at 37°C at 120 rpm for 1-4 h, and a fraction (50-200 µl) of the cell culture was plated out on an LB medium containing appropriate antibiotic(s).

2.2.2.2 Plasmid transformation into *A.tumefaciens*

Preparation of electro-competent *A.tumefaciens* cells

A single *A.tumefaciens* colony was picked and pre-cultured in 5 ml YEP medium containing 50 mg/l of carbenicillin and 200 mg/l rifampicin. The cells grew at 28°C at 120 rpm for 2 days. The cell culture was transferred into 200 ml of antibiotic-free YEP medium and grew for 4-5 h at 28°C with vigorous shaking until OD_{600} 0.5-0.6 was reached. The cells were chilled on ice for 15-30 min and collected by centrifugation at 4000 rpm at 4°C for 10 min. The cells were washed 3 times with 100 ml, 20 ml and 4 ml of ice-cold 10% glycerol. The

pelleted cells were suspended in 1.5 ml ice-cold 10% glycerol, dispensed into 50 µl aliquots, frozen in LN_2 and stored at -80°C.

Transformation of plasmids into *A.tumefaciens* via electroporation

20-50 ng of salt-free plasmid DNA were added in 50 µl of electro-competent C58C1 (pGV2260), and the mixture was transferred into a 1 mm electroporation cuvette. The cells were pulsed once at 440V for a few seconds and 250 µl of a SOC medium were immediately added. Then the cells were incubated overnight at 27°C at 120 rpm. A fraction (20-50 µl) of the cell culture was plated on LB medium containing 50 mg/l kanamycin, 250 mg/l rifampicin and 100 mg/l carbenicillin and incubated at 27 °C for 2 days.

2.2.3 Transgenic plant preparation

2.2.3.1 Plant cultivation

Col-0 and C24 seeds were sown on a 6:1 mixture of substrate 1 (Klasmann-Deilmann GmbH, Geeste, Germany) and sand. After stratification (3 days, 4°C in the dark), plants grew under short-day conditions (8h light/16h darkness) for 3 weeks and thereafter under long-day conditions (16h light/8h darkness).

2.2.3.2 Transformation of *Arabidopsis thaliana*

A fresh grown single *Agrobacterium* colony was picked and put into 5 ml of a YEP medium containing antibiotics (2 mg/l tetracycline, 50 mg/l carbenicillin and 200 mg/l rifampicin for cosmid and 50 mg/l kanamycin, 50 mg/l carbenicillin and 200 mg/l rifampicin for genomic clones) and cultured at 28°C for 2 days (plasmid strain), or 3 days (cosmid strain) at 120 rpm in the dark. The 5 ml *Agrobacterium* culture was transferred into 500 ml of an antibiotic-free YEP medium and grown at 28°C overnight. The *Agrobacterium* cells were collected at 3000 rpm at 4°C for 10 min. The cells were washed three times with 50 ml of an *Agrobacterium* infiltration buffer. The concentration of resuspended cells were adjusted with *Agrobacterium* infiltration buffer until OD_{600} 0.5-0.6 was reached. Then 0.05% (v/v) Silwet L-77 was added. *Arabidopsis* flowers were dipped twice in this *Agrobacterium* suspension and then placed in the dark overnight. On the following day, water was sprayed over the plants to rinse the residue of the infiltration buffer, and then the plants were grown under long-day conditions.

2.2.3.3 Transgenic plant selection

Selection of cosmid transgenic plants

Transgenic seeds were pre-sterilized with 70% EtOH for 1 min and then sterilized with 4% (v/v) NaHCl and 0.001% (v/v) Tween-20 for 20 min. The seeds were rinsed 5 times with autoclaved ddH_2O. Seeds were suspended in 0.01% agar solution and evenly spread out onto solid half-strength MS medium containing 50 mg/l kanamycin. Sown seeds were stratified at 4°C for 3 days in the dark and grew for 10-14 days in a phyto-room setting of 12 h light/ 12 h darkness at 28°C. The plants surviving on the selection medium were transplanted in soil in 6 cm pots and grown further under long-day conditions (16 h light/8 h darkness) at 28°C until the seed maturation stage was reached. The seed desiccation was done in a green house.

Selection of genomic clone transgenic plants

Transgenic seeds were evenly scattered on wet soil in pots, and stratified for 3 days at 4°C in the dark. After growing under short-day conditions (8 h light/ 16 h darkness) for 2 weeks, 20 mg/l basta (Bayer AG, Langenfeld, Germany) was sprayed over the seedlings. For the selection in T1, the first basta treatment was made onto 10 day-old seedlings, and repeated twice in a 3-day interval. For the selection in T2, basta was sprayed once onto 10 day-old seedlings. 10 days after the last basta treatment, surviving plants were transplanted into new soil in pots and grown under long-day conditions (16 h light/ 8 h darkness).

2.2.3.4 Analysis of transgenic plants

Resistant plants (to kanamycin for cosmid transgenic plants, to basta for genomic clone transgenic plants) were selected and propagated to the T2 generation. Copy numbers of T-DNA in the transgenic plants were estimated by determining the segregation ratio. More than 100 seeds of T2 were sown on a selection medium. 10 days after sowing, the numbers of surviving and dead plants were counted and their ratio was evaluated with Pearson's chi-squared test applying the Yates's correction (Yates 1934) using Microsoft Excel 2010. Transgenics following the Mendelian ratio 3:1 (antibiotic resistance:antibiotic susceptible) were presumed to have one T-DNA copy and used for the further experiments. For analysis of the integrity of inserts of T-DNA in transgenic plants, both sides of inserts were amplified using PCR. Plants with one T-DNA copy number and both ends of inserts were used for further analysis.

2.2.4 Polymerase chain reaction (PCR)

For the purpose of cloning with PCR products, the high-fidelity Velocity DNA Polymerase (Bioline, Luckenwalde, Germany) was used according to the manufacturer's protocol. Otherwise, PCR was done using a homemade *Taq* polymerase (Leelayuwat et al. 1997) or the Dream *Taq* DNA polymerase (Thermo Scientific, Schwerte, Germany).

PCR reaction mix

Reagent	Amount per reaction
Template DNA	10-100 ng
PCR amplification buffer (10x)	10% of reaction volume
dNTP mix (dATP, dGTP, dCTP, dTTP)	0.2 mM each
Forward primer (10µM)	0.5 µM
Reverse primer (10µM)	0.5 µM
Taq DNA polymerase	1 U
ddH2O	Up to final volume of 20 µl

Thermal profile

Stage	Temperature (^{o}C)	Time (sec)	No. of cycles
Initial denaturation	94	300	
Denaturation	94	30	
Annealing	59	20	35 cycles
Extension	72	60 sec /1 kb	
Final extension	72	300	

2.2.5 DNA analysis

2.2.5.1 Plasmid and cosmid DNA isolation

For the entire cosmid sequencing using Next Generation Sequencing on Roche GS FLX (454), the cosmid DNA was isolated according to the alkaline lysis method (Steuernagel et al. 2009). Otherwise, plasmid and cosmid DNA were isolated using the QIAprep Spin Miniprep Kit (Qiagen) according to the manufacturer's protocol.

2.2.5.2 Plant genomic DNA isolation

A.thaliana genomic DNA was extracted according to CTAB methods (Winnepenninckx et al. 1993).

2.2.5.3 DNA sequencing

For the sequencing of PCR amplicons, PCR products were purified with the Qiagen Extract Kit according to the manufacturer's protocol. DNA sequences were determined by the IPK internal sequencing service using Sanger sequencing on a 96-capillary ABI 3730 instrument. Entire cosmid sequencing was performed on the Roche GS FLX (454) through the IPK internal service (Steuernagel et al. 2009).

2.2.5.4 Analysis of polymorphisms between Col-0 and C24

The Col-0 sequence (TAIR10) was obtained from TAIR (http://www.arabidopsis.org). The C24 sequences used for this study were acquired from C24 cosmids harboring the corresponding C24 genomic DNA fragments. Sequence polymorphisms between C24 and Col-0 were analyzed using online blasting tools on NCBI and TAIR, and the DNASTAR lasergene 8 software. The ORF structure of the C24 sequence was analyzed with the online gene finding tool 'FGENESH' (http://linux1.softberry.com), then the acquired ORFs were blasted to a Col-0 peptide sequence using the BLASTX tool (http://blast.ncbi.nlm.nih.gov/) to find polymorphisms in peptide sequences.

2.2.6 Transcript analysis using HiSeq2000

To study the differences in transcription levels of Col-0, C24 and their reciprocal hybrids Col-0×C24 and C24×Col-0 at an early developmental stage, RNA was extracted from 5 day-old seedlings. A total of 250 seeds from each genotype were divided into 2 pools and sown on nylon mesh (pore size 200 µm) covering 80 ml of soil moistened with tap water. After stratification (1 day, 4°C in the dark), the plants grew in a controlled growth chamber [16 h light, 120 µmol s^{-1} m^{-2}, 20ºC and 60% relative humidity (RH) / 8 h darkness, 18ºC and 75% RH]. The plants were shuffled randomly every day during growth to minimize positional effects in the phytochamber. 5 day-old seedlings were harvested in the middle of the light period. The plants with nylon mesh were deep frozen in LN_2. The shoots were collected by brushing over frozen plants, and stored at -80°C. The total RNA of each genotype was isolated using a modified hot borate RNA extraction method (Wan and Wilkins 1994). Subsequent procedures, including quality controls for RNA, library preparation ligation of adaptors, gel purification and RNA sequencing were performed by the Nucleic Acid Analysis sub-group of the Heterosis group at IPK Gatersleben. The amount of RNA, 445 ng Col-0, 540 ng C24, 535 ng Col-0xC24 and 774 ng C24xCol-0 were used for RNA sequencing. A library preparation was carried out according the manufacturer's recommendation (Illumina TruSeq RNA Sample Prep Kit). After an agarose gel-based size selection, the average cDNA-fragment size was 220 bp with a range of 150 bp to 360 bp. The barcoded libraries from 4 samples were run on a single lane paired end 100 bp on a HiSeq2000H (Illumina, San Diego, CA, USA), which resulted in 40 million pass filter read pairs (40 million. 2x100bp = 8 Gigabases/sample). The RNA sequence was analyzed by Martin Mascher from the Bioinformatics and Information Technology research group at IPK Gatersleben. These

sequences were mapped against the reference genome (TAIR10). The mapping was conducted using the TopHat 1.2.0 software (http://tophat.cbcb.umd.edu). The Cufflinks online tool (http://cufflinks.cbcb.umd.edu/) was used for comparing the differential gene expressions between parents, their reciprocal hybrids and among each other.

2.2.7 Phenotyping of plant growth

2.2.7.1 Plant growth conditions

Each set of experiments was done in the same phytochamber equipped with an automatic phenotyping system, LemnaTec Scanalyzer (LemnaTec, Aachen, Germany). A total of 12 lanes were in the phytochamber, each lane has 4 blocks, each block was composed of 8 carriers, each carrier containing a 12-well tray. 12 wells in a tray were sown with the same plant line. The 8 carriers of each block were transported together for watering and taking pictures. Images were taken every day starting 4 h after the light. 50 ml of water was added at 2-day intervals. Seeds were stratified at 4°C for 2 days in the dark on moistened Whatman filter paper in Petri dishes and then placed individually in soil in 12 well trays. The trays were covered with plastic wrap to prevent drying out and placed on a carrier. The seeds were stratified one day more at 4°C in the dark in a phytochamber. On the following three days, the plants grew under low temperature conditions (16 h light, 120 µmol s^{-1} m^{-2}, 16ºC and 75% RH / 8 h darkness, 14ºC and 75% RH). From then until sampling, the plants grew under standard conditions (16 h light, 120 µmol s^{-1} m^{-2}, 20ºC and 60% RH / 8 h darkness, 18ºC and 75% RH).

2.2.7.2 Plant growth analysis

Plant images were made every day starting from 5 days after sowing (DAS, 1 DAS is defined as the day of seed sown on moistened Whatman filter paper in Petri dishes) until harvesting, using the automated imaging system. Until 8 DAS images were taken with fluorescence light only. Plastic wraps covering the top of pots were removed before imaging on 8^{th} DAS. From 8 to 12 DAS, images were taken with both visible and fluorescence light. Thereafter, images were taken under visible, fluorescence and infrared light. Image analysis was done by the Image Analysis research group at IPK Gatersleben. For this analysis, the fluorescence images were used. Pixel values from fluorescence images were extracted using the Integrated Analysis Platform (IAP) (Klukas et al. 2012; Klukas et al. 2014). In order to convert fluorescence-based area (pixel) data to leaf area (LA, mm^2), the given pixel values were

multiplied by 0.0081266. This value was estimated by comparison between pixel value of green object and area of the green object. The mean leaf area was estimated by the linear mixed model (Piepho et al. 2003) formulated by Jochen C. Reif and Yusheng Zhao from the Quantitive Genetic research group at IPK. Plant genotypes were considered as fixed factors. Combinatorial interactions between each set of experiments, replications within the experiments and blocks within the replications were considered as random factors. Plant growth is positively related to its innate seeds size (Meyer et al. 2004, Elwell et al. 2011). Thus for LA estimation in homozygous cosmid transgenic plants, the seed size was used as a cofactor. For the size determination, approximately 100 *Arabidopsis* seeds from each genotype were evenly scattered on PCR adhesive seals and fixed on A4 paper. Seeds were scanned using a scanner (11000XL, Epson, Japan) at 12000 dpi (Dots per inch). The images were saved as TIFF files which were subsequently exported into bitmap format. The measurement of the seed area was performed using the Evaluator software (developed by Dmitry Peschansky, IPK Gatersleben; Meyer et al., 2012) according to the software instructions.

The linear mixed model for LA in homozygous cosmid transgenics was as follows:

Fixed factor: Genotype + Seed area (mm^2)

Random factor:

Experiment+Experiment.Genotype+Replicate/Experiment+Block/Replicate.Experiment

After completing the phenotyping of plant growth in the segregating T2 generation, the transgenic plants were sorted into transgenic siblings and non-transgenic siblings. Transgenic plants harboring single candidate alleles were screened by spraying 20 mg/l basta onto the plants. Plants which were resistant to basta were regarded as transgenic siblings. To sort cosmid transgenic plants into non-transgenic and transgenic siblings, the genomic DNA of the transgenic plants were isolated using Extract-N-Amp™ (Sigma-Aldrich, Seelze, Germany). Transgenic and non-transgenic siblings were discriminated based on PCR analysis: Plants which have a *NPTII* were regarded as transgenic siblings. The linear mixed model for estimation of LA in T2 generation was as follows:

Fixed factor: Genotype

Random factor:

Experiment+Experiment.Genotype+Replicate/Experiment+Block/Replicate.Experiment.

The data were analyzed with REML in Genstat 14th edition.

2.2.8 Metabolic profiling

2.2.8.1 Plant sample preparation

For metabolic profiling, the four independent T3 homozygous lines from each of the nine genotypes (*Col+FUM2*$_{Col}$, *Col+FUM2*$_{C24}$, *Col+EV*, *C24+FUM2*$_{Col}$, *C24+FUM2*$_{C24}$, *C24+EV*, *fum2+FUM2*$_{Col}$, *fum2+FUM2*$_{C24}$ and *fum2+EV*) were used. Seeds of each line were sown in a pot (74 mm X 105 mm) and stratified at 4ºC for 2 days in the dark and grew in a phytochamber setting at low light and low temperature condition (16 h light, 120 µmol s^{-1} m^{-2}, 18ºC and 65% RH / 8 h dark, 16ºC and 75% RH) for 3days, then grew at standard condition (16 h light, 180 µmol s^{-1} m^{-2}, 20ºC and 65% RH / 8 h dark, 18ºC and 75% RH) until harvested. Ten days after sowing, the plants were transplanted into 9 cm pots. Five plants of a line were planted per pot and 10 plants composed one replicate. Six replicates were in one experiment. During the entire growing time, plants were shuffled randomly in 2 to 3 day intervals to minimize positional effects in the phytochamber. 19 day-old plants were harvested 4 hours prior to lights-out. The harvested plant materials were directly deep frozen in liquid nitrogen and stored at -80ºC.

2.2.8.2 Gas chromatography-mass spectrometry

1 ml of extraction buffer (55.5% of methanol, 22.15% of chloroform, 22.15% of dH_2O and 0.2% of internal standard L-Alanine-2,3,3,3-d_4) was added to 15±1 mg of pulverized deep frozen samples. After vigorous vortexing for 10 to 15 min at 4ºC, 400 µl dH_2O was added to facilitate phase separation. After another short vortexing, samples were centrifuged at 12,000 rpm at 4ºC for 10 min. 100 µl of the supernatant was transferred into a GC-MS vial and then dried using a vacuum concentrator (RVC 2-33 CD plus, Christ, Harz, Germany) for 3 h. Before crimping caps, vials were filled with argon. The polar metabolites were extracted in two batches in two consecutive days. The GC-MS was performed with both batches in one run. 1 µl out of 30 µl derivatized of samples at 230ºC was injected splitlessly using a MPS2-XL autosampler (Gerstel, Muehlheim, Germany) onto a DB-35ms capillary column (Agilent) which is equipped in an Agilent 7890 gas chromatograph (Agilent, CA, USA). The temperature gradient was set from 85ºC to 340ºC with a 15ºC per minute ramp using a gas flow of 2 ml per minute. The analytes were transferred into a Pegasus HT mass spectrometer (LECO, St. Joseph, MI, USA) setting at 250ºC. The recorded mass range was mass/charge (*m/z*) 50 to 600 at 20 scans per sec. The energy potential for ionization was -70 eV with a detector voltage of 1650V and a source temperature of 250ºC.

2.2.8.3 Data analysis

The analytes were identified with ChromaTOF software (LECO) and the Golm Metabolome Database library (GMD, http://gmd.mpimp-golm.mpg.de/). Metabolite levels were determined in a targeted fashion for 182 compounds using the R software package TargetSearch (Cuadros-Inostroza et al. 2009), using an *Arabidopsis* analyte reference generated previously (Riewe et al. 2012). Compounds with low average signal intensities (signal height<300) or with more than 80% missing values were discarded. Data was normalized with internal standard (L-Alanine-2,3,3,3-d_4) by multiplying individual metabolite abundances with the median internal standard abundance and dividing them by the internal standard abundance. Data was weight normalized accordingly. Data was median normalized for two balanced extraction days (Lisec et al. 2006). Outliers exceeding 2xSD were removed. Except unknown compounds, a total of 56 metabolites were analyzed. The data was log-transformed to improve normality (Steinfath et al. 2008). Statistical analysis was performed using R software (R Core Team 2013).

3 Results

3.1 Identification of biomass heterosis genes and their allelic variants

3.1.1 Sequence characteristics and deduced features of the 14 biomass heterosis candidate genes and their allelic variation

To study biological functions of the 14 candidate genes with potential impact on biomass heterosis, preliminary research on the genes was made based on published data (Table 1). All candidate genes were only tentatively annotated based on sequence similarities with characterized domains or characterized genes (Table 1). Polymorphisms of amino acid (aa) in exons were prevalent in 13 candidate genes, *At4g00335* only has 3 nucleotide changes in introns (data not shown). 37 of the total 50 peptide changes were located on non-conserved regions and 10 were in the DUF573 domain of unknown function (Table 2). The length of *At4g00280* differed between the two alleles: the $At4g00280_{Col}$ consisted of 72 aa, while $At4g00280_{C24}$ was shorter due to a stop codon at the 26th aa in the ML domain (Table 2). The most remarkable nucleotide polymorphisms between Col-0 and C24 in intergenic sequences of the 14 genes were the 491 bp and the 1925 bp InDels (Fig. 4): the 491 bp deletion was located in the intergenic sequences between $At4g00305_{C24}$ and $At4g00310_{C24,}$ and the 1925 bp insertion was located in the intergenic sequences between $At4g00270_{C24}$ and $At4g00280_{C24}$. The whole 491 bp polymorphic sequence was clearly deleted in C24 (the sequence of the deletion in Appendix 2), including 8 DNA binding sites (Table 3). The 1925 bp insertion in C24 was the sum of several genomic fragments which were embedded over the intergenic region between $At4g00270_{C24}$ and $At4g00280_{C24}$ (Fig. 3). One ORF which is composed of one exon (300 bp) was found in the 1925 bp insertion in C24 (Appendix 1). The ORF (ORF_{C24}) sequence did not match to any available sequences of organisms in NCBI (http://blast.ncbi.nlm.nih.gov/).

Figure 3: Alignment of the C24 intergenic region between *At4g00270* and *At4g00280* against to Col-0 reference sequence. The line in black indicates the ORF_{24}. The thin lines in gray represent the C24 intergenic region whose sequence does not match to the Col-0 reference sequence. The lines in pink and red represent the C24 intergenic regions which match to the corresponding locus in Col-0 reference sequence. The sequence positions of the red boxes are noted below. The colors of boxes represent the alignment scores: pink 80-200, red >=200.

Table 1: Published features of the 14 candidate genes

Gene[a]	Annotation[b]	Exon[c]	Position[d]	Cellular localization[e]	Protein domain[f]	Functionally characterized homolog	Promoter elements/motifs[g]
At4g00238.1	DNA-binding storekeeper (STK) protein-related transcriptional regulator	1	103826 ..105217	NC	DUF573	STK in potato	AtMYC2 BS in RD22, CCA1 M1 BS in CAB1, ATB2/AtbZIP53/AtbZIP44/GBF5 BS in ProDH, W-box PM, ARF1 BSM, ATHB6 BSM, CCA1 BSM, DPBF1&2 BSM, MYB BSP, MYB4 BSM, RAV1-A BSM, LFY CS BSM, ARF BSM, BoxII PM, GATA PM [LRE], Ibox PM, L1-box PM, T-box PM, SORLREP3, SORLIP5
At4g00240.1	Phospholipase D β2	10	106380 ..110718	IMBO, NC, PMB, VS	C2 Ca-DMT, C2 MT, C2 Ca/lipid-BD, CaLB, PLD, PLD/Transphosphatidylase	PLDβ2 (*At2g00240*)	AtMYC2 BS in RD22, CCA1 M1 BS in CAB1, BrBS1 IN AG, BrBS2 IN AG, BrBS3 IN AG, ATB2/AtbZIP53/AtbZIP44/GBF5 BS in ProDH, W-box PM, CArG PM, ARF1 BSM, ATHB6 BSM, CCA1 BSM, DPBF1&2 BSM, E2F BSM, MYB BSP, MYB4 BSM, RAV1-A BSM, RAV1-B BSM, LFY CS BSM, ARF BSM, GATA PM [LRE], Hexamer PM, Ibox PM, L1-box PM, T-box PM, SORLREP3, SORLIP2
At4g00250.1	STK -related transcriptional regulator	1	112705 ..113966	CP	DUF573	STK in potato	ATB2/AtbZIP53/AtbZIP44/GBF5 BS in ProDH, ARF1 BSM, DPBF1&2 BSM, MYB BSP, MYB4 BSM, RAV1-A BSM, LFY CS BSM, ARF BSM, BoxII PM, DRE-like PM, GATA PM [LRE], Hx PM, L1-box PM, T-box PM, TELO-box PM
At4g00260.1	MATERNAL EFFECT EMBRYO ARREST 45	7	114971 ..117051	NC	TF B3, DNA-binding pseudobarrel		DPBF1&2 BSM, Hx PM
At4g00270.1	STK-related transcriptional regulator	2	117152 ..118577	NC	DUF573	STK in potato	AtMYC2 BS in RD22, BrBS1 IN AG, BrBS2 IN AG, BrBS3 IN AG, ATB2/AtbZIP53/AtbZIP44/GBF5 BS in ProDH, W-box PM, ARF1 BSM, CCA1 BSM, DPBF1&2 BSM, MYB3 BSM, MYB4 BSM, RAV1-A BSM, ARF BSM, BoxII PM, DRE-like PM, GATA PM [LRE], Ibox PM, L1-box PM, LTRE PM, RY-repeat PM, SORLREP3
At4g00280.1	Leucine-rich repeat transmembrane protein kinase protein	3	122409 ..122851		Malectin_like		
At4g00290.1	Mechanosensitive ion channel protein	7	122851 ..125591	CP, MB, MCMB, MC, PMB	Msc, transmembrane-2, LSM-related		W-box PM, ARF1 BSM, ATHB2 BSM, RAV1-A BSM, LFY CS BSM, ARF BSM, GATA PM [LRE], T-box PM, SORLREP3, SORLIP2
At4g00300.2	Fringe related protein	1	128586 ..130399	ER, EX, NC	DUF604, Maletin	Drosophila genes fringe	RAV1-A BSM, LFY CS BSM
At4g00305.1	RING/U-box	1	131550	NC	ZF, RING-type, C3HC4		ATHB6 BSM, MYB BSP, MYB4 BSM, RAV1-A BSM,

Gene[a]	Annotation[b]	Exon[c]	Position[d]	Cellular localization[e]	Protein domain[f]	Functionally characterized homolog	Promoter elements/motifs[g]
	superfamily protein		..131930		RING-type, RING/FYVE/PHD-type		LFY CS BSM, VOZ BS, GATA PM [LRE], SORLREP3
At4g00310.1	Embryo Sac Development arrest 8 (EDA8)	3	133643 ..135469	NC			AtMYC2 BS in RD22, BellringerBS2 IN AG, BrBS3 IN AG, W-box PM, ABFs BSM, ARF1 BSM, CCA1 BSM, DPBF1&2 BSM, MYB BSP, MYB4 BSM, RAV1-A BSM, LFY CS BSM, ABRE BSM, ABRE-like BSM, ARF BSM, EE PM, GATA PM [LRE], G-box PM [LRE], GCC-box PM, Ibox PM, L1-box PM, Z-box PM, SORLIP2
At4g00315.1	F-box/RNI-like/FBD-like domains-containing protein	3	137404 ..138888	SCF ubiquitin ligase complex, CP, NC	FBD, cyclin-like, FBD-like, FBD, LRR 2, Skp2-like		AtMYC2 BS in RD22, BrBS1 IN AG, ARF1 BSM, CCA1 BSM, MYB BSP, MYB3 BSM, MYB4 BSM, RAV1-A BSM, LFY CS BSM, ARF BSM, BoxII PM, GATA PM [LRE], SORLIP5
At4g00320.1	F-box/RNI-like superfamily protein	3	139939 ..141643	MB, NC	FBD, Skp2-like, FBD-like, LRR 2		ATB2/AtbZIP53/AtbZIP44/GBF5 BS in ProDH, W-box PM, ARF1 BSM, DPBF1&2 BSM, MYB4 BSM, RAV1-A BSM, LFY CS BSM, ARF BSM, BoxII PM, GATA PM [LRE], Hx PM, T-box PM, SORLIP2
At4g00330.1	Calmodulin-binding receptor-like cytoplasmic kinase 2	6	142601 ..144523	NC	STPK, PK, ATP BS, PK -like, PK, catalytic, STPK-like	CRCK1 (*At5g58940*), (Yang et al. 2004).	BrBS1 IN AG, W-box PM, ATHB2 BSM, RAV1-A BSM, RAV1-B BSM, LFY CS BSM, BoxII PM, DRE-like PM, EE PM, GATA PM [LRE], GCC-box PM, T-box PM
At4g00335.1	RING-H2 finger B1A	4	145904 ..148339	NC	ZF, C3HC4 RING-type, RING/FYVE/PHD-type		BrBS1 IN AG, W-box PM, ATHB2 BSM, RAV1-A BSM, RAV1-B BSM, LFY CS BSM, BoxII PM, DRE-like PM, EE PM, GATA PM [LRE], GCC-box PM, T-box PM

[a] Splicing variant of the gene which was used for this analysis.
[b] Source: http://arabidopsis.org/
[c] Number of exons and introns
[d] Nucleotide positon from 5' UTR to 3'UTR, reference sequence Col-0 (TAIR 10, http://arabidopsis.org/)
[e] Source: http://arabidopsis.org/, BS: binding site, CP: chloroplasts, ER: endoplasmic reticulum, EX: extracellular region, IMBO: intracellular membrane bounded organelle, MB: membrane, MC: mitochondria, NC: nucleolus, PMB: plasma membrane, VS: vesicle
[f] Source: http://pfam.xfam.org/ (version Pfam 27.0), (Finn et al. 2014), (D)MT: (dependent) membrane targeting, LRR: leucine-rich repeat, LSM: like sm ribonucleoprotein, Msc: mechanosensitive ion channel, PK: protein kinase, STPK: Serine/threonine protein kinase, TF: transcriptional factor, ZF: zinc finger
[g] source: http://arabidopsis.med.ohio-state.edu/AtcisDB/, (Palaniswamy et al. 2006), BSM: binding site motif, BSP: binding site promoter, Br: bellringer, CS: consensus, EE: evening element, Hx: Hexamer, PM: promoter motif

Table 2: Encoded amino acid sequence variation of the Col-0 and C24 alleles of the 14 candidate genes

Gene[a]	Col-0[b]	C24[b]	Position[c]	Domain
At4g00238.1	E	G	20	ND
	K	E	26	ND
	N	K	98	ND
	V	M	208	DUF573
At4g00240.1	R	K	335	ND
	R	T	363	PLD δ
	Q	R	373	PLD δ
	N	T	447	PLD δ
	G	S	556	PLD δ
	R	K	484	PLD δ
	N	S	491	PLD δ
	G	E	857	PLD δ
At4g00250.1	F	S	23	ND
	K	I	74	ND
	SR	*D	96-97	ND
	A	P	113	DUF573
	H	N	163	DUF573
	RL	SF	172-173	DUF573
	N	R	175	DUF573
	VN	GD	179-180	DUF573
	NK	MR	183-184	DUF573
	G	V	186	DUF573
	N	D	189	DUF573
	P	R	212	ND
	V	I	221	ND
	*	V	242-243	ND
	L	F	245	ND
	N	S	263	ND
	L	V	280	ND
	K	R	285	ND
	V	L	292	ND
	E	K	304	ND
At4g00260.1	NE...FQ	*	430-440	ND
At4g00270.1	V	G	13	ND
	NGTLS	*	19-23	ND
	H	R	24	ND
	E	D	42	ND
	M	Q	50	ND
	M	K	52	ND
	KK	*	57-58	ND
	*	UFEIAQ	78-79	DUF573
	*	D	222-223	ND
	DEDN	EDNE	223-226	ND
At4g00280.1	ED	VQ	14-15	ND
	EEGRE	NFCCL	17-21	Malectin_like
	S	Y	23	Malectin_like
	VP...TI	*	26-71	Malectin_like
At4g00290.1	A	P	22	ND
	A	V	47	ND
	G	D	104	ND
	ND	*	105-106	ND
At4g00300.2	T	N	42	ND
	N	K	72	ND
At4g00305.1	I	T	44	ND
At4g00310.1	C	S	19	ND
At4g00320.1	D	E	407	ND
At4g00330.1	N	K	47	ND

[a] Splicing variant of the gene

[b] The letter in rows of Col-0 and C24 refer to the peptide sequences in one-letter amino acid abbreviations, *: deletion

[c] The polymorphism sites were plotted with the starting amino acid of the Col-0 alleles as starting point (1).

[d] ND: nonconserved domain.

Table 3: Cis-regulatory elements binding sites in the 491bp InDel

Name [a]	Genomic position [b]	Sequence [c]
GATA PM	132069-132074	tgataa
MYB4 BSM	132130-132136	aacaaac
RAV1-A BSM	132133-132137	caaca
MYB BSP	132157-132164	aaccaaac
MYB4 BSM	132157-132163	accaaac
Evening Element PM	132183-132191	aaaatatct
GATA PM	132456-132461	tgataa
AtMYC2 BS in RD22	132549-132554	cacatg

[a] BS: binding site, BSM: binding site motif, BSP: binding site promoter, PM: promoter motif.
[b] Start and end position of the corresponding binding sites. The positions match to the reference Col-0 position on chr.IV (TAIR 10).
[c] Source: http://arabidopsis.med.ohio-state.edu/AtcisDB/.

3.1.2 RNA expression of the 14 genes in parents and hybrids at 5 DAS

Of the 14 candidate genes, the expression levels of 10 genes were detected in all four genotypes (Table 4). Differential expression between genotypes was tested using pairwise comparison (Trapnell et al. 2012), but none of the candidate genes was significantly different between genotypes. Also, none of the genes in the hybrids were expressed greater than 1.5 times of the mid parental value (MPV) or less than one-fourth of the MPV, which are the thresholds applied for detecting hybrid specific transcription levels at an early growth in *Arabidopsis* (Fujimoto et al. 2012).

Table 4: Transcription levels of the 14 candidate genes in parents and hybrids at 5 DAS

Locus	Col-0	C24	Col-0×C24	C24×Col-0
AT4G00238	1.00 (9.34)	1.06 (9.93)	0.90 (8.40)	0.88 (8.18)
AT4G00240	1.00 (1.64)	2.00 (3.27)	1.71 (2.79)	1.27 (2.07)
AT4G00250	1.00 (1.27)	0.06 (0.08)	0.34 (0.43)	0.38 (0.48)
AT4G00260	NT	NT	NT	NT
AT4G00270	1.00 (18.58)	0.47 (8.67)	0.69 (12.9)	0.65 (12.03)
AT4G00280	NT	NT	NT	NT
AT4G00290	1.00 (9.76)	0.72 (7.00)	0.9 (8.74)	0.93 (9.06)
AT4G00300	1.00 (31.87)	1.16 (36.84)	1.16 (36.87)	1.11 (35.48)
AT4G00305	1.00 (0.76)	0.86 (0.65)	0.76 (0.58)	1.13 (0.86)
AT4G00310	1.00 (4.03)	0.7 (2.81)	0.69 (2.77)	0.71 (2.86)
AT4G00315	NT	NT	NT	NT
AT4G00320	NT	NT	NT	NT
AT4G00330	1.00 (28.91)	0.68 (19.67)	1.01 (29.23)	0.91 (26.31)
AT4G00335	1.00 (8.36)	0.81 (6.77)	0.84 (7.01)	0.91 (7.61)

All transcripts are relative to Col-0, Absolute transcription value in parenthesis, NT: Not tested because of insufficient reads. Significant differences between genotypes were tested using pairwise comparison according to the method (Trapnell et al. 2012).

Overall transcript levels were lower in C24 than in Col-0, except *At4g00240* whose expression levels in C24 were two times higher than in Col-0. The expression levels of 6 genes (*At4g00240*, *-250*, *-270*, *-290*, *-300* and *-335*) in the hybrids were in the range of MPV which is between lower and higher parent values (Table 4). The transcription levels of

At4g00238 and *At4g00310* in the hybrids were lower than the lower parent values (Table 4). The transcriptional patterns of genes belonging to the same family were similar: The two RING finger protein coding genes, *At4g00305* and *At4g00335* were expressed less in C24 than in Col-0, and their expression levels in the hybrids were similar to those of paternal parent values. *At4g00250* and *At4g00270* which encode STK-related transcriptional regulators were expressed lower in C24 than in Col-0 and the expression levels in the reciprocal hybrids were similar to each other.

Expression of several genes which have been described as key genes (*CCA1* and *LHY*) for biomass heterosis in interspecific hybrids and allopolyploid in *Arabidopsis* (Ni et al. 2009), and genes which are supposed to be involved in biomass heterosis in *Arabidopsis* hybrids were also analyzed (Shen et al. 2012) (Appendix 3). However none of the expression levels were significantly different between the 4 genotypes.

3.1.3 Evaluation of the candidate genes using cosmids

3.1.3.1 Analysis of the selected cosmids

Four Col-0 cosmids (Cos_013_{Col}, Cos_062_{Col}, Cos_125_{Col} and Cos_170_{Col}) and five C24 cosmids (Cos_395_{C24}, Cos_401_{C24}, Cos_411_{C24}, Cos_507_{C24} and Cos_523_{C24}) harboring the 14 candidate genes were selected from Col-0 and C24 genomic libraries constructed in pCLD04541 (Fig. 4).

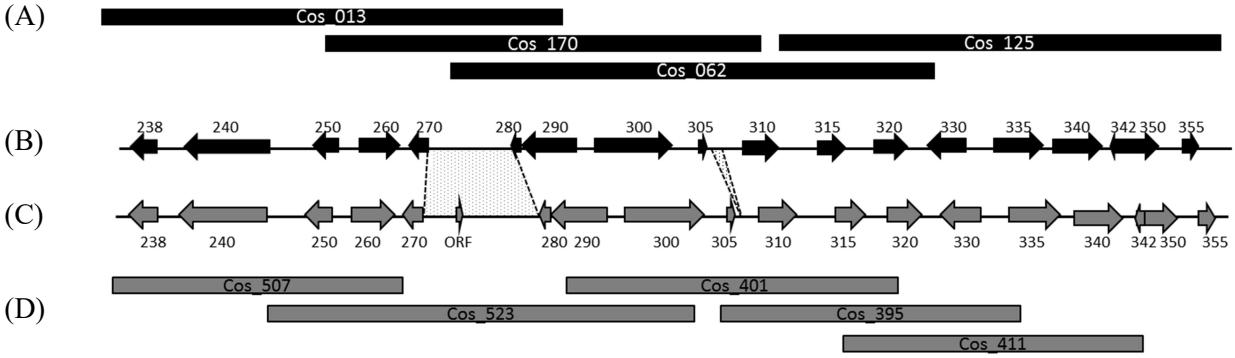

Figure 4: Schematic drawing of the inserts of the selected cosmids and their genomic region covered. It shows the four inserts of Col-0 cosmids (A), the genes of Col-0 (B), the five inserts of C24 cosmids (D) and the genes of C24 (C). The two zones in gray lying between (B) and (C) represent the two sequence polymorphisms, 1925 bp insertion in C24 (left) and the 491 bp deletion in C24 (right).

Every candidate gene with the upstream sequence (-2000 upstream of the TSS) and downstream sequence (1500 downstream of the stop codon) was completely included within one or two of the inserts (Fig. 4 and Appendix 4). The 1925 bp insertion lying between

At4g00270$_{C24}$ and *At4g00280*$_{C24}$ is harbored in the Cos_523$_{C24}$ insert. The 491 bp polymorphic sequence is included in the Cos_395$_{C24}$ and Cos_401$_{C24}$ inserts (Fig. 4).

3.1.3.2 Estimation of leaf area of cosmid transgenic lines in homozygous T3

Six independent homozygous T3 lines of each genotype and four controls (Col-0, C24, Col-0×C24 and C24×Col-0) were phenotyped using an automated phenotyping system, LemnaTec Scanalyzer (Junker et al. 2015). Because of low transformation efficiency with Cos_062, the genotypes '*Col+062*$_{Col}$' and '*C24+062*$_{Col}$' could not be phenotyped in T3. The experiments were carried out three times consecutively.

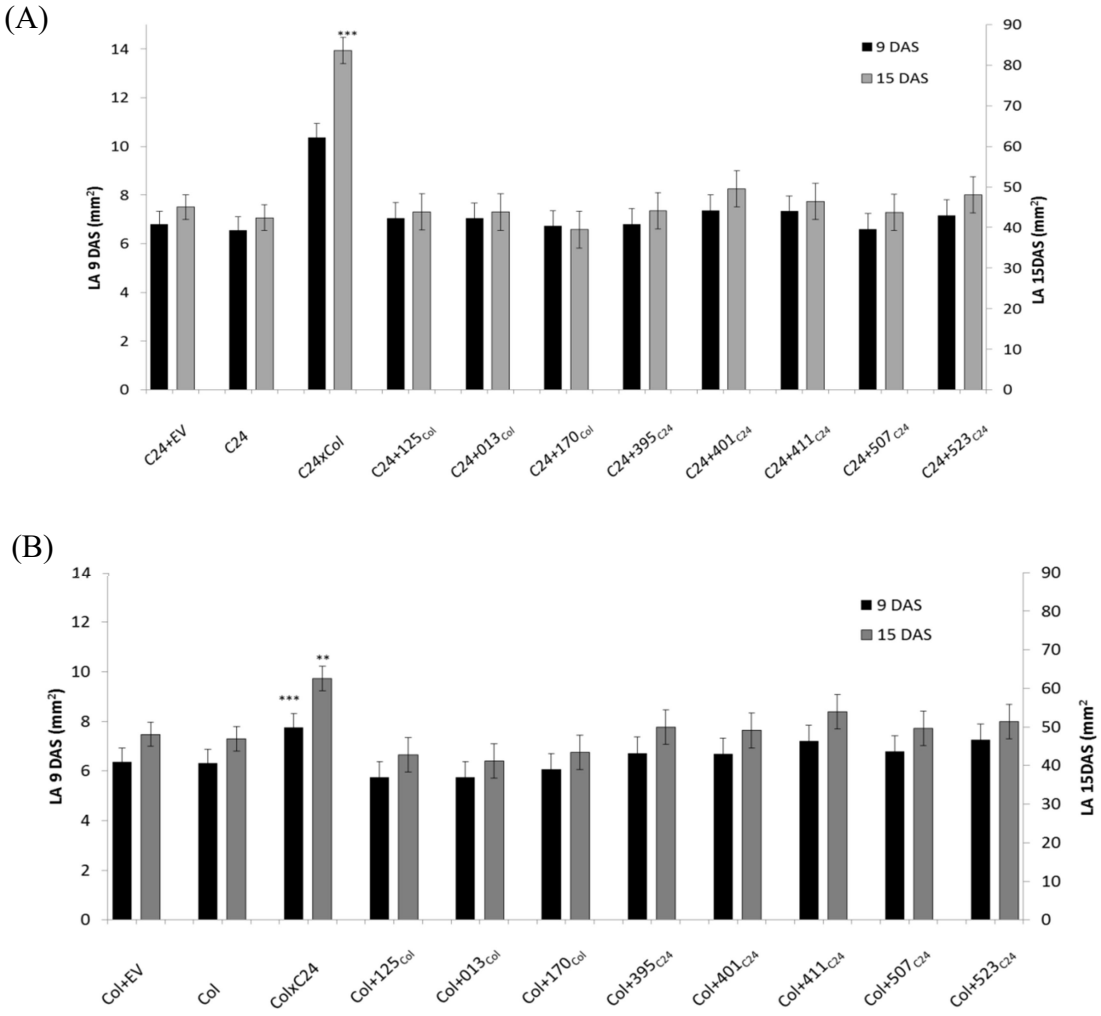

Figure 5: Mean leaf area of cosmid transgenic genotype. Mean value of Col-0 transgenic genotype. (A) and C24 transgenic genotype (B) at 9 DAS (left axis) and 15 DAS (right axis). Mean leaf area of a genotype was estimated from 6 individual lines belonging to the same genotype using the linear mixed model (REML). Error bars represent standard errors (SE). Significant differences between mean value of the transgenic genotype and mean value of the transgenic control genotype with the same plant genomic background were identified using Fisher's Least Significant Difference (LSD): **P < 0.01, ***P < 0.001

Even though all transgenic lines are progenies of plants which were propagated at the same time under identical environmental conditions, the mean values of leaf area within lines of the same genotype varied, even within lines of transgenic controls (Appendix 8). Plants which were germinated from large-sized seeds were relatively big (Appendix 9 and 14). Thus mean values of seed area, which were estimated from 100 seeds of the corresponding transgenic line, were used in the linear mixed model as a co-factor. Due to the varying mean leaf areas within the lines, to estimate the phenotypic change in leaf area by transgene, the data set was processed in two ways.

Firstly, every mean value of a genotype, which is composed of 6 individual transgenic lines, was compared to the mean value of the transgenic control genotype with the same plant genomic background (Appendix 9). The mean values of each reciprocal hybrid were compared to that of the cosmid transgenic control whose genomic background is identical with the maternal line of the hybrids. Only means of the reciprocal hybrids were significantly greater than the controls. No significant differences between transgenic and control genotypes were observed at any time point (Fig. 5 and Appendix 9). However, a phenotypic change by a transgene can be masked in the mean value of the genotype because of the large variation within lines.

Therefore, to monitor a phenotypic change by a transgene gene in individual lines, each mean leaf area of a line was compared to the mean leaf area of the transgenic control genotype (Appendix 10, Fig. 6). The majority of C24 transgenic lines harboring Col-0 genomic fragments (14 of 18 lines) did not differ in leaf area compared to transgenic control *C24+EV* (Fig. 6A). On the contrary, more than half of Col-0 transgenic lines harboring Col-0 genomic fragments, 9 lines at 9 DAS and 11 lines at 15 DAS, were smaller than the control *Col+EV* (Fig. 6B). Most of the C24 and Col-0 transgenic plants harboring C24 genomic fragments did not show a phenotypic change in size (Fig. 6C and 6D). Only three lines, $Col{+}411_{C24}$ *A, -B* and $Col{+}523_{C24}$ *B* were larger than the control at 9 DAS, but the large size was not sustained until 15 DAS. However, among cosmid transgenic genotypes, the $Col{+}411_{C24}$ was the only genotype (Fig. 6C), two lines of which displayed larger leaf area than the controls by an opponent genomic fragment.

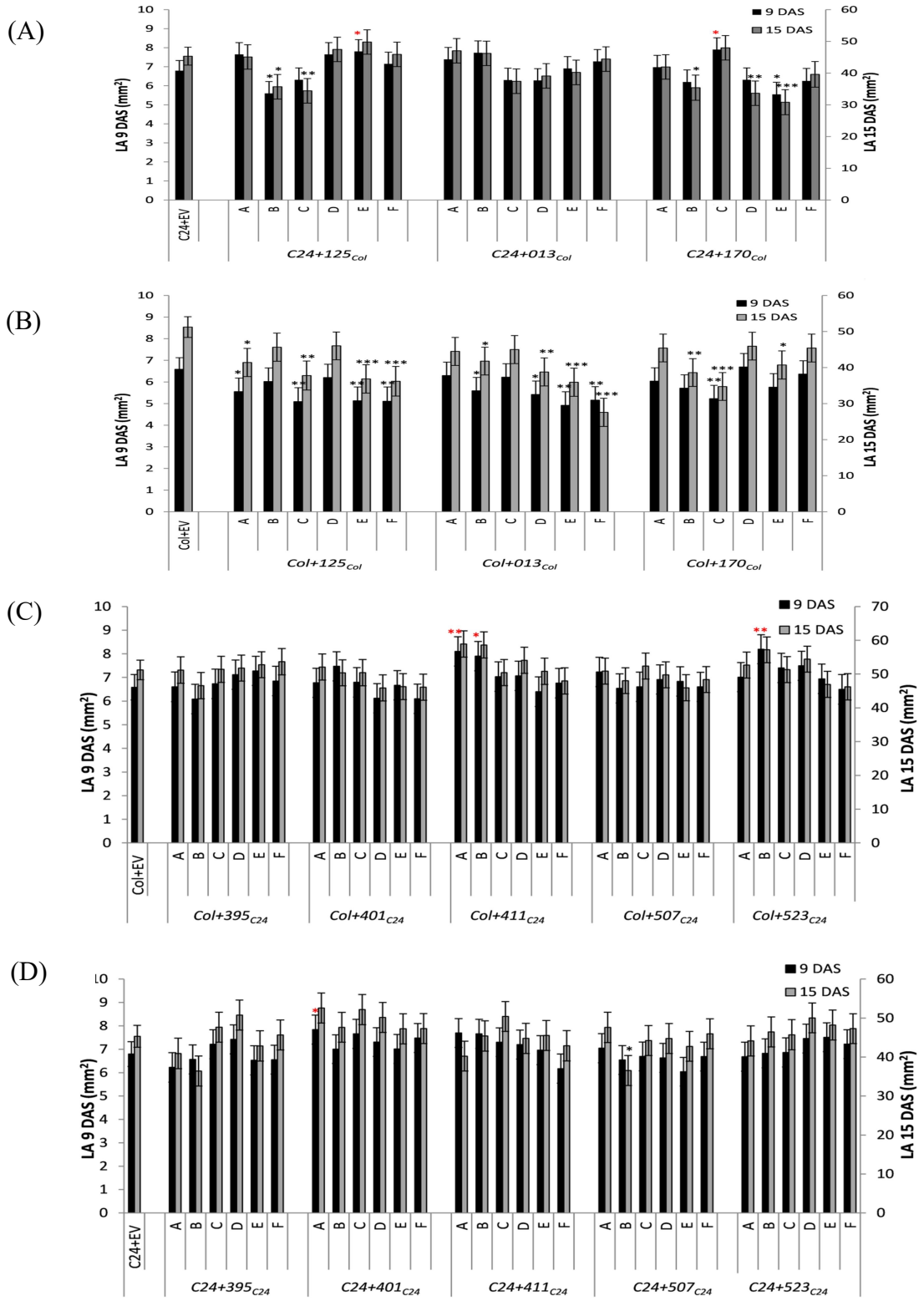

Figure 6: Mean leaf area of cosmid transgenic lines in T3 generation at 9 DAS and 15 DAS. The mean value was estimated using the linear mixed model (REML) from 3 replicates. Error bars represent standard errors. Significant differences between mean value of a transgenic line and mean value of the transgenic control genotype with the same plant genomic background was identified using the Fisher's Least Significant Difference (LSD): *P < 0.5, **P < 0.01, ***P < 0.001. Asterisks in red: transgenic lines larger transgenic control genotype; Asterisks in black: transgenic lines smaller than transgenic control genotype

3.1.3.3 Estimation of a change in leaf area using segregating T2 populations

To overcome within-line variation, which was observed in the phenotype of homozygous T3 lines, several genotypes were phenotyped again in T2 generation. These genotypes included *C24+411*$_{C24}$, *Col+411*$_{C24}$, *C24+062*$_{Col}$, *Col+062*$_{Col}$, *Col+395*$_{C24}$, *C24+395*$_{C24}$, *Col+401*$_{C24}$ and *C24+401*$_{C24}$ (Appendix 11). The segregating T2 seedlings were sorted into transgenic siblings and non-transgenic siblings (the method described in 2.2.7.2). The mean value of transgenic siblings was compared to the mean value of their non-transgenic siblings. The genotype *Col+411*$_{C24}$ was included in the phenotyping in the T2 generation because the two lines *Col+411* $_{C24}$*A* and *Col+411* $_{C24}$*B* exhibited a greater increase in leaf area than the transgenic control (Fig. 6C). However, unlike the homozygous T3, *Col+411* $_{C24}$*A* and *Col+411* $_{C24}$*B* in T2 did not exhibit an increase in size compared to their non-transgenic siblings, and they are also not bigger than other transgenic lines (Fig. 7A and 7B). The cosmid transgenic lines harboring *At4g00310* transgene *C24+062*$_{Col}$, *Col+062*$_{Col}$, *Col+395*$_{C24}$, *C24+395*$_{C24}$, *Col+401*$_{C24}$ and *C24+401*$_{C24}$ were phenotyped in T2. Because among transgenic plants harboring a single candidate gene, only transgenic lines harboring single transferred *At4g00310* exhibit a change in leaf area (section 3.1.4.2). Unlike the decreased size in transgenics harboring a single *At4g00310* transgene, all the cosmid transgenic genotypes harboring *At4g00310* transgene, except *C24+401E*, did not display any difference in leaf area compared to their non-transgenic siblings (Appendix 11 and Fig. 7).

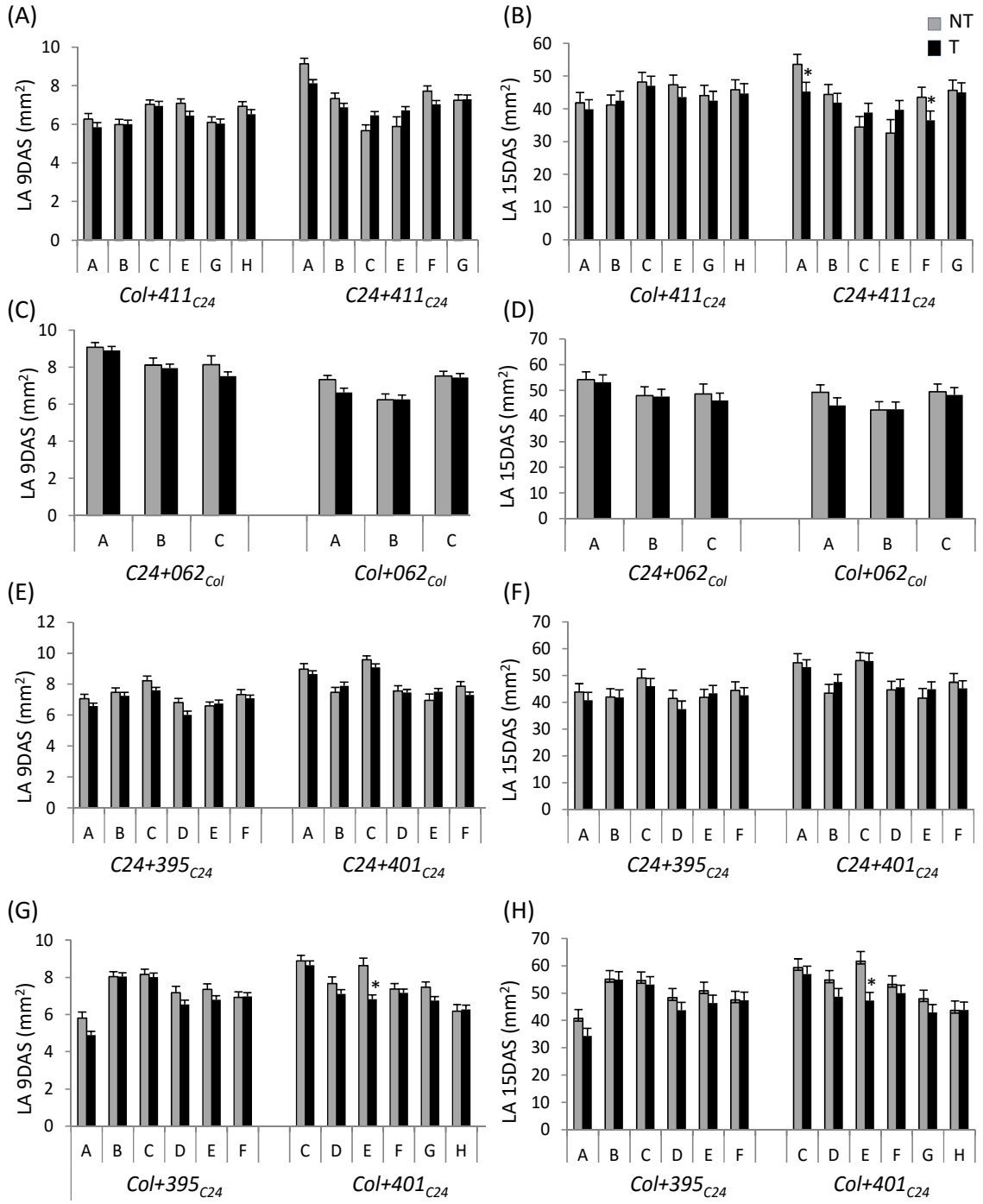

Figure 7: Mean leaf area of cosmid transgenic lines in T2 segregating generation at 9 DAS and 15 DAS. The mean value was calculated using the linear mixed model (REML) from 3 replicates. Error bars represent standard errors (SE). T: transgenic siblings. NT: non-transgenic siblings. Significant differences between mean value of T and NT were identified if the difference between means of T and NT is greater than 2×SE, and marked with an asterisk.

3.1.4 Evaluation of the individual 14 candidate alleles

3.1.4.1 Cloning of individual candidate alleles

To isolate causative gene and potential allelic variation on biomass heterosis, the Col-0 and C24 alleles of the 14 candidate genes, and ORF_{C24} were cloned individually (Fig. 8 and Appendix 5). To regulate the cloned alleles in plants under their own cis-regulatory elements, every allele was constructed with the upstream and downstream sequences. The detailed methods for deducing promoter and terminate sequences are described in 2.2.2. $p305_{Col}$, $p305_{C24}$, $p310_{Col}$ and $p310_{C24}$ include the 491 bp polymorphic sequence.

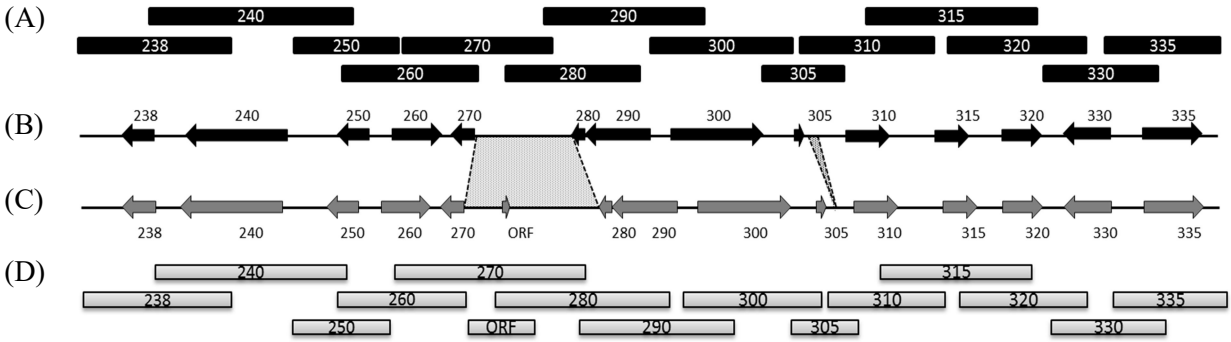

Figure 8: Schematic drawing of the genomic fragments of the individual genomic clones (A and D). Alignments of cloned Col-0 (A) and C24 (D) genomic fragments against the genomic region of Col-0 (B) and of C24 (C). The three-digits (XXX) in boxes are corresponding to the last three-digits (XXX) of the genomic clone names, pXXXCol (A) or pXXXC24 (D). The arrows in black and in gray represent the 14 genes of Col-0 (B) and C24 (C), respectively. The two zones in gray lying between (B) and (C), a trapezoid in left and triangle in right, refer to the polymorphic genomic regions of 1925 bp insertion in C24 (left) and the 491 bp deletion in C24 (right), respectively.

3.1.4.2 Evaluation of change in leaf area of transgenic line harboring single candidate genes

To overcome within-line variation which would make it difficult to detect a change in leaf area by transgenes, the whole set of transgenic plants harboring the individual candidate genes were phenotyped in T2 generation. Mean leaf area of transgenic siblings was compared to that of non-transgenic siblings (the method described in 2.2.7.2). Only lines harboring *At4g00310* did show any phenotypic change in leaf area (Appendix 12): Transgenic plants harboring introduced *At4g00310* transgene were smaller compared to their non-transgenic siblings (Appendix 12). Without distinction of *At4g00310* alleles and genetic backgrounds, transferred *At4g00310* induced a decrease in rosette leaves. The reducing leaf area was detectable at early vegetative stages (9 DAS) and the difference in leaf area compared to that of non-transgenic siblings was more noticeable as vegetative growth progressed (Fig. 9). To investigate a

general pattern of phenotypic change by introduced *At4g00310* alleles in the two genomic backgrounds, phenotyping was repeated with an increased number of transgenic lines (Appendix 15). But a uniform pattern of change by genotype was not observed (Fig. 9). Occurrence of phenotypic change was higher in $Col{+}310_{Col}$ and lower in $C24{+}310_{Col}$ (Fig. 9).

