# REPRODUCTIVE GENETICS AND EPIGENETICS OF F1 HYBRID TRIPLOIDS OF *ARABIDOPSIS THALIANA* L.

Volume I of I

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A thesis submitted to National University of Ireland, Galway
For the degree of Doctor of Philosophy

College of Science, School of Natural Sciences
Botany and Plant Science Discipline


Under the supervision of Prof. Charles Spillane
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December 2011

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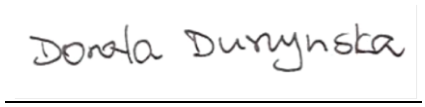
# DECLARATION

This thesis is my own work and has not been submitted for another degree, either at National University of Ireland, Galway or elsewhere.

Signed: 

Dorota Duszynska

# AKNOWLEDGMENTS


- To my supervisor, Prof Charlie Spillane for giving me the opportunity to pursue my PhD degree in the Plant Genetics and Biotechnology Lab, for guidance and support.
- To IRCSET and Thomas Crawford Hayes Trust Research Fund for financial support.
- To my project collaborators: Dr.Tim Sharbel for assistance with flow cytometry and for hosting me in his lab in IPK (Gatersleben, Germany) for several months, Dr. Tom Juenger (Uni Texas, Austin) for providing assistance with population genetics, and to Bjarni Vilhjalmsson (Prof. MagnusNordborg's lab, GMI Vienna) for running GWAS.
- To my GRC committee members – Prof. Brian McStay and Prof. Kevin Sullivan for keeping eye on my progress.
- To all my labmates for sharing your passion to science...and to the College Bar! ☺
- A special thanks to Dr. Peter for lab assistance, good advice, and for taking care of my English grammar! Thank you for your professionalism, optimism, and patience while proof reading and correcting my thesis, manuscripts, and other applications.
- To Kristina for help finding the best accommodation in Galway and for being a great neighbour, together with Gabriel, Eric and little Kili ☺
- To my best roomie – Aurélie, I will never forget those four months under one roof!
- To all my friends from Cork that are very special to me (Klaudia, Aisling & Bart, Camila & Tim, Kamila, Ania, Eduardo, Marie, Lesley & Sven and the Tyndall crew).
- To Pat and his love of volleyball.
- To my friends from Poland - Agnieszka, Żaba, Beti, Kasia, Magda, Karolina, Monika and Magda, because our friendships survived long distance.
- Szczególne podziękowania kieruję do moich Rodziców, brata Mateusza i Ani za siłę i odwagę. Mój sukces zawdzięczam przede wszystkim Waszej wierze we mnie. Dziękuję!
- To John M, Breda, Laura, Shane and Charlie, my Irish Family, for your love that made me feel like at home.
- I would like to dedicate this work to the One who has been supporting me from the first day of my PhD. Words cannot describe how grateful I am - Pádraig, tá a fhios agat is fearr, ní bheadh sé indéanta gan tú... buíochas a ghabháil leat go mór!

## COURSES ATTENDED

- **3rd International PhD School on Plant Development**
  Retzbach-Würzburg, Germany, October 2010

- **School on Apomixis: theory and practice**
  Wageningen, Holland, June 2010

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  UCC, Cork, April 2010

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  UCC, Cork, August – September 2009

## PRESENTATIONS

**2011**

- " Reproduction of F1 hybrid triploids of *Arabidopsis thaliana*" (oral presentation)
  **Botany and Plant Science (BPS) departmental seminar, NUI Galway**

**2010**

- "Genetics and epigenetics of F1 hybrid triploids of *Arabidopsis thaliana*"
  (oral presentation, awarded COST Action conference fellowship)
  **3rd International PhD School on Plant Development, Germany**

- "Screening for asexual reproduction in *Arabidopsis thaliana*"
  (oral presentation, awarded COST Action conference fellowship)
  **School on Apomixis: theory and practice - Training School, Holland**

- Genetics and epigenetics of F1 hybrid triploids of *Arabidopsis thaliana*" (oral presentation) –
  **Irish Plant Scientists Association Meeting (IPSAM), UCD Dublin**

**2009**

- "Natural genetic variation for reproductive characters in reciprocal triploids of *Arabidopsis thaliana"* (oral presentation)
  **Irish Network of Developmental Biologists Meeting (INDB), NUI Galway**

**2008**

- "Apomixis technology development using the model plant *Arabidopsis thaliana*" – (poster presentation)
  **Agricultural Biotechnology International Conference (ABIC), UCC Cork**

- "Screening for asexual reproduction in *Arabidopsis thaliana*" –
  (poster presentation)
  **Irish Plant Scientists Association Meeting (IPSAM), NUI Maynooth**

**2007**

- "Screening for asexual reproduction in *Arabidopsis thaliana"* – (poster presentation – awarded 1st poster prize**)**
  **Irish Plant Scientists Association Meeting (IPSAM), UCC Cork**

- "Screening for asexual reproduction in *Arabidopsis thaliana*" – (oral presentation)
  **Plant Research Platform – UCC Cork**

# ABBREVIATIONS

ABRC – Arabidopsis Biological Resource Centre

AFLP – Amplified Fragment Length Polymorphism

*A.thalina* (At) – *Arabidopsis thaliana*

ANU – (Aborted seeds/Normal seeds/Unfertilized ovules)

ANOVA – ANalysis Of VAriance

BAC – Bacterial Artificial Chromosome

BPH – Best-Parent Heterosis

CAPS – Cleaved Amplified Polymorphic Sequences

cm – centimetre

cM – centiMorgan

DH – Double Haploid

DNA – Deoxyribose Nucleic Acid

EBN – Endosperm Balance Number

GC – Guanine Cytosine

GWA – Genome-Wide Association

K – 1000

Kb – Kilobase

LD – Linkage Disequilibrium

m – Meter

Mb – Megabase

min – minute

mm – millimeter

MMC – Megaspore Mother Cell

MPH – Mid-Parent Heterosis

MSQT – Multiple SNP Query Tool

N – North

NASC – European Arabidopsis Stock Centre

ND – no data

NIL – Near Isogenic Line

PCR – Polymerase Chain Reaction

QTL – Quantitative Trait Locus

QTN – Quantitative Trait Nucleotide

RFLP – Restriction Fragment Length Polymorphism

RIL – Recombinant Inbred Line

RNA – Ribose Nucleic Acid

RNAi – interfering RNA

SASSC – Sendai Arabidopsis Seed Stock Center

SD – Standard Deviation

SE – Standard Error

siRNA – short interfering RNA

SNP – Single Nucleotide Polymorphism

TAIR - The Arabidopsis Information Resource

T-DNA – transferred DNA

TE – Transposable Element

WPH- Worst-Parent Heterosis

A x L – a cross, where diploid (A) accession was used as a seed parent and (L) L*er*-0 as a pollen parent

L x A – a cross, where (L) L*er*-0 was used as a seed parent and (A) accession as a pollen parent

2x X 4x – a cross, where diploid (2x) accession was used as a seed parent and tetraploid (4x) as a pollen parent

4x X 2x – a cross, where tetraploid (4x) accession was used as a seed parent and diploid (2x) as a pollen parent

# ABSTRACT

Polyploidy, the occurrence of more than two complete sets of chromosomes in a single nucleus, is an important process contributing to eukaryotic evolution. Polyploidy is also a widespread speciation mechanism and is common among plants, fish and amphibians. Polyploidy can be advantageous, and lead to heterosis, gene redundancy, and asexual reproduction (apomixis). However, polyploidy makes the completion of mitosis and meiosis more challenging, which often results in production of aneuploid cells and gametes. Improved understanding of the biology of polyploids is essential because many economically important crops are polyploid, including *Solanum tuberosum* (potato), *Triticum* (wheat) and *Gossypium* (cotton).

Polyploid crops also include triploid crops such as *Musa* (banana), *Malus domestica* (apple) and *Beta vulgaris* (sugar beet). Chromosome segregation during triploid meiosis is particularly complicated by the need for the three sets of chromosomes to be resolved to two poles which results in the production of gametes with frequent chromosome loss and fragmentation. Hence, the immediate progeny of triploids can have complex karyotypes, which can differ in the number of copies of each chromosome. Imbalanced dosage effects resulting from aneuploidy can be deleterious to development, and contribute to the dramatic reduction in fertility of triploids.

In this thesis, genome dosage and hybridity effects on reproduction, seed size heterosis and seed development were investigated using a set of F1 hybrid and isogenic triploids of *Arabidopsis thaliana*. These were generated by reciprocal inter-ploidy crosses, incorporating 90 different accessions that can differ genetically and epigenetically. Relative triploid reproductive traits of selfed F1 triploids were analysed by collecting ANU data (A – aborted F2 seeds, N – normal F2 seeds, U- unfertilized ovules) and by converting the datasets to %A, %N, %U with respect to the total number of ovules per silique ($\Sigma$ = A+N+U).

It was discovered that, depending on genotype, selfed F1 isogenic triploids and F1 hybrid triploids exhibit differing levels of fertility defects at the pre-fertilisation (unfertilised F1 ovules) and post-fertilisation stages (normal vs aborted F2 seeds). It was further discovered that epigenetic parent-of-origin effects on reproduction and seed development can occur between reciprocal F1 triploids which have differing contributions of maternal- vs paternal-derived chromosomes. Such epigenetic parent-of-origin effects were observed for both isogenic F1 triploids (Col-0) and for a wide range of hybrid F1 triploid genotypes. The epigenetic parent-of-origin effects either caused greater fertility defects in the maternal excess F1 triploid or in the paternal excess F1 triploid. Flow cytometric analysis of single seed indicated that the surviving F2 seed progeny comprised a wide range of DNA contents, including likely aneuploids with incomplete sets of chromosomes. This supports the proposed role for triploids as evolutionary bridges between plant populations of different ploidy levels.

The 89 diploid accessions used to generate the F1 hybrid triploids have been extensively characterised for SNPs (Single Nucleotide Polymorphisms) across the *Arabidopsis thaliana* genome. This allowed the use of genome wide association (GWA) mapping for the identification of loci involved in control of reciprocal F1 hybrid triploid reproduction and production of normal F2 seed sets (%N). Two candidate loci involved in the phenotypic variation in %N among F2 seeds produced by paternal excess F1 triploids and maternal excess F1 triploids were identified by GWA mapping and named *POT (Paternal Overdose Triploid)* and *MOT* (*Maternal Overdose Triploid)* respectively. Candidate protein-coding genes at the *POT* and *MOT* loci were selected based on their known involvement in the plant reproductive pathways and seed development. Some of these genes are involved in epigenetic processes including DNA methylation, histone modification, and siRNA mediated gene silencing and are strong candidates for explaining the epigenetic parent-of-origin effects which are controlling triploid reproductive success.

# CHAPTER 1 – General Introduction

## 1. *Arabidopsis thaliana* - a model species in plant biology and beyond

*Arabidopsis thaliana* (a mouse-ear cress) is a small annual dicotyledon in the mustard family (*Brassicaceae*) which in the mid-1980s emerged as a phenomenal model organism of choice for studying biology of flowering plants (Rhee et al., 2003). Its small size (~30 cm) and limited growth facilities requirements, also the short lifecycle (~2 months) and rapid production of swarms of seeds (up to 10 000 seeds per plant) by self-fertilization, made it very useful to deal with in the laboratory conditions. Moreover, the smallest plant genome size (125 Mb) amenable to detailed molecular analysis, and rapid advances in genetic transformation by *Agrobacterium*-mediated gene transfer contributed to establishing *A. thaliana,* a model system in plant genetics (Koornneef and Meinke, 2010). Although several other plants of agricultural or horticultural importance, including tomato, maize, pea, rice, barley, petunia, and snapdragon were close to being recognized as model systems, *A. thaliana*, a simple weed of no economic value is the world's most studied angiosperm so far (Meinke et al., 1998, Somerville and Koornneef, 2002) and has called "the botanical Drosophila" (Leonelli, 2007).

### 1.1 The first sequenced plant species

*A. thaliana* is the first plant species for which the sequence of all genes essential for flowering plant life-cycle has been identified (Arabidopsis, 2000). This was followed by a revolutionary new initiative driven by the goal to understand the function and every molecular interaction of all 25 000 protein-coding genes in the sequenced genome by the year 2010. An implicit goal of these international projects was to gain ultimate understanding of the molecular process in the model system and to use this knowledge to bring other plants forward as efficiently as possible (Chory et al., 2000). *A. thaliana* is closely related to hundreds of thousands of other plant species, and therefore the knowledge gathered over decades on this model organism can be applied in many aspects of plant biology in general, not only in crops from *Brassicaceae* family (Somerville and Koornneef, 2002). For example, some key aspects of plant physiology are highly conserved between *Arabidopsis thaliana* and economically important grasses like rice (Kobayashi and Weigel, 2007).

Elucidation of gene function requires detailed phenotypic characterization of genetic variation, which can be induced by mutations or can be found among natural accessions of *Arabidopsis thaliana* (Alonso-Blanco and Koornneef, 2000).

### 1.2 Identification of *Arabidopsis thaliana* gene function – analysis of mutants

The Arabidopsis genome is amenable to forward genetics by which genetic variation is artificially induced, often through radiation or insertional mutagenesis. Mutant plants are then screened for particular phenotypes. Availability of mutants for most of *Arabidopsis thaliana* genes greatly facilitated identification of gene functions and improved our understanding of plant biology (Page and Grossniklaus, 2002). It enabled determination of gene functions by phenotypical analysis of genotypes in which specific genes were disrupted by T-DNA or transposon insertion, or using RNAi technology. However, mutant collections are in a limited number of genetic backgrounds which account for only a small part of *Arabidopsis thaliana* natural variation. Mutant phenotype of genes for which the wild-type accession carries natural null allele or a weak allele might not be detected. Epistatic interactions can cause some phenotypes to appear only in certain genetic backgrounds (Alonso-Blanco and Koornneef, 2000). It was reported that about 9.4% of protein-coding genes of wild accessions of *A. thaliana* are affected by "major-effect change" SNPs which can lead to natural knock-outs (Clark et al., 2007). For example, *FRI* and *FLC* loci responsible for late flowering could not have been identified in mutant screens as the most standard laboratory accessions (L*er*-0, Col-0, Ws) for which mutants are available, have early flowering phenotype and they carry loss of function alleles at *FLC* and/or *FRI* (Koornneef et al., 2004). Moreover, most flowering variation among *Arabidopsis thaliana* accessions is multigenic and requires quantitative analysis of segregating populations for identification and mapping of corresponding QTLs. Natural variation among accessions provides a complementary resource to discover novel gene functions (such as flowering time) and allelic variants interacting with the genetic background or environment and also alleles of small effects on phenotype, particularly for traits related to plant adaptation (Benfey and Mitchell-Olds, 2008).

### 1.3 Natural genetic variation among *Arabidopsis thaliana* accessions

*Arabidopsis thaliana*'s natural habitat includes a wide range of varying environments, stretching along the Northern hemisphere in Europe, Asia, and Africa; from northern Scandinavia ($68^0$ N) to Kenya ($0^\circ$) and from sea level in The Netherlands to the Himalayas (4200 m). It has been introduced from its native Eurasia to North America, Australia, and Japan – **FIGURE 1** – (Hoffmann, 2002, Rédei, 1970, Alonso-Blanco and Koornneef, 2000, Sharbel et al., 2000). Many different natural accessions have been collected from wild populations since 1937 by the pioneers of *Arabidopsis thaliana* research – Prof. Friedrich Laibach and colleagues. They are available through seed stock centres – ABRC (Arabidopsis Biological Resource Center) in the USA, NASC (European Arabidopsis Stock Centre) in the UK and SASSC (Sendai Arabidopsis Seed Stock Center) in Japan, where they are propagated, preserved and distributed for research purposes (Scholl et al., 2000).

**FIGURE 1.** Geographical distribution of *A. thaliana* (Weigel and Mott, 2009).

Plants collected from different geographic locations often show considerable natural genetic variation. *A. thaliana*, like many other species, including crops such as maize, is highly polymorphic, while other species, such as soybean and melon are much less polymorphic (Shattuck-Eidens et al., 1990, Nordborg et al., 2005). Within-species, natural variation can be explained by spontaneously occurring mutations that have been maintained in nature by evolutionary processes including artificial and natural selection. For the past two decades there has been a rapid growth in research focused on the relationship between

phenotypic and genetic diversity. A number of genes and functional polymorphisms underlying natural variation in plant biological processes have been discovered, mostly in *Arabidopsis thaliana* (Weigel and Nordborg, 2005, Alonso-Blanco et al., 2009, Alonso-Blanco et al., 2005). It contributed to a better understanding of the molecular basis of adaptation to local conditions and determined the ecological and evolutionary processes that maintain this variation (Mitchell-Olds and Schmitt, 2006, Mitchell-Olds et al., 2007). Considerable variation has been described for many adaptive traits such as tolerance to abiotic stress, including temperature, draught, and metals (Murphy and Taiz, 1995, McKay et al., 2003, Hannah et al., 2006), and resistance to biotic stress, such as insects, fungi, bacteria, or viruses (Kunkel, 1996). Variation has also been found in many other traits, including developmental (flowering time, seed size, plant height) (Koornneef et al., 1998, Mitchell-Olds, 1996, Alonso-Blanco et al., 1999, Krannitz et al., 1991), physiological (seed dormancy) (Bentsink et al., 2006), biochemical (enzymatic activities) (Mitchell-Olds and Pedersen, 1998) and complex genetic mechanisms (chiasma frequency, DNA methylation, gene expression level) (Sanchez-Moran et al., 2002, Riddle and Richards, 2002, Cervera et al., 2002, Kehoe et al., 1999, Zhang et al., 2008). It has been reported that 20% of genes can have variation in expression in different accessions (Keurentjes et al., 2007b, Kliebenstein, 2009). However, in some cases these differences may not have a significant effect on phenotypical changes because of the buffering effect of molecular chaperones (Fu et al., 2009, Queitsch et al., 2002).

Natural genetic variation among *Arabidopsis thaliana* accessions, adapted to local natural conditions and their highly inbred nature makes this model species suitable for studying genome-wide associations (GWA) (Atwell et al., 2010) and genotype-environment interactions (G x E) (Jansen et al., 1995).

### 1.4 Polymorphism (SNPs) discovery among *Arabidopsis thaliana* natural accessions

Comparative sequencing, targeting thousands of regions across the entire genome of *Arabidopsis thaliana* natural accessions facilitated development of single nucleotide polymorphism (SNP) markers and revealed high level of genetic variation between accessions (Nordborg et al., 2005, Clark et al., 2007).

The first attempt to sequence the *Arabidopsis thaliana* genome was focused on the Columbia (Col-0) accession using a bacterial artificial chromosome (BAC)-by-BAC sequencing approach, to create a high-quality reference genome (Arabidopsis, 2000). Cereon Genomics LLC, a subsidiary of Monsanto Co., has successfully accomplished sequencing the second accession of *A.thaliana* - Landsberg *erecta* (L*er*) by the whole-genome shotgun approach (Rounsley and Last, 2010). Comparison of these two sequenced *Arabidopsis thaliana* accessions provided a first insight into the nature of large-scale genomic variation and led to identification of putative polymorphisms: single nucleotide polymorphisms (SNPs) and insertion-deletion polymorphisms (indels), that could be used as markers – **FIGURE 2 -** (Rounsley and Last, 2010). This also led to the generation of the first genome-wide polymorphism database for any plant species, freely accessible through TAIR (http://www.Arabidopsis.org) (Rounsley, 2003).


**FIGURE 2**. First genome-wide polymorphism (SNPs and indels) discovery between Col and L*er Arabidopsis thaliana* accessions (Rounsley and Last, 2010).

The next milestone in expanding our knowledge of *Arabidopsis thaliana* sequence variation was the identification of nearly 9,000 polymorphisms across 12 accessions (Schmid et al., 2003). To search for polymorphism in a panel of 96 accessions, a standard PCR-based dideoxy sequencing method has been used to sequence 1,500 short fragments (Nordborg et al., 2005). A subset of the 20 most diverse strains was then selected for a whole genome re-

sequencing using high-density microarray to construct the first-generation haplotype map (HapMap) for *A.thaliana* (Clark et al., 2007). Recent breakthroughs in resequencing had led to the development of a custom high-density Affymetrix 250K SNP-chip for genotyping over 1000 genetically diverse natural accessions of *A. thaliana* (Borevitz et al., 2007). Efforts are currently under way to characterize the whole-genome sequence variation in all those accessions (http://1001genomes.org/) using different technologies and different depths of sequencing coverage (Weigel and Mott, 2009). The availability of high-density SNP markers throughout the genome facilitated high-resolution mapping and association mapping approaches based on linkage disequilibrium (LD).

Several software tools have been developed to assist researchers in selecting appropriate markers for crossing particular accessions, e.g. MSQT (Multiple SNP Query Tool) (Warthmann et al., 2007) or TAIR SeqViewer (http://www.arabidopsis.org/servlets/ sv).

### 1.5 Mapping large-effect loci from qualitative variation

Some of the phenotypic natural variation existing between accessions is qualitative (discrete) due to single-gene allelic variants (monogenic). Genetic analysis of variation in these traits, similarly to variation between induced mutants and wild-types, includes detection of cosegregation with markers (Mendelian linkage mapping) and allelism tests by complementation. In *Arabidopsis thaliana*, several molecular marker techniques have been developed for mapping major effect allelic variation in specific traits between two accessions. These marker techniques include restriction fragment length polymorphisms (RFLPs), cleaved amplified polymorphic sequences (CAPSs), microsatellites and amplified fragment length polymorphisms (AFLPs) (Alonso-Blanco et al., 1998b). Availability of the complete genome sequence and the high density of polymorphisms between accessions makes these marker techniques very useful for the construction of high resolution genetic maps, for the analysis of genetic diversity, rapid and accurate linkage mapping of loci, as well as for applied diagnostic purposes, analysis of the genetic structure of the populations and phylogenetic analysis, etc (Rafalski, 2002). Many disease resistance genes (Ton et al., 1999, Deslandes et al., 1998) and large-effect alleles for flowering time (an otherwise typically quantitative trait) have been identified between accessions (Clarke and Dean, 1994).

## 1.6 Mapping loci from quantitative variation

Most of the genetic variation among accessions is quantitative, as effects of allelic variation at several loci (multigenic) together with the environmental effect, determines a continuous phenotypic distribution of the trait in segregating populations (Alonso-Blanco and Koornneef, 2000). Genomic regions of interest for complex quantitative traits can be identified by statistical association of specific trait variation with segregating molecular markers - quantitative trait loci (QTLs). QTL mapping (linkage mapping) takes advantage of the natural variation present within species and have been successfully used in various types of segregating populations. In *Arabidopsis thaliana* well characterized 'immortal' mapping populations consisting of homozygous individuals are widely used for this analysis. Examples of such populations are recombinant inbred lines (RILs) derived from crosses between divergent accessions and then successively selfed for several generations, doubled haploids (DH), and near isogenic lines (NILs) obtained through repeated backcrossing and extensive genotyping (Keurentjes et al., 2007a, Alonso-Blanco et al., 1998b, Balasubramanian et al., 2009). Increasing density of genetic maps resulting from decreasing costs of genotyping has contributed to the growing success of identifying individual loci (QTLs) responsible for some complex traits (Borevitz and Nordborg, 2003). A QTL is typically a genomic segment (5 to 50 cM) accounting a proportion of trait variation, which contains potentially many hundreds of individual genes and other chromosomal features from which one or few could be casual (Mauricio, 2001). Various NILs have been developed, where only one QTL region segregates in an otherwise isogenic genetic background, to confirm and fine map QTLs previously identified in RILs (Keurentjes et al., 2007a). Further analyses of these regions are necessary for the final identification and cloning of quantitative trait genes – QTGs and identification of the underlying nucleotide polymorphism (QTN) (Alonso-Blanco et al., 1998a, Juenger et al., 2005, Alonso-Blanco et al., 2003, Bentsink et al., 2003).

In 1923, Karl Sax identified a QTL for seed size in beans (*Phaseolus vulgaris*) which was mapped by statistical association with a Mendelian marker for seed pigmentation (Sax, 1923). More recently, the *FRIGIDA (FRI*) gene involved in the control of flowering time in *Arabidopsis thaliana* (Johanson et al., 2000) and the *DELAYED GERMINATION1 (DOG1*)

gene (Bentsink et al., 2006) have been identified by the means of QTL mapping. *ACD6* gene was confirmed to be responsible for the single major effect QTL for both leaf initiation rate and late-onset leaf necrosis, by fine mapping to 12 kb region and targeting with artificial microRNAs (Todesco et al., 2010). This was consistent with the identification of the same region responsible for variation in necrosis in the set of 96 accessions by genome-wide association mapping (Atwell et al., 2010).

QTL mapping has had an enormous impact on agriculture, manifested by the identification of locus for tomato fruit size (Frary et al., 2000), maize fruit architecture (Dorweiler et al., 1993), and length and quality of the cotton seed fibre (Jiang et al., 1998), yielding insight into the evolution of the domestication of these crops (Mauricio, 2001).

### 1.7 Genome-wide association (GWA) mapping - potentials and pitfalls

Genome-wide association mapping (LD mapping) determines the genetic basis of phenotypic variation by identification of sequence variants that correlate with a given phenotype across a large number of populations of unrelated individuals (Myles et al., 2009, Aranzana et al., 2005, Weigel and Nordborg, 2005). *A. thaliana* has been widely used for this approach because of the high density of molecular markers available (one SNP every 500 bp) and also the high level of homozygosity found in the naturally inbred accessions (Zhao et al., 2007, Nordborg and Weigel, 2008). Once genotyped, various *Arabidopsis thaliana* accessions can be phenotyped multiple times for many different traits in many different environments, or for the same trait to reduce environmental noise (Atwell et al., 2010, Aranzana et al., 2005). Increasing number of sequenced accessions and improved sequencing technologies has contributed to the increasing power of associations, and the growing success of GWA mapping in understanding the genetics of natural variation and in studying traits of agricultural importance (Weigel and Mott, 2009). Sequence variants identified by the means of LD mapping are expected to be very closely linked to QTL e.g. *FRIGIDA*. It has been demonstrated that this gene can be mapped to a roughly 30 kb region by searching directly for marker-trait associations (LD) in a population of individuals (Hagenblad et al., 2004).

However, association mapping can suffer from serious confounding effects and some marker-trait associations may not be due to casual relationships (LD), but because of the demographical factors such as strong population structure or complex genetics. This may occur as accessions collected from the same geographical regions are often more related than those coming from more distant locations which might produce increased rates of false-positive associations, especially when studying traits important for adaptation to local environments (Weigel and Nordborg, 2005, Nordborg et al., 2005, Sharbel et al., 2000). Statistical tools correcting for population structure are being developed to reduce rates of spurious genotype-phenotype associations (Pritchard et al., 2000, Devlin and Roeder, 1999, Zhao et al., 2007, Yu et al., 2005b). However, it seems impossible to reduce the false-positive rates without paying the price of introducing small rates of false negatives that are strongly correlated with population structure. This can decrease the power of association mapping, which is also determined both by the marker density and sample size. If the sample size is too small, only major QTLs can be detected. Association studies using *a priori* candidate genes within a particular region where a QTL is located have a higher potential, especially if they turn out to be significantly over-represented among SNPs associated with the phenotype (Atwell et al., 2010). Confounding results can best be overcome by further experimental validations using crosses, transgenic lines or complementary QTL mapping in controlled crosses. Also, the nested association mapping (NAM) strategy developed in maize, which involves generation of experimental populations in which members of different populations are crossed in a systematic way (Weigel and Mott, 2009, Yu et al., 2008), is being incorporated into Arabidopsis research.

### 1.7.1 Linkage Disequilibrium (LD)

Linkage Disequilibrium (LD), the basis for genome-wide association studies, is the non-random association of alleles at different loci explaining genetic variation in natural populations. Associations, known as linkage disequilibria, typically exist between very closely linked loci which tend to be inherited together (haplotypes) more often than alleles at unlinked loci. This means that LD mapping is useful for fine mapping e.g. after a gene has been roughly localized using linkage mapping by QTL (Borevitz and Nordborg, 2003). LD reflects the genetic force of recombination, and the average rate of decay depends on the recombination frequency along the genome, the mating system of the organism, and its population history, including the forms of selection acting up it (Rafalski, 2002). A high degree of inbreeding reduces the efficacy of recombination in breaking up linkage disequilibrium so makes LD more likely to be extensive. Given the selfing nature of *A. thaliana*, LD decays much more rapidly than it was initially predicted; within 10 kb (Plagnol et al., 2006, Kim et al., 2007, Nordborg et al., 2002, Nordborg, 2000). LD determines the marker density required for association mapping: if LD decays too rapidly, a very dense map is needed. If the LD is extensive, whole genome scans are feasible but the resolution might be limited (Nordborg et al., 2002).

## 2. *Arabidopsis thaliana* sexual reproduction and seed development

### 2.1 Plant life cycle

The angiosperm life-cycle alternates between a dominant diploid sporophytic generation and a reduced haploid gametophytic generation, restricted to only a few cells of female embryo sac and male pollen grains (Page and Grossniklaus, 2002). In angiosperms the life cycle begins with the process of double fertilization, which initiates seed development and ends with the meiotic division during gametogenesis – **FIGURE 3**.

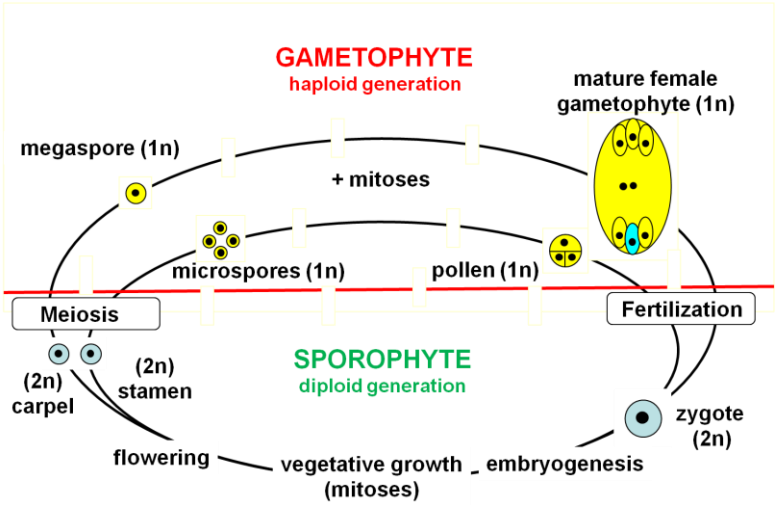


**FIGURE 3.** Higher plant life cycle.

### 2.2 Flowering time

It is essential for angiosperms (flowering plants) to flower under favourable environmental conditions to ensure successful reproduction. Therefore the time for transition from the vegetative to the reproductive phase is likely to reflect significant adaptations to different natural environments (Alonso-Blanco et al., 1998a). Flowering time is an important trait as earlier reproduction often translates into higher fitness (Kelly and Levin, 2000). Natural genetic variation for flowering time has been found among accessions of *Arabidopsis thaliana*, even when grown under the same conditions. Flowering time can be influenced by environmental factors such as photoperiod length and temperature. Certain plant species require a 3-8 week cold treatment for the induction of flowering, called vernalization (Michaels and Amasino, 2000, Nordborg and Bergelson, 1999)

### 2.3 Gametogenesis

During the process of gametogenesis, haploid spores in male and female parts of the flower undergo two or three mitotic divisions to generate the maternal megagametophyte (embryo sac) and paternal microgametophyte (pollen grain).

#### 2.3.1 Microsporogenesis

In male gametogenesis, meiosis produces four haploid microspores which subsequently undergo two rounds of mitosis to produce a pollen grain (male gametophyte). First mitosis asymmetrically divides the haploid spores to form a larger vegetative cell engulfing a smaller generative cell, which upon second mitosis generates two haploid sperm cells. Each pollen grain carries a pair of haploid sperm cells contained within the cytoplasm of the uninucleate vegetative cell – **FIGURE 4 -** (Scott et al., 2008).

#### 2.3.2 Megasporogenesis

The *Arabidopsis thaliana* developmental pattern of the female gametophyte (embryo sac) is the polygonum type which is present in 70% of all angiosperms (Coimbra et al., 2007). It is located in the core of the mature ovule, surrounded by the maternal tissue of the integuments, and attached to the carpel placental tissue by the funiculus. One cell in the sub-epidermal layer of the ovule (nucellus) develops into the megaspore mother cell (MMC) which undergoes meiotic reduction giving rise to four megaspores. Three megaspores degrade, and the single surviving one forms the embryo sac through three rounds of mitosis, producing eight nuclei. Before cytokinesis, each nuclei migrates to its own specific position in the embryo sac: two synergids flank the egg cell in the micropylar pole, three in the antipodal position in the opposite chalazal pole, and the two polar nuclei which form the homodiploid central cell remain in the centre – **FIGURE 4 -** (Christensen et al., 1997, Drews et al., 1998, Grossniklaus and Schneitz, 1998, Olmedo-Monfil et al., 2010, Yang and Sundaresan, 2000).

## 2.4 Double fertilization

Flowering plants comprise over 250,000 different species. The reason for their evolutionary success is partly due to the development of double fertilization, producing an embryo along with the second product of fertilization - endosperm. In sexually reproducing diploid plants, each pollen grain contains two sperm nuclei that are delivered by the pollen tube to the embryo sac upon germination of the vegetative cell. A pollen tube guidance system ensures that a single pollen tube penetrates each embryo sac and that each female gamete is fertilized by a maximum of two sperm, avoiding polyspermy (Scott et al., 2008, Higashiyama et al., 2001). Recognition and fusion of one of the sperm nuclei with the haploid egg cell results in the formation of a diploid zygote, which gives rise to the daughter plant, while fertilization of the homodiploid central cell by the other sperm nuclei gives raise to the triploid endosperm – **FIGURE 4** - (Sprunck and Dresselhaus, 2009, Dresselhaus, 2006, Faure et al., 2002). This tissue differs in principle from the embryo due to the genomic ratio, which is 2:1 maternal to paternal genome, and also due to its development, function and fate (Olsen, 2004). The fusion of the parental genomes during karyogamy reinitiates the plant life-cycle leading to a new zygotic generation (Berger, 2008).

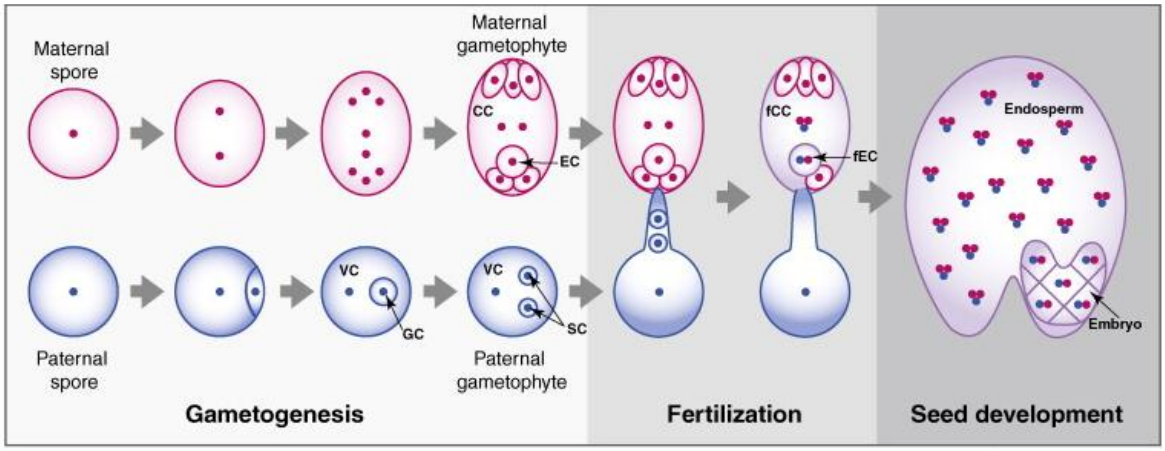


**FIGURE 4**. Gametogenesis, double fertilization and seed development in *Arabidopsis thaliana* (Mosher and Melnyk, 2010).

## 2.5 Seed development

The final product of double fertilization in diploid angiosperms such as *Arabidopsis thaliana* is a seed consisting of three parts that differ in their genetic composition: the diploid embryo carrying one copy of both maternal and paternal allele, the triploid endosperm carrying two copies of maternal and one copy of paternal allele, and the seed coat composed of diploid maternal tissue – **FIGURE 5 -** (House et al., 2010). Viable seed development requires integrated cell proliferation and cell elongation of these three components (Autran et al., 2011, Aw et al., 2010, Chaudhury et al., 2001).

Seed development of many dicots, including *Arabidopsis thaliana*, initially involves active endosperm proliferation and integument expansion followed by a second phase in which the embryo grows to its full size. Endosperm first grows through successive divisions of the triploid nuclei without cytokinesis as a syncitium for several mitotic cycles, and after that it is partitioned into individual cells during cellularization (Olsen, 2004, Berger, 1999). It functions as a sink tissue that acquires nutrients from the seed parent for the developing embryo and during germination, plays role in signalling among seed components and protects the embryo from physical and osmotic stress (Berger, 2003). The endosperm is transient and is consumed by the growing embryo during seed development, leaving only a single layer of cells at maturity that do not genetically contribute to the next generation.

Growth of the zygote is typically polar and the division asymmetric, leading to formation of a small apical cell which develops into the spherical proembryo, and a large basal cell, the founder of the feeding structure called the suspensor. The maternal ovule undergoes regulated growth and the integuments ultimately constitute the seed coat or testa (Sundaresan, 2005).

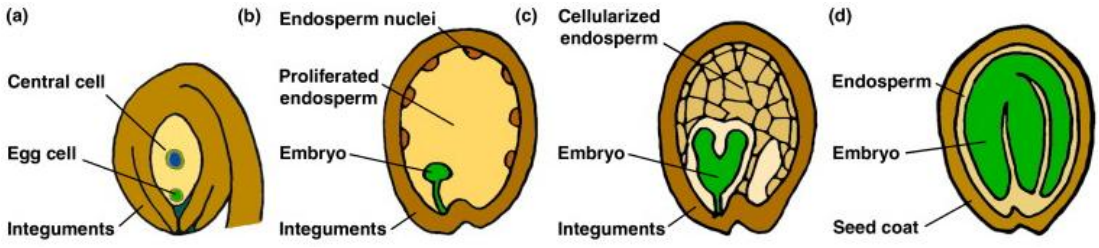


**FIGURE 5**. Arab*idopsis thaliana* seed development (Sun et al., 2010).

### 3. Seed size control

Seed size is a very important adaptive trait as larger seeds have often higher fitness than smaller seeds, and are expected to be under strong stabilization selection (Silvertown, 1989, Stanton, 1984). Larger seeds often have a higher probability of germinating, and seedlings emerging from larger seeds tend to have greater survival and better performance under a range of environments (Manning et al., 2009, Westoby et al., 2002, Krannitz et al., 1991). Variation in seed size can be attributed to environmental conditions that provide more or less resources for the maternal parent to invest in seeds (Roach and Wulff, 1987). Most grains consumed today have larger seeds than their wild relatives, subject to years of selection and breeding programs (Shomura et al., 2008). Identification of factors involved in seed size and number control can be very important in developing strategies to improve grain yield in crops, such as *Brassica napus* – close relative to *Arabidopsis thaliana* (Egli, 1998, Diepenbrock, 2000).

Genes controlling seed size can be discovered by either forward or reverse genetics, or by QTL mapping of loci underlying natural variation (Alonso-Blanco et al., 1999, Rowan et al., 2011). Extensive variation in seed size and weight (up to 3.5-fold) has been found among *Arabidopsis thaliana* natural accessions, providing opportunities for QTL mapping of loci controlling this trait (Krannitz et al., 1991, Alonso-Blanco et al., 1999, Stokes et al., 2007, Ungru et al., 2008, De Jong et al., 2009). A number of QTLs have been identified to control seed size, of which some collocate with QTLs for maternal traits, such as seed number and fruit size, suggesting that maternal tissues are important in determination of the seed size (Alonso-Blanco et al., 1999). Significant loci controlling seed weight have been identified using QTL mapping in crop plants, such as tomato, soybean, maize and rice, however not many of the corresponding genes have been cloned so far (Doganlar et al., 2000, Hyten et al., 2004, Song et al., 2007).

It has been observed that the same genotype can have different effects on the seed size and number depending if it is inherited maternally or paternally. This parent-of-origin effects can be explained by maternal/paternal effects, including sex-specific expression, differential dosage effects or/and genomic imprinting (uniparental gene expression)

(Kinoshita et al., 1999, Vielle-Calzada et al., 1994, FitzGerald et al., 2008, House et al., 2010, Dilkes and Comai, 2004, Hardenack et al., 1994, Köhler et al., 2004).

### 3.1 Parental effects

Many maternal effects on seed size have been found (Roach and Wulff, 1987, Mousseau and Fox, 1998, Galloway et al., 2009), while only rarely have paternal effects been observed (Andersson, 1990, Marshall and Whittaker, 1989, De Jong et al., 2009). Maternal control of seed size by integument elongation has been observed in crosses between *Arabidopsis thaliana* natural accessions where seeds of distinct sizes produced seeds similar to the size of the maternal accession (Alonso-Blanco et al., 1999, Garcia et al., 2005). The maternal genotype was found to explain a larger proportion of the variance in seed size and number in the natural populations of *Arabidopsis thaliana*, however paternal genotype also significantly affected resource allocation to seeds and explained approximately 10% of the variation (House et al., 2010). Feedback control from the maternal integument on endosperm growth could determine the final seed size.

Paternal effects were observed in crosses involving the L*er* accession, where a maternally inherited L*er* genome produced the smallest seeds, while a paternally transmitted L*er* genome always produced seeds with the largest size. This suggests that the L*er* paternal effect is independent of the maternal genotype (House et al., 2010, Alonso-Blanco et al., 1999). Paternal effect through sex-specific gene expression in *Arabidopsis thaliana* has been found for the *SSP (SHORT SUSPENSOR*) protein produced in mature pollen, which upon fertilization triggers zygotic *YDA (YODA*) activity, to promote elongation of the zygote and development of the suspensor, thus controlling embryonic patterning (Bayer et al., 2009).

### 3.1.1 Regulation of seed size through integument development

Only a handful of genes are known to be involved in determining *Arabidopsis thaliana* seed size (Jofuku et al., 2005, Adamski et al., 2009, Zhou et al., 2009, Wang et al., 2010, Mizukami and Fischer, 2000). Analysis of *Arabidopsis thaliana* mutants directly affecting seed size reveal that both endosperm and integument growth are involved in controlling this trait (Garcia et al., 2005, Sun et al., 2010).

Maternal control via integument cell elongation and proliferation is affected by WRKY transcription factor *TTG2 (TRANSPARENT TESTA GLABRA2*) promoting seed size (Johnson et al., 2002a, Debeaujon et al., 2000). *AINTEGUMENTA (ANT)* transcription factor plays a role in controlling cell proliferation and its ectopic expression results in an increase in size of various plant organs, including seeds (Krizek, 1999, Mizukami and Fischer, 2000). The cytochrome P450 *KLU (KLUH*) promotes seed size by generating a maternal mobile signal, possibly hormones, to control inner integument of a developing ovule (Adamski et al., 2009).

By contrast, a transcription factor mediating gene expression in response to auxin -*ARF2 (AUXIN RESPONSE FACTOR2*, also known as *MEGAINTEGUMENTA* - *MNT*), the floral homeotic gene AP2 (APETALA2), and the ubiquitin interaction motif-containing the DA1 protein limit seed size by restricting cell proliferation in the integuments (Schruff et al., 2006, Jofuku et al., 2005, Ohto et al., 2005, Li et al., 2008). Also Bsister class MADS-box proteins TT16/ABS (*TRANSPARENT TESTA16/ARABIDOPSIS BSISITER*) and *GORDITA/AGL63* are expressed in female reproductive organs and inhibit seed growth (Nesi et al., 2002, Prasad et al., 2010).

### 3.1.2 Regulation of seed size through endosperm development

Early endosperm proliferation and subsequent seed size at maturity is controlled by a successive action of *IKU1, IKU2 (HAIKU*) and *MINI3 (MINISEED)* genes (Garcia et al., 2003, Luo et al., 2005). Although mutations in these genes cause primarily reduced growth and precocious cellularization of the endosperm, the overall seed size is decreased, including the size of the integument. This provides evidence for a feedback communication from the filial generation of endosperm to the maternal seed integument (Garcia et al., 2003). Both *IKU2* and *MINI3* genes likely correspond to two seed size QTLs identified in L*er*/Cvi RIL lines (Alonso-Blanco et al., 1999, Luo et al., 2005). Although *TTG2* and *IKU2* genes act in independent genetic pathways, crossing of both mutants causes an additive effect, supporting a cross-talk between the integument and endosperm to determine the final seed size (Garcia et al., 2005). Mutations in *EXS (EXTRA SPOROGENOUS CELLS*) and *SHB1* genes (*SHORT HYPOCOTYL UNDER BLUE1*) also exhibit slow endosperm development and smaller seed phenotype implying that they are involved in seed size control (Canales et al., 2002, Zhou et al., 2009).

Several MADS-box proteins, a group of transcription factors involved in flower and fruit development, have been found to regulate seed size through maternal control of endosperm growth. AGAMOUS-LIKE proteins such as AGL80, AGL61 and AGL62 promote nuclear proliferation and delay endosperm cellularization (Kang et al., 2008b, Portereiko et al., 2006a, Steffen et al., 2008).

In maize, a *miniature 1* mutant has been found to produce smaller seeds with reduced endosperm growth, similar to *iku* mutants in *Arabidopsis thaliana* (Miller and Chourey, 1992). A *miniature 1* gene encodes a protein cell wall invertase2 which is supposedly involved in the coordination of the maternal nutrients supply to the seed (Cheng et al., 1996).

Moreover, phytohormones such as auxins, cytokinins, gibberellins, and brassinolides were also found to be involved in seed size regulation through the control of endosperm and embryo growth (Müller and Sheen, 2007, Richards et al., 2001, Divi and Krishna, 2009).

### 3.2 Parent-of-origin dosage effects

In balanced crosses (2x X 2x or 4x X 4x), the seed size increases with overall ploidy, but altering parental genome ratios has an even greater effect on the size of the mature seed (Scott et al., 1998). *Arabidopsis thaliana*, unlike many other plant species, readily produces viable seeds that contain triploid (3x) embryos from reciprocal crosses between diploid (2x) and tetraploid (4x) parents. Doubling the dosage of the paternal genome (in 2x X 4x crosses) results in increased and prolonged endosperm proliferation, delayed cellularization and abnormally larger seeds, while doubling the maternal contribution (in 4x X 2x crosses) produces abnormally smaller seeds comprising of fewer endosperm (Scott et al., 1998). Tripling the dose of maternal or paternal genomes (from 6x X 2x or 2x X 6x crosses, respectively) results in producing seeds with similar but more extreme phenotypes which eventually abort. Observed phenotypes strongly suggest that endosperm failure is the main cause of seed abortion following inter-ploidy crosses and the extent of endosperm growth is important for final seed size (Haig and Westoby, 1991, Birchler, 1993). According to the Endosperm Balance Number (EBN) hypothesis, the exact genome ratio of two maternal to one paternal genome (2m:1p) is crucial for the normal endosperm development, rather than the total ploidy level (Lin, 1984, Lin, 1982). Disruption of this ratio causes an imbalanced expression of maternally and paternally derived genomes leading to an endosperm breakdown or even failure of seed development (Dilkes and Comai, 2004). It has been proposed that inter-ploidy cross phenotypes are attributed to the disrupted balance of active copies of parentally imprinted genes in the seed (Haig and Westoby, 1991, Gehring et al., 2004, Baroux et al., 2002).

### 3.3 Epigenetic regulation of endosperm development

Epigenetics describes stable changes to gene expression that is beyond variation at the DNA sequence level, e.g. variation in cytosine methylation or covalent histone modification (Banaei Moghaddam et al., 2010). Chromatin can have a transcriptionally active form and have a low methylation level (euchromatin), or can be more condensed, trancriptionally inert and highly methylated (heterochromatin) (Kouzarides, 2007). Selected lysine (K) residues of histones are subjected to acetylation, methylation and other modifications (Tariq and Paszkowski, 2004, Demidov et al., 2006).

### 3.3.1 DNA methylation

DNA methylation is a heritable epigenetic modification of cytosine that regulates growth and development through transcriptional silencing of transposons and transgenes, genomic imprinting, defence against pathogens and endogenous gene silencing (Xiao et al., 2006, Bender, 2004, Martienssen and Colot, 2001, Gehring et al., 2004, Simon et al., 2005, Finnegan et al., 1996, Kakutani et al., 1996, Miura et al., 2001, Zhang et al., 2010, Paszkowski and Whitham, 2001). DNA methylation is associated with histone modifications causing chromatin rearrangements and accessibility of DNA to transcription factors (Soppe et al., 2002, Jackson et al., 2002, Johnson et al., 2002b, Fransz and de Jong, 2002). However, methylation-independent epigenetic regulation, such as *MOM*1 (*Mo*rpheus' *m*olecule 1) was also reported (Mittelsten Scheid et al., 2002, Yokthongwattana et al., 2009).

DNA methylation is regulated by two enzymatic activities – 'de novo' and 'maintenance' DNA methyltransferases (MTases), together with DNA demethylases, histone-modifying enzymes, chromatin remodelling factors, and the RNA interference machinery (Zhang et al., 2010). In *Arabidopsis thaliana*, *MET1 (METHYLTRASFERASE1*) primarily maintains CpG DNA methylation in the gametophyte and sporophyte generations (Finnegan and Kovac, 2000, Jean Finnegan and Dennis, 1993), while a smaller subset of DNA methylation (CpNpG and asymmetric CpNpN) is maintained by *CMT3 (CHROMOMETHYLASE3*) and the de novo *DRM1/DRM2 (DOMAINS RE-ARRANGED METHYL-TRANSFERASE*) methyltrasferases (Henikoff and Comai, 1998, Bartee et al., 2001, Lindroth et al., 2001, Cao and Jacobsen, 2002). *DDM1 (DECREASED DNA METHYLATION1*) is a SWI2/SNF2 chromatin remodelling protein also involved in DNA methylation patterning in *Arabidopsis thaliana* and has also been found to regulate genomic imprinting, transposons, gene silencing and paramutation (Vielle-Calzada et al., 1999, Singer et al., 2001, Hirochika et al., 2000, Stam and Mittelsten Scheid, 2005). Reduction of cytosine methylation is carried out by DNA glycosylases *ROS1* (*REPRESSOR OF SILENCING1*) or *DME* (*DEMETER*) (Choi et al., 2002, Gong et al., 2002). Reduced DNA methylation has pleiotropic effects on plant development, including delayed flowering, reduced fertility, abnormal gametogenesis and abnormal shoot and leaf

development (Saze et al., 2003, Ronemus et al., 1996, Finnegan et al., 1996, Kakutani et al., 1996).

DNA hypomethylation has a parent-of-origin effect on seed size, and reciprocal crosses between hypomethylated and WT plants result in similar seed phenotypes to those observed in inter-ploidy crosses (Adams et al., 2000, Scott et al., 1998). Hypomethylated plants produce gametes with imprinted alleles that lose most/all of their silencing, so that hypomethylated pollen provides active genes that were previously only maternally expressed in endosperm. This leads to an overexpression of maternally-specific growth inhibitors which in turn cause a phenocopy of maternal genome excess plants from inter-ploidy crosses and smaller seeds. Hypomethylated embryo sacs will result in an opposite phenotype of paternal excess plants from an inter-ploidy cross, i.e. larger seeds (Scott et al., 1998, Xiao et al., 2006). This shows that DNA methylation plays an important role in parent-of-origin effects on seed development via the action of imprinted genes in the endosperm, and disruption to the balance of expression of imprinted genes explains the observed phenotypes (FitzGerald et al., 2008, Adams et al., 2000).

Resemblance of *iku* seeds, seeds containing an excess of maternal dosage in endosperm and seeds obtained by fertilization of WT ovules by *met1* mutant pollen strongly suggests an epigenetic control of genes involved in endosperm growth, such as *IKU* (Garcia et al., 2005).

### 3.3.2 siRNAs

Changes in cytosine methylation patterns are associated with action of small interfering RNAs (siRNAs) that provide targets for methyltransferases (Matzke et al., 2009). Short interfering RNAs play crucial role in gene regulation. These 24 nt long RNAs, corresponding to transposable elements (TEs) and other repetitive elements, cause epigenetic gene silencing by de novo cytosine methylation. In the RdDM pathway (RNA-dependent DNA methylation) siRNAs are generated by the action of DNA-dependent RNA polymerase Pol IV, RNA-dependent RNA polymerase RdR2, and Dicer-like DCL3 (Chan et al., 2005, Matzke et al., 2009). They are loaded into ARGONAUTE proteins AGO4 and AGO6 to direct cytosine methylation by DRM2 MTase together with Pol V and the chromatin remodelling protein, DRD1 (Onodera et al., 2005, Wierzbicki et al., 2009).

The uniparental expression of PolIV-dependent siRNAs (p4-siRNAs ) is initiated in the female gametophyte and persists during seed development (Mosher et al., 2009). These maternally inherited and paternally silenced siRNAs contribute to the list of imprinted loci found in the *Arabidopsis thaliana* genome, and support the parental genome conflict hypothesis in which the maternal genome has a suppressive effect on hybrids. Interaction between maternal p4-siRNA and a paternal target is a unique property of hybrids, therefore it can affect post-zygotic failures in reciprocal inter-specific crosses, such as of *A.thaliana* and *A.arenosa* (Chen, 2010, Comai et al., 2000, Mosher and Melnyk, 2010). It could also contribute to phenotypes that are not observed in the parents, including growth enhancement through hybrid vigour and transgressive segregation (Mosher et al., 2009).

### 3.3.3 Genomic imprinting

Parental genomes are not functionally equivalent in endosperm, since each contributes a unique set of active alleles of imprinted genes (Adams et al., 2000). Kinship theory proposes that maternal and paternal parents are in conflict over the allocation of resources to their offspring (Haig and Westoby, 1989, Haig, 2000). Reproductive fitness of a maternal parent is greater when she distributes resources equally among her offspring, while a paternal parent benefits when maternal resources are concentrated in his own offspring. Therefore the role of paternal genes is to promote seed growth, even if it reduces the overall number of seeds produced by the mother, while female genes are acting antagonistic to those, reducing the growth (Scott et al., 1998). This suggests that the growth promoters are paternally active and maternally silenced while the growth inhibitors have the opposite expression patterns. Consistently with the limited resources available to a mother for allocation among her offspring, there is a trade-off between the size and the number of seeds that can be produced by a maternal plant (Sadras, 2007).

Conflict over optimum seed size between parents, and between parents and offspring may explain the evolution of imprinting in genes that affect seed size (de Jong and Scott, 2007, Haig and Westoby, 1991). Similar to the situation observed for the mammalian placenta, flowering plants can imprint some genes during endosperm development in a way that they are only expressed through either maternal or paternal allele, depending on the locus (Huh

et al., 2007, McKeown et al., 2011). These imprinted genes reflect different epigenetic marks acquired or lost during gametogenesis (Paszkowski and Grossniklaus, 2011).

Genomic imprinting is associated with differential DNA methylation (DMR) between parental alleles. *MEA, FIS2* and *FWA* imprinted genes are silenced from the paternal allele in endosperm by *MET1* mediated DNA methylation (Jullien et al., 2006b, Xiao et al., 2003), but active from the maternal allele owing to the methylation-removing activity of DEMETER (DME) during female gametogenesis (Kinoshita et al., 1999, Choi et al., 2002, Kinoshita et al., 2004). Both *MET1* and *DME* are essential for the control of seed development, although they function antagonistically (Xiao et al., 2003). Maternal control of MET1 over the seed size is mediated through its action on the maternal tissues such as integument (FitzGerald et al., 2008).

A direct relationship between DNA methylation and Polycomb group proteins (PcG) in the parent-of-origin regulation of seed size has been shown (Vinkenoog et al., 2000, Jullien et al., 2006b, Xiao et al., 2003). Mutations in PcG genes cause parent-of-origin effects on seed viability and endosperm cell proliferation (Hsieh et al., 2003, Kiyosue et al., 1999).

FIS-class genes (*FERTILIZATION INDEPENDENT SEED*) mediate genomic imprinting in flowering plants and maternal mutants (*medea*, *fis2*, and *fie*) show many features of the paternal excess phenotype, resulting in embryo lethality later in the seed development (Luo et al., 1999, Ohad et al., 1999, Grossniklaus et al., 1998). The shared phenotypes are supported by similar patterns of gene expression between paternal excess triploids and *fis* class mutants (Tiwari et al., 2010). The maternal mutants phenotype can be overcome by crosses to the hypomethylated (*met1*) pollen parent resulting in viable seeds of normal size (Adams et al., 2000, Luo et al., 2000).

FIS-class gene products participate in Polycomb Repressive Complex (PRC) which inhibits transcription of target genes through epigenetic modification of chromatin (Jullien et al., 2006a, Makarevich et al., 2006, Lund and van Lohuizen, 2004). FIS proteins repress transcription of loci in the maternally derived genome while the paternal genome is expressed normally, e.g repression of the maternal alleles of *PHERES1* (*PHE1*) by MEA (Spielman et al., 2001, Köhler et al., 2003). *PHERES1* has been identified to be

preferentially paternally expressed in *Arabidopsis thaliana* (Köhler et al., 2005), while majority of known imprinted genes are maternally expressed (Grossniklaus et al., 1998, Kinoshita et al., 2004, Gehring et al., 2009, Tiwari et al., 2008).

FIS class genes are involved in the control of endosperm proliferation, and fertilization triggers release of FIS-mediated repression (Chaudhury et al., 2001, Grossniklaus et al., 2001b). Mutants of the FIS genes show endosperm proliferation without fertilization, forming seed-like structures that eventually abort (Chaudhury et al., 1997, Chaudhury and Berger, 2001, Ohad et al., 1996). However, fertilized *fis* mutant egg cell can trigger autonomous central cell division giving raise to the diploid endosperm, and seed development can be completed with reduced size. A positive signal coming from the fertilization of the egg cell and the negative signal released by the FIS-class genes suggests that there is a cross-talk between embryo and endosperm coordinating seed development (Nowack et al., 2007).

## 4. Whole genome changes and their implications for plant reproduction

### 4.1 Polyploidy

Polyploidy refers to whole genome duplications resulting in the presence of more than two full chromosome sets (Comai, 2005). It can arise when rare mitotic or meiotic catastrophe causes the production of unreduced gametes. Unstable triploids can be formed when such diploid gametes fuse to the regular haploid gamete. It is less likely that two unreduced gametes can fuse together, giving rise to a more stable tetraploid zygote. Autopolyploidy results from duplicating a genome within the same species, such as potato (*Solanum tuberosum*), alfalfa (*Medicago sativa*), and sugarcane (*Saccharum*), while allopolyploidy is a consequence of hybridization of diverged genomes from different species followed by chromosome doubling. In an allopolyploid system, such as bread wheat (*Triticum aestivum*), cotton (*Gossypium hirsutum*), and oilseed rape (*Brassica napus* – 'canola'), or *Arabidopsis suecica*, diverged homoeologous genomes function together in a coordinated fashion (Riddle and Birchler, 2003, Chen, 2010).

Polyploidy is very common among plants, including many economically important crops, but it is also found among yeasts, fish, amphibians and reptiles (Gregory, 2005, Bogart, 1980, Ramsey and Schemske, 1998). Over 70% of flowering plants have experienced multiple ancestral whole-genome duplications resulting in polyploid formation and subsequent gradual process of diploidization, leading to gene loss with retention of selected gene categories (Soltis et al., 2004, Freeling and Thomas, 2006, Ramsey and Schemske, 2002, Otto and Whitton, 2000). This potentially led to neo- or sub-functionalization of genes and eventually to phenotypic diversification and adaptation (Comai, 2005, Adams and Wendel, 2005b). Polyploidy causes redundancy, a presence of multiple copies of the same gene, which allows for mutations, genetic drift and natural selection to alter some of those copies without deleterious effects on the whole organism (Comai, 2005). Other advantages of polyploidy, such as heterosis and asexual reproduction (apomixis) are covered in section 4.4 and 4.5 of this chapter.

A process of polyploidization causes large scale genomic reorganizations manifested by a wide variety of phenotypic alterations (Weiss and Maluszynska, 2001, Pontes et al., 2004, Madlung et al., 2002, Madlung et al., 2005, Pecinka et al., 2011). Studies in maize

demonstrated two sources of phenotypic response to ploidy change: one common to all genotypes, and one genotype specific (Riddle et al., 2006). The most evident phenotypical change due to polyploidy is associated with increased size of floral organs and fruits, pollen grains, leaves, and stomata (Blakeslee, 1941, Ramsey and Schemske, 2002, Altmann et al., 1994). Diploid, triploid and tetraploid *Arabidopsis thaliana* plants are morphologically quite similar, although there is an obvious positive correlation between flower size and ploidy (Henry et al., 2005). A strong genetic variation for the response to ploidy change has been reported for *Arabidopsis thaliana* triploids and their ability to successfully produce seeds, and seed size (Henry et al., 2005, Alonso-Blanco et al., 1999).

Challenges that polyploids face include nuclear enlargement that changes cellular architecture and alters the ratio between chromatin volume and nuclear envelope surface, potentially disturbing the interaction between components of chromatin and envelope-bound proteins (Riddle et al., 2006, Comai, 2005). Polyploids usually have difficulties in the normal completion of mitosis and meiosis. Meiosis that involves more than two sets of chromosomes can produce aneuploid gametes that differ in frequency and complexity depending on the type of polyploidy and species.

Ploidy effects on gene regulation could result from genome doubling and/or intergenomic interactions. An increase in the copy number of all chromosomes affects all genes equally, however expression of some genes have been found to increase proportionally with ploidy, while the others exhibit dosage compensation, uniparental- or biased gene expression (Guo et al., 1996, Auger et al., 2005, Birchler et al., 2001). Epigenetic alterations often accompany polyploidization and they include loss or gain of cytosine methylation, gene silencing, non-additive gene expression, and reactivation of transposable elements (Lee and Chen, 2001). Ploidy-dependent paramutation, where epigenetic state transmits from a suppressed allele to an active allele, resulting in non-Mendelian inheritance of silenced alleles, have been reported for a transgenic locus in tetraploid *Arabidopsis thaliana* line (Mittelsten Scheid et al., 2003, Mittelsten Scheid et al., 1996).

### 4.1.1 Effects of triploidy on plant reproduction

Triploids are polyploids carrying three complete sets of chromosomes. They can result from the fusion of an unreduced diploid gamete to a normal haploid gamete or from inter-ploidy crosses between diploid and tetraploid parents.

In animals, triploidy is almost always associated with sterility or lethality (Garnier Géré et al., 2002, Benfey, 1999), although triploid fish and amphibians are known to grow to maturity (Stöck et al., 2002). Plants usually tolerate triploidy, although some of them are effectively sterile, such as seedless watermelon, or have severely reduced fertility, like Datura (Satina et al., 1938) and maize (McClintock, 1929). Additionally, mild reductions in fertility have been found in triploid poplar (Johnsson, 1942), sugar beet (Levan, 1942) and Melandrium (Warmke and Blakeslee, 1940). *Arabidopsis thaliana* triploids are fertile but their fertility is reduced compared to diploids. This is a consequence of aneuploid gametes production and subsequent formation of aneuploid progeny composed of various karyotypes (Khush, 1973). The reason for the low frequency of viable progeny within triploid populations can be also explained by the phenomenon called triploid block (Köhler et al., 2010). Triploid embryos abort because of abnormal endosperm development and those who survive often exhibit reduced reproductive fitness (Satina et al., 1938, Vinkenoog et al., 2003).

Triploids are meiotically unstable and therefore transient. In triploid meiosis, trivalents are formed and the chromosomes are distributed onto both daughter cells. Very rarely one of them gets exactly the double amount (2x) of the complete single set (1x) and more often both daughter cells receive incomplete sets resulting in aneuploidy. In a species with only two chromosome types, only one out of four possible gametes will have the normal haploid complement of chromosomes – **FIGURE 6.** The number of possible combinations of chromosomes in gametes increases rapidly with the increasing number of chromosome types (5 in *Arabidopsis thaliana*). Therefore, the relative amount of balanced gametes decreases for genomes with larger numbers of chromosomes. The products of triploid meiosis give reise to diploid, triploid and tetraploid progeny, as well as a swarm of individuals spanning the whole range of aneuploidy types (Henry et al., 2005, Satina et al., 1938). Triploids are thought to act as intermediates in transition from diploidy to

polyploidy (Ramsey and Schemske, 1998). Analysis of triploid progeny suggests that there is a strong selection against aneuploidy; propagation of triploids for a few generations resolve into diploid and tetraploid cohorts (Henry et al., 2005, Henry et al., 2007).

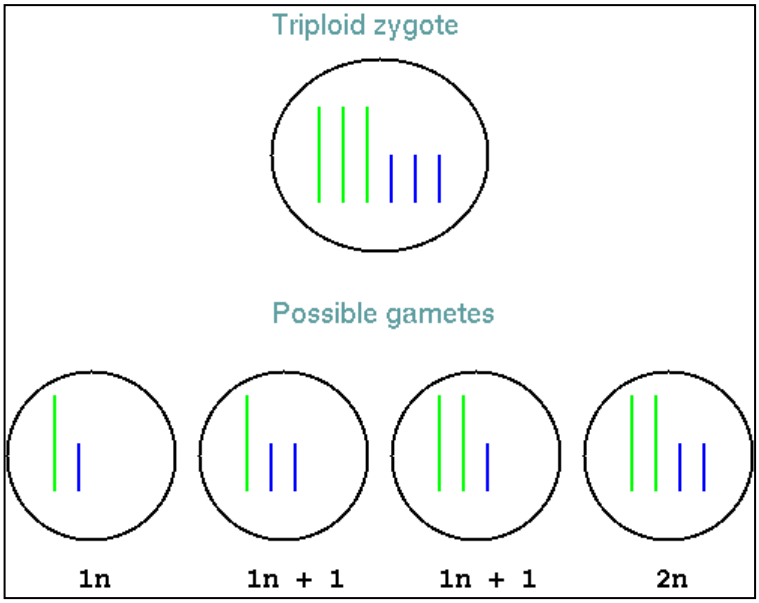


**FIGURE 6**. A schematic representation of triploid meiosis in a species with two chromosome types. Four possible gametes produced by such triploid include two euploid types: haploid and diploid, and two aneuploid types with one extra chromosome each: disomic for chromosome 1 and disomic for chromosome 2.

### 4.2 Aneuploidy

Aneuploidy is characterized by a relative excess or deficiency of some chromosome types or parts of chromosomes resulting in gene dosage imbalance (Henry et al., 2007, Blakeslee, 1922). Aneuploidy can arise via nondisjunction of chromatids and chromosomes during triploid meiosis, where three sets of chromosomes cannot be resolved to two poles (Henry et al., 2005, McClintock, 1929). It can be also artificially induced through colchicine treatment or breeding strategies.

Most animals, including humans do not tolerate aneuploidy and they are either developmentally abnormal or lethal (Matzke et al., 2003). However, plants and yeasts produce viable and fertile aneuploid swarms and the frequency of specific types of aneuploidy can be altered by the genotype (Fundyga et al., 2004, Henry et al., 2005, Rick and Notani, 1961). Aneuploids provide a pool of phenotypes, specific to the dosage of each chromosome type, that are not present within euploid populations (Huettel et al., 2008). These phenotypes include altered leaf colour and shape, dwarfism, or changes in flowering time (Henry et al., 2005, Henry et al., 2010). Dramatic phenotypical and developmental changes can be explained by the departure from the strict stoichiometry between the different dosage-sensitive gene products involved in proper functioning of protein complexes, and the consequences of this can be severe (Veitia, 2005, Birchler et al., 2001, Birchler et al., 2005). The most common form of aneuploidy is trisomy, characterized by the presence of one extra chromosome in an otherwise diploid genome (Koornneef and Veen, 1983, Khush, 1973, Blakeslee, 1922, Huettel et al., 2008). Aneuploids in polyploid backgrounds usually exhibit milder phenotypic alterations due to the overall higher chromosome copy number and reduction in relative chromosome imbalance (Ramsey and Schemske, 1998).

Aneuploidy can lead to epigenetic changes, because chromatin regulatory pathways are sensitive to the dosage of genes encoding regulatory factors, and can also alter imprinting patterns (Birchler et al., 2005). Aneuploids are also susceptible to silencing because of the exposure of unpaired chromatin regions to epigenetic remodelling mechanisms (Shiu et al., 2001). Long-term phenotypic consequences of aneuploidy has been reported, suggesting that epigenetic modifications of aneuploids can persist in their diploid or polyploid progeny (Henry et al., 2010, Comai, 2005).

A single locus, *SDI (SENSITIVE TO DOSAGE IMBALANCE*), has been identified by studying RIL populations derived from *Arabidopsis thaliana* intraspecific hybrid triploid of diploid Col and the natural tetraploid Wa accession (Schiff et al., 2001, Henry et al., 2007, Henry et al., 2005). These lines segregate into near-diploid and near-tetraploid individuals and the *SDI* locus was found to exhibit segregation distortion only in the latter population, particularly in the severe aneuploids. Thus, *SDI* is thought to be involved in aneuploid

survival by buffering the effects of dosage imbalance. Viable aneuploids can facilitate gene flow between diploids and tetraploids via triploid bridge and thus prevent polyploid speciation (Henry et al., 2007, Ramsey and Schemske, 1998). Parent-of-origin effects and dosage effects on the inheritance of each chromosome type have been studied in *Arabidopsis thaliana* aneuploids (Henry et al., 2009).

Karyotyping of aneuploids is complicated by the complex chromosomal composition due to multiple heterozygous combinations at loci present in more than two copies (Henry et al., 2006). Most available markers are co-dominant and can detect the presence of two different alleles; however they do not provide quantitative measure of relative allelic ratios. Several methods for description of variation in chromosome numbers have been developed, although mainly for the diagnostics of foetal aneuploidy in humans (in a diploid genome) (Dudarewicz et al., 2005). Quantitative genotyping of SNPs using microarrays or pyrosequencing, as well as quantitative fluorescent PCR (QF-PCR) are promising molecular methods for rapid detection of chromosome copy number variation in polyploid backgrounds (Rickert et al., 2002, Rickert et al., 2005, Henry et al., 2006).

### 4.3 Hybridization

Evolutionary, gene interactions are postulated to be functional within species, but incompatible or deleterious in hybrids (Dobzhansky, 1937, Muller, 1942). In animals, inter-specific hybrids and polyploids are usually sterile (e.g. mule, a hybrid between a horse and a donkey) or unviable (Clarke, 1984, Mable, 2004, Muller, 1925). This could be a result of incompatibilities related to abnormal expression patterns of imprinted genes, imbalanced dosage of sex chromosomes, or epigenetic activation of retroelements (Vrana et al., 2000, O'Neill et al., 1998). Nevertheless, a number of animal hybrids, such as fish, fruit flies and butterflies have been formed and found to grow even better than their parents (Dowling and Secor, 1997). Many crops, such as maize (*Zea mays*) and sorghum (*Sorghum bicolour*), are grown mainly as hybrids, as heterozygotes often have higher fitness than the homozygotes (Chen, 2010).

Functional divergence between duplicate genes can lead to incompatibilities between *Arabidopsis thaliana* accessions, therefore frequency of seed abortion in inter-accession crosses depends on the combination of maternal and paternal genotypes (Bikard et al., 2009, Bomblies et al., 2007). Epistatic incompatibility operates during seed development due to interactions between genes that have evolved independently (Fishman and Willis, 2001). Mate choice exists when mothers control seed development by selectively aborting seeds depending on the paternal genotype (Korbecka et al., 2002, Marshall and Ellstrand, 1988). Endosperm incompatibility in inter-specific crosses is one of the major barriers to hybridization in flowering plants, leading to seed abortion through disrupting proper endosperm development (Kinoshita, 2007). Post-germination incompatibilities as a result of autoimmune activity have been shown among *Arabidopsis thaliana* accessions (Bomblies et al., 2007).

### 4.3.1 Inter-ploidy hybridization

Dosage effects play an important role in endosperm, which acts as a sensor for genomic imbalance (Costa et al., 2004, Baroux et al., 2002, Birchler, 1993). Inter-ploidy crosses cause deviation from the normal 2:1 maternal to paternal genomic ratio and often lead to defective endosperm development (Scott et al., 1998). Frequency of seed abortion in *Arabidopsis thaliana* inter-ploidy crosses depends on the accessions, and it was shown that Col-0 used as a male parent in a 2x X 4x cross produces high rates of aborted seeds (Dilkes et al., 2008).