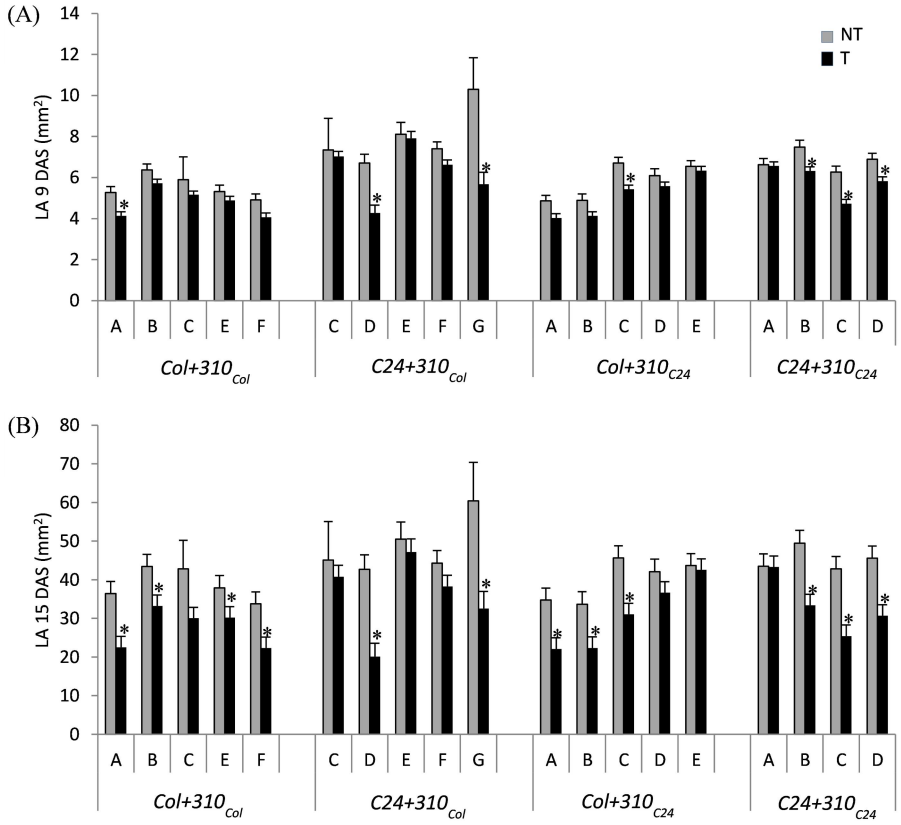

Figure 9: Analysis of leaf area of transgenics harboring transferred *At4g00310* in T2 segregating generation. Mean value was calculated using linear mixed model (REML) from 3 replicates, and error bars represent standard errors (SE). T: transgenic siblings, NT: non-transgenic siblings. Significant differences between mean value of T and NT were identified if the difference between means of T and NT was greater than 2×SE and marked with an asterisk.

3.1.4.3 Morphological changes in transgenic plants harboring transferred *At4g00310*

The decrease in leaf area in transgenic plants harboring transferred *At4g00310* was caused by shortened petioles and small leaves, not by delayed shoot development or tardy germination: Both transgenic siblings and their non-transgenic siblings germinated at the same time at 5 DAS (including 3 days of stratification) and had the same leaf number throughout in early vegetative stages (Fig. 10A and 10B). The difference in leaf area between transgenics sibling and their non-transgenic siblings was detectable at early vegetative stages (Fig. 10A) and became more pronounced throughout the development (Fig. 10B). Compared to the transgenic control *Col+EVA* (Fig. 10D), leaf blades of the transgenic plants harboring introduced *At4g00310* were serrated and leaves grew in a cluster by the shortened petioles in later vegetative stages (Fig. 10E).

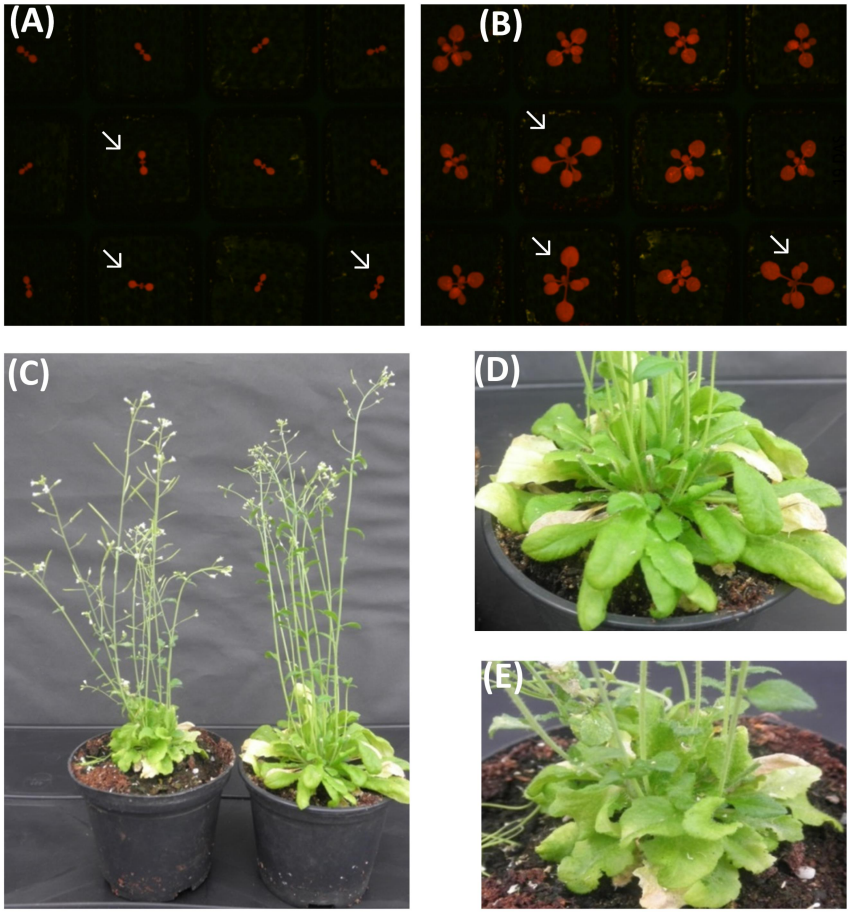

Figure 10: Phenotypes of transgenic plants harboring introduced *At4g00310* transgene. (A-B) Fluorescence images of the *Col+310*$_{C24}$*C* population at 8 DAS (A) and at 15 DAS (B). Arrows mark non-transgenic sibilings. (C) The morphology of transgenic *Col+310*$_{C24}$*C* (left) and transgenic control *Col+EVA* (right) at flowering stage. (D-E) Close up of the transgenic *Col+310*$_{C24}$*C* (E) and the transgenic control *Col+EVA* (D).

3.2 Assessment of *FUMARASE2* allelic variants

3.2.1 Analysis of the two cosmids, Cos_FUM2$_{Col}$ and Cos_FUM2$_{C24}$

Two cosmids, Cos_FUM2$_{Col}$ and Cos_ FUM2$_{C24}$ harboring the *FUM2$_{Col}$* and the *FUM2$_{C24}$* allele, respectively, were isolated from Col-0 and C24 genomic DNA libraries constructed in the binary cosmid pCLD04541. Both ends of the inserts were sequenced and blasted against the *Arabidopsis* reference sequence (Col-0, TAIR 10). The insert of the Cos_FUM2$_{Col}$ is 22,605 bp long, and the 5' and 3' of the insert-ends correspond to the reference Col-0 genomic positions chr.V:20720939 and chr.V:20743544, respectively (Table 5). Besides *FUM2$_{Col}$*, 7 additional genes (from *At5g50930* to *At5g51000*) are present in the insert (Table 5). The insert of the Cos_FUM2$_{C24}$ is 17,658 bp long, and the 5' and 3' of the insert-ends correspond to the reference Col-0 genomic positions chr.IV:1461692 and chr.V:20739760, respectively (Table 5). The insert of Cos_FUM2$_{C24}$ harbors 2 additional genes (*At5g50960* and *At5g50970* beside *FUM2$_{C24}$*.

Table 5: Genes in the inserts of Cos_FUM2$_{Col}$ and Cos_FUM2$_{C24}$

Gene[a]	Cos_FUM2$_{Col}$[b]	Cos_FUM2$_{C24}$[b]	Description
At5g50930	I	–	ATMHF1, Histone superfamily protein
At5g50940	I	–	RNA-binding KH domain-containing protein
At5g50950	I	I	FUMARASE 2
At5g50960	I	I	ATNBP35, NBP35
At5g50970	I	I	Transducin family protein / WD-40 repeat family protein
At5g50990	I	–	Tetratricopeptide repeat (TPR)-like superfamily protein
At5g50995	I	–	tRNA-Tyr (anticodon: GTA)
At5g51000	I	–	F-box and associated interaction domains-containing protein

[a] AGI code
[b] I: genes which are included in the cosmid, –: genes which are not included in the cosmid. Only genes whose start and end codons are in the insert were noted.

Tentative characteristics of the additional genes in the two cosmids which were predicted based on the information of conserved domains, genes sharing high sequence similarity and biological data: *AtMHF1*/*At5g50930* and *ATMHF2* are co-factors of *AtFANCM,* a meiotic anti-crossover factor, and limit crossover formation at meiosis (Girard et al. 2014). *At5g50940* contains the hnRNP K homology (KH) domain which has been identified as nucleic acid recognition motif in proteins and binds RNA or ssDNA (reviewed by Valverde, Edwards, & Regan, 2008). *At5g50940* is supposed to be involved in transcription from RNA polymerase II promoter (Carbon et al. 2009). *At5g50960* encodes ATNBP35 which is an ortholog of cytosolic Fe-S cluster assembly factor NBP35 in yeast *Saccharomyces cerevisiae* (Roy et al.

2003, Hausmann et al. 2005, Kohbushi et al. 2009). The *AtNbp35* is essential for life supporting, because AtNbp35-deficient *Arabidopsis* mutants were seedling-lethal mutants (Kohbushi et al. 2009). *At5g50970* was tentatively annotate a protein belonging to a WD-40 repeat family protein which are known to serve as platforms for the assembly of protein complexes or mediators of transient interplay among other proteins (Stirnimann et al. 2010). In *Arabidopsis*, several WD40-containing proteins act as key regulators of plant-specific developmental events (van Nocker and Ludwig 2003). *At5g50990* is annotated as a tetratricopeptide repeat (TPR)-like superfamily protein. TPR is a structural motif consisting of a degenerate 34 amino acid sequence motif identified in a wide variety of proteins. But a specific biological function of *At5g50990* has not been proved. *At5g50995* encodes a transfer RNA carrying tryptophan (anticodon: GTA). Considering the tentative characteristics of the additional genes in the two cosmids, they are expected to not directly influence fumarate and malate content in plants.

3.2.2 Polymorphisms between the Col-0 and C24 *FUMARASE 2* alleles

Sequences of the 5' and 3' ends of the Cos_FUM$_{C24}$ insert match to the different genome of Col-0 reference sequences (TAIR 10) chr.IV:1461692 and chr.V:20739760, respectively. In order to find out whether the sequence of 5' end of the insert which matches to the locus on chr.IV was caused by chimeric ligation which could occur during C24 genomic library preparation, the promoter sequence of *FUM2* was amplified using PCR with C24 genomic DNA and sequenced. The upstream sequence of *FUM2* in C24 was identical with that in Cos_FUM$_{C24}$, which prove that the upstream sequence of *FUM2* is polymorphic between *FUM2$_{Col}$* and *FUM2$_{C24}$*. The entire Cos_FUM$_{C24}$ was sequenced using Next Generation Sequencing on Roche GS FLX (454). The acquired Cos_FUM$_{C24}$ sequence was used for the further analysis on sequence polymorphism between *FUM2$_{Col}$* and *FUM2$_{C24}$*. Two insertions were found in the upstream region of *FUM2*$_{C24}$ and the insertions match to noncoding upstream sequences of *At4g03310* and *At2g07700* (Fig. 11). The coding sequences of *FUM2$_{Col}$* and *FUM2$_{C24}$* are composed of 16 exons and the full-length genomic DNA sequences (from 3' UTR to 5'UTR) of *FUM2$_{Col}$* and *FUM2$_{C24}$* share 98% sequence identity. Six SNPs were present in the exons between the two alleles; 4 SNPs cause synonymous substitutions and 2 SNPs caused nonsynonymous substitutions. The first nonsynonymous substitution occurs in the 1st exon at the genomic position chr.V:20731622. Because 'a' in Col-0 was changed into 'g', the isoleucine in Col-0 changed to the threonine in C24; the

second change occurs at the genomic position chr.V:20731739 in 7th exon, thus GAG (glutamine) in Col-0 is changed to AAG (lysine) in C24.

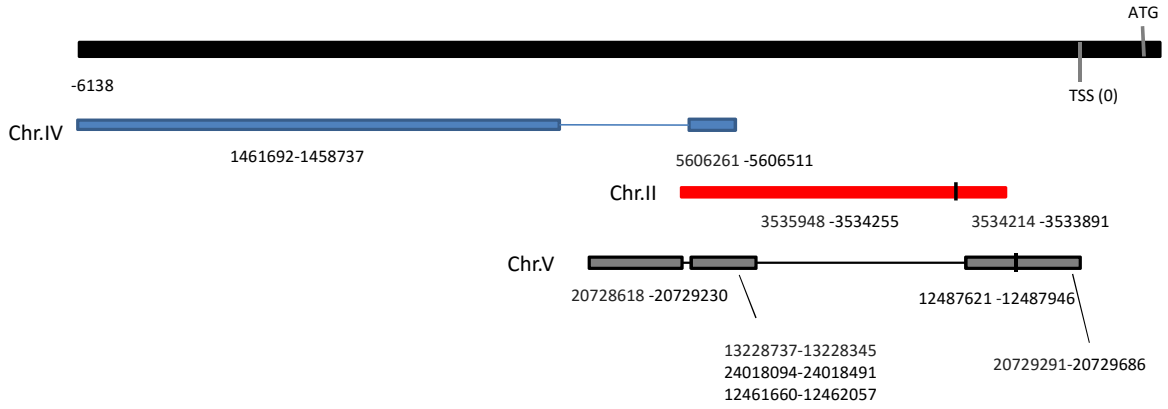

Figure 11: The upstream region of *FUM2*$_{C24}$. The bar in black on top: from upstream region of *FUM2*$_{C24}$ (-6138) to start codon (ATG). The sequences of upstream region of *FUM2*$_{C24}$ matches with Col-0 reference sequence (TAIR 10) on three different chromosomes, chr.IV (bars in blue), chr.II (bars in red) and Chr.V (bars in gray). The numbers noted under the boxes refer the genomic positions of both ends of the genomic fragments in the Col-0 reference sequence (TAIR 10). TSS: transcription start site, ATG, start codon.

3.2.3 Metabolite analysis in the transgenic plants

3.2.3.1 Metabolite profiling of the whole set of transgenic plants

FUM2$_{Col}$ and *FUM2*$_{C24}$ were separately introduced into the three genomic backgrounds Col-0, C24 and *fum2*. As controls, Cos_EV was transformed into the three genomic backgrounds. A total of 36 homozygous lines, four independent lines of each nine genotypes (*Col+FUM2*$_{Col}$, *Col+FUM2*$_{C24}$, *Col+EV*, *C24+FUM2*$_{Col}$, *C24+FUM2*$_{C24}$, *C24+EV*, *fum2+FUM2*$_{Col}$, *fum2+FUM2*$_{C24}$ and *fum2+EV*) were used for metabolite profiling. Metabolite abundances in each of lines were measured using gas chromatography-mass spectrometry (GC-MS). Fumarate levels with-in lines were similar to each other, except in *fum2+FUM2*$_{C24}$A whose level was deviated from the average level of the *fum2+FUM2*$_{C24}$ (Fig. 12). The line '*fum2+FUM2*$_{C24}$A' was omitted for further analysis.

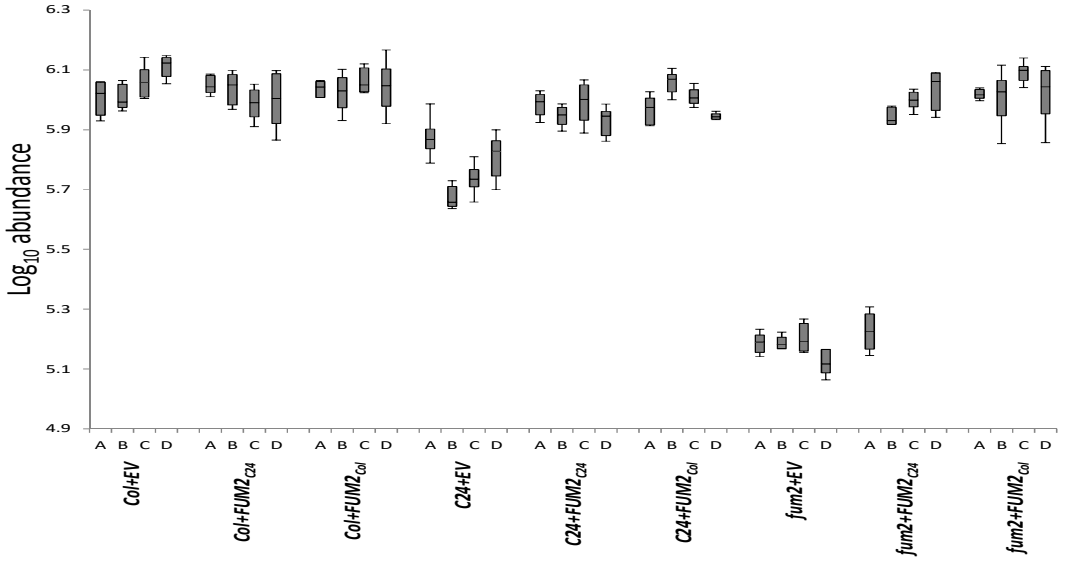

Figure 12: Fumarate levels in all tested transgenic lines. Data are displayed in box-and-whisker plots; horizontal line in a box: median values, the bottom and top of the box: first and third quartile, the whiskers: the lowest and highest values. X-axis: transgenic lines, four individual homozygous lines of the nine genotypes were noted in A, B, C and D and the genotypes were noted below. Y-axis: log10-transformed relative fumarate abundance. The fumarate abundance was adjusted with an internal standard amount and original sample weight.

3.2.3.2 Metabolites whose levels vary between Col-0 and C24

In a total of 56 targeted metabolites, levels of 34 metabolites were significantly different between *Col+EV* and *C24+EV* (Appendix 16 and Table 6): the metabolites include fumarate, malate, pyruvate, shikimate α-ketoglutaric acid and succinate. Fumarate level in *Col+EV* was 1.85 times higher than *C24+EV*; the malate level in *Col+EV* was 0.74% of the level in *C24+EV*. Levels of shikimate, pyruvate, α-ketoglutaric and succinate in *Col+EV* were lower than those in *C24+EV* (Table 6).

Table 6: Metabolite whose levels were different in Col-0 and C24

Metablite	*Col+EV*	*C24+EV*
α-Aminoadipic acid	2.65 ± 0.10	3.00 ± 0.06
Alanine	4.16 ± 0.05	4.22 ± 0.05
β-Alanine	3.00 ± 0.07	2.80 ± 0.07
Arginine	3.34 ± 0.07	3.51 ± 0.07
Ascorbate	3.76 ± 0.12	3.96 ± 0.09
Asparagine	3.74 ± 0.05	3.67 ± 0.06
2-Aminobutyric acid	2.83 ± 0.06	2.94 ± 0.05
Citrate	5.62 ± 0.07	5.70 ± 0.07
Erythritol	2.59 ± 0.05	2.75 ± 0.07
Fructose	3.47 ± 0.10	3.90 ± 0.16
Fumarate	6.05 ± 0.06	5.78 ± 0.09
Glucopyranose	4.63 ± 0.12	4.78 ± 0.12
Glucose	3.60 ± 0.17	3.98 ± 0.13
α-Ketoglutaric acid	2.89 ± 0.10	3.24 ± 0.08
Glyceric acid	3.74 ± 0.11	3.89 ± 0.10
Glycine	4.57 ± 0.11	5.05 ± 0.15
Homoserine	2.93 ± 0.05	2.84 ± 0.06
Indole-3-acetonitrile	3.78 ± 0.13	3.48 ± 0.13
Leucine	3.51 ± 0.07	3.60 ± 0.09
Malate	5.23 ± 0.06	5.37 ± 0.03
2-Methyl malic acid	3.57 ± 0.08	3.69 ± 0.06
Ornithine	3.00 ± 0.08	3.16 ± 0.12
Phenylalanine	3.72 ± 0.08	3.81 ± 0.06
Putrescine	3.59 ± 0.11	3.69 ± 0.09
Pyruvate	3.54 ± 0.06	3.70 ± 0.06
Ribitol	3.09 ± 0.08	3.28 ± 0.07
Serine	4.54 ± 0.10	4.86 ± 0.15
Shikimate	2.56 ± 0.05	2.61 ± 0.04
Succinate	3.36 ± 0.08	3.58 ± 0.08
Sucrose	5.39 ± 0.06	5.51 ± 0.04
Threonine	4.33 ± 0.05	4.47 ± 0.07
Tyramine	3.08 ± 0.07	2.86 ± 0.05
Tyrosine	2.83 ± 0.08	2.91 ± 0.04
Valine	4.23 ± 0.04	4.28 ± 0.05

Metabolite abundances were adjusted to an internal standard amount and original sample weight, and then $\log_{10}$-transformed. Data represent Mean ± SD. Significant differences in metabolites among the nine genotypes were identified using the Tukey Multiple Comparison test. The full metabolite data are in Appendix 16.

3.2.3.3 Metabolites whose levels were restored by complementation of *FUMARASE 2*

In order to study whether metabolite levels which changed by blocking FUM2 activity can be restored to the level of the wild-type by introduced $FUM2_{Col}$, abundances of a total of 56 metabolites in two genotypes, *fum2+*$FUM2_{Col}$ and *Col+EV,* were compared. Except two metabolites (methionine and succinate), the levels of the 54 metabolites were completely restored by $FUM2_{Col}$ (Appendix 16). But the different methionine levels were not caused by *FUM2* because the methionine level in *fum2+EV* was not different from the level in *Col+EV*.

3.2.3.4 Metabolites whose level changed in *fum2*

Among all 56 metabolites, the levels of 13 metabolites (fumarate, malate, succinate, α-ketoglutaric acid, shikimate, pyruvate, aspartate, threonic acid, arginine, leucine, glucose, α-aminoadipic acid and pipecolic acid) changed significantly in *fum2+EV* (Table 7 and Appendix 16). The levels of threonic acid, an OAA-derived aspartate and a pyruvate-derived leucine significantly increased in *fum2+EV*. The contents of α -aminoadipic acid and shikimate were higher and the content of piecolic acid was lower in *fum2+EV* compared to those in *Col+EV* (Table 7). α-Aminoadipic acid and piecolic acid are involved in catabolic pathways of L-lysine in higher plants (Moulin et al. 2006). But the content of lysine, the initial metabolite of the pathway, did not change in *fum2+EV* (Appendix 16). Shikimate is an intermediate of shikimate pathway which converts simple carbohydrate precursors derived from glycolysis and the pentose phosphate pathway to the aromatic amino acids phenylalanine, tryptophan and tyrosine (Herrmann and Weaver 1999). But none of the aromatic amino acids changed in *fum2+EV* (Appendix 16).

Table 7: Metabolites which changed in absence of *FUM2* and the restored levels by introduced *FUM2*

Metabolite	*fum2+EV*				*Col+EV*				*fum2+FUM2$_{Col}$*			
Glucose	3.78	±	0.28	b	3.60	±	0.17	c	3.60	±	0.19	c
Aspartate	5.23	±	0.04	a	5.17	±	0.04	bc	5.17	±	0.06	bc
Arginine	3.26	±	0.08	c	3.34	±	0.07	b	3.35	±	0.08	b
Leucine	3.58	±	0.09	bc	3.51	±	0.07	d	3.48	±	0.06	d
Threonic acid	3.35	±	0.04	a	3.27	±	0.04	bcd	3.28	±	0.07	bc
Fumarate	5.17	±	0.05	d	6.05	±	0.06	a	6.04	±	0.07	a
Malate	5.39	±	0.06	a	5.23	±	0.06	d	5.24	±	0.06	d
Pyruvate	3.68	±	0.07	a	3.54	±	0.06	c	3.52	±	0.04	c
Succinate	3.51	±	0.06	b	3.36	±	0.08	de	3.30	±	0.08	f
α-Ketoglutaric acid	3.15	±	0.05	b	2.89	±	0.10	cd	2.82	±	0.09	d
α -Aminoadipic acid	2.81	±	0.14	b	2.65	±	0.10	de	2.69	±	0.09	cd
Pipecolic acid	2.42	±	0.14	b	2.60	±	0.10	a	2.64	±	0.12	a
Shikimate	2.62	±	0.04	a	2.56	±	0.05	cd	2.56	±	0.05	cd

Metabolite abundance was adjusted for an internal standard amount and original sample weight and the values were $\log_{10}$ transformed. Data represent Mean ± SD. The significant differences between genotypes were identified using the Tukey Multiple Comparison test.

3.2.3.5 Fumarate and malate levels changed by *FUMARASE 2* alleles

Fumarate abundance in *fum2+EV* was approximately 14% of the level in *Col+EV*. To find out whether the two *FUM2* alleles (*FUM2$_{Col}$* and *FUM2$_{C24}$*) are responsible for the fumarate

content variation which was observed in the Col-0/C24 RIL population, the two alleles was separately transformed into *fum2* mutants.

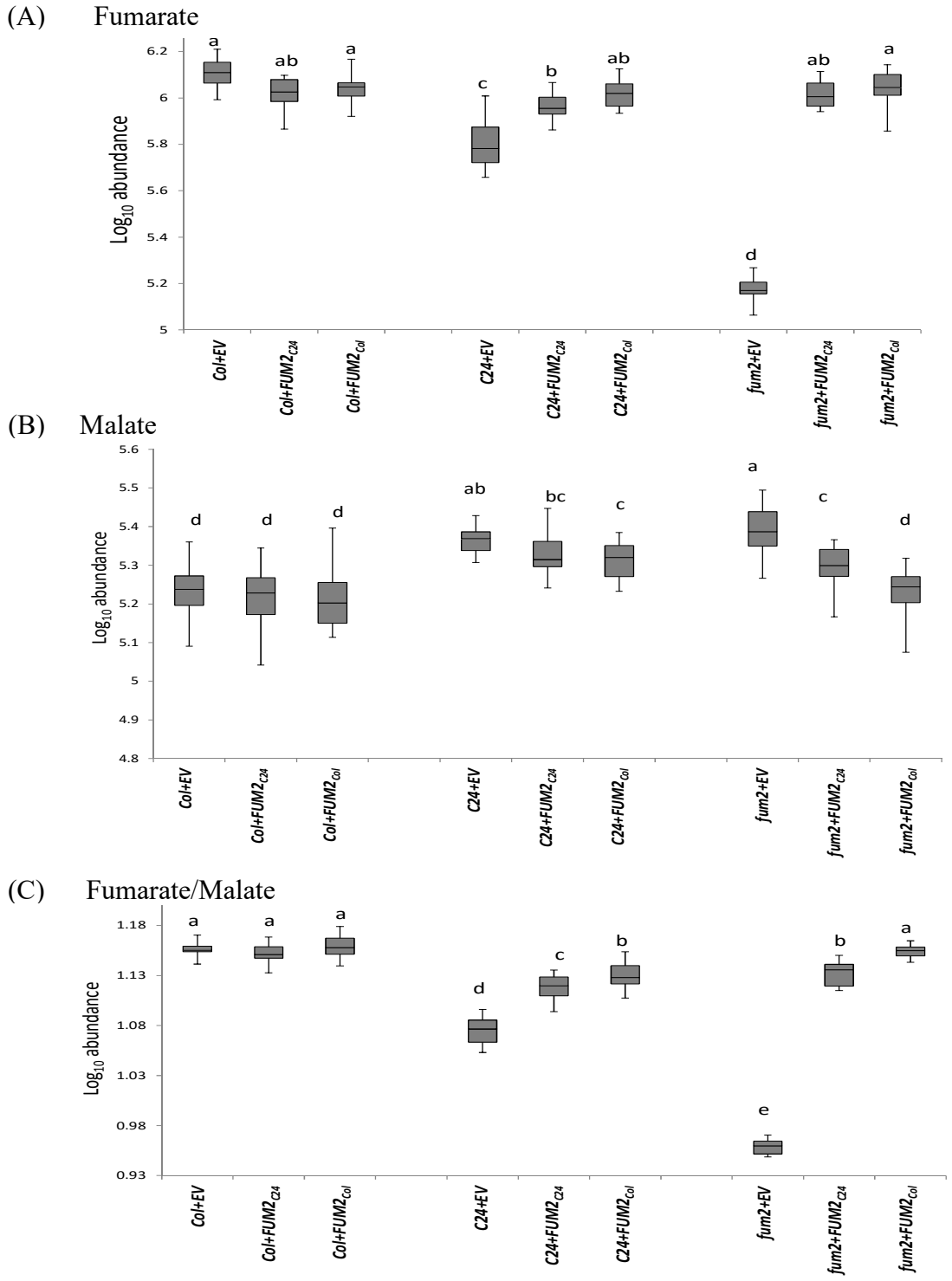

Figure 13: Fumarate, malate levels and fumarate/malate ratio in the genotypes. Data are displayed in box-and-whisker plot: horizontal line in a box: median values. The bottom and top of the box: first and third quartile. The whiskers: the lowest and highest values. X-axis: the nine transgenic genotypes. Y-axis: log_{10}-transformed relative fumarate (A) malate (B) abundance and fumarate/malate ratio (C). Metabolite abundance was adjusted for an internal standard amount and original sample weight and log_{10}-transformed. The significant differences between genotypes were identified using the Tukey Multiple Comparison test and signified above boxes.

As fumarate levels in *fum2+FUM2*$_{\text{Col}}$ and *fum2+FUM2*$_{C24}$ were 98% and 88% of *Col+EV*, respectively, the increased fumarate level by *FUM2*$_{Col}$ was higher than by *FUM2*$_{C24}$, but they were not significantly different from each other (Fig. 13A). On the contrary, restored levels of malate were significantly different by two types of transferred *FUM2* alleles: the malate level in *fum2+FUM2*$_{C24}$ was 15% higher compared to *fum2+FUM2*$_{Col}$ (Fig. 13B). Comparing the fumarate/malate ratio between *fum2+FUM2*$_{Col}$ and *fum2+FUM2*$_{C24}$, which may reflect the catalytic activities of FUM2, the ratio in *fum2+FUM2*$_{Col}$ was higher than *fum2+FUM2*$_{C24,}$ suggesting that malate was more converted to fumarate by *FUM2*$_{Col}$ than by *FUM2*$_{C24}$ in *fum2* (Fig. 13C).

3.2.3.6 Inheritance pattern of *FUMARASE 2* alleles and the dosage effect

To study the inheritance pattern of two *FUM2* alleles and the presence of the dosage effect, fumarate and malate levels in the six genotypes (*Col+FUM2*$_{Col,}$ *Col+FUM2*$_{C24,}$ *Col+EV,* *C24+FUM2*$_{Col,}$ *C24+FUM2*$_{C24}$ and *C24+EV*) were compared. Levels of fumarate and malate were altered only by transferred *FUM2*$_{\text{Col}}$ in a C24 background which contains a pair of endogenous *FUM2*$_{\text{C24}}$ (genotype *C24+FUM2*$_{Col}$) (Fig. 13). In contrast, neither of transferred *FUM2* alleles in a Col-0 background which harbor a pair of endogenous *FUM2*$_{\text{Col}}$ led any change of fumarate and malate levels. Therefore, *FUM2*$_{\text{Col}}$ is dominance over *FUM2*$_{\text{C24}}$. Gene dosage effect was only observed in fumarate level in C24 background: fumarate level in *C24+FUM2*$_{C24}$ which harbors two pairs of *FUM2*$_{\text{C24}}$ was higher than in *C24+EV* which harbors a pair of endogenous *FUM2*$_{\text{C24}}$.

3.2.3.7 Metabolites which were differentially affected by *FUMARASE 2* alleles

Among the 13 metabolites whose levels altered in *fum2+EV*, levels of four metabolites (succinate, α-ketoglutaric acid, shikimate and pyruvate) were different between the two genotypes*,* *fum2+FUM2*$_{Col}$ and *fum2+FUM2*$_{C24}$. From this result it can be inferred that the four metabolites are not only influenced by a *FUM2,* but also differently affected by a *FUM2* allelic variant (Fig. 14). The increased levels of the four metabolites in *fum2+EV* were fully replicated by transferred *FUM2*$_{Col}$ (genotype *fum2+FUM2*$_{Col}$) but less restored by transferred *FUM2*$_{C24}$ (genotype *fum2+FUM2*$_{C24}$) (Fig. 14). These of three metabolites (succinate, α-ketoglutaric acid and pyruvate) can be converted from fumarate and malate with several enzymatic steps: succinate can be converted to fumarate by the reversible reaction of the fumarate reductase/ succinate dehydrogenase complex (Iverson et al. 1999). α-Ketoglutaric

acid can be converted to succinyl-coenzyme A (CoA) and further converted to succinate by reactions of a ketoglutarate dehydrogenase and succinyl-CoA synthetase, respectively. Malate can be converted to oxaloacetate by malate dehydrogenase and further converted to pyruvate by pyruvate carboxylase.

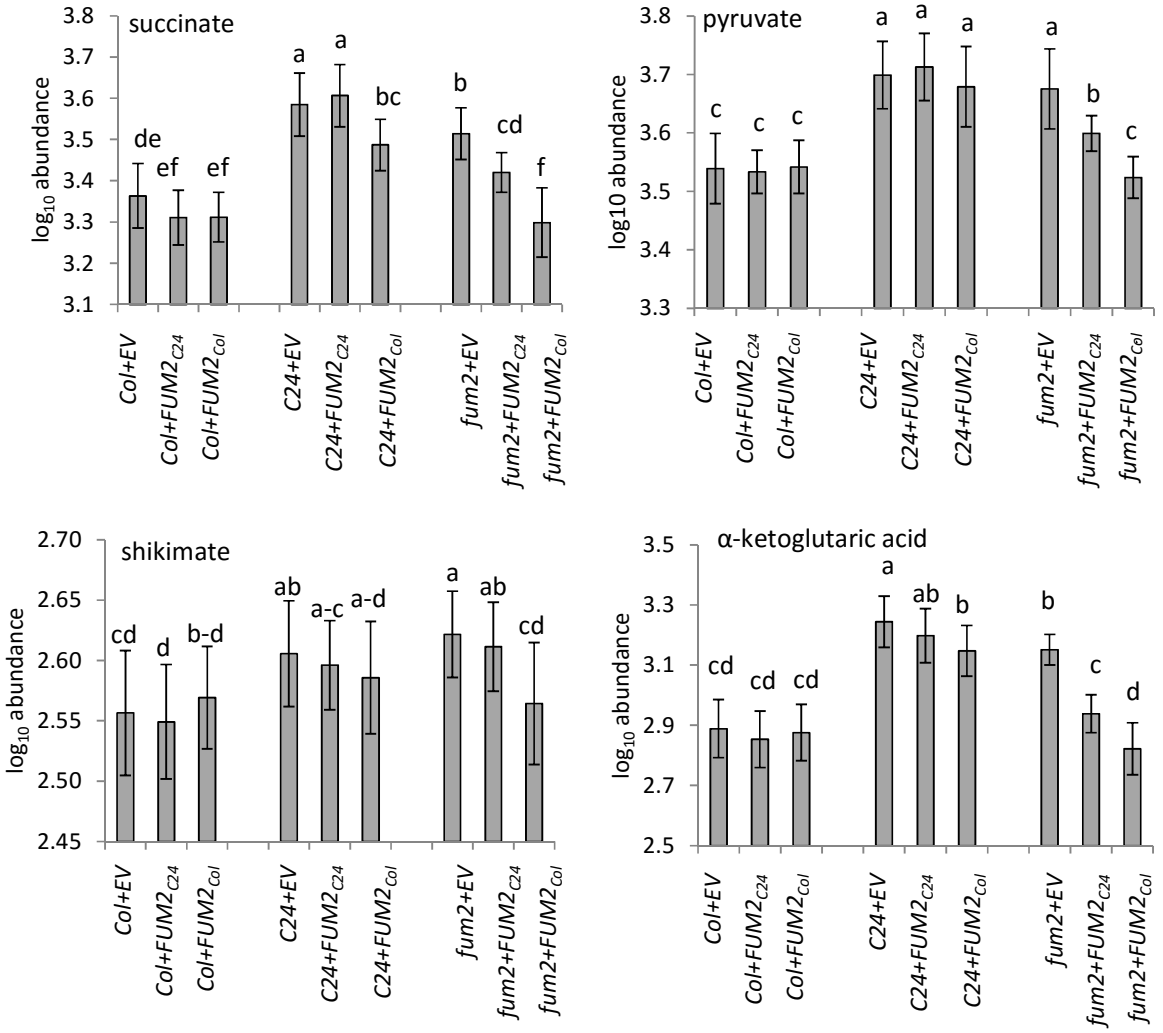

Figure 14: Metabolites whose levels were differently affected by *FUM2* alleles. Metabolite abundance was adjusted for an internal standard amount and original sample weight and log_{10}-transformed. Bars indicate Mean ± SD. For each metabolite significant differences between genotypes were identified using the Tukey Multiple Comparison test and signified above the bars.

To investigate whether alterations of the four metabolites correlate with the alteration of fumarate and malate contents or the alteration within the four metabolite contents, the metabolite levels in the transgenic plants were associated using pairwise correlations (Fig. 15). Fumarate content had only a moderately negative correlation with malate level, but a weak negative correlation with the four metabolites (succinate, α-ketoglutaric acid and pyruvate). The three metabolites (succinate, α-ketoglutaric acid and pyruvate) strongly positively

correlated with malate, and shikimate moderately positively correlated with malate. Especially α-ketoglutaric acid correlates very strongly and positively with malate, succinate and pyruvate. α-Ketoglutaric acid and succinate most strongly correlated with each other.

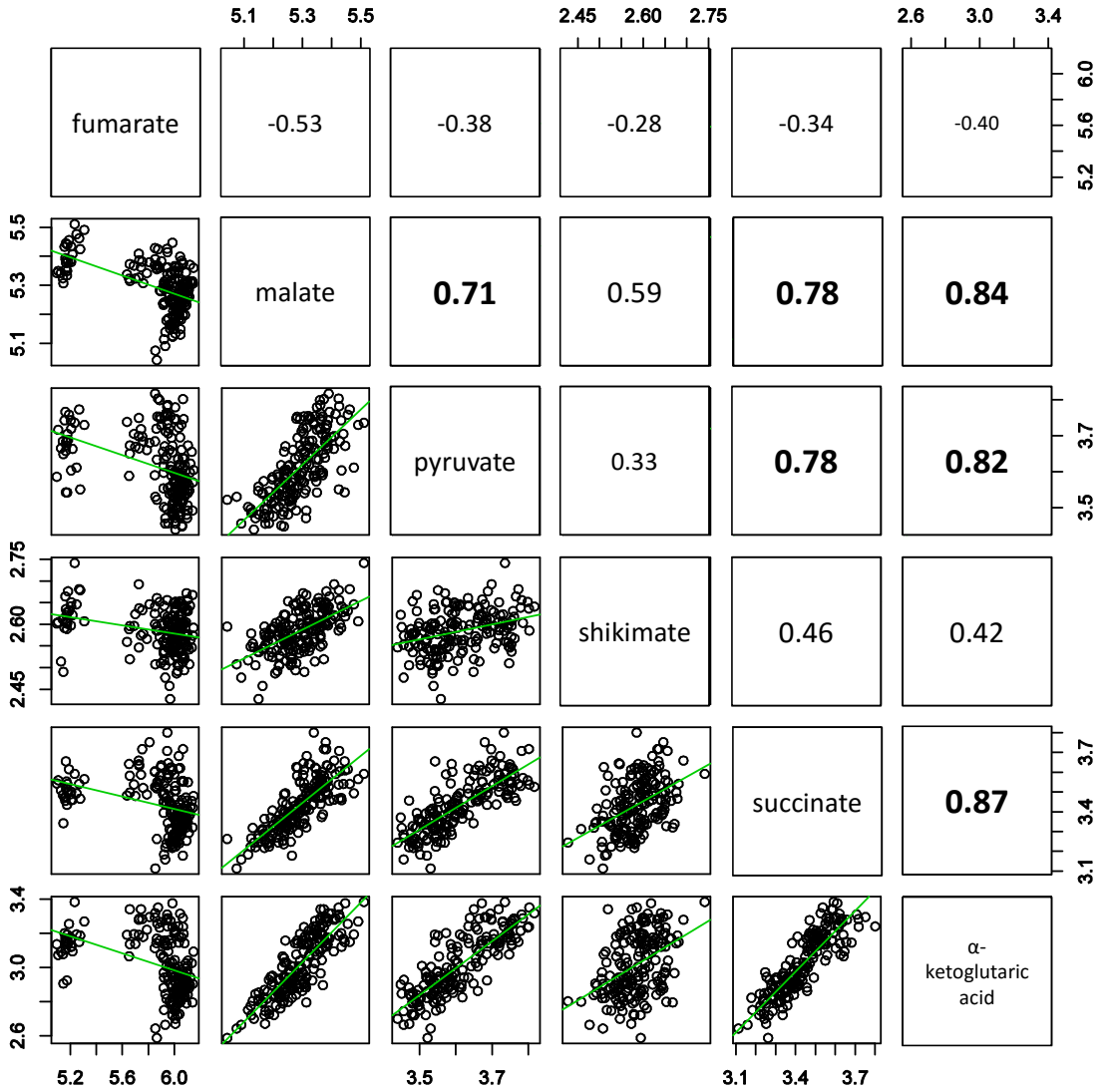

Figure 15: Comparison of the six metabolite abundances in the transgenic plants. Data were compiled from metabolite abundances of all investigated transgenic plants. X-and Y-axes show the $\log_{10}$-transformed metabolite abundance, which were adjusted for an internal standard amount and original sample weight. The graph in the bottom-right quadrant indicates scatter plots with linear regression lines showing pairwise comparisons of metabolite abundance in transgenic plants. Pearson's correlation coefficients of these indexes are indicated in the top-right quadrant.

4 Discussion

Based on knowledge from two related QTL analyses on biomass heterosis (Meyer et al. 2004) and metabolite content (Lisec et al. 2008), this study assesses the allelic variants of the investigated candidate genes and its relation to the observed trait variations.

4.1 Investigation of biomass heterosis candidate genes and their allelic variants

In a previous QTL study for heterosis biomass, Col-0 and C24 genomic fragments on top of chr.IV contributed differently to the trait: the variations from the Col-0 genomic segment were observed throughout the whole sampling points, from 6 to 15 DAS in the largest portion. On the other hand, the contribution of C24 genomic segments was detectable at 15 DAS with a small phenotypic contribution (Meyer et al. 2004). The QTL was fine mapped to 14 genes, from *At4g00238* to *At4g00335*. This observation led to the assumption that polymorphism(s) between alleles of the 14 genes may be the genetic basis for the differential phenotypic variation on biomass heterosis. To isolate causative genes and the associated allelic variation on biomass heterosis, all alleles were introduced into Col-0 and C24.

4.1.1 Deduced features of the candidate genes

To estimate presumable roles of each candidate gene, preliminary research on the genes was carried out. But none of the biological functions of the 14 candidate genes was proved by biological experiments. They were merely annotated based on sequence similarity with functionally characterized genes. The provided information of the genes is summarized below.

DNA-binding storekeeper protein-related transcriptional regulators: At4g00238, At4g00250 and At4g00270

The *Arabidopsis* genome encodes 29 DNA-binding storekeeper (STK) protein-related transcriptional regulators (https://www.arabidopsis.org/), which share homology of over 200 amino acid regions with the plant-specific DNA-binding protein coding gene *STK* in potato (*Solanum tuberosum*) (Zourelidou et al. 2002). The homologous region may function as a DNA-binding domain due to the presence of three α-helices, two of which might serve as transcriptional activation domains (Schwechheimer et al. 1998). Because the biological function of the *STK*-related transcriptional regulator in *Arabidopsis* is not reported yet, its

presumable role can only be inferred from the known features of STKs in potato. STKs regulate the expression of class I *patatin* genes (Zourelidou et al. 2002). *Patatin* genes in potato are involved in biosynthesis of patatin which is the major storage protein making up 40% of the soluble protein in the sink organ 'tuber' (Mignery et al. 1988). The *Patatin* genes are classified into class I and class II, based on conserved sequences (Bevan et al. 1986, Mignery et al. 1988). Promoters of *Patatin* class I have highly conserved 100 base pair regions which are comprised of two conserved motifs, the A-box and the B-box which confer tuber-specific and sucrose-inducible expressions (Grierson et al. 1994). The conserved B-box motif was also found in promoters of genes involved in sugar and starch metabolism. The SKTs specfically recognize the B-box motif (Grierson et al. 1994). Therefore, the STKs might function in control of transcription of *Patatin* class I by binding the B-box motif in the promoters, and regulate biosynthesis of patatin, sugar and starch (Zourelidou et al. 2002).

Contents of sugar and starch are tightly related to the biomass heterosis in *Arabidopsis*: Hybrid and allopolyploid *Arabidopsis* synthesize more starch and chlorophyll by regulating genes involved in chlorophyll and starch metabolic pathways (described in detail in chapter 1.1.4, Ni et al. 2009; Fujimoto et al. 2012). Sugar content in *Arabidopsis* influences vegetative growth by shifting the flowering time (Ohto 2001, Yang et al. 2013), and *Arabidopsis* growing on high sugar concentration increases its leaf number due to significantly delayed flowering time (Ohto 2001).

Phospholipase D β2: At4g00240

Phospholipase D (PLD) is an important signaling enzyme which hydrolyzes glycerophospholipids to a phosphatidic acid (PA) and a free-head group. Phospholipases can be found in a wide range of organisms, including bacteria, yeast, plants, animals, and viruses (Exton 2002, Jenkins and Frohman 2005). PLDs in plant play a pivotal role in regulating critical cellular functions, such as phytohormone-promoted senescence (Fan et al. 1997, Jacob et al. 1999), transmembrane signaling (Colley et al. 1997, Jones et al. 1999), cell proliferation (Daniel et al. 1999), plant-pathogen interactions (Wang 1999, 2000) and wound-induced lipid hydrolysis (Lee et al. 1997b, Ryu and Wang 1998). *A.thaliana* has 12 genes encoding PLDs, which are classified into 5 groups based on sequence similarities; PLDα, β, γ, δ *and* ζ (Qin and Wang 2002, Wang 2002). The overall domain structures of the PLDs are similar, but their distinct functions in the cell are caused by sequence variations in two motifs, a regulatory Ca^{2+}-dependent phospholipid-binding (C2) motif and a phosphatidylinositol 4,5-bisphosphate

(PPI) binding motif. Sequence variations in the C2 motif in *PLDs* confer their own unique calcium binding affinities, and those in the PPI-binding motif provide the specificities to substrates (Qin et al. 1997). PLDα is related to ABA signaling in senescence and freezing tolerance (Fan et al. 1997, Welti et al. 2002, Zhang et al. 2004). PLD ζ is involved in root elongation: the elongation of the primary root of double knockouts of PLD ζ 1 and PLD ζ 2 in *Arabidopsis* was slower than in the wild type (Li et al. 2006). PLDβ is evolutionarily divergent from PLDα, and the N terminus of PLDβ contains a regulatory C2 domain that was found in a number of signal transducing and membrane trafficking proteins (Pappan et al. 1997). The gene '*At4g00240*' was named '*PLDβ2*' for sharing approximately 75% amino acid sequence identity with *PLDβ* (*At2g42010*) (Wang 1999).

Mechanosensitive ion channel protein: At4g00290

Mechanosensitive ion channels are known as players in transducing mechanical stimuli into intracellular signals. One of the candidate genes, At4g00290, is annotated as a mechanosensitive ion channel protein encoding gene, for harboring two conserved domains, a small-conductance mechanosensitive channel and a mechanosensitive ion channel (Schallus et al. 2008).

Fringe related protein: At4g00300

At4g00300 was annotated as a fringe-related protein based on sequence identity with *DROSOPHILA GENES FRINGE* (*D-fing*) which encodes a boundary-specific cell signaling molecule which is only expressed in dorsal cells but not in ventral cells in *Drosophila* (Irvine and Wieschaus 1994). At4g00300 is expected to act as a glycosyltransferase due to having a maletin-like (ML) domain which is a short stretch of leucine-rich repeats (LRRs) (Schallus et al. 2008).

Leucine-rich repeat transmembrane protein kinase protein: At4g00280

At4g00280 is a hypothetical protein coding gene with unknown function. Its sequence best matches the leucine-rich repeat transmembrane protein kinase. Leucine-rich repeats (LRRs) which have 20–29 amino acid conserved residue sequence motifs play a crucial role in protein–protein interactions (Kobe and Deisenhofer 1993). LRR proteins participate in many biologically important processes, such as hormone–receptor interactions, enzyme inhibition, cell adhesion and cellular trafficking (reviewed by Kobe and Kajava 2001).

Embryogenesis related genes: At4g00260 and *At4g00310*

At4g00260 and *At4g00310* were named *MATERNAL EFFECT EMBRYO ARREST* (*MEE*) *44* and *MEE* 46, respectively, because two dissociation (Ds) mutants whose corresponding genes were abolished by a transposable element displayed delayed embryo development (Pagnussat et al. 2005). Both *At4g00260* and *At4g00310* are supposed to be involved in embryo development, but do not directly cause embryo lethality (Pagnussat et al. 2005). This study did not investigate the vegetative growth of the mutants. Thus, the functions of *At4g00260* and *At4g00310* in plant growth are still largely unknown. But the two genes may not belong to the same family for not sharing any sequence similarities: *At4g00260* has a plant-specific B3-DNA Binding Domain which might act as a transcription regulator (Waltner et al. 2005), while *At4g00310* does not have any known conserved domain.

F-box domain containing protein: At4g00315 and At4g00320

F-box protein members contain the F-box motif which consists of conserved 40~50 amino acids (Elledge and Harper 1998). Several F-box genes were identified from mutant screenings and play important roles in numerous biological progresses. These include *SUPPRESSOR OF NIM 1-1* (*SON1*) which is involved in pathogen resistance (Kim and Delaney 2002), *TRANSPORT INHIBITOR RESPONSE 1* (*TIR 1*) which regulates auxin responses (Ruegger et al. 1998), *CORONATINE-INSENSI-TIVE 1* (*COI1*) which plays a major role in jasmonate signaling (Xie et al. 1998), *UNUSUAL FLORAL ORGANS* (*UFO*) which is required for floral development (Samach et al. 1999), *ZEITLUPE* (*ZTL*) which is involved in photocontrol of circadian period (Somers et al. 2000), and *FLAVIN-BINDING, KELCH REPEAT, F-BOX 1* (*FKF1*) which controls flowering time (Nelson et al. 2000). In many cases, F-box proteins have several protein-protein interaction domains located in the downstream region of the F-box domain, and they confer the substrate specificity for ubiquitylation. A FBD domain is one of the downstream domains localized in the downstream region of the F-box. It is a plant-specific unknown domain found in an *in-silico* analysis and contains about 80 conserved amino acid residues (Schultz et al. 1998). This motif is usually present together with a F-box domain or a BRCA1 C Terminus (BRCT) domain (Schultz et al. 1998). A BRCT domain is a motif composed of 90-100 amino acids (Zhang et al. 1998), and genes harboring it are involved in cell cycle regulation and DNA damage repair (Bork et al. 1997, Callebaut and Mornon 1997).

In an *A.thaliana* genome analysis, at least 568 F-box protein genes were identified. 112 of them contain a FBD domain as well (Kuroda et al. 2002), 102 of these have an additional CRFA domain lying between F-box and FBD. Ten of all F-box protein genes only harbor FBD and F-box, and 11 genes only CRFA and F-box (Kuroda et al. 2002). *At4g00315* has a FBD-domain, and *At4g00320* contains both CRFA- and FBD-domains. Both FBD and CRFA domains have conserved leucin residues. Sequences of both motifs are dissimilar from LRR but their 3D-structure is alike. So both CRFA- and FBD-domains are supposed to be subgroups of LRR, and to act like LRR which is key motif for protein-protein interaction (Kuroda et al. 2002).

Zinc finger; RING-H2 finger B1A (RHB1A): At4g00305 and At4g00335

Zinc-finger proteins are the most abundant proteins in eukaryotes. Zinc-binding motifs in the zinc-finger proteins vary in structure, function and binding substrates (Laity et al. 2001). The *REALLY INTERESTING NEW GENE* (*RING*) finger motif belongs to a zinc-finger domain and has a cysteine-rich sequence (Freemont et al. 1991). The RING finger family can be subcategorized into C3-H-C4 type and C3-H2-C3 type, the latter also being called 'RING-H2' (Saurin et al. 1996). Recently RING finger proteins have been described to act as ubiquitin protein ligases (Freemont 2000, Joazeiro and Weissman 2000, Lechner et al. 2002). For example, Sinat5 (an *Arabidopsis* homologue of the RING-finger Drosophila protein SINA) has a ubiquitin protein ligase activity, and is involved in lateral root development (Xie et al. 2002). The two candidate genes *At4g00305* and *At4g00335* were annotated as *RING-H2 finger B1A* (*RHB1A*) for having Ring finger domains. RING-finger proteins are also known to be involved in hormone-mediated growth. *ATL2* is induced by auxin (Martínez-García et al. 1996), and *BRASSINOSTEROID-RESPONSIVE RING-H2* (*BRH*) is down-regulated by brassinosteroids (Molnár et al. 2002). Misexpressions of certain RING-finger proteins are connected to clear phenotypes but some of them are not. For instance, *RING-FINGER FOR EMBRYOGENESIS 1* (*RIE1*) plays a critical role in seed development, and abolishment of the gene leads to lethality (Xu and Li 2003). Suppression of *BRH1* caused thicker inflorescence stems and abnormal leaf shape, whereas there is no clear difference in morphology in overexpression of *BRH1* (Molnár et al. 2002). *Arabidopsis RING zinc finger* (*A-RZF*) is preferentially expressed in seed developmental stages and *RHA2b* is expressed in vascular tissues and styles of flowers, but abolishment of these genes did not lead to any specific morphological change. The authors conclude that because of the presence of other genes

whose functions are redundant with *RZF* and *RHA2b*, the knock-out mutants did not show any phenotypic change (Zou and Taylor 1997, Lechner et al. 2002).

Calmodulin-binding receptor-like cytoplasmic kinase 2: At4g00330

Calcium is one of the best documented second messengers and acts as a mediator which conveys external and internal stimuli into cells (reviewed by Reddy, 2001). At the cellular level, these stimuli change the calcium concentration which can be recognized by calcium receptors (Price et al. 1994, Knight 2000, Pei et al. 2000, Xiong et al. 2002)**.** The calmodulin (CaM), one of the calcium receptor proteins, has four EF-hand motifs which have conserved helix-loop-helix structures and bind to a single calcium ion. The active form of Ca^{2+}/CaM complex interacts with downstream target proteins, such as metabolic enzymes, structural proteins, transcriptional factors, ion channels and pumps (reviewed by Reddy, 2001). *At4g00330* is named *CRCK2* because of its sequence similarity with *CRCK1* (*At5g58940*). Both CRCK1 and CRCK2 have two conserved catalytic domains, Protein Kinases and Serine/Threonine protein kinases. CaM binds to CRCK1 in a calcium dependent manner, and its expression is increased in response to stress situations or the presence of stress related molecules. Therefore CRCK1 is supposed to play a role in stress signaling transduction in plants (Yang et al. 2004).

The remarkable polymorphic sequences, 491 bp and 1925 bp InDels

To detect sequence polymorphisms between Col-0 and C24 alleles of the candidate genes and the intergenic regions, the two obtained Col-0 and C24 sequences on top of chr.IV were compared. The most remarkable differences in nucleotide sequences were the presence of 491 bp and 1925 bp InDels. The 491 deletion is present in the putative promoter of *At4g00310* in C24. The 491 InDel includes 8 cis-regulatory elements binding sites, which are therefore excised in the promoter of $At4g00310_{C24}$. The 8 binding sites include 4 MYB binding sites, an Evening Element promoter motif, 2 GATA promoter motifs and a RAV1-A binding site motif. Evening elements are required for the circadian control of gene expression (Harmer et al. 2000). MYB proteins are key factors in development, metabolism and responses to biotic and abiotic stresses (reviewed by Dubos et al. 2010). GATA-binding proteins are responsible for light-responsive transcription (Teakle et al. 2002). Therefore, the presence or absence of the 8 cis-regulatory binding sites may become a source of *At4g00310* allelic variation in expression.

The newly found ORF_{C24} in the 1925 bp insertion in C24 was especially interesting because if it is transcribed, the hybrids possess one more hemizygous gene. Perhaps, the ORF_{C24} forms new network with Col-0 allele, which might lead to biomass heterosis in hybrid, like Fu and Dooner's (2002) speculation of genetic basis for heterosis in maize.

4.1.2 Expression of the 14 candidate genes at the transcript level

In a detailed study on early biomass heterosis, the time point of critical enhancement in RGR was narrowed down to 3-4 DAS (Meyer et al. 2012). In the global transcription analysis with 4-day-old hybrids (Col-0xC24, C24xCol-0) and their parental lines (Col-0 and C24) using microarrays (Affymetrix ATH1 GeneChips), overall transcript levels in the hybrids were mainly between parental transcript levels (Meyer et al. 2012). However, microarray based transcription analysis measure relative not absolute transcription values. Therefore, to investigate not only the transcription pattern but also absolute transcription levels of the 14 candidate genes in hybrids and the parents at the critical time point for growth vigor in hybrids, transcription analysis was carried out using RNASeq. Considering restricted amounts of seeds which are produced by manual crosses, the differences in germination time within the 4 genotypes (Fujimoto et al. 2012, Meyer et al. 2012) and minimal required quantity of the RNA, the earliest time point for the transcription analysis was decided at 5 DAS. Expression patterns and relative abundance of RNA in the 4 genotypes were similar between genes belonging to the same families: transcription patterns between two RING finger protein coding genes (*At4g00305*, and *At4g00335*) and between two STK-related transcriptional regulators (*At4g00250* and *At4g00270*) were similar to each other. This observation is consistent with a finding in global gene expression analysis in *Arabidopsis*: expression levels of genes in the same family show more similarity than would be expected by chance, which may be by caused by a co-opt gene for specific developmental processes (Schmid et al. 2005). The expressions of the two F-box genes, *At4g00315* and *At4g00320*, were not detectable in any of the 4 genotypes, consistent with results reported by Kuroda et al. (2002): The expression levels of the majority of cloned F-box genes (78 of 82 total cloned F-box protein genes) were very low compared to housekeeping genes. Also, in global gene expressions analyses in various tissue types and development stages, expression levels of most F-box genes were not detectable (Schmid et al. 2005). The authors explained that due to the highly specialized F-box functions in certain biological process, it is difficult to detect their individual expressions in transcription analyses targeting whole organs (Schmid et al. 2005).