Inter-specific hybridization between diploid *A.thaliana* and tetraploid *A.arenosa* leads to failure in viable seed production as triploid hybrid embryos abort shortly after reaching the globular stage (Bushell et al., 2003). The cytoplasmic-nucler incompatibilities or parent-specific imprinting may play a role in the tolerance to inter-specific hybridization (Scott et al., 1998, Price et al., 1994)

### 4.3.2 Allopolyploidy

The majority of polyploid angiosperms (~75%) are allopolyploids, and they include many important crops such as canola, wheat and cotton (Chen, 2010). They can be formed by inter-specific hybridisation and chromosome doubling (Hilu, 1993). The problem of sterility in many diploid hybrids can be overcome in allopolyploids where a proper pairing partner is available to each chromosome, resulting in fertility (Winge, 1917). A synthetic Arabidopsis allopolyploid line was produced by a genetic cross between two autotetraploids: *A. thaliana* (female parent) and *A. arenosa* (male parent), whereas the reciprocal cross fails (Comai et al., 2000, Chen et al., 2004).

Sudden reunification of divergent genomes may induce genomic and phenotypic instability; therefore neoallopolyploids must go through the stability bottleneck before they start producing fertile progeny and become adapted, like the natural allopolyploid *Arabidopsis suecica* (Comai et al., 2000, Madlung et al., 2005, Ramsey and Schemske, 2002). The instability syndrome of newly synthesised allopolyploids can be explained by parental regulatory divergence and inter-genomic incompatibilities. Gene regulatory hierarchies are dosage dependent, and when the diverged hierarchies are reunited in a hybrid, the expression of the target loci might exhibit novel patterns of expression (Birchler et al., 2001).

Polyploidization and/or hybridization are likely to cause genomic shock leading to widespread misregulation and activation of suppressed heterochromatic elements (McClintock, 1984, Comai et al., 2003). A consequence of this could be genetic and epigenetic changes resulting in altered gene expression, gene silencing, nucleolar dominance, novel tissue specificity or activation of transposable elements (Adams et al., 2003, Blanc and Wolfe, 2004, Riddle et al., 2010, Comai et al., 2000, Lee and Chen, 2001, Adams and Wendel, 2005a, Wang et al., 2004, Madlung et al., 2005). Gene silencing or activation in allopolyploids can either impede hybridization or have an opposite effect, providing changes in gene expression towards improved fitness (Comai et al., 2000). Nucleolar dominance in the allotetraploid of *A.suecica* has been revealed by differences in rRNA gene expression showing silencing of the *A.thaliana* genes (Chen et al., 1998, Chen and Pikaard, 1997). Epigenetically regulated genes responding to transition from

autopolyploidy to allopolyploidy have been identified in Arabidopsis (Wang et al., 2004, Comai et al., 2000, Wang et al., 2006) and wheat (Shaked et al., 2001). Non-additive gene regulation following inter-specific hybridization may result from the competition between the diverged regulatory pathways in allopolyploids of Arabidopsis and other species, such as cotton, wheat and Senecio (Wang et al., 2004, Adams et al., 2004, Hegarty et al., 2005, Kashkush et al., 2002). Gene expression changes detected in allopolyploids are likely caused by the inability of regulatory networks from the two parental genomes to successfully coordinate their actions. Non-additive gene expression in allopolyploids often depends on expression divergence between the parents, with more distantly related species inducing higher levels of gene expression changes in the hybrids. Diverged transcriptional regulators in the two genomes cause target genes from either parent to respond differentially to the combined regulatory signals (Riddle and Birchler, 2003). This provides a molecular basis for hybrid vigour and novel variation in the allopolyploid offspring (Birchler et al., 2003, Comai et al., 2000, Wang et al., 2004, Chen, 2007). Changes in genome organization and gene expression are thought to have played a role in the evolutionary success of allopolyploid plants (Osborn et al., 2003, Soltis and Soltis, 1995).

### 4.4 Heterosis

Heterosis (hybrid vigour) is the ability of F1 hybrid offspring to exhibit characteristics, such as biomass, stature, growth rate or fertility that lie outside the range of the both parents – **FIGURE 7 -** (Auger et al., 2005, Chen, 2007, Chen, 2010). Heterosis has been exploited extensively in plant breeding for centuries leading to new varieties of hybrid or allopolyploid crops, e.g. triticale, which combines the good grain quality and high yield of wheat with the disease and stress tolerance of rye (Guedes-Pinto et al., 1996, Birchler et al., 2003). Heterosis for yield in maize has been found to be positively correlated to genetic diversity of the progenitors (Guo et al., 2006). Consistently with this, viable inter-specific hybrids show more heterosis than intra-specific hybrids. Mating among siblings or self-fertilization, leads to accumulation of deleterious mutations and recessive alleles, called inbreeding depression (Charlesworth and Charlesworth, 1999).

Intra-specific hybrids of *Arabidopsis thaliana* have been employed as a model for understanding heterosis in hybrid crops (Banaei Moghaddam et al., 2010, Stokes et al., 2007, Meyer et al., 2004, Rohde et al., 2004, Barth et al., 2003, Meyer et al., 2010). Heterosis can be affected by genetic background, therefore not all *Arabidopsis thaliana* accession combinations give rise to hybrid vigour, however some hybrids have been found to have increased biomass or cold tolerance (Meyer et al., 2004, Rohde et al., 2004). *Arabidopsis thaliana* accessions have a high level of epigenetic variation, thus some inter-accession hybridisations could lead to modification of chromatin marks and expression of heterosis (Vaughn et al., 2007).

Heterosis was systematically characterized by Charles Darwin (Darwin, 1876), who observed that crossed plants of over 60 different species were more vigorous than the selfed ones. More recent studies pointed out the roles of three molecular models in controlling this phenomenon. Heterosis can be explained by the dominance model stating that slightly deleterious parental alleles are complemented in the hybrids by superior alleles, resulting in better performance (Xiao et al., 1995). The other classical hypothesis for hybrid vigour is overdominance, suggesting that genes have greater expression in heterozygous conditions (Li et al., 2001). Both heterosis and inbreeding depression can result from epistatic interactions between alleles in different loci in ways that they mask the action of each other (Yu et al., 1997, Birchler et al., 2006). A combination of dominance, overdominance and epistasis has been found to be involved in biomass heterosis in *Arabidopsis thaliana* intra-specific hybrids (Meyer et al., 2010).

Hybrid vigour results from genome-wide changes and interactions between maternal and paternal alleles. Hybrids/allopolyploids can exhibit two general modes of gene expression: allelic additive expression with mid parental expression level (*cis*), or non-additive expression with other than averaged parental expression levels (*trans*) (Springer and Stupar, 2007, Birchler et al., 2003). Molecular events underlying heterosis can involve a cumulative expression of each allele (dominance model) or, non-additive patterns of expression (overdominance, epistasis), repressing or activating progenitors' genes (Adams et al., 2003, Auger et al., 2005, Wang et al., 2004). Non-additive gene expression and epigenetic regulation is controlled by post-transcriptional mechanisms via RNA-mediated

pathways (including miRNAs and siRNAs), RNA-directed DNA methylation, and chromatin remodelling (Chen, 2007, Chen and Ni, 2006). Many miRNA targets are non-additively expressed in allotetraploids, suggesting that miRNAs produced during inter-specific hybridization or polyploidization serve as a buffer against genetic clashes between species, leading to growth vigour and adaptation (Wang et al., 2006, Ha et al., 2009).

**FIGURE 7.** Illustration of heterosis for biomass production in reciprocal hybrids of maize (two central individuals), compared to their parental inbred lines (two peripheral individuals) (Birchler et al., 2003).

Repeat-associated siRNAs are underrepresented during F1 and early stages of allopolyploid formation, allowing for genomic shock to cause meiotic disorders, activation of some transposable elements, genome instability and infertility (McClintock, 1984, Ha et al., 2009). The levels of siRNAs are restored in the late and natural allopolyploids to maintain chromatin and genome stability through siRNA-directed DNA methylation and chromatin modifications (Wang et al., 2004, Comai, 2000).

*Arabidopsis suecica* allotetraploids grow more vigorously, however the size of their seeds and flowers are an average of the two parents (Chen, 2010). Allopolyploidy leads to permanent, heritable fixation of divergent parental genomes because there is a limited pairing to homoeologous chromosomes during meiosis. It also shows increased vigour compared to corresponding diploid hybrids which lose their heterozygosity with every generation, due to genetic recombination (Auger et al., 2005, Birchler et al., 2003, Comai, 2005).

### 4.5 Apomixis

Apomixis is asexual plant reproduction through seeds, where emerging clonal seedlings retain the genotype of the maternal parent (Koltunow and Grossniklaus, 2003). Apomixis and sexual reproduction are closely related processes, where apomixis is considered as a special-temporary deregulation of the sexual pathway – **FIGURE 8**- (Nogler, 1984, Grossniklaus, 2001, Spillane et al., 2001a). In some instances apomictic species can revert to sexuality which prevents them from accumulation of deleterious mutations and from becoming evolutionary dead ends; they are called facultative apomicts (Verduijn et al., 2004). Apomixis is found in over 40 plant families represented by more than 400 species (Nogler, 1984, Carman, 1997).

Apomixis includes three components: female gametophyte formation without meiosis (apomeiosis), autonomous embryo development (parthenogenesis), and functional endosperm formation (Ozias-Akins and Van Dijk, 2007). Despite omission of meiosis and double-fertilization, apomicts produce fruits and viable seeds. Usually early in the ovule development, cells initiating apomixis undergo gametophytic apomixis (diplospory or apospory), and the unreduced embryo sacs are formed through mitotic divisions (d'Erfurth et al., 2009, Ravi et al., 2008). Embryogenesis occurs autonomously and endosperm formation may be autonomous as well, or may require fertilization of the central cell (pseudogamy) (Ohad et al., 1999, Gross-Hardt et al., 2002, Lotan et al., 1998, Schmidt et al., 1997, Shah et al., 2001). Later in the ovule development cells initiating apomixis may directly develop into embryos in the process called adventitious embryony (Boutelier et al., 2001, Kantama et al., 2006). In order to survive, it is essential for the adventitious embryo

to gain access to the nutrient endosperm from the adjacent meiotically derived embryo sac (Chaudhury et al., 1997, Grossniklaus et al., 1998, Spielman et al., 2003). Diplospory, apospory, and adventitious embryony can coexist in one apomictic species, such as *Beta*, and *Rosaceae* (Nybom, 1988).

Over the last century, a growing body of apomixis researchers has proposed many models for the control of apomictic events, given the diversity of this phenomenon (Dresselhaus and Colombo, 2001, Spillane et al., 2001b). One of the first models postulates that apomixis results from the hybridization of related species with differences in reproductive characters, as all naturally occurring apomicts are highly heterozygous (Ernst, 1918, Carman, 2001, Carman, 1997, Schranz et al., 2005). Asynchronous expression of genes controlling reproductive programs from the two hybridized genomes may be responsible for precocious embryo sac initiation and embryogenesis (Carman, 1997). Polyploidy is often associated with apomixis as a diploid or an aneuploid gamete is required for the apomictic reproduction (Sharbel and Mitchell-Olds, 2001, Quarin et al., 2001, van Dijk and Bakx-Schotman, 2004). In triploids, apomixis might be selected as it allows bypassing of the triploid block which leads to sterility in some species (Köhler et al., 2010, Noyes and Rieseberg, 2000). However, recovery of diploid apomicts suggests that polyploidy might be only a consequence of asexual reproduction, not a causative factor (Nogler, 1982, Bicknell, 1997, Grimanelli et al., 2001). The second model establishes that apomixis results from mutations at one or a few loci, so that genes conferring apomixis are mutated alleles of genes controlling sexual reproduction (Koltunow, 1993, Grimanelli et al., 2001, Grossniklaus et al., 2001a, Savidan, 2001). Epigenetic gene regulation model unites both the mutation and hybridization hypotheses as epigenetic changes may be a consequence of hybridization and polyploidization, and cause stable epimutations that are maintained even after a reduction in ploidy (Spillane et al., 2001a, Grossniklaus, 2001, Chandler and Stam, 2004, Lee and Chen, 2001, Kakutani et al., 1996, Grimanelli et al., 2001). An epigenetic model also fits the observation that in some cases simple chromosome doubling without hybridization or mutation, can lead to an apomictic mode of reproduction, like in *Paspalum notatum* (Quarin et al., 2001).

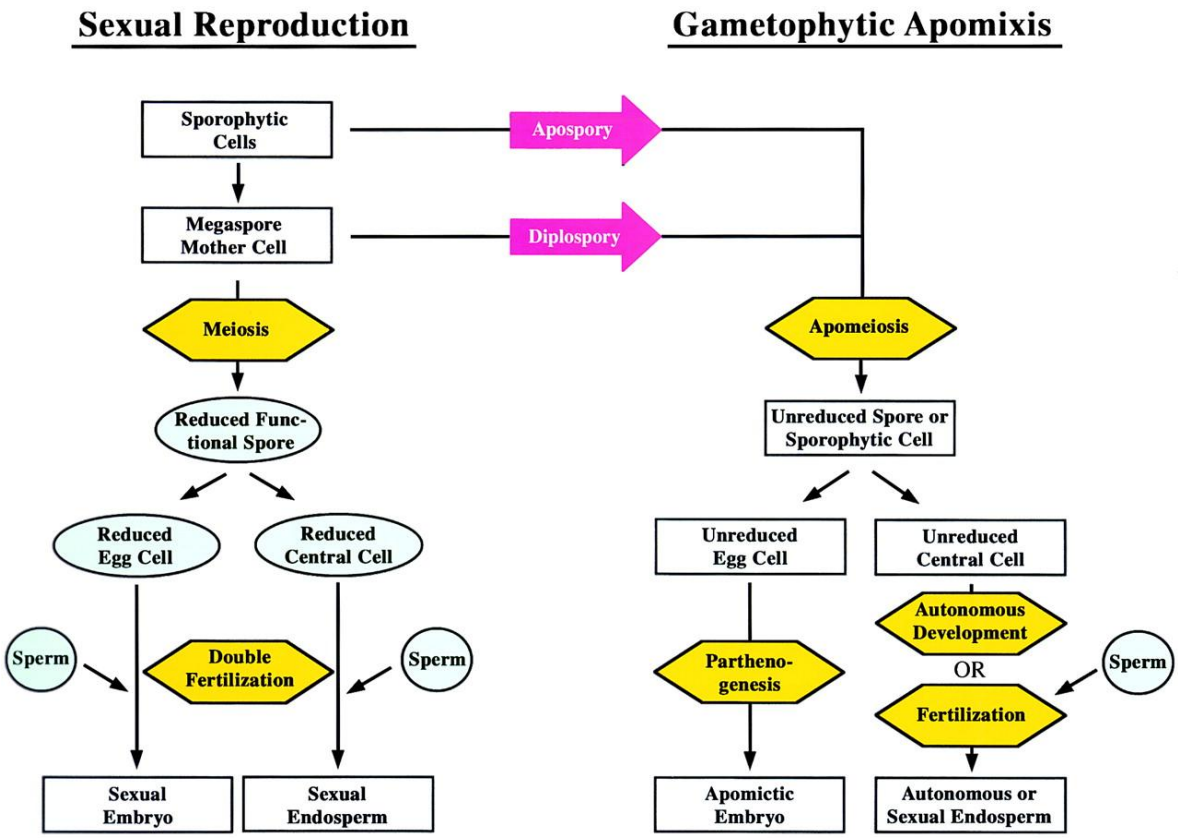


**FIGURE 8.** Sexual vs asexual plant reproduction (Grossniklaus et al., 2001a)

Harnessing apomixis technology for permanent fixation of hybrid vigour would have many agricultural benefits (Dresselhaus et al., 2001, van Dijk and van Damme, 2000, Siddiqi et al., 2009, Spillane et al., 2004, Spillane et al., 2001a).

## 5. Summary

In summary, the utility of natural genetic and epigenetic variation among *Arabidopsis thaliana* accessions for studying the biology of reproduction in flowering plants was outlined. For better understanding the phenomena of polyploidy evolutionary success, reproductive isolation, hybrid incompatibility, and heterosis, focus was put on the contributions and interactions of parental genomes during embryogenesis and seed development.

# CHAPTER 2 – Heterosis in *Arabidopsis thaliana* diploid and triploid seeds size

## 2.1 Introduction

Heterosis (hybrid vigour) refers to the ability of F1 hybrid offspring to exhibit more vigorous phenotypes, such as biomass, stature, growth rate or fertility, that lie outside the phenotypic range observed in both parents (Auger et al., 2005, Chen, 2007, Chen, 2010). Inbred lines of maize are found to show deterioration in yield and vigour, but the hybrids between two inbred lines immediately and completely recover, in many cases exceeding the yield of the parental varieties from which they were derived (Shull, 1908, Crow, 1998). Heterosis has been exploited extensively in plant breeding for centuries leading to new varieties of hybrid or allopolyploid crops, e.g. triticale, which combines the good grain quality and high yield of wheat, with the disease and stress tolerance of rye (Guedes-Pinto et al., 1996, Birchler et al., 2003). However, despite its great importance to agriculture, the genetic bases of this phenomenon remain obscure. Many models have been proposed to explain heterosis, although it cannot be explained by simple complementation of multiple unfavourable alleles. It is a complex phenomenon that involves interplay of alleles causing gene expression in the hybrid that deviates from the mid-parent value.

Intra-specific hybrids of *Arabidopsis thaliana* have been employed as a model for understanding heterosis in hybrid crops (Stokes et al., 2007, Meyer et al., 2004, Rohde et al., 2004, Banaei Moghaddam et al., 2010, Barth et al., 2003, Meyer et al., 2010). Heterosis can be affected by genetic background; therefore not all *Arabidopsis thaliana* accession combinations give rise to hybrid vigour. However some F1 hybrids were found to have increased biomass or cold tolerance (Meyer et al., 2004, Rohde et al., 2004). Several studies have suggested that epigenetic variation may play an important role in heterosis, e.g. altered transcription of a few regulatory genes through epigenetic variations was found to be associated with growth vigour in hybrids (He et al., 2010, Makarevitch et al., 2007, Ni et al., 2008, Shindo et al., 2006). *Arabidopsis thaliana* accessions have a high level of genetic and epigenetic variation, thus some inter-accession and/or inter-ploidy hybridizations could lead to modification of chromatin marks and expression of heterosis (Cao et al., 2011, Vaughn et al., 2007).

Seed size heterosis has been reported for intra-specific *Arabidopsis thaliana* F1 hybrid diploids, where the paternal genome was acting to promote the nutritional resource allocation to seeds (Stokes et al., 2007). Maternal effects were also found to have a significant impact on seed size by controlling nutrient supply and early endosperm development (Vielle-Calzada et al., 1994, Stokes et al., 2007, Chaudhury and Berger, 2001).

Heterosis is usually described as mid-parent heterosis (MPH), where the average trait value of the F1 hybrid is compared to the average trait value of the parents, however in the agricultural context, the hybrid must exceed the best parent to be useful (Meyer et al., 2004). Seed size best-parent heterosis (BPH), where the F1 offspring has bigger seeds than the best parent, and worst-parent heterosis (subtractive heterosis, WPH), where F1 offspring has smaller seeds than the worst parent represent extreme, or so called transgressive phenotypes (Rieseberg et al., 1999). They are both of great agricultural importance, e.g. larger seeds have been selected for many food grains for centuries, however, in certain species, smaller seeds are more desirable, such as grapes, watermelon or tomato (Milborrow, 1998).

### 2.1.1 Research Objectives

This chapter aims to determine heterotic effects on *Arabidopsis thaliana* F1 seed size in three types of genetic crosses: four inter-ploidy isogenic crosses, sixty inter-ploidy hybrid crosses, and five balanced diploid hybrid crosses. This experimental design allows the effects of parental genome dosage (in inter-ploidy isogenics) to be compared with the effects of hybridization (in balanced diploid hybrids) on heterosis in different genetic backgrounds (accessions).

Finally, parental genome dosage effects and inter-accession hybridizations (inter-ploidy hybrids) were combined to determine if both effects together are manifested by a stronger heterotic response.

## 2.2 Methodology and Materials

### 2.2.1 Plant material

Diploid plants of 60 wild inbred strains (accessions) of the 96 Nordborg *Arabidopsis thaliana* mapping panel (Nordborg et al., 2005) were grown from seeds provided by NASC (CS22564-CS22659). Tetraploid seeds originated from: L*er*-0 Ueli Grossniklaus's lab (originally from Cold Spring Harbour Labs), Col-0 and C24 (from Luca Comai's lab, University of Washington), Zurich (from Ortrun Mittelsten Scheid's lab, GMI Vienna). Seeds were surface sterilized by soaking in 5% v/v sodium hypochlorite, 0.05% Triton-X solution (5 minutes) and washed five times with sterile distilled water. Sterilized seeds were individually sown on plates of Murashige and Skoog medium containing 4.6 g $L^{-1}$ MS salts, 30 g $L^{-1}$ sucrose and 8 g $L^{-1}$ agarose (Murashige and Skoog, 1962). The seeded MS plates were incubated in a Percival Tissue Culture Cabinet under a 16:8 hr light: dark ($21^0$C/$18^0$C) regime until the fourth true leaf stage (Boyes et al., 2001b). Seedlings were transplanted to individual pots of soil (8 parts Westland multipurpose compost: 1 part perlite: 1 part vermiculite) and transferred to growth chambers to grow under fluorescent lamps at 200 µmol $m^{-2}$ $s^{-1}$ at $21^0$C/$18^0$C and a 16:8 hr light: dark photoperiod.

### 2.2.2 Genetic crosses

Mature floral buds of each of the female parents, prior to anthesis, were manually emasculated and reciprocally crossed by hand to the appropriate male parent under a Leica MZ6 dissecting microscope using fine tweezers. The pollinated gynoecia were left for up to 7 days to grow until the mature siliques became yellow-green. The generated F1 seeds were collected in Eppendorf tubes with pierced lids and stored in boxes containing desiccant silica gel at room temperature.

### 2.2.3 Seed size measurement

The size of reciprocal F1 seeds, as well as their male and female parents, was determined by placing samples of at least 10 seeds per line on a black microscope slide, imaging under a dissecting microscope (Leica MZ6) and measuring areas with DigiShape 1.9.217 software (Cortex Nova, Poland). Lines with less than 10 seeds or with seeds of shrivelled appearance were not used in the determination of seed size heterosis.

### 2.2.4 Calculation of heterosis

Mean size [$mm^2$] of at least 10 normal-looking seeds per line was used for determining the seed size heterosis of F1 offspring over their mid-parent (MP), best-parent (BP), or worst-parent (WP) values. The mid-parent heterosis (MPH) values were calculated to determine offspring that have seed size greater than the average of both parents; %MPH = (F1 – MP)/MP X 100%. Best-parent heterosis (BPH) determines offspring with increased seed size over the largest parent; %BPH = (F1 – BP)/BP X 100%, and the worst-parent heterosis determines offspring that has seeds smaller than the smallest parent; %WPH = (F1 – WP)/WP X 100% (Meyer et al., 2004).

### 2.2.5 Clearings of seeds

Siliques containing diploid hybrid seeds (Kas-1 x L*er*-0 and L*er*-0 x Kas-1) at different developmental stages (4/5/6/7/8 d.a.p.) were harvested and fixed in Carnoy's solution (3 EtOH: 1 acetic acid) at $4^0$C o/n. The following day they were rinsed with MQ water and incubated in Hoyer's solution (7.5g gum arabic, 100g chloral hydrate, 5ml glycerol, dissolved in 60ml $H_20$) at $4^0$C o/n. Cleared seeds were dissected from the siliques and mounted on the microscope slide with an additional drop of Hoyer's solution. A slide cover slip was gently added without applying hand pressure. Embryos at different developmental stages were observed using Nomarski microscopy with Differential Interference Contrast (DIC), and images taken using built-in digital camera (Olympus).

## 2.3 Results

### 2.3.1 Heterosis is observed in isogenic F1 triploid seeds of *Arabidopsis thaliana*

Reciprocal, inter-ploidy crosses in isogenic C24, L*er*-0 (Scott et al., 1998), and Col-0 (Rowan et al., 2011) genetic backgrounds have been shown to cause dosage-dependent phenotypes on seed size. To generate four sets of F1 isogenic reciprocal triploids in different genetic backgrounds, diploid accessions of L*er*-0, Col-0, C24, and Zu have been crossed to their isogenic tetraploids, in both directions. Each of the accessions differed in relation to the seed size of the parental 2x and 4x lines. For instance, Zu had the largest seeds, whilst L*er*-0 represented accession with the smallest seeds (**Figure 2.1, Figure 2.2**).

On average, tetraploid parents and F1 2x X 4x paternal excess triploid seeds were found to be approximately 1.3 times larger than diploid parents, whereas F1 4x X 2x maternal excess triploids were 0.8 times smaller (**Figure 2.2**). The accession Zu produced the largest reciprocal F1 triploid seeds, whereas L*er*-0 inter-ploidy crosses generated the smallest reciprocal F1 triploid seeds (only Col-0 F1 2x X 4x paternal excess triploid seeds had slightly smaller size than L*er*-0) (**Figure 2.1, Figure 2.2 Figure 2.3**).

| **24** | **2x4** | **4x2** | **4x4** | **Col-0** | **2x4** | **4x2** | **4x4** |
|---|---|---|---|---|---|---|---|
| **2x2** | 0.0001 | **0.266*** | 0.0001 | **2x2** | **0.006*** | 0.0001 | 0.0001 |
| **2x4** | | 0.0001 | **1*** | **2x4** | | 0.0001 | 0.0001 |
| **4x2** | | | 0.0001 | **4x2** | | | 0.0001 |
| **L*er*-0** | **2x4** | **4x2** | **4x4** | **Zu** | **2x4** | **4x2** | **4x4** |
| **2x2** | 0.0001 | 0.0001 | 0.0001 | **2x2** | 0.0001 | 0.0001 | 0.0001 |
| **2x4** | | 0.0001 | 0.0001 | **2x4** | | 0.0001 | **0.51*** |
| **4x2** | | | 0.0001 | **4x2** | | | 0.0001 |

**Table 2.1**. ANOVA - ANalysis Of VAriance in seed size between groups of different ploidies within four genetic backgrounds. Statistically non-significant differences in seed size are highlighted in bold and with an asterisks. Significance level is at $p < 0.001$.

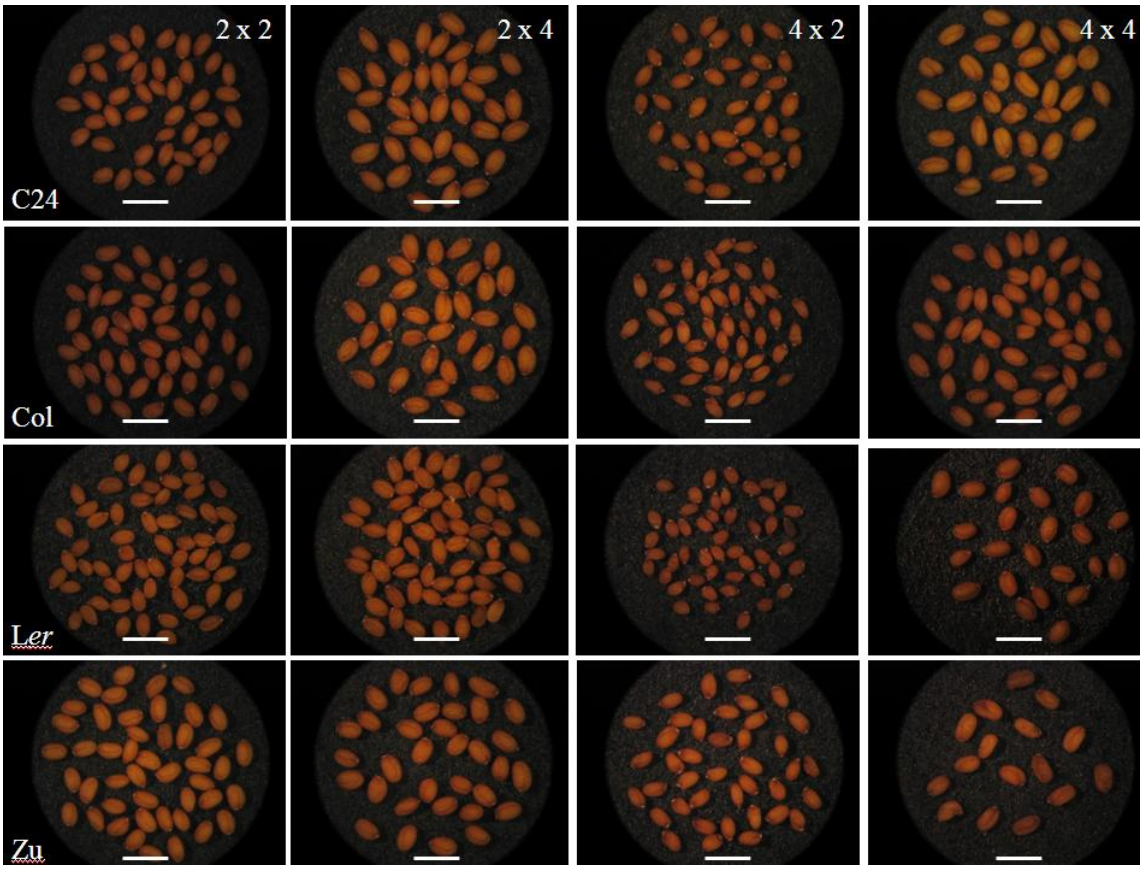


**Figure 2.1.** Dosage-dependent F1 seed size across isogenic ploidy series for four accessions (genetic backgrounds) of *Arabidopsis thaliana*. Diploids and tetraploids are represented by 2 X 2 and 4 x 4, respectively. F1 isogenic triploids are represented by 2 x 4 (paternal excess triploids) and 4 x 2 (maternal excess triploids). Scale bar = 1mm.

.

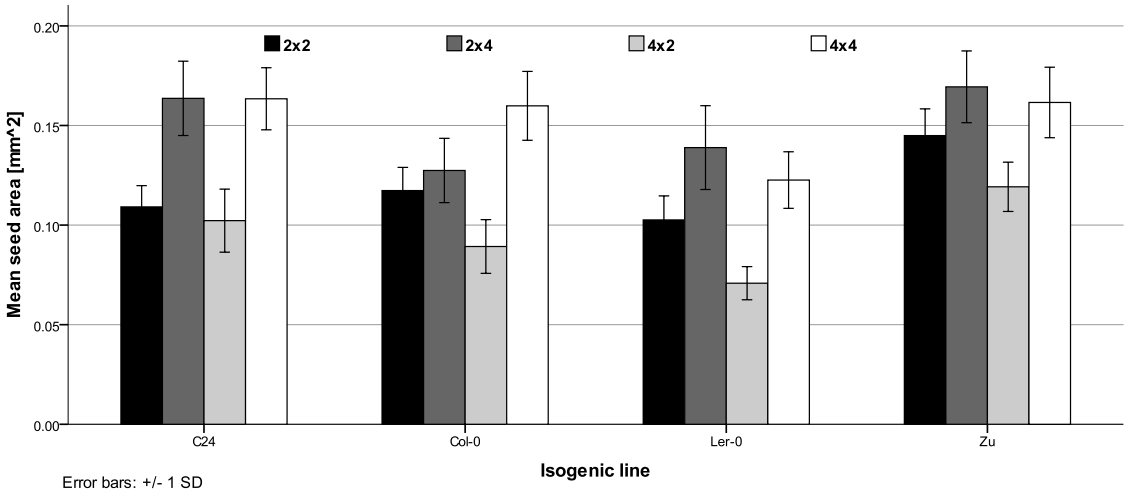


**Figure 2.3.** Mean seed area in four different isogenic lines (Ler-0, Zu, C24, Col-0) clustered by accession. 2x2 = diploid, 4x4 = tetraploid, 4x2 = maternal excess F1 isogenic triploid, 2x4 = paternal excess F1 isogenic triploid.

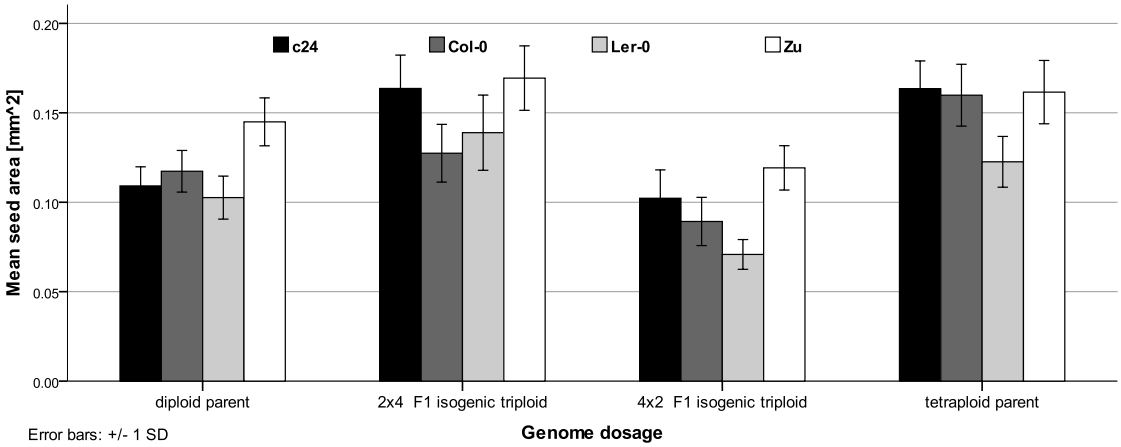


**Figure 2.2.** Mean seed area in four different isogenic lines (L*er*-0, Zu, C24, Col-0) clustered by genome dosage.

| Accession | 2x2 | 4x4 | 2x4 | 4x2 | MP | BP | WP | MPH%_2x4 | MPH%_4x2 | %BPH_2x4 | %BPH_4x2 | %WPH_2x4 | %WPH_4x2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **C24** | 0.1091 | 0.1634 | 0.1636 | 0.1022 | 0.1362 | 0.1634 | 0.1091 | 20.0734 | -24.991 | 0.1224 | -37.4541 | 49.9541 | -6.3245 |
| **Col-0** | 0.1173 | 0.1599 | 0.1274 | 0.0892 | 0.1386 | 0.1599 | 0.1173 | -8.0808 | -35.642 | -20.3252 | -44.2151 | 8.6104 | -23.9557 |
| **Ler-0** | 0.1026 | 0.1226 | 0.1389 | 0.0708 | 0.1126 | 0.1226 | 0.1026 | 23.357 | -37.123 | 13.2953 | -42.2512 | 35.3801 | -30.9941 |
| **Zu** | 0.1449 | 0.1616 | 0.1694 | 0.1192 | 0.1532 | 0.1616 | 0.1449 | 10.5383 | -22.219 | 4.82673 | -26.2376 | 16.9082 | -17.7364 |

**Table 2.2.** Mid-parent (MPH), best-parent (BPH), and worst-parent (WPH) heterosis for seed size in four reciprocal isogenic F1 triploid lines generated by inter-ploidy crosses.

Inter-ploidy crosses can lead to positive heterosis if F1 2x X 4x paternal excess triploids produce larger seeds than their bigger parent (best-parent heterosis, BPH), or subtractive heterosis if the seeds produced by F1 4x X 2x maternal excess triploids are smaller than their smaller parent (worst-parent heterosis, WPH). Three of four F1 2x X 4x paternal excess isogenic triploid lines display best-parent heterosis, with L*er*-0 having the best heterotic performance (BPH = 13%; MPH = 23.4%), followed by Zu (BPH = 4.3%; MPH = 10.5%), with minimal heterotic effect observed in C24 F1 triploid seeds (BPH = 0.1%; MPH = 20.1%). Only Col-0 did not produce heterotic F1 triploid progeny over best-parent (BPH = -20.6%), or mid-parent (MPH = -8.1%) (**Figure 2.4, Figure 2.5**).

All of the isogenic F1 maternal excess triploids exhibited subtractive heterosis over their worst-parent and always produced smaller seeds, with L*er*-0 (WPH = -31%) and Col-0 (WPH = -23.9%) having the strongest heterotic effect, followed by Zu (WPH = -17.7%) and C24 (WPH = -6.3%) (**Figure 2.6**). Notably, the Col-0 triploids that lost their heterotic effect on seed size in the 2x X 4x paternal excess F1 triploid were one of the best performing in the 4x X 2x maternal excess in terms of subtractive heterosis (**Table 2.2**, **Figure 2.6**). This means that in the Col-0 background, the 4x X 2x cross results in the expected WPH phenotype, but no BPH is observed in the isogenic F1 triploid seeds from the reciprocal 2x X 4x cross (**Figure 2.5, Figure 2.6**).

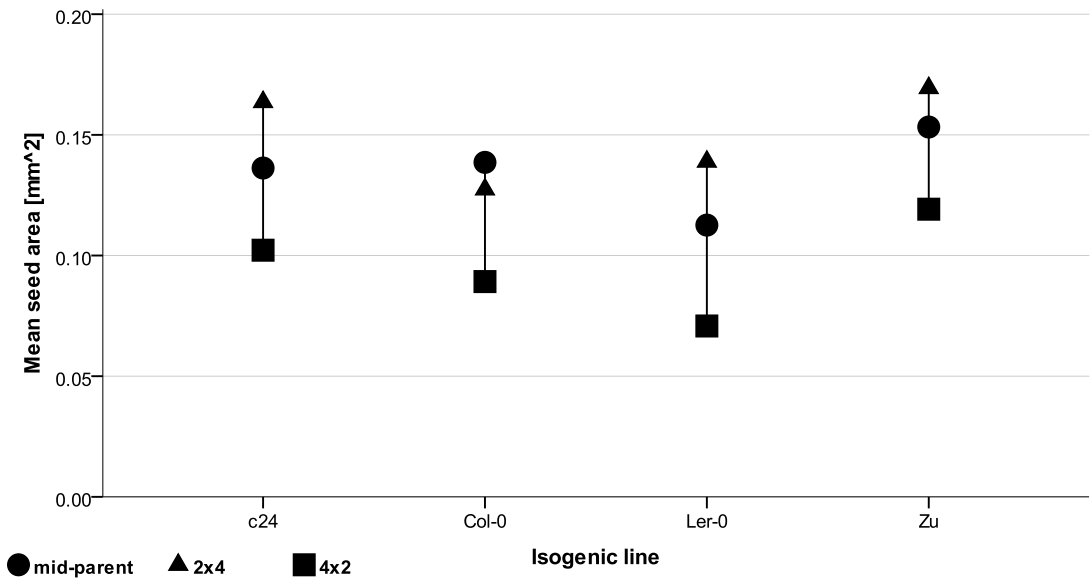


**Figure 2.4.** Mid-parent heterosis (MPH) for the F1 isogenic triploid seeds. The circle indicates the mid-parent value, the triangle indicates the 2x X 4x paternal excess triploid seed size, the square indicates the 4x X 2x maternal excess triploid seed size.

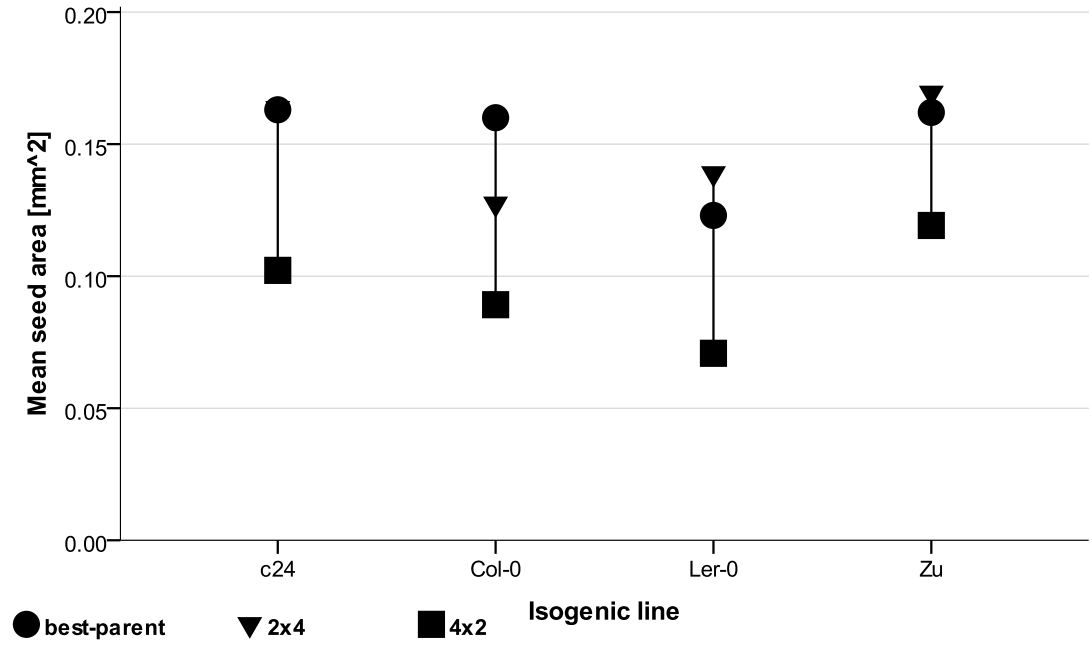


**Figure 2.5.** Best-parent heterosis (BPH) for the F1 isogenic triploid seed size. The circle indicates the best-parent value, the triangle indicates the 2x X 4x paternal excess triploid seed size, the square indicates the 4x X 2x maternal excess triploid seed size.

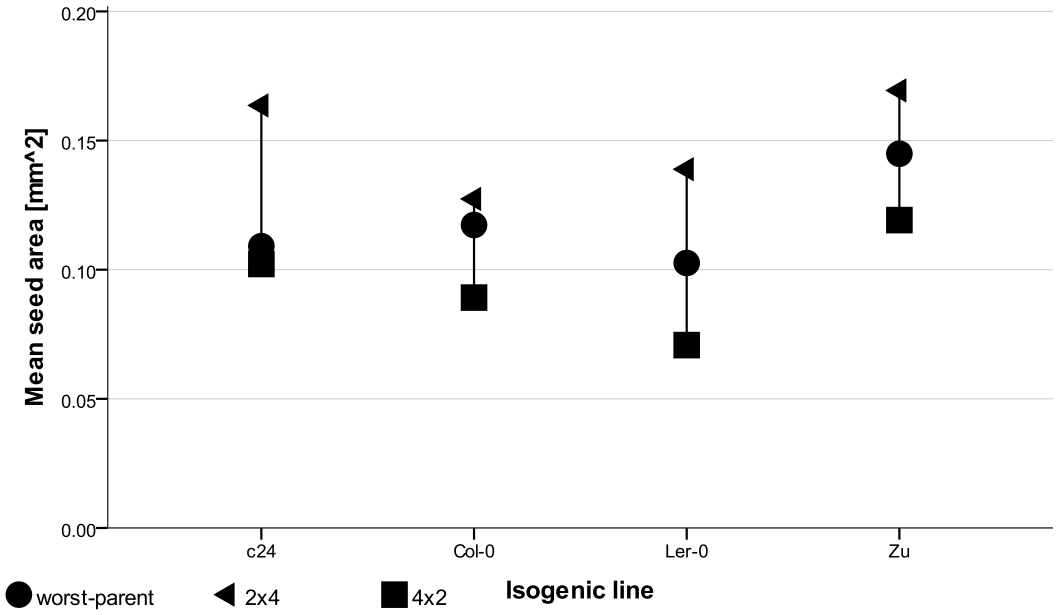


**Figure 2.6.** Worst-parent heterosis (WPH) for the F1 isogenic triploid seed size. The circle indicates the worst-parent value, the triangle indicates the 2x X 4x paternal excess triploid seed size, the square indicates the 4x X 2x maternal excess triploid seed size.

**2.3.2 Maternal genotypes explain the variation in the size of F1 hybrid triploid seeds.**

Natural variation in the seed size of 60 different inbred lines (accessions) of *Arabidopsis thaliana* is determined, and demonstrated to range from 0.092 $mm^2$ to 0.153 $mm^2$ (**Figure 2.7, Table 2.4**). Also, the average size of L*er*-0 tetraploid seeds was determined (0.146 $mm^2$) and it was found to be larger than the diploid seeds except for the following accessions: Mrk-0 (0.153 $mm^2$), Bur-0 (0.149 $mm^2$), Cvi-0 (0.147 $mm^2$), Mz-0 (0.147 $mm^2$), and Rmx-A02 (0.146 $mm^2$) (**Figure 2.8, Table 2.4**).

The L*er*-0 tetraploid line was used to generate two sets of F1 hybrid triploid seeds: 4x X 2x maternal excess F1 triploids (where tetraploid L*er*-0 was used as a seed parent) and 2x X 4x paternal excess triploids (where tetraploid L*er*-0 was used as a pollen donor), by reciprocal crosses to 60 different diploid accessions. In all cases, paternal excess F1 hybrid triploid seeds were found to be consistently larger than seeds with maternal genome excess (**Appendix A, Figure 2.8, Table 2.4**).

Phenotypical correlations were estimated by plotting seed size of the diploid accession, against the size of the 2x X 4x paternal excess F1 triploid seeds, and the 4x X 2x maternal excess F1 triploids (**Figure 2.9, Table 2.3**). There was a significant positive correlation between the diploid maternal accessions and the 2x X 4x paternal excess F1 triploid seed size ($r = 0.442$ p-value $< 0.001$), suggesting that seed size is a flexible trait that is strongly affected by maternal condition. There was no correlation between the seed size of diploid paternal accessions and the 4x X 2x maternal excess triploid seeds they sired, i.e. 2x accessions with bigger seeds did not sire bigger 3x seeds than 2x accessions with smaller seeds (**Figure 2.9, Table 2.3)**.

Regression of the 4x X 2x maternal excess triploid offspring on seed size of the tetraploid L*er*-0 mother was not possible as there was only one mother (L*er*-0 4x) used for crosses to 60 fathers. However, it was observed that there was a minimum variation in the size of 4x X 2x maternal excess triploid seeds sired by different fathers, ranging from 0.066 $mm^2$ in Spr1-2 to 0.108 $mm^2$ in Bay-0 ($\Delta = 0.042$ $mm^2$). Much greater variation in the size of the 2x X 4x paternal excess triploid seeds, ranging from 0.243 $mm^2$ in Kas-1 to 0.132 $mm^2$ in Ei-2 ($\Delta = 0.111$ $mm^2$) corresponds to the high natural variation found among diploid accessions used as female parents (**Figure 2.7**, **Figure 2.8).**

To determine the difference in size between pairs of reciprocal F1 triploid seeds, the mean size of 4x X 2x maternal excess F1 triploid seeds was subtracted from 2x X 4x paternal excess F1 triploid seeds. The positive values of all bars representing the differences in seed size confirm that all 2x X 4x paternal excess F1 triploid seeds were consistently larger than their reciprocal triploids. Differences in F1 seed size varied by line and ranged from 0.162mm$^2$ to 0.044 mm$^2$ (**Table 2.3, Figure 2.10**).

A strong positive correlation was found between the difference in size in reciprocal triploid seeds and the 2x X 4x paternal excess F1 triploid seeds ($r = 0.934$ p-value $< 0.001$). A negative correlation was found between the difference in size in reciprocal F1 triploid seeds and the 4x X 2x maternal excess triploid seeds ($r = -0.343$ p-value $< 0.01$). A positive correlation was also found between the differences in reciprocal F1 triploid seed size and the size of diploid seeds ($r = 0.404$ p-value $< 0.001$). If the 4x X 2x maternal excess triploid seed size is approximately constant (as the same L*er*-0 mother is used in each cross), the difference in size between the reciprocal F1 triploid seeds largely depends on the variation in the 2x X 4x paternal excess F1 triploid seed size, which in turn is controlled by the diploid parent. This confirms the previous observation that diploid parent affects 2x X 4x paternal excess F1 triploid seed size (**Figure 2.9, Table 2.3)**.

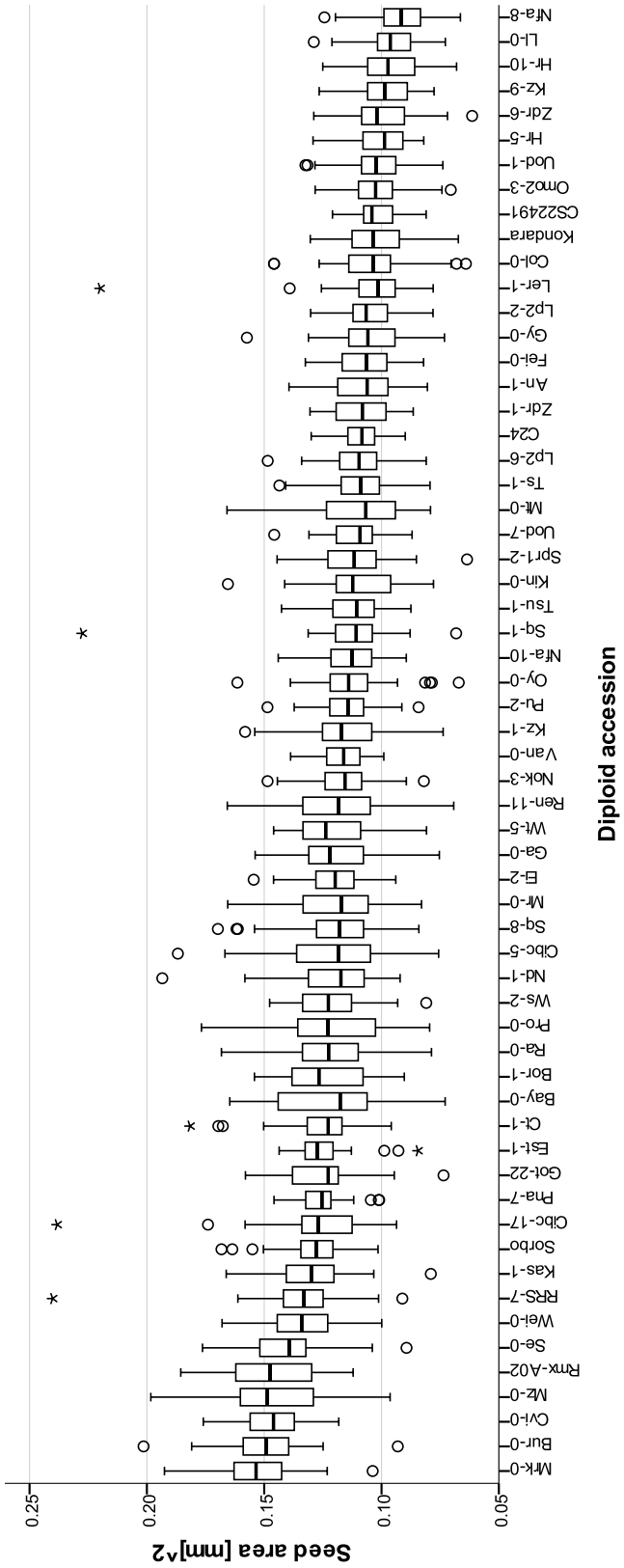


**Figure 2.7.** Natural variation in seed size across 60 diploid accessions of *Arabidopsis thaliana* represented as standard box-plots. The median values, inter-quartile ranges and outliers are displayed.

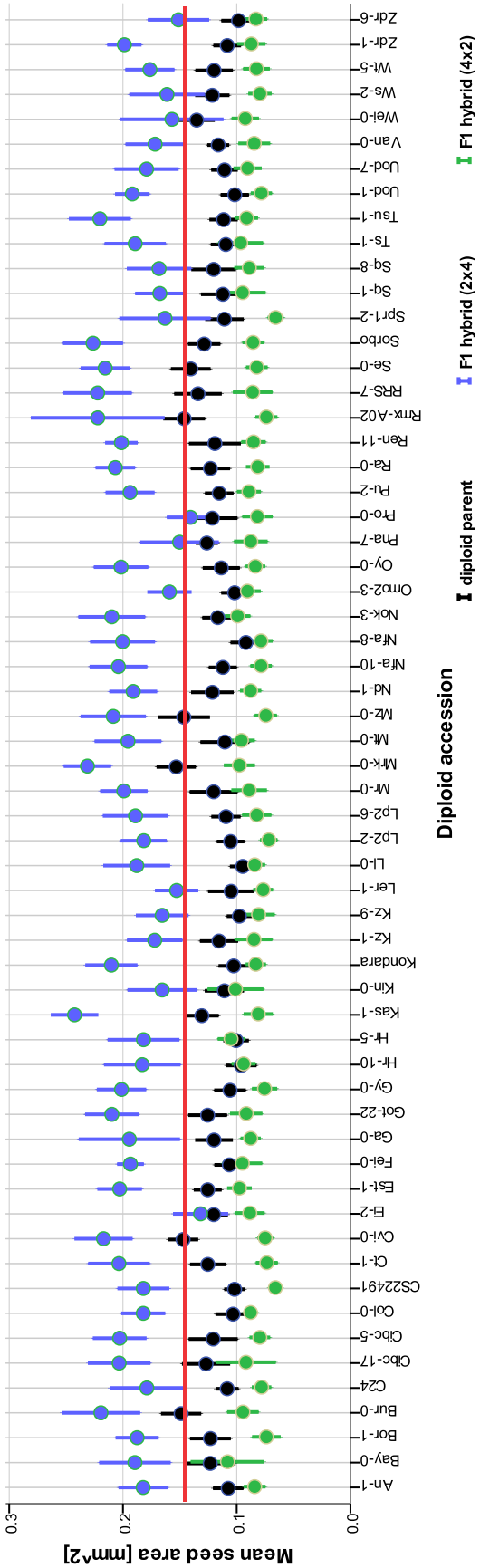


**Figure 2.8.** Mean seed size of tetraploid *Ler*-0 parent (red line), diploid parents (black dots), 2x X 4x paternal excess F1 hybrid triploids (blue dots) and 4x X 2x maternal excess F1 hybrid triploids (green dots). Error bars represent standard deviation (SD).

| | Difference | F1_2x4_triploid | F1_4x2_triploid |
|---|---|---|---|
| Diploid | r = 0.404 | r = 0.442 | r = 0.033 |
| | p-value < 0.001 | p-value < 0.001 | p-value = 0.8 |
| F1_4x2_triploid | r = -0.343 | r = 0.014 | |
| | p-value < 0.01 | p-value = 0.913 | |
| F1_2x4_triploid | r = 0.934 | | |
| | p-value < 0.001 | | |

**Table 2.3.** Pearson correlations between the size of diploid parents, F1 2x X 4x paternal excess hybrid triploids, F1 4x X 2x maternal excess hybrid triploids, and the difference in size between pairs of reciprocal triploids. Correlation is significant at the 0.05 level.

| 4x L*er*-0 | name | 2x | name | F1_2x4 | name | F1_4x2 | name | 2x4- 4x2 |
|---|---|---|---|---|---|---|---|---|
| **0.146** | **Mrk-0** | **0.153** | Kas-1 | 0.243 | CS22491 | 0.066 | Kas-1 | 0.162 |
| | **Bur-0** | **0.149** | Mrk-0 | 0.231 | Spr1-2 | 0.066 | Rmx-A02 | 0.148 |
| | **Cvi-0** | **0.147** | Sorbo | 0.226 | Lp2-2 | 0.072 | Cvi-0 | 0.142 |
| | **Mz-0** | **0.147** | Rmx-A02 | 0.222 | Bor-1 | 0.074 | Sorbo | 0.14 |
| | **Rmx-A02** | **0.146** | RRS-7 | 0.222 | Ct-1 | 0.074 | RRS-7 | 0.136 |
| | Se-0 | 0.14 | Tsu-1 | 0.22 | Mz-0 | 0.074 | Mz-0 | 0.135 |
| | Wei-0 | 0.135 | Bur-0 | 0.219 | Rmx-A02 | 0.074 | Se-0 | 0.134 |
| | RRS-7 | 0.134 | Cvi-0 | 0.217 | Cvi-0 | 0.075 | Mrk-0 | 0.133 |
| | Kas-1 | 0.131 | Se-0 | 0.216 | Gy-0 | 0.076 | Ct-1 | 0.13 |
| | Sorbo | 0.129 | Got-22 | 0.21 | Ler-1 | 0.077 | Tsu-1 | 0.129 |
| | Cibc-17 | 0.127 | Kondara | 0.21 | C24 | 0.078 | Kondara | 0.127 |
| | Got-22 | 0.126 | Nok-3 | 0.21 | Uod-1 | 0.078 | Gy-0 | 0.125 |
| | Pna-17 | 0.126 | Mz-0 | 0.209 | Nfa-10 | 0.079 | Nfa-10 | 0.125 |
| | Ct-1 | 0.125 | Ra-0 | 0.207 | Nfa-8 | 0.079 | Ra-0 | 0.125 |
| | Est-1 | 0.125 | Ct-1 | 0.204 | Cibc-5 | 0.08 | Bur-0 | 0.124 |
| | Bay-0 | 0.123 | Nfa-10 | 0.204 | Ws-2 | 0.08 | Cibc-5 | 0.123 |
| | Bor-1 | 0.123 | Cibc-17 | 0.203 | Kas-1 | 0.081 | Nfa-8 | 0.121 |
| | Ra-0 | 0.123 | Cibc-5 | 0.203 | Kz-9 | 0.081 | Oy-0 | 0.118 |
| | Cibc-5 | 0.121 | Est-1 | 0.203 | Lp2-6 | 0.082 | Got-22 | 0.118 |
| | Nd-1 | 0.121 | Oy-0 | 0.202 | Pro-0 | 0.082 | Ren-11 | 0.116 |
| | Pro-0 | 0.121 | Gy-0 | 0.201 | Ra-0 | 0.082 | CS22491 | 0.116 |
| | Ws-2 | 0.121 | Ren-11 | 0.201 | Se-0 | 0.082 | Bor-1 | 0.114 |
| | Ei-2 | 0.12 | Nfa-8 | 0.2 | Kondara | 0.083 | Uod-1 | 0.114 |
| | Ga-0 | 0.12 | Mr-0 | 0.199 | Wt-5 | 0.083 | Zdr-1 | 0.112 |
| | Mr-0 | 0.12 | Zdr-1 | 0.199 | Zdr-6 | 0.083 | Cibc-17 | 0.111 |
| | Sq-8 | 0.12 | Mt-0 | 0.196 | An-1 | 0.084 | Nok-3 | 0.111 |
| | Wt-5 | 0.12 | Ga-0 | 0.194 | Ll-0 | 0.084 | Mr-0 | 0.11 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Ren-11 | 0.119 | Pu-2 | 0.194 | Oy-0 | 0.084 | Lp2-2 | 0.11 |
| | Nok-3 | 0.117 | Fei-0 | 0.193 | Kz-1 | 0.085 | Lp2-6 | 0.107 |
| | Kz-1 | 0.116 | Uod-1 | 0.192 | Ren-11 | 0.085 | Ga-0 | 0.106 |
| | Van-0 | 0.116 | Nd-1 | 0.191 | Van-0 | 0.085 | Est-1 | 0.105 |
| | Pu-2 | 0.115 | Bay-0 | 0.189 | RRS-7 | 0.086 | Pu-2 | 0.105 |
| | Oy-0 | 0.114 | Lp2-6 | 0.189 | Sorbo | 0.086 | Ll-0 | 0.104 |
| | Nfa-10 | 0.112 | Ts-1 | 0.189 | Zdr-1 | 0.087 | Nd-1 | 0.103 |
| | Sq-1 | 0.112 | Bor-1 | 0.188 | Col-0 | 0.088 | C24 | 0.101 |
| | Tsu-1 | 0.112 | Ll-0 | 0.188 | Ei-2 | 0.088 | Mt-0 | 0.1 |
| | Kin-0 | 0.111 | Hr-10 | 0.183 | Ga-0 | 0.088 | Fei-0 | 0.098 |
| | Spr1-2 | 0.111 | An-1 | 0.182 | Nd-1 | 0.088 | An-1 | 0.098 |
| | Uod-7 | 0.111 | Col-0 | 0.182 | Pna-17 | 0.088 | Spr1-2 | 0.097 |
| | Mt-0 | 0.11 | CS22491 | 0.182 | Mr-0 | 0.089 | Col-0 | 0.094 |
| | Ts-1 | 0.11 | Hr-5 | 0.182 | Pu-2 | 0.089 | Wt-5 | 0.093 |
| | Lp2-6 | 0.109 | Lp2-2 | 0.182 | Sq-8 | 0.089 | Ts-1 | 0.092 |
| | C24 | 0.108 | C24 | 0.179 | Uod-7 | 0.09 | Hr-10 | 0.089 |
| | Zdr-1 | 0.108 | Uod-7 | 0.179 | Omo2-3 | 0.091 | Uod-7 | 0.089 |
| | An-1 | 0.107 | Wt-5 | 0.176 | Tsu-1 | 0.091 | Kz-1 | 0.087 |
| | Fei-0 | 0.106 | Kz-1 | 0.172 | Cibc-17 | 0.092 | Van-0 | 0.087 |
| | Gy-0 | 0.106 | Van-0 | 0.172 | Got-22 | 0.092 | Kz-9 | 0.084 |
| | Ler-1 | 0.105 | Sq-1 | 0.168 | Wei-0 | 0.092 | Bay-0 | 0.081 |
| | Lp2-2 | 0.105 | Sq-8 | 0.168 | Hr-10 | 0.094 | Ws-2 | 0.081 |
| | Col-0 | 0.103 | Kin-0 | 0.166 | Bur-0 | 0.095 | Sq-8 | 0.079 |
| | Kondara | 0.103 | Kz-9 | 0.165 | Fei-0 | 0.095 | Hr-5 | 0.077 |
| | CS22491 | 0.102 | Spr1-2 | 0.163 | Sq-1 | 0.095 | Ler-1 | 0.076 |
| | Omo2-3 | 0.102 | Ws-2 | 0.161 | Mt-0 | 0.096 | Sq-1 | 0.073 |
| | Uod-1 | 0.102 | Omo2-3 | 0.159 | Ts-1 | 0.097 | Omo2-3 | 0.068 |
| | Hr-5 | 0.101 | Wei-0 | 0.157 | Est-1 | 0.098 | Zdr-6 | 0.068 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Kz-9 | 0.098 | Ler-1 | 0.153 | Mrk-0 | 0.098 | Kin-0 | 0.065 |
| | Zdr-6 | 0.098 | Zdr-6 | 0.151 | Nok-3 | 0.099 | Wei-0 | 0.065 |
| | Hr-10 | 0.096 | Pna-17 | 0.15 | Kin-0 | 0.101 | Pna-17 | 0.062 |
| | Ll-0 | 0.095 | Pro-0 | 0.141 | Hr-5 | 0.105 | Pro-0 | 0.059 |
| | Nfa-8 | 0.092 | Ei-2 | 0.132 | Bay-0 | 0.108 | Ei-2 | 0.044 |

**Table 2.4.** Seed size of tetraploid L*er*, diploid accessions, F1 paternal and maternal excess hybrid triploids, and differences in seed size between reciprocal triploids, sorted in descending order.

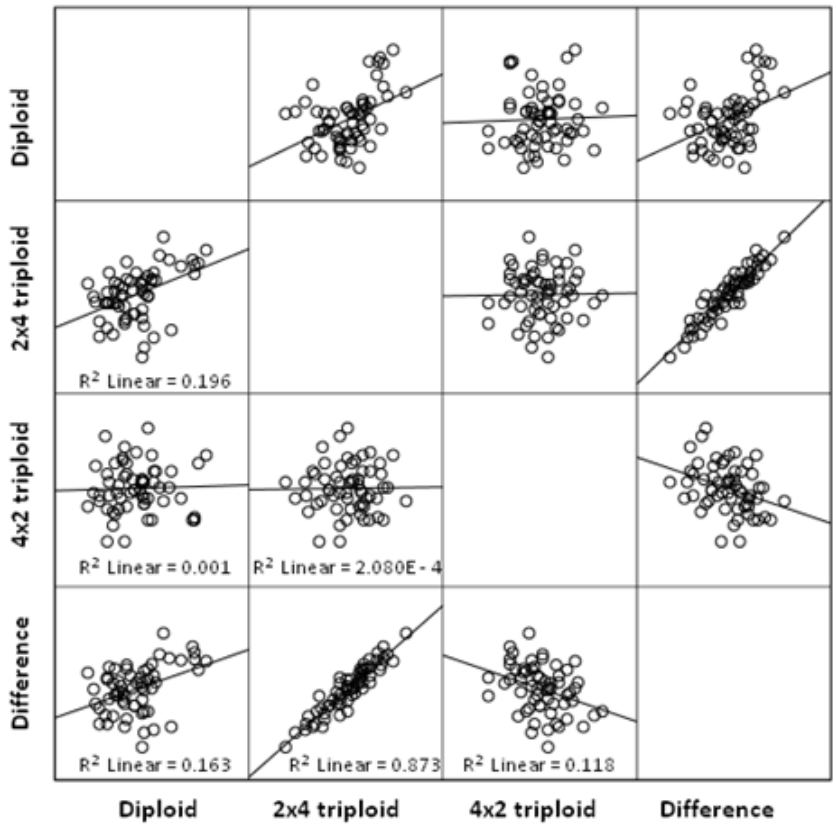


**Figure 2.9.** Scatter plot matrix representing phenotypical relationship between seed size of parental diploids, F1 reciprocal hybrid triploids (2x4 paternal excess F1 hybrid triploids and 4x2 maternal excess F1 hybrid triploids), and the difference in seed size of reciprocal triploids (2x4 – 4x2).

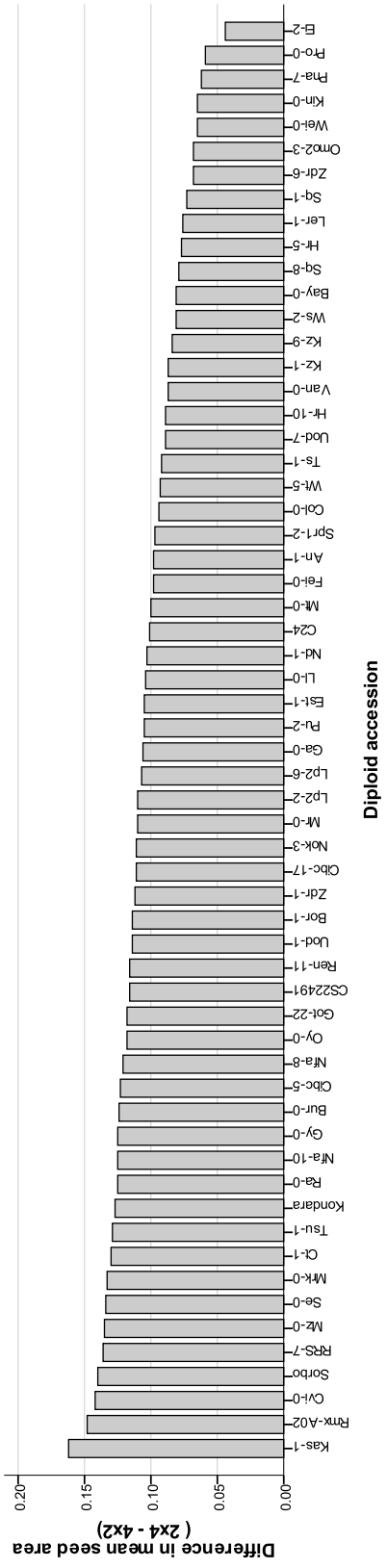


**Figure 2.10**. Difference in mean seed size between reciprocal F1 hybrid triploids (2x4 – 4x2, where 2x4 are paternal excess F1 hybrid triploids, and 4x2 are maternal excess F1 hybrid triploids ).

### 2.3.3 Reciprocal F1 hybrid triploids display parent-of-origin dependent heterosis for seed size.

For calculations of best-parent heterosis (BPH), L*er*-0 was considered as the best-parent (BP) except for the Bur-0, Cvi-0, Mrk-0, Mz-0 and Rmx-A02 diploid accessions which had seeds bigger or equal to the tetraploid L*er*-0 (**Table 2.4, Figure 2.8)**. Conversely, to determine the worst-parent heterosis (WPH), the worst-parents (WP) were the diploid accessions, except for: Bur-0, Cvi-0, Mrk-0, Mz-0 and Rmx-A02, which were larger than L*er*-0 (**Table 2.4, Figure 2.8)**. Best-parent heterosis was found in all paternal excess F1 hybrid triploids, except for Ei-2 (BPH = -9.8%) and Pro-0 (BPH = -3.7%). The highest BPH was attributed to: Kas-1, Sorbo, RRS-7, Rmx-A02, Mrk-0, and Tsu-1 which had seeds over 50% larger than their best-parent, and the lowest BPH heterosis found in this set of triploids was only 3% (Pna-17) (**Table 2.5, Figure 2.12**). All maternal excess F1 triploids displayed subtractive heterosis and consistently produced seeds smaller than the worst-parent, except for Hr-5 (WPH = 4%) (**Figure 2.13**). Triploids with the highest subtractive heterosis were: Rmx-A02, Mz-0, Cvi-0, Se-0, Ct-1, and Spr1-2, all producing seeds over 40% smaller than the worst-parent. Worst-parent heterosis was the lowest in Hr-10 maternal excess triploids, which had seeds only 2% smaller than the worst-parent (**Table 2.5, Figure 2.13)**.

The level of the best-parent heterosis in paternal excess triploids negatively correlates with the level of worst-parent heterosis in reciprocal maternal excess triploids (**Figure 2.14**). However, some accessions, such as Ei-2 and Pro-0 completely lost the heterotic effect in the 2x X 4x paternal excess, and Pna -7 had a very weak best-parent heterosis, but they still showed a high level of worst-parent heterosis (-27%, -32%, -30%, respectively) in the reciprocal direction (**Table 2.5, Figure 2.12, Figure 2.13**). Also, Hr-5 triploids lost their worst-parent heterosis in the 4x X 2x maternal excess but displayed 25% of best-parent heterosis in the 2x X 4x cross (**Table 2.5, Figure 2.12, Figure 2.13**).