4.1.3 Segregating T2 families of plants are ideally suited to study transgene effects on biomass accumulation

Usually, target phenotypes of transgenic plants are evaluated in homozygous T3 and compared to the wild types or transgenics with vector control. Like other experiments, phenotyping of cosmid transgenics was originally done in homozygous T3. But the mean values within transgenic lines varied even among the control lines. Thus phenotypic changes in transgenic lines by expression of transgene were hard to detect. We observed that vegetative growth of transgenic lines was positively correlated with seed size. This observation is consistent with a report by Elwell et al. (2011): *Arabidopsis* seedlings from large seeds grew bigger and faster than *Arabidopsis* seedlings from small seeds. Seed-size also influences the germination time, flowering time, primary root growth and early vegetative growth (Elwell et al. 2011). Therefore, mean seed sizes of individual transgenic lines were analyzed and used as a co-factor for estimating mean leaf area of the transgenic lines. Even though this correction for seed size reduced the within-line variations, they were still too large for evaluating morphological changes by transgene. Thus phenotypic changes in transgenic lines by expression of transgenes were hard to detect. Therefore, to set up a definite control, the mean value of the control was estimated from 6 individual transgenic control lines. Then the mean leaf area of individual transgenic genotypes was compared to the control. But convincing controls could not be set for comparing phenotypic change by transgene, therefore the transgenic lines were phenotyped in segregating generation T2. We found that non-transgenic siblings were a proper control to their transgenic siblings, because they originated from the same mother: the life history circumstances of the parental generation which can affect growth and development throughout the life cycle of the next generation were equalized over T2 seeds (Elwell et al. 2011).

4.1.4 Inserted single *At4g00310* transgene induced a decrease in leaf area

Among the 14 candidate genes only *At4g00310* led to a change in size of rosette leaves. The decrease in leaf area was caused by morphological change in shortened petioles and rosette leaves, not by delayed vegetative growth. Both transferred alleles, $At4g00310_{Col}$ and $At4g00310_{C24}$ induced a decrease in size in both Col-0 and C24 genomic backgrounds which have a pair of endogenous *At4g00310* alleles. This result shows that neither of the *At4g00310* alleles is dominant over the other, because the transferred recessive allele would not lead to

phenotypic change in the presence of an endogenous dominant allele. Because all the alleles induce the reduction in every background, the inheritance pattern of the *At4g00310* alleles may be co-dominant. Because transferred alleles induced the phenotype change in the presence of the same alleles in genomic background, the *At4g00310* have also dosage effect. However, each allele seems to be regulated differentially depending on plant genomic background. This differential regulation by plant genomic background was more clearly observed in the Col-0 allele: The occurrence rates of decrease in leaf area by $At4g00310_{Col}$ were higher in a Col-0 genomic background than in a C24 background (Fig. 9). On the other hand, the C24 allele led to a decrease in size at a similar rate in both genomic backgrounds (Fig. 9). If allelic variation is present, the possible genetic basis might be in the polymorphism sequence in the promoter sequence: The promoter of $At4g00310_{Col}$ has a 491 bp insertion which includes 8 key cis-regulatory elements binding sites (Table 3). Therefore the two *At4g00310* alleles might be expressed differently by the cis-regulatory elements.

4.1.5 Shortened rosette leaves by transferred *At4g00310* initiated during vegetative growth

At4g00310 was assumed to be involved in embryogenesis because of two observations: First, Ds insertion mutants which have transposable elements in the *At4g00310*, showed a defected growth at an embryo development stage (Pagnussat et al. 2005). Secondly, in a global gene expression analysis in different tissues and at various developmental stages, *At4g00310* was highly expressed in ovaries and in seeds during embryo development stages (Appendix 7). Despite these indications, the shortened rosette leaves which were observed in the four transgenic genotypes, $Col{+}310_{Col}$, $Col{+}310_{C24}$, $C24{+}310_{Col}$ and $C24{+}310_{C24}$, do not seem to be initiated during embryo development stages. But rather the decreased size of transgenic plants seems to start during cotyledons and rosette developmental stages which were initiated after germination. If *At4g00310* transgene affected embryo-development, thereby influencing rosette development, a decrease in leaf area could be monitored at an early vegetative stage when two cotyledons have emerged from seeds. However, several transgenic lines, $Col{+}310_{Col}$ *B*, *–E*, *–F* and $Col{+}310_{C24}$*A*, *–B* which showed a clear reduction in size at 15 DAS did not display any decrease in leaf area at 9 DAS. This implies that the decrease in leaf area in *At4g00310* transgenic plants was initiated during early vegetative stages after germination. In addition to that, there was no indication of an abnormality in the embryos of the transgenic lines. Usually transgenic plants with embryo defection can be indirectly verified by the low

germination ratio and an aberrant segregation ratio, which are the theoretical basis of the selection of embryo defect transgenics (Pagnussat et al. 2005). But all transgenic lines harboring *At4g00310* in T2 segregated at a 3:1 Mendelian ratio (transgenic plants: non-transgenic plants) which means that at least the gene does not affect embryo development. Also, seed sizes of the *At4g00310* transgenic lines which were measured in segregating T2 population did not differ from other transgenic lines; the standard deviation of the seed area was not larger than in other lines (Appendix 14). Even though the biological function of *At4g00310* is largely unknown, its presumable pleotropic role in plant development could be deduced from the co-expression data (Heyndrickx and Vandepoele 2012; http://arabidopsis.org/): expression of *At4g00310* was influenced by various external stimuli and changed differently at different developmental stages. If the decrease in leaf area in the *At4g00310* transgenic plants is related to the expression level of the gene, it would be noticeable: Since the time points at which leaf areas in transgenic plants start to decrease would be consistent with the time point at which *At4g00310* shows the lowest expression during the whole life cycle (Appendix 7). In our gene expression data at 5 DAS, the genes in hybrids were less expressed than the lower parent (C24) value (Table 4).

4.1.6 Overall discussion of results from the transgenic plants and two hypotheses

To find the causal gene(s) for biomass heterosis among the candidate genes and the potential allelic variance for heterosis biomass, all the alleles were introduced in plants. The transgenic plants were phenotyped in leaf area. To sum up the phenotyping results of two sets of transgenic plants, cosmid transgenic plants, and transgenic plants harboring single transgene, only transgenic lines harboring single *At4g00310* transgene showed a decrease in size. Expression of transferred $At4g00310_{Col}$ and $At4g00310_{C24}$ induced a reduction in size in both genomic backgrounds. The reduction in size of the transgenic plants was caused by shortened petioles and rosette leaves but not by delayed growth. However, none of the cosmid transgenic lines, even in cosmid transgenic lines harboring transferred *At4g00310*, show any change in leaf area. Nevertheless, the resulting morphological change by *At4g00310* transgene 'little rosette leaves' was contrary to our expected phenotype 'large leaves and vigorous growth at early development stages' which was observed in a reciprocal *Arabidopsis* hybrid, and in the QTL study for biomass heterosis (Meyer et al. 2010). To address these results, two hypotheses were formulated.

4.1.6.1 Hypothesis 1: A cooperative effect of several candidate genes might be the main contributor to an early vegetative growth vigor

The transgenic plants harboring *At4g00310* transgene show a decrease in shoot growth, while the cosmid transgenic plants harboring transferred *At4g00310* did not show any phenotypic change. However, there are no differences in sequence of *At4g00310* between genomic (pMDC123) and cosmid (pCLD04541) clones. Because $At4g00310_{Col}$ and $At4g00310_{C24}$ in pMDC123 were cloned with their own genomic sequence with extended upstream and downstream sequences, the sequences of them are fully included in the three cosmids vectors harboring *At4g00310*, Cos_062, Cos_401 and Cos_395. The only difference between genomic clones and inserts of the cosmid vectors is in the existence of neighboring genes: Besides *At4g00310*, the three cosmids (Cos_062, Cos_401 and Cos_395) share the same two neighboring genes *At4g00305* and *At4g00315*. Thus, the decreased leaf size by *At4g00310* transgene could be offset by one of the adjacent genes, even though transgenic lines harboring either *At4g00305* or *At4g00315* alone did not display any change in size. Based on sequence identities, *At4g00305* and *At4g00315* were annotated as a RING-H2 finger B1A and an F-box domain containing protein, respectively. F-box domain containing proteins and some RING-H2 finger proteins are known to be acting as ubiquitin protein ligases which play a central role to confer a selectivity to targeted proteins which will be degraded through ubiquitin mediated protein degradation (Freemont 2000, Joazeiro and Weissman 2000, Lechner et al. 2002). If either of the neighboring genes regulates the activity of *At4g00310* by mediating the degradation through ubiquitylation pathway, the negative effect on leaf elongation by *At4g00310* can be offset by coupling with either of *At4g00305* or *At4g00315*. One interesting feature of *At4g00310* is that *LEAFY* (*LFY*), a master regulator in floral organ specification (Maizel et al. 2005), binds to the 1.8 kb upstream of *At4g00310* (Moyroud et al. 2011), even though there is no evidence for the direct regulation of *At4g00310* by LFY. LFY is known to regulate a floral homeotic protein *APETALA 3* (*AP3*) which works for specification of stamens and petals by binding to a consensus site in the regulator regions of AP3 (Wagner et al. 1999, Lamb et al. 2002). To express AP3 by LFY, a F-box protein *UNUSUAL FLORAL ORGANS* (*UFO*) must be conjugated with LFY (Lee et al. 1997a). Like the regulation of AP3 by LFY coupling with a F-box protein UFO, expression of *At4g00310* could be controlled by LFY coupling with either of the neighboring genes, *At4g00305*, a RING-H2 finger protein, some of which are known to act like F-box proteins, or *At4g00315*, a F-box domain containing protein. Beside the co-activator of LFY for AP3 expression, UFO is also involved

in the establishment of the lateral meristems and primordia in the peripheral zone of the apical and floral meristems by enhancing the activity of LFY. For the biological process, LFY and SEPALLATA MADS-box transcription factors are required (Risseeuw et al. 2013). Whether one of the neighboring genes or *At4g00310* itself is involved in other biological processes is not known; however, based on co-expression data of *At4g00310*, it seems to be involved in many biological processes (Heyndrickx and Vandepoele 2012). Thus, coupling with other candidate genes also possibly contributes to vegetative growth vigor.

4.1.6.2 Hypothesis 2: Repression of *At4g00310* at early development stages is decisive for growth vigor

The second hypothesis is that *At4g00310* might be the causative gene for early vegetative growth, even though the resulting phenotype was small rosette leaves which are opposed to our expected phenotype 'vegetative growth vigor'. We assume that the small rosette leaves of transgenic lines were caused by overexpression of *At4g00310* at early vegetative stages. As was already mentioned previously, the smaller rosettes were initiated with leaf development after germination but not from embryo development stages. The early vegetative stages correspond to the development stages which show the lowest expression of *At4g00310* during the life cycle (Appendix 7). Also, in our RNA expression data carried out with 5 day-old plants the expressions of *At4g00310* in reciprocal hybrids were lower than the low parent value in C24 (Table 4). Thus we assume that the low expression of *At4g00310* at early vegetative stages might be related to the enhanced vegetative growth in hybrids. On the contrary, however, activation of *At4g00310* at the early vegetative stages might possibly cause a reduction in size like the phenotype of transgenic lines harboring *At4g00310*.

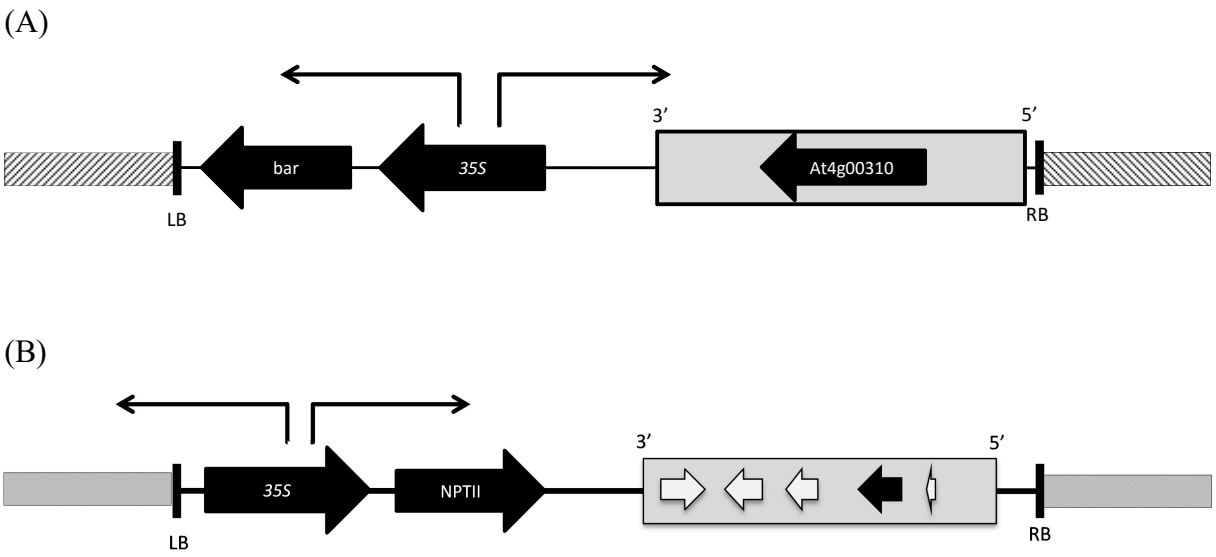

Figure 16: Hypothesis on the differential expressions of cloned *At4g00310* by the dissimilar structures of cloned vectors. (A-B) Schematic drawing of genomic contents arranged in T-DNA of pMDC123 (A) and of pCLD04541 (B) which are integrated in plant genome (boxes with lines) (A) A 35S enhancer in 35S promoter is located between insert (box in gray) and bar. The 35S enhancer can drive bi-directionally: toward *bar* as it is originally intended and toward an insert inadvertently. Thus a cloned gene in an insert of pMDC123 might be overexpressed in the plant genome by neighboring 35S enhancer and the overdrives *At4g00310* might result in a decreased rosette leave. (B) A 35S enhancer in a 35S promoter is located upstream of NPTII. *At4g00310* (black arrow in a gray box) in cosmid insert (box in gray) is far away from the 35S promoter and blocked by neighboring genes (white arrows in gray box). Thus *At4g00310* in a cosmid can be expressed only by its native promoter.

The small rosette of transgenic plants might be caused by overexpression of *At4g00310* because of the structure of T-DNA of vector pMDC123. The insert constructed in pMDC123 is directly adjacent to a 35S promoter which drives the basta resistance gene (*BAR*) (Fig.16). The 35S promoter sequence includes an orientation-independent 35S enhancer (Yoo et al. 2005), thus not only *BAR* but also a gene in the insert can be driven by the neighboring 35S enhancer. Consequently, the *At4g00310* transgene in plant genome could be driven by the 35S enhancer. Several similar observations have been reported in the literature. The overexpression of FT with the root specific promoter LRP1 (Lateral Root Primordia 1) was observed when it was constructed next to the 35S promoter (Yoo et al. 2005). The unintentional overexpression was also observed in GUS when it was located next to the 35S promoter (Yoo et al. 2005, Singer et al. 2010). *Arabidopsis* mutants which were transformed with several types of vectors showed abnormal morphology by endogenous genes which were overexpressed by neighboring 35S promoter of T-DNAs (Weigel et al. 2000). All the authors agree that the overexpressed genes were caused by the 35S enhancer in the neighboring 35S promoter (Yoo et al. 2005, Singer et al. 2010); however, opinions concerning expression patterns of the genes driven by an adjacent 35S enhancer diverge: It has been explained as a

consequence of overexpressed endogenous expression or as a consequence of constitutive overexpression. Weigel et al (2000) consider on endogenous overexpression more plausible than constitutive ectopic expression for the following observations. Expression of endogenous FT which is next to the 35S enhancer was only detected in the shoot part like endogenous FT but in higher expression levels (Weigel et al. 2000). In addition to that, because the abnormal phenotypes of the mutants by adjacent 35S promoters were exactly opposite to that of loss-of-function mutants, the expressions of genes by 35S enhancer are presumably followed by enhanced-endogenous expression, otherwise the overexpression would result in unexpected phenotypes (Kardailsky et al. 1999, Weigel et al. 2000). On the contrary, Yoo et al and Singer et al observed that cloned genes were constitutively non-tissue-specifically overexpressed by a neighboring 35S enhancer, even if the genes were constructed to be driven under certain tissue specific promoters (Yoo et al. 2005, Singer et al. 2010).

One remarkable difference between the $At4g00310_{Col}$ and $At4g00310_{C24}$ is the presence or absence of the 8 key binding sites on their promoter sequence. The key binding sites may provide a source of allelic variation in expression under distinct genomic background or certain developmental stages. From phenotyping results in the four genotypes $Col{+}310_{Col}$, $Col{+}310_{C24}$, $C24{+}310_{Col}$ and $C24{+}310_{C24}$, there were indications of different regulations depending on 310 allele types and the plant genomic background, such as variances in degree of leaf area reduction and occurrence rates of the reduction: however, the differences were not clear enough to draw firm conclusions. Unlike the differences in the promoter sequences and transcription levels between $At4g00310_{Col}$ and $At4g00310_{C24}$, the insignificant difference in phenotypic change in the genotypes might also be caused by influence of the 35S enhancer sequence. If the alleles were constitutively overexpressed by the 35S enhancer (Yoo et al. 2005, Singer et al. 2010), the presence of allelic variation in transcription regulation could be masked. Therefore, the relationship between the expression pattern and levels of transferred *At4g00310* alleles and the resulted phenotype should be studied further.

On the other hand, *At4g00310*s in T-DNA of cosmids were not directly adjacent to the 35S promoter (Fig.16). *At4g00310* was always surrounded by neighboring genes, *At4g00305* and *At4g00315* and a NPTII lay between a 35S promoter and an insert. Therefore, *At4g00310* introduced by cosmid might be expressed under its own promoter and that did not lead to any change in size.

To sum up the second hypothesis, *At4g00310* is supposed to be the causative gene for early growth vigor in hybrids, but the reversed phenotype ‘shorten rosette’ from our expected phenotype can be caused by overexpression of *At4g00310* by an adjacent 35S promoter at

early vegetative stages. The unchanged phenotype in cosmid transgenic harboring *At4g00310* might be caused by expression of *At4g00310* within its native chromosomal context including cis-regulatory sequences.

4.2 Analysis of $FUM2_{Col}$ and $FUM2_{C24}$ alleles

In a metabolite QTL study using Col/C24 RILs, an mQTL on chr.V was identified. The lines with Col-0 allele displayed a higher fumarate level (Lisec et al. 2008). In the confidence interval, *FUM2* (*At5g50950)* was identified. In a following study, the *FUM2* was responsible for the major fumarate content in *Arabidopsis* (Pracharoenwattana et al. 2010). Under the hypothesis that a variation between $FUM2_{Col}$ and $FUM2_{C24}$ might be the causal genetic basis for the fumarate content variation, the two alleles were introduced in Col-0, C24 and *fum2.* Fumarate and other metabolite levels in the obtained transgenic plants were analyzed.

4.2.1 Promoter of *FUMARASE 2* is critical for the proper expression

Compared to the case of not fully restored fumarate levels by complementation of *FUM2* driven by a double 35S promoter (Pracharoenwattana et al. 2010), reducing fumarate level in *fum2* was completely restored by transferred $FUM2_{Col}$ which is driven by its own promoter and terminator sequences (genotype *fum2+*$FUM2_{Col}$). The 35S promoter drives gene expression constitutively and non-tissue specifically (Benfey and Chua 1990). But fumarate contents vary depending on tissue types (Chia et al. 2000) and the level in leaves follow diurnal changes: increasing during the day and decreasing during the night (Fahnenstich et al. 2007, Zell et al. 2010, Pracharoenwattana et al. 2010). The fully restored fumarate level by transferred *FUM2* driven under its native promoter implies that cis-regulatory sequences in the promoter of *FUM2* are critical for the proper spatio-temporal expression of *FUM2*.

4.2.2 Levels of amino acids and soluble sugars changed by *FUMARASE 2*

The levels of threonic acid, an OAA-derived aspartate and a pyruvate-derived leucine significantly increased in *fum2+EV*. The increasing levels of the two amino acids, aspartate and leucine may be caused due to increasing levels of the corresponding organic acids, pyruvate and OAA in *fum2+EV*. Even though the level of OAA was not examined in this study, estimates from increased malate content which can be converted to OAA by malate dehydrogenase (MDH) suggest that the OAA level may have increased in *fum2+EV*. This

result is consistent with Fahnenstich et al. (2007): Significantly altered levels of the amino acids according to the increased levels of their corresponding organic acids were also observed in the *MEm* transgenic *Arabidopsis* which harbors the maize C_4 NADP-malic enzyme and grew under long-day conditions (16h light/8h darkness) (Fahnenstich et al. 2007). However, an inconsistent result was found in a study which used the same *fum2* mutants in similar ages but grew them under shorter day length (12 h light/12 h darkness) (Pracharoenwattana et al. 2010): altered levels of the amino acids and type of amino acids changed in the *fum2* were largely different from our data set. The contrasting outcomes may be caused by the differently synthesized levels of the corresponding organic acids: Organic acids are synthesized and accumulated during the day and decrease during dark period. Day length is critical for the accumulation of the organic acids (Gibon et al., 2004). Thus different day-lengths in the experiments may explain difference in the contents of organic acids and consequently result in different levels of the organic acid-derived amino acids. Glucose was increased in *fum2+EV* compared to *Col+EV*, which implies that fumarate or malate content is tightly linked with the content of soluble sugars. Both fumarate and sucrose serve as a storage form of fixed carbon and are metabolically accessible to each other (Chia et al. 2000, Fahnenstich et al. 2007, Pracharoenwattana et al. 2010) and in some cases the supplement of sucrose can compensate for fumarate and malate deficiency (Fahnenstich et al. 2007).

4.2.3 *FUMARASE 2* allelic variation is not the major contributor for the variation in fumarate content between Col-0 and C24

To study whether *FUM2* allelic variation is the main genetic determinant of the fumarate content variation which was observed in Col-0/C24 RIL population, each of the two alleles was transformed into the *fum2* mutant. Then, fumarate and malate levels and fumarate/malate ratio were compared in the two genotypes, *fum2+FUM2*$_{Col}$ and *fum2+FUM2*$_{C24}$. The malate level was significantly lower and the fumarate/malate ratio was significantly higher in *fum2+FUM2*$_{Col}$ than in *fum2+FUM2*$_{C24}$, thus it can be inferred that more malate may be converted to fumarate by *FUM2*$_{Col}$ than by *FUM2*$_{C24}$. Also the *FUM2* allelic variation can be indirectly estimated from altered levels of other metabolites such as succinate, α-ketoglutaric acid, shikimate and pyruvate, which significantly changed not only by blocked FUM2 activity but also by type of *FUM2* alleles: Those altered levels in *fum2+EV* were completely restored by transferred *FUM2*$_{Col}$ but less restored by *FUM2*$_{C24}$. However, the difference in fumarate content between *fum2+FUM2*$_{Col}$ and *fum2+FUM2*$_{C24}$ was statistically insignificant. It appears

that the allelic variation is not the decisive genetic factor for the variation on fumarate levels. Otherwise, considering significantly more reduced malate level by $FUM2_{Col}$ to $FUM2_{C24}$, more fumarate may be produced by $FUM2_{Col}$, but the converted fumarate cannot be maintained at a high level by rapid conversion to other organic acids. In any case, *FUM2* allelic variation is not the decisive genetic factor for the fumarate content variation in the Col-0/C24 RIL population. Rather, the explanation for the fumarate content variation could be found on a balance of other metabolite levels such as malate, pyruvate, succinate and α-ketoglutaric acid which are interconvertible to fumarate by several steps of enzyme reactions. Contents of many metabolites whose levels are sensitively changed by fumarase activities, such as malate, pyruvate, shikimate, succinate, α-ketoglutaric acid and sucrose, are already significantly enriched in C24 compared to Col-0. Enzymes activities between C24 and Col-0 are largely different (Cross et al. 2006). Also subtle changes in metabolite concentrations, not only direct substrates but also other metabolites, can largely affect enzyme activities (Wheeler et al. 2008). Therefore, the variation in fumarate contents between Col-0 and C24 might be an integrated outcome of several factors, such as innate metabolite levels and activities of several enzymes and their interactions.

4.2.4 NADPH accumulation might trigger organic acid changes for nitrogen assimilation

Like malate content, the levels of the four organic acids (succinate, α-ketoglutaric acid, shikimate and pyruvate) were not only significantly increased by blocking FUM2 activity, but the decreased levels in *fum2* were differently restored by the two *FUM2* alleles: The altered levels in *fum2+EV* were completely restored to wild-type levels by transferred $FUM2_{Col}$, but only partially restored by transferred $FUM2_{C24}$. In the correlation test, malate level strongly positively correlated with levels of the four metabolites (succinate, α-ketoglutaric acid and pyruvate) and was moderately positively correlated with shikimate level. These results imply that the four metabolites might be tightly related to malate, a substrate of FUM2. Interestingly, the altered level of succinate which can be interconverted to fumarate by succinate dehydrogenase did not significantly correlate with alteration of fumarate content, but is related to malate which cannot be directly changed to succinate via an enzyme. It suggests that other factor(s) might mediate in the series of metabolite changes, and the mediator can be estimated by a study: The opposite experimental outcome concerning malate and succinate levels was found in the metabolite changes in *MEm*s, *Arabidopsis* transgenic plants harboring

an overexpressed maize NADP-malic enzyme which catalyzes the oxidative decarboxylation of malate, producing pyruvate, CO_2, and NAD(P)H in the presence of a divalent cation (Chang and Tong 2003, Fahnenstich et al. 2007). By activation of the NADP-malic enzyme in *MEm*s, malate and fumarate levels were decreased, and pyruvate and three organic acids (succinate, α-ketoglutaric acid, shikimate) levels were increased (Fahnenstich et al. 2007). This study clearly shows that malate level change itself is not the direct factor for the other four metabolite (pyruvate, succinate, α-ketoglutaric acid, shikimate) level changes and also implies that another factor mediates the metabolite changes. We supposed that the factor might be an increased level of NADP by activation of the NADP-malic enzyme. The link between NADP-malic enzyme reaction and accumulation of the organic acids was identified in *Hymenolepis diminuta:* The accumulated NADPH which was produced by the conversion of malate to pyruvate by malic enzyme (ME) was preferentially utilized in the reaction of the electron transport-coupled fumarate reductase which converts fumarate to succinate, consequently the succinate level increased (Scheibel and Saz 1966, Scheibel et al. 1968, Saz et al. 1972). Even though the cases found in *Hymenolepis diminuta* are not reported in plants, several studies report that NADP(H) is largely used as a cofactor in the enzyme reaction for metabolite conversion in cytosol: In plants, NAD-dependent MEs isoforms function predominantly in mitochondria in the TCA cycle (Artus and Edwards 1985), while NADP-dependent MEs are found in cytosol and plastids (Edwards and Andreo 1992, Drincovich et al. 2001). In *Arabidopsis*, there are four NADP-dependent malic enzyme coding genes and one is localized in plastids whereas the other isoforms are in cytosols (Wheeler et al. 2008). Also the activations of NADP-dependent malic enzymes in *Arabidopsis* are largely influenced by succinate concentration (Wheeler et al. 2008).

Interestingly, the production of the three metabolites, α-ketoglutaric acid, shikimate and pyruvate, is also related to oxidation of NADPH and reduction of $NADP^+$: α-Ketoglutarate is produced by isocitrate dehydrogenase (IDH) which catalyzes the oxidative decarboxylation of isocitrate, producing α-ketoglutarate and CO_2. During this process $NAD(P)^+$ is reduced to NAD(P)H. In *Arabidopsis*, two types of IDH are present, NAD-dependent IDH (NAD-IDH) and NADP-dependent IDH (NADP-IDH). NAD-IDH is involved in TCA cycles but not in nitrogen assimilation (Lemaitre et al. 2007), while NADP-ICD is involved in nitrogen metabolism in cytosol in plants (Scheible et al. 2000, Hodges et al. 2003, Schneidereit et al. 2006), and is expressed in *Arabidopsis* leaves and roots (Leterrier et al. 2012). Therefore, accumulated $NADP^+$ by activation of fumarase reductase may be utilized in the reaction of cytosolic NADP-ICD and accordingly accumulate α-ketoglutarate in *fum2+EV*. Therefore, in

the correlation test, succinate and α-ketoglutarate may show a strong positive correlation. Shikimate is an intermediate product of the shikimate pathway which links metabolism of carbohydrates to biosynthesis of aromatic amino compounds such as phenylalanine, tyrosine, and tryptophan (reviewed by Herrmann and Weaver 1999). When shikimate is converted from 3-dehydroshikimic acid by shikimate dehydrogenase, $NADP^+$ is used as a cofactor (Bentley 1990).

The synthesis of the two metabolites α-aminoadipic acid and piecolic acid which were significantly changed in *fum2+EV* are also related to the oxidation of NADPH. The level of α-aminoadipic acid was higher and that of piecolic acid was lower in *fum2+EV* relative to the *Col+EV*. Three metabolites, pipecolic acid, α-aminoadipic acid and α-ketoglutarate are involved in a catabolic pathway of L-lysine in higher plants (Moulin et al. 2006). The first step in this pathway is the condensation of lysine and α-ketoglutarate catalyzed by lysine α-ketoglutarate reductase using NADPH as a cofactor and producing saccharopine (Stepansky and Galili 2003, Moulin et al. 2006). Then the saccharopine is converted to α-aminoadipic-semialdehyde and it is converted into either α-aminoadipic acid by aminoadipate reductase or piecolic acid by several steps of enzymatic reactions (Moulin et al. 2006). Thus, if α-aminoadipic-semialdehyde is preferentially converted into the direction of α-aminoadipic acid synthesis, the piecolic acid level should conversely decrease. Therefore, significantly higher levels of α-aminoadipic acid and lower levels of piecolic in *fum2+EV* can be influenced by increased levels of α-ketoglutarate and NADP accumulation in *fum2+EV*.

Accumulation of fumarate during the day by *FUM2* is necessary for rapid plant growth under high nitrogen conditions (Pracharoenwattana et al. 2010). Also, malate, NADP-ICD and lysine-ketoglutarate reductase/saccharopine dehydrogenase which are involved in lysine catabolism are also known to be involved in nitrogen assimilation in plants (Zioni et al. 1971, Touraine et al. 1988, 1992, Hamilton et al. 1996, Scheible et al. 2000, Hodges et al. 2003, Stepansky and Galili 2003, Schneidereit et al. 2006): Malate acts as a counter-anion for pH regulation, and α-ketoglutaric acid serves as a nitrogen acceptor when large amounts of nitrogen are accumulated in ammonium and glutamine (Scheible et al. 2000). Unlike severe defection in vegetative growth under high nitrogen conditions, *fum2* did not show any symptom of nitrogen deficiency during vegetative growth under normal growing conditions (Pracharoenwattana et al. 2010), which implies that even though fumarate level is low in *fum2*, the plants still assimilate nitrogen by other metabolites. We suppose that increased malate level by blocking FUM2 activity may trigger increasing levels of other metabolites involved

in nitrogen assimilation, therefore *fum2* might grow normally under normal conditions without any disadvantages.

4.2.5 Conclusions of the *FUMARASE 2* alleles investigation

Our study provides a good approach for completely restoring metabolite levels by introducing corresponding genes with their own genomic contents using cosmid transformation. Also, by transformation of the two different *FUM2* alleles in three genomic backgrounds, Col-0, C24 and *fum2,* we could identify the inheritance pattern of the alleles and the presence of the dosage effect. By comparison of metabolite levels in the genotypes, we also observed other metabolites changes which are not substrates of *FUM2* and their presumable role as substitutes of fumarate.

Even though we could not observe a clear difference between the two *FUM2* alleles in fumarate level, considering the fact that a cytosolic fumarase is required for rapid growth under a nitrogen rich soil and the several-fold increased level only observed under the high nitrogen condition (Pracharoenwattana et al. 2010), the *FUM2* allele difference on fumarate content might be clearly observed only under high nitrogen conditions. The possibility should be examined further.

4.3 Overall conclusion

This study was conducted in order to investigate whether allelic variations in candidate genes are the genetic basis for variation in the targeted traits. The individual alleles of investigated genes were evaluated with complementation approaches. Even though allelic variation of investigated genes was not connected to the differences of traits, by analyzing resulting phenotype and metabolite changes in several transgenic genotypes, we could test and observe not only allele-dependent regulation, but also interaction between transgene and genomic background, interaction within alleles and gene dosage effects.

5 Summary

Naturally occurring allelic diversity in plants is an important genetic source for morphological diversity and evolutionary fitness. In this study we investigated whether allelic variants of candidate genes are responsible for variability in biomass heterosis and fumarate content using two *Arabidopsis* accessions, Col-0 and C24.

Heterosis describes the phenomenon that hybrids have superior traits compared to the parents. From previous QTL studies on biomass heterosis using Col-0/C24 RIL, a QTL on top of chr.IV was responsible for the largest percentage of phenotypic variation, and the locus was fine mapped to 14 genes, from *At4g00238* to *At4g00335*. In order to identify the causative gene(s) and the potential allelic variant for the variability on early vegetative growth, every allele was investigated in a transgenic approach. Only transgenic plants harboring a single *At4g00310* transgene showed a decrease in leaf area by shortened petioles and rosette leaves without distinction of *At4g00310* alleles and genetic backgrounds. Occurrence of phenotypic change was higher in $Col{+}310_{Col}$, than in $C24{+}310_{Col}$, which implies that transferred 310_{Col} might interact with genomic background. The 491 bp InDel which harbors 8 key cis-regulatory binding sites was deleted in the promoter sequence of 310_{C24}. Therefore, the presence or absence of the 8 cis-regulatory binding sites may become a source of *At4g00310* allelic variation in expression. Interestingly, cosmid transgenic lines harboring *At4g00310* transgene did not display any change in leaf area. The possible reasons for the different phenotypic change between two groups of transgenic plants harboring *At4g00310* were discussed in detail.

The enzyme fumarase catalyzes the reversible hydration/dehydration of fumarate to malate. *FUMARASE 2* (*At5g50950*) was found within the confidence interval of a fumarate QTL. Lines with the Col-0 alleles showed higher fumarate levels compared to the lines with C24 alleles. The causal variation between $FUM2_{Col}$ and $FUM2_{C24}$ influencing fumarate content was investigated. The two *FUM2* alleles differed by two insertions in the promoter of $FUM2_{C24}$. The transferred $FUM2_{Col}$ driven under its native promoter in *fum2* fully restored fumarate level, which implies that cis-regulatory sequences in the promoter of *FUM2* are critical for the proper spatiotemporal expression of *FUM2*. Malate was more converted to fumarate by $FUM2_{Col}$ than by $FUM2_{C24}$ in *fum2*. However, fumarate levels do not differ between *FUM2* alleles in *fum2*, which implies that *FUM2* allele variant is not the major genetic factor for fumarase content variation. Rather, fumarate content might be decided on a

balance of other metabolite levels which are interconvertible to fumarate by several steps of enzyme reactions and significantly different between Col-0 and C24.

By testing different alleles in several genomic backgrounds, inheritance patterns of alleles, allele- and background-dependent dosage effect and allelic and non-allelic interactions were proved. However, the allelic variants of investigated genes did not lead to the target trait variations. We concluded that the target traits are collective outcomes of allelic variants and genetic background.

6 Zusammenfassung

Natürlich vorkommende allelische Diversität in Pflanzen ist eine wichtige genetische Quelle für morphologische Diversität und evolutionäre Fitness. In dieser Arbeit wurde untersucht, ob allelische Varianten von Kandidatengenen für Variabilität in Biomasse-Heterosis und Fumaratgehalt verantwortlich sind. Die Untersuchungen wurden an zwei *Arabidopsis*-Akzessionen, Col-0 und C24, durchgeführt.

Heterosis beschreibt das Phänomen, dass Hybride stärkere Merkmalsausprägungen gegenüber ihren Eltern haben. In früheren QTL Analysen von Biomasse-Heterosis mit Col-0/C24 RILs war ein QTL oben auf Chr.IV verantwortlich für den größten Teil der phänotypischen Variation, und der Locus konnte durch Feinkartierung auf 14 Gene, von *At4g00238* bis *At4g00335,* eingegrenzt werden. Um das ursächliche Gen und die allelische Variante, für die Variabilität des frühen vegetativen Wachstums zu identifizieren, wurde jedes Allel in einem transgenen Ansatz untersucht. Nur transgene Pflanzen, die ein einzelnes *At4g00310*-Transgen enthielten, zeigten eine Verringerung der Blattfläche durch verkürzte Blattstiele und Rosettenblätter, ohne Unterscheidung der *At4g00310* Allele oder des genetischen Hintergrunds. Phänotypische Unterschiede waren häufiger in $Col{+}310_{Col}$ als in $C24{+}310_{Col}$ zu beobachten, was darauf hindeutet, dass das transferierte 310_{Col} mit dem genetischen Hintergrund interagiert. Das 491 bp InDel, das 8 wichtige cis-regulatorische Bindungsstellen enthält, fehlte in der Promotersequenz von 310_{C24}. Daher könnte die Anwesenheit oder das Fehlen der 8 cis-regulatorischen Bindungsstellen eine Quelle für die allelische Expressionsvariation von *At4g00310* sein.

Interessanterweise zeigten mit Cosmiden transformierte Linien, die das *At4g00310* Transgen enthielten, keine Veränderung der Blattfläche. Die möglichen Gründe für die unterschiedlichen phänotypischen Veränderungen der beiden Gruppen von transgenen Pflanzen mit *At4g00310* wurden diskutiert.

Das Enzym Fumarase katalysiert die reversible Hydration/Dehydration von Fumarat zu Malat. *FUMARASE 2* (*At5g50950*) wurde innerhalb des Konfidenzintervalls eines Fumarat QTL gefunden. Linien mit dem Col-0 Allel enthielten mehr Fumarat als Linien mit dem C24 Allel. Wir untersuchten die ursächliche Variation zwischen $FUM2_{Col}$ und $FUM2_{C24}$, die den Fumaratgehalt beeinflusst. Die beiden *FUM2* Allele unterscheiden sich durch zwei Insertionen im Promoter von $FUM2_{C24}$. Das von seinem eigenen Promoter gesteuerte $FUM2_{Col}$ in *fum2* konnte den Fumaratgehalt wiederherstellen, was darauf hindeutet, dass cis-regulatorische Sequenzen im *FUM2* Promoter entscheidend sind für eine räumlich und

zeitlich korrekte Expression von *FUM2*. $FUM2_{Col}$ in *fum2* verwandelte mehr Malat zu Fumarat als $FUM2_{C24}$ in *fum2*. Der Fumaratgehalt zwischen den beiden Allelen in *fum2* war jedoch nicht unterschiedlich, was andeutet, dass die *FUM2* Allelvariante nicht der entscheidende genetische Faktor für die Variation des Fumaratgehalts ist. Vielmehr könnte der Fumaratgehalt durch ein Gleichgewicht anderer Metabolite bestimmt werden, die in mehreren enzymatischen Schritten zu Fumarat konvertiert werden können, und deren Gehalt in Col-0 und C24 signifikant verschieden ist.

Durch die Analyse verschiedener Allele in mehreren genomischen Hintergründen konnten Vererbungsmuster der Allele, Allel- und Hintergrund-spezifische Dosis-Effekte sowie allelische und nicht-allelische Interaktionen aufgezeigt werden. Die allelischen Varianten der untersuchten Gene führten jedoch nicht zu Unterschieden in den entsprechenden Merkmalen. Wir schließen daraus, dass die untersuchten Merkmale das Ergebnis eines gemeinsamen Wirkens von allelischen Varianten und genetischem Hintergrund sind.

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8 Appendixes

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cacacaatagaatggattcgcaaacatattcagcaatgacaagagttctaaatagc

Appendix 1: The sequence of the intergenic region between *At4g00270* and *At4g00280* in C24. The first and the last sequence are corresponded with the positions, chr.IV:118478 and chr.IV:122408 in Col-0, respectively (TAIR 10). The ORF_{C24} sequence is underlined. The start codon (ATG) and the stop codon (TGA) of the ORF_{C24} were marked in bold capital.

Ttatcatagtcctggacgttcggttatccattcgatttcggttatattcattcagttatcggtttgttgttctaaaggtattcattcagtttggtttcgattctttcg
gttttcagatatttttttgattattttcaagtatttttattattttatatatattctcaagtatttttaattatttactttagaaataactaatacttttaaaatttttgattgat
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tgggataaacaactacaagaaaattttgccttaatcgacgtcacatgtaaaca

Appendix 2: The sequence of the 491 bp deletion located between *At4g00305*$_{C24}$ and *Atg00310*$_{C24}$. The position of the sequence corresponds to the Col-0 reference sequence (TAIR 10), chr. IV:132070-132560.

Appendix 3: The transcription of key known genes for biomass heterosis at 5DAS

AGI [a]	Name [a]	Col-0	C24 [b]	Col-0×C24 [b]	C24×Col-0 [b]
AT2G46830	CCA1	1.0	2.3	1.2	1.2
AT1G01060	LHY	1.0	2.4	1.3	1.7
AT5G61380	TOC 1	1.0	0.9	1.1	1.0
AT1G22770	GI	1.0	0.8	1.0	1.1
AT5G13930	CHS	1.0	2.6	1.9	1.1
AT3G51240	F3H	1.0	2.1	2.0	1.3
AT5G08640	FLS	1.0	2.3	1.8	0.9
AT1G15690	AVP1	1.0	0.8	0.9	1.0
AT1G56010	NAC1	1.0	0.4	0.9	0.7
AT3G59900	ARGOS	1.0	1.0	0.8	0.9

[a] The genes which were suggested to be involved in biomass heterosis in *Arabidopsis* (Ni et al. 2009, Shen et al. 2012)was selected

[b] All measurements are relative to Col-0

Appendix 4: Genomic positions of the inserts of selected cosmids and the genes in the inserts

Cosmids	Start[a]	End[b]	Size (bp)[c]	Gene[d]
Cos_013$_{Col}$	102,345	124,942	22,597	*At4g00238-At4g00280*
Cos_062$_{Col}$	119,496	143,122	23,626	*At4g00280-At4g00320*
Cos_125$_{Col}$	135,687	156,730	21,043	*At4g00315-At4g00355*
Cos_170$_{Col}$	113,353	134,609	21,256	*At4g00260-At4g00305*
Cos_395$_{C24}$	131,021	146,499	15,478	*At4g00305-At4g00330*
Cos_401$_{C24}$	123,534	140,333	16,799	*At4g00300-At4g00315*
Cos_411$_{C24}$	137,811	152,329	14,518	*At4g00320-At4g00340*
Cos_507$_{C24}$	102,915	117,389	14,474	*At4g00238-At4g00260*
Cos_523$_{C24}$	110,627	129,789	19,162	*At4g00250-At4g00290*

[a] Genomic position of 5' end of the insert

[b] Genomic position of 3' end of the insert; note: both insert-end positions match to the reference Col-0 position on chr.IV (TAIR 10).

[c] The total length of the insert and the lengths of Col-0 and C24 cosmids were calculated from Col-0 and C24 sequences, respectively.

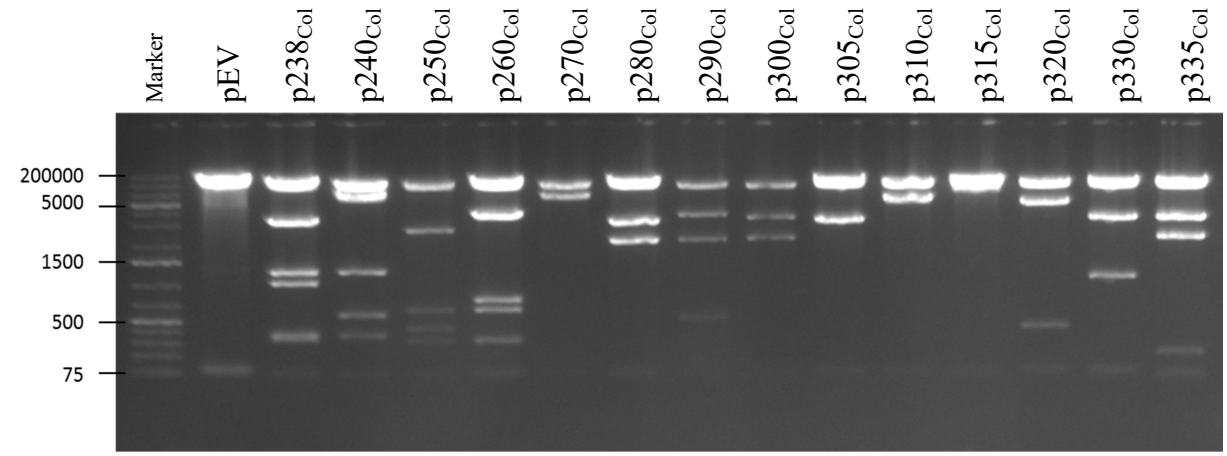
[d] Only genes whose start and end codons are in the insert were noted.

Appendix 5: Length of promoter and terminator sequences of the candidate genes of Col-0 and C24 which were estimated for cloning

Construct	Insert (bp)[a]	Promoter (bp)[b]	Gene body (bp)[c]	Terminator (bp)[d]
$p238_{Col}$	6351	3312 (3088)	1038	2001
$p240_{Col}$	8167	2594 (1985)	4339	1234
$p250_{Col}$	4166	1297 (1198)	960	1909
$p260_{Col}$	5536	2001 (102)	2081	1454
$p270_{Col}$	6049	3017 (3000)	992	2040
$p280_{Col}$	5381	2170 (NA)	443	2768
$p290_{Col}$	6476	2446 (1207)	2204	1826
$p300_{Col}$	5747	860 (101)	3618	1269
$p305_{Col}$	3263	1339 (384)	381	1543
$p310_{Col}$	5399	2095 (1928)	1379	1925
$p315_{Col}$	7041	2990 (1487)	1485	2566
$p320_{Col}$	5535	2084 (1051)	1705	1746
$p330_{Col}$	4745	2003 (1500)	1641	1101
$p335_{Col}$	6128	2566 (2441)	1097	2465
$p238_{C24}$	6252	3218 (2994)	1038	1996
$p240_{C24}$	8023	2566 (1957)	4317	1140
$p250_{C24}$	4135	1300 (1201)	957	1878
$p260_{C24}$	5457	2001 (101)	2091	1365
$p270_{C24}$	7966	4942 (4925)	974	2050
$p280_{C24}$	7290	2204 (NA)	438	4648
$p290_{C24}$	6502	2449 (1196)	2238	1815
$p300_{C24}$	5745	845 (136)	3629	1271
$p305_{C24}$	2789	1353 (396)	381	1055
$p310_{C24}$	4890	1607 (1440)	1379	1904
$p315_{C24}$	7032	2969 (1467)	1485	2578
$p320_{C24}$	5545	2096 (1063)	1705	1744
$p330_{C24}$	4766	2025 (1518)	1642	1099
$p335_{C24}$	6268	2600 (2475)	1203	2465
$pORF_{C24}$	2773	1625 (NA)	300	848

[a] From 5' end to 3' end of an insert.
[b] From 5'end of an insert to the transcript start site (TSS) (From end of promoter position which is estimated by AGRIS to TSS)
[c] From a start codon to a stop codon.
[d] From a transcript stop site to the 3'end of an insert.

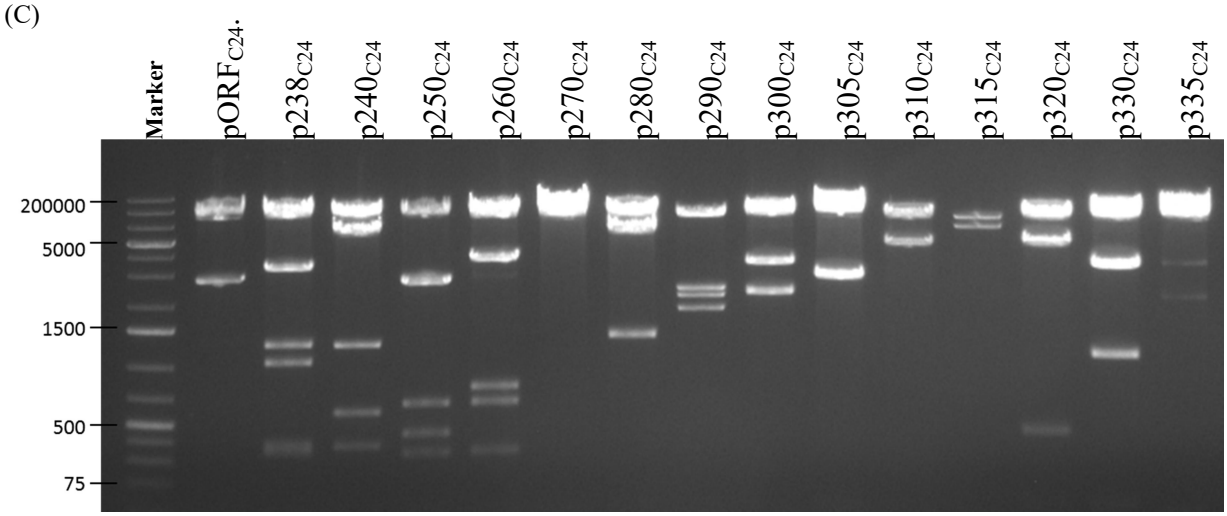
(A)



(B)

pEV	p238Col	p240Col	p250Col	p260Col	p270Col	p280Col	p290Col	p300Col	p305Col	p310Col	p315Col	p320Col	p330Col	p335Col
8477	8477	8477	8477	8477	8477	8477	8477	8477	8477	8477	8477	8477	8477	8477
85	3304	5918	2712	3746	6049	3209	3725	3492	3263	5399	7041	4972	3492	3538
	1280	1280	666	784	85	2172	2209	2255	85	85	85	450	1140	2296
	1047	717	448	666		85	542	85				113	113	231
	384	585	340	340			85					85	85	85
	336	85	85	85										63
	85													

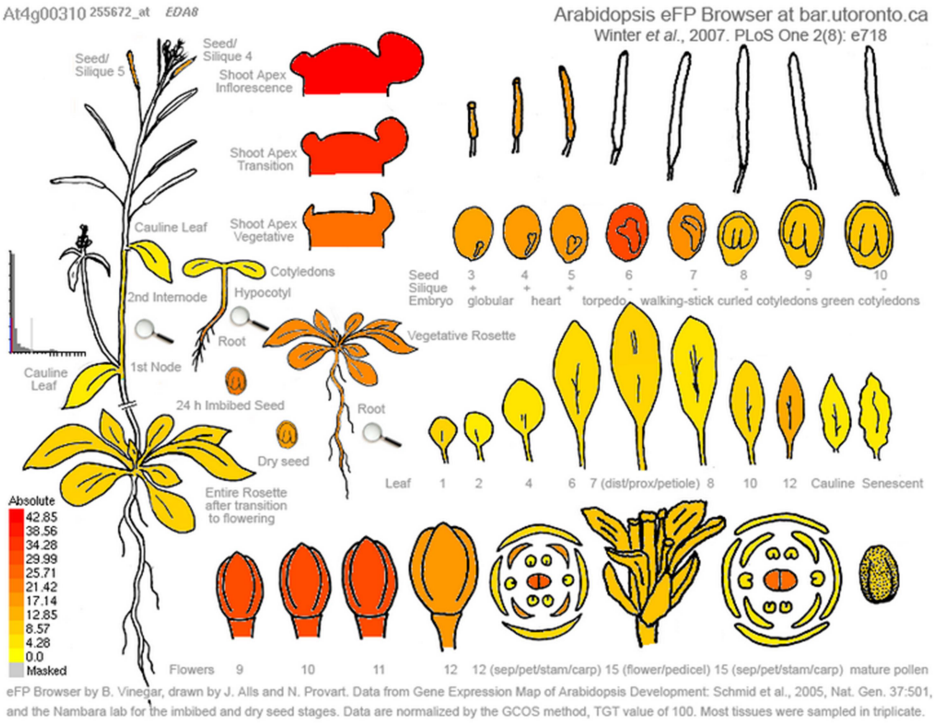
(C)



(D)

pORFC24.	p238C24	p240C24	p250C24	p260C24	p270C24	p280C24	p290C24	p300C24	p305C24	p310C24	p315C24	p320C24	p330C24	p335C24
8477	8477	8477	8477	8477	8477	8477	8477	8477	8477	8477	8477	8477	8477	8477
2773	3316	5868	2678	3667	7966	5937	2421	3488	2789	4890	7032	4982	3515	3678
85	1280	1280	666	781	85	1353	2211	2257	85	85	85	450	1138	2278
	1047	602	448	666		85	1870	85				113	113	231
	336	273	343	343			85					85	85	85
	273	85	85	85										63
	85													

Appendix 6: The genomic clone and the *EcoRI* restriction fragment-lengths. The Col-0 clones (A) and the C24 clones (C) in pMDC123 were digested with *EcoR*I, and their resulted fragment sizes (bp) of the corresponding Col-0 clone and the C24 clone were denoted in (B) and (D) respectively.



Appendix 7: Transcription levels of *At4g00310* in Col-0 from different tissues and from various develpmental stages (Source: http://bar.utoronto.ca/efp_arabidopsis/cgi-bin/efpWeb.cgi, the *At4g00310* expression data was selected from develpmental MAP). The color code indicate the absolute expression levels of the genes and noted in bottom left.