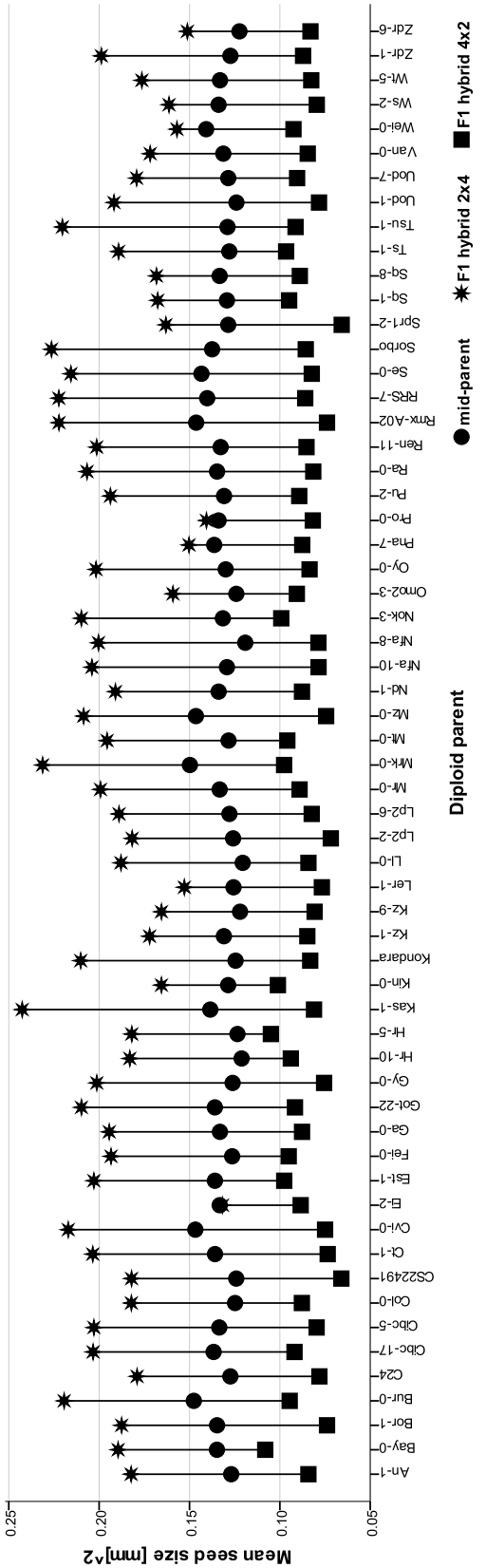


**Figure 2.11.** Mid-parent heterosis (MPH) for the F1 hybrid triploid seeds generated in 2x X 4x and 4x X 2x crosses. The circle indicates the mid-parent value, the star indicates the 2x X 4x paternal excess F1 hybrid triploid seed size, the square indicates the 4x X 2x maternal excess F1 hybrid triploid seed size

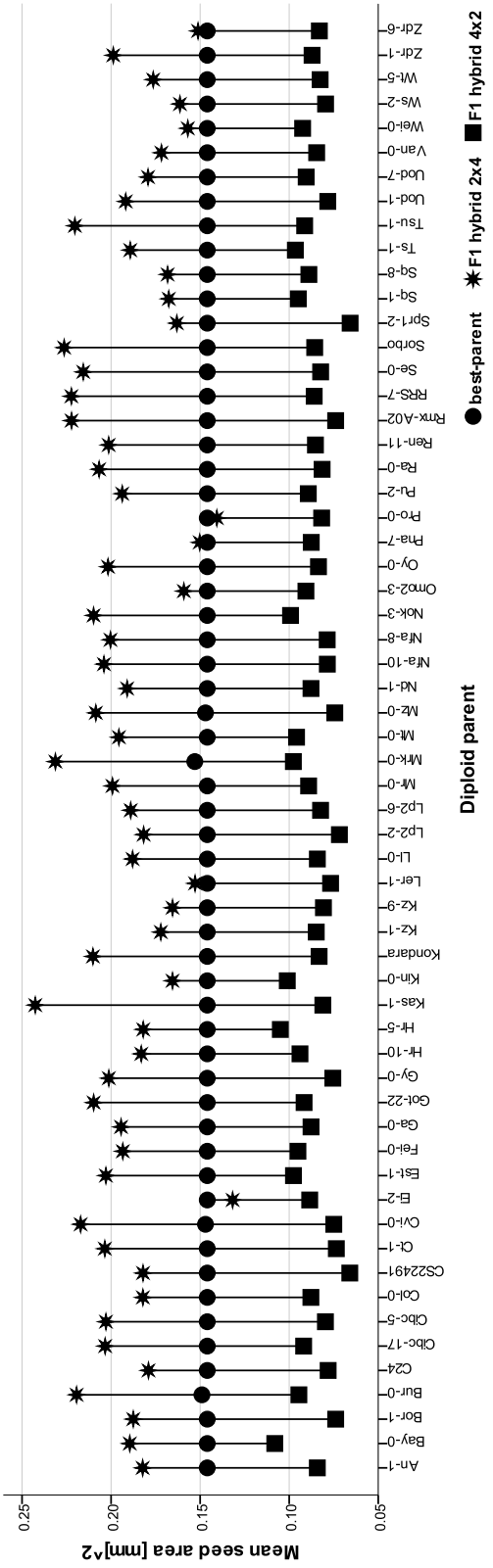


**Figure 2.12.** Best-parent heterosis (BPH) for the F1 hybrid triploid seeds generated in 2x X 4x and 4x X 2x crosses. The circle indicates the best-parent value, the star indicates the 2x X 4x paternal excess F1 hybrid triploid seed size, the square indicates the 4x X 2x maternal excess F1 hybrid triploid seed size

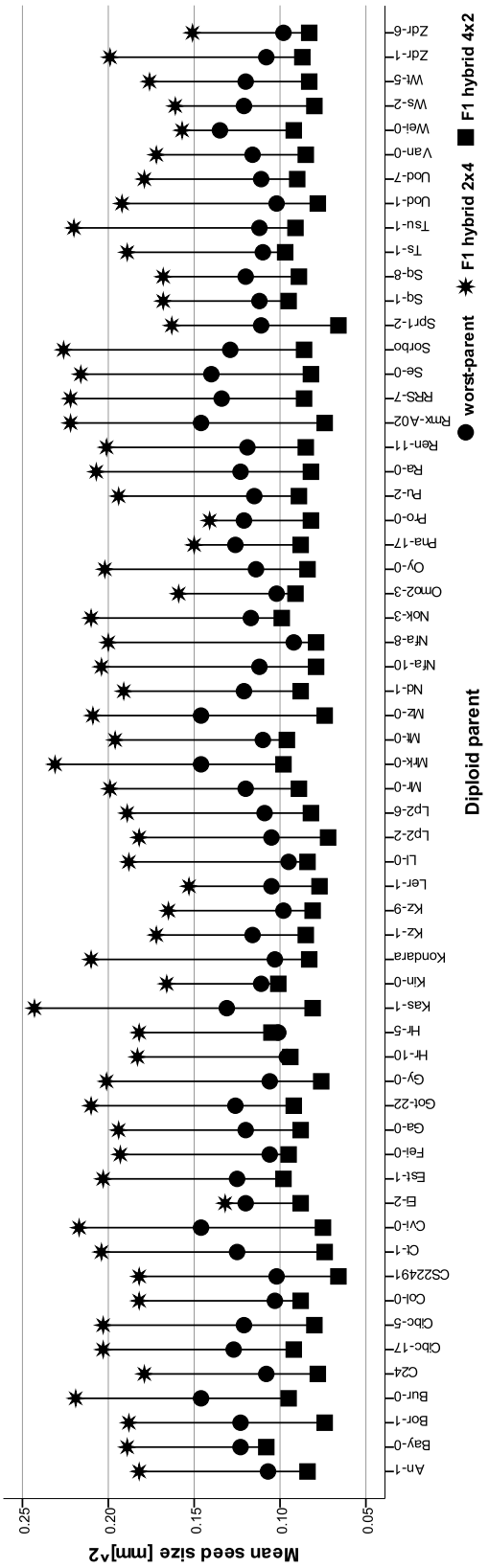


**Figure 2.13.** Worst-parent heterosis (WPH) for the F1 hybrid triploid seeds generated in 2x X 4x and 4x X 2x crosses. The circle indicates the worst-parent value, the star indicates the 2x X 4x paternal excess F1 hybrid triploid seed size, the square indicates the 4x X 2x maternal excess F1 hybrid triploid seed size.

| Diploid mother | MPH%_ 2x4 | Diploid father | MPH%_ 4x2 | Diploid mother | BPH%_ 2x4 | Diploid father | BPH%_ 4x2 | Diploid mother | WPH%_ 2x4 | Diploid father | WPH%_ 4x2 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Kas-1 | 75,352 | Rmx-A02 | -49,409 | Kas-1 | 66,134 | CS22491 | -54,795 | Nfa-8 | 117.391 | Mz-0 | -49.315 |
| Tsu-1 | 71,092 | Mz-0 | -49,129 | Sorbo | 55,027 | Spr1-2 | -54,795 | Kondara | 103.883 | Rmx-A02 | -49.315 |
| Kondara | 69,079 | Cvi-0 | -48,853 | RRS-7 | 52,259 | Lp2-2 | -50,685 | LI-0 | 97.895 | Cvi-0 | -48.63 |
| Nfa-8 | 68,423 | Spr1-2 | -48,76 | Rmx-A02 | 52,188 | Rmx-A02 | -49,315 | Tsu-1 | 96.429 | Se-0 | -41.429 |
| Sorbo | 64,853 | CS22491 | -46,765 | Mrk-0 | 50,97 | Ct-1 | -49,315 | Hr-10 | 90.625 | Ct-1 | -40.8 |
| Gy-0 | 59,821 | Ct-1 | -45,814 | Tsu-1 | 50,907 | Bor-1 | -49,315 | Gy-0 | 89.623 | Spr1-2 | -40.541 |
| Nok-3 | 59,739 | Bor-1 | -45,077 | Se-0 | 47,685 | Mz-0 | -49,135 | Uod-1 | 88.235 | Bor-1 | -39.837 |
| RRS-7 | 58,755 | Lp2-2 | -42,87 | Cvi-0 | 47,507 | Cvi-0 | -48,922 | Kas-1 | 85.496 | Kas-1 | -38.168 |
| Nfa-10 | 58,007 | Se-0 | -42,496 | Bur-0 | 47,352 | Gy-0 | -47,945 | Zdr-1 | 84.259 | RRS-7 | -35.821 |
| Zdr-1 | 56,306 | Kas-1 | -41,368 | Kondara | 43,972 | Ler-1 | -47,26 | Nfa-10 | 82.143 | CS22491 | -35.294 |
| LI-0 | 56,103 | Ws-2 | -40,501 | Nok-3 | 43,717 | Uod-1 | -46,575 | Fei-0 | 82.075 | Bur-0 | -34.932 |
| Oy-0 | 55,427 | Cibc-5 | -40,259 | Got-22 | 43,689 | C24 | -46,575 | Hr-5 | 80.198 | Cibc-5 | -33.884 |
| Uod-1 | 54,841 | Gy-0 | -40,036 | Mz-0 | 42,345 | Nfa-10 | -45,89 | Nok-3 | 79.487 | Ws-2 | -33.884 |
| Mrk-0 | 54,598 | Ra-0 | -39,416 | Ra-0 | 41,578 | Nfa-8 | -45,89 | CS22491 | 78.431 | Ra-0 | -33.333 |
| Got-22 | 54,508 | Nfa-10 | -39,126 | Nfa-10 | 39,725 | Cibc-5 | -45,205 | Mt-0 | 78.182 | Sorbo | -33.333 |
| Ra-0 | 53,623 | Pro-0 | -38,859 | Ct-1 | 39,401 | Ws-2 | -45,205 | Oy-0 | 77.193 | Mrk-0 | -32.877 |
| Fei-0 | 53,237 | Ler-1 | -38,813 | Cibc-17 | 39,272 | Kas-1 | -44,521 | Col-0 | 76.699 | Pro-0 | -32.231 |
| Mt-0 | 52,568 | RRS-7 | -38,605 | Est-1 | 38,981 | Kz-9 | -44,521 | Sorbo | 75.194 | Wei-0 | -31.852 |
| Cibc-5 | 52,155 | C24 | -38,571 | Cibc-5 | 38,967 | Se-0 | -43,836 | Lp2-6 | 73.394 | Lp2-2 | -31.429 |
| Rmx-A02 | 52,073 | Wt-5 | -37,844 | Oy-0 | 38,159 | Ra-0 | -43,836 | Lp2-2 | 73.333 | Wt-5 | -30.833 |
| Ren-11 | 51,875 | Sorbo | -37,6 | Ren-11 | 37,923 | Lp2-6 | -43,836 | Ts-1 | 71.818 | Pna-17 | -30.159 |
| Hr-10 | 51,31 | Uod-1 | -36,753 | Gy-0 | 37,862 | Pro-0 | -43,836 | An-1 | 70.093 | Nfa-10 | -29.464 |
| Se-0 | 50,58 | Bur-0 | -35,862 | Nfa-8 | 37,231 | Kondara | -43,151 | Ren-11 | 68.908 | Ren-11 | -28.571 |
| Ct-1 | 49,983 | Ren-11 | -35,693 | Mr-0 | 36,467 | Wt-5 | -43,151 | Pu-2 | 68.696 | Gy-0 | -28.302 |
| Mr-0 | 49,661 | Pna-17 | -35,64 | Zdr-1 | 36,148 | Zdr-6 | -43,151 | Kz-9 | 68.367 | C24 | -27.778 |
| Est-1 | 49,475 | Oy-0 | -35,614 | Mt-0 | 33,972 | Oy-0 | -42,466 | Ra-0 | 68.293 | Cibc-17 | -27.559 |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cibc-17 | 48,997 | Lp2-6 | -35,499 | Ga-0 | 33,15 | Ll-0 | -42,466 | Cibc-5 | 67.769 | Nd-1 | -27.273 |
| Bur-0 | 48,787 | Van-0 | -35,497 | Pu-2 | 32,705 | An-1 | -42,466 | Got-22 | 66.667 | Got-22 | -26.984 |
| Pu-2 | 48,26 | Kz-1 | -35,156 | Fei-0 | 32,472 | Ren-11 | -41,781 | Mr-0 | 65.833 | Kz-1 | -26.724 |
| Cvi-0 | 48,098 | Mrk-0 | -34,724 | Uod-1 | 31,351 | Kz-1 | -41,781 | C24 | 65.741 | Van-0 | -26.724 |
| Ts-1 | 48,079 | Nd-1 | -34,357 | Nd-1 | 30,815 | Van-0 | -41,781 | RRS-7 | 65.672 | Ei-2 | -26.667 |
| Lp2-6 | 47,969 | Wei-0 | -34,236 | Bay-0 | 29,792 | Sorbo | -41,096 | Ct-1 | 63.2 | Ga-0 | -26.667 |
| Hr-5 | 47,57 | Ga-0 | -34,023 | Ts-1 | 29,619 | RRS-7 | -41,096 | Est-1 | 62.4 | Ler-1 | -26.667 |
| CS22491 | 46,888 | Nfa-8 | -33,869 | Lp2-6 | 29,441 | Zdr-1 | -40,411 | Ga-0 | 61.667 | Oy-0 | -26.316 |
| Ga-0 | 46,192 | Kz-9 | -33,71 | Ll-0 | 28,704 | Ga-0 | -39,726 | Uod-7 | 61.261 | Mr-0 | -25.833 |
| Col-0 | 46,18 | An-1 | -33,556 | Bor-1 | 28,458 | Nd-1 | -39,726 | Cibc-17 | 59.843 | Sq-8 | -25.833 |
| Lp2-2 | 44,579 | Ei-2 | -33,521 | Hr-10 | 25,364 | Col-0 | -39,726 | Mrk-0 | 58.219 | Lp2-6 | -24.771 |
| An-1 | 43,815 | Sq-8 | -33,195 | An-1 | 24,842 | Pna-17 | -39,726 | Nd-1 | 57.851 | Uod-1 | -23.529 |
| Nd-1 | 42,856 | Mr-0 | -33,08 | Col-0 | 24,761 | Ei-2 | -39,726 | Omo2-3 | 55.882 | Pu-2 | -22.609 |
| Mz-0 | 42,605 | Kondara | -33,064 | CS22491 | 24,699 | Mr-0 | -39,041 | Se-0 | 54.286 | Est-1 | -21.6 |
| Bay-0 | 40,75 | Cibc-17 | -32,668 | Hr-5 | 24,614 | Pu-2 | -39,041 | Zdr-6 | 54.082 | An-1 | -21.495 |
| C24 | 40,726 | Got-22 | -32,51 | Lp2-2 | 24,466 | Sq-8 | -39,041 | Bay-0 | 53.659 | Zdr-1 | -19.444 |
| Uod-7 | 39,634 | Zdr-6 | -32,018 | Uod-7 | 22,774 | Uod-7 | -38,356 | Bor-1 | 52.846 | Kondara | -19.417 |
| Bor-1 | 39,377 | Pu-2 | -31,686 | C24 | 22,605 | Tsu-1 | -37,671 | Rmx-A02 | 52.055 | Uod-7 | -18.919 |
| Kz-9 | 35,819 | Zdr-1 | -31,47 | Wt-5 | 20,782 | Omo2-3 | -37,671 | Bur-0 | 50 | Tsu-1 | -18.75 |
| Wt-5 | 32,65 | Ll-0 | -30,059 | Kz-1 | 17,859 | Got-22 | -36,986 | Sq-1 | 50 | Kz-9 | -17.347 |
| Kz-1 | 31,565 | Uod-7 | -29,532 | Van-0 | 17,631 | Cibc-17 | -36,986 | Kin-0 | 49.55 | Nok-3 | -15.385 |
| Van-0 | 30,988 | Col-0 | -29,52 | Sq-8 | 15,257 | Wei-0 | -36,986 | Cvi-0 | 48.63 | Zdr-6 | -15.306 |
| Sq-1 | 29,832 | Tsu-1 | -29,086 | Sq-1 | 14,795 | Mrk-0 | -36,556 | Kz-1 | 48.276 | Sq-1 | -15.179 |
| Kin-0 | 28,883 | Est-1 | -28,112 | Kin-0 | 13,366 | Bur-0 | -36,273 | Van-0 | 48.276 | Col-0 | -14.563 |
| Omo2-3 | 28,436 | Omo2-3 | -26,876 | Kz-9 | 13,349 | Hr-10 | -35,616 | Spr1-2 | 46.847 | Nfa-8 | -14.13 |
| Spr1-2 | 27,01 | Sq-1 | -26,508 | Spr1-2 | 11,693 | Fei-0 | -34,932 | Wt-5 | 46.667 | Mt-0 | -12.727 |
| Sq-8 | 26,387 | Mt-0 | -25,217 | Ws-2 | 10,502 | Sq-1 | -34,932 | Ler-1 | 45.714 | Bay-0 | -12.195 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Zdr-6 | 23,735 | Fei-0 | -24,651 | Omo2-3 | 9,001 | Mt-0 | -34,247 | Mz-0 | 43.151 | Ts-1 | -11.818 |
| Ler-1 | 21,819 | Ts-1 | -24,464 | Wei-0 | 7,484 | Ts-1 | -33,562 | Sq-8 | 40 | Ll-0 | -11.579 |
| Ws-2 | 20,636 | Nok-3 | -24,451 | Ler-1 | 4,679 | Est-1 | -32,877 | Ws-2 | 33.058 | Omo2-3 | -10.784 |
| Wei-0 | 11,613 | Hr-10 | -22,342 | Zdr-6 | 3,547 | Nok-3 | -32,192 | Pna-17 | 19.048 | Fei-0 | -10.377 |
| Pna-17 | 10,375 | Kin-0 | -21,291 | Pna-17 | 2,915 | Kin-0 | -30,822 | Pro-0 | 16.529 | Kin-0 | -9.009 |
| Pro-0 | 5,144 | Bay-0 | -19,713 | Pro-0 | -3,685 | Hr-5 | -28,082 | Wei-0 | 16.296 | Hr-10 | -2.083 |
| Ei-2 | -1,016 | Hr-5 | -14,862 | Ei-2 | -9,759 | Bay-0 | -26,027 | Ei-2 | 10 | Hr-5 | 3.96 |

**Table 2.5.** F1 hybrid triploids sorted by highest mid-parent heterosis (MPH), best-parent heterosis (BPH), and worst-parent heterosis (WPH) for seed size. BPH for paternal excess F1 hybrid triploids (2x X 4x) and WPH for maternal excess F1 hybrid triploids (4x X 2x) are highlighted in green.

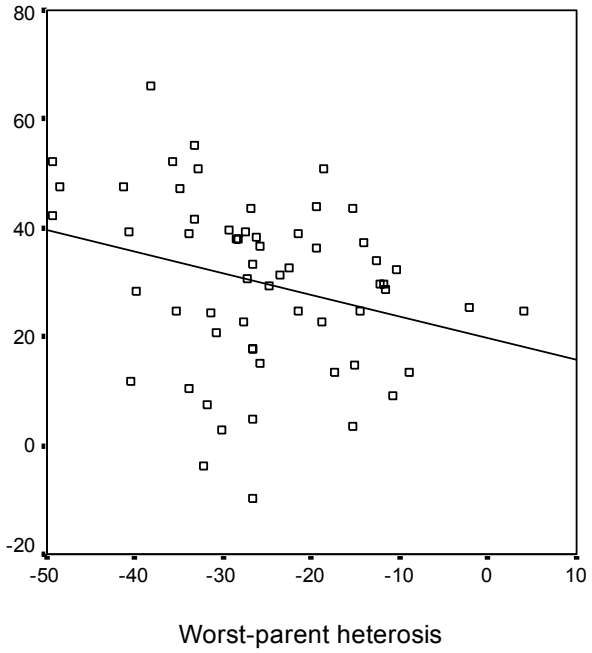


**Figure 2.14** Negative correlation between worst-parent heterosis (WPH) in F1 4x X 2x maternal excess hybrid triploids and best-parent heterosis (BPH) in F1 2x X 4x paternal excess hybrid triploids ($r = -0.282$ p-value $< 0.05$). Correlation is significant at the 0.05 level.

### 2.3.4 Parent-of-origin dependent heterosis in F1 seed size is observed in reciprocal F1 hybrid diploid seeds.

It is possible that the heterosis effects observed in F1 hybrid triploids is due to hybridity and/or genome dosage effects. To determine whether crosses of particular genetic backgrounds tend to generate heterosis effects independently of genome dosage effects, a series of inter-accession crosses to generate F1 hybrid diploid seeds were conducted. To generate F1 hybrid diploids, selected diploid accessions (Wei-0, Pro-0, Ga-0, Ren-1, and Fei-0) were reciprocally crossed to diploid tester line L*er*-0, and the size of their seeds were measured (**Table 2.6, Figure 2.17**).

The L*er*-0 diploid had the smallest parental seeds (0.097 $mm^2$), similar to Fei-0, Ga-0 and Pro-0 accessions (0.107 $mm^2$, 0.105 $mm^2$, and 0.109 $mm^2$, respectively). Ren-1 and Wei-0 had considerably larger seeds (0.132 $mm^2$, and 0.128 $mm^2$, respectively). The small-seeded L*er*-0 produced small F1 hybrid diploid progeny as a female when sired by five different pollen donors. The reciprocal crosses showed that the L*er*-0 males always sired bigger seeds (**Figure 2.15, Figure 2.16**).

To determine which reciprocal F1 hybrid diploids have the largest parent-of-origin effect in seed size, the size of the smaller seeds (L*er*-0 used as a maternal parent) was subtracted from the larger seeds (L*er*-0 used as a paternal parent). As shown in **Figure 2.19**, Pro-0 when crossed to L*er*-0, produced F1 hybrid diploid offspring with the smallest differences in size between reciprocal F1 hybrids ($\Delta = 0.03mm^2$). This was also observed in the triploid background, where Pro-0 that was used as a maternal parent in a cross to a tetraploid L*er*-0, produced the F1 2x X 4x triploid hybrids whose size did not significantly increase (**Figure 2.4, Figure 2.10**). The accessions with the highest difference in seed size between reciprocal F1 diploid hybrids were: Fei-0 and Ga-0 (both $\Delta = 0.046mm^2$) (**Figure 2.19**).

A series of seed clearings at different developmental stages (4-8 days after pollination – d.a.p.) were performed to visualize the three main components of the seed: embryo, endosperm and seed coat. Microscope images of reciprocal F1 hybrid diploids indicated that differences in seed size were due to the primary endosperm and ovule integument growth but not the embryo, which had a similar size in both smaller and larger types of seeds (**Figure 2.18**). The endosperm would eventually be consumed, being replaced by the

growing embryo, which constitutes most of the mature seed; therefore mature bigger seeds will have bigger embryos than smaller seeds (data not shown).

The impact of cross direction on seed size heterosis was determined by identifying mid-parent (MP), best-parent (BP) and worst-parent (WP) values for the diploid hybrid crosses (**Table 2.6**). Best-parents were the five different diploid accessions crossed to L*er*-0 which had the smallest seeds; therefore L*er*-0 was always the worst-parent (**Table 2.6)**. All F1 hybrid diploids sired by the L*er*-0 father (A x L) showed strong best-parent heterosis, ranging from 12% (Pro-0) to 38% (Ga-0) (**Figure 2.21**). While best-parent heterosis indicates if F1 offspring has superior performance over the largest parent, subtractive heterosis identifies F1 offspring having worse performance than the smaller parent. It was found that, when L*er*-0 was used as a female parent, Pro-0 and Fei-0 F1 hybrids had smaller seeds than the worst-parent (WPH = -5.1%, WPH = -1.8%, respectively) (**Figure 2.22, Table 2.6**). Although Ga-0 and Wei-0 did not display worst-parent heterosis when they sired hybrid seeds from the L*er*-0 mother, the seeds were smaller than the mid-parent value (**Figure 2.20**). Ren-1 did not show worst-parent heterosis but it did show mid-parent heterosis in L x A cross direction (**Figure 2.20, Figure 2.22, Table 2.6**).

| L*er*-0_parent | Accession | A parent | AXL*er*-0 | L*er*-0xA | MP | BP | WP | %MPH_AxL | %MPH_LxA | %BPH_AxL | %BPH_LxA | %WPH_AxL | %WPH_LxA |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.0971 | Wei-0 | 0.1285 | 0.1512 | 0.1115 | 0.1128 | 0.1285 | 0.0971 | 34.0661 | -1.1351 | 17.6654 | -13.2296 | 55.7792 | 14.8769 |
| | Fei-0 | 0.1071 | 0.1412 | 0.0953 | 0.1021 | 0.1071 | 0.0971 | 38.3095 | -6.6019 | 31.8156 | -10.9871 | 45.4764 | -1.7622 |
| | Ga-0 | 0.1051 | 0.1453 | 0.0997 | 0.1011 | 0.1051 | 0.0971 | 43.7340 | -1.3492 | 38.2149 | -5.1372 | 49.7122 | 2.7538 |
| | Ren-1 | 0.1321 | 0.155 | 0.1181 | 0.1146 | 0.1322 | 0.0971 | 35.2224 | 3.01283 | 17.2677 | -10.6651 | 59.669 | 21.6364 |
| | Pro-0 | 0.109 | 0.1225 | 0.0921 | 0.103 | 0.109 | 0.0971 | 18.92 | -10.619 | 12.4095 | -15.51207 | 26.231 | -5.1237 |

**Table 2.6.** Calculations of mid-parent heterosis (%MPH), best-parent heterosis (%BPH), and worst-parent heterosis (%WPH) for seed size in five reciprocal F1 diploid hybrids.

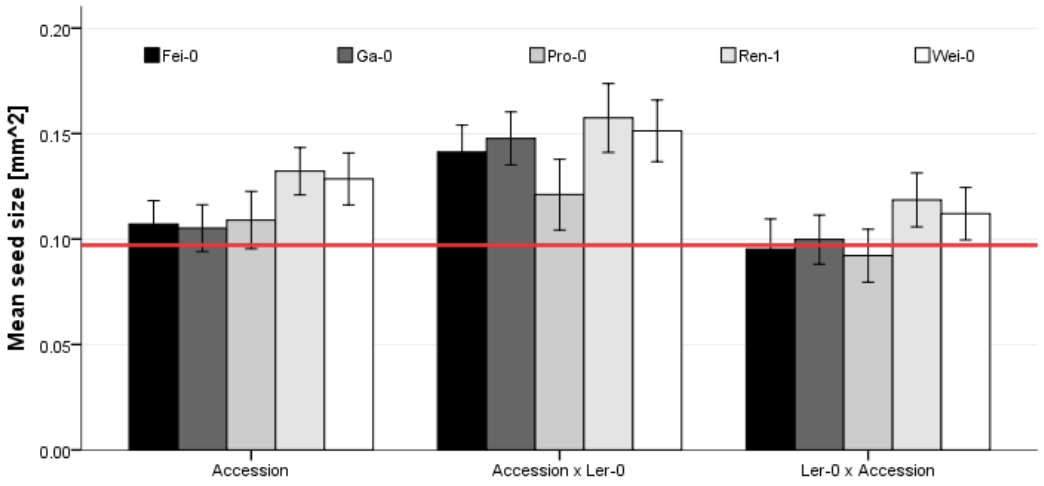


**Figure 2.15.** Mean seed size of five different diploid accessions (A) crossed to diploid L*er*-0 (red line), and their reciprocal F1 hybrid diploid offspring (AxL and LxA). LxA – a cross where L*er*-0 was used as a maternal parent and other diploid accession (A) was used as a paternal parent, AxL - a cross where a diploid accession (A) was used as a maternal parent and the L*er*-0 was used as a paternal parent. Error bars represent SD.

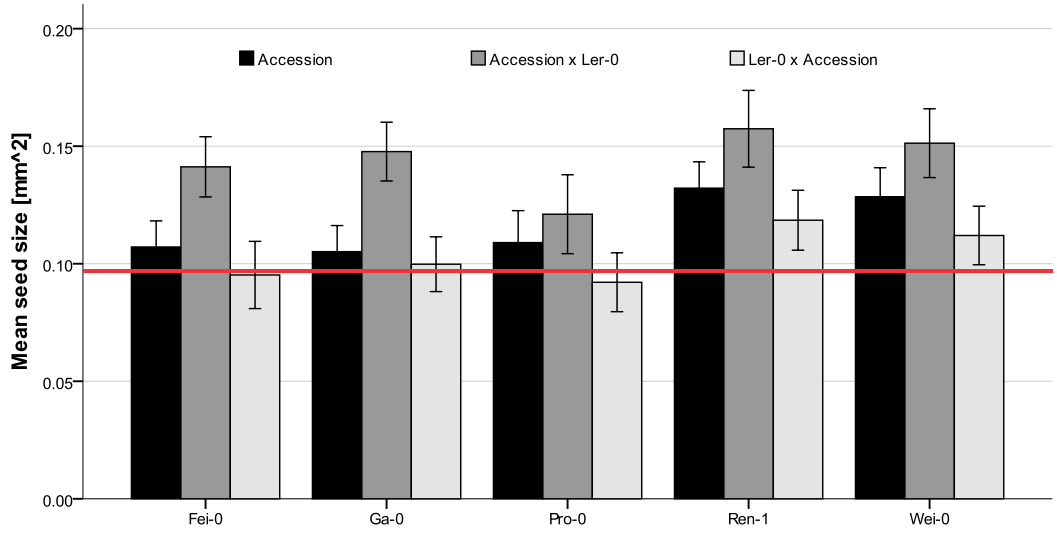


**Figure 2.16.** Mean seed size of five different diploid Accessions crossed to diploid L*er*-0 (red line), and their reciprocal F1 hybrid diploid offspring (AxL and LxA). LxA – a cross where L*er*-0 was used as a maternal parent and other diploid accession (A) was used as a paternal parent, AxL - a cross where a diploid accession (A) was used as a maternal parent and the L*er*-0 was used as a paternal parent. Error bars represent SD.

| Fei-0 | **AxL** | **LxA** | **L** | **A** |
|---|---|---|---|---|
| **AxL** | | 0.0001 | 0.0001 | 0.0001 |
| **LxA** | | | **0.433*** | 0.0001 |
| **L** | | | | 0.0001 |
| Ga-0 | AxL | LxA | L | A |
| **AxL** | | 0.0001 | 0.0001 | 0.0001 |
| **LxA** | | | **0.054*** | **0.008*** |
| **L** | | | | 0.0001 |
| Pro-0 | AxL | LxA | L | A |
| **AxL** | | 0.0001 | 0.0001 | 0.0001 |
| **LxA** | | | **0.001*** | 0.0001 |
| **L** | | | | 0.0001 |
| Ren-1 | AxL | LxA | L | A |
| AxL | | 0.0001 | 0.0001 | 0.0001 |
| LxA | | | 0.0001 | 0.0001 |
| L | | | | 0.0001 |
| Wei-0 | AxL | LxA | L | A |
| **AxL** | | 0.0001 | 0.0001 | 0.0001 |
| **LxA** | | | 0.0001 | 0.0001 |
| **L** | | | | 0.0001 |

**Table 2.7.** ANOVA - ANalysis Of VAriance in seed size between groups of different diploid hybrids and their parental lines. Statistically non-significant differences in seed size are highlighted in bold and with an asterisks. Significance level is at $p < 0.001$. A – accession, L – L*er*-0 (diploid), LxA – a cross where L*er*-0 was used as a maternal parent and other diploid accession (A) was used as a paternal parent, AxL - a cross where a diploid accession (A) was used as a maternal parent and a L*er*-0 was used as a paternal parent.

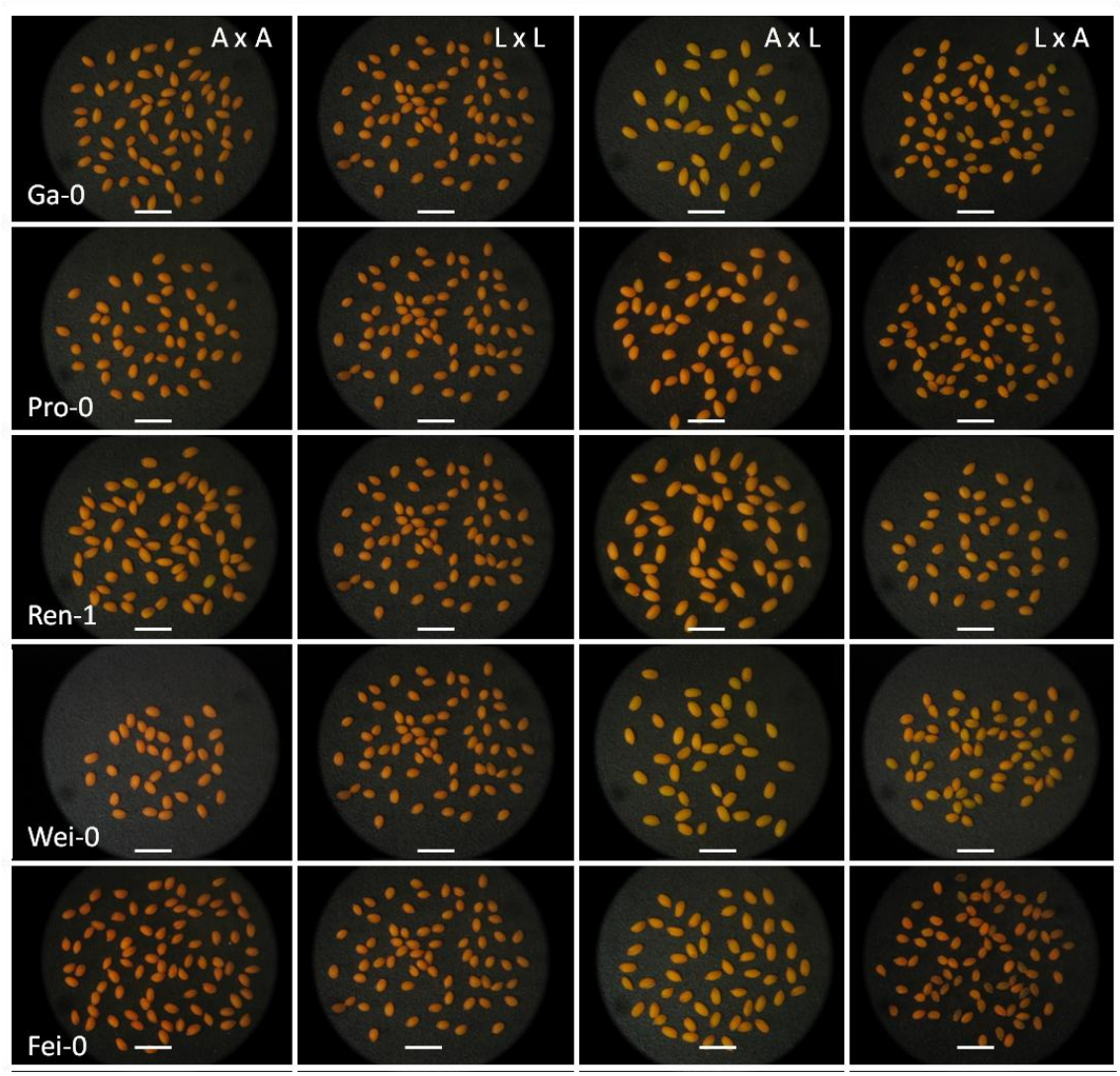


**Figure 2.17. S**eed size of F1 hybrid diploids and their diploid parents (L – L*er*-0, A – Accessions: Ga-0, Pro-0, Ren-1, Wei-0, Fei-0). LxA – F1 hybrid diploid where L*er*-0 was used as a maternal parent and other diploid accession (A) was used as a paternal parent, AxL – F1 hybrid diploid where a diploid accession (A) was used as a maternal parent and a L*er*-0 was used as a paternal parent. Scale bar = 1mm

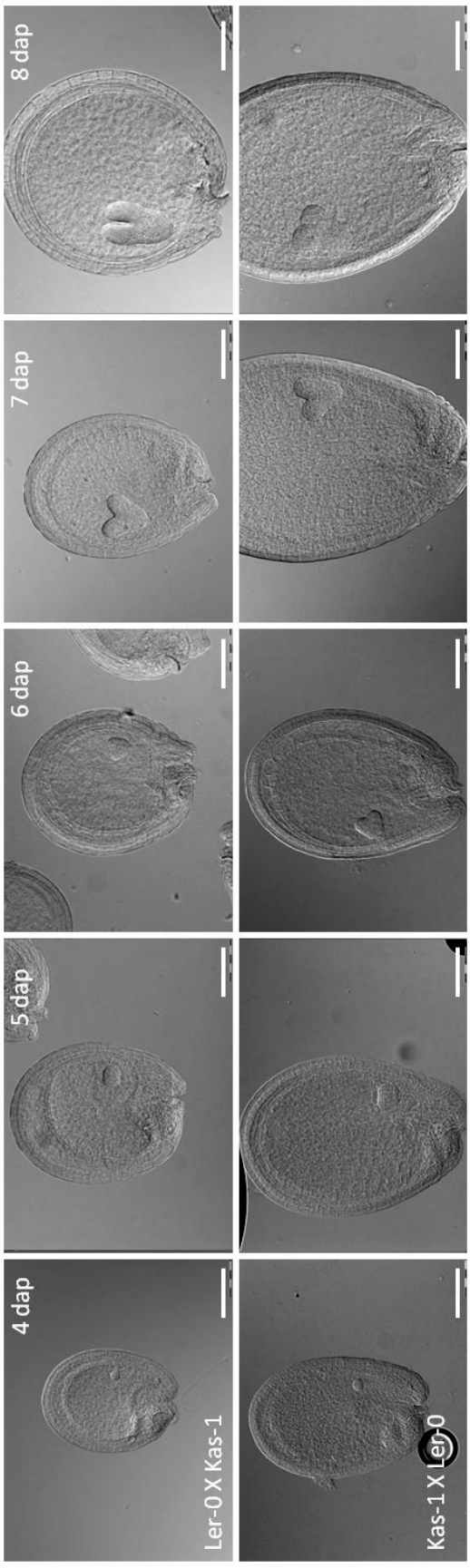


**Figure 2.18.** Microscope images showing differences in size of F1 hybrid reciprocal diploid seeds at different developmental stages. Columns represent different time points: 4, 5, 6, 7 and 8 days after pollination (d.a.p.), rows represent; L*er*-0 x Kas-1 and Kas-1 x L*er*-0 F1 hybrids diploids. Scale bar = 100µm.