Appendix 8: Mean leaf area of individual cosmid transgenic lines in T3 homozygous generation

Genotype	9 DAS		10 DAS		11 DAS		12 DAS		13 DAS		14 DAS		15 DAS		16 DAS		17 DAS	
	LA	SE	LA	SE	LA	SE	LA	SE	LA	SE	LA	SE	LA	SE	LA	SE	LA	SE
C24	6.6	0.68	8.8	0.91	12.3	1.31	16.7	1.78	23.8	2.48	33	3.4	46	4.6	58	6.0	74	7.9
C24 X Col-0	10.3	1.39	13.2	1.89	19.0	2.77	27.8	3.89	36.8	5.36	52	7.7	72	10.9	102	15.1	134	20.8
C24+EV A	7.2	0.68	9.5	0.92	13.9	1.30	19.4	1.79	25.7	2.50	35	3.4	46	4.7	59	6.2	75	8.1
C24+EV B	7.5	0.62	10.0	0.84	14.4	1.17	19.8	1.60	27.6	2.25	38	3.0	50	4.1	64	5.2	82	6.7
C24+EV C	6.3	0.56	8.3	0.75	11.7	1.04	16.7	1.40	22.7	1.98	31	2.6	42	3.4	54	4.1	67	5.0
C24+EV D	6.5	0.62	8.6	0.84	12.2	1.17	16.7	1.60	22.3	2.24	31	3.0	42	4.1	55	5.2	70	6.7
C24+EV E	7.3	0.55	9.8	0.74	13.9	1.02	19.2	1.36	26.2	1.93	36	2.5	48	3.3	63	3.9	81	4.7
C24+EV F	6.6	0.63	8.7	0.84	12.3	1.19	17.1	1.62	24.4	2.27	34	3.0	47	4.1	60	5.3	76	6.8
C24+125 $_{Col}$ A	7.6	0.62	9.8	0.83	13.8	1.17	19.2	1.59	25.7	2.23	35	3.0	45	4.0	58	5.1	72	6.6
C24+125 $_{Col}$ B	5.7	0.66	7.6	0.89	10.7	1.26	15.1	1.72	21.4	2.42	29	3.3	40	4.5	52	5.8	66	7.7
C24+125 $_{Col}$ C	6.4	0.65	8.3	0.88	11.5	1.25	15.7	1.70	21.4	2.39	29	3.2	38	4.4	50	5.7	63	7.5
C24+125 $_{Col}$ D	7.6	0.62	10.0	0.83	14.1	1.18	20.1	1.59	27.2	2.23	37	3.0	48	4.0	61	5.2	78	6.6
C24+125 $_{Col}$ E	7.6	0.64	9.9	0.86	13.9	1.21	19.7	1.66	26.5	2.32	36	3.1	47	4.2	60	5.5	76	7.1
C24+125 $_{Col}$ F	7.1	0.62	9.3	0.83	13.1	1.17	18.7	1.59	25.3	2.23	35	3.0	46	4.0	60	5.1	73	6.6
C24+013 $_{Col}$ A	7.4	0.62	9.7	0.83	13.8	1.17	19.4	1.59	25.9	2.24	36	3.0	48	4.1	62	5.2	79	6.7
C24+013 $_{Col}$ B	7.6	0.62	9.9	0.84	13.6	1.18	19.2	1.61	25.0	2.26	34	3.0	45	4.1	58	5.2	73	6.7
C24+013 $_{Col}$ C	6.3	0.63	8.2	0.85	10.7	1.20	15.6	1.63	21.3	2.29	29	3.1	40	4.2	53	5.4	68	7.0
C24+013 $_{Col}$ D	6.4	0.65	8.3	0.88	11.6	1.24	16.3	1.69	22.9	2.37	32	3.2	43	4.4	56	5.7	70	7.4
C24+013 $_{Col}$ F	7.0	0.64	9.1	0.86	12.5	1.23	17.6	1.67	23.8	2.34	33	3.2	44	4.3	55	5.5	70	7.3
C24+013 $_{Col}$ E	7.3	0.62	9.4	0.84	13.0	1.19	18.5	1.61	24.9	2.25	34	3.0	46	4.1	58	5.2	74	6.8
C24+170 $_{Col}$ A	6.9	0.62	9.0	0.83	12.4	1.17	17.5	1.59	23.3	2.23	32	3.0	42	4.0	53	5.1	66	6.6
C24+170 $_{Col}$ B	6.3	0.67	8.1	0.90	11.0	1.29	14.9	1.75	20.6	2.44	29	3.3	39	4.5	52	5.9	65	7.8
C24+170 $_{Col}$ C	7.7	0.65	9.7	0.87	13.4	1.24	18.7	1.69	24.5	2.36	34	3.2	45	4.3	58	5.6	72	7.3
C24+170 $_{Col}$ D	6.2	0.64	7.9	0.86	10.1	1.22	14.4	1.66	18.3	2.33	24	3.1	31	4.3	40	5.5	49	7.1
C24+170 $_{Col}$ E	5.6	0.64	7.1	0.86	9.7	1.22	13.5	1.64	18.1	2.30	24	3.1	33	4.2	44	5.4	55	7.0
C24+170 $_{Col}$ F	6.3	0.68	8.1	0.91	11.3	1.30	15.7	1.75	22.3	2.44	31	3.3	43	4.5	57	5.8	71	7.7
C24+395 $_{C24}$ A	6.4	0.65	8.5	0.87	11.9	1.23	16.2	1.68	23.2	2.36	32	3.2	44	4.3	57	5.5	74	7.2
C24+395 $_{C24}$ B	6.6	0.62	8.1	0.84	11.1	1.17	14.5	1.60	20.3	2.25	28	3.0	37	4.1	48	5.2	61	6.7
C24+395 $_{C24}$ C	7.2	0.62	9.5	0.84	13.7	1.17	18.8	1.60	25.6	2.24	35	3.0	47	4.1	60	5.2	77	6.7
C24+395 $_{C24}$ D	7.4	0.62	9.9	0.84	14.1	1.17	19.4	1.60	26.4	2.24	37	3.0	49	4.1	65	5.2	83	6.7
C24+395 $_{C24}$ E	6.8	0.70	9.1	0.94	12.7	1.34	17.2	1.84	24.7	2.57	35	3.5	48	4.8	62	6.3	80	8.3
C24+395 $_{C24}$ F	6.5	0.63	8.6	0.84	12.3	1.18	16.9	1.61	23.4	2.26	33	3.0	44	4.1	59	5.2	77	6.8
C24+401 $_{C24}$ A	8.0	0.64	10.5	0.85	15.2	1.20	21.2	1.64	29.0	2.30	41	3.1	55	4.2	72	5.4	93	7.0
C24+401 $_{C24}$ B	7.1	0.62	9.3	0.84	13.2	1.17	18.3	1.60	25.7	2.24	36	3.0	49	4.1	65	5.2	85	6.7
C24+401 $_{C24}$ C	7.6	0.68	9.9	0.91	14.2	1.29	20.0	1.78	25.8	2.48	36	3.4	47	4.6	62	6.1	77	8.1
C24+401 $_{C24}$ D	7.5	0.64	10.1	0.86	14.4	1.22	19.8	1.66	28.4	2.33	40	3.1	53	4.3	69	5.5	88	7.1
C24+401 $_{C24}$ E	7.2	0.66	9.6	0.89	13.5	1.26	18.5	1.72	26.7	2.41	38	3.3	51	4.4	66	5.7	85	7.5
C24+401 $_{C24}$ F	7.7	0.67	9.9	0.90	13.8	1.27	18.8	1.73	27.0	2.43	38	3.3	51	4.5	66	5.8	85	7.6
C24+411 $_{C24}$ A	7.8	0.62	9.8	0.83	13.6	1.17	17.7	1.59	23.5	2.24	31	3.0	41	4.0	52	5.2	65	6.6
C24+411 $_{C24}$ B	8.0	0.80	10.7	1.08	14.9	1.56	20.1	2.16	28.0	3.00	39	4.2	54	5.8	68	7.8	86	10.4
C24+411 $_{C24}$ C	7.4	0.62	9.8	0.83	13.9	1.17	19.4	1.59	27.3	2.24	38	3.0	52	4.1	67	5.2	86	6.7
C24+411 $_{C24}$ D	7.5	0.73	10.0	0.98	14.1	1.40	19.3	1.92	26.9	2.69	38	3.7	51	5.1	66	6.7	84	8.9
C24+411 $_{C24}$ E	7.2	0.70	9.6	0.95	13.7	1.35	18.9	1.85	26.7	2.59	38	3.6	51	4.9	66	6.4	85	8.4
C24+411 $_{C24}$ F	6.2	0.63	8.3	0.84	11.4	1.17	16.0	1.60	22.4	2.25	31	3.0	42	4.1	56	5.2	70	6.7
C24+507 $_{C24}$ A	7.0	0.67	9.0	0.90	12.8	1.27	17.9	1.74	24.0	2.43	33	3.3	43	4.5	57	5.9	72	7.8
C24+507 $_{C24}$ B	6.7	0.66	8.8	0.89	11.7	1.27	15.8	1.73	21.8	2.42	30	3.3	40	4.5	53	5.8	69	7.6
C24+507 $_{C24}$ C	6.8	0.63	9.0	0.84	12.5	1.18	17.1	1.61	24.0	2.26	34	3.0	46	4.1	61	5.2	79	6.7
C24+507 $_{C24}$ D	6.8	0.68	9.2	0.91	13.1	1.29	18.2	1.76	25.7	2.47	36	3.4	49	4.6	64	6.0	82	7.8
C24+507 $_{C24}$ E	6.2	0.63	8.4	0.85	11.8	1.20	16.5	1.64	23.6	2.30	33	3.1	45	4.2	59	5.4	74	7.0
C24+507 $_{C24}$ F	6.8	0.62	9.0	0.84	12.6	1.17	17.3	1.60	24.8	2.25	35	3.0	47	4.1	61	5.2	77	6.7
C24+523 $_{C24}$ A	6.9	0.67	9.1	0.90	12.9	1.28	17.6	1.75	25.6	2.46	36	3.3	48	4.6	61	5.9	79	7.7
C24+523 $_{C24}$ B	7.0	0.68	9.4	0.92	13.4	1.31	18.4	1.79	26.7	2.51	38	3.4	51	4.7	66	6.1	85	8.0
C24+523 $_{C24}$ C	7.1	0.71	9.7	0.95	13.7	1.36	18.8	1.86	26.8	2.60	38	3.6	51	4.9	67	6.4	87	8.5
C24+523 $_{C24}$ D	7.5	0.62	9.9	0.83	14.1	1.16	19.5	1.58	26.8	2.23	37	3.0	49	4.0	64	5.1	82	6.6
C24+523 $_{C24}$ E	7.6	0.63	10.0	0.84	14.4	1.19	19.8	1.62	27.6	2.27	38	3.0	50	4.1	64	5.3	82	6.8
C24+523 $_{C24}$ F	7.3	0.63	9.8	0.85	13.8	1.20	19.0	1.64	26.4	2.30	37	3.1	49	4.2	64	5.4	83	6.9
Col-0	6.3	0.64	8.6	0.86	12.2	1.23	16.5	1.65	24.5	2.32	35	3.1	50	4.2	68	5.4	90	7.0
Col-0 X C24	7.7	0.72	10.3	0.97	14.7	1.37	20.9	1.89	29.0	2.64	42	3.6	58	5.0	80	6.6	108	8.7
Col+EV A	6.7	0.67	9.0	0.90	12.4	1.28	17.6	1.74	24.6	2.44	34	3.3	48	4.5	67	5.9	88	7.7
Col+EV B	5.7	0.93	7.0	1.26	9.7	1.83	13.7	2.54	17.6	3.52	25	5.0	35	7.0	51	9.4	68	12.7
Col+EV C	6.1	0.68	8.2	0.91	11.5	1.29	16.2	1.77	22.3	2.48	32	3.4	44	4.6	62	6.0	82	7.8
Col+EV D	5.6	0.91	7.4	1.23	10.3	1.79	15.2	2.48	20.3	3.43	28	4.8	39	6.7	56	9.1	74	12.3
Col+EV E	6.1	0.62	8.2	0.83	11.7	1.18	16.3	1.59	23.7	2.24	34	3.0	49	4.0	68	5.2	92	6.7
Col+EV F	6.5	0.79	8.7	1.06	12.2	1.53	17.2	2.11	23.3	2.93	33	4.1	45	5.6	63	7.5	84	10.0
Col+125 $_{Col}$ A	5.6	0.65	7.5	0.87	10.4	1.23	14.6	1.68	21.4	2.35	31	3.2	45	4.3	62	5.6	83	7.3
Col+125 $_{Col}$ B	5.9	0.63	8.0	0.84	11.0	1.20	15.5	1.61	22.0	2.26	32	3.0	45	4.1	63	5.2	86	6.7
Col+125 $_{Col}$ C	5.2	0.72	7.2	0.97	10.1	1.40	13.8	1.91	20.6	2.66	30	3.7	44	5.0	61	6.7	84	8.9
Col+125 $_{Col}$ D	6.1	0.62	8.1	0.84	11.3	1.18	15.8	1.60	22.3	2.25	32	3.0	45	4.1	63	5.2	84	6.7
Col+125 $_{Col}$ E	5.0	0.67	6.4	0.90	8.9	1.27	12.7	1.73	17.4	2.41	24	3.3	34	4.4	47	5.7	63	7.5
Col+125 $_{Col}$ F	5.1	0.67	6.7	0.90	8.9	1.28	12.6	1.73	18.3	2.40	27	3.2	39	4.4	54	5.7	74	7.4
Col+013 $_{Col}$ A	6.3	0.65	8.6	0.87	11.8	1.24	16.0	1.68	23.1	2.36	33	3.2	48	4.4	67	5.6	89	7.4
Col+013 $_{Col}$ B	5.5	0.63	7.2	0.84	10.3	1.19	14.2	1.61	19.5	2.26	28	3.0	40	4.1	57	5.3	76	6.8
Col+013 $_{Col}$ C	6.3	0.66	8.5	0.89	12.0	1.27	16.4	1.72	23.7	2.42	34	3.3	49	4.5	68	5.8	92	7.7
Col+013 $_{Col}$ D	5.5	0.66	7.5	0.89	10.4	1.27	14.5	1.72	20.9	2.41	30	3.3	43	4.5	59	5.8	81	7.7
Col+013 $_{Col}$ E	5.0	0.66	6.7	0.88	9.4	1.25	12.9	1.70	18.6	2.39	27	3.2	40	4.4	55	5.7	76	7.5
Col+013 $_{Col}$ F	5.3	0.69	7.0	0.92	9.4	1.32	12.0	1.80	15.9	2.51	23	3.4	33	4.7	45	6.2	61	8.2
Col+170 $_{Col}$ A	5.9	0.62	8.1	0.83	11.3	1.18	15.9	1.59	22.1	2.23	32	3.0	45	4.0	63	5.1	86	6.6
Col+170 $_{Col}$ B	5.6	0.62	7.5	0.83	10.5	1.17	14.1	1.59	19.3	2.24	27	3.0	39	4.0	54	5.2	73	6.7
Col+170 $_{Col}$ C	5.2	0.63	6.9	0.84	9.3	1.20	12.9	1.62	18.1	2.27	26	3.1	37	4.1	51	5.3	69	6.9
Col+170 $_{Col}$ D	6.7	0.62	8.9	0.84	12.3	1.19	16.7	1.60	23.7	2.25	34	3.0	48	4.1	65	5.2	87	6.8
Col+170 $_{Col}$ E	5.8	0.66	7.9	0.89	11.0	1.27	15.1	1.72	21.9	2.40	32	3.3	45	4.5	62	5.8	84	7.6
Col+170 $_{Col}$ F	6.4	0.62	8.6	0.83	11.6	1.18	16.1	1.59	22.9	2.24	33	3.0	46	4.1	65	5.2	87	6.7
Col+395 $_{C24}$ A	6.5	0.69	8.5	0.93	12.3	1.32	17.2	1.81	23.8	2.53	33	3.5	46	4.8	64	6.2	83	8.3
Col+395 $_{C24}$ B	6.0	0.64	8.1	0.85	11.6	1.20	16.0	1.63	22.6	2.29	32	3.1	44	4.2	61	5.4	80	7.0
Col+395 $_{C24}$ C	6.7	0.63	9.2	0.84	13.1	1.18	18.3	1.61	25.4	2.27	36	3.0	50	4.1	68	5.3	89	6.8
Col+395 $_{C24}$ D	7.1	0.62	9.3	0.84	13.2	1.17	18.4	1.60	25.5	2.24	36	3.0	50	4.1	69	5.2	91	6.7
Col+395 $_{C24}$ E	7.4	0.63	10.2	0.85	14.4	1.20	19.7	1.63	27.9	2.29	40	3.1	55	4.2	75	5.4	99	6.9
Col+395 $_{C24}$ F	6.8	0.67	8.9	0.90	13.0	1.26	18.2	1.74	25.3	2.43	36	3.3	49	4.5	68	5.9	90	7.8
Col+401 $_{C24}$ A	6.7	0.63	9.0	0.85	13.0	1.20	18.2	1.64	25.4	2.30	36	3.1	49	4.2	68	5.4	89	7.0
Col+401 $_{C24}$ B	7.7	0.65	10.2	0.88	14.5	1.24	19.3	1.70	27.4	2.38	39	3.2	54	4.4	73	5.7	97	7.4
Col+401 $_{C24}$ C	7.0	0.66	9.3	0.89	13.3	1.26	18.4	1.72	27.0	2.41	39	3.3	54	4.4	74	5.8	98	7.5
Col+401 $_{C24}$ D	6.4	0.71	8.7	0.95	12.4	1.36	17.2	1.86	25.2	2.60	37	3.6	51	4.9	71	6.4	94	8.5
Col+401 $_{C24}$ E	6.9	0.68	9.3	0.92	13.0	1.30	17.6	1.78	25.5	2.49	36	3.4	51	4.6	68	6.0	91	7.9
Col+401 $_{C24}$ F	6.1	0.62	8.1	0.84	11.7	1.18	16.3	1.60	23.7	2.25	34	3.0	47	4.1	64	5.2	86	6.7
Col+411 $_{C24}$ A	8.4	0.73	11.5	0.99	16.2	1.41	22.2	1.94	32.7	2.71	47	3.7	65	5.1	88	6.8	118	9.0
Col+411 $_{C24}$ B	8.1	0.68	11.0	0.92	15.6	1.30	21.3	1.78	31.2	2.49	45	3.4	63	4.6	86	6.0	115	7.9
Col+411 $_{C24}$ C	7.3	0.74	10.0	1.00	14.2	1.43	19.3	1.97	28.2	2.74	41	3.8	57	5.2	76	6.9	103	9.2
Col+411 $_{C24}$ D	7.0	0.66	9.2	0.89	13.1	1.25	18.5	1.71	25.4	2.40	36	3.3	50	4.4	70	5.8	93	7.6
Col+411 $_{C24}$ E	6.2	0.71	8.1	0.96	11.8	1.36	16.6	1.89	22.8	2.63	32	3.6	45	5.0	62	6.6	81	8.8
Col+411 $_{C24}$ F	7.1	0.79	9.8	1.07	13.8	1.54	18.6	2.12	27.5	2.95	40	4.1	56	5.7	76	7.6	101	10.2
Col+507 $_{C24}$ A	7.4	0.66	10.0	0.89	14.4	1.26	19.3	1.72	27.8	2.41	39	3.3	55	4.5	74	5.8	98	7.5
Col+507 $_{C24}$ B	6.6	0.62	9.0	0.83	12.7	1.17	17.4	1.59	24.8	2.24	35	3.0	49	4.1	67	5.2	89	6.7
Col+507 $_{C24}$ C	6.5	0.67	8.7	0.90	12.5	1.26	17.5	1.74	24.7	2.43	35	3.3	48	4.5	64	5.9	86	7.8
Col+507 $_{C24}$ D	7.2	0.69	9.7	0.94	13.8	1.33	19.1	1.82	27.0	2.55	39	3.5	55	4.8	75	6.2	100	8.2
Col+507 $_{C24}$ E	7.0	0.64	9.4	0.86	13.3	1.21	18.5	1.65	24.3	2.31	35	3.1	48	4.2	66	5.4	88	7.0
Col+507 $_{C24}$ F	6.8	0.67	9.3	0.90	13.3	1.28	18.2	1.75	26.6	2.46	38	3.3	52	4.6	72	5.9	95	7.8
Col+523 $_{C24}$ A	7.0	0.63	9.4	0.85	13.4	1.19	18.6	1.63	26.0	2.29	36	3.1	50	4.2	69	5.4	91	7.0
Col+523 $_{C24}$ B	8.2	0.62	11.0	0.84	15.5	1.17	21.4	1.60	28.6	2.25	40	3.0	55	4.1	76	5.2	99	6.8
Col+523 $_{C24}$ C	7.5	0.62	9.7	0.83	14.2	1.17	19.7	1.59	26.4	2.24	37	3.0	51	4.0	70	5.2	91	6.6
Col+523 $_{C24}$ D	7.5	0.62	10.1	0.83	14.3	1.17	19.8	1.59	27.4	2.24	39	3.0	53	4.1	73	5.2	94	6.7
Col+523 $_{C24}$ E	7.1	0.69	9.8	0.92	13.6	1.31	18.7	1.80	25.9	2.51	37	3.4	51	4.7	69	6.1	91	8.0
Col+523 $_{C24}$ F	6.8	0.76	9.2	1.02	13.0	1.47	17.5	2.02	26.1	2.81	38	3.9	53	5.4	73	7.1	98	9.5

Mean leaf ares was estimated using linear mixed model.

LA: Leaf Area in mm2, SE: Standard error, ns: not significant

Appendix 9: Mean leaf area of cosmid transgenic genotypes in T3 homozygous generation

Genotype	9 DAS			10 DAS			11 DAS			12 DAS			13 DAS			14 DAS			15 DAS			16 DAS			17 DAS		
	LA	SE	Sig.	LA	SE	Sig.	LA	SE	Sig.	LA	SE	Sig.	LA	SE	Sig.	LA	SE	Sig.	LA	SE	Sig.	LA	SE	Sig.	LA	SE	Sig.
C24+EV	6.8	0.55	ns	9.0	0.71	ns	12.7	0.98	ns	17.8	1.37	ns	24.3	1.86	ns	34	3.1	ns	45	3.1	ns	58	3.7	ns	74	4.4	ns
C24	6.6	0.56	ns	8.5	0.73	ns	12.1	1.01	ns	16.8	1.41	ns	22.8	1.91	ns	31	3.2	ns	42	3.2	ns	54	3.9	ns	68	4.6	ns
C24 X Col-0	10.4	0.57	***	14.0	0.74	***	19.8	1.03	***	27.8	1.43	***	40.4	1.94	***	59	3.2	***	84	3.3	***	116	4.0	***	155	4.7	***
C24+125 $_{Col}$	7.0	0.63	ns	9.2	0.85	ns	12.9	1.19	ns	18.2	1.70	ns	24.5	2.37	ns	33	*3.8*	ns	44	4.5	ns	56	5.9	ns	70	7.7	ns
C24+013 $_{Col}$	7.0	0.63	ns	9.1	0.85	ns	12.6	1.19	ns	18.0	1.70	ns	23.9	2.37	ns	33	3.8	ns	44	4.5	ns	56	5.9	ns	71	7.7	ns
C24+170 $_{Col}$	6.7	0.64	ns	8.6	0.85	ns	11.8	1.20	ns	16.3	1.70	ns	21.7	2.37	ns	30	3.8	ns	39	4.5	ns	51	5.9	ns	63	7.7	ns
C24+395 $_{C24}$	6.8	0.63	ns	8.9	0.84	ns	12.5	1.19	ns	17.0	1.70	ns	23.6	2.36	ns	33	3.8	ns	44	4.5	ns	58	5.9	ns	74	7.7	ns
C24+401 $_{C24}$	7.4	0.63	ns	9.6	0.84	ns	13.7	1.19	ns	19.1	1.70	ns	26.5	2.36	ns	37	3.8	ns	49	4.5	ns	65	5.9	ns	83	7.7	ns
C24+411 $_{C24}$	7.3	0.63	ns	9.6	0.84	ns	13.4	1.19	ns	18.5	1.70	ns	25.2	2.36	ns	35	*3.8*	ns	46	4.5	ns	60	5.9	ns	76	7.7	ns
C24+507 $_{C24}$	6.6	0.63	ns	8.7	0.84	ns	12.1	1.19	ns	16.8	1.70	ns	23.3	2.36	ns	32	3.8	ns	44	4.5	ns	57	5.9	ns	73	7.7	ns
C24+523 $_{C24}$	7.2	0.63	ns	9.5	0.84	ns	13.4	1.19	ns	18.6	1.70	ns	26.0	2.36	ns	36	3.8	ns	48	4.5	ns	62	5.9	ns	80	7.7	ns
Col+EV	6.4	0.55	ns	8.6	0.72	ns	12.0	0.98	ns	16.7	1.37	ns	24.0	1.87	ns	34	*3.1*	ns	48	3.1	ns	67	3.7	ns	89	4.4	ns
Col-0	6.3	0.56	ns	8.4	0.73	ns	12.0	1.02	ns	16.5	1.41	ns	23.6	1.91	ns	34	3.2	ns	47	3.2	ns	64	3.9	ns	85	4.6	ns
Col-0 X C24	7.8	0.56	***	10.5	0.73	**	15.0	1.02	**	21.0	1.41	**	30.4	1.91	**	44	3.2	**	63	3.2	**	86	3.9	**	116	4.6	**
Col+125 $_{Col}$	5.7	0.64	ns	7.6	0.85	ns	10.5	1.20	ns	14.8	1.70	ns	21.0	2.37	ns	30	3.8	ns	43	4.5	ns	60	5.9	ns	80	7.7	ns
Col+013 $_{Col}$	5.7	0.64	ns	7.6	0.85	ns	10.7	1.19	ns	14.7	1.70	ns	20.2	2.37	ns	29	3.8	ns	41	4.5	ns	57	5.9	ns	77	7.7	ns
Col+170 $_{Col}$	6.1	0.63	ns	8.1	0.85	ns	11.3	1.19	ns	15.5	1.70	ns	21.6	2.37	ns	31	3.8	ns	43	4.5	ns	60	5.9	ns	81	7.7	ns
Col+395 $_{C24}$	6.7	0.63	ns	9.1	0.84	ns	12.9	1.19	ns	17.8	1.70	ns	25.3	2.36	ns	36	*3.8*	ns	50	4.5	ns	69	5.9	ns	91	7.7	ns
Col+401 $_{C24}$	6.7	0.63	ns	8.9	0.84	ns	12.7	1.19	ns	17.5	1.70	ns	24.9	2.36	ns	35	3.8	ns	49	4.5	ns	67	5.9	ns	89	7.7	ns
Col+411 $_{C24}$	7.2	0.63	ns	9.7	0.84	ns	13.7	1.19	ns	19.1	1.70	ns	27.1	2.36	ns	39	3.8	ns	54	4.5	ns	74	5.9	ns	98	7.7	ns
Col+507 $_{C24}$	6.8	0.63	ns	9.1	0.84	ns	13.0	1.19	ns	18.0	1.70	ns	25.2	2.36	ns	36	3.8	ns	50	4.5	ns	68	5.9	ns	90	7.7	ns
Col+523 $_{C24}$	7.3	0.63	ns	9.7	0.84	ns	13.7	1.19	ns	19.0	1.70	ns	26.3	2.36	ns	37	3.8	ns	51	4.5	ns	70	5.9	ns	92	7.7	ns

Mean leaf ares was estimated using linear mixed model.

Each mean value of genotype was compared to the mean value of transgenic control genotype in the same genomic background.

Significance tested by Fisher's Least Significant Difference (LSD): *,$P < 0.5$; **,$P < 0.01$; ***,$P < 0.001$.

LA: Leaf Area in mm^2, SE: Standard error, ns: not significant

Appendix 10: Comparison of mean leaf area between transgenic lines and transgenic control genotype

Genotype	9 DAS			10 DAS			11 DAS			12 DAS			13 DAS			14 DAS			15 DAS			16 DAS			17 DAS		
	LA	SE	Sig.	LA	SE	Sig.	LA	SE	Sig.	LA	SE	Sig.	LA	SE	Sig.	LA	SE	Sig.	LA	SE	Sig.	LA	SE	Sig.	LA	SE	Sig.
C24+EV	6.8	0.53	ns	9.0	0.69	ns	12.8	0.92	ns	17.9	1.26	ns	24.5	1.73	ns	33.8	2.17	ns	45	2.8	ns	59	3.3	ns	74	3.8	ns
C24	6.4	0.55	ns	8.2	0.72	ns	11.3	0.97	ns	15.7	1.32	ns	21.1	1.80	*	28.9	2.29	*	39	3.0	ns	50	3.6	ns	62	4.2	ns
C24 X Col-0	11.0	0.63	***	15.2	0.82	***	22.1	1.17	***	31.2	1.55	***	45.7	2.08	***	66.6	2.72	***	94	3.6	***	128	4.5	***	171	5.5	***
C24+125 $_{Col}$ A	7.6	0.62	ns	9.8	0.81	ns	13.8	1.12	ns	19.3	1.55	ns	25.6	2.13	ns	34.6	2.84	ns	45	3.9	ns	58	5.0	ns	71	6.4	ns
C24+125 $_{Col}$ B	5.6	0.62	*	7.3	0.82	*	10.2	1.13	**	14.5	1.56	*	19.6	2.15	*	26.6	2.86	*	36	3.9	*	47	5.0	*	59	6.5	ns
C24+125 $_{Col}$ C	6.3	0.62	ns	8.0	0.82	ns	11.0	1.13	ns	15.2	1.56	ns	19.8	2.14	*	26.5	2.86	*	34	3.9	**	45	5.0	*	57	6.5	*
C24+125 $_{Col}$ D	7.6	0.62	ns	10.0	0.81	ns	14.0	1.13	ns	20.1	1.55	ns	26.9	2.13	ns	36.6	2.84	ns	47	3.9	ns	60	5.0	ns	77	6.4	ns
C24+125 $_{Col}$ E	7.8	0.62	*	10.2	0.81	ns	14.4	1.12	ns	20.3	1.55	ns	27.7	2.13	ns	37.8	2.84	ns	50	3.9	ns	63	5.0	ns	80	6.4	ns
C24+125 $_{Col}$ F	7.1	0.62	ns	9.3	0.81	ns	13.1	1.13	ns	18.7	1.55	ns	25.2	2.13	ns	34.7	2.84	ns	46	3.9	ns	59	5.0	ns	72	6.4	ns
C24+013 $_{Col}$ A	7.4	0.62	ns	9.7	0.81	ns	13.8	1.12	ns	19.3	1.55	ns	25.5	2.13	ns	35.5	2.85	ns	47	3.9	ns	61	5.0	ns	77	6.4	ns
C24+013 $_{Col}$ B	7.7	0.62	ns	10.1	0.81	ns	13.9	1.12	ns	19.6	1.55	ns	25.6	2.13	ns	34.9	2.84	ns	46	3.9	ns	59	5.0	ns	76	6.4	ns
C24+013 $_{Col}$ C	6.3	0.62	ns	8.1	0.82	ns	10.5	1.13	*	15.3	1.56	ns	20.3	2.14	*	27.6	2.86	*	37	3.9	ns	49	5.0	ns	63	6.5	ns
C24+013 $_{Col}$ D	6.3	0.62	ns	8.0	0.82	ns	11.1	1.13	ns	15.8	1.56	ns	21.3	2.14	ns	29.0	2.86	ns	39	3.9	ns	51	5.0	ns	64	6.5	ns
C24+013 $_{Col}$ F	6.9	0.62	ns	8.8	0.82	ns	12.1	1.14	ns	17.1	1.56	ns	22.5	2.14	ns	30.6	2.86	ns	40	3.9	ns	51	5.0	ns	64	6.5	ns
C24+013 $_{Col}$ E	7.3	0.62	ns	9.3	0.82	ns	13.0	1.14	ns	18.4	1.56	ns	24.4	2.14	ns	33.3	2.86	ns	44	3.9	ns	57	5.0	ns	72	6.5	ns
C24+170 $_{Col}$ A	7.0	0.62	ns	9.1	0.81	ns	12.5	1.12	ns	17.6	1.55	ns	23.3	2.13	ns	32.1	2.84	ns	42	3.9	ns	53	5.0	ns	65	6.4	ns
C24+170 $_{Col}$ B	6.2	0.64	ns	7.8	0.84	ns	10.5	1.17	*	14.4	1.60	*	19.0	2.19	**	26.0	2.93	**	35	4.0	*	47	5.2	*	58	6.7	ns
C24+170 $_{Col}$ C	7.9	0.62	*	10.1	0.81	ns	13.9	1.13	ns	19.3	1.55	ns	25.9	2.13	ns	35.7	2.84	ns	48	3.9	ns	61	5.0	ns	77	6.4	ns
C24+170 $_{Col}$ D	6.3	0.62	ns	8.1	0.81	ns	10.6	1.13	*	14.9	1.55	*	19.4	2.13	**	25.8	2.85	**	34	3.9	**	43	5.0	**	53	6.4	**
C24+170 $_{Col}$ E	5.6	0.63	*	7.0	0.83	**	9.4	1.16	*	13.2	1.59	*	17.2	2.17	***	22.8	2.91	***	31	3.9	***	41	5.1	**	51	6.6	**
C24+170 $_{Col}$ F	6.3	0.65	ns	7.8	0.85	ns	10.9	1.21	ns	15.2	1.63	ns	20.9	2.23	ns	29.0	2.99	ns	40	4.1	ns	52	5.3	ns	64	6.8	ns
C24+395 $_{C24}$ A	6.2	0.63	ns	8.1	0.83	ns	11.3	1.14	ns	15.6	1.58	ns	22.0	2.17	ns	30.6	2.90	ns	41	3.9	ns	54	5.1	ns	69	6.6	ns
C24+395 $_{C24}$ B	6.6	0.62	ns	8.0	0.81	ns	10.9	1.12	ns	14.4	1.55	*	20.0	2.13	*	27.7	2.84	*	36	3.8	*	47	5.0	ns	60	6.4	ns
C24+395 $_{C24}$ C	7.2	0.62	ns	9.6	0.81	ns	13.7	1.12	ns	18.9	1.55	ns	25.9	2.13	ns	35.8	2.84	ns	48	3.9	ns	62	5.0	ns	79	6.4	ns
C24+395 $_{C24}$ D	7.4	0.62	ns	9.9	0.81	ns	14.2	1.12	ns	19.5	1.55	ns	26.8	2.13	ns	37.5	2.85	ns	51	3.9	ns	67	5.0	ns	85	6.4	ns
C24+395 $_{C24}$ E	6.5	0.62	ns	8.6	0.82	ns	11.8	1.13	ns	16.3	1.57	ns	22.5	2.15	ns	31.5	2.87	ns	43	3.9	ns	56	5.0	ns	71	6.5	ns
C24+395 $_{C24}$ F	6.6	0.62	ns	8.7	0.81	ns	12.4	1.12	ns	17.1	1.55	ns	24.1	2.13	ns	33.8	2.84	ns	46	3.9	ns	62	5.0	ns	80	6.4	ns
C24+401 $_{C24}$ A	7.8	0.62	*	10.3	0.81	ns	14.8	1.12	*	20.8	1.55	*	28.1	2.13	ns	39.5	2.84	ns	53	3.9	ns	69	5.0	ns	90	6.4	ns
C24+401 $_{C24}$ B	7.0	0.61	ns	9.1	0.81	ns	12.9	1.11	ns	18.0	1.54	ns	25.1	2.12	ns	34.9	2.82	ns	48	3.8	ns	63	4.9	ns	83	6.4	ns
C24+401 $_{C24}$ C	7.7	0.62	ns	10.3	0.82	ns	14.7	1.13	ns	20.6	1.56	ns	27.6	2.14	ns	39.0	2.86	ns	52	3.9	ns	68	5.0	ns	85	6.5	ns
C24+401 $_{C24}$ D	7.3	0.62	ns	9.8	0.81	ns	13.9	1.11	ns	19.2	1.55	ns	27.1	2.12	ns	38.0	2.83	ns	50	3.8	ns	66	5.0	ns	84	6.4	ns
C24+401 $_{C24}$ E	7.0	0.62	ns	9.2	0.82	ns	12.8	1.12	ns	17.8	1.56	ns	25.1	2.14	ns	35.4	2.85	ns	47	3.9	ns	62	5.0	ns	79	6.4	ns
C24+401 $_{C24}$ F	7.5	0.62	ns	9.5	0.81	ns	13.2	1.12	ns	18.1	1.55	ns	25.3	2.13	ns	35.2	2.84	ns	47	3.8	ns	62	5.0	ns	79	6.4	ns
C24+411 $_{C24}$ A	7.7	0.62	ns	9.7	0.81	ns	13.4	1.11	ns	17.5	1.55	ns	23.1	2.12	ns	30.7	2.83	ns	40	3.8	ns	51	5.0	ns	64	6.4	ns
C24+411 $_{C24}$ B	7.7	0.63	ns	9.8	0.82	ns	13.6	1.14	ns	18.6	1.57	ns	24.4	2.16	ns	33.7	2.88	ns	45	3.9	ns	58	5.0	ns	72	6.5	ns
C24+411 $_{C24}$ C	7.3	0.61	ns	9.7	0.81	ns	13.6	1.11	ns	19.1	1.54	ns	26.7	2.12	ns	37.6	2.82	ns	50	3.8	ns	66	4.9	ns	84	6.4	ns
C24+411 $_{C24}$ D	7.2	0.62	ns	9.3	0.81	ns	13.0	1.12	ns	18.2	1.56	ns	24.3	2.14	ns	33.5	2.85	ns	45	3.9	ns	58	5.0	ns	74	6.4	ns
C24+411 $_{C24}$ E	7.0	0.62	ns	9.1	0.81	ns	12.8	1.12	ns	17.9	1.55	ns	24.4	2.13	ns	34.2	2.85	ns	46	3.9	ns	59	5.0	ns	76	6.4	ns
C24+411 $_{C24}$ F	6.2	0.62	ns	8.3	0.82	ns	11.4	1.13	ns	16.0	1.57	ns	22.6	2.15	ns	31.5	2.87	ns	43	3.9	ns	56	5.0	ns	71	6.5	ns
C24+507 $_{C24}$ A	7.0	0.62	ns	9.3	0.82	ns	13.3	1.13	ns	18.4	1.56	ns	25.7	2.14	ns	35.6	2.86	ns	48	3.9	ns	63	5.0	ns	80	6.5	ns
C24+507 $_{C24}$ B	6.5	0.62	ns	8.4	0.81	ns	11.1	1.12	ns	15.1	1.55	*	20.2	2.13	*	27.6	2.83	*	37	3.8	*	49	5.0	ns	62	6.4	ns
C24+507 $_{C24}$ C	6.7	0.62	ns	8.8	0.81	ns	12.2	1.12	ns	16.8	1.55	ns	23.3	2.13	ns	32.7	2.83	ns	44	3.8	ns	59	5.0	ns	77	6.4	ns
C24+507 $_{C24}$ D	6.6	0.62	ns	8.8	0.81	ns	12.3	1.12	ns	17.3	1.55	ns	23.7	2.13	ns	32.9	2.83	ns	45	3.8	ns	59	5.0	ns	74	6.4	ns
C24+507 $_{C24}$ E	6.0	0.61	ns	8.1	0.81	ns	11.3	1.11	ns	15.9	1.54	ns	22.4	2.12	ns	31.2	2.82	ns	43	3.8	ns	56	4.9	ns	70	6.4	ns
C24+507 $_{C24}$ F	6.7	0.62	ns	8.8	0.81	ns	12.3	1.12	ns	17.0	1.55	ns	24.2	2.13	ns	34.0	2.83	ns	46	3.8	ns	60	5.0	ns	76	6.4	ns
C24+523 $_{C24}$ A	6.7	0.62	ns	8.7	0.81	ns	12.1	1.12	ns	16.8	1.56	ns	23.8	2.14	ns	32.7	2.85	ns	44	3.9	ns	56	5.0	ns	72	6.4	ns
C24+523 $_{C24}$ B	6.8	0.62	ns	9.0	0.81	ns	12.7	1.12	ns	17.5	1.56	ns	24.7	2.13	ns	34.5	2.85	ns	46	3.9	ns	61	5.0	ns	77	6.4	ns
C24+523 $_{C24}$ C	6.9	0.62	ns	9.1	0.82	ns	12.8	1.13	ns	17.7	1.56	ns	24.5	2.14	ns	33.9	2.86	ns	46	3.9	ns	60	5.0	ns	77	6.5	ns
C24+523 $_{C24}$ D	7.5	0.62	ns	9.9	0.81	ns	14.1	1.11	ns	19.5	1.55	ns	27.0	2.12	ns	37.6	2.83	ns	50	3.8	ns	65	5.0	ns	83	6.4	ns
C24+523 $_{C24}$ E	7.5	0.62	ns	9.8	0.81	ns	14.0	1.11	ns	19.4	1.55	ns	26.8	2.12	ns	36.6	2.83	ns	48	3.8	ns	62	5.0	ns	79	6.4	ns
C24+523 $_{C24}$ F	7.2	0.62	ns	9.5	0.81	ns	13.4	1.12	ns	18.5	1.55	ns	25.4	2.13	ns	35.2	2.83	ns	47	3.8	ns	62	5.0	ns	79	6.4	ns
Col+EV	6.6	0.54	ns	8.9	0.70	ns	12.8	0.94	ns	17.8	1.29	ns	25.6	1.76	ns	36.6	2.21	ns	51	2.9	ns	71	3.4	ns	93	4.0	ns
Col-0	6.1	0.55	ns	8.1	0.71	ns	11.4	0.97	ns	15.6	1.32	ns	22.3	1.79	ns	31.8	2.27	ns	44	3.0	ns	61	3.6	ns	81	4.2	ns
Col-0 X C24	8.0	0.56	***	10.9	0.72	***	15.8	0.99	***	22.2	1.33	***	32.2	1.81	***	47.0	2.31	***	66	3.0	***	90	3.6	***	121	4.3	***
Col+125 $_{Col}$ A	5.6	0.63	*	7.3	0.82	*	10.0	1.14	**	14.2	1.57	*	20.1	2.15	**	29.2	2.88	*	41	3.9	*	58	5.1	*	77	6.5	ns
Col+125 $_{Col}$ B	6.0	0.62	ns	8.1	0.82	ns	11.2	1.15	ns	15.7	1.57	ns	22.3	2.15	ns	32.3	2.88	ns	46	3.9	ns	64	5.0	ns	86	6.5	ns
Col+125 $_{Col}$ C	5.1	0.64	**	6.7	0.85	**	9.3	1.19	**	13.0	1.62	**	18.1	2.22	***	26.3	2.97	**	38	4.0	**	54	5.2	**	74	6.7	*
Col+125 $_{Col}$ D	6.2	0.62	ns	8.3	0.81	ns	11.5	1.13	ns	16.0	1.55	ns	22.7	2.13	ns	32.6	2.84	ns	46	3.9	ns	64	5.0	ns	85	6.4	ns
Col+125 $_{Col}$ E	5.1	0.64	**	6.7	0.83	**	9.4	1.16	**	13.2	1.59	**	18.6	2.18	***	26.1	2.91	***	37	3.9	***	51	5.1	**	68	6.6	**
Col+125 $_{Col}$ F	5.1	0.66	**	6.6	0.87	**	8.6	1.21	***	12.3	1.66	***	17.4	2.26	***	25.2	3.04	***	36	4.1	***	51	5.3	**	70	6.9	**
Col+013 $_{Col}$ A	6.3	0.62	ns	8.4	0.82	ns	11.4	1.14	ns	15.6	1.56	ns	21.7	2.14	ns	31.2	2.86	ns	44	3.9	ns	62	5.0	ns	83	6.5	ns
Col+013 $_{Col}$ B	5.6	0.62	*	7.4	0.81	*	10.6	1.13	*	14.6	1.55	*	20.2	2.13	**	29.3	2.84	*	42	3.9	*	58	5.0	*	78	6.4	ns
Col+013 $_{Col}$ C	6.2	0.62	ns	8.2	0.82	ns	11.5	1.14	ns	15.9	1.57	ns	22.0	2.15	ns	31.7	2.87	ns	45	3.9	ns	63	5.0	ns	85	6.5	ns
Col+013 $_{Col}$ D	5.4	0.62	*	7.2	0.82	*	9.9	1.14	**	13.9	1.56	**	19.2	2.15	**	27.5	2.86	**	39	3.9	**	54	5.0	**	74	6.5	*
Col+013 $_{Col}$ E	4.9	0.63	**	6.4	0.82	***	9.0	1.14	***	12.4	1.57	***	17.1	2.16	***	24.9	2.88	***	36	3.9	***	51	5.1	**	69	6.5	**
Col+013 $_{Col}$ F	5.2	0.63	**	6.6	0.82	**	8.8	1.15	***	11.3	1.57	***	13.9	2.16	***	19.8	2.88	***	28	3.9	***	38	5.1	**	52	6.5	***
Col+170 $_{Col}$ A	6.0	0.62	ns	8.2	0.81	ns	11.5	1.13	ns	16.1	1.55	ns	22.3	2.13	ns	32.1	2.84	ns	45	3.9	ns	63	5.0	ns	86	6.4	ns
Col+170 $_{Col}$ B	5.7	0.62	ns	7.6	0.81	ns	10.6	1.13	*	14.2	1.56	*	19.4	2.14	**	27.5	2.85	**	39	3.9	**	54	5.0	**	73	6.4	*
Col+170 $_{Col}$ C	5.2	0.62	**	6.8	0.81	**	9.1	1.13	***	12.7	1.55	***	17.3	2.13	***	24.5	2.85	***	35	3.9	***	49	5.0	***	65	6.4	***
Col+170 $_{Col}$ D	6.7	0.62	ns	8.9	0.81	ns	12.2	1.13	ns	16.5	1.55	ns	23.1	2.13	ns	32.9	2.85	ns	46	3.9	ns	63	5.0	ns	85	6.4	ns
Col+170 $_{Col}$ E	5.8	0.63	ns	7.6	0.82	ns	10.5	1.15	*	14.5	1.57	*	20.3	2.16	**	29.0	2.88	*	41	3.9	*	57	5.1	*	77	6.5	ns
Col+170 $_{Col}$ F	6.4	0.62	ns	8.5	0.81	ns	11.5	1.14	ns	16.0	1.56	ns	22.5	2.14	ns	32.0	2.86	ns	45	3.9	ns	63	5.0	ns	86	6.5	ns
Col+395 $_{C24}$ A	6.6	0.63	ns	8.9	0.83	ns	12.9	1.14	ns	17.9	1.58	ns	25.8	2.16	ns	36.7	2.89	ns	51	3.9	ns	70	5.1	ns	92	6.5	ns
Col+395 $_{C24}$ B	6.1	0.62	ns	8.2	0.82	ns	11.9	1.13	ns	16.3	1.57	ns	23.6	2.15	ns	33.9	2.87	ns	47	3.9	ns	64	5.0	ns	85	6.5	ns
Col+395 $_{C24}$ C	6.7	0.62	ns	9.3	0.82	ns	13.2	1.13	ns	18.5	1.57	ns	26.0	2.15	ns	37.2	2.87	ns	51	3.9	ns	70	5.0	ns	92	6.5	ns
Col+395 $_{C24}$ D	7.1	0.62	ns	9.4	0.81	ns	13.3	1.12	ns	18.5	1.55	ns	26.1	2.13	ns	37.3	2.84	ns	52	3.9	ns	71	5.0	ns	93	6.4	ns
Col+395 $_{C24}$ E	7.3	0.62	ns	9.9	0.81	ns	13.9	1.12	ns	19.2	1.55	ns	26.9	2.13	ns	38.1	2.84	ns	53	3.8	ns	72	5.0	ns	95	6.4	ns
Col+395 $_{C24}$ F	6.8	0.62	ns	9.3	0.82	ns	13.5	1.13	ns	18.8	1.56	ns	27.1	2.15	ns	38.6	2.86	ns	54	3.9	ns	74	5.0	ns	98	6.5	ns
Col+401 $_{C24}$ A	6.8	0.62	ns	9.2	0.81	ns	13.3	1.12	ns	18.5	1.56	ns	26.4	2.14	ns	37.7	2.85	ns	52	3.9	ns	71	5.0	ns	94	6.4	ns
Col+401 $_{C24}$ B	7.5	0.62	ns	9.9	0.81	ns	13.8	1.12	ns	18.6	1.55	ns	25.9	2.13	ns	36.5	2.84	ns	50	3.9	ns	69	5.0	ns	91	6.4	ns
Col+401 $_{C24}$ C	6.8	0.62	ns	8.9	0.82	ns	12.7	1.13	ns	17.7	1.56	ns	25.5	2.15	ns	36.1	2.86	ns	50	3.9	ns	69	5.0	ns	92	6.5	ns
Col+401 $_{C24}$ D	6.1	0.62	ns	8.1	0.81	ns	11.5	1.12	ns	16.1	1.56	ns	22.8	2.14	ns	33.1	2.85	ns	46	3.9	ns	64	5.0	ns	85	6.4	ns
Col+401 $_{C24}$ E	6.7	0.62	ns	8.8	0.81	ns	12.2	1.12	ns	16.7	1.56	ns	23.6	2.13	ns	33.3	2.85	ns	46	3.9	ns	63	5.0	ns	84	6.4	ns
Col+401 $_{C24}$ F	6.1	0.62	ns	8.1	0.81	ns	11.6	1.12	ns	16.1	1.55	ns	23.4	2.13	ns	33.4	2.85	ns	46	3.9	ns	64	5.0	ns	85	6.4	ns
Col+411 $_{C24}$ A	8.1	0.62	**	10.9	0.82	**	15.2	1.13	*	21.0	1.56	*	30.0	2.15	*	42.3	2.86	ns	59	3.9	ns	80	5.0	ns	107	6.5	ns
Col+411 $_{C24}$ B	7.9	0.62	*	10.5	0.82	*	14.9	1.12	*	20.5	1.56	ns	29.4	2.14	ns	42.0	2.85	ns	59	3.9	ns	80	5.0	ns	107	6.4	ns
Col+411 $_{C24}$ C	7.0	0.62	ns	9.3	0.82	ns	13.1	1.13	ns	18.1	1.57	ns	25.4	2.15	ns	36.2	2.87	ns	50	3.9	ns	69	5.0	ns	92	6.5	ns
Col+411 $_{C24}$ D	7.1	0.62	ns	9.5	0.82	ns	13.5	1.13	ns	19.0	1.56	ns	26.9	2.14	ns	39.0	2.86	ns	54	3.9	ns	75	5.0	ns	99	6.5	ns
Col+411 $_{C24}$ E	6.4	0.63	ns	8.6	0.82	ns	12.6	1.14	ns	17.5	1.58	ns	25.3	2.16	ns	36.5	2.88	ns	51	3.9	ns	69	5.1	ns	92	6.5	ns

Appendix 10. continued

Genotype	9 DAS			10 DAS			11 DAS			12 DAS			13 DAS			14 DAS			15 DAS			16 DAS			17 DAS		
	LA	SE	Sig.	LA	SE	Sig.	LA	SE	Sig.	LA	SE	Sig.	LA	SE	Sig.	LA	SE	Sig.	LA	SE	Sig.	LA	SE	Sig.	LA	SE	Sig.
Col+411 $_{C24}$ F	6.8	0.63	ns	9.0	0.82	ns	12.5	1.14	ns	17.2	1.57	ns	24.1	2.16	ns	35.0	2.88	ns	48	3.9	ns	66	5.1	ns	88	6.5	ns
Col+507 $_{C24}$ A	7.2	0.62	ns	9.6	0.81	ns	13.7	1.12	ns	18.6	1.55	ns	26.1	2.13	ns	36.8	2.84	ns	51	3.8	ns	70	5.0	ns	92	6.4	ns
Col+507 $_{C24}$ B	6.5	0.62	ns	8.8	0.81	ns	12.5	1.11	ns	17.1	1.55	ns	24.4	2.12	ns	34.5	2.83	ns	48	3.8	ns	66	5.0	ns	87	6.4	ns
Col+507 $_{C24}$ C	6.6	0.62	ns	9.1	0.81	ns	13.0	1.12	ns	18.2	1.56	ns	26.5	2.14	ns	37.8	2.85	ns	52	3.9	ns	69	5.0	ns	93	6.4	ns
Col+507 $_{C24}$ D	6.9	0.62	ns	9.2	0.81	ns	12.9	1.12	ns	18.0	1.55	ns	24.7	2.13	ns	35.3	2.85	ns	50	3.9	ns	68	5.0	ns	91	6.4	ns
Col+507 $_{C24}$ E	6.8	0.62	ns	9.1	0.82	ns	12.8	1.12	ns	18.0	1.56	ns	23.3	2.14	ns	33.0	2.85	ns	46	3.9	ns	63	5.0	ns	85	6.4	ns
Col+507 $_{C24}$ F	6.6	0.62	ns	8.9	0.81	ns	12.5	1.12	ns	17.4	1.55	ns	24.8	2.13	ns	35.1	2.84	ns	48	3.8	ns	67	5.0	ns	88	6.4	ns
Col+523 $_{C24}$ A	7.0	0.62	ns	9.6	0.81	ns	13.7	1.12	ns	18.9	1.55	ns	27.0	2.13	ns	38.2	2.84	ns	53	3.8	ns	72	5.0	ns	96	6.4	ns
Col+523 $_{C24}$ B	8.2	0.62	**	11.1	0.81	**	15.6	1.11	**	21.5	1.55	**	29.3	2.12	ns	41.3	2.83	ns	57	3.8	ns	78	5.0	ns	102	6.4	ns
Col+523 $_{C24}$ C	7.4	0.62	ns	9.7	0.81	ns	14.1	1.12	ns	19.6	1.56	ns	26.3	2.14	ns	37.2	2.85	ns	51	3.9	ns	70	5.0	ns	91	6.4	ns
Col+523 $_{C24}$ D	7.5	0.62	ns	10.2	0.81	ns	14.4	1.12	ns	20.0	1.55	ns	27.9	2.13	ns	39.6	2.84	ns	54	3.8	ns	74	5.0	ns	97	6.4	ns
Col+523 $_{C24}$ E	6.9	0.62	ns	9.3	0.82	ns	12.9	1.13	ns	17.9	1.57	ns	24.0	2.15	ns	34.0	2.86	ns	47	3.9	ns	63	5.0	ns	83	6.5	ns
Col+523 $_{C24}$ F	6.5	0.63	ns	8.5	0.82	ns	11.9	1.14	ns	16.2	1.57	ns	23.1	2.16	ns	33.4	2.88	ns	46	3.9	ns	64	5.0	ns	87	6.5	ns

Mean leaf ares was estimated using linear mixed model.

Each mean value of transgenic line was compared to the mean value of transgenic control genotype in the same genomic background.

Significance tested by Fisher's Least Significant Difference (LSD): *,P < 0.5; **,P < 0.01; ***,P < 0.001.

LA: Leaf Area in mm^2, SE: Standard error, ns: not significant

Appendix 11: Mean leaf area of cosmid transgenic lines in T2 segregating generation

Genotype	6 DAS					7 DAS					8 DAS					9 DAS					10 DAS					11 DAS					12 DAS				
	NT		T			NT		T			NT		T			NT		T			NT		T			NT		T			NT		T		
	Mean	SE	Mean	SE	Sig.	Mean	SE	Mean	SE	Sig.	Mean	SE	Mean	SE	Sig.	Mean	SE	Mean	SE	Sig.	Mean	SE	Mean	SE	Sig.	Mean	SE	Mean	SE	Sig.	Mean	SE	Mean	SE	Sig.
C24+062 $_{c24}$ A	0.23	0.045	0.29	0.041	ns	1.74	0.159	1.61	0.148	ns	5.37	0.291	5.35	0.274	ns	9.08	0.245	8.90	0.211	ns	12.09	0.303	11.98	0.259	ns	16.8	0.52	16.7	0.47	ns	23.5	0.99	23.5	0.94	ns
C24+062 $_{c24}$ B	0.29	0.065	0.31	0.039	ns	2.07	0.213	1.82	0.146	ns	4.84	0.367	4.74	0.270	ns	8.11	0.371	7.95	0.204	ns	10.61	0.465	10.60	0.249	ns	14.7	0.72	14.8	0.46	ns	20.9	1.21	20.6	0.93	ns
C24+062 $_{c24}$ C	0.31	0.086	0.32	0.046	ns	1.92	0.252	1.85	0.156	ns	5.06	0.434	4.25	0.288	ns	8.14	0.471	7.51	0.238	ns	11.09	0.592	10.16	0.293	ns	15.0	0.89	14.0	0.51	ns	21.1	1.41	19.6	0.98	ns
Col+062 $_{c24}$ A	0.42	0.045	0.39	0.046	ns	2.13	0.154	2.04	0.152	ns	3.95	0.284	3.92	0.281	ns	7.32	0.230	6.64	0.225	ns	9.61	0.284	8.78	0.276	ns	13.5	0.50	12.2	0.49	ns	18.6	0.96	16.9	0.96	ns
Col+062 $_{c24}$ B	0.57	0.052	0.56	0.042	ns	2.05	0.181	2.19	0.154	ns	3.58	0.320	3.74	0.283	ns	6.25	0.300	6.26	0.230	ns	8.23	0.374	8.26	0.284	ns	11.7	0.60	11.6	0.50	ns	16.1	1.08	16.0	0.96	ns
Col+062 $_{c24}$ C	0.50	0.044	0.42	0.040	ns	2.47	0.160	2.39	0.152	ns	4.48	0.290	4.47	0.280	ns	7.53	0.243	7.43	0.223	ns	10.06	0.300	9.85	0.274	ns	13.9	0.52	13.7	0.49	ns	19.2	0.98	18.7	0.95	ns
Col+395 $_{c24}$ A	0.22	0.081	0.23	0.047	ns	1.46	0.188	1.27	0.150	ns	3.50	0.331	2.89	0.274	ns	5.82	0.314	4.89	0.211	ns	7.78	0.391	6.58	0.259	ns	10.9	0.63	9.4	0.47	ns	15.1	1.10	13.0	0.94	ns
Col+395 $_{c24}$ B	0.40	0.054	0.32	0.040	ns	2.26	0.172	2.16	0.145	ns	4.77	0.309	4.58	0.269	ns	8.03	0.275	8.05	0.201	ns	10.72	0.342	10.79	0.246	ns	15.1	0.57	15.2	0.45	ns	20.6	1.04	20.8	0.92	ns
Col+395 $_{c24}$ C	0.36	0.065	0.22	0.045	ns	1.99	0.168	2.09	0.148	ns	4.78	0.306	4.86	0.275	ns	8.16	0.270	8.02	0.212	ns	10.72	0.336	10.59	0.261	ns	15.2	0.56	14.9	0.47	ns	20.8	1.03	20.3	0.94	ns
Col+395 $_{c24}$ D	0.34	0.063	0.23	0.048	ns	1.84	0.207	1.77	0.157	ns	4.35	0.348	4.01	0.286	ns	7.17	0.348	6.54	0.235	ns	9.42	0.436	8.68	0.290	ns	13.5	0.68	12.2	0.50	ns	18.7	1.16	16.8	0.97	ns
Col+395 $_{c24}$ E	0.31	0.059	0.28	0.047	ns	1.70	0.173	1.58	0.150	ns	4.20	0.312	3.90	0.276	ns	7.36	0.282	6.80	0.216	ns	9.83	0.351	9.09	0.265	ns	13.8	0.58	12.8	0.47	ns	19.1	1.05	17.7	0.94	ns
Col+395 $_{c24}$ F	0.31	0.057	0.39	0.044	ns	1.79	0.180	1.85	0.145	ns	4.03	0.322	4.09	0.270	ns	6.92	0.301	6.97	0.203	ns	9.15	0.374	9.34	0.248	ns	13.1	0.61	13.3	0.46	ns	18.0	1.08	18.1	0.92	ns
C24+395 $_{c24}$ A	0.26	0.058	0.20	0.044	ns	1.88	0.175	1.62	0.144	ns	4.34	0.311	3.90	0.269	ns	7.06	0.279	6.58	0.200	ns	9.52	0.347	8.84	0.245	ns	13.1	0.57	12.0	0.45	ns	18.5	1.04	16.9	0.92	ns
C24+395 $_{c24}$ B	0.36	0.054	0.24	0.042	ns	1.96	0.178	1.73	0.145	ns	4.73	0.318	4.37	0.269	ns	7.46	0.292	7.26	0.200	ns	9.63	0.363	9.48	0.245	ns	13.1	0.59	12.9	0.45	ns	18.0	1.07	17.8	0.92	ns
C24+395 $_{c24}$ C	0.29	0.056	0.25	0.039	ns	2.07	0.181	1.69	0.142	ns	4.98	0.324	4.53	0.267	ns	8.22	0.303	7.59	0.196	ns	10.87	0.377	10.07	0.239	ns	14.9	0.61	13.8	0.45	ns	21.1	1.08	19.5	0.92	ns
C24+395 $_{c24}$ D	0.23	0.069	0.19	0.067	ns	1.52	0.174	1.18	0.166	ns	3.93	0.308	3.57	0.295	ns	6.80	0.276	6.01	0.251	ns	9.15	0.343	8.17	0.310	ns	12.6	0.57	11.3	0.53	ns	17.6	1.04	15.6	1.00	ns
C24+395 $_{c24}$ E	0.14	0.063	0.21	0.054	ns	1.36	0.160	1.50	0.155	ns	3.90	0.293	3.94	0.283	ns	6.60	0.248	6.74	0.229	ns	8.80	0.307	9.01	0.282	ns	12.0	0.52	12.4	0.50	ns	17.0	0.99	17.5	0.96	ns
C24+395 $_{c24}$ F	0.26	0.059	0.25	0.044	ns	1.94	0.186	1.83	0.149	ns	4.43	0.329	4.14	0.271	ns	7.33	0.311	7.09	0.206	ns	9.77	0.388	9.53	0.252	ns	13.5	0.62	13.0	0.46	ns	18.9	1.10	18.1	0.93	ns
Col+401 $_{c24}$ C	0.25	0.058	0.29	0.045	ns	2.02	0.178	1.78	0.153	ns	5.44	0.316	5.00	0.280	ns	8.88	0.293	8.65	0.224	ns	11.95	0.365	11.80	0.276	ns	16.8	0.59	16.4	0.49	ns	22.7	1.06	22.1	0.96	ns
Col+401 $_{c24}$ D	0.40	0.071	0.24	0.048	ns	2.09	0.208	1.74	0.154	ns	4.57	0.358	4.20	0.282	ns	7.66	0.365	7.11	0.229	ns	10.32	0.457	9.63	0.282	ns	14.8	0.71	13.6	0.49	ns	20.5	1.19	18.9	0.96	ns
Col+401 $_{c24}$ E	0.24	0.067	0.27	0.055	ns	1.93	0.222	1.64	0.165	ns	5.35	0.370	3.94	0.289	*	8.63	0.392	6.82	0.242	*	11.43	0.492	9.21	0.299	*	16.3	0.75	13.1	0.51	*	22.6	1.24	18.0	0.98	*
Col+401 $_{c24}$ F	0.26	0.061	0.24	0.045	ns	1.87	0.186	1.66	0.147	ns	4.50	0.320	4.44	0.273	ns	7.37	0.295	7.17	0.209	ns	9.88	0.367	9.64	0.256	ns	14.0	0.60	13.6	0.47	ns	19.3	1.07	18.7	0.93	ns
Col+401 $_{c24}$ G	0.33	0.056	0.28	0.045	ns	2.00	0.173	1.54	0.155	ns	4.37	0.311	3.93	0.283	ns	7.47	0.281	6.74	0.230	ns	10.03	0.349	8.97	0.283	ns	13.9	0.58	12.5	0.50	ns	19.0	1.05	17.2	0.96	ns
Col+401 $_{c24}$ H	0.27	0.070	0.21	0.049	ns	1.32	0.202	1.51	0.154	ns	3.63	0.351	3.89	0.283	ns	6.18	0.353	6.28	0.230	ns	8.31	0.442	8.45	0.283	ns	11.7	0.69	11.9	0.50	ns	16.4	1.17	16.5	0.96	ns
C24+401 $_{c24}$ A	0.22	0.066	0.24	0.041	ns	2.03	0.207	2.02	0.144	ns	5.41	0.360	5.03	0.269	ns	8.96	0.366	8.66	0.200	ns	11.93	0.457	11.57	0.245	ns	16.5	0.71	15.9	0.45	ns	23.2	1.20	22.3	0.92	ns
C24+401 $_{c24}$ B	0.35	0.067	0.25	0.047	ns	1.68	0.194	1.78	0.153	ns	4.44	0.344	4.60	0.281	ns	7.46	0.335	7.90	0.226	ns	9.80	0.419	10.47	0.278	ns	13.5	0.66	14.5	0.49	ns	18.8	1.14	20.2	0.96	ns
C24+401 $_{c24}$ C	0.27	0.052	0.31	0.045	ns	2.21	0.165	2.10	0.151	ns	5.54	0.297	5.26	0.278	ns	9.58	0.256	9.09	0.219	ns	12.62	0.317	12.16	0.270	ns	17.4	0.54	16.8	0.48	ns	24.3	1.01	24.0	0.95	ns
C24+401 $_{c24}$ D	0.45	0.060	0.28	0.041	*	2.02	0.187	1.93	0.144	ns	4.62	0.330	4.50	0.268	ns	7.57	0.317	7.46	0.198	ns	10.01	0.395	9.84	0.242	ns	13.7	0.63	13.6	0.45	ns	19.2	1.11	19.1	0.92	ns
C24+401 $_{c24}$ E	0.28	0.065	0.24	0.038	ns	1.96	0.228	1.91	0.142	ns	4.46	0.381	4.42	0.266	ns	6.95	0.408	7.52	0.195	ns	9.03	0.512	9.92	0.238	ns	12.6	0.78	13.7	0.45	ns	17.5	1.27	19.3	0.92	ns
C24+401 $_{c24}$ F	0.21	0.069	0.27	0.042	ns	1.73	0.183	1.62	0.144	ns	4.50	0.326	4.21	0.268	ns	7.86	0.307	7.30	0.200	ns	10.35	0.383	9.72	0.244	ns	14.2	0.62	13.4	0.45	ns	20.3	1.09	18.9	0.92	ns
Col+411 $_{c24}$ A	0.43	0.053	0.47	0.044	ns	1.87	0.176	1.93	0.157	ns	3.65	0.317	3.63	0.289	ns	6.27	0.290	5.84	0.240	ns	8.24	0.361	7.74	0.296	ns	11.4	0.59	10.8	0.51	ns	15.9	1.06	14.8	0.98	ns
Col+411 $_{c24}$ B	0.23	0.052	0.22	0.046	ns	1.30	0.169	1.50	0.152	ns	3.50	0.303	3.72	0.279	ns	6.00	0.267	6.01	0.220	ns	7.97	0.331	8.01	0.271	ns	11.3	0.55	11.4	0.48	ns	15.6	1.02	15.7	0.95	ns
Col+411 $_{c24}$ C	0.30	0.048	0.23	0.053	ns	1.79	0.157	1.72	0.159	ns	4.14	0.286	3.92	0.291	ns	7.03	0.235	6.95	0.243	ns	9.35	0.290	9.25	0.301	ns	13.2	0.50	13.0	0.52	ns	18.3	0.97	18.0	0.99	ns
Col+411 $_{c24}$ E	0.37	0.043	0.32	0.046	ns	2.01	0.152	1.77	0.157	ns	4.25	0.281	3.91	0.288	ns	7.09	0.224	6.44	0.239	ns	9.50	0.276	8.61	0.294	ns	13.3	0.49	12.1	0.51	ns	18.2	0.96	16.6	0.98	ns
Col+411 $_{c24}$ G	0.35	0.056	0.31	0.046	ns	1.69	0.178	1.64	0.154	ns	3.68	0.320	3.52	0.282	ns	6.10	0.296	6.04	0.227	ns	8.19	0.368	8.11	0.280	ns	11.7	0.60	11.5	0.49	ns	16.4	1.07	16.0	0.96	ns
Col+411 $_{c24}$ H	0.43	0.045	0.40	0.044	ns	2.23	0.159	2.09	0.156	ns	3.86	0.290	3.81	0.286	ns	6.93	0.243	6.52	0.235	ns	9.06	0.300	8.70	0.290	ns	12.7	0.52	12.2	0.50	ns	17.4	0.98	16.7	0.97	ns
C24+411 $_{c24}$ A	0.33	0.054	0.29	0.040	ns	2.26	0.172	1.79	0.143	ns	5.71	0.311	4.80	0.267	*	9.13	0.280	8.12	0.197	ns	12.01	0.347	10.76	0.240	ns	16.7	0.57	14.8	0.45	*	23.4	1.04	20.5	0.92	*
C24+411 $_{c24}$ B	0.34	0.059	0.32	0.043	ns	1.81	0.171	1.78	0.147	ns	4.35	0.307	4.20	0.271	ns	7.34	0.273	6.88	0.205	ns	9.78	0.339	9.20	0.251	ns	13.5	0.56	12.7	0.46	ns	19.0	1.03	17.8	0.93	ns
C24+411 $_{c24}$ C	0.26	0.091	0.31	0.044	ns	1.26	0.192	1.59	0.146	ns	3.26	0.323	3.81	0.271	ns	5.67	0.304	6.47	0.205	ns	7.65	0.379	8.48	0.251	ns	10.3	0.61	11.5	0.46	ns	14.3	1.08	16.3	0.93	ns
C24+411 $_{c24}$ E	0.18	0.108	0.27	0.045	*	1.33	0.273	1.55	0.145	ns	3.75	0.460	4.08	0.268	ns	5.88	0.515	6.72	0.198	ns	7.84	0.648	9.02	0.242	ns	10.8	0.96	12.3	0.45	ns	14.8	1.50	17.2	0.92	ns
C24+411 $_{c24}$ F	0.32	0.051	0.29	0.041	ns	2.03	0.167	1.84	0.145	ns	4.90	0.303	4.45	0.269	ns	7.72	0.267	7.03	0.200	ns	10.00	0.331	9.19	0.244	ns	13.6	0.55	12.3	0.45	ns	19.1	1.02	16.9	0.92	ns
C24+411 $_{c24}$ G	0.30	0.055	0.34	0.044	ns	1.84	0.177	1.85	0.148	ns	4.61	0.313	4.36	0.275	ns	7.25	0.290	7.30	0.213	ns	9.63	0.361	9.64	0.262	ns	13.2	0.59	13.2	0.47	ns	18.6	1.06	18.7	0.94	ns
Col+EV B	0.21	0.059	0.17	0.056	ns	1.41	0.164	1.32	0.159	ns	3.99	0.296	3.83	0.289	ns	6.80	0.255	6.48	0.240	ns	9.06	0.316	8.68	0.296	ns	12.7	0.53	12.2	0.51	ns	17.5	1.00	16.8	0.98	ns
Col+EV C	0.32	0.049	0.32	0.044	ns	2.16	0.164	2.18	0.153	ns	3.92	0.298	4.02	0.282	ns	6.94	0.259	6.67	0.227	ns	9.06	0.320	8.81	0.280	ns	12.9	0.54	12.6	0.49	ns	17.6	1.01	17.4	0.96	ns
Col+EV D	0.32	0.054	0.30	0.047	ns	1.88	0.163	1.76	0.156	ns	4.03	0.297	3.63	0.285	ns	6.97	0.256	6.20	0.233	ns	9.17	0.317	8.25	0.288	ns	12.9	0.54	11.5	0.50	ns	17.8	1.00	16.0	0.97	ns
C24+EV A	0.17	0.058	0.28	0.048	ns	1.38	0.180	1.45	0.149	ns	4.01	0.318	4.18	0.277	ns	7.09	0.293	7.11	0.217	ns	9.39	0.364	9.57	0.266	ns	12.9	0.59	13.2	0.48	ns	18.3	1.07	18.7	0.94	ns
C24+EV B	0.30	0.055	0.30	0.047	ns	1.76	0.167	1.89	0.151	ns	4.87	0.302	5.04	0.277	ns	8.61	0.265	8.70	0.217	ns	11.48	0.329	11.73	0.267	ns	15.8	0.55	16.2	0.48	ns	21.9	1.02	22.6	0.95	ns
C24+EV E	0.25	0.053	0.27	0.050	ns	1.37	0.173	1.36	0.154	ns	3.44	0.297	3.54	0.279	ns	6.18	0.256	5.97	0.222	ns	8.41	0.318	8.15	0.272	ns	11.6	0.54	11.1	0.48	ns	15.9	1.01	15.2	0.95	ns

Each mean value of transgenic lines was compared to the mean value of their non-transgenic siblings.

Significance differences estimated if the difference between means of T and NT is greater than 2×SE and marked with an asterisk.

Mean: Leaf Area in mm^2, SE: Standard error, ns: not significant,T: transgenic plant, NT: non-transgenic siblings

Appendix 11. contnued

Genotype	13 DAS					14 DAS					15 DAS					16 DAS					17 DAS				
	NT		T			NT		T			NT		T			NT		T			NT		T		
	Mean	SE	Mean	SE	Sig.	Mean	SE	Mean	SE	Sig.	Mean	SE	Mean	SE	Sig.	Mean	SE	Mean	SE	Sig.	Mean	SE	Mean	SE	Sig.
C24+062 $_{C24}$ A	32.3	1.32	32.2	1.25	ns	41.1	1.51	41.1	1.39	ns	54.2	3.0	53.1	2.88	ns	67	3.4	65	3.2	ns	84	3.8	81	3.6	ns
C24+062 $_{C24}$ B	28.0	1.64	28.0	1.24	ns	36.9	1.98	36.0	1.37	ns	47.9	3.4	47.5	2.86	ns	61	4.0	60	3.2	ns	75	4.8	77	3.6	ns
C24+062 $_{C24}$ C	28.1	1.92	27.0	1.31	ns	35.9	2.39	34.9	1.48	ns	48.5	3.9	46.0	2.95	ns	61	4.6	60	3.3	ns	74	5.7	74	3.8	ns
Col+062 $_{C24}$ A	26.2	1.29	24.0	1.28	ns	36.1	1.45	32.6	1.43	ns	49.3	2.9	44.1	2.91	ns	67	3.3	60	3.3	ns	90	3.7	81	3.7	ns
Col+062 $_{C24}$ B	22.6	1.45	22.6	1.29	ns	30.8	1.70	30.9	1.45	ns	42.4	3.2	42.6	2.93	ns	58	3.6	58	3.3	ns	80	4.2	79	3.7	ns
Col+062 $_{C24}$ C	27.2	1.32	26.2	1.27	ns	36.4	1.50	35.3	1.43	ns	49.4	3.0	48.2	2.91	ns	66	3.4	65	3.3	ns	89	3.8	86	3.7	ns
Col+395 $_{C24}$ A	21.2	1.49	18.0	1.25	ns	29.3	1.76	24.7	1.39	ns	40.8	3.2	34.3	2.88	ns	56	3.7	48	3.2	*	77	4.3	66	3.6	*
Col+395 $_{C24}$ B	29.3	1.39	29.5	1.23	ns	40.2	1.61	40.0	1.36	ns	55.1	3.1	55.0	2.85	ns	75	3.5	75	3.2	ns	102	4.0	101	3.5	ns
Col+395 $_{C24}$ C	29.4	1.38	28.7	1.25	ns	39.9	1.59	38.9	1.39	ns	54.6	3.1	53.2	2.88	ns	73	3.5	72	3.2	ns	99	4.0	97	3.6	ns
Col+395 $_{C24}$ D	26.3	1.57	23.4	1.30	ns	35.7	1.89	32.1	1.47	ns	48.3	3.4	43.7	2.95	ns	66	3.9	60	3.3	ns	90	4.6	81	3.8	ns
Col+395 $_{C24}$ E	27.1	1.41	24.8	1.26	ns	36.7	1.64	33.8	1.40	ns	50.9	3.1	46.4	2.89	ns	70	3.5	64	3.2	ns	94	4.1	86	3.6	ns
Col+395 $_{C24}$ F	25.2	1.45	25.5	1.23	ns	34.2	1.71	34.7	1.36	ns	47.5	3.2	47.4	2.85	ns	64	3.6	65	3.2	ns	87	4.2	88	3.6	ns
C24+395 $_{C24}$ A	25.6	1.40	23.5	1.23	ns	33.2	1.63	30.7	1.36	ns	43.9	3.1	40.8	2.85	ns	55	3.5	53	3.2	ns	69	4.1	66	3.5	ns
C24+395 $_{C24}$ B	25.2	1.43	24.5	1.23	ns	32.3	1.67	31.9	1.36	ns	41.9	3.1	41.8	2.85	ns	53	3.6	53	3.2	ns	69	4.2	65	3.5	ns
C24+395 $_{C24}$ C	28.8	1.46	26.8	1.22	ns	37.1	1.71	35.0	1.34	ns	49.1	3.2	46.0	2.83	ns	61	3.6	58	3.2	ns	79	4.3	73	3.5	ns
C24+395 $_{C24}$ D	24.1	1.39	21.8	1.33	ns	31.4	1.61	28.1	1.52	ns	41.4	3.1	37.5	3.00	ns	53	3.5	49	3.4	ns	67	4.1	63	3.9	ns
C24+395 $_{C24}$ E	24.1	1.33	24.6	1.29	ns	31.6	1.52	32.5	1.45	ns	41.9	3.0	43.4	2.93	ns	55	3.4	56	3.3	ns	70	3.9	72	3.7	ns
C24+395 $_{C24}$ F	25.7	1.48	25.1	1.24	ns	33.3	1.74	32.6	1.37	ns	44.5	3.2	42.6	2.86	ns	58	3.7	55	3.2	ns	73	4.3	70	3.6	ns
Col+401 $_{C24}$ C	31.8	1.43	30.6	1.28	ns	42.7	1.68	41.2	1.43	ns	59.4	3.1	57.0	2.91	ns	82	3.6	77	3.3	ns	110	4.2	104	3.7	ns
Col+401 $_{C24}$ D	29.0	1.62	26.5	1.29	ns	39.9	1.95	36.0	1.45	ns	54.8	3.4	48.7	2.93	ns	75	4.0	66	3.3	ns	102	4.8	90	3.7	ns
Col+401 $_{C24}$ E	32.1	1.69	25.2	1.31	*	44.2	2.06	34.5	1.49	*	61.7	3.5	47.3	2.97	*	84	4.2	64	3.3	*	114	5.0	87	3.8	*
Col+401 $_{C24}$ F	27.6	1.44	26.2	1.25	ns	38.1	1.68	36.3	1.38	ns	53.2	3.1	50.0	2.87	ns	73	3.6	69	3.2	ns	99	4.2	93	3.6	ns
Col+401 $_{C24}$ G	26.6	1.41	24.0	1.29	ns	35.6	1.63	32.2	1.45	ns	48.0	3.1	42.9	2.93	ns	65	3.5	58	3.3	ns	88	4.1	78	3.7	ns
Col+401 $_{C24}$ H	23.2	1.59	23.3	1.29	ns	31.8	1.90	31.8	1.45	ns	43.7	3.4	43.8	2.93	ns	61	3.9	60	3.3	ns	83	4.7	82	3.7	ns
C24+401 $_{C24}$ A	31.5	1.62	30.4	1.23	ns	40.8	1.95	39.8	1.36	ns	54.7	3.4	53.1	2.85	ns	68	4.0	67	3.2	ns	88	4.8	86	3.5	ns
C24+401 $_{C24}$ B	25.5	1.54	27.5	1.28	ns	32.6	1.84	35.8	1.44	ns	43.4	3.3	47.5	2.92	ns	55	3.8	61	3.3	ns	73	4.5	78	3.7	ns
C24+401 $_{C24}$ C	32.8	1.35	32.3	1.27	ns	42.5	1.54	42.0	1.42	ns	55.5	3.0	55.5	2.90	ns	70	3.4	71	3.2	ns	90	3.9	90	3.7	ns
C24+401 $_{C24}$ D	26.1	1.49	26.5	1.22	ns	34.3	1.76	34.3	1.35	ns	44.6	3.2	45.7	2.84	ns	56	3.7	58	3.2	ns	69	4.4	73	3.5	ns
C24+401 $_{C24}$ E	23.8	1.73	26.4	1.22	ns	31.1	2.12	34.1	1.34	ns	41.6	3.6	44.8	2.83	ns	51	4.2	56	3.2	ns	65	5.2	71	3.5	ns
C24+401 $_{C24}$ F	27.6	1.47	26.4	1.23	ns	35.5	1.73	34.3	1.35	ns	47.5	3.2	45.2	2.84	ns	60	3.7	57	3.2	ns	74	4.3	72	3.5	ns
Col+411 $_{C24}$ A	22.5	1.43	20.9	1.31	ns	30.7	1.67	29.0	1.49	ns	41.8	3.1	39.8	2.96	ns	57	3.6	55	3.3	ns	78	4.2	75	3.8	ns
Col+411 $_{C24}$ B	22.0	1.37	22.4	1.27	ns	30.2	1.58	30.8	1.42	ns	41.2	3.0	42.5	2.90	ns	56	3.5	58	3.3	ns	77	4.0	79	3.7	ns
Col+411 $_{C24}$ C	25.7	1.30	25.3	1.32	ns	35.2	1.47	34.5	1.50	ns	48.2	2.9	47.0	2.97	ns	65	3.3	64	3.4	ns	89	3.8	87	3.8	ns
Col+411 $_{C24}$ E	25.5	1.28	23.3	1.31	ns	34.7	1.43	31.8	1.48	ns	47.4	2.9	43.6	2.96	ns	65	3.3	60	3.3	ns	87	3.7	81	3.8	ns
Col+411 $_{C24}$ G	23.0	1.44	22.6	1.28	ns	31.4	1.69	30.7	1.44	ns	44.1	3.2	42.4	2.92	ns	61	3.6	59	3.3	ns	83	4.2	80	3.7	ns
Col+411 $_{C24}$ H	24.4	1.32	23.5	1.30	ns	33.3	1.50	32.4	1.47	ns	45.9	3.0	44.7	2.95	ns	63	3.4	62	3.3	ns	84	3.8	83	3.8	ns
C24+411 $_{C24}$ A	31.8	1.40	27.6	1.22	*	40.8	1.63	35.0	1.34	*	53.6	3.1	45.3	2.84	*	67	3.5	56	3.2	*	85	4.1	70	3.5	*
C24+411 $_{C24}$ B	26.1	1.39	24.4	1.24	ns	33.9	1.60	31.5	1.37	ns	44.4	3.1	41.9	2.86	ns	56	3.5	53	3.2	ns	71	4.0	67	3.6	ns
C24+411 $_{C24}$ C	20.2	1.46	22.6	1.24	ns	26.3	1.72	29.4	1.37	ns	34.4	3.2	38.8	2.86	ns	46	3.7	50	3.2	ns	59	4.3	63	3.6	ns
C24+411 $_{C24}$ E	19.7	2.05	23.3	1.22	ns	25.6	2.57	30.2	1.35	ns	32.6	4.1	39.7	2.84	ns	43	4.9	52	3.2	ns	55	6.1	66	3.5	ns
C24+411 $_{C24}$ F	26.2	1.37	22.8	1.23	*	33.8	1.58	28.7	1.35	*	43.5	3.0	36.5	2.84	*	56	3.5	46	3.2	*	70	4.0	57	3.5	*
C24+411 $_{C24}$ G	25.7	1.43	25.7	1.25	ns	33.8	1.66	33.3	1.40	ns	45.7	3.1	45.0	2.88	ns	59	3.6	58	3.2	ns	75	4.2	73	3.6	ns
Col+EV B	24.3	1.34	23.1	1.31	ns	32.8	1.54	31.2	1.49	ns	44.6	3.0	42.7	2.96	ns	61	3.4	58	3.3	ns	83	3.9	79	3.8	ns
Col+EV C	25.0	1.35	24.4	1.28	ns	34.3	1.55	33.3	1.44	ns	47.6	3.0	46.1	2.92	ns	65	3.4	63	3.3	ns	88	3.9	85	3.7	ns
Col+EV D	24.8	1.35	22.2	1.30	ns	33.9	1.54	30.4	1.46	ns	47.1	3.0	42.3	2.94	ns	64	3.4	58	3.3	ns	87	3.9	78	3.8	ns
C24+EV A	24.8	1.43	25.5	1.26	ns	32.4	1.67	33.2	1.41	ns	42.4	3.1	44.0	2.89	ns	53	3.6	56	3.2	ns	68	4.2	71	3.6	ns
C24+EV B	29.5	1.37	30.5	1.26	ns	37.9	1.58	39.4	1.41	ns	49.0	3.0	51.7	2.89	ns	62	3.5	65	3.2	ns	78	4.0	82	3.6	ns
C24+EV E	21.5	1.35	21.2	1.27	ns	28.1	1.55	27.4	1.42	ns	37.0	3.0	35.9	2.91	ns	48	3.4	47	3.3	ns	62	3.9	60	3.7	ns

Appendix 12: Mean leaf area of transgenic lines harboring single candidate genes and their segregating siblings

Genotype	9 DAS					10 DAS					11 DAS					12 DAS					13 DAS					14 DAS					15 DAS					16 DAS					17 DAS				
	NT		T			NT		T			NT		T			NT		T			NT		T			NT		T			NT		T			NT		T			NT		T		
	Mean	SE	Mean	SE	Sig.	Mean	SE	Mean	SE	Sig.	Mean	SE	Mean	SE	Sig.	Mean	SE	Mean	SE	Sig.	Mean	SE	Mean	SE	Sig.	Mean	SE	Mean	SE	Sig.	Mean	SE	Mean	SE	Sig.	Mean	SE	Mean	SE	Sig.	Mean	SE	Mean	SE	Sig.
C24 X Col-0	11.8	0.68				16.1	0.83				22.6	1.12				31.5	1.61				45.9	2.25				67	3.0				94	3.8				128	4.4			ns	175	5.5			
Col-0 X C24	9.1	0.68				12.5	0.83				18.0	1.13				25.2	1.61				37.0	2.25				55	3.0				78	3.8				107	4.5				146	5.5			
Col-0	6.4	0.68				8.9	0.83				12.7	1.13				17.7	1.62				26.0	2.26				38	3.0				53	3.8				74	4.5				100	5.5			
C24	6.5	0.68				8.7	0.83				12.3	1.12				17.6	1.61				24.8	2.25				34	3.0				46	3.8				60	4.4				76	5.4			
Col+EV A	6.5	0.68	6.1	0.69	ns	9.0	0.83	8.3	0.84	ns	13.0	1.13	11.9	1.16	ns	18.0	1.62	16.6	1.65	ns	25.9	2.26	24.1	2.30	ns	37	3.0	34	3.1	ns	51	3.8	48	3.9	ns	71	4.5	66	4.6	ns	96	5.5	90	5.7	ns
Col+EV B	7.1	0.70	6.8	0.68	ns	9.6	0.85	9.4	0.83	ns	13.8	1.17	13.2	1.12	ns	18.8	1.67	18.2	1.61	ns	27.1	2.33	26.4	2.25	ns	39	3.1	38	3.0	ns	55	3.9	55	3.8	ns	77	4.7	76	4.4	ns	103	5.8	103	5.4	ns
Col+EV C	7.8	0.70	7.7	0.68	ns	10.8	0.85	10.5	0.83	ns	15.4	1.16	14.9	1.12	ns	21.5	1.66	20.5	1.61	ns	31.2	2.32	29.8	2.25	ns	45	3.1	43	3.0	ns	63	3.9	61	3.8	ns	86	4.6	83	4.4	ns	115	5.7	113	5.4	ns
C24+EV A	8.7	0.70	8.8	0.68	ns	11.6	0.86	11.6	0.82	ns	16.4	1.17	16.5	1.12	ns	23.1	1.67	23.1	1.61	ns	31.8	2.34	32.1	2.25	ns	44	3.1	44	3.0	ns	59	4.0	59	3.8	ns	76	4.7	77	4.4	ns	98	5.9	99	5.4	ns
C24+EV B	8.8	0.93	7.7	0.67	*	11.7	1.17	10.4	0.82	ns	16.6	1.63	15.0	1.12	ns	24.1	2.30	21.3	1.61	ns	33.9	3.23	29.8	2.24	ns	45	4.4	42	3.0	ns	65	5.8	56	3.8	ns	81	7.3	73	4.4	ns	104	9.6	94	5.4	ns
C24+EV C	8.0	0.71	8.0	0.68	ns	10.7	0.86	10.7	0.82	ns	15.2	1.19	15.5	1.12	ns	21.6	1.69	21.5	1.61	ns	30.1	2.36	29.9	2.24	ns	42	3.2	42	3.0	ns	57	4.0	56	3.8	ns	73	4.8	72	4.4	ns	96	5.9	94	5.4	ns
Col+238 $_{Col}$ *A*	5.7	0.78	5.9	0.72	ns	8.0	0.96	8.1	0.89	ns	11.3	1.32	11.5	1.22	ns	15.5	1.89	15.4	1.74	ns	22.2	2.65	22.5	2.47	ns	32	3.6	33	3.3	ns	45	4.7	46	4.2	ns	63	5.7	64	5.1	ns	84	7.3	86	6.4	ns
Col+238 $_{Col}$ *B*	6.6	0.79	6.5	0.72	ns	9.1	0.98	9.2	0.88	ns	12.9	1.35	13.2	1.21	ns	17.7	1.92	18.0	1.74	ns	26.0	2.70	26.2	2.43	ns	38	3.7	38	3.3	ns	52	4.8	52	4.2	ns	73	5.9	73	5.1	ns	99	7.5	98	6.4	ns
Col+238 $_{Col}$ *C*	6.3	0.77	5.8	0.73	ns	8.3	0.94	8.1	0.90	ns	12.0	1.29	11.6	1.22	ns	16.7	1.85	16.0	1.76	ns	24.2	2.60	23.1	2.47	ns	35	3.5	33	3.3	ns	50	4.5	47	4.3	ns	69	5.6	65	5.2	ns	93	7.1	89	6.5	ns
C24+238 $_{Col}$ *A*	7.9	0.76	7.6	0.71	ns	10.6	0.94	10.0	0.87	ns	15.2	1.29	14.3	1.19	ns	21.8	1.84	20.5	1.71	ns	30.8	2.58	28.6	2.40	ns	44	3.5	40	3.2	ns	60	4.5	54	4.1	ns	79	5.5	73	5.0	ns	102	7.0	92	6.2	ns
C24+238 $_{Col}$ *B*	7.4	0.74	7.1	0.71	ns	10.1	0.91	9.5	0.87	ns	14.5	1.25	13.8	1.19	ns	20.5	1.80	19.7	1.72	ns	29.5	2.52	27.9	2.40	ns	42	3.4	39	3.2	ns	56	4.4	53	4.1	ns	73	5.3	71	5.0	ns	91	6.8	89	6.2	ns
C24+238 $_{Col}$ *C*	6.0	0.74	5.8	0.71	ns	8.1	0.91	8.0	0.88	ns	11.7	1.25	11.4	1.20	ns	16.9	1.80	16.4	1.72	ns	24.9	2.52	23.6	2.41	ns	35	3.4	33	3.3	ns	47	4.4	45	4.2	ns	63	5.3	60	5.0	ns	80	6.8	77	6.3	ns
Col+238 $_{C24}$ *A*	6.5	0.77	6.1	0.72	ns	9.2	0.95	8.4	0.89	ns	13.0	1.30	12.0	1.21	ns	18.0	1.87	16.3	1.74	ns	24.7	2.62	22.8	2.44	ns	35	3.6	32	3.3	ns	49	4.6	44	4.2	ns	69	5.6	61	5.1	ns	92	7.2	82	6.4	ns
Col+238 $_{C24}$ *B*	5.1	0.76	4.7	0.73	ns	7.1	0.93	6.5	0.89	ns	10.3	1.28	9.5	1.22	ns	14.3	1.83	12.9	1.75	ns	20.6	2.57	18.5	2.45	ns	30	3.5	27	3.3	ns	42	4.5	38	4.2	ns	60	5.5	53	5.1	ns	82	7.0	73	6.5	ns
Col+238 $_{C24}$ *C*	4.2	0.76	4.0	0.73	ns	6.0	0.93	5.5	0.89	ns	8.6	1.27	7.9	1.22	ns	11.8	1.82	10.8	1.75	ns	17.1	2.56	15.3	2.45	ns	25	3.5	23	3.3	ns	35	4.5	31	4.2	ns	50	5.5	45	5.1	ns	69	6.9	62	6.4	ns
C24+238 $_{C24}$ *A*	5.6	0.84	5.4	0.72	ns	7.5	1.03	7.4	0.89	ns	10.7	1.41	10.2	1.21	ns	15.3	2.01	14.4	1.74	ns	22.0	2.82	20.6	2.44	ns	32	3.9	29	3.3	ns	44	5.0	40	4.2	ns	58	6.2	54	5.1	ns	76	8.0	69	6.4	ns
C24+238 $_{C24}$ *B*	6.0	0.91	5.8	0.71	ns	8.3	1.14	8.0	0.87	ns	11.8	1.58	11.2	1.19	ns	16.8	2.24	15.6	1.71	ns	23.6	3.15	22.8	2.40	ns	34	4.3	32	3.2	ns	46	5.7	44	4.1	ns	63	7.1	59	5.0	ns	77	9.3	74	6.2	ns
C24+238 $_{C24}$ *C*	6.8	0.77	6.7	0.73	ns	9.4	0.95	9.2	0.90	ns	13.3	1.30	13.0	1.23	ns	18.9	1.86	18.2	1.76	ns	26.8	2.62	26.2	2.47	ns	39	3.6	37	3.3	ns	54	4.6	51	4.3	ns	74	5.6	70	5.2	ns	94	7.2	91	6.5	ns
Col+240 $_{Col}$ *A*	6.5	0.72	6.3	0.72	ns	9.1	0.89	8.8	0.88	ns	13.1	1.21	12.6	1.20	ns	18.3	1.74	17.6	1.73	ns	26.5	2.44	25.4	2.43	ns	39	3.3	37	3.3	ns	53	4.2	51	4.2	ns	72	5.1	70	5.1	ns	99	6.4	95	6.3	ns
Col+240 $_{Col}$ *B*	6.7	0.78	6.6	0.71	ns	9.3	0.96	8.9	0.87	ns	13.2	1.32	12.8	1.19	ns	17.9	1.89	17.2	1.71	ns	26.3	2.66	25.2	2.40	ns	38	3.6	36	3.2	ns	53	4.7	50	4.1	ns	73	5.8	68	5.0	ns	99	7.3	92	6.2	ns
Col+240 $_{Col}$ *C*	7.2	0.76	6.9	0.71	ns	9.9	0.94	9.5	0.87	ns	14.3	1.29	13.6	1.19	ns	19.9	1.84	18.8	1.71	ns	28.8	2.60	27.3	2.42	ns	42	3.5	40	3.2	ns	59	4.5	56	4.1	ns	82	5.5	77	5.0	ns	112	7.0	105	6.2	ns
C24+240 $_{Col}$ *A*	7.6	0.76	6.8	0.71	ns	10.2	0.94	9.1	0.87	ns	14.5	1.28	13.2	1.19	ns	20.8	1.84	19.3	1.71	ns	29.8	2.58	27.5	2.40	ns	42	3.5	39	3.2	ns	57	4.5	53	4.1	ns	75	5.5	72	5.0	ns	98	7.0	92	6.2	ns
C24+240 $_{Col}$ *B*	7.8	0.81	6.1	0.71	**	10.3	0.99	8.2	0.87	**	14.8	1.37	11.4	1.19	**	20.5	1.96	15.7	1.72	**	28.5	2.75	21.4	2.41	***	40	3.7	29	3.3	***	54	4.9	38	4.1	***	69	6.0	50	5.0	***	88	7.7	63	6.3	***
C24+240 $_{Col}$ *C*	6.0	0.77	5.6	0.72	ns	8.2	0.94	7.6	0.88	ns	11.5	1.30	10.7	1.20	ns	16.5	1.86	15.7	1.73	ns	23.6	2.61	22.5	2.42	ns	33	3.5	32	3.3	ns	45	4.6	43	4.2	ns	60	5.6	58	5.0	ns	77	7.1	75	6.3	ns
Col+240 $_{C24}$ *A*	5.3	0.75	5.3	0.71	ns	7.5	0.92	7.4	0.88	ns	10.7	1.26	10.6	1.19	ns	14.7	1.81	14.7	1.72	ns	21.4	2.53	21.2	2.41	ns	31	3.4	31	3.3	ns	43	4.4	42	4.1	ns	61	5.4	59	5.0	ns	82	6.8	80	6.3	ns
Col+240 $_{C24}$ *B*	4.9	0.76	5.4	0.71	ns	6.8	0.93	7.3	0.87	ns	9.6	1.28	10.3	1.19	ns	13.1	1.83	14.1	1.71	ns	19.1	2.56	20.6	2.40	ns	29	3.5	30	3.2	ns	40	4.5	42	4.1	ns	55	5.5	58	5.0	ns	75	6.9	80	6.2	ns
Col+240 $_{C24}$ *C*	5.5	0.74	5.3	0.72	ns	7.6	0.91	7.6	0.88	ns	10.9	1.25	10.8	1.21	ns	14.9	1.79	14.8	1.73	ns	21.7	2.51	21.4	2.43	ns	31	3.4	31	3.3	ns	44	4.4	43	4.2	ns	61	5.3	60	5.1	ns	84	6.7	81	6.4	ns
C24+240 $_{C24}$ *A*	6.7	0.74	6.3	0.73	ns	8.8	0.91	8.3	0.89	ns	12.1	1.24	11.4	1.22	ns	17.1	1.78	16.2	1.75	ns	24.2	2.50	23.1	2.46	ns	34	3.4	33	3.3	ns	47	4.3	45	4.2	ns	65	5.3	62	5.2	ns	84	6.8	84	6.5	ns
C24+240 $_{C24}$ *B*	9.1	0.75	9.0	0.71	ns	11.8	0.92	12.1	0.87	ns	17.3	1.26	17.4	1.19	ns	24.9	1.81	24.5	1.71	ns	34.6	2.54	34.3	2.40	ns	49	3.4	48	3.2	ns	66	4.4	64	4.1	ns	82	5.4	82	5.0	ns	112	6.8	108	6.2	ns
C24+240 $_{C24}$ *C*	7.0	0.73	6.4	0.72	ns	9.5	0.90	9.0	0.88	ns	13.6	1.23	12.6	1.20	ns	19.4	1.77	17.9	1.73	ns	27.5	2.48	25.5	2.43	ns	38	3.4	36	3.3	ns	52	4.3	49	4.2	ns	69	5.2	66	5.1	ns	87	6.6	83	6.3	ns
Col+250 $_{Col}$ *A*	5.6	0.75	5.4	0.71	ns	7.7	0.93	7.4	0.87	ns	11.1	1.27	10.5	1.19	ns	14.9	1.82	14.5	1.72	ns	21.7	2.55	21.2	2.41	ns	31	3.5	31	3.3	ns	44	4.4	43	4.1	ns	60	5.4	59	5.0	ns	82	6.9	81	6.3	ns
Col+250 $_{Col}$ *B*	4.5	0.76	4.7	0.71	ns	6.3	0.93	6.5	0.87	ns	9.1	1.26	9.4	1.19	ns	12.3	1.81	12.9	1.71	ns	18.0	2.54	18.9	2.40	ns	27	3.4	28	3.2	ns	37	4.4	38	4.1	ns	52	5.4	53	5.0	ns	71	6.8	72	6.2	ns
Col+250 $_{Col}$ *C*	4.7	0.78	4.6	0.71	ns	6.5	0.96	6.3	0.87	ns	9.3	1.31	9.1	1.19	ns	13.0	1.88	12.7	1.71	ns	19.0	2.64	18.7	2.40	ns	28	3.6	27	3.2	ns	39	4.6	38	4.1	ns	55	5.7	54	5.0	ns	75	7.2	74	6.2	ns
C24+250 $_{Col}$ *A*	5.7	0.75	6.0	0.71	ns	8.0	0.92	8.5	0.87	ns	11.3	1.27	12.1	1.19	ns	15.9	1.82	17.0	1.72	ns	22.5	2.55	24.4	2.40	ns	31	3.5	35	3.2	ns	43	4.4	48	4.1	ns	58	5.4	66	5.0	ns	77	6.9	87	6.2	ns
C24+250 $_{Col}$ *B*	6.4	0.74	5.9	0.72	ns	8.7	0.90	7.8	0.88	ns	12.3	1.24	11.1	1.20	ns	17.3	1.78	15.7	1.73	ns	24.4	2.49	22.1	2.43	ns	35	3.4	31	3.3	ns	49	4.3	44	4.2	ns	66	5.3	59	5.1	ns	84	6.6	76	6.3	ns
C24+250 $_{Col}$ *C*	6.4	0.75	5.9	0.71	ns	8.8	0.93	7.9	0.87	ns	12.6	1.28	11.1	1.19	ns	18.1	1.83	16.1	1.71	ns	25.7	2.57	22.8	2.40	ns	36	3.5	31	3.2	ns	49	4.5	43	4.1	ns	63	5.5	57	5.0	ns	83	7.0	72	6.2	ns
Col+250 $_{C24}$ *A*	6.4	0.75	5.9	0.71	ns	8.7	0.92	8.1	0.87	ns	12.4	1.27	11.5	1.19	ns	17.2	1.82	15.9	1.71	ns	24.3	2.55	22.8	2.40	ns	35	3.5	33	3.2	ns	48	4.4	45	4.1	ns	66	5.4	63	5.0	ns	88	6.9	84	6.2	ns
Col+250 $_{C24}$ *B*	6.7	0.76	6.5	0.71	ns	9.1	0.93	8.9	0.87	ns	12.9	1.27	12.7	1.19	ns	17.6	1.82	17.4	1.72	ns	25.3	2.55	25.1	2.41	ns	37	3.5	36	3.3	ns	51	4.5	50	4.1	ns	70	5.4	70	5.0	ns	94	6.9	93	6.2	ns
Col+250 $_{C24}$ *C*	7.3	0.84	6.9	0.71	ns	10.3	1.05	9.6	0.87	ns	14.6	1.45	13.5	1.19	ns	20.0	2.06	18.7	1.71	ns	28.7	2.89	26.7	2.39	ns	42	4.0	39	3.2	ns	59	5.2	54	4.1	ns	81	6.4	75	5.0	ns	110	8.3	100	6.2	ns
C24+250 $_{C24}$ *A*	5.8	0.76	6.0	0.71	ns	8.1	0.93	8.1	0.87	ns	11.5	1.28	11.6	1.19	ns	16.8	1.83	16.6	1.71	ns	24.1	2.57	23.8	2.40	ns	34	3.5	33	3.2	ns	46	4.5	45	4.1	ns	61	5.5	59	5.0	ns	79	7.0	76	6.2	ns
C24+250 $_{C24}$ *B*	6.3	0.75	6.7	0.71	ns	8.6	0.92	8.9	0.87	ns	12.3	1.27	12.8	1.19	ns	17.4	1.82	18.2	1.71	ns	25.0	2.65	26.3	2.41	ns	36	3.5	37	3.2	ns	48	4.4	50	4.1	ns	64	5.4	66	5.0	ns	80	6.9	84	6.3	ns
C24+250 $_{C24}$ *C*	6.9	0.75	6.8	0.71	ns	9.4	0.92	9.3	0.87	ns	13.8	1.26	13.4	1.19	ns	19.9	1.81	19.0	1.71	ns	28.3	2.53	27.4	2.40	ns	40	3.4	38	3.2	ns	54	4.4	52	4.1	ns	71	5.4	70	5.0	ns	93	6.8	90	6.2	ns
Col+260 $_{Col}$ *A*	3.7	0.75	4.0	0.72	ns	5.3	0.92	5.7	0.88	ns	7.8	1.26	8.1	1.20	ns	10.6	1.81	11.1	1.72	ns	15.4	2.54	16.2	2.41	ns	23	3.4	24	3.3	ns	32	4.4	33	4.2	ns	45	5.4	47	5.0	ns	61	6.8	65	6.3	ns
Col+260 $_{Col}$ *B*	7.2	0.77	7.5	0.72	ns	10.5	0.95	10.4	0.89	ns	14.9	1.31	14.7	1.21	ns	20.7	1.87	20.4	1.74	ns	29.6	2.63	29.4	2.44	ns	43	3.6	43	3.3	ns	60	4.6	60	4.2	ns	83	5.7	84	5.1	ns	114	7.2	113	6.4	ns
Col+260 $_{Col}$ *C*	5.0	0.75	5.2	0.71	ns	7.0	0.93	7.1	0.87	ns	10.1	1.26	10.2	1.19	ns	13.8	1.81	13.9	1.71	ns	20.1	2.54	20.2	2.40	ns	30	3.5	30	3.2	ns	42	4.4	42	4.1	ns	59	5.4	59	5.0	ns	81	6.8	81	6.2	ns
C24+260 $_{Col}$ *A*	5.7	0.74	6.2	0.71	ns	7.7	0.92	8.4	0.87	ns	10.8	1.25	11.8	1.19	ns	15.2	1.80	16.8	1.72	ns	21.8	2.52	24.0	2.40	ns	31	3.4	34	3.2	ns	41	4.4	45	4.1	ns	56	5.4	59	5.0	ns	71	6.8	75	6.2	ns
C24+260 $_{Col}$ *B*	6.3	0.75	6.1	0.71	ns	8.6	0.92	8.3	0.87	ns	12.2	1.26	11.7	1.19	ns	17.2	1.81	16.6	1.71	ns	24.7	2.54	23.8	2.39	ns	35	3.4	34	3.2	ns	48	4.4	46	4.1	ns	64	5.4	62	5.0	ns	83	6.8	79	6.2	ns
C24+260 $_{Col}$ *C*	6.5	0.75	6.2	0.71	ns	9.0	0.93	8.4	0.87	ns	12.9	1.28	12.2	1.19	ns	18.6	1.83	17.4	1.72	ns	27.0	2.57	25.2	2.41	ns	38	3.5	35	3.3	ns	49	4.5	48	4.1	ns	65	5.5	64	5.0	ns	84	7.0	82	6.3	ns
Col+260 $_{C24}$ *A*	6.4	0.74	5.8	0.71	ns	8.9	0.91	7.9	0.87	ns	12.5	1.25	11.3	1.19	ns	17.3	1.80	15.6	1.72	ns	25.0	2.52	22.3	2.41	ns	36	3.4	33	3.3	ns	50	4.4	46	4.1	ns	69	5.3	64	5.0	ns	93	6.8	86	6.3	ns
Col+260 $_{C24}$ *B*	6.1	0.75	6.0	0.71	ns	8.7	0.92	8.4	0.87	ns	12.4	1.26	11.9	1.19	ns	16.8	1.81	16.0	1.71	ns	23.9	2.57	23.2	2.41	ns	35	3.5	33	3.2	ns	48	4.4	46	4.1	ns	66	5.4	64	5.0	ns	89	6.8	87	6.2	ns
Col+260 $_{C24}$ *C*	7.1	0.76	6.3	0.71	ns	9.9	0.93	8.7	0.88	ns	13.8	1.28	12.2	1.19	ns	19.3	1.83	16.4	1.72	*	27.9	2.57	23.6	2.41	ns	40	3.5	34	3.3	ns	55	4.5	46	4.1	*	77	5.5	64	5.0	**	103	7.0	86	6.3	*
C24+260 $_{C24}$ *A*	6.9	0.75	7.1	0.71	ns	9.3	0.92	9.5	0.87	ns	13.4	1.26	13.6	1.19	ns	18.9	1.81	19.4	1.71	ns	27.3	2.56	27.8	2.42	ns	39	3.4	39	3.2	ns	54	4.4	53	4.1	ns	74	5.4	73	5.0	ns	97	6.8	94	6.2	ns
C24+260 $_{C24}$ *B*	6.3	0.75	6.8	0.71	ns	8.6	0.92	9.3	0.88	ns	12.2	1.27	13.0	1.20	ns	17.4	1.81	18.3	1.72	ns	24.8	2.54	26.2	2.41	ns	35	3.5	37	3.3	ns	48	4.4	51	4.1	ns	64	5.4	68	5.0	ns	80	6.9	89	6.3	ns
C24+260 $_{C24}$ *C*	8.9	0.77	9.4	0.71	ns	12.4	0.95	13.1	0.87	ns	17.5	1.31	18.4	1.19	ns	24.5	1.87	25.7	1.71	ns	34.1	2.62	35.9	2.40	ns	48	3.6	50	3.2	ns	67	4.6	69	4.1	ns	88	5.6	91	5.0	ns	114	7.2	118	6.2	ns
Col+270 $_{Col}$ *A*	7.5	0.77	7.3	0.71	ns	10.3	0.95	10.0	0.87	ns	14.9	1.33	14.2	1.19	ns	20.0	1.86	19.5	1.71	ns	29.1	2.61	28.5	2.39	ns	42	3.5	41	3.2	ns	59	4.6	58	4.1	ns	81	5.6	80	5.0	ns	112	7.1	108	6.2	ns
Col+270 $_{Col}$ *B*	7.1	0.89	6.6	0.71	ns	9.7	1.11	9.2	0.87	ns	13.7	1.60	12.7	1.19	ns	18.8	2.18	17.8	1.70	ns	27.5	3.06	25.9	2.38	ns	40	4.2	37	3.2	ns	56	5.5	53	4.1	ns	78	6.9	73	4.9	ns	105	9.0	99	6.1	ns
Col+270 $_{Col}$ *C*	6.9	0.78	6.8	0.71	ns	9.6	0.96	9.5	0.87	ns	12.8	1.48	13.2	1.20	ns	18.6	1.89	18.2	1.71	ns	28.2	2.65	26.8	2.39	ns	40	3.6	38	3.2	ns	56	4.7	54	4.1	ns	77	5.7	75	5.0	ns	103	7.3	101	6.2	ns
C24+270 $_{Col}$ *A*	6.7	0.74	6.6	0.71	ns	8.9	0.91	8.8	0.88	ns	12.9	1.26	12.4	1.21	ns	18.5	1.79	17.9	1.72	ns	25.5	2.51	24.9	2.41	ns	36	3.4	35	3.3	ns	48	4.4	47	4.2	ns	62	5.3	61	5.0	ns	79	6.7	76	6.3	ns
C24+270 $_{Col}$ *B*	9.4	0.76	9.3	0.71	ns	12.5	0.94	12.4	0.88	ns	17.7	1.31	17.4	1.20	ns	24.4	1.84	24.5	1.72	ns	33.3	2.58	33.3	2.41	ns	46	3.5	45	3.3	ns	61	4.5	60	4.1	ns	77	5.5	76	5.0	ns	99	7.0	99	6.3	ns
C24+270 $_{Col}$ *C*	12.8	0.79	11.1	0.71	***	16.7	0.98	14.7	0.87	**	23.3	1.37	20.7	1.19	*	30.2	1.94	27.8	1.71	ns	40.9	2.72	37.4	2.39	ns	53	3.7	50	3.2	ns	68	4.8	65	4.1	ns	88	5.9	81	5.0	ns	113	7.6	105	6.2	ns
Col+270 $_{C24}$ *A*	6.0	0.76	5.2	0.72	ns	8.1	0.94	7.1	0.88	ns	11.2	1.29	10.1	1.21	ns	15.6	1.83	14.1	1.73	ns	22.5	2.57	20.4	2.43	ns	32	3.5	29	3.3	ns	45	4.5	42	4.2	ns	62	5.5	58	5.1	ns	84	6.9	79	6.4	ns