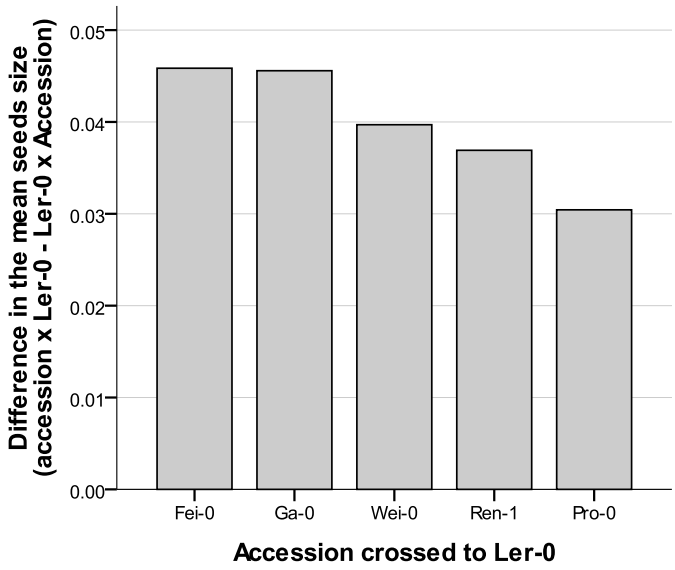


**Figure 2.19**. Differences in seed size between reciprocal F1 hybrid diploids (AxL – LxA). AxL - a cross where a diploid accession (A) was used as a maternal parent and the L*er*-0 was used as a paternal parent; LxA – a cross where L*er*-0 was used as a maternal parent and other diploid accession (A) was used as a paternal parent.

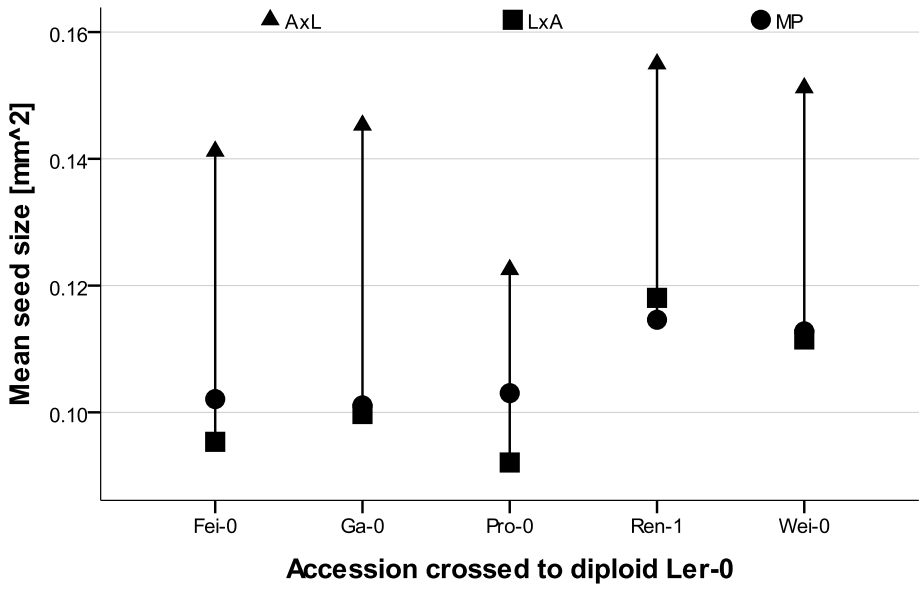


**Figure 2.20.** Mid-parent heterosis for the F1 hybrid diploid seeds generated from AxL and LxA crosses, where A refers to diploid accession and L refers to diploid L*er*-0. The circle indicates the mid-parent value, the triangle indicates the AxL F1 hybrid diploid seed size, the square indicates the LxA F1 hybrid diploid seed size.

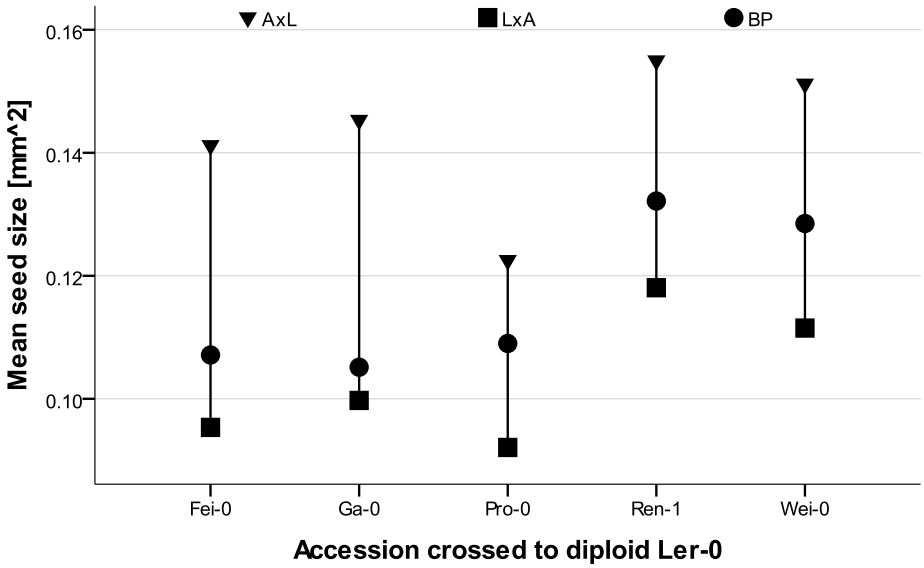


**Figure 2.21.** Best-parent heterosis for the F1 hybrid diploid seeds generated from AxL and LxA crosses, where A refers to diploid accession and L refers to diploid L*er*-0. The circle indicates the best-parent value, the triangle indicates the AxL F1 hybrid diploid seed size, the square indicates the LxA F1 hybrid diploid seed size.

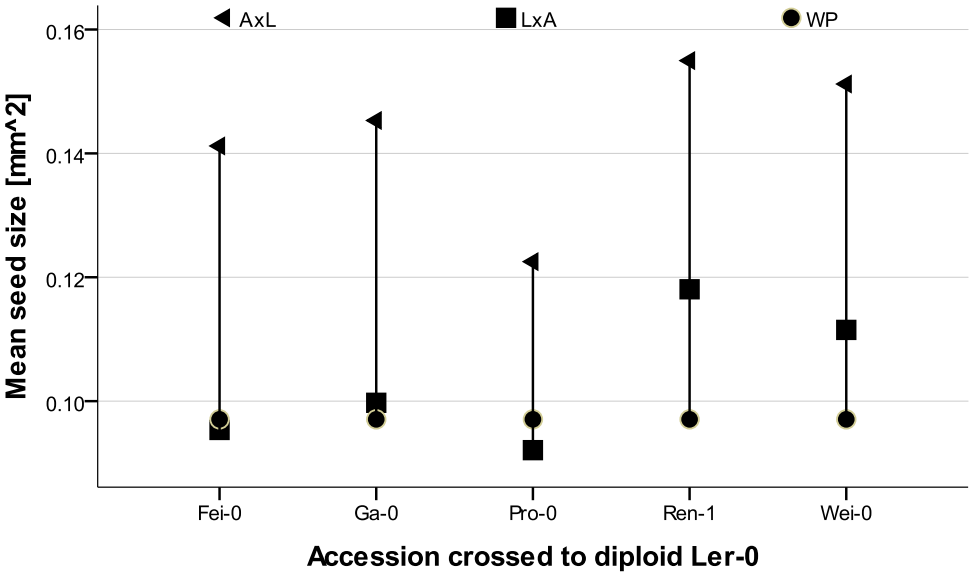


**Figure 2.22.** Worst-parent heterosis for the F1 hybrid diploid seeds generated from AxL and LxA crosses, where A refers to diploid accession and L refers to diploid L*er*-0. The circle indicates the worst-parent value, the triangle indicates the AxL F1 hybrid diploid seed size, the square indicates the LxA F1 hybrid diploid seed size.

## 2.4 Discussion

Seed size is an important agronomical trait, and both smaller and larger seeds could be of economic interest. Many food grains have been selected and bred for their size for centuries (Sundaresan, 2005). However, in certain species, smaller seeds are more desirable than bigger ones, e.g. in grapes, watermelon or tomato. Therefore, understanding mechanisms governing modulations in seed size is of highly practical value.

One of the approaches to identify genes involved in the control of seed size and heterosis in *Arabidopsis thaliana* is to utilize the considerable natural variation in seed size in different accessions (**Figure 2.7)**. Seed size has been determined for a large number of *Arabidopsis thaliana* accessions (Rowan et al., 2011, De Jong et al., 2009), and most recently 80 accessions have been measured for seed size as a part of the 1001 genomes project (www.1001genomes.org) (Cao et al., 2011). Seeds with the most extreme differences in size are ideal for the generation of RIL populations for identifying underlying QTLs with a major effect on seed size (Rowan et al., 2011, Alonso-Blanco et al., 1999). Those regions become targets for further fine-mapping in order to identify the underlying genes. Alternatively, genome-wide association mapping tools can be applied to search for DNA polymorphism associated with natural variation in seed size and heterosis (Atwell et al., 2010), however the sample size of 60 accessions as part of this research was not powerful enough to detect any significant SNPs (data not shown).

Seed size is a result of a coordinated growth of sporophytic maternal tissue, and the two products of double fertilization: triploid endosperm and a diploid embryo. The rate and duration of endosperm proliferation in the early stage of seed development is one of the crucial determinants of the final seed size (Chaudhury et al., 2001). Crosses between diploid and tetraploid parents cause deviation from the normal 2:1 maternal : paternal genome ratio in the endosperm, leading to genomic imbalance and abnormal endosperm development (Dilkes and Comai, 2004). Seeds with paternal genome excess have endosperm that is characterized by accelerated mitosis and delayed cellularization, whereas doubled maternal genome causes reduced mitosis and precocious cellularization of endosperm (Scott et al., 1998).

In the inter-ploidy isogenic crosses, paternal excess triploids were larger than their diploid parents in all four genetic backgrounds (C24, Col-0, L*er*-0, and Zu); however the size of Col-0 triploids did not increase significantly compared to the other accessions (**Figure 2.1, Table 2.1)**. Consistently with these results, it has been previously demonstrated that the average seed size of 2x X 4x paternal genome excess triploids is surprisingly similar to the diploid seed size in Col-0 background (Rowan et al., 2011). This could be explained by the fact that Col-0 accession used as a tetraploid pollen parent in inter-ploidy crosses causes a high rate of seed abortion (Dilkes et al., 2008). The maternally expressed *TTG2* gene has been found to control seed viability in those crosses involving Col-0 tetraploid parent (Dilkes et al., 2008).

Triploid seeds with doubled dosage of paternal genome are usually more vigorous than either parent. The role of endosperm in early seed development is to acquire maternal nutrients and transmit them to the embryo. Therefore, larger endosperm with over-proliferated cells can accumulate more resources from the maternal parent and be more effective in provisioning the growing embryo (Scott et al., 1998). In this research, L*er*-0 2x X 4x paternal excess triploids were found to be over 13% larger than the largest parent, followed by Zu (5%) and C24 (only 0,1%). Col-0 triploids did not have a heterotic effect over the best-parent, or the mid-parent (**Table 2.2, Figure 2.4, Figure 2.5)**. Poor development of endosperm tissue in 4x X 2x maternal excess triploids leads to impaired resource acquisition and transmission to the embryo, which is manifested by decreased vigour and subtractive heterosis for seed size found in all four accessions. Notably, the Col-0 triploids, which totally lost their heterotic effect in paternal excess, had a high level of subtractive heterosis (-24%) in maternal excess, and were the second best performing triploids after L*er*-0 (WPH = -31%) (**Figure 2.6**).

Inter-ploidy inter-accession crosses showed that both hybridization and polyploidization events have a strong impact on seed development manifested in altered seed size. These whole-genome changes lead to transgressive phenotypes, when F1 hybrid triploid seeds grow 66% larger than the best-parent or over 49% smaller than the worst-parent (**Table 2.5)**. Parental dosage effects led to expected seed size phenotypes, where a double dose of maternal genome resulted in reduced seed size, and increased paternal genome dosage

caused enlarged seeds (**Figure 2.8**). Moreover, a strong accession-specific response to inter-ploidy hybridizations has been observed, where some hybrids displayed better heterosis than the others, with two cases where best-parent heterotic effects were completely lost (Pro-0 BPH = -3.7%, Ei-2 BPH = -9.76) (**Table 2.5, Figure 2.12**). The strength of heterotic response varies between hybrids and depends on the cross direction of inter-ploidy crosses (**Table 2.5).**

A negative correlation ($r = -0.282$ p-value $< 0.05$) between WPH and BPH suggest that there is a general relationship between expression of transgressive phenotypes in reciprocal inter-ploidy triploid (**Figure 2.14**). This means that the stronger best-parent heterosis in 2x X 4x triploids, the stronger worst-parent heterosis in 4x X 2x triploids. Exception from this was found in inter-ploidy isogenic cross (Col-0) and inter-ploidy hybrid crosses (Pro-0, Ei-2, both crossed to L*er*-0 tetraploid) where 4x X 2x maternal excess triploid seeds were significantly smaller than the smaller parent (WPH); however 2x X 4x paternal excess triploids did not display best-parent heterosis (BPH) (**Table 2.2, Table 2.5)**. There was also one example of the F1 hybrid triploid (Hr-5) that lost its BPH but was still expressing high level of WPH **(Table 2.5**). This suggests that paternal excess effects can be decoupled from maternal excess effects and that both effects can be regulated by independent regulatory pathways.

Inter-ploidy inter-accession hybridizations also showed that the maternal genotype largely explains the variation in seed size, as a significant correlation has been found between diploid accession and 2x X 4x paternal excess triploids (**Figure 2.9, Table 2.3)** (Meyer et al., 2004, Alonso-Blanco et al., 1999, de Jong and Scott, 2007). There was no relationship between seed size of the paternal parent and the seeds they sired - 4x X 2x maternal excess triploids (**Figure 2.9)** (De Jong et al., 2009). These results suggested that, although the increased size of the 2x X 4x hybrid triploid seeds is mediated by the excess of paternal genome dosage in endosperm, the maternal parent controls the extent to which the size is increased, i.e. the bigger the maternal diploid seed, the bigger the 2x X 4x paternal excess triploid offspring. This effect is not observed when the diploid accession is used as a paternal parent; therefore it is a 'maternal effect'.

It has been previously proposed that the variation in heterotic response is not entirely due to maternal effects during seed development or genetic distance between the parents but it is rather the result of interactions between the alleles of a subset of genes (Stokes et al., 2007). (Stokes et al., 2007) further reported that even accessions with higher relative genetic distance can have low or even subtractive level of heterosis, and in opposite, less related accessions can have increased vigour. This supports the finding that some inter-ploidy isogenic crosses produced more vigorous offspring than some inter-ploidy hybrids, e.g. F1 2x X 4x isogenic L*er*-0 triploid had BPH = 13%, while F1 2x X 4x hybrid Pna-17 triploid had only 3% BPH (**Table 2.2, Table 2.5**).

In the balanced 2x X 2x crosses, the same genotypes can have different effects on seed size, depending if they are inherited maternally or paternally (**Figure 2.16)** (Dilkes and Comai, 2004). Suppression of paternal genes during seed development leads to the very commonly observed maternal effects (Kinoshita et al., 1999, Vielle-Calzada et al., 2000). More rarely, silencing of maternal genes can also be observed, suggesting that paternal genotype can also affect seed size (Köhler et al., 2005). Paternal effects are small and difficult to detect, however, it was shown that crosses involving L*er*-0 display particularly prominent paternal effects (House et al., 2010). L*er*-0 used as a maternal parent produced on average the smallest seeds of all four different accessions, while L*er*-0 crossed as a pollen donor always produced seeds with the largest size (House et al., 2010). A similar observation was reported by (Alonso-Blanco et al., 1999) who studied parent-of-origin effects on seed size using reciprocal crosses of accession L*er*-0 and Cvi-0. This is consistent with the results from this research showing that L*er*-0 mothers always give rise to smaller seeds than L*er*-0 fathers (**Figure 2.15)**. This could be explained by the fact that the five different accessions used as a female parent were always bigger than the L*er*-0 father and the increased size of the F1 AxL hybrid is due to maternal effects (**Figure 2.15**, **Table 2.6**). However, Fei-0 and Ga-0 accessions had very similar seed size to L*er*-0, therefore a significantly increased seed size of those two F1 AxL hybrids could not be entirely explained by maternal effects. In fact, there is a possibility that the L*er*-0 accession controls seed size through paternal effects. All L*er*-0 fathers sired F1 diploid hybrid seeds showing best-parent heterosis, whereas in the reciprocal direction, no best-parent heterosis has been found **(Figure 2.21)**.

In fact, there were two cases of subtractive heterosis (Fei-0, Pro-0) where F1 hybrid seeds were even smaller than the smaller parent (**Figure 2.22)**.

Epigenetic perspective complementary to the conventional models for the molecular basis of heterosis can be applied to explain the heterotic effects observed in crosses carried out during this research (Chandler and Stam, 2004). Heterosis in *Arabidopsis thaliana* seeds can be driven by the action of imprinted genes in endosperm, that are functionally non-equivalent in maternal and paternal genomes (Haig and Westoby, 1991). Parental conflict over resource allocation has been proposed to account for the maternally controlled seed growth inhibition, and paternally induced endosperm overproliferation (Haig, 2000, de Jong and Scott, 2007). *PHERES1* and *MEDEA* are imprinted genes that act antagonistically and show parent-specific gene expression (Grossniklaus et al., 1998, Köhler et al., 2003, Köhler et al., 2005). DNA methylation is essential for embryogenesis and seed development, and regulates genomic imprinting by repressing or activating gene transcription (Gehring et al., 2004). It has been shown that hypomethylation of either parental genome has a parent-of-origin effect on the seed size mimicking inter-ploidy phenotypes (Xiao et al., 2006). Maternal genome hypomethylation causes phenocopy of paternal excess triploid seeds and paternal genome hypomethylation leads to maternal excess triploid seeds phenotype (Adams et al., 2000). DNA methylation may be particularly important for regulation of genes and pathways that influence seed size as it is the case for the imprinted genes in the endosperm (Jullien et al., 2006b). The relationship between DNA methylation and epigenetic regulators (Polycomb group proteins) has been found in the parent-of-origin regulation of seed size (Hsieh et al., 2003).

Heterosis in the size of hybrid maize embryo and endosperm has been demonstrated, and a number of genes that showed non-additive expression have been hypothesized to potentially underlie hybrid vigour (Jahnke et al., 2010, Stupar et al., 2007). Some of those genes corresponded to the histone variants and genes coding for chromatin modifying proteins, indicating possible relation of the hybrid state to epigenetic processes (Jahnke et al., 2010). *Arabidopsis thaliana* accessions display a high level of natural epigenetic variation, thus inter-accession and/or inter-ploidy crosses could potentially induce

chromatin mark modifications leading to heterosis (Banaei Moghaddam et al., 2010, Vaughn et al., 2007).

## 2.5 Conclusions

In this study, it has been demonstrated that a combination of certain genotypes and/or parental genome dosage can lead to a significant increase or decrease in seed size. Isogenic inter-ploidy crosses caused changes in seed development that led to altered seed size phenotypes. The results showed that vigorous offspring could be produced only through ploidy changes, and the extent to which seed size increases/decreases is genotype (accession) specific.

Diploid inter-accession crosses showed that seed size can be controlled solely by parental genotypes, without altering genome dosage. They also demonstrated parent-of-origin dependent positive and subtractive heterosis.

Inter-ploidy inter-accession crosses showed that both hybridization and polyploidization events have an impact on seed development manifested in altered seed size. However, only some triploid hybrids showed additive expression of heterosis (caused by altered genome dosage and hybridization), and some F1 offspring had milder heterosis than isogenic triploids or hybrid diploids alone.

The maternal genotype largely explained the variation in the seed size, but evidence of paternal effects has also been presented. The overall pattern of heterosis observed in crosses carried out as part of this research is likely to be a result of allelic interactions from both maternal and paternal genomes.

# CHAPTER 3 – Pre-fertilization effects on reproduction of *Arabidopsis thaliana* F1 triploids

## 3.1 Introduction

Polyploid organisms frequently display serious reproductive defects which can vary in severity between organisms (Henry et al., 2007). Differences in polyploid reproduction are of evolutionary importance as polyploids can act as both evolutionary bridges between lineages of different ploidy or as reproductive barriers potentially underlying speciation events (Otto, 2007, Henry et al., 2007). Triploidy, in particular, is not tolerated in plants such as maize and poplar and is a leading cause of miscarriage in humans (Sankaranarayanan, 1979) but in certain plants and fish it is well-tolerated and may even be fixed in citrus and amphibians (Stöck et al., 2002). There are a number of important triploid crops where lack of fertility of F1 triploids is a key agronomic trait that generates seedless fruits. Such crops include banana, cucumber, watermelon and pineapple. While seedless fruits are a desirable trait, this can also represent a constraint to the breeding and improvement of triploid crops where fertility is a requirement for successful breeding programs (Lora et al., 2011).

Ploidy alone can affect the fertility of polyploid plants, for example by control of the transition to flowering, meiotic efficiency, gametogenesis and the dosage tolerance of gametes, embryos and endosperms (Chen, 2007, Comai et al., 2003). Triploid plants of some species are largely infertile (e.g. triploid banana). However, there are also plant species where triploids can generate some viable gametes e.g. *Arabidopsis thaliana*, which produces viable seeds. While many wild and crop plants are polyploid (or have undergone ancestral paleopolyploidy events), understanding of the impact of genetic variation on the fertility of polyploid plants remains limited (Henry et al., 2005, Wendel, 2000). There is a need for greater understanding of how the interplay between hybridisation and polyploidy can differentially affect the fertility of polyploids. In particular, little is known regarding the tolerance of the gametes of selfed F1 triploid plants to genome and/or allelic dosage effects that could perturb transmission of dosage-sensitive regions of the genome to the next generation (Henry et al., 2007, McClintock, 1984, Birchler et al., 2001, Comai et al., 2003).

It has also been observed that parent-of-origin dependent genome-dosage effects can affect the development of F1 triploid seeds obtained from inter-ploidy crosses of diploid and tetraploid *A. thaliana* (Lin, 1984, Scott et al., 1998, Johnston and Hanneman Jr, 1995). However, it is not known whether epigenetic parent-of-origin dependent effects on phenotype also affect later stages of development of F1 triploid plants. *A. thaliana* provides a useful model to investigate the fertility of both isogenic and hybrid F1 triploids, by facilitating dissection of the relative roles of triploidy (genome dosage) versus hybridity (allelic dosage).

### 3.1.1 Research Objectives

This chapter is focused on investigating the fertility of *Arabidopsis thaliana* reciprocal F1 hybrid triploids. Given the variation in tolerance to ploidy changes and hybridization which can exist between plant species, it was hypothesised that genetic differences between *Arabidopsis thaliana* accessions could alter their fertility when such genomic changes were induced.

The proportion of unfertilized ovules (%U) relative to the sum of all ovules in the mature silique (Σ) was screened in the self-pollinated reciprocal F1 hybrid triploids generated through inter-ploidy crosses between a tetraploid L*er*-0 and 89 different diploid accessions. Additionally, %U and Σ arising from reproduction of four different reciprocal F1 isogenic triploids and five different reciprocal F1 hybrid diploids was also investigated.

To determine the extent by which aberrant male gametogenesis could be linked to altered fertility in reciprocal F1 hybrid triploids, the viability of pollen grains produced by triploids with the highest and lowest fertility was tested.

## 3.2 Methodology and Materials

### 3.2.1 Plant material

Diploid plants of 89 wild inbred strains (accessions) of the 96 Nordborg *Arabidopsis thaliana* mapping panel (Nordborg et al., 2005) were grown from seeds provided by NASC (CS22564-CS22659). Tetraploid seeds originated from: L*er*-0 Ueli Grossniklaus's lab (originally from Cold Spring Harbour Labs), Col-0 and C24 (from Luca Comai's lab, University of Washington), Zurich (from Ortrun Mittelsten Scheid's lab, GMI Vienna). All tetraploids were generated by colchicine doubling (Blakeslee, 1922). Seeds were surface sterilized by soaking in 5% v/v sodium hypochlorite, 0.05% v/v Triton-X solution (5 minutes) and washed five times with sterile distilled water. Sterilized seeds were individually sown on plates of Murashige and Skoog medium containing 4.6 g $L^{-1}$ MS salts, 30 g $L^{-1}$ sucrose and 8 g $L^{-1}$ agarose (Murashige and Skoog, 1962). The seeded MS plates were incubated in a Percival Tissue Culture Cabinet under a 16:8 hr light: dark ($21^0$C/$18^0$C) regime until the fourth true leaf stage (Boyes et al., 2001b). Seedlings were transplanted to individual pots of soil (8 parts Westland multipurpose compost: 1 part perlite: 1 part vermiculite) and transferred to growth chambers to grow under fluorescent lamps at 200 μmol $m^{-2}$ $s^{-1}$ at $21^0$C/$18^0$C and a 16:8 hr light: dark photoperiod.

### 3.2.2 Reciprocal crosses

Plants were crossed reciprocally by manual emasculation and cross-pollination prior to anthesis under a Leica MZ6 dissecting microscope using Dumostar No. 5 tweezers. Mature siliques were harvested after 7 days. F1 plants were grown in randomized positions to minimize genotype-by-environment (G x E) interactions.

### 3.2.3 Flow cytometry

All F1 triploids used in this study were confirmed to be eutriploid by flow cytometry using PAI PARTEC ploidy analyzer. Fresh leaf tissue (~300 mg) was macerated in a round petri dish with a razor blade for 1 min and incubated in nuclei extraction buffer (Cystain UV Precise P, Partec) for 5 min and the suspensions were filtered through nylon filters of 30 µm mesh width. Nuclei were labelled with fluorescence dye (Cystain UV Precise P, Partec, according to manufacturer's instructions) and ploidy levels determined by direct comparison with standards.

### 3.2.4 Reproductive phenotypes

All ploidy-confirmed F1 triploids and F1 hybrid diploids were allowed to self–pollinate and nearly-mature siliques were dissected under a Leica MZ6 microscope and photographed using a Canon Power Shot S50 camera. Five siliques were analyzed per F1 plant (for each of three individual F1 hybrid plants) and the silique contents scored for % unfertilized ovules (%U), which were identified as very small, white bodies (Meinke, 1994) as a proportion of all possible ovules ($\sum$ of ovules), fertilized or not.

### 3.2.5 Alexander staining of pollen grains

Anthers from young flowers were dissected and mounted on slides and a few drops of Alexander stain* (Alexander, 1969) added. A slide cover slip was added and light hand pressure applied. Pollen grains were observed after 24 hr under a Leica MZ6 light microscope and viable and inviable pollen grains scored (n =100 grains for each of three flowers from three biological replicates). The cytoplasm of viable pollen grains displays a red/purple colour, while that of the aborted grains is pale blue.

*Alexander stain : Ethanol 95% (10 ml), Malachite green (1% in 95% Ethanol) (1 ml), Fuchsin acid (1% in water) (5 ml), Orange G (1% in water) (0.5 ml), Chloral hydrate 5g, Glacial acetic acid 2 ml, Glycerol 25 ml, Distilled water 50 ml.

### 3.2.6 Statistical analyses of heritability of triploid reproductive trait (U)

Data for %U and ∑ were analyzed using linear mixed models in PROC Mixed in SAS (Littel et al., 1996) with accession and cross-direction as fixed factors and including their interaction. In this framework, (a) the contribution of parental genetic variation in triploid traits is indicated by a significant accession term, (b) parent-of-origin effects indicated by a significant cross direction term, and (c) genetic variation in parent-of-origin effects by significant accession X cross direction interaction. The significance of model terms was determined using standard Type 3 analysis and F-ratios (Littel et al., 1996). The contribution of parental genetic effects to triploid variability was also quantified under additional models considering accession as a random term in data split by cross-direction. Proportion of the triploid reproductive variation explained by the single-dose contribution of the natural accessions was calculated as the ratio of the among-accession variance component to the total phenotypic variability in triploid traits. This allowed the determination of the broad-sense heritability although this differs from standard broad-sense heritability given the unequal parental genomic contributions. Similarly, single dose genetic correlations among triploid reproductive characters (in both cross directions) were estimated by the standard Pearson product-moment correlation of triploid line means. The significance of each genetic correlation was determined using a t-test after a Z transformation of the correlation coefficient. Levels of significance for phenotypic and genetic correlations were not adjusted for multiple tests.

## 3.3 Results

### 3.3.1 Fertility (%U) of F1 isogenic triploids of *A. thaliana* varies by genotype

It is known that isogenic F1 triploids of *Arabidopsis thaliana* in the accession Col-0 are sub-fertile (Henry et al., 2005). Sub-fertility (unfertilized ovules) also occurs in isogenic F1 triploids in the L*er*-0, C24 and Zu genetic backgrounds, and the sub-fertility in the Col-0 genetic background has been confirmed (**Figure 3.1**). Depending on genetic background, 41 % to 62 % of ovules formed in the siliques of these selfed isogenic triploids remained unfertilized with L*er*-0 and C24 triploids having the highest proportions of unfertilized ovules (%U) (**Figure 3.1, Table 3.1**). Within each genetic background, no statistically significant difference in fertility was found between any of the reciprocal pairs of F1 triploids indicating no parent-of-origin effects (**Figure 3.1**). This demonstrates that in *Arabidopsis thaliana,* genetic background affects isogenic triploid fertility via mechanisms which do not vary by cross direction.

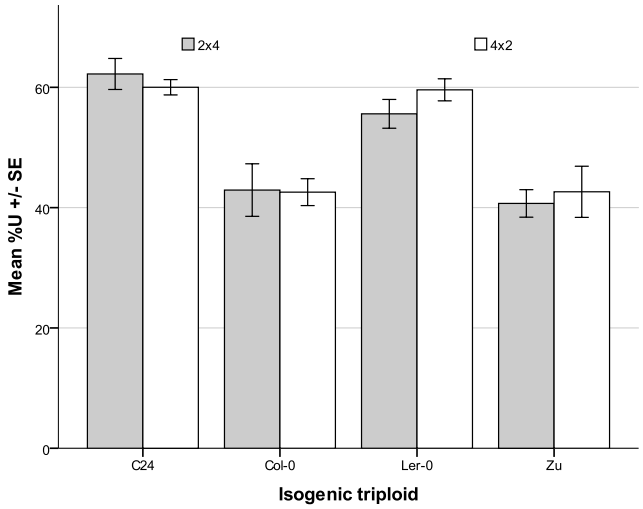


**Figure 3.1**. Fertility of *A. thaliana* F1 isogenic triploids is determined by cross direction as well as parental genotype. Bars represent mean %U in four reciprocal F1 isogenic triploid lines (Col-0, Zu, C24 and L*er*-0). Error bars represent standard error. Mean values were compared using Student's t-test and no significant differences were found in %U between pairs of reciprocal triploids (p-value < 0.05).

| | %U_2 x 4 | %U_4 x 2 | %U_Δ | ∑_2 x 4 | ∑_4 x 2 | ∑_Δ |
|---|---|---|---|---|---|---|
| **Col-0** | 42.9 ± 9.8 | 42.6 ± 8.6 | 0.3 | 58.6 ± 7.4 | 42.5 ± 6.6 | **16.1*** |
| **C24** | 62.2 ± 10 | 60 ± 4.9 | 2.2 | 48 ± 4.4 | 46.8 ± 5.1 | 1.1 |
| **Zu** | 40.7 ± 7.2 | 42.6 ± 16.5 | -1.9 | 23.8 ± 3.7 | 38.6 ± 12 | **-14.8*** |
| **Ler-0** | 59.6 ± 7.1 | 55.6 ± 9.3 | 4 | 45.9 ± 4.9 | 42.7 ± 4.6 | 3.2 |

**Table 3.1.** Ovule fertility rates (%U) and total ovule number (∑) of reciprocal F1 isogenic triploids of *A. thaliana* illustrated by cross direction and as parental genotype. Mean values of %U and ∑ between pairs of reciprocal F1 triploids were compared using Student's t-test and significant differences found in ∑ are highlighted in bold and with an asterisks (p-value < 0.05). No significant differences were found in %U.

### 3.3.2 Fertility (%U) of F1 hybrid triploids display epigenetic parent-of-origin effects

To investigate the effect of hybridity (allelic variation) on the fertility of F1 hybrid triploids, a tetraploid L*er*-0 line (LLLL) was reciprocally crossed to diploid lines of 89 different accessions (AA). Where the tetraploid L*er*-0 line was used as the female (seed) parent the resulting F1 hybrid triploid offspring had a 4x X 2x maternal genome excess (2m: 1p). In the reciprocal direction, the tetraploid L*er*-0 line was used as the male (pollen) parent, producing a 2x X 4x paternal genome excess F1 hybrid triploid (1m: 2p). It should be noted that each reciprocal pair of F1 hybrid triploids have the same genetic composition (LLA in the former case, ALL in the latter; L refers to the tetraploid L*er*-0 and A to a diploid accession), differing only according to whether the L*er*-0 genomes were transmitted via the pollen or the ovules. 166 different F1 hybrid lines were validated as triploid by flow cytometry (data not shown). These were grown in triplicate and allowed to self-fertilize. Siliques were dissected to assess the relative proportions of unfertilized ovules (%U) in each line.

Similar to the analysis of F1 isogenic triploids, all F1 hybrid triploids produced many ovules which were unfertilized within the silique (**Figure 3.2**). The proportion of unfertilized ovules varied significantly by genotype, that is, between F1 hybrid triploids produced from parents of different diploid accessions (**Table 3.2, Figure 3.3**). In the most extreme case, 74.2% of the ovules formed by 1m: 2p F1 hybrid triploids generated from diploid Tamm-27 were unfertilized whilst only 39.7% of those formed by 1m: 2p F1 triploids generated from diploid Pro-0 were unfertilized (**Figure 3.3A).** Triploids generated from diploid Tamm-27 in the reciprocal cross direction also had the highest rates of unfertilized ovules of the 2m: 1p triploids (76.4%) but in this case the highest ovule fertility was found in the 2m: 1p F1 hybrid triploids generated from the RMX-A02 accession (44.4%). In the 4x X 2x cross direction (2m: 1p), triploids generated from Pro-0 only had 53.9% unfertilized ovules (**Figure 3.3B**). Depending on genotype, the F1 hybrid triploids generated in both cross directions displayed rates of unfertilized ovules that were both higher and lower than the isogenic F1 triploids generated only in the L*er*-0 genetic background (**Figure 3.3**).


**Figure 3.2.** Siliques produced by selfed inter-accession *Arabidopsis thaliana* triploids show high variability in the seed set. (A) Most of the ovules remain unfertilized - small, white atrophied bodies indicated by arrows. (B) Normal seeds, as set in the silique of the typical non-hybrid diploid accession are shown as a comparison.