Appendix 12. continued

	9 DAS					10 DAS					11 DAS					12 DAS					13 DAS					14 DAS					15 DAS					16 DAS					17 DAS				
Genotype	NT		T			NT		T			NT		T			NT		T			NT		T			NT		T			NT		T			NT		T			NT		T		
	Mean	SE	Mean	SE	Sig.	Mean	SE	Mean	SE	Sig.	Mean	SE	Mean	SE	Sig.	Mean	SE	Mean	SE	Sig.	Mean	SE	Mean	SE	Sig.	Mean	SE	Mean	SE	Sig.	Mean	SE	Mean	SE	Sig.	Mean	SE	Mean	SE	Sig.	Mean	SE	Mean	SE	Sig.
Col+270$_{C24}$ B	4.4	0.78	4.8	0.72	ns	6.1	0.96	6.6	0.88	ns	9.1	1.34	9.6	1.21	ns	12.5	1.89	13.6	1.72	ns	18.2	2.66	19.4	2.42	ns	27	3.6	28	3.3	ns	39	4.7	40	4.2	ns	54	5.7	56	5.0	ns	75	7.3	77	6.3	ns
Col+270$_{C24}$ C	5.8	0.76	5.9	0.71	ns	8.1	0.94	8.2	0.87	ns	11.8	1.34	11.9	1.21	ns	16.4	1.84	16.2	1.72	ns	23.9	2.59	23.5	2.41	ns	34	3.5	34	3.3	ns	49	4.5	48	4.1	ns	68	5.5	67	5.0	ns	93	7.0	91	6.2	ns
C24+270$_{C24}$ A	9.1	0.79	9.1	0.71	ns	12.2	0.98	12.0	0.87	ns	17.5	1.36	17.5	1.20	ns	24.1	1.93	24.0	1.72	ns	33.1	2.71	32.8	2.40	ns	45	3.7	45	3.2	ns	60	4.8	60	4.1	ns	76	5.9	77	5.0	ns	97	7.5	99	6.2	ns
C24+270$_{C24}$ B	11.8	0.75	11.7	0.71	ns	15.0	0.93	15.3	0.88	ns	21.2	1.35	21.5	1.22	ns	28.7	1.82	28.7	1.72	ns	38.9	2.56	38.8	2.42	ns	54	3.5	53	3.3	ns	70	4.5	69	4.2	ns	89	5.4	88	5.0	ns	117	6.9	116	6.3	ns
C24+270$_{C24}$ C	6.2	0.78	6.3	0.73	ns	8.3	0.95	8.3	0.89	ns	11.7	1.31	11.9	1.23	ns	16.8	1.87	17.0	1.75	ns	23.6	2.63	23.9	2.45	ns	32	3.6	33	3.3	ns	44	4.6	45	4.2	ns	58	5.7	57	5.1	ns	75	7.2	75	6.5	ns
Col+280$_{Col}$ A	6.8	0.75	6.8	0.71	ns	9.4	0.93	9.4	0.87	ns	13.1	1.28	13.1	1.19	ns	17.9	1.83	17.9	1.72	ns	25.8	2.57	25.3	2.41	ns	37	3.5	36	3.3	ns	51	4.5	49	4.1	ns	70	5.5	68	5.0	ns	93	6.9	90	6.3	ns
Col+280$_{Col}$ B	5.1	0.75	4.7	0.72	ns	7.0	0.93	6.4	0.88	ns	10.0	1.27	9.2	1.20	ns	13.8	1.82	12.6	1.72	ns	19.9	2.55	18.2	2.42	ns	29	3.5	27	3.3	ns	42	4.5	38	4.2	ns	58	5.5	52	5.0	ns	79	6.9	71	6.3	ns
Col+280$_{Col}$ C	5.3	0.75	5.2	0.71	ns	7.4	0.93	7.3	0.87	ns	10.7	1.27	10.4	1.19	ns	14.8	1.82	14.3	1.71	ns	21.7	2.59	21.1	2.42	ns	31	3.5	30	3.2	ns	44	4.5	42	4.1	ns	61	5.4	59	5.0	ns	83	6.9	79	6.2	ns
C24+280$_{Col}$ A	7.2	0.74	6.8	0.71	ns	10.1	0.92	9.3	0.88	ns	14.2	1.26	13.2	1.20	ns	19.4	1.80	18.1	1.72	ns	27.8	2.53	26.0	2.42	ns	40	3.4	37	3.3	ns	55	4.4	51	4.2	ns	76	5.4	70	5.0	ns	102	6.8	94	6.3	ns
C24+280$_{Col}$ B	5.8	0.76	5.6	0.72	ns	7.8	0.93	7.7	0.88	ns	11.4	1.28	11.2	1.20	ns	15.7	1.83	15.6	1.72	ns	23.1	2.57	22.6	2.41	ns	33	3.5	33	3.3	ns	47	4.5	46	4.2	ns	66	5.5	63	5.0	ns	90	7.0	87	6.3	ns
C24+280$_{Col}$ C	6.5	0.76	6.4	0.71	ns	9.0	0.93	8.7	0.87	ns	12.9	1.28	12.3	1.19	ns	17.8	1.84	16.8	1.71	ns	25.6	2.58	24.1	2.40	ns	37	3.5	34	3.2	ns	51	4.5	48	4.1	ns	71	5.5	66	5.0	ns	96	7.0	89	6.2	ns
Col+280$_{C24}$ A	6.1	0.77	5.9	0.75	ns	8.5	0.95	8.4	0.92	ns	11.4	1.29	11.4	1.26	ns	15.1	1.85	15.2	1.80	ns	20.8	2.60	20.8	2.53	ns	29	3.5	28	3.4	ns	40	4.6	37	4.4	ns	54	5.6	49	5.4	ns	67	7.1	64	6.8	ns
Col+280$_{C24}$ B	5.1	0.78	5.3	0.72	ns	6.7	0.94	7.4	0.88	ns	9.2	1.29	10.1	1.21	ns	12.9	1.85	14.6	1.73	ns	18.7	2.59	20.5	2.43	ns	27	3.5	29	3.3	ns	37	4.5	40	4.2	ns	50	5.5	53	5.1	ns	63	7.0	66	6.4	ns
Col+280$_{C24}$ C	6.0	0.76	6.0	0.71	ns	8.3	0.93	8.3	0.87	ns	11.7	1.28	11.8	1.19	ns	16.4	1.83	16.8	1.71	ns	23.4	2.57	23.5	2.40	ns	33	3.5	33	3.2	ns	45	4.5	45	4.1	ns	59	5.5	61	5.0	ns	74	7.0	76	6.2	ns
C24+280$_{C24}$ A	9.4	0.83	8.7	0.71	ns	13.4	1.03	12.3	0.87	ns	19.0	1.42	17.4	1.19	ns	26.8	2.03	24.5	1.71	ns	38.7	2.85	35.4	2.40	ns	57	3.9	51	3.2	ns	83	5.1	75	4.1	ns	113	6.3	106	5.0	ns	153	8.1	146	6.2	ns
C24+280$_{C24}$ B	7.0	0.72	7.1	0.72	ns	9.5	0.89	9.6	0.89	ns	13.3	1.22	13.7	1.21	ns	19.0	1.75	19.5	1.74	ns	26.8	2.45	27.6	2.44	ns	38	3.3	40	3.3	ns	52	4.2	55	4.2	ns	70	5.1	73	5.1	ns	90	6.5	95	6.4	ns
C24+280$_{C24}$ C	7.7	0.78	7.9	0.71	ns	10.3	0.97	10.7	0.88	ns	14.5	1.33	15.3	1.20	ns	20.5	1.90	21.6	1.72	ns	29.0	2.67	30.1	2.41	ns	41	3.6	42	3.3	ns	54	4.7	56	4.2	ns	70	5.8	73	5.0	ns	89	7.4	93	6.3	ns
Col+290$_{Col}$ A	4.9	0.79	5.1	0.71	ns	7.0	0.97	7.2	0.87	ns	10.0	1.34	10.4	1.19	ns	13.6	1.91	14.2	1.71	ns	20.1	2.73	20.4	2.40	ns	29	3.7	30	3.2	ns	41	4.7	43	4.1	ns	57	5.8	60	5.0	ns	77	7.4	82	6.2	ns
Col+290$_{Col}$ B	5.1	0.76	5.3	0.71	ns	7.1	0.94	7.4	0.87	ns	10.2	1.29	10.4	1.19	ns	14.0	1.85	14.4	1.71	ns	20.3	2.59	21.0	2.39	ns	30	3.5	31	3.2	ns	42	4.5	43	4.1	ns	58	5.6	60	5.0	ns	80	7.1	82	6.2	ns
Col+290$_{Col}$ C	4.6	0.76	4.9	0.71	ns	6.2	0.93	6.8	0.87	ns	9.0	1.27	9.9	1.19	ns	12.1	1.82	13.5	1.71	ns	17.5	2.55	19.5	2.40	ns	25	3.5	28	3.2	ns	36	4.5	40	4.1	ns	51	5.4	56	5.0	ns	69	6.9	76	6.2	ns
C24+290$_{Col}$ A	5.8	0.74	5.6	0.71	ns	8.0	0.92	7.6	0.87	ns	11.3	1.25	10.6	1.19	ns	15.9	1.80	14.9	1.71	ns	22.7	2.52	21.4	2.40	ns	32	3.4	30	3.2	ns	44	4.4	42	4.1	ns	62	5.3	57	5.0	ns	82	6.8	75	6.2	ns
C24+290$_{Col}$ B	6.3	0.74	6.3	0.71	ns	8.6	0.91	8.5	0.87	ns	11.9	1.25	11.8	1.19	ns	16.8	1.79	16.9	1.72	ns	24.5	2.50	24.1	2.41	ns	35	3.4	34	3.3	ns	48	4.4	47	4.1	ns	65	5.3	64	5.0	ns	84	6.7	83	6.3	ns
C24+290$_{Col}$ C	5.7	0.79	5.6	0.71	ns	8.1	0.97	7.9	0.87	ns	11.4	1.34	11.2	1.19	ns	16.1	1.91	15.7	1.71	ns	23.3	2.68	22.7	2.39	ns	33	3.7	32	3.2	ns	45	4.7	44	4.1	ns	61	5.8	60	5.0	ns	79	7.4	76	6.2	ns
Col+290$_{C24}$ A	4.3	0.89	5.0	0.71	ns	6.3	1.11	7.1	0.87	ns	9.2	1.58	10.2	1.19	ns	13.2	2.18	14.6	1.70	ns	19.2	3.06	21.1	2.38	ns	28	4.2	30	3.2	ns	39	5.5	43	4.1	ns	55	6.9	59	4.9	ns	74	9.0	80	6.1	ns
Col+290$_{C24}$ B	5.4	0.78	5.1	0.71	ns	7.4	0.96	7.2	0.88	ns	10.8	1.31	10.6	1.21	ns	15.0	1.88	14.7	1.72	ns	21.6	2.64	21.8	2.41	ns	31	3.6	31	3.3	ns	44	4.6	44	4.1	ns	61	5.7	62	5.0	ns	83	7.3	84	6.3	ns
Col+290$_{C24}$ C	5.0	0.74	4.9	0.72	ns	6.8	0.92	6.7	0.88	ns	10.0	1.33	9.8	1.22	ns	13.9	1.79	13.7	1.73	ns	20.4	2.51	20.1	2.42	ns	30	3.4	29	3.3	ns	42	4.4	42	4.2	ns	58	5.3	58	5.0	ns	81	6.7	81	6.3	ns
C24+290$_{C24}$ A	5.8	0.79	6.4	0.72	ns	7.8	0.98	8.6	0.89	ns	10.9	1.38	12.7	1.22	ns	16.2	1.93	17.9	1.74	ns	23.4	2.71	25.3	2.44	ns	33	3.7	35	3.3	ns	44	4.8	47	4.2	ns	59	5.9	62	5.1	ns	77	7.5	80	6.4	ns
C24+290$_{C24}$ B	8.0	0.81	8.0	0.72	ns	10.8	1.01	10.8	0.88	ns	16.2	1.46	15.8	1.23	ns	22.6	1.99	22.4	1.73	ns	31.5	2.79	32.2	2.43	ns	44	3.8	45	3.3	ns	58	4.9	59	4.2	ns	76	6.1	76	5.1	ns	103	7.9	102	6.3	ns
C24+290$_{C24}$ C	9.2	0.74	9.2	0.71	ns	12.6	0.91	12.5	0.88	ns	18.4	1.25	18.1	1.20	ns	25.6	1.79	25.0	1.72	ns	34.7	2.51	34.5	2.41	ns	49	3.4	49	3.3	ns	66	4.4	66	4.1	ns	84	5.3	86	5.0	ns	113	6.7	115	6.3	ns
Col+300$_{Col}$ A	7.8	0.75	8.2	0.71	ns	10.7	0.93	11.2	0.87	ns	15.9	1.32	15.9	1.21	ns	21.0	1.82	21.4	1.72	ns	30.6	2.55	31.0	2.40	ns	44	3.5	44	3.2	ns	61	4.5	62	4.1	ns	83	5.4	84	5.0	ns	112	6.9	112	6.2	ns
Col+300$_{Col}$ B	10.6	0.76	10.5	0.71	ns	14.7	0.94	14.4	0.87	ns	20.8	1.31	20.6	1.20	ns	28.8	1.84	28.4	1.71	ns	42.1	2.59	41.2	2.40	ns	61	3.5	60	3.2	ns	84	4.5	83	4.1	ns	114	5.5	112	5.0	ns	153	7.0	152	6.2	ns
Col+300$_{Col}$ C	6.7	0.76	6.8	0.71	ns	9.3	0.93	9.6	0.87	ns	13.3	1.30	13.7	1.20	ns	18.6	1.83	18.9	1.71	ns	27.0	2.56	27.2	2.40	ns	39	3.5	38	3.2	ns	54	4.5	54	4.1	ns	74	5.5	73	5.0	ns	99	6.9	98	6.2	ns
C24+300$_{Col}$ A	5.5	0.74	5.8	0.72	ns	7.5	0.92	7.9	0.88	ns	10.8	1.26	11.3	1.21	ns	15.4	1.80	16.2	1.73	ns	21.7	2.53	22.7	2.42	ns	29	3.4	31	3.3	ns	40	4.4	42	4.2	ns	53	5.4	55	5.0	ns	68	6.8	71	6.3	ns
C24+300$_{Col}$ B	5.3	0.79	5.4	0.72	ns	7.0	0.98	7.0	0.88	ns	10.3	1.39	10.0	1.23	ns	14.7	1.92	14.4	1.72	ns	21.2	2.69	20.5	2.41	ns	29	3.7	28	3.3	ns	40	4.8	38	4.2	ns	52	5.9	50	5.0	ns	66	7.5	64	6.3	ns
C24+300$_{Col}$ C	5.4	0.76	5.0	0.72	ns	7.0	0.94	6.7	0.88	ns	10.0	1.31	9.5	1.21	ns	14.4	1.85	13.4	1.74	ns	20.6	2.59	19.3	2.43	ns	28	3.5	26	3.3	ns	38	4.5	36	4.2	ns	52	5.6	49	5.1	ns	67	7.1	65	6.4	ns
Col+300$_{C24}$ A	5.2	0.74	5.2	0.71	ns	7.3	0.92	7.2	0.87	ns	10.4	1.25	10.5	1.19	ns	14.1	1.79	14.5	1.71	ns	20.6	2.52	21.7	2.40	ns	30	3.4	31	3.2	ns	41	4.4	44	4.1	ns	56	5.3	61	5.0	ns	76	6.7	83	6.2	ns
Col+300$_{C24}$ B	5.4	0.75	5.1	0.71	ns	7.3	0.93	7.2	0.88	ns	10.5	1.26	10.2	1.19	ns	14.3	1.81	13.9	1.72	ns	21.5	2.54	20.4	2.41	ns	31	3.4	30	3.3	ns	44	4.4	42	4.1	ns	62	5.4	59	5.0	ns	84	6.9	82	6.3	ns
Col+300$_{C24}$ C	6.5	0.76	7.2	0.72	ns	9.5	0.93	10.1	0.88	ns	13.6	1.28	14.2	1.20	ns	18.2	1.84	19.4	1.73	ns	26.3	2.59	28.3	2.42	ns	40	3.5	41	3.3	ns	55	4.5	58	4.2	ns	75	5.5	79	5.0	ns	101	7.0	104	6.3	ns
C24+300$_{C24}$ A	7.2	0.79	8.4	0.71	ns	10.3	0.98	11.6	0.88	ns	14.3	1.34	16.4	1.20	ns	20.1	1.92	22.7	1.73	ns	28.9	2.69	31.3	2.42	ns	41	3.7	45	3.3	ns	56	4.8	62	4.2	ns	79	5.8	86	5.0	ns	107	7.9	112	6.4	ns
C24+300$_{C24}$ B	4.1	0.76	4.8	0.72	ns	5.7	0.93	6.4	0.87	ns	8.0	1.27	8.9	1.19	ns	11.4	1.82	12.6	1.71	ns	16.6	2.56	18.6	2.40	ns	24	3.5	27	3.2	ns	32	4.5	36	4.1	ns	45	5.4	49	5.0	ns	60	6.9	64	6.2	ns
C24+300$_{C24}$ C	5.1	0.77	4.7	0.71	ns	7.0	0.94	6.6	0.87	ns	9.8	1.29	9.3	1.19	ns	13.4	1.85	12.8	1.72	ns	19.1	2.59	18.3	2.40	ns	27	3.5	26	3.2	ns	37	4.5	36	4.1	ns	52	5.6	50	5.0	ns	68	7.1	68	6.2	ns
Col+305$_{Col}$ A	5.6	0.74	5.4	0.72	ns	7.8	0.92	7.4	0.88	ns	11.1	1.26	10.7	1.20	ns	15.5	1.80	14.4	1.73	ns	22.5	2.53	20.8	2.42	ns	33	3.4	30	3.3	ns	46	4.4	41	4.2	ns	64	5.4	58	5.0	ns	85	7.0	77	6.3	ns
Col+305$_{Col}$ B	10.5	0.76	10.7	0.71	ns	14.4	0.95	14.5	0.87	ns	20.2	1.30	20.6	1.19	ns	28.3	1.86	28.5	1.71	ns	41.6	2.61	41.5	2.39	ns	60	3.5	61	3.2	ns	83	4.6	85	4.1	ns	115	5.6	118	5.0	ns	158	7.1	161	6.2	ns
Col+305$_{Col}$ C	7.6	0.74	7.8	0.71	ns	10.5	0.92	10.7	0.87	ns	14.4	1.25	14.9	1.19	ns	20.1	1.80	20.6	1.71	ns	29.1	2.52	29.7	2.40	ns	41	3.4	42	3.2	ns	57	4.4	58	4.1	ns	79	5.4	80	5.0	ns	106	6.8	107	6.2	ns
C24+305$_{Col}$ A	7.4	0.74	6.9	0.71	ns	9.8	0.91	9.2	0.87	ns	14.0	1.24	13.2	1.19	ns	19.8	1.78	18.6	1.71	ns	28.0	2.50	26.0	2.40	ns	39	3.4	36	3.2	ns	52	4.3	50	4.1	ns	68	5.3	66	5.0	ns	87	6.7	82	6.2	ns
C24+305$_{Col}$ B	7.6	0.75	7.1	0.71	ns	9.8	0.92	9.5	0.87	ns	14.2	1.26	13.5	1.19	ns	20.2	1.80	19.3	1.71	ns	28.9	2.53	27.5	2.40	ns	40	3.4	38	3.2	ns	55	4.4	52	4.1	ns	72	5.4	67	5.0	ns	91	6.8	87	6.2	ns
C24+305$_{Col}$ C	5.4	1.09	5.6	0.71	ns	6.6	1.25	7.4	0.87	ns	9.7	1.75	10.4	1.19	ns	13.8	2.46	14.2	1.71	ns	19.1	3.47	20.0	2.39	ns	27	4.8	28	3.2	ns	40	6.3	38	4.1	ns	55	8.0	51	5.0	ns	73	10.5	65	6.2	ns
Col+305$_{C24}$ A	6.0	0.74	5.9	0.71	ns	8.3	0.91	8.2	0.87	ns	11.8	1.24	11.6	1.19	ns	16.3	1.78	16.0	1.72	ns	23.5	2.53	23.2	2.42	ns	34	3.4	34	3.3	ns	47	4.3	47	4.1	ns	66	5.3	66	5.0	ns	88	6.7	89	6.2	ns
Col+305$_{C24}$ B	7.0	0.74	7.3	0.71	ns	9.8	0.91	10.3	0.87	ns	13.7	1.25	14.3	1.19	ns	18.5	1.79	19.5	1.71	ns	27.0	2.51	28.0	2.40	ns	39	3.4	40	3.2	ns	55	4.4	56	4.1	ns	75	5.3	76	5.0	ns	101	6.7	103	6.2	ns
Col+305$_{C24}$ C	5.6	0.77	5.5	0.71	ns	7.5	0.94	7.7	0.87	ns	10.8	1.29	10.9	1.19	ns	14.9	1.84	14.8	1.71	ns	22.0	2.59	21.7	2.39	ns	33	3.5	32	3.2	ns	46	4.5	45	4.1	ns	64	5.5	62	5.0	ns	89	7.1	85	6.2	ns
C24+305$_{C24}$ A	6.9	0.75	7.4	0.71	ns	9.4	0.93	10.1	0.87	ns	13.5	1.27	14.7	1.19	ns	19.6	1.82	20.5	1.72	ns	28.3	2.55	28.5	2.40	ns	40	3.5	39	3.2	ns	55	4.5	52	4.1	ns	75	5.4	65	5.0	*	96	6.9	79	6.2	*
C24+305$_{C24}$ B	12.0	0.77	11.9	0.71	ns	16.2	0.96	16.1	0.87	ns	23.3	1.32	23.0	1.19	ns	33.0	1.88	32.5	1.71	ns	48.1	2.64	47.3	2.40	ns	71	3.6	69	3.2	ns	101	4.6	98	4.1	ns	136	5.7	133	5.0	ns	187	7.2	181	6.2	ns
C24+305$_{C24}$ C	8.5	0.77	8.4	0.71	ns	11.4	0.95	11.2	0.87	ns	16.6	1.31	16.0	1.19	ns	24.1	1.87	22.5	1.71	ns	34.4	2.63	30.8	2.40	ns	48	3.6	42	3.2	*	66	4.6	55	4.1	**	87	5.6	71	5.0	**	113	7.2	90	6.2	***
Col+310$_{Col}$ A	6.3	0.74	5.2	0.72	*	8.7	0.92	6.7	0.88	**	12.3	1.27	9.5	1.20	**	16.8	1.80	13.0	1.72	**	24.4	2.53	18.6	2.41	**	35	3.4	26	3.3	***	49	4.4	35	4.2	***	68	5.4	47	5.0	***	92	6.8	62	6.3	***
Col+310$_{Col}$ B	6.8	0.76	6.3	0.72	ns	9.4	0.93	8.4	0.88	ns	13.4	1.32	11.6	1.21	ns	18.2	1.83	16.2	1.73	ns	26.3	2.57	23.0	2.42	ns	38	3.5	32	3.3	ns	53	4.5	44	4.2	**	72	5.5	57	5.1	**	97	7.0	74	6.3	***
Col+310$_{Col}$ C	8.2	1.24	6.1	0.71	ns	11.5	1.57	8.4	0.87	*	15.9	2.20	11.2	1.20	*	21.8	3.09	15.6	1.70	*	32.5	4.35	22.5	2.39	*	46	6.0	31	3.2	**	65	8.1	43	4.1	**	89	10.4	57	4.9	**	122	13.8	77	6.2	***
C24+310$_{Col}$ A	9.0	0.83	8.7	0.72	ns	11.5	1.03	11.3	0.89	ns	15.9	1.43	15.6	1.23	ns	19.9	2.00	21.7	1.74	ns	26.1	2.81	30.0	2.44	ns	35	3.8	42	3.3	ns	46	5.0	56	4.2	ns	59	6.2	71	5.1	ns	78	8.0	93	6.4	ns
C24+310$_{Col}$ B	6.2	0.82	4.6	0.76	*	8.1	1.00	5.9	0.94	**	11.5	1.40	8.2	1.29	**	16.2	1.96	11.1	1.85	**	22.9	2.76	15.3	2.59	***	32	3.8	21	3.5	***	42	4.9	27	4.6	***	57	6.0	34	5.6	***	77	7.7	42	7.1	***
C24+310$_{Col}$ C	11.0	0.94	9.1	0.72	*	14.2	1.18	12.1	0.89	*	20.5	1.64	17.4	1.23	*	28.3	2.33	23.6	1.74	*	38.0	3.28	32.5	2.45	ns	51	4.5	45	3.3	ns	67	6.0	58	4.2	ns	85	7.6	75	5.1	ns	110	9.9	96	6.4	ns
Col+310$_{C24}$ A	6.4	0.79	5.1	0.71	*	8.9	0.98	6.8	0.87	**	12.7	1.34	9.7	1.19	**	17.8	1.92	13.1	1.71	***	25.5	2.70	18.1	2.40	***	37	3.7	26	3.2	***	51	4.8	34	4.1	***	71	5.9	45	5.0	***	99	7.5	60	6.2	***
Col+310$_{C24}$ B	7.6	0.76	5.4	0.71	***	10.3	0.93	7.1	0.87	***	14.6	1.27	9.9	1.19	***	20.1	1.83	13.4	1.72	***	28.9	2.56	18.4	2.41	***	41	3.5	25	3.3	***	58	4.5	34	4.1	***	79	5.5	45	5.0	***	107	6.9	59	6.3	***
Col+310$_{C24}$ C	8.1	0.75	6.3	0.71	***	11.4	0.92	8.5	0.87	***	15.8	1.26	11.9	1.19	***	22.0	1.81	16.0	1.71	***	31.6	2.54	22.4	2.40	***	46	3.5	31	3.2	***	63	4.4	41	4.1	***	87	5.4	54	5.0	***	118	6.8	71	6.2	***
C24+310$_{C24}$ A	6.8	0.73	6.9	0.72	ns	9.1	0.90	9.3	0.88	ns	13.2	1.23	13.2	1.20	ns	18.9	1.77	19.1	1.73	ns	27.2	2.48	27.1	2.43	ns	38	3.4	38	3.3	ns	51	4.3	52	4.2	ns	67	5.2	69	5.1	ns	87	6.6	88	6.3	ns
C24+310$_{C24}$ B	7.9	0.74	6.3	0.71	***	10.6	0.92	8.5	0.87	***	15.0	1.26	11.6	1.19	***	21.6	1.80	15.9	1.71	***	30.6	2.57	21.8	2.42	***	42	3.4	30	3.2	***	56	4.4	39	4.1	***	73	5.4	52	5.0	***	93	6.8	63	6.2	***
C24+310$_{C24}$ C	6.1	0.74	5.4	0.72	ns	8.2	0.91	7.2	0.88	ns	11.7	1.25	10.0	1.20	*	17.0	1.80	13.6	1.72	**	24.4	2.52	18.8	2.41	**	35	3.4	26	3.3	**	47	4.4	34	4.2	***	63	5.3	46	5.0	***	80	6.7	56	6.3	***
Col+315$_{Col}$ A	9.7	0.75	9.7	0.71	ns	13.5	0.93	13.5	0.87	ns	18.8	1.27	18.7	1.19	ns	25.9	1.82	25.9	1.71	ns	37.5	2.55	37.6	2.40	ns	55	3.5	54	3.2	ns	77	4.5	75	4.1	ns	107	5.4	104	5.0	ns	144	6.9	141	6.2	ns
Col+315$_{Col}$ B	5.9	0.77	5.4	0.71	ns	7.8	0.94	7.5	0.87	ns	11.2	1.29	10.7	1.19	ns	15.5	1.84	14.7	1.71	ns	22.6	2.64	21.1	2.41	ns	33	3.5	31	3.2	ns	45	4.5	43	4.1	ns	62	5.5	60	5.0	ns	86	7.0	82	6.2	ns

Appendix 12. continued

Genotype	9 DAS					10 DAS					11 DAS					12 DAS					13 DAS					14 DAS					15 DAS					16 DAS					17 DAS				
	NT		T			NT		T			NT		T			NT		T			NT		T			NT		T			NT		T			NT		T			NT		T		
	Mean	SE	Mean	SE	Sig.	Mean	SE	Mean	SE	Sig.	Mean	SE	Mean	SE	Sig.	Mean	SE	Mean	SE	Sig.	Mean	SE	Mean	SE	Sig.	Mean	SE	Mean	SE	Sig.	Mean	SE	Mean	SE	Sig.	Mean	SE	Mean	SE	Sig.	Mean	SE	Mean	SE	Sig.
Col+315 $_{Col}$ C	5.3	0.79	4.8	0.71	ns	7.4	0.97	6.6	0.87	ns	10.7	1.34	9.6	1.19	ns	14.7	1.91	13.2	1.71	ns	21.4	2.68	19.0	2.40	ns	32	3.7	28	3.2	ns	45	4.7	39	4.1	ns	62	5.8	54	5.0	ns	85	7.4	75	6.2	ns
C24+315 $_{Col}$ A	9.2	0.76	9.1	0.71	ns	12.5	0.94	12.3	0.87	ns	17.7	1.30	17.3	1.19	ns	24.8	1.86	24.3	1.71	ns	35.0	2.61	34.1	2.39	ns	50	3.5	48	3.2	ns	69	4.6	66	4.1	ns	89	5.6	87	5.0	ns	112	7.1	112	6.2	ns
C24+315 $_{Col}$ B	7.7	0.76	7.2	0.71	ns	10.3	0.94	9.7	0.87	ns	14.8	1.29	14.0	1.19	ns	20.9	1.85	20.3	1.71	ns	30.1	2.61	28.1	2.42	ns	42	3.5	40	3.2	ns	57	4.5	54	4.1	ns	75	5.6	70	5.0	ns	95	7.1	90	6.2	ns
C24+315 $_{Col}$ C	6.7	0.74	6.8	0.71	ns	8.9	0.92	8.9	0.87	ns	12.7	1.26	12.7	1.19	ns	18.0	1.81	18.2	1.71	ns	25.2	2.53	25.2	2.40	ns	35	3.4	36	3.2	ns	48	4.4	48	4.1	ns	64	5.4	64	5.0	ns	81	6.8	79	6.2	ns
Col+315 $_{C24}$ A	6.8	0.75	6.8	0.71	ns	9.5	0.93	9.6	0.87	ns	13.3	1.27	13.3	1.19	ns	18.3	1.82	18.3	1.71	ns	26.5	2.55	26.8	2.39	ns	38	3.5	38	3.2	ns	53	4.5	54	4.1	ns	74	5.4	74	5.0	ns	100	6.9	100	6.2	ns
Col+315 $_{C24}$ B	7.9	0.75	7.4	0.71	ns	10.9	0.93	10.2	0.87	ns	15.4	1.27	14.6	1.19	ns	21.3	1.82	20.0	1.71	ns	30.7	2.57	28.7	2.41	ns	44	3.5	41	3.2	ns	61	4.5	58	4.1	ns	85	5.4	80	5.0	ns	113	6.9	106	6.2	ns
Col+315 $_{C24}$ C	7.8	0.77	7.8	0.71	ns	10.5	0.96	10.7	0.87	ns	14.7	1.32	14.9	1.19	ns	20.1	1.89	20.3	1.72	ns	29.0	2.65	29.2	2.41	ns	42	3.6	42	3.3	ns	58	4.7	58	4.1	ns	79	5.7	79	5.0	ns	108	7.3	105	6.3	ns
C24+315 $_{C24}$ A	7.6	0.73	7.3	0.71	ns	10.1	0.91	9.7	0.88	ns	14.4	1.24	13.8	1.20	ns	20.6	1.78	19.6	1.73	ns	28.7	2.49	27.4	2.42	ns	40	3.4	39	3.3	ns	55	4.3	53	4.2	ns	73	5.3	70	5.0	ns	93	6.6	90	6.3	ns
C24+315 $_{C24}$ B	5.8	0.76	5.9	0.71	ns	7.9	0.93	7.9	0.87	ns	11.0	1.27	11.1	1.19	ns	15.8	1.83	16.0	1.71	ns	22.9	2.58	22.7	2.41	ns	33	3.5	32	3.2	ns	44	4.5	44	4.1	ns	59	5.5	60	5.0	ns	77	6.9	79	6.2	ns
C24+315 $_{C24}$ C	6.8	0.75	6.8	0.71	ns	9.4	0.92	9.3	0.87	ns	13.1	1.26	12.9	1.19	ns	18.9	1.81	18.3	1.72	ns	27.0	2.53	26.1	2.40	ns	38	3.4	37	3.2	ns	53	4.4	51	4.1	ns	73	5.4	69	5.0	ns	92	6.8	89	6.2	ns
Col+320 $_{Col}$ A	5.8	0.83	5.8	0.71	ns	7.7	1.02	7.9	0.87	ns	11.1	1.41	11.0	1.18	ns	15.1	2.00	14.9	1.70	ns	21.9	2.81	21.0	2.40	ns	31	3.8	30	3.2	ns	43	5.0	42	4.1	ns	59	6.2	60	4.9	ns	78	8.0	80	6.2	ns
Col+320 $_{Col}$ B	5.9	0.75	5.4	0.71	ns	8.1	0.92	7.3	0.87	ns	11.5	1.26	10.5	1.19	ns	15.6	1.81	14.3	1.71	ns	22.6	2.53	20.5	2.40	ns	33	3.4	30	3.2	ns	46	4.4	42	4.1	ns	63	5.4	59	5.0	ns	86	6.8	79	6.2	ns
Col+320 $_{Col}$ C	6.4	0.76	5.5	0.71	ns	8.8	0.93	7.8	0.87	ns	12.5	1.28	10.8	1.19	ns	17.3	1.83	15.0	1.71	ns	25.3	2.56	21.2	2.40	ns	37	3.5	30	3.2	ns	51	4.5	42	4.1	*	70	5.5	58	5.0	*	95	6.9	79	6.2	*
C24+320 $_{Col}$ A	5.5	0.76	5.1	0.72	ns	7.7	0.94	7.1	0.88	ns	11.0	1.29	10.0	1.21	ns	15.9	1.85	14.2	1.74	ns	22.6	2.59	20.7	2.43	ns	32	3.5	29	3.3	ns	44	4.5	40	4.2	ns	60	5.6	55	5.1	ns	77	7.1	69	6.5	ns
C24+320 $_{Col}$ B	8.1	0.75	8.0	0.72	ns	10.4	0.93	10.7	0.88	ns	14.6	1.27	15.3	1.20	ns	20.6	1.82	21.5	1.73	ns	29.0	2.56	29.9	2.43	ns	41	3.5	42	3.3	ns	54	4.5	56	4.2	ns	69	5.5	70	5.1	ns	90	6.9	93	6.3	ns
C24+320 $_{Col}$ C	7.8	0.75	7.6	0.71	ns	10.4	0.92	10.1	0.87	ns	14.9	1.26	14.4	1.19	ns	21.1	1.81	20.5	1.71	ns	29.6	2.53	28.9	2.40	ns	42	3.4	41	3.2	ns	57	4.4	54	4.1	ns	73	5.4	68	5.0	ns	96	6.9	87	6.3	ns
Col+320 $_{C24}$ A	6.0	0.74	6.0	0.71	ns	8.1	0.91	8.2	0.87	ns	11.6	1.25	11.7	1.19	ns	16.0	1.79	16.1	1.72	ns	23.4	2.51	23.4	2.40	ns	34	3.4	34	3.3	ns	48	4.4	48	4.1	ns	66	5.3	66	5.0	ns	88	6.7	89	6.2	ns
Col+320 $_{C24}$ B	4.9	0.74	4.1	0.71	ns	6.7	0.90	5.9	0.88	ns	9.7	1.23	8.6	1.19	ns	13.1	1.77	11.6	1.72	ns	18.5	2.52	17.2	2.42	ns	28	3.4	25	3.3	ns	39	4.3	35	4.1	ns	54	5.2	50	5.0	ns	76	6.6	69	6.2	ns
Col+320 $_{C24}$ C	4.7	0.75	4.9	0.71	ns	6.6	0.92	6.7	0.87	ns	9.4	1.26	9.6	1.19	ns	13.0	1.81	13.1	1.71	ns	19.0	2.54	19.0	2.40	ns	27	3.4	28	3.2	ns	38	4.4	38	4.1	ns	53	5.4	53	5.0	ns	72	6.8	72	6.2	ns
C24+320 $_{C24}$ A	5.5	0.75	5.5	0.71	ns	7.6	0.92	7.6	0.87	ns	10.5	1.27	10.4	1.19	ns	14.7	1.81	14.2	1.72	ns	20.5	2.54	19.6	2.40	ns	29	3.5	27	3.2	ns	38	4.4	37	4.1	ns	51	5.4	49	5.0	ns	61	6.8	60	6.2	ns
C24+320 $_{C24}$ B	5.1	0.76	5.0	0.73	ns	6.9	0.93	6.9	0.89	ns	9.5	1.28	9.6	1.21	ns	13.3	1.84	13.4	1.75	ns	19.6	2.58	19.7	2.45	ns	28	3.5	28	3.3	ns	39	4.5	39	4.2	ns	55	5.5	55	5.1	ns	72	7.0	72	6.4	ns
C24+320 $_{C24}$ C	5.5	0.76	5.1	0.72	ns	7.7	0.94	7.2	0.88	ns	11.0	1.29	10.2	1.20	ns	15.2	1.85	14.3	1.72	ns	21.5	2.59	20.4	2.41	ns	31	3.5	29	3.3	ns	43	4.5	40	4.2	ns	61	5.6	56	5.0	ns	80	7.1	72	6.3	ns
Col+330 $_{Col}$ A	5.3	0.75	5.4	0.72	ns	7.3	0.92	7.3	0.88	ns	10.4	1.29	10.4	1.20	ns	14.3	1.81	14.6	1.72	ns	21.2	2.54	21.5	2.41	ns	31	3.4	31	3.3	ns	44	4.4	45	4.2	ns	62	5.4	63	5.0	ns	84	6.8	85	6.3	ns
Col+330 $_{Col}$ B	5.9	0.76	5.6	0.71	ns	8.1	0.94	7.8	0.87	ns	11.5	1.31	11.2	1.21	ns	16.2	1.84	15.4	1.72	ns	24.1	2.58	22.2	2.41	ns	35	3.5	32	3.3	ns	51	4.5	46	4.1	ns	71	5.5	65	5.0	ns	97	7.0	88	6.2	ns
Col+330 $_{Col}$ C	5.1	0.75	5.2	0.71	ns	7.1	0.92	7.2	0.87	ns	10.3	1.31	10.3	1.20	ns	14.3	1.81	14.2	1.72	ns	21.1	2.54	20.9	2.41	ns	30	3.5	30	3.3	ns	43	4.4	43	4.1	ns	60	5.4	60	5.0	ns	81	6.8	82	6.2	ns
C24+330 $_{Col}$ A	8.1	0.77	7.4	0.72	ns	10.8	0.95	9.7	0.89	ns	15.5	1.37	14.0	1.23	ns	21.6	1.87	19.8	1.75	ns	30.3	2.62	27.1	2.45	ns	41	3.6	37	3.3	ns	54	4.6	49	4.2	ns	69	5.6	63	5.1	ns	87	7.2	82	6.4	ns
C24+330 $_{Col}$ B	7.6	0.76	7.8	0.71	ns	10.0	0.93	10.3	0.87	ns	14.8	1.36	14.9	1.21	ns	20.6	1.84	21.0	1.71	ns	28.6	2.58	28.7	2.40	ns	39	3.5	39	3.2	ns	52	4.5	52	4.1	ns	67	5.5	67	5.0	ns	84	7.0	85	6.2	ns
C24+330 $_{Col}$ C	5.9	0.81	6.5	0.72	ns	7.8	1.01	8.6	0.88	ns	11.3	1.39	12.6	1.21	ns	16.1	1.98	17.8	1.73	ns	22.8	2.78	24.7	2.42	ns	31	3.8	34	3.3	ns	43	4.9	46	4.2	ns	55	6.1	60	5.1	ns	71	7.8	78	6.3	ns
Col+330 $_{C24}$ A	5.2	0.76	5.2	0.72	ns	7.2	0.94	7.1	0.88	ns	10.4	1.32	10.2	1.21	ns	14.3	1.85	14.2	1.73	ns	21.0	2.59	20.8	2.42	ns	30	3.5	30	3.3	ns	43	4.5	42	4.2	ns	60	5.5	59	5.0	ns	82	7.0	80	6.3	ns
Col+330 $_{C24}$ B	7.6	0.79	7.4	0.73	ns	10.8	0.98	10.1	0.90	ns	15.0	1.37	14.6	1.26	ns	21.2	1.92	20.0	1.76	ns	30.3	2.70	28.5	2.47	ns	44	3.7	41	3.3	ns	62	4.8	58	4.3	ns	87	5.9	80	5.2	ns	118	7.5	109	6.5	ns
Col+330 $_{C24}$ C	5.3	0.75	5.1	0.73	ns	7.5	0.92	6.8	0.90	ns	10.7	1.28	9.7	1.23	ns	14.8	1.80	13.5	1.76	ns	21.7	2.53	19.8	2.46	ns	31	3.4	28	3.3	ns	44	4.4	40	4.3	ns	60	5.4	56	5.2	ns	83	6.8	76	6.5	ns
C24+330 $_{C24}$ A	6.6	0.76	6.7	0.71	ns	8.5	0.93	8.8	0.87	ns	12.3	1.29	12.6	1.20	ns	17.5	1.83	18.1	1.72	ns	25.0	2.57	25.1	2.40	ns	35	3.5	35	3.2	ns	47	4.5	47	4.1	ns	63	5.5	62	5.0	ns	80	7.0	78	6.2	ns
C24+330 $_{C24}$ B	8.1	1.08	6.4	0.71	ns	11.3	1.36	8.5	0.87	ns	15.7	1.90	12.1	1.21	ns	22.0	2.67	17.3	1.71	ns	32.0	3.76	24.4	2.40	ns	44	5.2	34	3.2	ns	60	6.9	46	4.1	*	79	8.8	60	5.0	*	104	11.6	77	6.2	*
C24+330 $_{C24}$ C	7.7	0.77	7.6	0.71	ns	10.0	0.95	9.8	0.87	ns	14.5	1.32	14.1	1.20	ns	20.8	1.86	20.5	1.71	ns	29.1	2.61	29.3	2.40	ns	41	3.6	41	3.2	ns	55	4.6	56	4.1	ns	72	5.6	72	5.0	ns	93	7.1	93	6.2	ns
Col+335 $_{Col}$ A	10.8	0.76	9.8	0.71	*	14.7	0.93	13.4	0.87	*	19.9	1.28	18.3	1.20	ns	27.2	1.83	24.6	1.72	ns	38.4	2.57	35.3	2.41	ns	54	3.5	50	3.3	ns	73	4.5	69	4.1	ns	97	5.5	93	5.0	ns	129	7.0	125	6.2	ns
Col+335 $_{Col}$ B	9.0	1.01	9.3	0.71	ns	12.3	1.27	12.5	0.87	ns	17.1	1.78	17.4	1.19	ns	23.3	2.51	23.8	1.70	ns	32.9	3.54	33.9	2.39	ns	46	4.9	48	3.2	ns	64	6.5	66	4.1	ns	86	8.2	89	4.9	ns	118	10.8	119	6.2	ns
Col+335 $_{Col}$ C	8.3	0.75	8.4	0.71	ns	11.4	0.92	11.5	0.87	ns	16.0	1.29	16.1	1.20	ns	22.1	1.81	22.1	1.71	ns	31.6	2.53	32.0	2.40	ns	45	3.4	45	3.2	ns	63	4.4	64	4.1	ns	84	5.4	86	5.0	ns	113	6.8	116	6.2	ns
C24+335 $_{Col}$ A	8.9	0.75	9.0	0.71	ns	11.8	0.92	11.9	0.87	ns	16.9	1.28	17.4	1.19	ns	23.9	1.81	23.9	1.71	ns	33.2	2.54	33.1	2.39	ns	46	3.5	46	3.2	ns	63	4.4	63	4.1	ns	81	5.4	82	5.0	ns	106	6.8	106	6.2	ns
C24+335 $_{Col}$ B	9.1	0.81	8.5	0.71	ns	12.0	1.00	11.1	0.87	ns	16.9	1.56	16.2	1.20	ns	23.5	1.98	22.9	1.70	ns	34.1	2.78	31.8	2.39	ns	48	3.8	44	3.2	ns	67	4.9	61	4.1	ns	87	6.1	81	4.9	ns	113	7.8	104	6.2	ns
C24+335 $_{Col}$ C	9.3	0.89	8.9	0.71	ns	11.5	1.11	11.5	0.87	ns	15.7	1.54	16.6	1.20	ns	21.0	2.19	23.1	1.70	ns	28.4	3.09	31.8	2.39	ns	37	4.3	44	3.2	ns	50	5.6	58	4.1	ns	64	7.1	76	4.9	ns	102	10.0	101	6.2	ns
Col+335 $_{C24}$ A	8.7	0.75	8.8	0.71	ns	12.1	0.93	12.2	0.88	ns	16.8	1.29	17.1	1.21	ns	23.1	1.82	23.1	1.72	ns	33.5	2.56	33.6	2.41	ns	48	3.5	48	3.3	ns	68	4.5	67	4.2	ns	93	5.5	91	5.0	ns	123	6.9	121	6.3	ns
Col+335 $_{C24}$ B	10.6	1.09	9.4	0.72	ns	14.1	1.38	13.0	0.89	ns	18.5	2.27	17.8	1.23	ns	26.9	2.72	24.5	1.74	ns	38.7	3.83	35.1	2.44	ns	54	5.3	50	3.3	ns	74	7.1	70	4.2	ns	102	9.0	95	5.1	ns	134	11.9	127	6.4	ns
Col+335 $_{C24}$ C	5.2	0.75	5.2	0.72	ns	7.0	0.92	7.2	0.88	ns	10.0	1.28	10.3	1.21	ns	14.5	1.81	14.8	1.73	ns	21.0	2.55	21.6	2.42	ns	31	3.5	31	3.3	ns	44	4.4	45	4.2	ns	61	5.4	64	5.0	ns	83	6.9	87	6.3	ns
C24+335 $_{C24}$ A	5.9	0.74	5.9	0.72	ns	7.7	0.91	8.0	0.88	ns	11.0	1.27	11.2	1.21	ns	16.2	1.79	16.1	1.73	ns	23.1	2.51	22.9	2.42	ns	32	3.4	32	3.3	ns	44	4.4	43	4.2	ns	58	5.3	56	5.0	ns	73	6.7	71	6.3	ns
C24+335 $_{C24}$ B	7.6	0.75	7.8	0.71	ns	10.0	0.93	10.3	0.87	ns	14.1	1.29	14.6	1.20	ns	20.0	1.83	20.9	1.71	ns	26.9	2.56	28.3	2.40	ns	37	3.5	39	3.2	ns	49	4.5	52	4.1	ns	64	5.5	66	5.0	ns	84	6.9	85	6.2	ns
C24+335 $_{C24}$ C	9.1	0.75	8.8	0.72	ns	12.0	0.93	11.7	0.88	ns	17.3	1.30	16.8	1.23	ns	23.8	1.83	22.9	1.73	ns	32.7	2.57	31.2	2.42	ns	45	3.5	42	3.3	ns	60	4.5	56	4.2	ns	75	5.5	71	5.0	ns	100	7.0	94	6.3	ns
Col+ORF $_{C24}$ A	7.0	0.78	6.6	0.71	ns	9.7	0.96	9.0	0.87	ns	13.8	1.32	12.9	1.19	ns	19.5	1.89	17.8	1.71	ns	28.3	2.65	25.9	2.40	ns	41	3.6	37	3.2	ns	56	4.7	52	4.1	ns	79	5.7	73	5.0	ns	106	7.3	99	6.2	ns
Col+ORF $_{C24}$ B	5.0	0.75	4.9	0.71	ns	7.2	0.91	6.9	0.87	ns	10.3	1.25	9.9	1.19	ns	14.0	1.80	13.6	1.71	ns	20.2	2.52	19.7	2.40	ns	29	3.4	29	3.2	ns	41	4.4	40	4.1	ns	57	5.3	56	5.0	ns	78	6.7	76	6.2	ns
Col+ORF $_{C24}$ C	6.2	0.77	6.1	0.71	ns	8.5	0.95	8.4	0.87	ns	12.2	1.30	12.1	1.18	ns	16.7	1.86	16.4	1.71	ns	24.2	2.61	23.5	2.39	ns	35	3.6	34	3.2	ns	49	4.6	47	4.1	ns	69	5.6	65	5.0	ns	94	7.1	89	6.2	ns
C24+ORF $_{C24}$ A	5.6	0.83	5.2	0.71	ns	7.1	1.00	7.0	0.88	ns	10.0	1.38	9.9	1.20	ns	13.6	1.97	14.0	1.72	ns	19.4	2.76	19.8	2.43	ns	28	3.8	28	3.3	ns	38	4.9	38	4.2	ns	51	6.0	50	5.0	ns	66	7.8	65	6.3	ns
C24+ORF $_{C24}$ B	5.6	0.79	5.8	0.72	ns	7.4	0.96	7.9	0.88	ns	10.4	1.32	11.1	1.20	ns	14.9	1.89	16.1	1.73	ns	21.5	2.65	23.2	2.43	ns	31	3.6	32	3.3	ns	42	4.7	44	4.2	ns	57	5.7	59	5.1	ns	72	7.3	75	6.4	ns
C24+ORF $_{C24}$ C	6.1	0.73	5.5	0.74	ns	8.1	0.89	7.3	0.91	ns	11.5	1.22	10.4	1.24	ns	16.6	1.76	15.2	1.79	ns	24.2	2.46	22.0	2.51	ns	34	3.3	31	3.4	ns	46	4.3	42	4.4	ns	61	5.2	56	5.3	ns	77	6.5	71	6.7	ns

Mean leaf ares was estimated using linear mixed model.

Each mean value of transgenic lines was compared to the mean value of their non-transgenic siblings.

Significance tested by Fisher's Least Significant Difference (LSD): *,P < 0.5; **,P < 0.01; ***,P < 0.001.

LA: Leaf Area in mm^2, SE: Standard error, ns: not significant

Appendix 13: Relative growth rates (RGR) of transgenic lines harboring single candidate gene and the differences in RGRs between transgenic siblings and their non-transgenic siblings

Genotype	RGR 09-10			RGR 10-11			RGR 11-12			RGR 12-13			RGR 13-14			RGR 14-15			RGR 15-16			RGR 16-17		
	NT	T	Sig.	NT	T	Sig.	NT	T	Sig.	**NT**	**T**	Sig.	**NT**	**T**	Sig.	**NT**	**T**	Sig.	**NT**	**T**	Sig.	**NT**	**T**	Sig.
Col+EV A	0.332 ± 0.017	0.317 ± 0.018	ns	0.364 ± 0.015	0.362 ± 0.015	ns	0.32 ± 0.008	0.326 ± 0.009	ns	0.368 ± 0.014	0.371 ± 0.014	ns	0.358 ± 0.007	0.355 ± 0.008	ns	0.338 ± 0.011	0.338 ± 0.012	ns	0.333 ± 0.015	0.331 ± 0.015	ns	0.307 ± 0.012	0.307 ± 0.013	ns
Col+EV B	0.314 ± 0.018	0.332 ± 0.017	ns	0.346 ± 0.016	0.341 ± 0.014	ns	0.303 ± 0.01	0.31 ± 0.008	ns	0.363 ± 0.014	0.374 ± 0.014	ns	0.363 ± 0.009	0.375 ± 0.007	ns	0.353 ± 0.012	0.357 ± 0.011	ns	0.338 ± 0.016	0.341 ± 0.015	ns	0.301 ± 0.013	0.309 ± 0.012	ns
Col+EV C	0.333 ± 0.018	0.331 ± 0.017	ns	0.359 ± 0.015	0.346 ± 0.014	ns	0.326 ± 0.009	0.326 ± 0.008	ns	0.375 ± 0.014	0.374 ± 0.014	ns	0.365 ± 0.008	0.367 ± 0.007	ns	0.343 ± 0.012	0.353 ± 0.011	ns	0.318 ± 0.015	0.323 ± 0.015	ns	0.299 ± 0.013	0.304 ± 0.012	ns
C24+EV A	0.293 ± 0.018	0.288 ± 0.017	ns	0.358 ± 0.016	0.348 ± 0.014	ns	0.328 ± 0.01	0.334 ± 0.008	ns	0.322 ± 0.015	0.332 ± 0.014	ns	0.324 ± 0.009	0.321 ± 0.007	ns	0.304 ± 0.012	0.3 ± 0.011	ns	0.24 ± 0.016	0.257 ± 0.015	ns	0.253 ± 0.013	0.255 ± 0.012	ns
C24+EV B	0.298 ± 0.032	0.31 ± 0.017	ns	0.349 ± 0.026	0.362 ± 0.014	ns	0.369 ± 0.022	0.346 ± 0.008	ns	0.344 ± 0.024	0.339 ± 0.013	ns	0.289 ± 0.02	0.33 ± 0.007	*	0.365 ± 0.02	0.312 ± 0.011	**	0.229 ± 0.026	0.266 ± 0.014	ns	0.223 ± 0.025	0.246 ± 0.012	ns
C24+EV C	0.302 ± 0.019	0.293 ± 0.017	ns	0.34 ± 0.016	0.365 ± 0.014	ns	0.343 ± 0.011	0.32 ± 0.008	ns	0.338 ± 0.015	0.333 ± 0.013	ns	0.332 ± 0.009	0.331 ± 0.007	ns	0.308 ± 0.012	0.302 ± 0.011	ns	0.268 ± 0.016	0.252 ± 0.015	ns	0.256 ± 0.014	0.262 ± 0.012	ns
Col+238 $_{Col}$ A	0.366 ± 0.024	0.337 ± 0.02	ns	0.345 ± 0.019	0.355 ± 0.017	ns	0.316 ± 0.015	0.312 ± 0.012	ns	0.36 ± 0.018	0.376 ± 0.016	ns	0.371 ± 0.013	0.381 ± 0.011	ns	0.345 ± 0.015	0.345 ± 0.013	ns	0.345 ± 0.019	0.341 ± 0.017	ns	0.306 ± 0.018	0.31 ± 0.015	ns
Col+238 $_{Col}$ B	0.326 ± 0.024	0.353 ± 0.02	ns	0.352 ± 0.02	0.364 ± 0.017	ns	0.308 ± 0.016	0.305 ± 0.012	ns	0.391 ± 0.019	0.383 ± 0.016	ns	0.364 ± 0.014	0.359 ± 0.01	ns	0.337 ± 0.016	0.331 ± 0.013	ns	0.333 ± 0.02	0.339 ± 0.017	ns	0.312 ± 0.018	0.31 ± 0.015	ns
Col+238 $_{Col}$ C	0.315 ± 0.023	0.335 ± 0.021	ns	0.373 ± 0.019	0.366 ± 0.017	ns	0.332 ± 0.014	0.328 ± 0.012	ns	0.385 ± 0.018	0.368 ± 0.016	ns	0.384 ± 0.012	0.369 ± 0.011	ns	0.354 ± 0.014	0.345 ± 0.013	ns	0.327 ± 0.019	0.348 ± 0.017	ns	0.305 ± 0.017	0.313 ± 0.015	ns
Col+238 $_{C24}$ A	0.353 ± 0.023	0.347 ± 0.02	ns	0.346 ± 0.019	0.367 ± 0.017	ns	0.317 ± 0.015	0.304 ± 0.012	ns	0.33 ± 0.018	0.35 ± 0.016	ns	0.345 ± 0.013	0.346 ± 0.01	ns	0.344 ± 0.015	0.327 ± 0.013	ns	0.348 ± 0.019	0.335 ± 0.017	ns	0.295 ± 0.017	0.311 ± 0.015	ns
Col+238 $_{C24}$ B	0.333 ± 0.022	0.326 ± 0.02	ns	0.371 ± 0.018	0.376 ± 0.017	ns	0.334 ± 0.014	0.324 ± 0.012	ns	0.38 ± 0.017	0.374 ± 0.016	ns	0.378 ± 0.012	0.384 ± 0.01	ns	0.346 ± 0.014	0.346 ± 0.013	ns	0.363 ± 0.019	0.341 ± 0.017	ns	0.314 ± 0.017	0.326 ± 0.015	ns
Col+238 $_{C24}$ C	0.368 ± 0.022	0.366 ± 0.021	ns	0.378 ± 0.018	0.354 ± 0.017	ns	0.329 ± 0.014	0.316 ± 0.012	ns	0.383 ± 0.017	0.375 ± 0.016	ns	0.371 ± 0.012	0.388 ± 0.01	ns	0.363 ± 0.014	0.343 ± 0.013	ns	0.354 ± 0.018	0.355 ± 0.017	ns	0.333 ± 0.016	0.336 ± 0.015	ns
C24+238 $_{Col}$ A	0.308 ± 0.022	0.289 ± 0.019	ns	0.347 ± 0.018	0.351 ± 0.016	ns	0.362 ± 0.014	0.349 ± 0.011	ns	0.349 ± 0.017	0.345 ± 0.015	ns	0.346 ± 0.012	0.337 ± 0.01	ns	0.337 ± 0.014	0.319 ± 0.013	ns	0.278 ± 0.019	0.289 ± 0.016	ns	0.254 ± 0.017	0.248 ± 0.014	ns
C24+238 $_{Col}$ B	0.319 ± 0.021	0.311 ± 0.02	ns	0.362 ± 0.018	0.366 ± 0.016	ns	0.345 ± 0.013	0.343 ± 0.011	ns	0.372 ± 0.017	0.358 ± 0.015	ns	0.34 ± 0.011	0.334 ± 0.01	ns	0.303 ± 0.014	0.314 ± 0.013	ns	0.277 ± 0.018	0.288 ± 0.017	ns	0.222 ± 0.016	0.232 ± 0.014	ns
C24+238 $_{Col}$ C	0.314 ± 0.022	0.322 ± 0.02	ns	0.354 ± 0.018	0.34 ± 0.016	ns	0.371 ± 0.013	0.359 ± 0.012	ns	0.397 ± 0.017	0.374 ± 0.016	ns	0.328 ± 0.011	0.341 ± 0.01	ns	0.315 ± 0.014	0.311 ± 0.013	ns	0.298 ± 0.018	0.307 ± 0.017	ns	0.249 ± 0.016	0.261 ± 0.014	ns
C24+238 $_{C24}$ A	0.356 ± 0.027	0.34 ± 0.02	ns	0.337 ± 0.021	0.321 ± 0.017	ns	0.34 ± 0.017	0.342 ± 0.012	ns	0.363 ± 0.02	0.373 ± 0.016	ns	0.37 ± 0.015	0.35 ± 0.01	ns	0.319 ± 0.017	0.323 ± 0.013	ns	0.294 ± 0.021	0.312 ± 0.017	ns	0.271 ± 0.02	0.261 ± 0.015	ns
C24+238 $_{C24}$ B	0.32 ± 0.031	0.324 ± 0.019	ns	0.351 ± 0.025	0.339 ± 0.016	ns	0.344 ± 0.021	0.329 ± 0.011	ns	0.347 ± 0.023	0.385 ± 0.015	ns	0.361 ± 0.019	0.336 ± 0.01	ns	0.308 ± 0.02	0.325 ± 0.013	ns	0.323 ± 0.025	0.307 ± 0.016	ns	0.206 ± 0.024	0.24 ± 0.014	ns
C24+238 $_{C24}$ C	0.329 ± 0.023	0.343 ± 0.021	ns	0.341 ± 0.019	0.341 ± 0.017	ns	0.346 ± 0.015	0.343 ± 0.012	ns	0.363 ± 0.018	0.374 ± 0.016	ns	0.36 ± 0.013	0.331 ± 0.011	ns	0.344 ± 0.015	0.347 ± 0.013	ns	0.316 ± 0.019	0.316 ± 0.017	ns	0.245 ± 0.017	0.265 ± 0.015	ns
Col+240 $_{Col}$ A	0.342 ± 0.02	0.343 ± 0.02	ns	0.355 ± 0.017	0.354 ± 0.017	ns	0.326 ± 0.012	0.328 ± 0.012	ns	0.377 ± 0.016	0.376 ± 0.016	ns	0.366 ± 0.01	0.358 ± 0.01	ns	0.34 ± 0.013	0.339 ± 0.013	ns	0.311 ± 0.017	0.325 ± 0.017	ns	0.318 ± 0.015	0.314 ± 0.015	ns
Col+240 $_{Col}$ B	0.328 ± 0.024	0.324 ± 0.019	ns	0.337 ± 0.019	0.359 ± 0.016	ns	0.303 ± 0.015	0.291 ± 0.011	ns	0.39 ± 0.018	0.382 ± 0.015	ns	0.361 ± 0.013	0.351 ± 0.01	ns	0.351 ± 0.015	0.341 ± 0.013	ns	0.312 ± 0.02	0.319 ± 0.016	ns	0.313 ± 0.018	0.311 ± 0.014	ns
Col+240 $_{Col}$ C	0.323 ± 0.023	0.311 ± 0.02	ns	0.372 ± 0.018	0.361 ± 0.016	ns	0.328 ± 0.014	0.319 ± 0.011	ns	0.383 ± 0.018	0.384 ± 0.016	ns	0.369 ± 0.013	0.372 ± 0.01	ns	0.353 ± 0.014	0.342 ± 0.013	ns	0.326 ± 0.019	0.333 ± 0.017	ns	0.319 ± 0.017	0.312 ± 0.014	ns
Col+240 $_{C24}$ A	0.342 ± 0.022	0.341 ± 0.02	ns	0.351 ± 0.018	0.355 ± 0.016	ns	0.323 ± 0.013	0.325 ± 0.012	ns	0.384 ± 0.017	0.377 ± 0.016	ns	0.368 ± 0.012	0.37 ± 0.01	ns	0.344 ± 0.014	0.334 ± 0.013	ns	0.349 ± 0.018	0.334 ± 0.017	ns	0.312 ± 0.016	0.307 ± 0.014	ns
Col+240 $_{C24}$ B	0.337 ± 0.023	0.321 ± 0.02	ns	0.344 ± 0.018	0.342 ± 0.016	ns	0.317 ± 0.014	0.32 ± 0.011	ns	0.377 ± 0.017	0.384 ± 0.015	ns	0.399 ± 0.012	0.38 ± 0.01	ns	0.342 ± 0.014	0.345 ± 0.013	ns	0.335 ± 0.018	0.333 ± 0.017	ns	0.323 ± 0.016	0.319 ± 0.014	ns
Col+240 $_{C24}$ C	0.34 ± 0.021	0.349 ± 0.02	ns	0.358 ± 0.018	0.348 ± 0.017	ns	0.321 ± 0.013	0.319 ± 0.012	ns	0.379 ± 0.017	0.374 ± 0.016	ns	0.367 ± 0.011	0.369 ± 0.01	ns	0.343 ± 0.014	0.339 ± 0.013	ns	0.346 ± 0.018	0.342 ± 0.017	ns	0.323 ± 0.016	0.306 ± 0.015	ns
C24+240 $_{Col}$ A	0.331 ± 0.023	0.317 ± 0.019	ns	0.345 ± 0.018	0.36 ± 0.016	ns	0.364 ± 0.014	0.375 ± 0.011	ns	0.37 ± 0.017	0.365 ± 0.015	ns	0.347 ± 0.012	0.341 ± 0.01	ns	0.323 ± 0.014	0.328 ± 0.013	ns	0.289 ± 0.019	0.316 ± 0.016	ns	0.264 ± 0.017	0.251 ± 0.014	ns
C24+240 $_{Col}$ B	0.323 ± 0.026	0.316 ± 0.02	ns	0.361 ± 0.02	0.323 ± 0.016	ns	0.324 ± 0.016	0.322 ± 0.012	ns	0.334 ± 0.019	0.311 ± 0.016	ns	0.347 ± 0.014	0.302 ± 0.01	**	0.302 ± 0.016	0.279 ± 0.013	ns	0.264 ± 0.021	0.273 ± 0.017	ns	0.25 ± 0.019	0.245 ± 0.014	ns
C24+240 $_{Col}$ C	0.335 ± 0.023	0.329 ± 0.02	ns	0.326 ± 0.019	0.332 ± 0.017	ns	0.361 ± 0.014	0.372 ± 0.012	ns	0.369 ± 0.018	0.37 ± 0.016	ns	0.339 ± 0.013	0.357 ± 0.01	ns	0.309 ± 0.015	0.309 ± 0.013	ns	0.297 ± 0.019	0.298 ± 0.017	ns	0.252 ± 0.017	0.266 ± 0.014	ns
C24+240 $_{C24}$ A	0.335 ± 0.021	0.323 ± 0.021	ns	0.318 ± 0.017	0.328 ± 0.017	ns	0.343 ± 0.013	0.341 ± 0.012	ns	0.347 ± 0.017	0.363 ± 0.016	ns	0.341 ± 0.011	0.353 ± 0.011	ns	0.337 ± 0.014	0.328 ± 0.013	ns	0.318 ± 0.018	0.328 ± 0.017	ns	0.272 ± 0.016	0.278 ± 0.015	ns
C24+240 $_{C24}$ B	0.278 ± 0.022	0.3 ± 0.019	ns	0.377 ± 0.018	0.364 ± 0.016	ns	0.354 ± 0.013	0.333 ± 0.011	ns	0.337 ± 0.017	0.344 ± 0.015	ns	0.337 ± 0.012	0.334 ± 0.01	ns	0.317 ± 0.014	0.303 ± 0.013	ns	0.224 ± 0.018	0.243 ± 0.017	ns	0.305 ± 0.016	0.266 ± 0.014	*
C24+240 $_{C24}$ C	0.318 ± 0.021	0.356 ± 0.02	ns	0.351 ± 0.017	0.327 ± 0.017	ns	0.349 ± 0.013	0.35 ± 0.012	ns	0.357 ± 0.016	0.364 ± 0.016	ns	0.322 ± 0.011	0.334 ± 0.01	ns	0.32 ± 0.013	0.322 ± 0.013	ns	0.29 ± 0.017	0.31 ± 0.017	ns	0.225 ± 0.015	0.23 ± 0.015	ns
Col+250 $_{Col}$ A	0.312 ± 0.022	0.324 ± 0.02	ns	0.37 ± 0.018	0.363 ± 0.016	ns	0.303 ± 0.014	0.317 ± 0.012	ns	0.381 ± 0.017	0.39 ± 0.016	ns	0.365 ± 0.012	0.371 ± 0.01	ns	0.343 ± 0.014	0.339 ± 0.013	ns	0.328 ± 0.018	0.339 ± 0.017	ns	0.311 ± 0.016	0.322 ± 0.014	ns
Col+250 $_{Col}$ B	0.344 ± 0.022	0.332 ± 0.02	ns	0.355 ± 0.018	0.366 ± 0.016	ns	0.309 ± 0.013	0.309 ± 0.011	ns	0.383 ± 0.017	0.388 ± 0.015	ns	0.39 ± 0.012	0.374 ± 0.01	ns	0.346 ± 0.014	0.346 ± 0.013	ns	0.348 ± 0.018	0.337 ± 0.017	ns	0.313 ± 0.016	0.316 ± 0.014	ns
Col+250 $_{Col}$ C	0.346 ± 0.024	0.333 ± 0.02	ns	0.357 ± 0.019	0.368 ± 0.016	ns	0.334 ± 0.015	0.333 ± 0.011	ns	0.376 ± 0.018	0.395 ± 0.015	ns	0.397 ± 0.013	0.371 ± 0.01	ns	0.345 ± 0.015	0.363 ± 0.013	ns	0.344 ± 0.019	0.345 ± 0.017	ns	0.324 ± 0.018	0.322 ± 0.014	ns
Col+250 $_{C24}$ A	0.315 ± 0.022	0.322 ± 0.02	ns	0.352 ± 0.018	0.349 ± 0.016	ns	0.333 ± 0.014	0.322 ± 0.011	ns	0.347 ± 0.017	0.362 ± 0.015	ns	0.358 ± 0.012	0.361 ± 0.01	ns	0.336 ± 0.014	0.33 ± 0.013	ns	0.328 ± 0.018	0.341 ± 0.017	ns	0.286 ± 0.016	0.299 ± 0.014	ns
Col+250 $_{C24}$ B	0.327 ± 0.022	0.323 ± 0.02	ns	0.347 ± 0.018	0.358 ± 0.016	ns	0.309 ± 0.014	0.306 ± 0.012	ns	0.366 ± 0.017	0.377 ± 0.016	ns	0.373 ± 0.012	0.364 ± 0.01	ns	0.35 ± 0.014	0.347 ± 0.013	ns	0.325 ± 0.018	0.325 ± 0.017	ns	0.297 ± 0.016	0.292 ± 0.014	ns
Col+250 $_{C24}$ C	0.339 ± 0.027	0.325 ± 0.019	ns	0.349 ± 0.022	0.343 ± 0.016	ns	0.299 ± 0.018	0.32 ± 0.011	ns	0.368 ± 0.021	0.361 ± 0.015	ns	0.372 ± 0.016	0.365 ± 0.01	ns	0.369 ± 0.017	0.346 ± 0.012	ns	0.309 ± 0.022	0.329 ± 0.016	ns	0.305 ± 0.021	0.297 ± 0.014	ns
C24+250 $_{Col}$ A	0.374 ± 0.022	0.376 ± 0.02	ns	0.336 ± 0.018	0.342 ± 0.016	ns	0.337 ± 0.014	0.334 ± 0.011	ns	0.364 ± 0.017	0.37 ± 0.015	ns	0.325 ± 0.012	0.35 ± 0.01	ns	0.325 ± 0.014	0.338 ± 0.013	ns	0.322 ± 0.018	0.325 ± 0.017	ns	0.285 ± 0.016	0.273 ± 0.014	ns
C24+250 $_{Col}$ B	0.332 ± 0.021	0.306 ± 0.02	ns	0.333 ± 0.017	0.339 ± 0.017	ns	0.334 ± 0.013	0.34 ± 0.012	ns	0.349 ± 0.017	0.36 ± 0.016	ns	0.37 ± 0.011	0.342 ± 0.01	ns	0.333 ± 0.014	0.343 ± 0.013	ns	0.322 ± 0.018	0.312 ± 0.017	ns	0.245 ± 0.016	0.264 ± 0.015	ns
C24+250 $_{Col}$ C	0.326 ± 0.022	0.307 ± 0.019	ns	0.347 ± 0.018	0.334 ± 0.016	ns	0.362 ± 0.014	0.373 ± 0.011	ns	0.361 ± 0.017	0.358 ± 0.015	ns	0.346 ± 0.012	0.325 ± 0.01	ns	0.325 ± 0.014	0.325 ± 0.013	ns	0.26 ± 0.019	0.303 ± 0.016	*	0.277 ± 0.017	0.248 ± 0.014	ns
C24+250 $_{C24}$ A	0.346 ± 0.022	0.319 ± 0.019	ns	0.342 ± 0.018	0.351 ± 0.016	ns	0.377 ± 0.014	0.361 ± 0.011	ns	0.371 ± 0.017	0.369 ± 0.015	ns	0.347 ± 0.012	0.346 ± 0.01	ns	0.313 ± 0.014	0.304 ± 0.013	ns	0.299 ± 0.018	0.294 ± 0.016	ns	0.258 ± 0.017	0.261 ± 0.014	ns
C24+250 $_{C24}$ B	0.315 ± 0.022	0.308 ± 0.019	ns	0.349 ± 0.018	0.349 ± 0.016	ns	0.349 ± 0.014	0.345 ± 0.011	ns	0.382 ± 0.018	0.367 ± 0.016	ns	0.346 ± 0.013	0.339 ± 0.01	ns	0.323 ± 0.014	0.323 ± 0.013	ns	0.285 ± 0.018	0.295 ± 0.017	ns	0.238 ± 0.016	0.24 ± 0.014	ns
C24+250 $_{C24}$ C	0.326 ± 0.022	0.347 ± 0.02	ns	0.376 ± 0.018	0.353 ± 0.016	ns	0.368 ± 0.013	0.344 ± 0.011	ns	0.361 ± 0.017	0.379 ± 0.015	ns	0.339 ± 0.012	0.335 ± 0.01	ns	0.312 ± 0.014	0.326 ± 0.013	ns	0.292 ± 0.018	0.297 ± 0.017	ns	0.261 ± 0.016	0.261 ± 0.014	ns
Col+260 $_{Col}$ A	0.367 ± 0.022	0.347 ± 0.02	ns	0.374 ± 0.018	0.359 ± 0.017	ns	0.321 ± 0.013	0.328 ± 0.012	ns	0.386 ± 0.017	0.373 ± 0.016	ns	0.379 ± 0.012	0.379 ± 0.01	ns	0.354 ± 0.014	0.363 ± 0.013	ns	0.355 ± 0.018	0.358 ± 0.017	ns	0.336 ± 0.016	0.341 ± 0.014	ns
Col+260 $_{Col}$ B	0.401 ± 0.023	0.346 ± 0.02	*	0.354 ± 0.019	0.342 ± 0.017	ns	0.317 ± 0.015	0.323 ± 0.012	ns	0.352 ± 0.018	0.373 ± 0.016	ns	0.368 ± 0.013	0.385 ± 0.01	ns	0.348 ± 0.015	0.349 ± 0.013	ns	0.337 ± 0.019	0.33 ± 0.017	ns	0.319 ± 0.017	0.307 ± 0.015	ns
Col+260 $_{Col}$ C	0.339 ± 0.022	0.34 ± 0.02	ns	0.375 ± 0.018	0.364 ± 0.016	ns	0.314 ± 0.014	0.317 ± 0.011	ns	0.383 ± 0.017	0.382 ± 0.015	ns	0.394 ± 0.012	0.387 ± 0.01	ns	0.369 ± 0.014	0.367 ± 0.013	ns	0.348 ± 0.018	0.346 ± 0.017	ns	0.317 ± 0.016	0.317 ± 0.014	ns
Col+260 $_{C24}$ A	0.347 ± 0.022	0.341 ± 0.02	ns	0.332 ± 0.018	0.351 ± 0.016	ns	0.323 ± 0.013	0.326 ± 0.012	ns	0.369 ± 0.017	0.359 ± 0.016	ns	0.373 ± 0.011	0.378 ± 0.01	ns	0.335 ± 0.014	0.354 ± 0.013	ns	0.326 ± 0.018	0.339 ± 0.017	ns	0.301 ± 0.016	0.303 ± 0.014	ns
Col+260 $_{C24}$ B	0.351 ± 0.022	0.355 ± 0.02	ns	0.351 ± 0.018	0.341 ± 0.016	ns	0.31 ± 0.014	0.297 ± 0.011	ns	0.366 ± 0.017	0.36 ± 0.016	ns	0.357 ± 0.012	0.368 ± 0.01	ns	0.336 ± 0.014	0.34 ± 0.013	ns	0.333 ± 0.018	0.336 ± 0.017	ns	0.301 ± 0.016	0.312 ± 0.014	ns
Col+260 $_{C24}$ C	0.341 ± 0.022	0.323 ± 0.02	ns	0.349 ± 0.018	0.334 ± 0.016	ns	0.326 ± 0.014	0.293 ± 0.012	ns	0.379 ± 0.017	0.366 ± 0.016	ns	0.363 ± 0.012	0.357 ± 0.01	ns	0.334 ± 0.014	0.327 ± 0.013	ns	0.336 ± 0.018	0.327 ± 0.017	ns	0.289 ± 0.017	0.304 ± 0.014	ns
C24+260 $_{Col}$ A	0.317 ± 0.022	0.31 ± 0.02	ns	0.35 ± 0.018	0.338 ± 0.016	ns	0.349 ± 0.013	0.346 ± 0.011	ns	0.376 ± 0.017	0.362 ± 0.015	ns	0.334 ± 0.011	0.35 ± 0.01	ns	0.307 ± 0.014	0.303 ± 0.013	ns	0.309 ± 0.018	0.296 ± 0.017	ns	0.24 ± 0.016	0.24 ± 0.014	ns
C24+260 $_{Col}$ B	0.326 ± 0.022	0.316 ± 0.019	ns	0.332 ± 0.018	0.328 ± 0.016	ns	0.338 ± 0.013	0.349 ± 0.011	ns	0.368 ± 0.017	0.37 ± 0.015	ns	0.344 ± 0.012	0.347 ± 0.01	ns	0.325 ± 0.014	0.32 ± 0.013	ns	0.302 ± 0.018	0.305 ± 0.016	ns	0.265 ± 0.016	0.244 ± 0.014	ns
C24+260 $_{Col}$ C	0.331 ± 0.022	0.33 ± 0.02	ns	0.355 ± 0.018	0.355 ± 0.016	ns	0.363 ± 0.014	0.348 ± 0.012	ns	0.386 ± 0.017	0.374 ± 0.016	ns	0.345 ± 0.012	0.34 ± 0.01	ns	0.297 ± 0.014	0.315 ± 0.013	ns	0.304 ± 0.019	0.297 ± 0.017	ns	0.255 ± 0.017	0.257 ± 0.014	ns
C24+260 $_{C24}$ A	0.309 ± 0.022	0.308 ± 0.02	ns	0.359 ± 0.018	0.357 ± 0.016	ns	0.341 ± 0.013	0.345 ± 0.011	ns	0.387 ± 0.017	0.365 ± 0.016	ns	0.348 ± 0.012	0.341 ± 0.01	ns	0.317 ± 0.014	0.311 ± 0.013	ns	0.327 ± 0.018	0.316 ± 0.017	ns	0.27 ± 0.016	0.26 ± 0.014	ns
C24+260 $_{C24}$ B	0.336 ± 0.022	0.319 ± 0.02	ns	0.336 ± 0.018	0.325 ± 0.016	ns	0.351 ± 0.014	0.337 ± 0.012	ns	0.358 ± 0.017	0.366 ± 0.016	ns	0.345 ± 0.012	0.35 ± 0.01	ns	0.331 ± 0.014	0.329 ± 0.013	ns	0.291 ± 0.018	0.296 ± 0.017	ns	0.232 ± 0.016	0.251 ± 0.015	ns
C24+260 $_{C24}$ C	0.355 ± 0.023	0.348 ± 0.019	ns	0.339 ± 0.019	0.341 ± 0.016	ns	0.328 ± 0.015	0.325 ± 0.011	ns	0.339 ± 0.018	0.342 ± 0.015	ns	0.343 ± 0.013	0.341 ± 0.01	ns	0.336 ± 0.015	0.329 ± 0.013	ns	0.286 ± 0.019	0.289 ± 0.017	ns	0.257 ± 0.017	0.251 ± 0.014	ns
Col+270 $_{Col}$ A	0.325 ± 0.023	0.336 ± 0.019	ns	0.341 ± 0.019	0.336 ± 0.016	ns	0.32 ± 0.015	0.32 ± 0.011	ns	0.385 ± 0.018	0.382 ± 0.015	ns	0.367 ± 0.013	0.369 ± 0.01	ns	0.349 ± 0.015	0.348 ± 0.012	ns	0.322 ± 0.019	0.327 ± 0.016	ns	0.328 ± 0.017	0.307 ± 0.014	ns
Col+270 $_{Col}$ B	0.311 ± 0.029	0.33 ± 0.019	ns	0.33 ± 0.025	0.344 ± 0.016	ns	0.331 ± 0.021	0.32 ± 0.011	ns	0.375 ± 0.022	0.371 ± 0.015	ns	0.367 ± 0.018	0.367 ± 0.009	ns	0.344 ± 0.019	0.349 ± 0.012	ns	0.335 ± 0.024	0.333 ± 0.016	ns	0.3 ± 0.023	0.302 ± 0.014	ns
Col+270 $_{Col}$ C	0.339 ± 0.023	0.335 ± 0.019	ns	0.339 ± 0.023	0.33 ± 0.017	ns	0.331 ± 0.019	0.314 ± 0.011	ns	0.429 ± 0.018	0.385 ± 0.015	*	0.348 ± 0.013	0.362 ± 0.01	ns	0.343 ± 0.015	0.356 ± 0.012	ns	0.323 ± 0.02	0.326 ± 0.016	ns	0.3 ± 0.018	0.3 ± 0.014	ns
Col+270 $_{C24}$ A	0.318 ± 0.023	0.336 ± 0.02	ns	0.336 ± 0.019	0.339 ± 0.017	ns	0.324 ± 0.014	0.323 ± 0.011	ns	0.371 ± 0.017	0.369 ± 0.016	ns	0.351 ± 0.012	0.358 ± 0.01	ns	0.351 ± 0.014	0.359 ± 0.013	ns	0.327 ± 0.019	0.341 ± 0.017	ns	0.306 ± 0.017	0.312 ± 0.015	ns
Col+270 $_{C24}$ B	0.346 ± 0.023	0.351 ± 0.02	ns	0.396 ± 0.02	0.371 ± 0.017	ns	0.308 ± 0.015	0.335 ± 0.011	ns	0.374 ± 0.018	0.366 ± 0.016	ns	0.378 ± 0.013	0.371 ± 0.01	ns	0.362 ± 0.015	0.369 ± 0.013	ns	0.349 ± 0.02	0.335 ± 0.017	ns	0.323 ± 0.018	0.318 ± 0.014	ns
Col+270 $_{C24}$ C	0.346 ± 0.023	0.341 ± 0.02	ns	0.359 ± 0.02	0.354 ± 0.017	ns	0.33 ± 0.015	0.312 ± 0.012	ns	0.382 ± 0.017	0.377 ± 0.015	ns	0.361 ± 0.012	0.362 ± 0.01	ns	0.369 ± 0.014	0.362 ± 0.013	ns	0.337 ± 0.019	0.333 ± 0.017	ns	0.315 ± 0.017	0.311 ± 0.014	ns
C24+270 $_{Col}$ A	0.293 ± 0.021	0.3 ± 0.02	ns	0.352 ± 0.018	0.352 ± 0.017	ns	0.36 ± 0.013	0.349 ± 0.011	ns	0.32 ± 0.017	0.326 ± 0.015	ns	0.334 ± 0.011	0.329 ± 0.01	ns	0.303 ± 0.014	0.303 ± 0.013	ns	0.253 ± 0.018	0.258 ± 0.017	ns	0.229 ± 0.016	0.225 ± 0.014	ns
C24+270 $_{Col}$ B	0.282 ± 0.022	0.278 ± 0.02	ns	0.332 ± 0.019	0.344 ± 0.017	ns	0.317 ± 0.014	0.327 ± 0.011	ns	0.314 ± 0.017	0.307 ± 0.015	ns	0.331 ± 0.012	0.311 ± 0.01	ns	0.292 ± 0.014	0.274 ± 0.013	ns	0.221 ± 0.019	0.242 ± 0.017	ns	0.24 ± 0.017	0.254 ± 0.014	ns
C24+270 $_{Col}$ C	0.258 ± 0.024	0.285 ± 0.019	ns	0.334 ± 0.02	0.333 ± 0.016	ns	0.257 ± 0.016	0.288 ± 0.011	ns	0.322 ± 0.019	0.299 ± 0.015	ns	0.262 ± 0.014	0.286 ± 0.01	ns	0.26 ± 0.016	0.271 ± 0.012	ns	0.262 ± 0.02	0.22 ± 0.016	*	0.247 ± 0.019	0.25 ± 0.014	ns
C24+270 $_{C24}$ A	0.294 ± 0.024	0.284 ± 0.019	ns	0.365 ± 0.02	0.369 ± 0.016	ns	0.315 ± 0.016	0.314 ± 0.011	ns	0.319 ± 0.019	0.316 ± 0.015	ns	0.315 ± 0.014	0.314 ± 0.01	ns	0.286 ± 0.016	0.288 ± 0.013	ns	0.227 ± 0.02	0.254 ± 0.017	ns	0.234 ± 0.018	0.252 ± 0.014	ns
C24+270 $_{C24}$ B	0.272 ± 0.022	0.269 ± 0.02	ns	0.355 ± 0.02	0.337 ± 0.017	ns	0.309 ± 0.016	0.287 ± 0.012	ns	0.304 ± 0.017	0.302 ± 0.016	ns	0.328 ± 0.012	0.323 ± 0.01	ns	0.263 ± 0.014	0.248 ± 0.013	ns	0.248 ± 0.018	0.257 ± 0.017	ns	0.276 ± 0.016	0.264 ± 0.014	ns

Appendix 13. continued

Genotype	RGR 09-10			RGR 10-11			RGR 11-12			RGR 12-13			RGR 13-14			RGR 14-15			RGR 15-16			RGR 16-17		
	NT	T	Sig.	NT	T	Sig.	NT	T	Sig.	**NT**	**T**	Sig.	**NT**	**T**	Sig.	**NT**	**T**	Sig.	**NT**	**T**	Sig.	**NT**	**T**	Sig.
C24+270$_{C24}$ C	0.317 ± 0.023	0.31 ± 0.02	ns	0.331 ± 0.019	0.346 ± 0.017	ns	0.349 ± 0.014	0.346 ± 0.012	ns	0.346 ± 0.018	0.341 ± 0.016	ns	0.308 ± 0.013	0.315 ± 0.01	ns	0.309 ± 0.015	0.309 ± 0.013	ns	0.291 ± 0.019	0.258 ± 0.017	ns	0.238 ± 0.017	0.258 ± 0.015	ns
Col+280$_{Col}$ A	0.337 ± 0.022	0.332 ± 0.02	ns	0.327 ± 0.018	0.329 ± 0.016	ns	0.304 ± 0.014	0.306 ± 0.012	ns	0.37 ± 0.017	0.351 ± 0.016	ns	0.348 ± 0.012	0.35 ± 0.01	ns	0.342 ± 0.014	0.333 ± 0.013	ns	0.328 ± 0.018	0.329 ± 0.017	ns	0.294 ± 0.017	0.286 ± 0.014	ns
Col+280$_{Col}$ B	0.329 ± 0.022	0.313 ± 0.02	ns	0.349 ± 0.018	0.371 ± 0.016	ns	0.319 ± 0.014	0.319 ± 0.012	ns	0.37 ± 0.017	0.369 ± 0.016	ns	0.394 ± 0.012	0.396 ± 0.01	ns	0.36 ± 0.014	0.35 ± 0.013	ns	0.344 ± 0.018	0.332 ± 0.017	ns	0.318 ± 0.016	0.309 ± 0.014	ns
Col+280$_{Col}$ C	0.334 ± 0.022	0.336 ± 0.02	ns	0.368 ± 0.018	0.372 ± 0.016	ns	0.315 ± 0.014	0.313 ± 0.011	ns	0.381 ± 0.018	0.38 ± 0.016	ns	0.374 ± 0.012	0.376 ± 0.01	ns	0.339 ± 0.014	0.344 ± 0.013	ns	0.34 ± 0.018	0.338 ± 0.017	ns	0.316 ± 0.016	0.307 ± 0.014	ns
Col+280$_{C24}$ A	0.336 ± 0.023	0.38 ± 0.022	ns	0.293 ± 0.019	0.32 ± 0.018	ns	0.276 ± 0.014	0.276 ± 0.013	ns	0.321 ± 0.018	0.309 ± 0.017	ns	0.329 ± 0.012	0.317 ± 0.012	ns	0.292 ± 0.015	0.292 ± 0.014	ns	0.305 ± 0.019	0.287 ± 0.018	ns	0.226 ± 0.017	0.275 ± 0.016	*
Col+280$_{C24}$ B	0.347 ± 0.023	0.36 ± 0.02	ns	0.312 ± 0.019	0.323 ± 0.017	ns	0.33 ± 0.014	0.351 ± 0.012	ns	0.389 ± 0.018	0.356 ± 0.016	*	0.347 ± 0.012	0.347 ± 0.01	ns	0.342 ± 0.014	0.334 ± 0.013	ns	0.324 ± 0.019	0.311 ± 0.017	ns	0.25 ± 0.017	0.235 ± 0.015	ns
Col+280$_{C24}$ C	0.337 ± 0.022	0.345 ± 0.02	ns	0.325 ± 0.018	0.333 ± 0.016	ns	0.344 ± 0.014	0.352 ± 0.011	ns	0.358 ± 0.017	0.34 ± 0.015	ns	0.354 ± 0.012	0.342 ± 0.01	ns	0.304 ± 0.014	0.317 ± 0.013	ns	0.284 ± 0.019	0.298 ± 0.017	ns	0.225 ± 0.017	0.24 ± 0.014	ns
C24+280$_{Col}$ A	0.343 ± 0.022	0.318 ± 0.02	ns	0.334 ± 0.018	0.343 ± 0.016	ns	0.308 ± 0.013	0.318 ± 0.012	ns	0.364 ± 0.017	0.368 ± 0.016	ns	0.361 ± 0.012	0.35 ± 0.01	ns	0.334 ± 0.014	0.326 ± 0.013	ns	0.314 ± 0.018	0.321 ± 0.017	ns	0.296 ± 0.016	0.301 ± 0.014	ns
C24+280$_{Col}$ B	0.314 ± 0.022	0.349 ± 0.02	ns	0.362 ± 0.018	0.374 ± 0.016	ns	0.315 ± 0.014	0.322 ± 0.012	ns	0.387 ± 0.017	0.379 ± 0.016	ns	0.373 ± 0.012	0.368 ± 0.01	ns	0.358 ± 0.014	0.344 ± 0.013	ns	0.337 ± 0.019	0.341 ± 0.017	ns	0.324 ± 0.017	0.318 ± 0.014	ns
C24+280$_{Col}$ C	0.343 ± 0.022	0.321 ± 0.019	ns	0.341 ± 0.018	0.341 ± 0.016	ns	0.328 ± 0.014	0.304 ± 0.011	ns	0.365 ± 0.017	0.36 ± 0.015	ns	0.358 ± 0.012	0.351 ± 0.01	ns	0.335 ± 0.014	0.343 ± 0.013	ns	0.347 ± 0.019	0.333 ± 0.016	ns	0.305 ± 0.017	0.302 ± 0.014	ns
C24+280$_{C24}$ A	0.365 ± 0.026	0.354 ± 0.019	ns	0.352 ± 0.021	0.351 ± 0.016	ns	0.332 ± 0.018	0.331 ± 0.011	ns	0.37 ± 0.02	0.373 ± 0.015	ns	0.38 ± 0.016	0.378 ± 0.01	ns	0.377 ± 0.017	0.376 ± 0.013	ns	0.316 ± 0.022	0.34 ± 0.016	ns	0.291 ± 0.02	0.315 ± 0.014	ns
C24+280$_{C24}$ B	0.31 ± 0.02	0.319 ± 0.02	ns	0.339 ± 0.017	0.352 ± 0.017	ns	0.349 ± 0.012	0.345 ± 0.012	ns	0.349 ± 0.016	0.352 ± 0.016	ns	0.347 ± 0.01	0.363 ± 0.01	ns	0.328 ± 0.013	0.325 ± 0.013	ns	0.306 ± 0.017	0.304 ± 0.017	ns	0.25 ± 0.015	0.262 ± 0.015	ns
C24+280$_{C24}$ C	0.3 ± 0.024	0.323 ± 0.02	ns	0.34 ± 0.019	0.351 ± 0.016	ns	0.34 ± 0.015	0.338 ± 0.012	ns	0.357 ± 0.018	0.344 ± 0.016	ns	0.333 ± 0.013	0.33 ± 0.01	ns	0.305 ± 0.015	0.31 ± 0.013	ns	0.267 ± 0.02	0.277 ± 0.017	ns	0.24 ± 0.018	0.255 ± 0.014	ns
Col+290$_{Col}$ A	0.338 ± 0.024	0.354 ± 0.02	ns	0.355 ± 0.02	0.36 ± 0.016	ns	0.31 ± 0.015	0.313 ± 0.011	ns	0.388 ± 0.019	0.383 ± 0.015	ns	0.366 ± 0.014	0.38 ± 0.01	ns	0.354 ± 0.015	0.359 ± 0.013	ns	0.336 ± 0.02	0.343 ± 0.016	ns	0.314 ± 0.018	0.32 ± 0.014	ns
Col+290$_{Col}$ B	0.348 ± 0.023	0.353 ± 0.019	ns	0.356 ± 0.019	0.341 ± 0.016	ns	0.311 ± 0.014	0.322 ± 0.011	ns	0.378 ± 0.018	0.381 ± 0.015	ns	0.384 ± 0.012	0.376 ± 0.01	ns	0.35 ± 0.014	0.356 ± 0.012	ns	0.345 ± 0.019	0.349 ± 0.016	ns	0.323 ± 0.017	0.317 ± 0.014	ns
Col+290$_{Col}$ C	0.32 ± 0.023	0.333 ± 0.02	ns	0.365 ± 0.018	0.37 ± 0.016	ns	0.304 ± 0.014	0.313 ± 0.011	ns	0.373 ± 0.017	0.375 ± 0.015	ns	0.364 ± 0.012	0.368 ± 0.01	ns	0.367 ± 0.014	0.35 ± 0.013	ns	0.347 ± 0.018	0.347 ± 0.016	ns	0.317 ± 0.016	0.314 ± 0.014	ns
Col+290$_{C24}$ A	0.397 ± 0.029	0.366 ± 0.019	ns	0.391 ± 0.025	0.369 ± 0.016	ns	0.348 ± 0.021	0.347 ± 0.011	ns	0.379 ± 0.022	0.367 ± 0.015	ns	0.374 ± 0.018	0.36 ± 0.009	ns	0.356 ± 0.019	0.355 ± 0.012	ns	0.354 ± 0.024	0.332 ± 0.016	ns	0.288 ± 0.023	0.295 ± 0.014	ns
Col+290$_{C24}$ B	0.355 ± 0.024	0.367 ± 0.02	ns	0.376 ± 0.019	0.367 ± 0.017	ns	0.327 ± 0.014	0.327 ± 0.011	ns	0.361 ± 0.018	0.392 ± 0.015	ns	0.366 ± 0.013	0.369 ± 0.01	ns	0.355 ± 0.015	0.349 ± 0.013	ns	0.33 ± 0.019	0.333 ± 0.017	ns	0.299 ± 0.018	0.305 ± 0.014	ns
Col+290$_{C24}$ C	0.327 ± 0.021	0.334 ± 0.02	ns	0.379 ± 0.02	0.396 ± 0.017	ns	0.334 ± 0.015	0.337 ± 0.012	ns	0.399 ± 0.017	0.387 ± 0.016	ns	0.368 ± 0.011	0.374 ± 0.01	ns	0.365 ± 0.014	0.363 ± 0.013	ns	0.325 ± 0.018	0.337 ± 0.017	ns	0.329 ± 0.016	0.333 ± 0.014	ns
C24+290$_{Col}$ A	0.336 ± 0.021	0.33 ± 0.02	ns	0.328 ± 0.018	0.321 ± 0.016	ns	0.344 ± 0.013	0.344 ± 0.011	ns	0.363 ± 0.017	0.367 ± 0.015	ns	0.352 ± 0.011	0.346 ± 0.01	ns	0.33 ± 0.014	0.336 ± 0.013	ns	0.342 ± 0.018	0.327 ± 0.017	ns	0.288 ± 0.016	0.276 ± 0.014	ns
C24+290$_{Col}$ B	0.303 ± 0.021	0.321 ± 0.02	ns	0.324 ± 0.018	0.327 ± 0.016	ns	0.341 ± 0.013	0.345 ± 0.012	ns	0.363 ± 0.017	0.364 ± 0.016	ns	0.343 ± 0.011	0.343 ± 0.01	ns	0.329 ± 0.014	0.334 ± 0.013	ns	0.323 ± 0.018	0.317 ± 0.017	ns	0.261 ± 0.016	0.267 ± 0.014	ns
C24+290$_{Col}$ C	0.345 ± 0.024	0.353 ± 0.019	ns	0.332 ± 0.02	0.335 ± 0.016	ns	0.344 ± 0.015	0.341 ± 0.011	ns	0.375 ± 0.019	0.377 ± 0.015	ns	0.343 ± 0.014	0.348 ± 0.01	ns	0.321 ± 0.015	0.314 ± 0.013	ns	0.32 ± 0.02	0.314 ± 0.016	ns	0.261 ± 0.018	0.247 ± 0.014	ns
C24+290$_{C24}$ A	0.305 ± 0.024	0.295 ± 0.02	ns	0.333 ± 0.021	0.363 ± 0.017	ns	0.368 ± 0.016	0.358 ± 0.012	ns	0.381 ± 0.019	0.357 ± 0.016	ns	0.334 ± 0.014	0.315 ± 0.01	ns	0.299 ± 0.016	0.307 ± 0.013	ns	0.313 ± 0.02	0.286 ± 0.017	ns	0.253 ± 0.018	0.247 ± 0.015	ns
C24+290$_{C24}$ B	0.301 ± 0.026	0.294 ± 0.02	ns	0.39 ± 0.022	0.395 ± 0.017	ns	0.347 ± 0.018	0.345 ± 0.012	ns	0.347 ± 0.02	0.369 ± 0.016	ns	0.336 ± 0.015	0.333 ± 0.01	ns	0.289 ± 0.016	0.286 ± 0.013	ns	0.285 ± 0.021	0.256 ± 0.017	ns	0.297 ± 0.02	0.285 ± 0.015	ns
C24+290$_{C24}$ C	0.308 ± 0.021	0.301 ± 0.02	ns	0.373 ± 0.018	0.359 ± 0.017	ns	0.325 ± 0.012	0.322 ± 0.011	ns	0.314 ± 0.017	0.326 ± 0.015	ns	0.341 ± 0.011	0.349 ± 0.01	ns	0.305 ± 0.014	0.308 ± 0.013	ns	0.243 ± 0.018	0.271 ± 0.017	ns	0.287 ± 0.016	0.28 ± 0.014	ns
Col+300$_{Col}$ A	0.33 ± 0.022	0.329 ± 0.02	ns	0.347 ± 0.019	0.335 ± 0.017	ns	0.318 ± 0.015	0.299 ± 0.012	ns	0.379 ± 0.017	0.376 ± 0.015	ns	0.362 ± 0.012	0.355 ± 0.01	ns	0.338 ± 0.014	0.341 ± 0.013	ns	0.323 ± 0.018	0.313 ± 0.017	ns	0.299 ± 0.016	0.296 ± 0.014	ns
Col+300$_{Col}$ B	0.334 ± 0.022	0.316 ± 0.019	ns	0.337 ± 0.019	0.339 ± 0.016	ns	0.332 ± 0.014	0.328 ± 0.011	ns	0.379 ± 0.017	0.372 ± 0.015	ns	0.372 ± 0.012	0.372 ± 0.01	ns	0.328 ± 0.014	0.333 ± 0.013	ns	0.307 ± 0.019	0.307 ± 0.016	ns	0.297 ± 0.017	0.302 ± 0.014	ns
Col+300$_{Col}$ C	0.35 ± 0.022	0.347 ± 0.019	ns	0.357 ± 0.019	0.348 ± 0.016	ns	0.332 ± 0.014	0.334 ± 0.011	ns	0.379 ± 0.017	0.367 ± 0.015	ns	0.366 ± 0.012	0.347 ± 0.01	ns	0.338 ± 0.014	0.347 ± 0.013	ns	0.325 ± 0.018	0.311 ± 0.017	ns	0.284 ± 0.017	0.298 ± 0.014	ns
Col+300$_{C24}$ A	0.331 ± 0.021	0.334 ± 0.02	ns	0.344 ± 0.018	0.377 ± 0.016	*	0.31 ± 0.013	0.318 ± 0.011	ns	0.386 ± 0.017	0.41 ± 0.015	ns	0.377 ± 0.011	0.365 ± 0.01	ns	0.328 ± 0.014	0.347 ± 0.013	ns	0.325 ± 0.018	0.336 ± 0.017	ns	0.301 ± 0.016	0.319 ± 0.014	ns
Col+300$_{C24}$ B	0.322 ± 0.022	0.343 ± 0.02	ns	0.368 ± 0.018	0.354 ± 0.016	ns	0.3 ± 0.014	0.317 ± 0.012	ns	0.408 ± 0.017	0.386 ± 0.016	ns	0.38 ± 0.012	0.378 ± 0.01	ns	0.365 ± 0.014	0.354 ± 0.013	ns	0.341 ± 0.018	0.343 ± 0.017	ns	0.334 ± 0.016	0.325 ± 0.015	ns
Col+300$_{C24}$ C	0.368 ± 0.023	0.339 ± 0.02	ns	0.348 ± 0.018	0.337 ± 0.017	ns	0.334 ± 0.014	0.324 ± 0.012	ns	0.369 ± 0.018	0.379 ± 0.016	ns	0.379 ± 0.012	0.369 ± 0.01	ns	0.337 ± 0.014	0.35 ± 0.013	ns	0.326 ± 0.018	0.315 ± 0.017	ns	0.307 ± 0.017	0.284 ± 0.015	ns
C24+300$_{Col}$ A	0.325 ± 0.022	0.321 ± 0.02	ns	0.357 ± 0.018	0.352 ± 0.017	ns	0.352 ± 0.013	0.351 ± 0.011	ns	0.346 ± 0.017	0.348 ± 0.016	ns	0.305 ± 0.012	0.306 ± 0.01	ns	0.324 ± 0.014	0.305 ± 0.013	ns	0.287 ± 0.018	0.281 ± 0.017	ns	0.238 ± 0.016	0.252 ± 0.014	ns
C24+300$_{Col}$ B	0.335 ± 0.024	0.303 ± 0.02	ns	0.354 ± 0.021	0.349 ± 0.017	ns	0.368 ± 0.016	0.355 ± 0.012	ns	0.371 ± 0.019	0.361 ± 0.015	ns	0.329 ± 0.014	0.319 ± 0.01	ns	0.309 ± 0.015	0.303 ± 0.013	ns	0.282 ± 0.02	0.275 ± 0.017	ns	0.224 ± 0.018	0.236 ± 0.014	ns
C24+300$_{Col}$ C	0.327 ± 0.023	0.321 ± 0.02	ns	0.348 ± 0.019	0.337 ± 0.017	ns	0.353 ± 0.014	0.336 ± 0.011	ns	0.354 ± 0.018	0.367 ± 0.016	ns	0.31 ± 0.012	0.313 ± 0.01	ns	0.321 ± 0.014	0.323 ± 0.013	ns	0.322 ± 0.019	0.312 ± 0.017	ns	0.251 ± 0.017	0.27 ± 0.015	ns
C24+300$_{C24}$ A	0.37 ± 0.024	0.337 ± 0.02	ns	0.33 ± 0.02	0.344 ± 0.016	ns	0.335 ± 0.016	0.311 ± 0.012	ns	0.374 ± 0.019	0.353 ± 0.016	ns	0.349 ± 0.014	0.356 ± 0.01	ns	0.327 ± 0.015	0.336 ± 0.013	ns	0.346 ± 0.02	0.311 ± 0.017	ns	0.268 ± 0.02	0.286 ± 0.015	ns
C24+300$_{C24}$ B	0.345 ± 0.023	0.347 ± 0.02	ns	0.344 ± 0.018	0.332 ± 0.016	ns	0.342 ± 0.014	0.342 ± 0.011	ns	0.4 ± 0.017	0.385 ± 0.015	ns	0.358 ± 0.012	0.35 ± 0.01	ns	0.328 ± 0.014	0.332 ± 0.013	ns	0.352 ± 0.018	0.319 ± 0.017	ns	0.297 ± 0.016	0.277 ± 0.014	ns
C24+300$_{C24}$ C	0.339 ± 0.023	0.353 ± 0.02	ns	0.337 ± 0.019	0.337 ± 0.016	ns	0.316 ± 0.014	0.322 ± 0.011	ns	0.355 ± 0.018	0.36 ± 0.015	ns	0.369 ± 0.012	0.354 ± 0.01	ns	0.319 ± 0.014	0.328 ± 0.013	ns	0.351 ± 0.019	0.356 ± 0.017	ns	0.289 ± 0.017	0.317 ± 0.014	ns
Col+305$_{Col}$ A	0.336 ± 0.022	0.321 ± 0.02	ns	0.35 ± 0.018	0.355 ± 0.017	ns	0.327 ± 0.013	0.321 ± 0.012	ns	0.375 ± 0.017	0.368 ± 0.016	ns	0.375 ± 0.012	0.361 ± 0.01	ns	0.343 ± 0.014	0.342 ± 0.013	ns	0.34 ± 0.018	0.334 ± 0.017	ns	0.312 ± 0.017	0.311 ± 0.015	ns
Col+305$_{Col}$ B	0.329 ± 0.023	0.323 ± 0.019	ns	0.348 ± 0.019	0.349 ± 0.016	ns	0.32 ± 0.014	0.312 ± 0.011	ns	0.383 ± 0.018	0.379 ± 0.015	ns	0.369 ± 0.013	0.377 ± 0.01	ns	0.342 ± 0.015	0.345 ± 0.012	ns	0.332 ± 0.019	0.324 ± 0.016	ns	0.308 ± 0.017	0.306 ± 0.014	ns
Col+305$_{Col}$ C	0.326 ± 0.021	0.316 ± 0.019	ns	0.323 ± 0.018	0.336 ± 0.016	ns	0.325 ± 0.013	0.313 ± 0.011	ns	0.379 ± 0.017	0.379 ± 0.015	ns	0.35 ± 0.011	0.347 ± 0.01	ns	0.341 ± 0.014	0.34 ± 0.013	ns	0.328 ± 0.018	0.317 ± 0.017	ns	0.293 ± 0.016	0.295 ± 0.014	ns
Col+305$_{C24}$ A	0.34 ± 0.021	0.334 ± 0.02	ns	0.349 ± 0.017	0.356 ± 0.016	ns	0.325 ± 0.013	0.315 ± 0.012	ns	0.379 ± 0.017	0.389 ± 0.016	ns	0.357 ± 0.012	0.367 ± 0.01	ns	0.337 ± 0.014	0.338 ± 0.013	ns	0.338 ± 0.018	0.346 ± 0.017	ns	0.295 ± 0.016	0.308 ± 0.014	ns
Col+305$_{C24}$ B	0.349 ± 0.021	0.339 ± 0.019	ns	0.339 ± 0.018	0.323 ± 0.016	ns	0.297 ± 0.013	0.307 ± 0.011	ns	0.38 ± 0.017	0.366 ± 0.015	ns	0.378 ± 0.011	0.361 ± 0.01	ns	0.343 ± 0.014	0.336 ± 0.013	ns	0.326 ± 0.018	0.327 ± 0.017	ns	0.31 ± 0.016	0.3 ± 0.014	ns
Col+305$_{C24}$ C	0.313 ± 0.023	0.346 ± 0.019	ns	0.366 ± 0.018	0.354 ± 0.016	ns	0.318 ± 0.014	0.308 ± 0.011	ns	0.39 ± 0.018	0.384 ± 0.015	ns	0.39 ± 0.012	0.386 ± 0.01	ns	0.358 ± 0.014	0.352 ± 0.013	ns	0.343 ± 0.019	0.346 ± 0.016	ns	0.325 ± 0.017	0.312 ± 0.014	ns
C24+305$_{Col}$ A	0.31 ± 0.021	0.309 ± 0.019	ns	0.348 ± 0.017	0.345 ± 0.016	ns	0.343 ± 0.013	0.34 ± 0.011	ns	0.357 ± 0.017	0.343 ± 0.015	ns	0.329 ± 0.011	0.34 ± 0.01	ns	0.305 ± 0.014	0.328 ± 0.013	ns	0.279 ± 0.018	0.291 ± 0.017	ns	0.243 ± 0.016	0.217 ± 0.014	ns
C24+305$_{Col}$ B	0.285 ± 0.022	0.303 ± 0.019	ns	0.369 ± 0.018	0.344 ± 0.016	ns	0.34 ± 0.013	0.353 ± 0.011	ns	0.373 ± 0.017	0.371 ± 0.015	ns	0.331 ± 0.012	0.326 ± 0.01	ns	0.31 ± 0.014	0.311 ± 0.013	ns	0.274 ± 0.018	0.273 ± 0.016	ns	0.232 ± 0.016	0.258 ± 0.014	ns
C24+305$_{Col}$ C	0.309 ± 0.04	0.31 ± 0.019	ns	0.367 ± 0.028	0.335 ± 0.016	ns	0.351 ± 0.025	0.32 ± 0.011	ns	0.334 ± 0.027	0.34 ± 0.015	ns	0.345 ± 0.022	0.33 ± 0.01	ns	0.385 ± 0.022	0.311 ± 0.012	***	0.319 ± 0.028	0.31 ± 0.016	ns	0.283 ± 0.028	0.258 ± 0.014	ns
C24+305$_{C24}$ A	0.314 ± 0.022	0.316 ± 0.02	ns	0.359 ± 0.018	0.37 ± 0.016	ns	0.367 ± 0.014	0.328 ± 0.011	ns	0.381 ± 0.017	0.331 ± 0.015	**	0.328 ± 0.012	0.307 ± 0.01	ns	0.332 ± 0.014	0.287 ± 0.013	***	0.321 ± 0.018	0.237 ± 0.017	***	0.232 ± 0.016	0.193 ± 0.014	*
C24+305$_{C24}$ B	0.318 ± 0.023	0.316 ± 0.019	ns	0.358 ± 0.019	0.355 ± 0.016	ns	0.335 ± 0.015	0.336 ± 0.011	ns	0.381 ± 0.018	0.373 ± 0.015	ns	0.383 ± 0.013	0.375 ± 0.01	ns	0.355 ± 0.015	0.351 ± 0.013	ns	0.294 ± 0.019	0.299 ± 0.016	ns	0.311 ± 0.018	0.296 ± 0.014	ns
C24+305$_{C24}$ C	0.303 ± 0.023	0.299 ± 0.019	ns	0.376 ± 0.019	0.352 ± 0.016	ns	0.369 ± 0.015	0.331 ± 0.011	ns	0.36 ± 0.018	0.316 ± 0.015	*	0.337 ± 0.013	0.303 ± 0.01	*	0.319 ± 0.015	0.268 ± 0.013	***	0.27 ± 0.019	0.239 ± 0.017	ns	0.263 ± 0.017	0.237 ± 0.014	ns
Col+310$_{Col}$ A	0.321 ± 0.022	0.271 ± 0.02	*	0.341 ± 0.018	0.335 ± 0.017	ns	0.306 ± 0.013	0.304 ± 0.011	ns	0.381 ± 0.017	0.368 ± 0.015	ns	0.351 ± 0.012	0.334 ± 0.01	ns	0.354 ± 0.014	0.293 ± 0.013	***	0.331 ± 0.018	0.293 ± 0.017	*	0.311 ± 0.016	0.265 ± 0.014	**
Col+310$_{Col}$ B	0.329 ± 0.022	0.306 ± 0.02	ns	0.339 ± 0.019	0.326 ± 0.017	ns	0.319 ± 0.014	0.318 ± 0.011	ns	0.369 ± 0.017	0.348 ± 0.016	ns	0.362 ± 0.012	0.333 ± 0.01	ns	0.345 ± 0.014	0.31 ± 0.013	*	0.314 ± 0.019	0.285 ± 0.017	ns	0.293 ± 0.017	0.261 ± 0.015	ns
Col+310$_{Col}$ C	0.335 ± 0.047	0.311 ± 0.019	ns	0.31 ± 0.037	0.313 ± 0.017	ns	0.293 ± 0.034	0.29 ± 0.011	ns	0.412 ± 0.035	0.362 ± 0.015	ns	0.352 ± 0.03	0.329 ± 0.009	ns	0.349 ± 0.03	0.308 ± 0.012	ns	0.317 ± 0.037	0.3 ± 0.016	ns	0.316 ± 0.037	0.281 ± 0.014	ns
Col+310$_{C24}$ A	0.331 ± 0.024	0.286 ± 0.02	ns	0.359 ± 0.02	0.339 ± 0.016	ns	0.33 ± 0.016	0.304 ± 0.011	ns	0.363 ± 0.019	0.328 ± 0.015	*	0.368 ± 0.014	0.339 ± 0.01	ns	0.341 ± 0.016	0.294 ± 0.013	**	0.334 ± 0.02	0.291 ± 0.016	*	0.324 ± 0.018	0.294 ± 0.014	ns
Col+310$_{C24}$ B	0.332 ± 0.022	0.283 ± 0.02	*	0.344 ± 0.018	0.326 ± 0.016	ns	0.313 ± 0.014	0.303 ± 0.012	ns	0.361 ± 0.017	0.336 ± 0.016	ns	0.362 ± 0.012	0.312 ± 0.01	***	0.353 ± 0.014	0.301 ± 0.013	***	0.316 ± 0.018	0.301 ± 0.017	ns	0.31 ± 0.016	0.289 ± 0.014	ns
Col+310$_{C24}$ C	0.34 ± 0.022	0.304 ± 0.02	ns	0.329 ± 0.018	0.328 ± 0.016	ns	0.318 ± 0.013	0.293 ± 0.011	ns	0.36 ± 0.017	0.338 ± 0.015	ns	0.36 ± 0.012	0.338 ± 0.01	ns	0.335 ± 0.014	0.292 ± 0.013	**	0.327 ± 0.018	0.288 ± 0.017	*	0.298 ± 0.016	0.278 ± 0.014	ns
C24+310$_{Col}$ A	0.241 ± 0.026	0.275 ± 0.02	ns	0.319 ± 0.022	0.34 ± 0.017	ns	0.296 ± 0.017	0.32 ± 0.012	ns	0.3 ± 0.02	0.323 ± 0.016	ns	0.29 ± 0.015	0.33 ± 0.01	*	0.275 ± 0.017	0.296 ± 0.013	ns	0.256 ± 0.021	0.242 ± 0.017	ns	0.258 ± 0.02	0.256 ± 0.015	ns
C24+310$_{Col}$ B	0.309 ± 0.026	0.275 ± 0.023	ns	0.331 ± 0.021	0.312 ± 0.019	ns	0.322 ± 0.016	0.296 ± 0.014	ns	0.357 ± 0.019	0.312 ± 0.018	*	0.329 ± 0.014	0.285 ± 0.012	*	0.302 ± 0.016	0.265 ± 0.014	*	0.298 ± 0.021	0.221 ± 0.019	***	0.31 ± 0.019	0.163 ± 0.017	*
C24+310$_{Col}$ C	0.257 ± 0.033	0.292 ± 0.02	ns	0.372 ± 0.026	0.354 ± 0.017	ns	0.337 ± 0.023	0.315 ± 0.012	ns	0.295 ± 0.025	0.324 ± 0.016	ns	0.279 ± 0.02	0.318 ± 0.011	ns	0.287 ± 0.021	0.279 ± 0.013	ns	0.227 ± 0.026	0.256 ± 0.017	ns	0.252 ± 0.025	0.241 ± 0.015	ns
C24+310$_{C24}$ A	0.32 ± 0.021	0.306 ± 0.02	ns	0.355 ± 0.017	0.345 ± 0.017	ns	0.355 ± 0.013	0.358 ± 0.012	ns	0.37 ± 0.016	0.356 ± 0.016	ns	0.341 ± 0.011	0.345 ± 0.01	ns	0.301 ± 0.013	0.308 ± 0.013	ns	0.29 ± 0.017	0.3 ± 0.017	ns	0.257 ± 0.015	0.248 ± 0.015	ns
C24+310$_{C24}$ B	0.307 ± 0.022	0.305 ± 0.02	ns	0.341 ± 0.018	0.308 ± 0.016	*	0.35 ± 0.013	0.305 ± 0.011	ns	0.331 ± 0.017	0.313 ± 0.016	ns	0.339 ± 0.012	0.322 ± 0.01	ns	0.304 ± 0.014	0.281 ± 0.013	ns	0.283 ± 0.018	0.279 ± 0.017	ns	0.24 ± 0.016	0.195 ± 0.014	*
C24+310$_{C24}$ C	0.295 ± 0.021	0.303 ± 0.02	ns	0.345 ± 0.018	0.309 ± 0.016	*	0.357 ± 0.013	0.313 ± 0.012	ns	0.369 ± 0.017	0.332 ± 0.016	*	0.353 ± 0.011	0.328 ± 0.01	ns	0.315 ± 0.014	0.287 ± 0.013	*	0.303 ± 0.018	0.303 ± 0.017	ns	0.243 ± 0.016	0.23 ± 0.014	ns
Col+315$_{Col}$ A	0.334 ± 0.022	0.339 ± 0.019	ns	0.335 ± 0.018	0.325 ± 0.016	ns	0.311 ± 0.014	0.313 ± 0.011	ns	0.375 ± 0.017	0.379 ± 0.015	ns	0.378 ± 0.012	0.367 ± 0.01	ns	0.341 ± 0.014	0.33 ± 0.013	ns	0.331 ± 0.018	0.326 ± 0.017	ns	0.299 ± 0.016	0.297 ± 0.014	ns
Col+315$_{Col}$ B	0.328 ± 0.023	0.338 ± 0.02	ns	0.36 ± 0.019	0.351 ± 0.016	ns	0.327 ± 0.014	0.32 ± 0.011	ns	0.376 ± 0.018	0.371 ± 0.016	ns	0.362 ± 0.013	0.372 ± 0.01	ns	0.35 ± 0.014	0.347 ± 0.013	ns	0.336 ± 0.019	0.33 ± 0.017	ns	0.32 ± 0.017	0.316 ± 0.014	ns
Col+315$_{Col}$ C	0.344 ± 0.024	0.328 ± 0.02	ns	0.351 ± 0.02	0.369 ± 0.016	ns	0.32 ± 0.015	0.327 ± 0.011	ns	0.384 ± 0.019	0.37 ± 0.015	ns	0.394 ± 0.014	0.378 ± 0.01	ns	0.358 ± 0.015	0.345 ± 0.013	ns	0.332 ± 0.02	0.347 ± 0.017	ns	0.324 ± 0.018	0.322 ± 0.014	ns
Col+315$_{C24}$ A	0.349 ± 0.022	0.343 ± 0.019	ns	0.34 ± 0.018	0.338 ± 0.016	ns	0.309 ± 0.014	0.317 ± 0.011	ns	0.369 ± 0.017	0.372 ± 0.015	ns	0.364 ± 0.012	0.358 ± 0.01	ns	0.341 ± 0.014	0.348 ± 0.012	ns	0.34 ± 0.018	0.337 ± 0.016	ns	0.305 ± 0.016	0.304 ± 0.014	ns

Appendix 13. continued

Genotype	RGR 09-10			RGR 10-11			RGR 11-12			RGR 12-13			RGR 13-14			RGR 14-15			RGR 15-16			RGR 16-17		
	NT	T	Sig.	NT	T	Sig.	NT	T	Sig.	NT	T	Sig.	NT	T	Sig.	NT	T	Sig.	NT	T	Sig.	NT	T	Sig.
Col+315 $_{C24}$ B	0.331 ± 0.022	0.328 ± 0.019	ns	0.348 ± 0.018	0.355 ± 0.016	ns	0.319 ± 0.014	0.307 ± 0.011	ns	0.378 ± 0.017	0.376 ± 0.016	ns	0.345 ± 0.012	0.348 ± 0.01	ns	0.343 ± 0.014	0.34 ± 0.013	ns	0.334 ± 0.018	0.326 ± 0.016	ns	0.29 ± 0.016	0.291 ± 0.014	ns
Col+315 $_{C24}$ C	0.292 ± 0.023	0.322 ± 0.02	ns	0.344 ± 0.019	0.331 ± 0.016	ns	0.308 ± 0.015	0.302 ± 0.012	ns	0.367 ± 0.018	0.368 ± 0.016	ns	0.357 ± 0.013	0.347 ± 0.01	ns	0.347 ± 0.015	0.34 ± 0.013	ns	0.316 ± 0.019	0.317 ± 0.017	ns	0.309 ± 0.018	0.291 ± 0.014	ns
C24+315 $_{Col}$ A	0.315 ± 0.023	0.314 ± 0.019	ns	0.345 ± 0.019	0.331 ± 0.016	ns	0.332 ± 0.014	0.335 ± 0.011	ns	0.353 ± 0.018	0.348 ± 0.015	ns	0.354 ± 0.013	0.339 ± 0.01	ns	0.332 ± 0.015	0.327 ± 0.013	ns	0.258 ± 0.019	0.278 ± 0.016	ns	0.227 ± 0.017	0.25 ± 0.014	ns
C24+315 $_{Col}$ B	0.3 ± 0.023	0.321 ± 0.02	ns	0.348 ± 0.019	0.35 ± 0.016	ns	0.341 ± 0.014	0.369 ± 0.011	ns	0.349 ± 0.018	0.346 ± 0.016	ns	0.347 ± 0.013	0.329 ± 0.01	ns	0.322 ± 0.015	0.317 ± 0.013	ns	0.28 ± 0.019	0.276 ± 0.017	ns	0.243 ± 0.017	0.248 ± 0.014	ns
C24+315 $_{Col}$ C	0.273 ± 0.022	0.287 ± 0.019	ns	0.354 ± 0.018	0.354 ± 0.016	ns	0.343 ± 0.013	0.348 ± 0.011	ns	0.344 ± 0.017	0.335 ± 0.015	ns	0.338 ± 0.012	0.338 ± 0.01	ns	0.315 ± 0.014	0.307 ± 0.013	ns	0.291 ± 0.018	0.294 ± 0.017	ns	0.236 ± 0.016	0.224 ± 0.014	ns
C24+315 $_{C24}$ A	0.292 ± 0.021	0.296 ± 0.02	ns	0.352 ± 0.017	0.349 ± 0.017	ns	0.346 ± 0.013	0.347 ± 0.012	ns	0.339 ± 0.017	0.339 ± 0.016	ns	0.334 ± 0.011	0.341 ± 0.01	ns	0.327 ± 0.014	0.323 ± 0.013	ns	0.288 ± 0.018	0.287 ± 0.017	ns	0.241 ± 0.016	0.255 ± 0.014	ns
C24+315 $_{C24}$ B	0.309 ± 0.022	0.328 ± 0.019	ns	0.328 ± 0.018	0.33 ± 0.016	ns	0.354 ± 0.014	0.353 ± 0.011	ns	0.374 ± 0.017	0.37 ± 0.016	ns	0.358 ± 0.012	0.343 ± 0.01	ns	0.324 ± 0.014	0.319 ± 0.013	ns	0.29 ± 0.018	0.315 ± 0.016	ns	0.274 ± 0.016	0.279 ± 0.014	ns
C24+315 $_{C24}$ C	0.34 ± 0.022	0.329 ± 0.02	ns	0.326 ± 0.018	0.333 ± 0.016	ns	0.36 ± 0.013	0.349 ± 0.011	ns	0.368 ± 0.017	0.364 ± 0.015	ns	0.345 ± 0.012	0.353 ± 0.01	ns	0.324 ± 0.014	0.332 ± 0.013	ns	0.326 ± 0.018	0.315 ± 0.017	ns	0.247 ± 0.016	0.258 ± 0.014	ns
Col+320 $_{Col}$ A	0.303 ± 0.026	0.335 ± 0.019	ns	0.35 ± 0.021	0.328 ± 0.016	ns	0.308 ± 0.017	0.296 ± 0.011	ns	0.364 ± 0.02	0.35 ± 0.015	ns	0.345 ± 0.015	0.361 ± 0.01	ns	0.327 ± 0.017	0.339 ± 0.012	ns	0.336 ± 0.021	0.338 ± 0.016	ns	0.282 ± 0.02	0.29 ± 0.014	ns
Col+320 $_{Col}$ B	0.318 ± 0.022	0.33 ± 0.02	ns	0.339 ± 0.018	0.348 ± 0.016	ns	0.302 ± 0.013	0.31 ± 0.011	ns	0.374 ± 0.017	0.362 ± 0.015	ns	0.365 ± 0.012	0.363 ± 0.01	ns	0.355 ± 0.014	0.356 ± 0.013	ns	0.329 ± 0.018	0.351 ± 0.017	ns	0.312 ± 0.016	0.312 ± 0.014	ns
Col+320 $_{Col}$ C	0.353 ± 0.023	0.351 ± 0.019	ns	0.349 ± 0.018	0.328 ± 0.016	ns	0.326 ± 0.014	0.333 ± 0.011	ns	0.388 ± 0.017	0.338 ± 0.015	**	0.364 ± 0.012	0.361 ± 0.01	ns	0.339 ± 0.014	0.327 ± 0.013	ns	0.328 ± 0.018	0.335 ± 0.016	ns	0.306 ± 0.017	0.312 ± 0.014	ns
Col+320 $_{C24}$ A	0.347 ± 0.022	0.338 ± 0.02	ns	0.36 ± 0.018	0.359 ± 0.016	ns	0.31 ± 0.013	0.316 ± 0.011	ns	0.387 ± 0.017	0.381 ± 0.015	ns	0.377 ± 0.011	0.381 ± 0.01	ns	0.35 ± 0.014	0.349 ± 0.013	ns	0.329 ± 0.018	0.335 ± 0.017	ns	0.302 ± 0.016	0.306 ± 0.014	ns
Col+320 $_{C24}$ B	0.304 ± 0.021	0.368 ± 0.02	**	0.386 ± 0.017	0.368 ± 0.016	ns	0.304 ± 0.013	0.307 ± 0.012	ns	0.383 ± 0.017	0.38 ± 0.016	ns	0.373 ± 0.011	0.382 ± 0.01	ns	0.361 ± 0.013	0.361 ± 0.013	ns	0.335 ± 0.017	0.353 ± 0.017	ns	0.334 ± 0.015	0.331 ± 0.014	ns
Col+320 $_{C24}$ C	0.327 ± 0.022	0.33 ± 0.02	ns	0.355 ± 0.018	0.355 ± 0.016	ns	0.324 ± 0.013	0.311 ± 0.011	ns	0.38 ± 0.017	0.374 ± 0.015	ns	0.364 ± 0.012	0.366 ± 0.01	ns	0.351 ± 0.014	0.352 ± 0.013	ns	0.34 ± 0.018	0.335 ± 0.017	ns	0.309 ± 0.016	0.307 ± 0.014	ns
C24+320 $_{Col}$ A	0.338 ± 0.023	0.351 ± 0.02	ns	0.345 ± 0.019	0.341 ± 0.017	ns	0.368 ± 0.014	0.349 ± 0.012	ns	0.358 ± 0.018	0.381 ± 0.016	ns	0.338 ± 0.012	0.356 ± 0.01	ns	0.337 ± 0.014	0.32 ± 0.013	ns	0.325 ± 0.019	0.334 ± 0.017	ns	0.256 ± 0.017	0.273 ± 0.015	ns
C24+320 $_{Col}$ B	0.292 ± 0.022	0.303 ± 0.02	ns	0.338 ± 0.018	0.351 ± 0.017	ns	0.337 ± 0.014	0.335 ± 0.012	ns	0.353 ± 0.017	0.334 ± 0.016	ns	0.335 ± 0.012	0.351 ± 0.01	ns	0.298 ± 0.014	0.291 ± 0.013	ns	0.257 ± 0.018	0.252 ± 0.017	ns	0.268 ± 0.016	0.278 ± 0.015	ns
C24+320 $_{Col}$ C	0.308 ± 0.022	0.306 ± 0.019	ns	0.35 ± 0.018	0.351 ± 0.016	ns	0.34 ± 0.013	0.345 ± 0.011	ns	0.339 ± 0.017	0.347 ± 0.015	ns	0.356 ± 0.012	0.339 ± 0.01	ns	0.314 ± 0.014	0.304 ± 0.013	ns	0.255 ± 0.018	0.236 ± 0.017	ns	0.27 ± 0.016	0.25 ± 0.014	ns
C24+320 $_{C24}$ A	0.351 ± 0.022	0.349 ± 0.02	ns	0.317 ± 0.018	0.322 ± 0.016	ns	0.334 ± 0.014	0.31 ± 0.011	ns	0.344 ± 0.017	0.328 ± 0.015	ns	0.325 ± 0.012	0.33 ± 0.01	ns	0.31 ± 0.014	0.305 ± 0.013	ns	0.287 ± 0.018	0.295 ± 0.017	ns	0.198 ± 0.016	0.223 ± 0.014	ns
C24+320 $_{C24}$ B	0.339 ± 0.023	0.332 ± 0.021	ns	0.322 ± 0.018	0.325 ± 0.017	ns	0.328 ± 0.014	0.336 ± 0.012	ns	0.4 ± 0.017	0.395 ± 0.016	ns	0.354 ± 0.012	0.357 ± 0.01	ns	0.329 ± 0.014	0.332 ± 0.013	ns	0.356 ± 0.019	0.343 ± 0.017	ns	0.292 ± 0.017	0.293 ± 0.015	ns
C24+320 $_{C24}$ C	0.354 ± 0.023	0.397 ± 0.02	ns	0.354 ± 0.019	0.349 ± 0.016	ns	0.32 ± 0.014	0.328 ± 0.012	ns	0.357 ± 0.018	0.366 ± 0.016	ns	0.373 ± 0.012	0.353 ± 0.01	ns	0.341 ± 0.015	0.344 ± 0.013	ns	0.347 ± 0.019	0.335 ± 0.017	ns	0.289 ± 0.017	0.276 ± 0.014	ns
Col+330 $_{Col}$ A	0.331 ± 0.022	0.346 ± 0.02	ns	0.347 ± 0.018	0.346 ± 0.016	ns	0.325 ± 0.014	0.329 ± 0.011	ns	0.408 ± 0.017	0.387 ± 0.015	ns	0.386 ± 0.012	0.384 ± 0.01	ns	0.356 ± 0.014	0.357 ± 0.013	ns	0.359 ± 0.018	0.349 ± 0.017	ns	0.306 ± 0.016	0.306 ± 0.014	ns
Col+330 $_{Col}$ B	0.323 ± 0.022	0.333 ± 0.02	ns	0.36 ± 0.019	0.348 ± 0.017	ns	0.312 ± 0.014	0.315 ± 0.011	ns	0.393 ± 0.017	0.37 ± 0.015	ns	0.385 ± 0.012	0.379 ± 0.01	ns	0.368 ± 0.014	0.355 ± 0.013	ns	0.334 ± 0.019	0.34 ± 0.017	ns	0.31 ± 0.017	0.312 ± 0.014	ns
Col+330 $_{Col}$ C	0.324 ± 0.022	0.338 ± 0.02	ns	0.361 ± 0.019	0.361 ± 0.016	ns	0.318 ± 0.014	0.322 ± 0.011	ns	0.392 ± 0.017	0.379 ± 0.015	ns	0.359 ± 0.012	0.366 ± 0.01	ns	0.358 ± 0.014	0.363 ± 0.013	ns	0.327 ± 0.018	0.339 ± 0.017	ns	0.302 ± 0.016	0.308 ± 0.014	ns
Col+330 $_{C24}$ A	0.323 ± 0.022	0.333 ± 0.02	ns	0.341 ± 0.019	0.37 ± 0.017	ns	0.325 ± 0.015	0.316 ± 0.011	ns	0.396 ± 0.017	0.381 ± 0.016	ns	0.36 ± 0.012	0.358 ± 0.01	ns	0.358 ± 0.014	0.359 ± 0.013	ns	0.342 ± 0.019	0.336 ± 0.017	ns	0.306 ± 0.017	0.3 ± 0.014	ns
Col+330 $_{C24}$ B	0.367 ± 0.024	0.328 ± 0.021	ns	0.347 ± 0.02	0.373 ± 0.018	ns	0.326 ± 0.016	0.307 ± 0.013	ns	0.362 ± 0.019	0.36 ± 0.016	ns	0.37 ± 0.014	0.368 ± 0.011	ns	0.354 ± 0.015	0.355 ± 0.013	ns	0.331 ± 0.02	0.327 ± 0.017	ns	0.314 ± 0.018	0.312 ± 0.015	ns
Col+330 $_{C24}$ C	0.348 ± 0.022	0.335 ± 0.021	ns	0.347 ± 0.018	0.357 ± 0.017	ns	0.322 ± 0.013	0.315 ± 0.012	ns	0.39 ± 0.017	0.388 ± 0.016	ns	0.355 ± 0.012	0.362 ± 0.011	ns	0.355 ± 0.014	0.348 ± 0.013	ns	0.327 ± 0.018	0.33 ± 0.017	ns	0.321 ± 0.016	0.312 ± 0.015	ns
C24+330 $_{Col}$ A	0.288 ± 0.023	0.273 ± 0.02	ns	0.363 ± 0.02	0.356 ± 0.017	ns	0.336 ± 0.016	0.347 ± 0.012	ns	0.341 ± 0.018	0.321 ± 0.016	ns	0.295 ± 0.013	0.302 ± 0.01	ns	0.286 ± 0.015	0.285 ± 0.013	ns	0.244 ± 0.019	0.258 ± 0.017	ns	0.232 ± 0.017	0.265 ± 0.015	ns
C24+330 $_{Col}$ B	0.279 ± 0.022	0.288 ± 0.019	ns	0.374 ± 0.02	0.365 ± 0.017	ns	0.332 ± 0.016	0.345 ± 0.011	ns	0.339 ± 0.017	0.311 ± 0.015	ns	0.313 ± 0.012	0.302 ± 0.01	ns	0.298 ± 0.014	0.29 ± 0.013	ns	0.256 ± 0.019	0.252 ± 0.017	ns	0.216 ± 0.017	0.243 ± 0.014	ns
C24+330 $_{Col}$ C	0.284 ± 0.025	0.299 ± 0.02	ns	0.359 ± 0.021	0.369 ± 0.017	ns	0.341 ± 0.016	0.345 ± 0.011	ns	0.348 ± 0.02	0.323 ± 0.016	ns	0.302 ± 0.015	0.315 ± 0.01	ns	0.336 ± 0.016	0.31 ± 0.013	ns	0.252 ± 0.021	0.267 ± 0.017	ns	0.247 ± 0.019	0.263 ± 0.015	ns
C24+330 $_{C24}$ A	0.277 ± 0.022	0.287 ± 0.02	ns	0.365 ± 0.019	0.367 ± 0.016	ns	0.34 ± 0.014	0.337 ± 0.011	ns	0.359 ± 0.017	0.327 ± 0.015	*	0.322 ± 0.012	0.319 ± 0.01	ns	0.324 ± 0.014	0.321 ± 0.013	ns	0.285 ± 0.019	0.265 ± 0.017	ns	0.232 ± 0.017	0.232 ± 0.014	ns
C24+330 $_{C24}$ B	0.344 ± 0.039	0.306 ± 0.02	ns	0.321 ± 0.031	0.352 ± 0.017	ns	0.334 ± 0.027	0.366 ± 0.011	ns	0.399 ± 0.029	0.345 ± 0.015	ns	0.305 ± 0.025	0.321 ± 0.01	ns	0.316 ± 0.025	0.316 ± 0.013	ns	0.297 ± 0.031	0.273 ± 0.017	ns	0.288 ± 0.031	0.245 ± 0.014	ns
C24+330 $_{C24}$ C	0.281 ± 0.023	0.27 ± 0.019	ns	0.348 ± 0.019	0.347 ± 0.016	ns	0.362 ± 0.014	0.369 ± 0.011	ns	0.35 ± 0.018	0.362 ± 0.015	ns	0.329 ± 0.013	0.326 ± 0.01	ns	0.317 ± 0.015	0.319 ± 0.013	ns	0.266 ± 0.019	0.262 ± 0.016	ns	0.258 ± 0.017	0.25 ± 0.014	ns
Col+335 $_{Col}$ A	0.308 ± 0.022	0.31 ± 0.02	ns	0.3 ± 0.018	0.308 ± 0.016	ns	0.3 ± 0.013	0.277 ± 0.011	ns	0.357 ± 0.017	0.365 ± 0.015	ns	0.342 ± 0.012	0.349 ± 0.01	ns	0.318 ± 0.014	0.329 ± 0.013	ns	0.297 ± 0.019	0.306 ± 0.017	ns	0.287 ± 0.017	0.295 ± 0.014	ns
Col+335 $_{Col}$ B	0.31 ± 0.036	0.302 ± 0.019	ns	0.322 ± 0.029	0.326 ± 0.016	ns	0.339 ± 0.025	0.306 ± 0.011	ns	0.338 ± 0.027	0.358 ± 0.015	ns	0.331 ± 0.023	0.341 ± 0.009	ns	0.33 ± 0.023	0.333 ± 0.012	ns	0.296 ± 0.029	0.302 ± 0.016	ns	0.337 ± 0.028	0.291 ± 0.014	ns
Col+335 $_{Col}$ C	0.316 ± 0.022	0.316 ± 0.019	ns	0.322 ± 0.018	0.329 ± 0.016	ns	0.32 ± 0.014	0.304 ± 0.011	ns	0.364 ± 0.017	0.373 ± 0.015	ns	0.346 ± 0.012	0.344 ± 0.01	ns	0.34 ± 0.014	0.344 ± 0.013	ns	0.303 ± 0.018	0.303 ± 0.017	ns	0.293 ± 0.016	0.301 ± 0.014	ns
Col+335 $_{C24}$ A	0.333 ± 0.022	0.337 ± 0.02	ns	0.324 ± 0.019	0.329 ± 0.017	ns	0.308 ± 0.014	0.302 ± 0.011	ns	0.377 ± 0.017	0.377 ± 0.015	ns	0.362 ± 0.012	0.356 ± 0.01	ns	0.345 ± 0.014	0.343 ± 0.013	ns	0.321 ± 0.018	0.314 ± 0.017	ns	0.286 ± 0.016	0.284 ± 0.014	ns
Col+335 $_{C24}$ B	0.297 ± 0.04	0.329 ± 0.02	ns	0.32 ± 0.039	0.311 ± 0.017	ns	0.341 ± 0.036	0.341 ± 0.012	ns	0.359 ± 0.03	0.358 ± 0.016	ns	0.331 ± 0.026	0.35 ± 0.01	ns	0.323 ± 0.025	0.346 ± 0.013	ns	0.322 ± 0.032	0.313 ± 0.017	ns	0.278 ± 0.032	0.3 ± 0.015	ns
Col+335 $_{C24}$ C	0.358 ± 0.022	0.345 ± 0.02	ns	0.358 ± 0.018	0.367 ± 0.017	ns	0.354 ± 0.013	0.347 ± 0.011	ns	0.37 ± 0.017	0.385 ± 0.016	ns	0.375 ± 0.012	0.373 ± 0.01	ns	0.366 ± 0.014	0.364 ± 0.013	ns	0.335 ± 0.018	0.35 ± 0.017	ns	0.296 ± 0.016	0.313 ± 0.014	ns
C24+335 $_{Col}$ A	0.289 ± 0.022	0.283 ± 0.019	ns	0.363 ± 0.018	0.367 ± 0.016	ns	0.338 ± 0.014	0.315 ± 0.011	ns	0.331 ± 0.017	0.331 ± 0.015	ns	0.331 ± 0.012	0.322 ± 0.01	ns	0.317 ± 0.014	0.323 ± 0.012	ns	0.264 ± 0.018	0.271 ± 0.016	ns	0.27 ± 0.016	0.26 ± 0.014	ns
C24+335 $_{Col}$ B	0.268 ± 0.025	0.27 ± 0.019	ns	0.373 ± 0.025	0.377 ± 0.016	ns	0.337 ± 0.021	0.346 ± 0.011	ns	0.377 ± 0.02	0.329 ± 0.015	**	0.345 ± 0.015	0.338 ± 0.009	ns	0.322 ± 0.016	0.323 ± 0.012	ns	0.276 ± 0.021	0.28 ± 0.016	ns	0.25 ± 0.019	0.249 ± 0.014	ns
C24+335 $_{Col}$ C	0.223 ± 0.03	0.25 ± 0.019	ns	0.285 ± 0.024	0.362 ± 0.016	***	0.258 ± 0.021	0.323 ± 0.011	ns	0.254 ± 0.023	0.32 ± 0.015	**	0.144 ± 0.018	0.326 ± 0.009	***	0.237 ± 0.019	0.283 ± 0.012	*	0.142 ± 0.024	0.267 ± 0.016	***	0.231 ± 0.025	0.282 ± 0.014	*
C24+335 $_{C24}$ A	0.292 ± 0.021	0.297 ± 0.02	ns	0.351 ± 0.018	0.355 ± 0.017	ns	0.37 ± 0.013	0.348 ± 0.011	ns	0.371 ± 0.017	0.369 ± 0.016	ns	0.33 ± 0.011	0.321 ± 0.01	ns	0.31 ± 0.014	0.298 ± 0.013	ns	0.28 ± 0.018	0.277 ± 0.017	ns	0.215 ± 0.016	0.223 ± 0.014	ns
C24+335 $_{C24}$ B	0.289 ± 0.022	0.28 ± 0.019	ns	0.335 ± 0.019	0.35 ± 0.016	ns	0.354 ± 0.014	0.354 ± 0.011	ns	0.296 ± 0.017	0.305 ± 0.015	ns	0.307 ± 0.012	0.307 ± 0.01	ns	0.287 ± 0.014	0.294 ± 0.013	ns	0.264 ± 0.018	0.248 ± 0.017	ns	0.254 ± 0.017	0.237 ± 0.014	ns
C24+335 $_{C24}$ C	0.28 ± 0.022	0.286 ± 0.02	ns	0.35 ± 0.019	0.343 ± 0.017	ns	0.325 ± 0.014	0.319 ± 0.012	ns	0.323 ± 0.017	0.308 ± 0.016	ns	0.305 ± 0.012	0.307 ± 0.01	ns	0.293 ± 0.014	0.282 ± 0.013	ns	0.233 ± 0.019	0.243 ± 0.017	ns	0.286 ± 0.017	0.276 ± 0.014	ns
Col+ORF $_{C24}$ A	0.354 ± 0.024	0.34 ± 0.02	ns	0.352 ± 0.019	0.352 ± 0.016	ns	0.337 ± 0.015	0.323 ± 0.011	ns	0.382 ± 0.018	0.382 ± 0.015	ns	0.363 ± 0.013	0.37 ± 0.01	ns	0.339 ± 0.015	0.342 ± 0.013	ns	0.336 ± 0.019	0.337 ± 0.016	ns	0.299 ± 0.018	0.311 ± 0.014	ns
Col+ORF $_{C24}$ B	0.357 ± 0.022	0.351 ± 0.02	ns	0.353 ± 0.018	0.354 ± 0.016	ns	0.311 ± 0.013	0.314 ± 0.011	ns	0.368 ± 0.017	0.374 ± 0.015	ns	0.37 ± 0.011	0.368 ± 0.01	ns	0.353 ± 0.014	0.343 ± 0.013	ns	0.336 ± 0.018	0.347 ± 0.017	ns	0.319 ± 0.016	0.315 ± 0.014	ns
Col+ORF $_{C24}$ C	0.31 ± 0.023	0.34 ± 0.019	ns	0.342 ± 0.019	0.364 ± 0.016	ns	0.319 ± 0.014	0.296 ± 0.011	ns	0.374 ± 0.018	0.363 ± 0.015	ns	0.364 ± 0.013	0.36 ± 0.01	ns	0.355 ± 0.015	0.351 ± 0.012	ns	0.341 ± 0.019	0.326 ± 0.016	ns	0.324 ± 0.017	0.312 ± 0.014	ns
C24+ORF $_{C24}$ A	0.291 ± 0.026	0.322 ± 0.02	ns	0.345 ± 0.021	0.326 ± 0.016	ns	0.298 ± 0.016	0.345 ± 0.012	ns	0.362 ± 0.019	0.366 ± 0.016	ns	0.36 ± 0.015	0.33 ± 0.01	ns	0.318 ± 0.016	0.315 ± 0.013	ns	0.315 ± 0.021	0.31 ± 0.017	ns	0.277 ± 0.019	0.263 ± 0.014	ns
C24+ORF $_{C24}$ B	0.309 ± 0.024	0.325 ± 0.02	ns	0.34 ± 0.019	0.339 ± 0.017	ns	0.346 ± 0.015	0.366 ± 0.012	ns	0.372 ± 0.018	0.379 ± 0.016	ns	0.369 ± 0.013	0.336 ± 0.01	*	0.317 ± 0.015	0.314 ± 0.013	ns	0.318 ± 0.019	0.31 ± 0.017	ns	0.251 ± 0.018	0.249 ± 0.015	ns
C24+ORF $_{C24}$ C	0.298 ± 0.02	0.299 ± 0.021	ns	0.34 ± 0.017	0.343 ± 0.018	ns	0.361 ± 0.012	0.38 ± 0.013	ns	0.384 ± 0.016	0.372 ± 0.017	ns	0.341 ± 0.011	0.338 ± 0.012	ns	0.302 ± 0.013	0.317 ± 0.014	ns	0.301 ± 0.017	0.314 ± 0.018	ns	0.236 ± 0.015	0.238 ± 0.016	ns