In contrast to the isogenic triploids, it was found that the rate of ovule infertility frequently varied between genetically identical F1 hybrid triploids (LLA vs. ALL) generated in the two reciprocal cross directions. Such epigenetic parent-of-origin effects on the rate of ovule infertility were statistically significant ($p < 0.05$) in 26 pairs of reciprocal F1 hybrid triploids whilst the remainder had no significant difference (**Figure 3.3C**). However, the parent of origin difference observed was not always in the same direction: i.e. eleven F1 hybrid triploids were more fertile as maternal excess 2m: 1p plants (e.g. CS22941), whilst fifteen F1 hybrid triploids displayed the opposite trend and were more fertile in the paternal excess 1m: 2p triploid (e.g. Fei-0) (**Figure 3.3C, Table 3.3**). Hence, not only is the range of F1 hybrid triploid fertility/infertility greater than indicated by analysis of isogenic F1 triploid plants (**Table 3.1**) but the ovule fertility of inter-accession F1 hybrid triploids can also vary significantly via unknown epigenetic effects depending on whether the supernumerary L*er*-0 genome was maternally or paternally inherited. To the author's knowledge, these represent the first evidence of epigenetic parent-of-origin effects controlling ovule fertility of selfed F1 hybrid triploid plants.

| Accession | Σ_2 x 4 | | SD | Accession | %U_2 x 4 | | SD | Accession | Σ_4 x 2 | | SD | Accession | %U -4 x 2 | | SD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Tamm-27 | 77.2 | ± | 11.3 | Tamm-27 | 74.2 | ± | 7.7 | CT-1 | 79 | ± | 5.1 | Tamm-27 | 76.4 | ± | 7.9 |
| CIBC-17 | 72.3 | ± | 10.6 | Uod-1 | 71.1 | ± | 8.1 | SORBO | 74.2 | ± | 4.7 | Ws-0 | 75.5 | ± | 13.2 |
| PU2-23 | 70.4 | ± | 1.7 | RMX-A180 | 70.4 | ± | 7.3 | HR-10 | 73.5 | ± | 8 | GA-0 | 74.5 | ± | 8.4 |
| VAR2-1 | 69.9 | ± | 8.9 | REN-1 | 68.9 | ± | 9.8 | FEI-0 | 73.5 | ± | 4.4 | Lov-5 | 74.3 | ± | 3.5 |
| BOR-4 | 69.5 | ± | 11.2 | TS-1 | 68.6 | ± | 9.2 | LP2-2 | 72.4 | ± | 8.3 | CVI-0 | 72.7 | ± | 6.6 |
| RMX-A180 | 66.5 | ± | 6.4 | CIBC-17 | 68.3 | ± | 8.5 | SHAHDARA | 71.5 | ± | 6.8 | FEI-0 | 71.7 | ± | 4.5 |
| SHAHDARA | 66.4 | ± | 5.4 | TAMM-2 | 68.3 | ± | 8.9 | LL-0 | 70 | ± | 5.2 | Ull2-5 | 69.9 | ± | 6.5 |
| KZ-9 | 65.1 | ± | 3.1 | CS22491 | 68.2 | ± | 4.8 | Ull2-5 | 69.5 | ± | 4.9 | SHAHDARA | 69.7 | ± | 8.2 |
| Kas-1 | 64.8 | ± | 5.2 | SHAHDARA | 67.3 | ± | 3.8 | Uod-1 | 69.5 | ± | 3.7 | MR-0 | 68.8 | ± | 9.6 |
| MR-0 | 64.7 | ± | 6.3 | Bil-5 | 67.1 | ± | 8.5 | KIN-0 | 68.9 | ± | 6.6 | EDEN-1 | 68.2 | ± | 8.5 |
| Ra-0 | 63.9 | ± | 3.4 | NFA-8 | 66.5 | ± | 12.6 | C24 | 68.8 | ± | 5.4 | Omo2-1 | 67.9 | ± | 8 |
| Bil-5 | 62.8 | ± | 4.5 | Kas-1 | 65.8 | ± | 10.6 | VAR2-1 | 68.6 | ± | 7.2 | SORBO | 67.9 | ± | 5.9 |
| LP2-6 | 62.8 | ± | 10 | WS-2 | 64.7 | ± | 8 | OY-0 | 67.9 | ± | 10.5 | Uod-1 | 66.6 | ± | 6 |
| Br-0 | 62.4 | ± | 5.8 | MT-0 | 64.6 | ± | 9 | Edi-0 | 67.7 | ± | 4.5 | KZ-1 | 66.5 | ± | 7.7 |
| SQ-1 | 62.1 | ± | 13.1 | SQ-8 | 64.4 | ± | 7 | WS-2 | 66.5 | ± | 7.5 | Pu-2-7 | 65.3 | ± | 6.3 |
| LZ-0 | 61.9 | ± | 9.9 | PU2-23 | 64 | ± | 6.5 | TSU-1 | 65.7 | ± | 5 | Br-0 | 65 | ± | 9.9 |
| ZDR-1 | 61.7 | ± | 14.6 | SQ-1 | 63.9 | ± | 8.6 | SE-0 | 65.6 | ± | 5.1 | ZDR-1 | 64.9 | ± | 14.2 |
| NFA-10 | 61.1 | ± | 9 | Pna-17 | 63.5 | ± | 11.3 | CVI-0 | 65.4 | ± | 6.3 | LL-0 | 64 | ± | 7.4 |
| Uod-1 | 61.1 | ± | 8.8 | Bay-0 | 63.4 | ± | 6.5 | Tamm-27 | 65.1 | ± | 4.3 | CT-1 | 63.6 | ± | 7.5 |
| Knox-10 | 60.4 | ± | 3.3 | RMX-A02 | 63.3 | ± | 9.5 | Br-0 | 64.7 | ± | 7.5 | TSU-1 | 63.6 | ± | 8.4 |
| Ei-2 | 60 | ± | 6.6 | KNOX-18 | 63.2 | ± | 11.1 | Omo2-1 | 64.5 | ± | 6.8 | CIBC-5 | 63.5 | ± | 7.3 |
| CT-1 | 60 | ± | 5.3 | CVI-0 | 63.2 | ± | 10.1 | MT-0 | 64 | ± | 9.5 | ND-1 | 63.1 | ± | 8.1 |
| CS22491 | 59.9 | ± | 8.3 | NOK-3 | 62.9 | ± | 14.9 | PRO-0 | 63.3 | ± | 5 | HR-10 | 63 | ± | 9.3 |
| Bor-1 | 59.7 | ± | 10.7 | KIN-0 | 62.7 | ± | 5.8 | OMO2-3 | 62.3 | ± | 7.3 | KONDARA | 63 | ± | 9.5 |

| | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| LL-0 | 59.3 | ± | 12.8 | KZ-9 | 62.4 | ± | 5.4 | MR-0 | 62.1 | ± | 9.5 | LZ-0 | 62.8 | ± | 5.4 |
| SQ-8 | 59 | ± | 11.4 | SORBO | 62.4 | ± | 8.6 | Pna-17 | 61.7 | ± | 11 | Bor-1 | 62.5 | ± | 5.1 |
| LP2-2 | 58.9 | ± | 6.1 | Pu-2-7 | 62 | ± | 8.7 | KONDARA | 61.5 | ± | 8.4 | MrK-0 | 62.5 | ± | 8.1 |
| PNA-10 | 58.7 | ± | 12.1 | CT-1 | 61.2 | ± | 7.8 | Pu-2-7 | 61.2 | ± | 7.9 | Edi-0 | 62.5 | ± | 5.3 |
| HR-10 | 58.4 | ± | 17 | GU-0 | 60.6 | ± | 9.1 | MS-0 | 61.2 | ± | 6.6 | TAMM-2 | 62.1 | ± | 14.8 |
| GA-0 | 58.3 | ± | 7.3 | MS-0 | 60.4 | ± | 12 | BOR-4 | 61.1 | ± | 5 | KIN-0 | 61.7 | ± | 7.7 |
| CVI-0 | 57.9 | ± | 4.5 | EDEN-2 | 60.3 | ± | 15.2 | Est-1 | 61 | ± | 9.1 | WS-2 | 61.5 | ± | 9.6 |
| RRS-7 | 57.1 | ± | 8.3 | Est-1 | 59.7 | ± | 9.2 | BUR-0 | 60.8 | ± | 7.4 | Bay-0 | 61.2 | ± | 8.7 |
| NFA-8 | 56.9 | ± | 10.2 | MrK-0 | 59.4 | ± | 11.9 | NOK-3 | 60.5 | ± | 9.1 | MT-0 | 61 | ± | 10.5 |
| TSU-1 | 56.9 | ± | 4.7 | VAR2-1 | 59.2 | ± | 9.8 | Spr1-2 | 59.7 | ± | 7.5 | OY-0 | 60.8 | ± | 10.9 |
| TS-5 | 56.8 | ± | 5.9 | LP2-6 | 59 | ± | 8 | Bor-1 | 59.5 | ± | 8.1 | UOD-7 | 60.4 | ± | 9.5 |
| KIN-0 | 56.2 | ± | 8.2 | NFA-10 | 58.5 | ± | 9.5 | EDEN-1 | 59.2 | ± | 5.6 | RRS-7 | 60 | ± | 10.5 |
| MT-0 | 56.1 | ± | 9.4 | ND-1 | 58.5 | ± | 9.9 | Ag-0 | 59.1 | ± | 7.8 | Ei-2 | 59.9 | ± | 12 |
| TAMM-2 | 55.6 | ± | 13.8 | GA-0 | 58.2 | ± | 11 | UII2-3 | 58.6 | ± | 12 | SE-0 | 59.9 | ± | 7.7 |
| FEI-0 | 55.5 | ± | 6.3 | CIBC-5 | 58 | ± | 9.7 | Kas-1 | 58.5 | ± | 6.8 | LP2-2 | 59.7 | ± | 8.3 |
| Pu-2-7 | 55.4 | ± | 5.4 | Bor-1 | 57.9 | ± | 10.1 | Knox-10 | 58.1 | ± | 5.3 | HR-5 | 59.7 | ± | 8.5 |
| GU-0 | 55.2 | ± | 7.6 | C24 | 57.8 | ± | 11.9 | REN-1 | 58.1 | ± | 9.5 | Ler-0 | 59.6 | ± | 7.1 |
| CIBC-5 | 55 | ± | 4.6 | KZ-1 | 57.6 | ± | 11.2 | RMX-A180 | 57.9 | ± | 8.4 | SQ-1 | 58.9 | ± | 10.4 |
| SORBO | 54.7 | ± | 11.8 | BUR-0 | 57.4 | ± | 6.8 | PNA-10 | 57.2 | ± | 7.1 | TS-1 | 58.6 | ± | 6.8 |
| OMO2-3 | 54.5 | ± | 7.5 | Ra-0 | 57.2 | ± | 7.6 | HR-5 | 56.7 | ± | 8.1 | PNA-10 | 58.5 | ± | 5.8 |
| ND-1 | 54.5 | ± | 6.8 | OMO2-3 | 56.2 | ± | 9.2 | CIBC-5 | 56.5 | ± | 6.7 | VAR2-1 | 58.5 | ± | 10.4 |
| OY-0 | 54.5 | ± | 6.4 | BOR-4 | 55.9 | ± | 13.8 | UOD-7 | 56.5 | ± | 11.1 | LP2-6 | 58.5 | ± | 13.6 |
| Van-0 | 54.4 | ± | 7.7 | HR-5 | 55.8 | ± | 10 | Yo-0 | 56.5 | ± | 8.4 | BUR-0 | 58 | ± | 5.6 |
| SE-0 | 54.4 | ± | 4 | HR-10 | 55.6 | ± | 11.9 | RRS-7 | 56.4 | ± | 4.8 | Ag-0 | 57.9 | ± | 9.6 |
| WS-2 | 54.2 | ± | 16.2 | Ler-0 | 55.6 | ± | 9.3 | TAMM-2 | 56.1 | ± | 9.4 | CIBC-17 | 57.5 | ± | 8.9 |
| NOK-3 | 54.1 | ± | 11.6 | LP2-2 | 55.4 | ± | 19.7 | Ws-0 | 56.1 | ± | 8.3 | REN-11 | 57 | ± | 14.3 |
| REN-1 | 53.9 | ± | 5.7 | REN-11 | 55.2 | ± | 11.8 | WEI-0 | 56 | ± | 8.6 | UII2-3 | 56.7 | ± | 7.2 |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Pna-17 | 53.8 | ± | 7.5 | SE-0 | 55.1 | ± | 10 | LP2-6 | 55.1 | ± | 7.4 | MS-0 | 56.7 | ± | 8.1 |
| REN-11 | 53.8 | ± | 9.5 | TS-5 | 55.1 | ± | 7.8 | GU-0 | 54.9 | ± | 9.5 | BOR-4 | 56.6 | ± | 11.8 |
| Bay-0 | 53.7 | ± | 8.4 | KONDARA | 55.1 | ± | 9.8 | TS-1 | 54.9 | ± | 9.7 | GU-0 | 56.5 | ± | 10.9 |
| WT-5 | 53.4 | ± | 13.6 | Knox-10 | 54.8 | ± | 5.8 | KZ-1 | 54.2 | ± | 6.7 | TS-5 | 56.5 | ± | 8.6 |
| BUR-0 | 53.1 | ± | 3.6 | LL-0 | 54.4 | ± | 7.4 | Got-22 | 54.1 | ± | 8.1 | PU2-23 | 56.3 | ± | 12.8 |
| KZ-1 | 52.8 | ± | 14.7 | RRS-10 | 54.3 | ± | 14.9 | TS-5 | 53.9 | ± | 6.8 | C24 | 56.1 | ± | 8.3 |
| HR-5 | 52.5 | ± | 5.3 | Gy-0 | 54.2 | ± | 14 | LZ-0 | 53.8 | ± | 8.4 | Kas-1 | 55.9 | ± | 10.8 |
| Est-1 | 52.1 | ± | 9.1 | RRS-7 | 54 | ± | 9.6 | RRS-10 | 53.1 | ± | 11.6 | ZDR-6 | 55.5 | ± | 12.6 |
| Gy-0 | 50.9 | ± | 10.5 | MR-0 | 54 | ± | 12.1 | MrK-0 | 52.8 | ± | 5.4 | WEI-0 | 55.5 | ± | 11.5 |
| TS-1 | 50.9 | ± | 4.5 | Ei-2 | 52.7 | ± | 8.9 | RMX-A02 | 52.7 | ± | 8.4 | Col-0 | 55.5 | ± | 14.6 |
| KONDARA | 50.9 | ± | 19.6 | TSU-1 | 52.5 | ± | 10.4 | ZDR-1 | 52.1 | ± | 9.1 | Yo-0 | 55.3 | ± | 8.8 |
| RRS-10 | 50.8 | ± | 15.2 | FEI-0 | 52.3 | ± | 16.2 | GA-0 | 51.7 | ± | 4.9 | Knox-10 | 55.2 | ± | 7.1 |
| MrK-0 | 50.6 | ± | 5.4 | Got-22 | 51.8 | ± | 18.5 | ZDR-6 | 50.9 | ± | 14.9 | NFA-10 | 54.6 | ± | 5.7 |
| RMX-A02 | 50.2 | ± | 7.2 | ZDR-6 | 51 | ± | 9.3 | Ei-2 | 50.9 | ± | 10 | RMX-A180 | 54.4 | ± | 10.7 |
| KNOX-18 | 49.9 | ± | 14.2 | PNA-10 | 50.4 | ± | 11.2 | Bay-0 | 50.3 | ± | 8.4 | PRO-0 | 53.9 | ± | 11.6 |
| LER-1 | 49.9 | ± | 4.3 | LZ-0 | 49.3 | ± | 10.1 | PU2-23 | 49.7 | ± | 13 | Est-1 | 53.8 | ± | 10.7 |
| C24 | 49.4 | ± | 11.9 | Br-0 | 49.3 | ± | 15.7 | Col-0 | 49.1 | ± | 12.6 | Gy-0 | 53.7 | ± | 9.2 |
| MS-0 | 46.9 | ± | 9.8 | Van-0 | 49.2 | ± | 9.7 | NFA-10 | 48.9 | ± | 6 | Van-0 | 53.5 | ± | 7.2 |
| Got-22 | 44.9 | ± | 10.4 | OY-0 | 49 | ± | 9.6 | CS22491 | 48.6 | ± | 11.2 | WT-5 | 53.4 | ± | 9.2 |
| UOD-7 | 44.3 | ± | 14.1 | WT-5 | 47.8 | ± | 12.2 | AN-1 | 48.4 | ± | 12.3 | NFA-8 | 53.3 | ± | 9.8 |
| EDEN-2 | 44 | ± | 10.1 | ZDR-1 | 46.2 | ± | 23.5 | CIBC-17 | 48.1 | ± | 6.9 | Got-7 | 52.9 | ± | 13.6 |
| AN-1 | 43.4 | ± | 21.1 | UOD-7 | 44.5 | ± | 20.8 | Van-0 | 48.1 | ± | 7 | Spr1-2 | 52.6 | ± | 8.3 |
| Ler-0 | 42.7 | ± | 4.6 | Col-0 | 44.2 | ± | 25.1 | KNOX-18 | 47.3 | ± | 17.9 | OMO2-3 | 52.6 | ± | 8.6 |
| MZ-0 | 42.6 | ± | 4.9 | LER-1 | 44.1 | ± | 8.3 | KZ-9 | 47.2 | ± | 14.5 | KZ-9 | 52.6 | ± | 16.2 |
| Col-0 | 42.3 | ± | 9.7 | MZ-0 | 43.7 | ± | 6.9 | LER-1 | 46.1 | ± | 7.1 | KNOX-18 | 52 | ± | 19.3 |
| ZDR-6 | 39.5 | ± | 13.3 | WEI-0 | 41.7 | ± | 15.5 | Ler-0 | 45.9 | ± | 4.9 | RRS-10 | 51.6 | ± | 9.6 |
| WEI-0 | 39 | ± | 4.9 | AN-1 | 41.5 | ± | 17 | ND-1 | 45.6 | ± | 9.7 | Ra-0 | 51.4 | ± | 12.1 |

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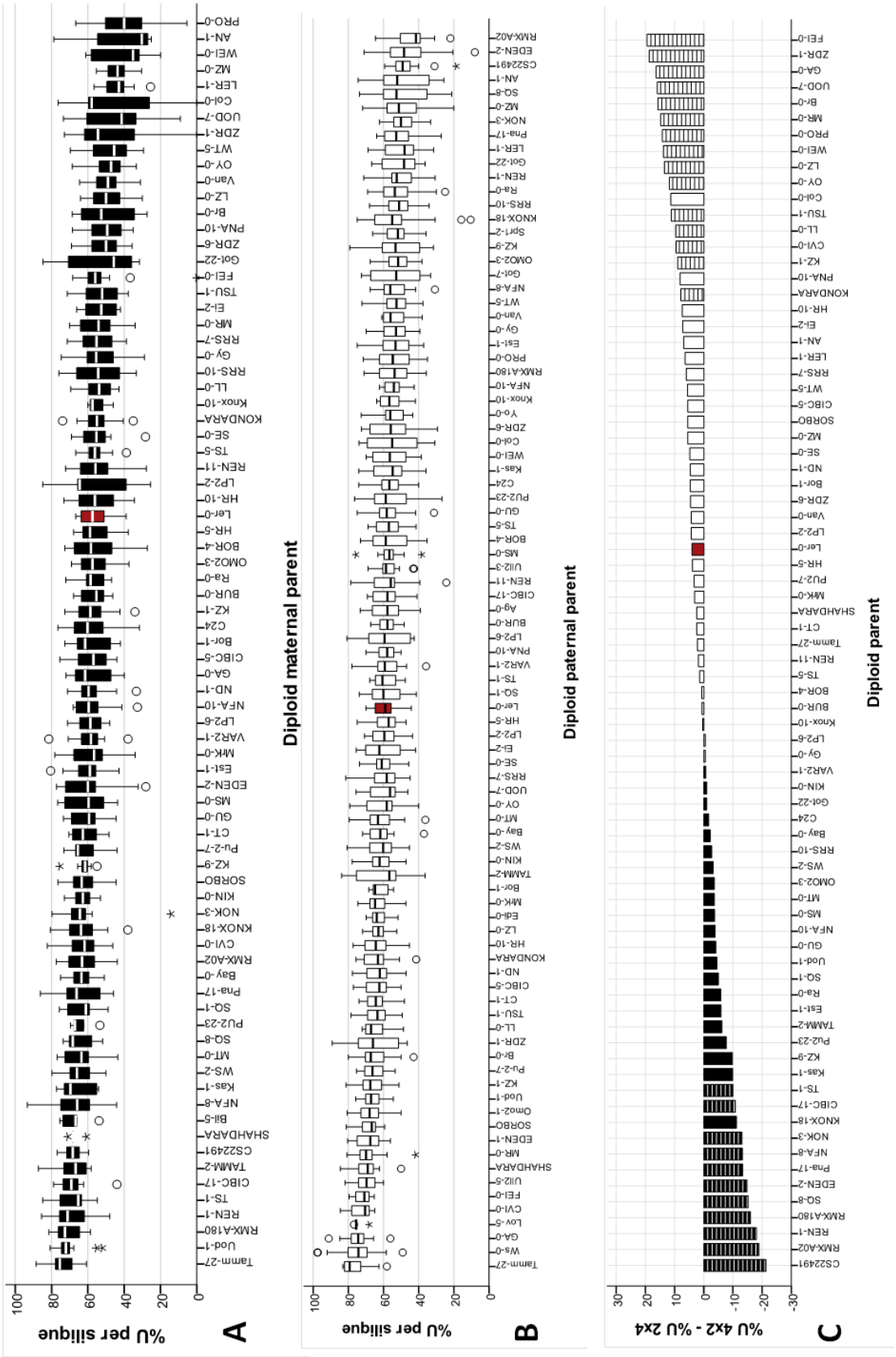
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PRO-0 | 38.3 | ± | 6.2 | PRO-0 | 39.7 | ± | 17.9 | Lov-5 | 45.4 | ± | 4.5 | REN-1 | 50.9 | ± | 9.7 |
| EDEN-1 | N.D. | | | EDEN-1 | N.D. | | | MZ-0 | 44.8 | ± | 14.3 | Got-22 | 50.8 | ± | 10.9 |
| Lov-5 | N.D. | | | Lov-5 | N.D. | | | Got-7 | 44.4 | ± | 11.6 | LER-1 | 50.5 | ± | 11.8 |
| Spr1-2 | N.D. | | | Spr1-2 | N.D. | | | Ra-0 | 43.7 | ± | 13.2 | Pna-17 | 50.2 | ± | 11.8 |
| Omo2-1 | N.D. | | | Omo2-1 | N.D. | | | NFA-8 | 43.3 | ± | 9.4 | NOK-3 | 49.8 | ± | 7.5 |
| Ull2-5 | N.D. | | | Ull2-5 | N.D. | | | SQ-8 | 42.3 | ± | 13.4 | SQ-8 | 49.2 | ± | 15.1 |
| Ull2-3 | N.D. | | | Ull2-3 | N.D. | | | EDEN-2 | 40.2 | ± | 13.1 | MZ-0 | 49.2 | ± | 13.7 |
| Got-7 | N.D. | | | Got-7 | N.D. | | | WT-5 | 40 | ± | 9.4 | AN-1 | 48.4 | ± | 16 |
| Ws-0 | N.D. | | | Ws-0 | N.D. | | | Gy-0 | 39.5 | ± | 6.6 | CS22491 | 46.9 | ± | 10.5 |
| Yo-0 | N.D. | | | Yo-0 | N.D. | | | REN-11 | 35.8 | ± | 10.3 | EDEN-2 | 45.5 | ± | 16.3 |
| Ag-0 | N.D. | | | Ag-0 | N.D. | | | SQ-1 | 33.3 | ± | 12.3 | RMX-A02 | 44.4 | ± | 11.2 |
| Edi-0 | N.D. | | | Edi-0 | N.D. | | | Bil-5 | N.D. | | | Bil-5 | N.D. | | |

**Table 3.2.** Siliques produced by Arabidopsis F1 hybrid triploids contain variable percentages of unfertilized ovules (%U) and total numbers of ovules (∑). Mean %U and ∑ values were calculated for each F1 hybrid triploid genotype from each reciprocal cross direction (i.e. maternal diploid 2x X 4x vs paternal diploid 4x X 2x crosses) and sorted in descending order for each trait. Data from isogenic L*er*-0 triploids is included and highlighted in grey colour. Standard deviation (SD) from mean is included for all values.

**Figure 3.3.** Fertility of *Arabidopsis thaliana* F1 hybrid triploids is determined by cross direction as well as parental genotype. Box-plots of %U are shown for (A) 78 paternal excess (2x X 4x) triploids and (B) 88 maternal excess (4x X 2x) triploids, each sorted in descending order of fertility. The median values, inter-quartile ranges and outliers are displayed. (C) Variation in fertility between the 77 pairs of hybrid triploids showing differences in mean %U per silique between triploids generated in the 4x X 2x and 2x X 4x cross direction. Accessions showing decreased fertility in the 2x X 4x cross direction (higher %U) are coloured in black and those with improved fertility (lower %U) are represented by white bars. Accessions in the centre of the figure show little difference in fertility between cross directions, and include the isogenic L*er*-0 triploid (highlighted in red colour). Those with statistically significant ($p < 0.05$) differences between cross directions (non-parametric Mann-Whitney Test) are shaded (next page).


A
%U per silique
Diploid maternal parent
B
%U per silique
Diploid paternal parent
C
%U 4x2 - %U 2x4
Diploid parent

| Accession | ∑4 x 2 | p-value | ∑2 x 4 | Accession | %U 4 x 2 | p-value | %U 2 x 4 |
|---|---|---|---|---|---|---|---|
| BUR-0 | **60.8*** | < 0.001 | 53.1 | CS22491 | 46.9 | < 0.001 | **68.2*** |
| CIBC-17 | 48.1 | < 0.001 | **72.3*** | FEI-0 | **71.7*** | < 0.001 | 52.3 |
| CT-1 | **79*** | < 0.001 | 60 | GA-0 | **74.5*** | < 0.001 | 58.2 |
| FEI-0 | **73.5*** | < 0.001 | 55.5 | LZ-0 | **62.8*** | < 0.001 | 49.3 |
| KIN-0 | **68.9*** | < 0.001 | 56.2 | NOK-3 | 49.8 | < 0.001 | **62.9*** |
| KZ-9 | 47.2 | < 0.001 | **65.1*** | REN-1 | 50.9 | < 0.001 | **68.9*** |
| LP2-2 | **72.4*** | < 0.001 | 58.9 | RMX-A02 | 44.4 | < 0.001 | **63.3*** |
| PRO-0 | **63.3*** | < 0.001 | 38.3 | RMX-A180 | 54.4 | < 0.001 | **70.4*** |
| Ra-0 | 43.7 | < 0.001 | **63.9*** | CIBC-17 | 57.5 | 0.001 | **68.3*** |
| REN-11 | 35.8 | < 0.001 | **53.8*** | MR-0 | **68.8*** | 0.001 | 54 |
| SE-0 | **65.6*** | < 0.001 | 54.4 | LL-0 | **64*** | 0.002 | 54.4 |
| SORBO | **74.2*** | < 0.001 | 54.7 | NFA-8 | 53.3 | 0.002 | **66.5*** |
| SQ-1 | 33.3 | < 0.001 | **62.1*** | SQ-8 | 49.2 | 0.002 | **64.4*** |
| WEI-0 | **56*** | < 0.001 | 39 | CVI-0 | **72.7*** | 0.005 | 63.2 |
| C24 | **68.8*** | 0.001 | 49.4 | OY-0 | **60.8*** | 0.005 | 49 |
| HR-10 | **73.5*** | 0.001 | 58.4 | TS-1 | 58.6 | 0.006 | **68.6*** |
| NFA-10 | 48.9 | 0.001 | **61.1*** | TSU-1 | **63.6*** | 0.007 | 52.5 |
| PU2-23 | 49.7 | 0.001 | **70.4*** | Br-0 | **65*** | 0.011 | 49.3 |
| Tamm-27 | 65.1 | 0.002 | **77.2*** | Pna-17 | 50.2 | 0.011 | **63.5*** |
| CS22491 | 48.6 | 0.003 | **59.9*** | WEI-0 | **55.5*** | 0.012 | 41.7 |
| WT-5 | 40 | 0.003 | **53.4*** | EDEN-2 | 45.5 | 0.013 | **60.3*** |
| Ei-2 | 50.9 | 0.004 | **60*** | KZ-1 | **66.5*** | 0.016 | 57.6 |
| LP2-6 | 55.1 | 0.004 | **62.8*** | KONDARA | **63*** | 0.024 | 55.1 |
| Gy-0 | 39.5 | 0.005 | **50.9*** | UOD-7 | **60.4*** | 0.033 | 44.5 |
| Pna-17 | **61.7*** | 0.005 | 53.8 | ZDR-1 | **64.9*** | 0.04 | 46.2 |
| Uod-1 | **69.5*** | 0.008 | 61.1 | PRO-0 | **53.9*** | 0.044 | 39.7 |
| OMO2-3 | **62.3*** | 0.011 | 54.5 | Uod-1 | 66.6 | 0.049 | 71.1 |

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|---|---|---|---|---|---|---|---|
| MS-0 | **61.2*** | 0.012 | 46.9 | NFA-10 | 54.6 | 0.052 | 58.5 |
| NFA-8 | 43.3 | 0.012 | **56.9*** | PNA-10 | 58.5 | 0.056 | 50.4 |
| UOD-7 | **56.5*** | 0.014 | 44.3 | KZ-9 | 52.6 | 0.059 | 62.4 |
| WS-2 | **66.5*** | 0.014 | 54.2 | Kas-1 | 55.9 | 0.074 | 65.8 |
| TSU-1 | **65.7*** | 0.016 | 56.9 | SORBO | 67.9 | 0.074 | 62.4 |
| Est-1 | **61*** | 0.017 | 52.1 | HR-10 | 63 | 0.077 | 55.6 |
| LL-0 | **70*** | 0.031 | 59.3 | KNOX-18 | 52 | 0.078 | 63.2 |
| Pu-2-7 | **61.2*** | 0.032 | 55.4 | Est-1 | 53.8 | 0.08 | 59.7 |
| ND-1 | 45.6 | 0.036 | **54.5*** | MZ-0 | 49.2 | 0.085 | 43.7 |
| SQ-8 | 42.3 | 0.038 | **59*** | CIBC-5 | 63.5 | 0.089 | 58 |
| Got-22 | **54.1*** | 0.039 | 44.9 | TAMM-2 | 62.1 | 0.104 | 68.3 |
| Kas-1 | 58.5 | 0.04 | **64.8*** | WT-5 | 53.4 | 0.11 | 47.8 |
| CVI-0 | 65.4 | 0.048 | 57.9 | Ei-2 | 59.9 | 0.134 | 52.7 |
| Ler-0 | 45.9 | 0.05 | 42.7 | LER-1 | 50.5 | 0.141 | 44.1 |
| GA-0 | 51.7 | 0.056 | 58.3 | AN-1 | 48.4 | 0.158 | 41.5 |
| LZ-0 | 53.8 | 0.056 | 61.9 | PU2-23 | 56.3 | 0.197 | 64 |
| KONDARA | 61.5 | 0.062 | 50.9 | RRS-7 | 60 | 0.213 | 54 |
| LER-1 | 46.1 | 0.064 | 49.9 | SE-0 | 59.9 | 0.213 | 55.1 |
| ZDR-1 | 52.1 | 0.068 | 61.7 | Ler-0 | 59.6 | 0.229 | 55.6 |
| RMX-A180 | 57.9 | 0.071 | 66.5 | OMO2-3 | 52.6 | 0.234 | 56.2 |
| REN-1 | 58.1 | 0.074 | 53.9 | SQ-1 | 58.9 | 0.245 | 63.9 |
| BOR-4 | 61.1 | 0.089 | 69.5 | WS-2 | 61.5 | 0.25 | 64.7 |
| AN-1 | 48.4 | 0.101 | 43.4 | ND-1 | 63.1 | 0.254 | 58.5 |
| OY-0 | 67.9 | 0.105 | 54.5 | Van-0 | 53.5 | 0.254 | 49.2 |
| MT-0 | 64 | 0.129 | 56.1 | Bor-1 | 62.5 | 0.272 | 57.9 |
| ZDR-6 | 50.9 | 0.135 | 39.5 | Tamm-27 | 76.4 | 0.281 | 74.2 |
| TS-1 | 54.9 | 0.145 | 50.9 | Col-0 | 55.5 | 0.29 | 44.2 |
| Col-0 | 49.1 | 0.205 | 42.3 | SHAHDARA | 69.7 | 0.321 | 67.3 |

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|---|---|---|---|---|---|---|---|
| EDEN-2 | 40.2 | 0.28 | 44 | Ra-0 | 51.4 | 0.323 | 57.2 |
| MrK-0 | 52.8 | 0.289 | 50.6 | MT-0 | 61 | 0.33 | 64.6 |
| RMX-A02 | 52.7 | 0.299 | 50.2 | ZDR-6 | 55.5 | 0.351 | 51 |
| HR-5 | 56.7 | 0.326 | 52.5 | MS-0 | 56.7 | 0.375 | 60.4 |
| Van-0 | 48.1 | 0.35 | 54.4 | Pu-2-7 | 65.3 | 0.418 | 62 |
| Br-0 | 64.7 | 0.373 | 62.4 | GU-0 | 56.5 | 0.419 | 60.6 |
| Bay-0 | 50.3 | 0.429 | 53.7 | CT-1 | 63.6 | 0.43 | 61.2 |
| Knox-10 | 58.1 | 0.457 | 60.4 | C24 | 56.1 | 0.457 | 57.8 |
| MR-0 | 62.1 | 0.467 | 64.7 | HR-5 | 59.7 | 0.52 | 55.8 |
| TS-5 | 53.9 | 0.5 | 56.8 | MrK-0 | 62.5 | 0.561 | 59.4 |
| NOK-3 | 60.5 | 0.533 | 54.1 | Bay-0 | 61.2 | 0.589 | 63.4 |
| SHAHDARA | 71.5 | 0.563 | 66.4 | REN-11 | 57 | 0.596 | 55.2 |
| KZ-1 | 54.2 | 0.569 | 52.8 | RRS-10 | 51.6 | 0.619 | 54.3 |
| MZ-0 | 44.8 | 0.589 | 42.6 | KIN-0 | 61.7 | 0.678 | 62.7 |
| CIBC-5 | 56.5 | 0.62 | 55 | Got-22 | 50.8 | 0.74 | 51.8 |
| VAR2-1 | 68.6 | 0.649 | 69.9 | VAR2-1 | 58.5 | 0.752 | 59.2 |
| GU-0 | 54.9 | 0.724 | 55.2 | LP2-6 | 58.5 | 0.775 | 59 |
| KNOX-18 | 47.3 | 0.771 | 49.9 | Gy-0 | 53.7 | 0.803 | 54.2 |
| Bor-1 | 59.5 | 0.835 | 59.7 | LP2-2 | 59.7 | 0.803 | 55.4 |
| PNA-10 | 57.2 | 0.852 | 58.7 | TS-5 | 56.5 | 0.848 | 55.1 |
| RRS-10 | 53.1 | 0.884 | 50.8 | BOR-4 | 56.6 | 0.852 | 55.9 |
| RRS-7 | 56.4 | 0.95 | 57.1 | BUR-0 | 58 | 0.852 | 57.4 |
| TAMM-2 | 56.1 | 0.97 | 55.6 | Knox-10 | 55.2 | 0.896 | 54.8 |

**Table 3.3.** Pairs of reciprocal F1 hybrid triploids ranked in descending order according to the significance of the parent-of-origin effect on ovule number (∑) and % unfertilized ovules (%U). F1 hybrid triploids with significantly higher %U and ∑ in given cross direction ($p < 0.05$, Mann-Whitney test) are highlighted in bold type and with an asterisks. A reciprocal pair of L*er*-0 isogenic F1 triploids is listed among the non-significantly different pairs of triploid F1 hybrids and is highlighted in grey colour.

### 3.3.3 Ovule number (Σ) shows genotype-dependent and parent-of-origin effects in *Arabidopsis thaliana* F1 triploids

Previous studies in *A. thaliana* have shown differences in ovule number between diploid accessions of Cvi-0 and L*er*-0, and their reciprocal F1 hybrid offspring (Alonso-Blanco et al., 1999). To investigate whether the range of differences detected in ovule fertility could be related to differences in the ability of triploid F1 hybrid genotypes to generate ovules, the sum of the fertilized and unfertilized ovules (∑) produced per silique for each isogenic (**Table 3.1**) and hybrid F1 triploid genotype (**Table 3.3**) has been calculated. The four different isogenic F1 triploid genotypes displayed some differences in ovule number. Similar ovule numbers were present in the siliques of the isogenic F1 triploids for three of the four genetic backgrounds tested, although they were lower in the Zu genetic background (e.g. in Zu genetic background, averaging *c.*24 ovules in siliques of the 1m: 2p triploid and 39 in the 2m: 1p triploid, as opposed to *c.*46 and 43 in the equivalent L*er*-0 triploids; **Table 3.1**). In two backgrounds, Col-0 and Zu, the reciprocal isogenic F1 triploids displayed statistically significant difference in the number of ovules produced per silique ($p < 0.05$), with Col-0 producing more ovules in 1m:2p siliques and Zu in 2m:1p siliques (**Figure 3.4, Table 3.1**). Hence, for these two genetic backgrounds there is an epigenetic parent-of-origin effect on total ovule number obtained from selfed reciprocal F1 triploids which are isogenic.