RGR, relative growth rate (in mm2) during the time period (DAS)

T: transgenic siblings, NT: non-transgenic siblings, RGR: Relative growth rate, SE: Standard error, ns: not significant

Significant differences between T and NT with in the same transgenics line and tested by LSD: *,P < 0.5; **,P < 0.01; ***,P < 0.001

Appendix 14: Mean seed area of transgenic lines harboring single genomic clone

Transgenic line	Mean	SD
C24	0.099	0.0127
C24 X Col-0	0.153	0.0154
Col-0	0.100	0.0117
Col-0 X C24	0.123	0.0112
Col+EV A	0.107	0.0224
Col+EV B	0.118	0.0206
Col+EV C	0.127	0.0207
C24+EV A	0.120	0.0171
C24+EV B	0.109	0.0194
C24+EV C	0.110	0.0096
Col+ORF $_{C24}$ A	0.119	0.0226
Col+ORF $_{C24}$ B	0.089	0.0152
Col+ORF $_{C24}$ C	0.115	0.0141
C24+ORF $_{C24}$ A	0.093	0.0151
C24+ORF $_{C24}$ B	0.099	0.0189
C24+ORF $_{C24}$ C	0.098	0.0146
Col+238 $_{Col}$ A	0.100	0.0153
Col+238 $_{Col}$ B	0.111	0.0202
Col+238 $_{Col}$ C	0.104	0.0191
Col+238 $_{C24}$ A	0.114	0.0172
Col+238 $_{C24}$ B	0.103	0.0206
Col+238 $_{C24}$ C	0.083	0.0114
C24+238 $_{Col}$ A	0.113	0.0209
C24+238 $_{Col}$ B	0.107	0.0163
C24+238 $_{Col}$ C	0.092	0.0148
C24+238 $_{C24}$ A	0.101	0.0175
C24+238 $_{C24}$ B	0.096	0.0177
C24+238 $_{C24}$ C	0.121	0.0176
Col+240 $_{Col}$ A	0.099	0.0156
Col+240 $_{Col}$ B	0.103	0.0195
Col+240 $_{Col}$ C	0.116	0.0193
Col+240 $_{C24}$ A	0.099	0.0143
Col+240 $_{C24}$ B	0.085	0.0141
Col+240 $_{C24}$ C	0.084	0.0164
C24+240 $_{Col}$ A	0.110	0.0188
C24+240 $_{Col}$ B	0.111	0.0235
C24+240 $_{Col}$ C	0.086	0.0173
C24+240 $_{C24}$ A	0.084	0.0228
C24+240 $_{C24}$ B	0.110	0.0137
C24+240 $_{C24}$ C	0.117	0.0198
Col+250 $_{Col}$ A	0.081	0.0136
Col+250 $_{Col}$ B	0.077	0.0130
Col+250 $_{Col}$ C	0.083	0.0123
Col+250 $_{C24}$ A	0.108	0.0185
Col+250 $_{C24}$ B	0.092	0.0159
Col+250 $_{C24}$ C	0.113	0.0234
C24+250 $_{Col}$ A	0.104	0.0195
C24+250 $_{Col}$ B	0.088	0.0140
C24+250 $_{Col}$ C	0.095	0.0170
C24+250 $_{C24}$ A	0.095	0.0142
C24+250 $_{C24}$ B	0.104	0.0174
C24+250 $_{C24}$ C	0.096	0.0177
Col+260 $_{Col}$ A	0.079	0.0123
Col+260 $_{Col}$ B	0.145	0.0159
Col+260 $_{Col}$ C	0.091	0.0135
Col+260 $_{C24}$ A	0.103	0.0226
Col+260 $_{C24}$ B	0.104	0.0176
Col+260 $_{C24}$ C	0.125	0.0250
C24+260 $_{Col}$ A	0.102	0.0131
C24+260 $_{Col}$ B	0.102	0.0162
C24+260 $_{Col}$ C	0.098	0.0161
C24+260 $_{C24}$ A	0.111	0.0211
C24+260 $_{C24}$ B	0.110	0.0209
C24+260 $_{C24}$ C	0.150	0.0190
Col+270 $_{Col}$ A	0.106	0.0131
Col+270 $_{Col}$ B	0.108	0.0142
Col+270 $_{Col}$ C	0.097	0.0137
Col+270 $_{C24}$ A	0.092	0.0129
Col+270 $_{C24}$ B	0.099	0.0108
Col+270 $_{C24}$ C	0.116	0.0135
C24+270 $_{Col}$ A	0.098	0.0123
C24+270 $_{Col}$ B	0.120	0.0145
C24+270 $_{Col}$ C	0.143	0.0207
C24+270 $_{C24}$ A	0.115	0.0124
C24+270 $_{C24}$ B	0.149	0.0177
C24+270 $_{C24}$ C	0.093	0.0153
Col+280 $_{Col}$ A	0.115	0.0221
Col+280 $_{Col}$ B	0.089	0.0140
Col+280 $_{Col}$ C	0.100	0.0166
Col+280 $_{C24}$ A	0.113	0.0245
Col+280 $_{C24}$ B	0.090	0.0127
Col+280 $_{C24}$ C	0.107	0.0166
C24+280 $_{Col}$ A	0.118	0.0203
C24+280 $_{Col}$ B	0.109	0.0175
C24+280 $_{Col}$ C	0.114	0.0161
C24+280 $_{C24}$ A	0.137	0.0196
C24+280 $_{C24}$ B	0.107	0.0197
C24+280 $_{C24}$ C	0.114	0.0212
Col+290 $_{Col}$ A	0.088	0.0142
Col+290 $_{Col}$ B	0.087	0.0126
Col+290 $_{Col}$ C	0.092	0.0132
Col+290 $_{C24}$ A	0.088	0.0171
Col+290 $_{C24}$ B	0.095	0.0161

Appendix 14. **continued**

Transgenic line	Mean	SD
Col+290 $_{C24}$ C	0.094	0.0185
C24+290 $_{Col}$ A	0.103	0.0219
C24+290 $_{Col}$ B	0.102	0.0222
C24+290 $_{Col}$ C	0.104	0.0177
C24+290 $_{C24}$ A	0.100	0.0150
C24+290 $_{C24}$ B	0.109	0.0117
C24+290 $_{C24}$ C	0.112	0.0225
Col+300 $_{Col}$ A	0.110	0.0137
Col+300 $_{Col}$ B	0.151	0.0239
Col+300 $_{Col}$ C	0.103	0.0184
Col+300 $_{C24}$ A	0.097	0.0205
Col+300 $_{C24}$ B	0.087	0.0161
Col+300 $_{C24}$ C	0.097	0.0131
C24+300 $_{Col}$ A	0.087	0.0154
C24+300 $_{Col}$ B	0.079	0.0083
C24+300 $_{Col}$ C	0.076	0.0177
C24+300 $_{C24}$ A	0.135	0.0326
C24+300 $_{C24}$ B	0.078	0.0136
C24+300 $_{C24}$ C	0.086	0.0172
Col+305 $_{Col}$ A	0.083	0.0143
Col+305 $_{Col}$ B	0.151	0.0204
Col+305 $_{Col}$ C	0.116	0.0182
Col+305 $_{C24}$ A	0.118	0.0170
Col+305 $_{C24}$ B	0.113	0.0222
Col+305 $_{C24}$ C	0.098	0.0177
C24+305 $_{Col}$ A	0.100	0.0192
C24+305 $_{Col}$ B	0.112	0.0201
C24+305 $_{Col}$ C	0.088	0.0150
C24+305 $_{C24}$ A	0.097	0.0160
C24+305 $_{C24}$ B	0.148	0.0250
C24+305 $_{C24}$ C	0.122	0.0201
Col+310 $_{Col}$ A	0.100	0.0149
Col+310 $_{Col}$ B	0.106	0.0147
Col+310 $_{Col}$ C	0.127	0.0177
Col+310 $_{C24}$ A	0.095	0.0150
Col+310 $_{C24}$ B	0.091	0.0187
Col+310 $_{C24}$ C	0.117	0.0189
C24+310 $_{Col}$ A	0.130	0.0142
C24+310 $_{Col}$ B	0.076	0.0239
C24+310 $_{Col}$ C	0.123	0.0208
C24+310 $_{C24}$ A	0.102	0.0208
C24+310 $_{C24}$ B	0.101	0.0168
C24+310 $_{C24}$ C	0.107	0.0165
Col+315 $_{Col}$ A	0.139	0.0213
Col+315 $_{Col}$ B	0.081	0.0164
Col+315 $_{Col}$ C	0.091	0.0169
Col+315 $_{C24}$ A	0.107	0.0195
Col+315 $_{C24}$ B	0.111	0.0217
Col+315 $_{C24}$ C	0.113	0.0234
C24+315 $_{Col}$ A	0.128	0.0234
C24+315 $_{Col}$ B	0.106	0.0247
C24+315 $_{Col}$ C	0.092	0.0140
C24+315 $_{C24}$ A	0.108	0.0181
C24+315 $_{C24}$ B	0.101	0.0158
C24+315 $_{C24}$ C	0.109	0.0180
Col+320 $_{Col}$ A	0.108	0.0221
Col+320 $_{Col}$ B	0.094	0.0165
Col+320 $_{Col}$ C	0.108	0.0145
Col+320 $_{C24}$ A	0.090	0.0165
Col+320 $_{C24}$ B	0.086	0.0153
Col+320 $_{C24}$ C	0.087	0.0161
C24+320 $_{Col}$ A	0.094	0.0173
C24+320 $_{Col}$ B	0.115	0.0178
C24+320 $_{Col}$ C	0.099	0.0133
C24+320 $_{C24}$ A	0.095	0.0187
C24+320 $_{C24}$ B	0.084	0.0177
C24+320 $_{C24}$ C	0.098	0.0162
Col+330 $_{Col}$ A	0.088	0.0099
Col+330 $_{Col}$ B	0.104	0.0112
Col+330 $_{Col}$ C	0.095	0.0087
Col+330 $_{C24}$ A	0.100	0.0139
Col+330 $_{C24}$ B	0.130	0.0294
Col+330 $_{C24}$ C	0.102	0.0138
C24+330 $_{Col}$ A	0.116	0.0240
C24+330 $_{Col}$ B	0.104	0.0190
C24+330 $_{Col}$ C	0.101	0.0129
C24+330 $_{C24}$ A	0.097	0.0113
C24+330 $_{C24}$ B	0.101	0.0165
C24+330 $_{C24}$ C	0.118	0.0151
Col+335 $_{Col}$ A	0.169	0.0254
Col+335 $_{Col}$ B	0.134	0.0279
Col+335 $_{Col}$ C	0.142	0.0187
Col+335 $_{C24}$ A	0.146	0.0203
Col+335 $_{C24}$ B	0.142	0.0255
Col+335C24C	0.118	0.0141
C24+335 $_{Col}$ A	0.139	0.0164
C24+335 $_{Col}$ B	0.123	0.0126
C24+335 $_{Col}$ C	0.130	0.0120
C24+335 $_{C24}$ A	0.097	0.0111
C24+335 $_{C24}$ B	0.104	0.0167
C24+335 $_{C24}$ C	0.132	0.0108

Mean seed area was estimated with 100 seeds of each transgenic lines in segragating T2 population.

Appendix 15: Mean leaf area of cosmid transgenic lines harboring transferred At4g00310 in T2 segregating generation

Genotype	6 DAS					7 DAS					8 DAS					9 DAS				
	NT		T			NT		T			NT		T			NT		T		
	Mean	SE	Mean	SE	Sig.	Mean	SE	Mean	SE	Sig.	Mean	SE	Mean	SE	Sig.	Mean	SE	Mean	SE	Sig.
Col+EV A	0.18	0.074	0.21	0.045	ns	1.08	0.177	1.10	0.144	ns	2.92	0.315	2.85	0.266	ns	4.70	0.288	4.94	0.195	ns
Col+EV B	0.18	0.088	0.26	0.050	ns	1.31	0.207	1.18	0.143	ns	3.94	0.355	3.39	0.266	ns	6.44	0.354	5.84	0.194	ns
Col+EV C	0.17	0.108	0.12	0.058	ns	0.81	0.184	1.12	0.149	ns	3.27	0.326	3.85	0.273	ns	5.75	0.305	6.39	0.210	ns
C24+EV A	0.15	0.087	0.18	0.057	ns	1.49	0.218	1.22	0.153	ns	4.63	0.382	4.14	0.280	ns	7.81	0.394	6.99	0.223	ns
C24+EV B	0.16	0.073	0.20	0.051	ns	1.37	0.186	1.44	0.149	ns	4.33	0.329	4.83	0.275	ns	7.44	0.314	8.07	0.214	ns
C24+EV C	0.22	0.081	0.21	0.045	ns	1.34	0.192	1.55	0.147	ns	4.63	0.338	4.95	0.273	ns	7.85	0.326	8.29	0.210	ns
Col+310 $_{Col}$ A	0.17	0.066	0.16	0.046	ns	1.25	0.176	0.73	0.147	ns	3.29	0.315	2.61	0.266	ns	5.28	0.287	4.14	0.194	*
Col+310 $_{Col}$ B	0.18	0.063	0.17	0.048	ns	1.42	0.177	1.22	0.146	ns	3.94	0.318	3.59	0.268	ns	6.37	0.293	5.72	0.200	ns
Col+310 $_{Col}$ C	0.32	0.235	0.14	0.050	*	0.92	0.551	0.94	0.139	ns	3.46	0.905	3.11	0.259	ns	5.89	1.110	5.16	0.179	ns
Col+310 $_{Col}$ E	0.19	0.063	0.20	0.044	ns	1.52	0.188	1.32	0.142	ns	3.24	0.332	2.85	0.266	ns	5.31	0.315	4.89	0.194	ns
Col+310 $_{Col}$ F	0.21	0.058	0.14	0.053	ns	1.21	0.177	0.84	0.153	ns	3.09	0.313	2.54	0.273	ns	4.92	0.286	4.06	0.209	ns
C24+310 $_{Col}$ C	0.06	0.235	0.29	0.057	*	1.54	0.755	1.45	0.162	ns	4.09	1.245	4.08	0.291	ns	7.34	1.540	7.03	0.244	ns
C24+310 $_{Col}$ D	0.21	0.098	0.24	0.082	ns	1.65	0.236	0.85	0.218	*	3.82	0.409	2.44	0.368	*	6.71	0.435	4.28	0.372	*
C24+310 $_{Col}$ E	0.15	0.168	0.22	0.066	ns	1.41	0.300	1.54	0.197	ns	4.65	0.513	4.84	0.348	ns	8.11	0.581	7.91	0.342	ns
C24+310 $_{Col}$ F	0.20	0.078	0.21	0.048	ns	1.32	0.196	1.17	0.152	ns	4.31	0.343	3.97	0.279	ns	7.40	0.333	6.63	0.223	ns
C24+310 $_{Col}$ G	0.55	0.234	0.12	0.139	*	3.20	0.755	1.19	0.323	ns	7.11	1.245	3.12	0.513	*	10.30	1.541	5.68	0.581	*
Col+310 $_{C24}$ A	0.10	0.065	0.10	0.067	ns	1.01	0.171	0.68	0.151	ns	2.94	0.305	2.63	0.273	ns	4.87	0.269	4.03	0.210	ns
Col+310 $_{C24}$ B	0.15	0.063	0.18	0.054	ns	1.30	0.186	1.00	0.148	ns	3.12	0.332	2.66	0.270	ns	4.89	0.316	4.13	0.203	ns
Col+310 $_{C24}$ C	0.28	0.062	0.22	0.055	ns	1.46	0.173	0.91	0.146	*	4.19	0.310	3.26	0.270	*	6.71	0.278	5.43	0.203	*
Col+310 $_{C24}$ D	0.24	0.066	0.28	0.044	ns	1.64	0.192	1.52	0.145	ns	4.00	0.340	3.52	0.268	ns	6.10	0.330	5.58	0.200	ns
Col+310 $_{C24}$ E	0.41	0.061	0.27	0.044	ns	1.79	0.171	1.62	0.145	ns	4.15	0.307	4.00	0.269	ns	6.55	0.273	6.34	0.200	ns
C24+310 $_{C24}$ A	0.15	0.074	0.24	0.049	ns	1.07	0.185	1.20	0.145	ns	3.49	0.320	3.93	0.268	ns	6.63	0.296	6.57	0.199	ns
C24+310 $_{C24}$ B	0.23	0.072	0.18	0.047	ns	1.33	0.199	0.96	0.142	ns	4.27	0.343	3.59	0.264	ns	7.49	0.333	6.33	0.190	*
C24+310 $_{C24}$ C	0.11	0.091	0.17	0.054	*	0.74	0.184	0.58	0.151	ns	3.51	0.322	2.59	0.268	*	6.26	0.300	4.73	0.200	*
C24+310 $_{C24}$ D	0.14	0.078	0.18	0.051	ns	1.12	0.179	0.86	0.149	ns	3.87	0.312	3.31	0.270	ns	6.90	0.282	5.83	0.203	*

Each mean value of transgenic lines was compared to the mean value of their non-transgenic siblings.

Significance tested by Fisher's Least Significant Difference (LSD): *,P < 0.5; **,P < 0.01; ***,P < 0.001.

LA: Leaf Area in mm^2, SE: Standard error, ns: not significant

Appendix 15. continued

Genotype	10 DAS					11 DAS					12 DAS					13 DAS				
	NT		T			NT		T			NT		T			NT		T		
	Mean	SE	Mean	SE	Sig.	Mean	SE	Mean	SE	Sig.	Mean	SE	Mean	SE	Sig.	Mean	SE	Mean	SE	Sig.
Col+EV A	6.3	0.36	6.7	0.24	ns	8.9	0.59	9.4	0.45	ns	12.4	1.06	13.0	0.92	ns	17.2	1.42	18.2	1.22	ns
Col+EV B	8.6	0.44	7.8	0.24	ns	12.0	0.69	10.9	0.44	ns	16.6	1.18	14.9	0.91	ns	22.7	1.59	20.3	1.22	ns
Col+EV C	8.0	0.38	8.7	0.26	ns	11.4	0.61	12.3	0.47	ns	16.3	1.09	17.2	0.94	ns	22.8	1.46	23.9	1.25	ns
C24+EV A	10.5	0.49	9.4	0.27	ns	14.5	0.76	12.9	0.49	ns	20.4	1.26	17.8	0.95	ns	27.2	1.70	24.0	1.27	ns
C24+EV B	9.8	0.39	10.8	0.26	ns	13.3	0.63	14.6	0.47	ns	18.3	1.10	20.4	0.94	ns	24.3	1.49	27.2	1.26	ns
C24+EV C	10.5	0.41	11.2	0.26	ns	14.2	0.65	15.4	0.47	ns	19.7	1.13	21.5	0.94	ns	26.2	1.52	28.5	1.25	ns
Col+310 $_{Col}$ A	6.9	0.36	5.4	0.24	*	9.9	0.59	7.2	0.44	*	13.6	1.06	9.8	0.91	*	18.9	1.42	12.9	1.22	*
Col+310 $_{Col}$ B	8.5	0.36	7.5	0.24	ns	12.0	0.59	10.2	0.45	*	16.5	1.07	14.1	0.92	ns	23.1	1.43	19.0	1.23	*
Col+310 $_{Col}$ C	8.4	1.40	6.9	0.22	ns	12.1	2.01	9.5	0.42	ns	16.4	2.89	12.9	0.89	ns	22.7	3.97	16.9	1.19	ns
Col+310 $_{Col}$ E	7.1	0.39	6.4	0.24	ns	9.8	0.63	8.5	0.44	ns	13.7	1.11	11.8	0.91	ns	19.2	1.49	16.0	1.22	ns
Col+310 $_{Col}$ F	6.6	0.36	5.4	0.26	ns	9.1	0.58	7.1	0.47	*	12.7	1.06	9.7	0.93	*	17.3	1.42	12.8	1.25	*
C24+310 $_{Col}$ C	9.8	1.95	9.4	0.30	ns	13.4	2.78	12.9	0.52	ns	18.3	3.94	17.7	0.99	ns	25.0	5.42	24.2	1.32	ns
C24+310 $_{Col}$ D	9.0	0.55	5.7	0.47	*	12.2	0.83	7.4	0.72	*	17.4	1.34	9.8	1.21	*	24.3	1.82	12.8	1.64	*
C24+310 $_{Col}$ E	11.0	0.73	10.6	0.43	ns	15.1	1.08	14.4	0.68	ns	21.9	1.66	20.3	1.16	ns	30.4	2.25	28.0	1.56	ns
C24+310 $_{Col}$ F	10.1	0.42	8.7	0.27	ns	13.7	0.66	11.9	0.49	ns	19.1	1.14	16.5	0.95	ns	26.0	1.54	22.7	1.28	ns
C24+310 $_{Col}$ G	13.0	1.95	7.7	0.73	*	18.9	2.79	10.4	1.08	*	27.6	3.95	14.3	1.65	*	37.0	5.42	19.2	2.25	*
Col+310 $_{C24}$ A	6.5	0.33	5.2	0.26	*	9.2	0.56	7.0	0.47	*	12.8	1.03	9.7	0.94	*	17.8	1.38	12.8	1.25	*
Col+310 $_{C24}$ B	6.7	0.39	5.4	0.25	ns	9.3	0.63	7.1	0.46	*	12.8	1.11	9.9	0.93	*	17.5	1.49	13.0	1.23	*
Col+310 $_{C24}$ C	9.0	0.34	7.2	0.25	*	12.5	0.57	9.7	0.46	*	17.2	1.04	13.2	0.92	*	23.8	1.40	17.7	1.23	*
Col+310 $_{C24}$ D	8.1	0.41	7.3	0.24	ns	11.3	0.65	10.1	0.45	ns	15.5	1.13	13.9	0.92	ns	21.7	1.53	19.2	1.23	ns
Col+310 $_{C24}$ E	8.7	0.34	8.5	0.24	ns	12.0	0.56	11.7	0.45	ns	16.5	1.03	16.0	0.92	ns	22.5	1.39	22.0	1.23	ns
C24+310 $_{C24}$ A	9.1	0.37	9.0	0.24	ns	12.5	0.60	12.2	0.45	ns	17.6	1.07	17.5	0.92	ns	24.8	1.44	24.7	1.23	ns
C24+310 $_{C24}$ B	10.2	0.42	8.5	0.23	*	14.1	0.66	11.3	0.44	*	20.0	1.14	15.5	0.91	*	27.9	1.54	20.5	1.21	*
C24+310 $_{C24}$ C	8.6	0.37	6.5	0.24	*	11.8	0.61	8.6	0.45	*	17.0	1.08	11.7	0.92	*	23.9	1.45	15.9	1.23	*
C24+310 $_{C24}$ D	9.4	0.35	7.9	0.25	*	13.1	0.58	10.6	0.46	*	18.5	1.05	14.2	0.93	*	25.7	1.41	18.0	1.23	*

Appendix 15. continued

Genotype	14 DAS					15 DAS					16 DAS					17 DAS				
	NT		T			NT		T			NT		T			NT		T		
	Mean	SE	Mean	SE	Sig.	Mean	SE	Mean	SE	Sig.	Mean	SE	Mean	SE	Sig.	Mean	SE	Mean	SE	Sig.
Col+EV A	24.1	1.66	25.5	1.34	ns	33	3.1	35	2.8	ns	47	3.6	49	3.2	ns	64	4.1	67	3.5	ns
Col+EV B	31.0	1.92	27.9	1.34	ns	43	3.4	39	2.8	ns	59	3.9	53	3.2	ns	80	4.7	73	3.5	ns
Col+EV C	31.6	1.72	32.8	1.39	ns	44	3.2	45	2.9	ns	62	3.7	63	3.2	ns	85	4.3	87	3.6	ns
C24+EV A	35.1	2.07	30.9	1.43	ns	47	3.5	41	2.9	ns	60	4.2	53	3.3	ns	79	5.0	69	3.7	ns
C24+EV B	30.6	1.75	35.3	1.40	ns	41	3.2	47	2.9	ns	52	3.7	60	3.2	ns	65	4.4	75	3.6	ns
C24+EV C	33.2	1.80	36.5	1.39	ns	43	3.3	48	2.9	ns	55	3.8	61	3.2	ns	68	4.4	77	3.6	ns
Col+310 $_{Col}$ A	26.2	1.66	16.9	1.34	*	36	3.1	23	2.8	*	50	3.6	30	3.1	*	68	4.1	39	3.5	*
Col+310 $_{Col}$ B	31.3	1.68	25.0	1.36	*	43	3.1	33	2.8	*	60	3.6	44	3.2	*	82	4.2	58	3.5	*
Col+310 $_{Col}$ C	31.1	5.21	22.5	1.29	ns	43	7.4	30	2.8	ns	60	9.2	40	3.1	*	80	11.9	54	3.4	*
Col+310 $_{Col}$ E	26.7	1.76	21.6	1.34	*	38	3.2	30	2.8	*	53	3.7	42	3.2	*	72	4.3	59	3.5	*
Col+310 $_{Col}$ F	24.2	1.66	16.8	1.39	*	34	3.1	22	2.9	*	47	3.6	30	3.2	*	64	4.1	39	3.6	*
C24+310 $_{Col}$ C	33.7	7.17	31.1	1.50	ns	45	9.9	41	3.0	ns	63	12.5	52	3.4	ns	78	16.3	67	3.8	ns
C24+310 $_{Col}$ D	31.5	2.24	15.9	1.98	*	43	3.7	20	3.5	*	55	4.4	26	4.0	*	69	5.4	31	4.8	*
C24+310 $_{Col}$ E	37.9	2.86	35.5	1.87	ns	51	4.4	47	3.3	ns	64	5.4	60	3.9	ns	81	6.7	75	4.6	ns
C24+310 $_{Col}$ F	34.0	1.83	29.3	1.43	ns	44	3.3	38	2.9	ns	56	3.8	50	3.3	ns	69	4.5	61	3.7	ns
C24+310 $_{Col}$ G	50.8	7.17	24.9	2.86	*	60	9.9	33	4.4	*	73	12.5	42	5.4	*	95	16.3	54	6.7	*
Col+310 $_{C24}$ A	24.8	1.59	16.7	1.39	*	35	3.1	22	2.9	*	48	3.5	29	3.2	*	67	4.0	39	3.6	*
Col+310 $_{C24}$ B	24.4	1.76	17.0	1.37	*	34	3.2	22	2.9	*	47	3.7	30	3.2	*	66	4.4	40	3.6	*
Col+310 $_{C24}$ C	33.0	1.62	23.1	1.36	*	46	3.1	31	2.9	*	63	3.5	41	3.2	*	87	4.1	54	3.6	*
Col+310 $_{C24}$ D	29.8	1.82	26.4	1.36	ns	42	3.3	37	2.9	ns	58	3.8	50	3.2	ns	80	4.5	69	3.6	ns
Col+310 $_{C24}$ E	31.3	1.60	30.5	1.36	ns	44	3.1	43	2.8	ns	60	3.5	58	3.2	ns	83	4.0	80	3.5	ns
C24+310 $_{C24}$ A	32.6	1.69	32.6	1.35	ns	44	3.2	43	2.8	ns	58	3.6	58	3.2	ns	73	4.2	74	3.5	ns
C24+310 $_{C24}$ B	36.7	1.83	26.3	1.33	*	49	3.3	33	2.8	*	65	3.8	43	3.1	*	81	4.5	54	3.5	*
C24+310 $_{C24}$ C	32.0	1.70	20.0	1.35	*	43	3.2	25	2.8	*	57	3.6	34	3.2	*	71	4.2	44	3.5	*
C24+310 $_{C24}$ D	33.7	1.64	23.7	1.37	*	46	3.1	31	2.9	*	61	3.5	40	3.2	*	77	4.1	51	3.6	*

Appendix 16: Identified metabolite levels in the nine transgenic genotypes

Metabolite	*Col+FUM2 $_{Col}$*	*Col+FUM2 $_{C24}$*	*Col+EV*	*C24+FUM2 $_{Col}$*	*C24+FUM2 $_{C24}$*	*C24+EV*	*fum2+FUM2 $_{Col}$*	*fum2+FUM2 $_{C24}$*	*fum2+EV*
Fructose	3.42 ± 0.13 c	3.46 ± 0.10 c	3.47 ± 0.10 bc	3.83 ± 0.20 a	3.95 ± 0.14 a	3.90 ± 0.16 a	3.45 ± 0.11 c	3.47 ± 0.11 bc	3.60 ± 0.17 b
Sucrose	5.40 ± 0.05 b	5.38 ± 0.05 b	5.39 ± 0.06 b	5.52 ± 0.06 a	5.53 ± 0.06 a	5.51 ± 0.04 a	5.35 ± 0.05 b	5.40 ± 0.05 b	5.40 ± 0.05 b
Glucose	3.53 ± 0.20 c	3.58 ± 0.17 c	3.60 ± 0.17 c	3.97 ± 0.20 a	4.04 ± 0.17 a	3.98 ± 0.13 a	3.60 ± 0.19 c	3.55 ± 0.18 c	3.78 ± 0.28 b
Fumarate	6.04 ± 0.06 a	6.02 ± 0.06 ab	6.05 ± 0.06 a	6.00 ± 0.06 ab	5.96 ± 0.05 b	5.78 ± 0.09 c	6.04 ± 0.07 a	5.99 ± 0.06 ab	5.17 ± 0.05 d
Malate	5.22 ± 0.07 d	5.22 ± 0.07 d	5.23 ± 0.06 d	5.31 ± 0.05 c	5.33 ± 0.05 bc	5.37 ± 0.03 ab	5.24 ± 0.06 d	5.30 ± 0.05 c	5.39 ± 0.06 a
Pyruvate	3.54 ± 0.05 c	3.53 ± 0.04 c	3.54 ± 0.06 c	3.68 ± 0.07 a	3.71 ± 0.06 a	3.70 ± 0.06 a	3.52 ± 0.04 c	3.60 ± 0.03 b	3.68 ± 0.07 a
alpha-Ketoglutarate	2.88 ± 0.09 cd	2.85 ± 0.09 cd	2.89 ± 0.10 cd	3.15 ± 0.08 b	3.20 ± 0.09 ab	3.24 ± 0.08 a	2.82 ± 0.09 d	2.94 ± 0.06 c	3.15 ± 0.05 b
alpha-Aminoadipic acid	2.60 ± 0.10 e	2.72 ± 0.10 cd	2.65 ± 0.10 de	2.99 ± 0.08 a	2.99 ± 0.07 a	3.00 ± 0.06 a	2.69 ± 0.09 cd	2.77 ± 0.07 bc	2.81 ± 0.14 b
Pipecolic acid	2.59 ± 0.13 a	2.60 ± 0.11 a	2.60 ± 0.10 a	2.66 ± 0.09 a	2.66 ± 0.09 a	2.58 ± 0.11 a	2.64 ± 0.12 a	2.65 ± 0.16 a	2.42 ± 0.14 b
Shikimate	2.57 ± 0.04 b-d	2.55 ± 0.05 d	2.56 ± 0.05 cd	2.59 ± 0.05 a-d	2.60 ± 0.04 a-c	2.61 ± 0.04 ab	2.56 ± 0.05 cd	2.61 ± 0.04 ab	2.62 ± 0.04 a
Succinate	3.31 ± 0.06 ef	3.31 ± 0.07 ef	3.36 ± 0.08 de	3.49 ± 0.06 bc	3.61 ± 0.08 a	3.58 ± 0.08 a	3.30 ± 0.08 f	3.42 ± 0.05 cd	3.51 ± 0.06 b
cis-Aconitic acid	2.98 ± 0.06 b	2.98 ± 0.08 b	3.00 ± 0.08 ab	3.03 ± 0.06 ab	3.03 ± 0.05 ab	3.05 ± 0.04 a	2.99 ± 0.08 ab	3.02 ± 0.07 ab	3.01 ± 0.06 ab
beta-Alanine	2.98 ± 0.05 a	3.01 ± 0.06 a	3.00 ± 0.07 a	2.81 ± 0.06 b	2.83 ± 0.08 b	2.80 ± 0.07 b	3.03 ± 0.09 a	3.00 ± 0.04 a	3.03 ± 0.05 a
Ascorbate	3.79 ± 0.16 c	3.76 ± 0.15 c	3.76 ± 0.12 c	4.01 ± 0.16 a	4.03 ± 0.14 a	3.96 ± 0.09 ab	3.83 ± 0.13 c	3.84 ± 0.12 bc	3.83 ± 0.11 c
2-Aminobutanoic acid	2.84 ± 0.07 b	2.85 ± 0.05 b	2.83 ± 0.06 b	2.95 ± 0.06 a	2.95 ± 0.05 a	2.94 ± 0.05 a	2.83 ± 0.05 b	2.86 ± 0.04 b	2.85 ± 0.05 b
4-Aminobutanoic acid	3.78 ± 0.19 a	3.74 ± 0.19 a	3.67 ± 0.14 a	3.76 ± 0.16 a	3.77 ± 0.10 a	3.75 ± 0.16 a	3.68 ± 0.13 a	3.74 ± 0.19 a	3.80 ± 0.16 a
Citrate	5.59 ± 0.07 b	5.61 ± 0.08 b	5.62 ± 0.07 b	5.69 ± 0.05 a	5.70 ± 0.06 a	5.70 ± 0.07 a	5.62 ± 0.08 b	5.63 ± 0.08 ab	5.65 ± 0.07 ab
Dehydroascorbic acid	4.88 ± 0.12 a	4.87 ± 0.11 a	4.88 ± 0.11 a	4.87 ± 0.09 a	4.87 ± 0.12 a	4.89 ± 0.10 a	4.89 ± 0.12 a	4.90 ± 0.14 a	4.92 ± 0.15 a
Erythritol	2.57 ± 0.07 b	2.57 ± 0.08 b	2.59 ± 0.05 b	2.76 ± 0.09 a	2.79 ± 0.06 a	2.75 ± 0.07 a	2.58 ± 0.13 b	2.58 ± 0.08 b	2.57 ± 0.13 b
Galactonic.acid	2.58 ± 0.10 a	2.60 ± 0.12 a	2.61 ± 0.11 a	2.66 ± 0.09 a	2.64 ± 0.11 a	2.69 ± 0.12 a	2.65 ± 0.14 a	2.65 ± 0.16 a	2.69 ± 0.19 a
Glucopyranose	4.64 ± 0.12 b	4.61 ± 0.09 b	4.63 ± 0.12 b	4.81 ± 0.11 a	4.78 ± 0.15 a	4.78 ± 0.12 a	4.61 ± 0.08 b	4.65 ± 0.06 b	4.68 ± 0.07 b
1,6-Anhydro-β-D-glucose	4.25 ± 0.17 ab	4.19 ± 0.12 b	4.24 ± 0.11 ab	4.35 ± 0.14 a	4.30 ± 0.22 ab	4.30 ± 0.17 ab	4.22 ± 0.14 ab	4.25 ± 0.08 ab	4.28 ± 0.10 ab
Glutamic acid	5.12 ± 0.05 a	5.10 ± 0.06 a	5.13 ± 0.05 a	5.11 ± 0.04 a	5.11 ± 0.05 a	5.12 ± 0.05 a	5.10 ± 0.06 a	5.11 ± 0.04 a	5.12 ± 0.05 a
Glycerate	3.75 ± 0.13 b	3.72 ± 0.08 b	3.74 ± 0.11 b	3.81 ± 0.04 ab	3.87 ± 0.09 a	3.89 ± 0.10 a	3.75 ± 0.13 b	3.74 ± 0.09 b	3.76 ± 0.08 b
Homoserine	2.91 ± 0.04 a-c	2.91 ± 0.05 a-c	2.93 ± 0.05 a	2.87 ± 0.07 cd	2.83 ± 0.06 d	2.84 ± 0.06 d	2.92 ± 0.05 ab	2.88 ± 0.04 a-d	2.87 ± 0.06 b-d
Indole-3-acetonitrile	3.74 ± 0.17 a	3.77 ± 0.14 a	3.78 ± 0.13 a	3.43 ± 0.11 b	3.44 ± 0.13 b	3.48 ± 0.13 b	3.79 ± 0.15 a	3.78 ± 0.12 a	3.79 ± 0.10 a
Myo-Inositol	4.47 ± 0.08 ab	4.43 ± 0.05 b	4.44 ± 0.04 ab	4.46 ± 0.06 ab	4.49 ± 0.04 a	4.49 ± 0.05 a	4.47 ± 0.07 ab	4.47 ± 0.07 ab	4.48 ± 0.05 ab
2-Methyl malic acid	3.58 ± 0.13 b	3.54 ± 0.05 b	3.57 ± 0.08 b	3.67 ± 0.05 a	3.68 ± 0.04 a	3.69 ± 0.06 a	3.56 ± 0.06 b	3.58 ± 0.06 b	3.60 ± 0.05 b
Nicotinamide	2.55 ± 0.51 a	2.61 ± 0.39 a	2.74 ± 0.46 a	2.85 ± 0.49 a	2.56 ± 0.53 a	2.92 ± 0.48 a	2.55 ± 0.44 a	2.80 ± 0.40 a	2.54 ± 0.49 a
Phenylalanine	3.70 ± 0.08 b	3.73 ± 0.07 b	3.72 ± 0.08 b	3.81 ± 0.08 a	3.82 ± 0.06 a	3.81 ± 0.06 a	3.69 ± 0.07 b	3.72 ± 0.06 b	3.73 ± 0.07 b
Phosphoric acid	4.38 ± 0.10 bc	4.38 ± 0.11 bc	4.40 ± 0.10 a-c	4.44 ± 0.09 ab	4.45 ± 0.08 ab	4.44 ± 0.09 ab	4.32 ± 0.09 c	4.43 ± 0.09 ab	4.49 ± 0.10 a
Putrescine	3.51 ± 0.08 b	3.57 ± 0.10 b	3.59 ± 0.11 b	3.74 ± 0.10 a	3.74 ± 0.07 a	3.69 ± 0.09 a	3.53 ± 0.10 b	3.53 ± 0.07 b	3.59 ± 0.09 b
Ribitol	3.13 ± 0.07 b	3.10 ± 0.09 b	3.09 ± 0.08 b	3.28 ± 0.08 a	3.27 ± 0.08 a	3.28 ± 0.07 a	3.10 ± 0.09 b	3.11 ± 0.10 b	3.15 ± 0.1 b
cis-Sinapic acid	3.15 ± 0.11 a	3.13 ± 0.10 a	3.14 ± 0.12 a	3.13 ± 0.09 a	3.12 ± 0.09 a	3.12 ± 0.08 a	3.14 ± 0.09 a	3.16 ± 0.09 a	3.15 ± 0.08 a
Spermidine	3.80 ± 0.06 ab	3.79 ± 0.06 ab	3.79 ± 0.08 ab	3.78 ± 0.05 ab	3.77 ± 0.06 b	3.78 ± 0.07 ab	3.77 ± 0.06 b	3.84 ± 0.04 a	3.82 ± 0.08 ab
Trehalose	2.94 ± 0.06 a	2.86 ± 0.07 b	2.90 ± 0.07 ab	2.89 ± 0.07 ab	2.91 ± 0.07 ab	2.91 ± 0.07 ab	2.89 ± 0.07 ab	2.91 ± 0.07 ab	2.89 ± 0.05 ab
Urea	2.53 ± 0.13 a	2.56 ± 0.16 a	2.53 ± 0.12 a	2.56 ± 0.11 a	2.57 ± 0.10 a	2.55 ± 0.11 a	2.53 ± 0.13 a	2.52 ± 0.13 a	2.59 ± 0.09 a
Ornithine	2.99 ± 0.15 b	2.96 ± 0.11 b	3.00 ± 0.08 b	3.20 ± 0.12 a	3.13 ± 0.12 a	3.16 ± 0.12 a	2.99 ± 0.12 b	2.90 ± 0.18 b	2.89 ± 0.18 b
Methionine	3.27 ± 0.14 a	3.17 ± 0.04 c	3.26 ± 0.08 ab	3.24 ± 0.07 ab	3.25 ± 0.06 ab	3.22 ± 0.07 a-c	3.16 ± 0.05 c	3.19 ± 0.05 bc	3.21 ± 0.04 a-c
Glutamine	4.83 ± 0.05 bc	4.83 ± 0.05 bc	4.86 ± 0.06 a-c	4.90 ± 0.08 a	4.89 ± 0.06 a	4.88 ± 0.07 ab	4.81 ± 0.06 c	4.83 ± 0.05 bc	4.85 ± 0.09 a-c
Isoleucine	3.53 ± 0.04 c	3.56 ± 0.05 bc	3.56 ± 0.05 bc	3.59 ± 0.06 ab	3.64 ± 0.05 a	3.60 ± 0.07 ab	3.56 ± 0.04 bc	3.57 ± 0.04 bc	3.60 ± 0.06 ab
Leucine	3.44 ± 0.06 d	3.51 ± 0.07 d	3.51 ± 0.07 d	3.62 ± 0.09 ab	3.68 ± 0.07 a	3.60 ± 0.09 b	3.48 ± 0.06 d	3.51 ± 0.06 cd	3.58 ± 0.09 bc
Lysine	3.67 ± 0.05 a	3.67 ± 0.06 a	3.68 ± 0.05 a	3.67 ± 0.05 a	3.66 ± 0.04 a	3.68 ± 0.04 a	3.69 ± 0.04 a	3.67 ± 0.04 a	3.66 ± 0.07 a
Glycine	4.53 ± 0.12 bc	4.54 ± 0.12 bc	4.57 ± 0.11 bc	5.06 ± 0.12 a	5.04 ± 0.13 a	5.05 ± 0.15 a	4.50 ± 0.16 c	4.51 ± 0.10 c	4.65 ± 0.12 b
Asparagine	3.71 ± 0.07 a-c	3.74 ± 0.05 a	3.74 ± 0.05 a	3.70 ± 0.08 a-c	3.68 ± 0.06 bc	3.67 ± 0.06 c	3.72 ± 0.06 ab	3.72 ± 0.04 a-c	3.71 ± 0.07 a-c
Aspartate	5.19 ± 0.06 ab	5.14 ± 0.04 cd	5.17 ± 0.04 bc	5.12 ± 0.05 d	5.11 ± 0.05 d	5.15 ± 0.04 b-d	5.17 ± 0.06 bc	5.17 ± 0.04 bc	5.23 ± 0.04 a
Threonic acid	3.30 ± 0.06 b	3.25 ± 0.05 c-e	3.27 ± 0.04 b-d	3.23 ± 0.06 de	3.21 ± 0.05 e	3.26 ± 0.06 b-e	3.28 ± 0.07 bc	3.27 ± 0.05 b-d	3.35 ± 0.04 a
Threonine	4.34 ± 0.05 b	4.34 ± 0.04 b	4.33 ± 0.05 b	4.46 ± 0.08 a	4.45 ± 0.05 a	4.47 ± 0.07 a	4.36 ± 0.08 b	4.32 ± 0.04 b	4.35 ± 0.07 b
Proline	4.40 ± 0.10 c	4.44 ± 0.12 bc	4.45 ± 0.09 a-c	4.55 ± 0.12 a	4.55 ± 0.10 a	4.54 ± 0.11 ab	4.53 ± 0.14 ab	4.39 ± 0.09 c	4.52 ± 0.10 ab
Alanine	4.16 ± 0.05 c	4.18 ± 0.05 a-c	4.16 ± 0.05 c	4.23 ± 0.06 a	4.22 ± 0.06 a	4.22 ± 0.05 ab	4.17 ± 0.05 bc	4.19 ± 0.05 a-c	4.22 ± 0.06 ab
Arginine	3.36 ± 0.09 b	3.31 ± 0.10 bc	3.34 ± 0.07 b	3.56 ± 0.10 a	3.52 ± 0.09 a	3.51 ± 0.07 a	3.35 ± 0.08 b	3.31 ± 0.07 bc	3.26 ± 0.08 c
Tryptophan	2.87 ± 0.09 b	2.89 ± 0.10 ab	2.89 ± 0.09 ab	2.88 ± 0.08 ab	2.89 ± 0.09 ab	2.90 ± 0.07 ab	2.90 ± 0.09 ab	2.92 ± 0.10 ab	2.95 ± 0.10 a
Tyramine	3.07 ± 0.08 b	3.14 ± 0.06 a	3.08 ± 0.07 b	2.90 ± 0.05 c	2.87 ± 0.06 c	2.86 ± 0.05 c	3.12 ± 0.06 ab	3.10 ± 0.04 ab	3.11 ± 0.04 ab
Tyrosine	2.79 ± 0.07 d	2.82 ± 0.07 cd	2.83 ± 0.08 cd	2.91 ± 0.08 ab	2.94 ± 0.07 a	2.91 ± 0.04 ab	2.80 ± 0.07 cd	2.84 ± 0.09 b-d	2.87 ± 0.08 bc
Serine	4.54 ± 0.14 b	4.53 ± 0.10 b	4.54 ± 0.10 b	4.87 ± 0.10 a	4.86 ± 0.09 a	4.86 ± 0.15 a	4.54 ± 0.15 b	4.51 ± 0.12 b	4.50 ± 0.12 b
Valine	4.21 ± 0.06 c	4.23 ± 0.04 bc	4.23 ± 0.04 bc	4.28 ± 0.05 a	4.30 ± 0.04 a	4.28 ± 0.05 a	4.21 ± 0.03 c	4.22 ± 0.03 bc	4.26 ± 0.04 ab

Metabolite abundances were adjusted for an internal standard amount and original sample weight, and then $\log_{10}$ transformed.

All values represent in Mean ± SD. Significant differences in metabolites among the 9 genotypes were identified using the Tukey Multiple Comparison test.

9 Curriculum Vitae

Personal data

Name:	Hea-Jung, Jeon
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Education

2010 - 1015 PhD in Natural Sciences, Group Heterosis, Molecular Genetics department, Leibniz Institute for Plant Genetics and Crop Plant Research (IPK), Gatersleben; Martin Luther University Halle-Wittenberg, Germany. Title of thesis: "Analysis of allelic gene effects in *Arabidopsis thaliana* biomass heterosis and metabolism". Supervisors: Dr. Rhonda Meyer, Dr. David Riewe, and Dr. Thomas Altmann

2005 - 2007 MSc in Horticulture (Plant Breeding), Kyung Hee University, Republic of Korea. Title of thesis: "Transformation of chicory with γ-tocopherol methyltransferase and homogentisate phytyltransferase genes". Supervisor: Geun-Won Choi. GPA: 4.263/4.5 (95.25/100)

2001 - 2005 BSc in Horticulture, Kyung Hee University, Republic of Korea. GPA: 3.785/4.2 (93.85/100

Work Experience

Feb. 2008 - Sep. 2009 Institute of Life Science & Resources in Kyung Hee University

Research technician

Feb. 2005 - Feb. 2007 Department of Horticulture, Kyung Hee University

Research Assistant in Plant Genetics and Breeding Laboratory

Poster Presentation at conferences

H.-J. Jeon and G.-W. Choi. 2006. Optimization of plant growth regulator composition and culture temperature for shoot formation from cotyledon explants in *Cichorium intybus var. sativum*. 27th International Horticultural Congress & Exhibition

H.-J. Jeon, RC Meyer, D Riewe, and T Altmann. 2012. Molecular identification and characterization of loci causing biomass heterosis in *Arabidopsis thaliana.* Plant Biology Congress, Freiburg

10 Declaration

Ich erkläre an Eides statt, dass ich die Arbeit selbstständig und ohne fremde Hilfe verfasst, keine anderen als die von mir angegebenen Quellen und Hilfsmittel benutzt und die den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht habe.

I declare under penalty of perjury that this thesis is my own work entirely and has been written without any help from other people. I used only the sources mentioned and included all the citations correctly both in word or content.

09.06.2015 *Hea-Jung, Jeon*