Variation in total ovule number was found across F1 hybrid triploids generated from crossing diploid accessions with the L*er*-0 tetraploid (**Figure 3.5**). As with %U, the total number of ovules (∑) varied significantly between F1 hybrid triploids produced from parents of different genetic backgrounds (**Figure 3.5**) with 39 reciprocal crosses indicating significant parent-of-origin effects on total ovule number between the reciprocal F1 hybrid triploids (**Figure 3.5C**). This indicates that genetic background of an F1 hybrid triploid can affect the number of ovules formed in carpels of triploid plants, and that some genetic backgrounds (both isogenic and hybrid) also show significant epigenetic parent-of-origin effects on ovule formation (**Figure 3.5C**). The F1 hybrid triploids generated in both cross directions displayed total ovule numbers that were both higher and lower than the isogenic F1 triploids in the L*er*-0 genetic background (**Figure 3.5**).

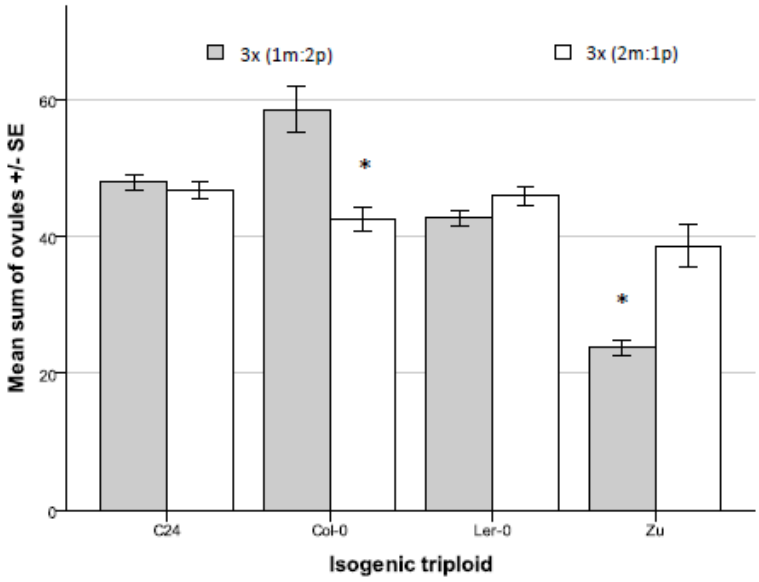
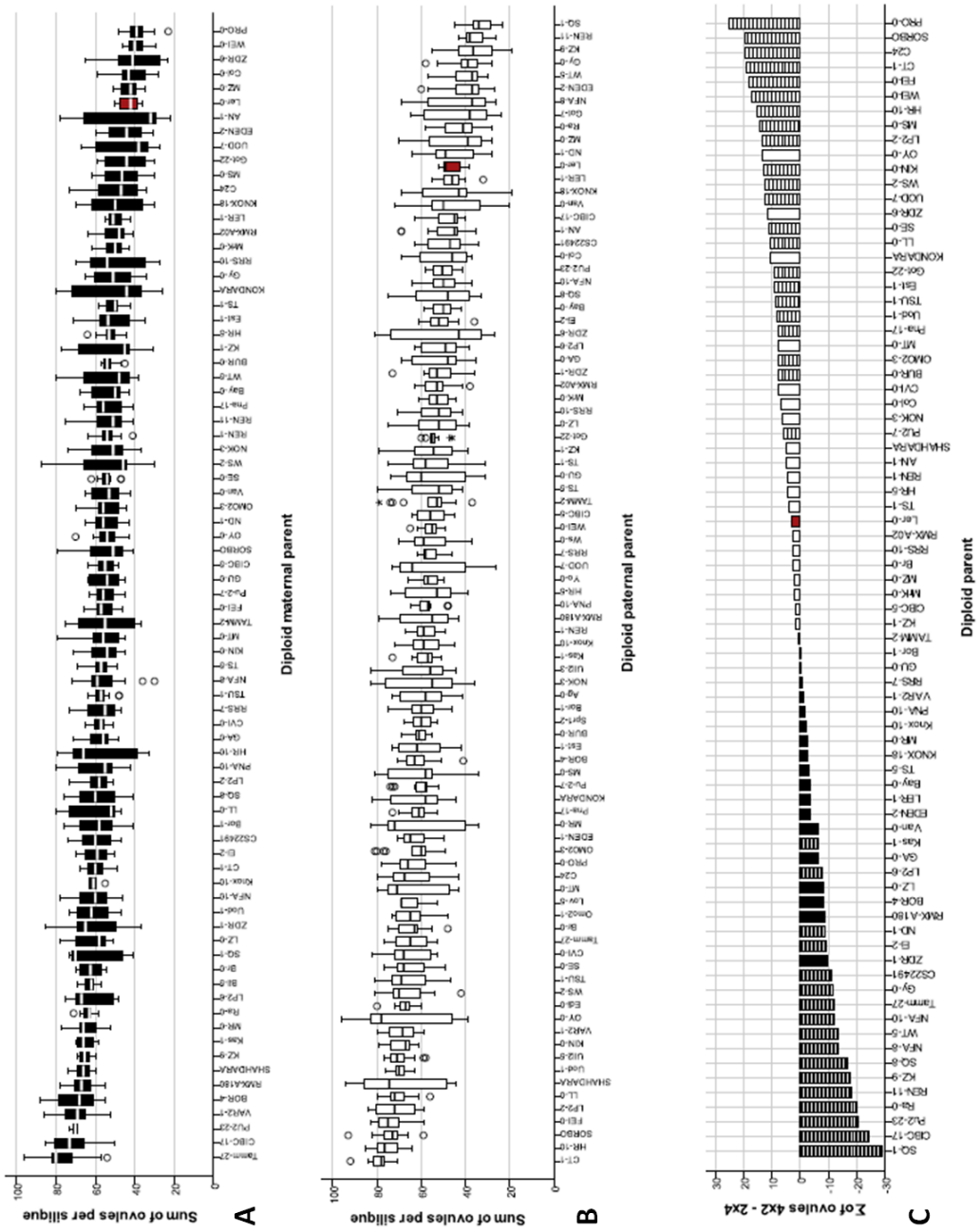



**Figure 3.4.** Sum of ovules of *Arabidopsis thaliana* F1 isogenic triploids is determined by cross direction as well as parental genotype. Bars represent mean sum of ovules in four reciprocal F1 isogenic triploid lines (C24, Col-0, L*er*-0, and Zu). Error bars represent standard error. Mean values were compared using Student's t-test and significant differences were marked by asterisks (p-value < 0.05).

**Figure 3.5**. Sum of ovules (∑) for *Arabidopsis thaliana* F1 hybrid triploids is determined by cross direction as well as parental genotype. Box-plots of ∑ are shown for (A) 78 paternal excess (2x X 4x) triploids and (B) 88 maternal excess (4x X 2x) triploids, each sorted in descending order of fertility. The median values, inter-quartile ranges and outliers are displayed. (C) Variation in ∑ between the 77 pairs of hybrid triploids showing differences in ∑ between reciprocal triploids. Accessions showing increased ∑ in the 2x X 4x cross direction are coloured in black and those with decreased ∑ are represented by white bars. Accessions in the centre of the figure show little difference in ∑ between cross directions, and include the isogenic L*er*-0 triploid (highlighted in red colour). Those with statistically significant ($p < 0.05$) differences between cross directions (non-parametric Mann-Whitney Test) are shaded (next page).


A
Sum of ovules per silique
Diploid maternal parent
B
Sum of ovules per silique
Diploid paternal parent
C
Σ of ovules 4x2 - 2x4
Diploid parent

### 3.3.4 Fertility (%U) of triploid F1 hybrids involves a trade-off with ovule number (Σ)

To determine the underlying basis of the variable reproductive success of *Arabidopsis thaliana* F1 hybrid triploids, phenotypic and genotypic associations between the traits were measured. As expected from the variation in fertility between different genetic backgrounds, mixed models detected significant genetic variation in both %U (proportion of unfertilised ovules) and the sum of ovules (∑). Significant associations with genetic variation were observed at $p < 0.0001$, with broad-sense heritabilities ranging from 0.15 - 0.44 (**Table 3.4**). Importantly, there was considerable support for cross direction ($p < 0.10$) and accession X cross direction interaction effects ($p < 0.001$) for the measured phenotypes (**Table 3.5, Figure 3.6**). Also, the pattern of correlation of triploid reproductive characters within and among parental cross directions was investigated. For phenotypic correlations, there was a similar pattern under both cross directions consisting of a significant positive correlation between %U and the ∑ ($r = 0.38$). This suggests that a trade-off exists between the formation of ovules and their ability to be fertilized. For genetic correlations, there again was a similar pattern under both cross direction consisting of a positive genetic correlation between %U and the sum of ovules. Finally, despite highly similar patterns of genetic correlation among reproductive traits within a cross directions, generally low genetic correlations across directions were observed (**Table 3.5**). This result is not surprising given the fact that the consistent significant accessions X cross direction interaction effects on the reproductive traits was observed (Tom Juenger – personal communication).

| Trait | 2 x 4 | 4 x 2 |
|---|---|---|
| % U | 0.15 | 0.30 |
| ∑ | 0.35 | 0.44 |

**Table 3.4**. Broad-sense heritabilities of proportions of Unfertilized ovules (%U) and the total number of ovules per silique (∑ of ovules) demonstrating the high level of heritability for variation in fertility.

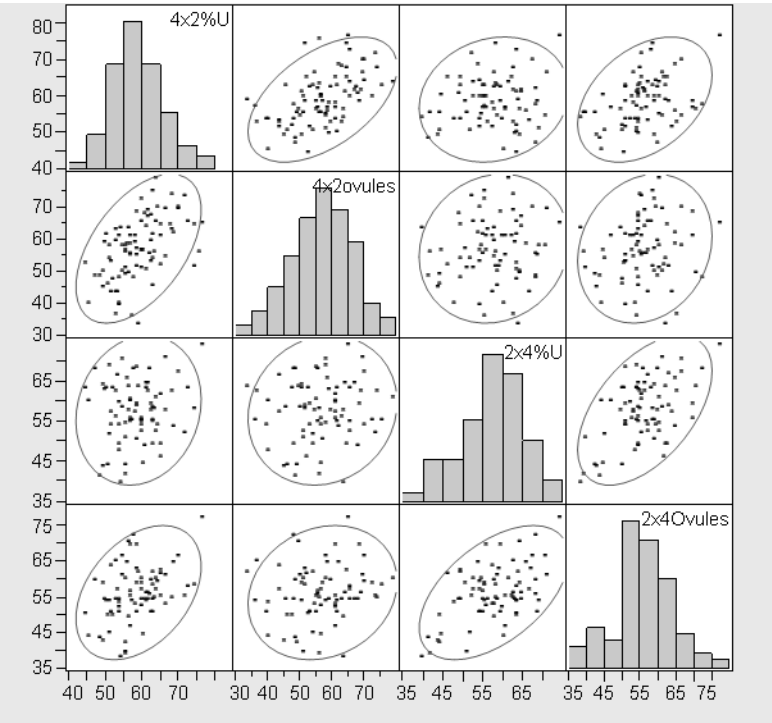



**Figure 3.6.** Diagonal matrix presenting the phenotypic and genetic correlations among the %U and ∑ of ovules traits, split by cross direction. % U (female) refers to proportion of unfertilized ovules from selfed 2x X 4x paternal excess triploids, (male) – 4x X 2x maternal excess triploids. Ovules (female) refers to sum of ovules (fertilized or not) produced by 2x X 4x paternal excess triploids, (male) - 4x X 2x maternal excess triploids.

| **Phenotypic Correlations** | | | |
|---|---|---|---|
| | 2x4 | ∑ | %U |
| | ∑ | 1 | 0.39 |
| | 4x2 | ∑ | %U |
| | ∑ | 1 | 0.37 |
| **Genetic Correlations** | | | |
| | 2x4 | ∑ | %U |
| | ∑ | 1 | 0.5 |
| | 4x2 | ∑ | %U |
| | ∑ | 1 | 0.52 |
| | **Across Direction** | | |
| | | ∑_2x4 | %U_2x4 |
| | ∑ _4 x2 | 0.21 | 0.12 |
| | %U_4x2 | 0.43 | 0.14 |

**Table 3.5.** Phenotypic and genetic correlations between %U and the total number of ovules (∑) and the effect of parental origin in inter-accession hybrid *Arabidopsis thaliana.*

### 3.3.5 Differences in F1 hybrid triploid fertility (%U) are not due to hybrid dysgenesis manifest in diploids

As differences in ovule formation and fertilization rates in F1 hybrid triploids could be a consequence of hybrid dysgenesis between L*er*-0 and certain other accessions (Bomblies et al., 2010), it was necessary to determine if F1 hybrid diploids displayed any evidence of similar hybrid dysgenesis effects. These were derived from reciprocal crosses between diploid L*er*-0 and five accessions: Wei-0, Pro-0, Ga-0 and Fei-0, representing the extremes of the fertility defect observed in 2m: 1p F1 hybrid triploids; and Ren-1 representing the most extreme fertility detects amongst the 1m: 2p F1 hybrid triploids (**Figure 3.3**). The rate of fertility defects was almost entirely ablated when the hybridisation occurred without a concomitant change in ploidy level. In fact, in all five F1 hybrid diploids at least 90% of ovules were successfully fertilized (**Figure 3.7, Table 3.6**). This indicates that the genotypic and parent-of-origin effects on fertility seen in the F1 hybrid triploids is due to a genome dosage effect rather than being a consequence of hybridity.

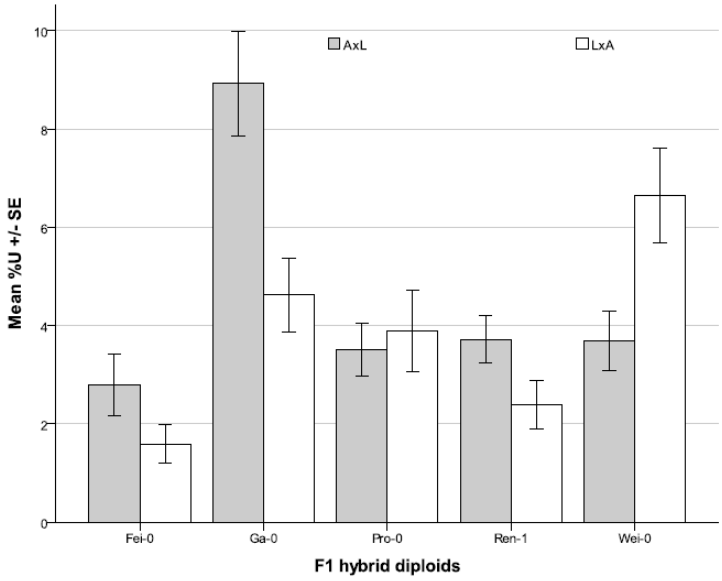



**Figure 3.7.** Fertility of *Arabidopsis thaliana* F1 hybrid diploids is determined by cross direction as well as parental genotype. Bars represent mean %U produced by selfed F1 hybrid diploids, generated by crosses between diploid (L) L*er*-0 and five different diploid (A) accessions (Fei-0, Ga-0, Pro-0, Ren-1, Wei-0). Error bars represent standard error.

| Accession | %U_AxL | | SD | %U_LxA | | SD | AxL -LxA | p-value |
|---|---|---|---|---|---|---|---|---|
| Wei-0 | 3.9 | ± | 4.3 | 6.7 | ± | 6.4 | -2.8 | **0.031*** |
| Ga-0 | 8.9 | ± | 7.1 | 4.6 | ± | 5.1 | 4.3 | **0.002*** |
| Pro-0 | 3.5 | ± | 3.6 | 3.9 | ± | 5.5 | -0.4 | 0.686 |
| Fei-0 | 2.8 | ± | 3.4 | 1.6 | ± | 2.1 | 1.2 | 0.1 |
| Ren-1 | 3.7 | ± | 3.3 | 2.4 | ± | 2.7 | 1.3 | 0.13 |

**Table 3.6.** Mean percentage of unfertilized ovules (%U) per silique in five pairs of reciprocal F1 hybrid diploids generated by crosses between diploid (L) L*er*-0 and five different diploid (A) accessions. Differences between mean %U in two cross directions was tested by Mann-Whitney U test and the significance level was in 0.05. Significant differences between reciprocal crosses are highlighted in bold type and an asterisk.

### 3.3.6 High proportion of unfertilized ovules (%U) identified in F1 triploids due to aberrant megagametogenesis or ovule development

F1 *A. thaliana* triploids reproduce through gametes with a high tendency to aneuploidy, which leads to subsequent infertility (Henry et al., 2005). Especially, F1 hybrid triploid infertility could result from impaired pollen production as observed in *A. thaliana* X *A. arenosa* interspecific interploidy crosses (Comai et al., 2003). To determine the extent by which aberrant female and/or male gametogenesis was linked to altered fertility in reciprocal F1 hybrid triploids, the viability of pollen grains produced by triploids with the highest and lowest fertility was tested (**Figure 3.8**). These were the triploids with the lowest fertility (2m:1p triploids derived from Uod-1 and RMX-A180 (i) and 1m:2p derived from Cvi-0 and Ga-0 (iii)); those with the highest fertility (2m:1p derived from Wei-0, An-1, and Pro-0 (ii) and 1m:2p derived from RMX-A02 (iv)); those with the greatest fertility difference between reciprocal crosses (derived from CS22491, Fei-0 and Zdr-1 (v & vi); and those with the lowest such differences (derived from Bur-0 and Gy-0 (vii)). Triploids produced from the reciprocal crosses of these lines were also tested to identify parent-of-origin effects in identical genetic backgrounds (**Figure 3.3**).

In most cases, pollen viability was reduced (**Figure 3.8**), suggesting that gametogenesis is indeed disrupted in triploids. However, no correlation was found between inviability of pollen and %U ($r = -0.24$ in 1m: 2p triploids; $r = -0.263$ 2m: 1p triploids, $p > 0.05$) (**Figure 3.9**). For example, the triploids generated from accession Fei-0 have the largest observed difference in fertility of any pair of reciprocal triploids, but both had similar pollen viability of *c.* 97 % (**Figure 3.3C, Figure 3.8**). Furthermore, the proportion of viable pollen was never less than 50 % so was still in high excess over the number of available ovules. This suggests that there is no causal link between pollen viability and triploid fertility; although Alexander staining only distinguishes the presence of correct cytoplasm so may underestimate the rate of nuclear defects. It does, however, preclude the existence of pollen abortion following widespread meiotic catastrophe. Therefore, the high proportion of unfertilized ovules identified in triploids is most likely to occur due to aberrant megagametogenesis or ovule development.

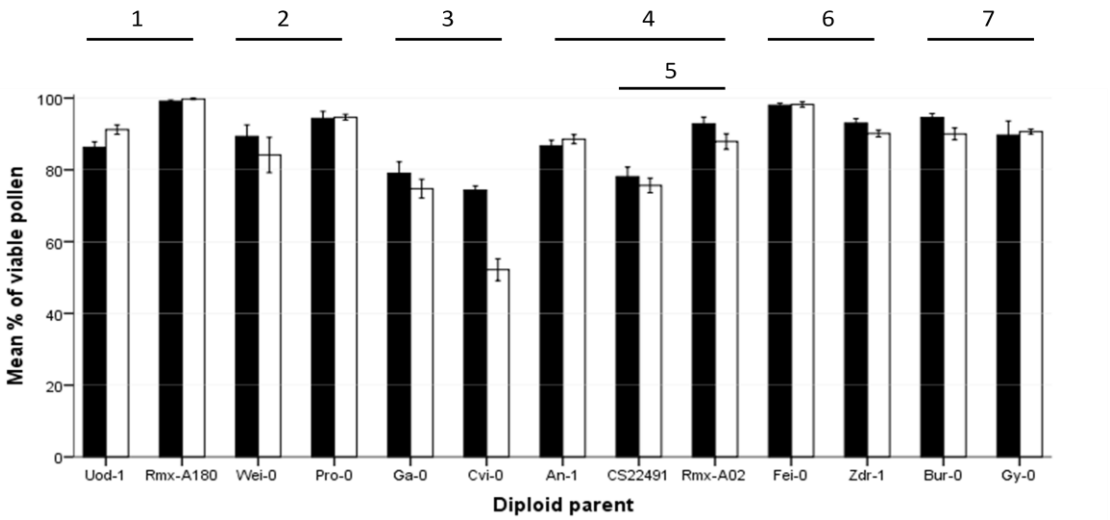



**Figure 3.8.** Pollen viability was determined for 13 reciprocal inter-accession *Arabidopsis thaliana* hybrid triploids. Data for male excess (2 x 4) triploids are represented by black bars, data for female excess (4 x 2) triploids are represented by white bars. Standard errors are shown.

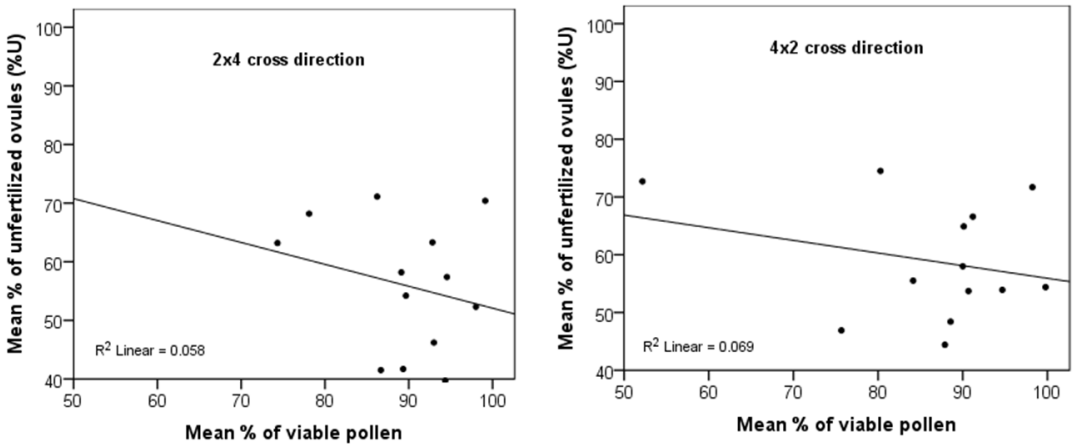



**Figure 3.9.** Pollen viability was correlated against %U and the association found to be non-significant (r = -0.24 in the 2 x 4 cross direction and r = -0.262 in the 4 x 2 cross direction, $p > 0.05$). Lines of best fit are shown.

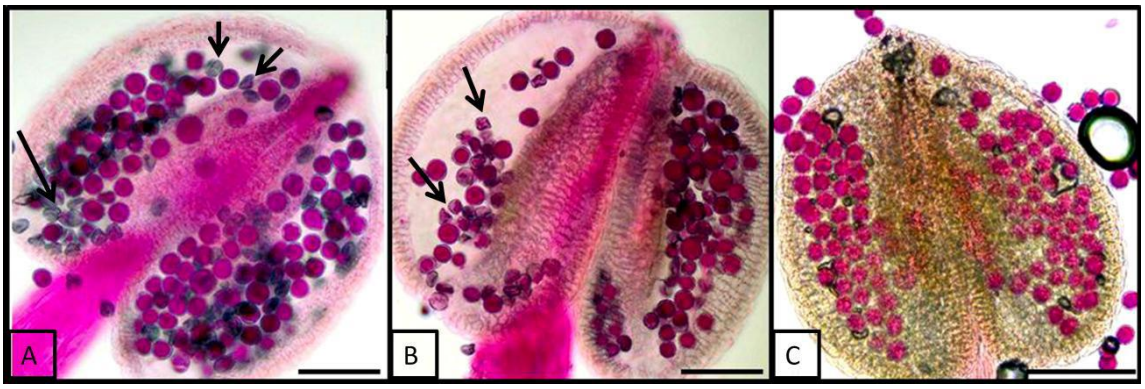



**Figure 3.10.** Alexander staining indicates rates of pollen inviability in inter-accession hybrid *Arabidopsis thaliana* triploids. Inviable pollen is visible as pale, shrunken grains (indicated by the arrow) in A – 4x X 2x and B – 2x X 4x hybrid triploids. C - anther from diploid accession shows only viable pollen which appears as plump, purple grains. Scale bars = 100 µm.

## 3.4 Discussion

In this chapter, fertility of F1 *Arabidopsis thaliana* triploids has been examined incorporating many different genotypes and it was found that there are strong genetic and heritable affects on their reproductive success (**Figure 3.3, Table 3.2, Table 3.4**). Strikingly, many of the pairs of triploids generated by crossing the same accessions in two reciprocal directions differ in reproductive success (**Figure 3.3C, Table 3.3**). These parent-of-origin effects may cause greater fertility in either the paternal excess (1m : 2p) or maternal excess (2m : 1p) triploids. This may result from the impact of altered genome balance in ways encountered by triploids but not by polyploids with equal parental contributions. These could include altered response to maternal affects (Grossniklaus et al., 1998) involving either the embryo and the endosperm, epigenetic effects such as differential DNA methylation (Simon et al., 2005) or misregulation of transposable elements, or result from imbalance of dosage-sensitive genes (Birchler et al., 2001). Dosage effects are known to alter endosperm of *A. thaliana* polyploids (Scott et al., 1998) and could therefore affect the fitness of the resulting plant, including its subsequent fertility. In addition, both maternal and paternal genomes have been shown to influence seed development in diploids, and such phenomena would presumably be altered by changes to the parental genome dosage. For example, maternal affects control endosperm development via regulation of the rate of cell cycling during the cell division stage in *Z. mays*, *A. thaliana* and *P. sativum* (Leblanc et al., 2002, Lemontey et al., 2000). Less well-defined maternal effects have been reported to alter aspects of phenotype throughout the plant's life cycle, such as tiller architecture in some grasses (Roach and Wulff, 1987). *A. thaliana* also exhibits paternal effects over embryogenesis due to the delivery of untranslated paternal mRNA encoding the SHORT SUSPENSOR protein kinase (Bayer et al., 2009).

It has been identified that under conditions in which ovule production is limited, presumably by the tendency of triploid organisms to produce aneuploid gametes, there is a trade-off between rate of ovule production and the success of fertilization (**Table 3.5**). This could occur due to a reduction in the vigour of triploid *A. thaliana* and consequent reduction in photosynthate. Additionally, the female gametophytes formed by triploids may require higher resource investment during their development and therefore compete with

each other. Competition between fertilized offspring has frequently been described (Greenway and Harder, 2007) but in triploids similar effects may occur between developing ovules as well. As many wild plants produce higher ovule numbers in order to take advantage of variable pollen supplies (Burd et al., 2009), such competition effects could also be widespread in diploid species under certain conditions. This could be particularly relevant for reproductive success if, as expected, subsequent seed size is also to be affected by ovule number (Alonso-Blanco et al., 1999). It is likely that the relationship between female gametophytes and the parental plant are of key importance as it has been determined that the pollen grains produced by triploid *A. thaliana* are still largely viable, and that this trait cannot explain variation in fecundity of different triploids (**Figure 3.8**).

A consequence of the described variation in reproductive success of different triploids (**Figure 3.3**) is that polyploidy could act differently on populations within a species, supporting the link between polyploidy and speciation (Rick and Barton, 1954). Such variation also occurs in agriculturally important species – for example, cherry tomato produce more aneuploids than triploids of the San Marzano variety (Rick and Barton, 1954, Rick and Notani, 1961). Such triploid tomatoes are normally sterile, but when seed fertility is recovered in parthenocarpic lines, fruit dry-mass is significantly increased (Habashy et al., 2004). The parent-of-origin dependent phenotypes observed in this study could also shed light on similar phenomena in cucumber in which desirable triploids can only be generated if the excess genome is paternally inherited and show decreased pollen fertility and seedlessness (Mackiewicz et al., 1998). As greater effects on reproduction were observed in hybrid than isogenic triploids, our data also provide a model for understanding of transgressive variation (Rick and Notani, 1961) and potentially heterosis (Birchler et al., 2003, Lippman and Zamir, 2007). Being able to select for altered fertility could allow the adaptation of apomixis for agricultural use (Spillane et al., 2004) or aid the breeding of triploid Musa varieties which vary greatly in fertility (Ortiz and Vuylsteke, 2004). Discussed results provide a framework for studying the genetics of fertility in triploids crops such as these. Understanding fertility of hybrid triploids will allow a greater understanding of speciation events involving polyploids, and how parental genomes interact during sexual reproduction of organisms of any ploidy level.

## 3.5 Conclusions

In this chapter, F1 triploid plants generated from inter-ploidy crosses of different accessions of *Arabidopsis thaliana* were shown to be fertile but reproduce with reduced and varied rates of success. Also, identification of novel parent-of-origin dependent genome dosage effects which control both fertility and ovule number in selfed F1 hybrid triploids was demonstrated. It was further shown that the variation in F1 triploid fertility is heritable and involves a trade-off between ovule number (Σ) and ovule fertility (%U) which is largely unaffected by pollen viability.

# CHAPTER 4 – Post-zygotic lethality in *Arabidopsis thaliana* F1 triploids

## 4.1 Introduction

Triploidy can result from the spontaneous fusion of an unreduced 2x gamete with a regular 1x gamete in the diploid individual, or from inter-ploidy hybridization between diploid and tetraploid individuals (Ramsey and Schemske, 1998). Flowering plants easily tolerate polyploidy but inter-ploidy crosses result in parent-of-origin dependent abnormal seed development, often followed by abortion (Leitch and Bennett, 1997, Scott et al., 1998, Birchler, 1993). The reason for low frequency of viable progeny in some triploid species can be explained by the phenomenon of triploid block (Köhler et al., 2010, Marks, 1966), in which triploid embryos abort because of abnormal endosperm development. Those which survive often exhibit reduced reproductive fitness (Satina et al., 1938, Vinkenoog et al., 2003). In other species, such as *Arabidopsis thaliana*, inter-ploidy crosses produce viable and sub-fertile triploids which may act as evolutionary bridges between different ploidy levels (Henry et al., 2005). Genetic control of inter-ploidy and intra-specific hybrid lethality has been dissected in *Arabidopsis thaliana* and *Drosophila melanogaster* (Bomblies and Weigel, 2007, Brideau et al., 2006).

Post-fertilization barriers encountered by *Arabidopsis thaliana* triploids are sensitive to ploidy and to the epigenetic status of the mating plants (Martienssen, 2010). Triploid seed phenotypes strongly suggest that endosperm failure is the main cause of post-zygotic lethality following inter-ploidy crosses and extent of endosperm growth determines final seed size (Haig and Westoby, 1991, Birchler, 1993). The Endosperm Balance Number (EBN) term describes the pattern of dosage-dependent incompatibility. Each species has a specific EBN, and all successful crosses require a 2:1 maternal to paternal EBN ratio in the hybrid endosperm. EBN varies independently of ploidy, and inter-specific crossability was found to depend on EBN, not ploidy (Lin, 1984, Johnston and Hanneman Jr, 1995, Carputo et al., 1997). Disruption of this ratio causes imbalanced expression of maternally and paternally derived genomes leading to endosperm breakdown and failure of seed development (Dilkes and Comai, 2004). According to the differential dosage hypothesis, any differential parental contribution of a dosage-dependent regulator of viability can affect the triploid block (Dilkes and Comai, 2004).

It has been proposed that inter-ploidy cross phenotypes can be attributed to the disrupted balance of active copies of parentally imprinted genes in the seed (Haig and Westoby, 1991, Gehring et al., 2004, Baroux et al., 2002). There is strong support for the *MEDEA* maternally expressed imprinted gene to control seed development defects following 2x X 4x inter-ploidy crosses, providing the molecular basis for dosage sensitivity (Erilova et al., 2009). The disruption of imprinting via DNA hypomethylation can lead to inter-ploidy failure by destroying coordinated parental programs in the seed and in the integument (Adams et al., 2000, Baroux et al., 2007) . Also, maternal and paternal genomes contribute differing small interfering RNA (siRNA) that play role in heterochromatin reprogramming in the reproductive cells, as well as in the products of double fertilization, the embryo and the endosperm (Martienssen, 2010). Imprinting and reprogramming are associated with the activity of transposable elements (TE) that can influence the expression of neighbouring genes, and thus explain aspects of hybridization failure (Slotkin and Martienssen, 2007).

### 4.1.1 Research Objectives

In this chapter, four reciprocal inter-ploidy isogenic crosses (Col-0, C24, L*er*-0, and Zu) and 77 reciprocal inter-ploidy, intra-specific hybridizations were carried out to generate a panel of F1 triploids (isogenic and hybrid). All F1 hybrid and isogenic triploids were allowed for self-fertilization and their F2 offspring was screened for post-zygotic seed lethality. It was sought to determine if natural variation found in diploid accessions of *Arabidopsis thaliana* can significantly affect triploid post-zygotic lethality. It was also investigated if the rates of F2 seed abortion differ between pairs of reciprocal triploids, subject to epigenetic parent-of-origin effects.

## 4.2 Methodology and Materials

### 4.2.1 Plant material

Diploid plants of 89 wild inbred accessions of *Arabidopsis thaliana* were grown from seeds provided by NASC (CS22564-CS22659). Tetraploid seeds originated from: L*er*-0 Ueli Grossniklaus's lab (originally from Cold Spring Harbour Labs), Col-0 and C24 (from Luca Comai's lab, University of Washington), Zurich (from Ortrun Mittelsten Scheid's lab, GMI Vienna). All tetraploids were generated by colchicine doubling (Blakeslee, 1922). Seeds were surface sterilized by soaking in 5% v/v sodium hypochlorite, 0.05% v/v Triton-X solution (5 minutes) and washed five times with sterile distilled water. Sterilized seeds were individually sown on plates of Murashige and Skoog medium containing 4.6 g $L^{-1}$ MS salts, 30 g $L^{-1}$ sucrose and 8 g $L^{-1}$ agarose (Murashige and Skoog, 1962). The seeded MS plates were incubated in a Percival Tissue Culture Cabinet under a 16:8 hr light: dark ($21^0$C/$18^0$C) regime until the fourth true leaf stage (Boyes et al., 2001b). Seedlings were transplanted to individual pots of soil (8 parts Westland multipurpose compost: 1 part perlite: 1 part vermiculite) and transferred to growth chambers to grow under fluorescent lamps at 200 µmol $m^{-2}$ $s^{-1}$ at $21^0$C/$18^0$C and a 16:8 hr light: dark photoperiod.

### 4.2.2 Reciprocal crosses

Plants were crossed reciprocally by manual emasculation and cross-pollination prior to anthesis under a Leica MZ6 dissecting microscope using Dumostar No. 5 tweezers. Mature siliques were harvested after 7 days. F1 plants were grown in randomized positions to minimize genotype-by-environment (GxE) interactions.

### 4.2.3 Flow cytometry

All F1 triploids used in this study were confirmed to be eutriploid by flow cytometry using the PAI PARTEC ploidy analyzer. Fresh leaf tissue (~300 mg) was macerated in a round petri dish with a razor blade for 1 min and incubated in a nuclei extraction buffer (Cystain UV Precise P, Partec) for 5 min and the suspensions filtered through nylon filters of 30 µm mesh width. Nuclei were labeled with fluorescence dye (Cystain UV Precise P, Partec, according to manufacturer's instructions) and ploidy levels determined by direct comparison with diploid standard.

### 4.2.4 Reproductive phenotypes

All ploidy-confirmed F1 triploids and F1 hybrid diploids were allowed to self–pollinate and nearly-mature siliques were dissected with fine tweezers under a Leica MZ6 microscope. Five siliques were analyzed per F1 plant (for each of three individual F1 hybrid plants) and the silique contents scored for % aborted seeds (%A), calculated as a proportion of aborted seeds to all possible ovules in the silique ($A/\sum$). Aborted seeds (A) were identified as those with a shrunken appearance, brown coloration and, usually, reduced size in the maturing silique, when compared with normal seeds (N) (Meinke, 1994).

### 4.2.5 Statistical analyses of heritability of triploid reproductive trait (A)

Data for %A and $\sum$ were analyzed using linear mixed models in PROC Mixed in SAS (Littel et al., 1996) with accession and cross-direction as fixed factors including their interaction. In this framework, the contribution of parental genetic variation in triploid traits is indicated by a significant accession term, parent-of-origin effects indicated by a significant cross direction term, and genetic variation in parent-of-origin effects by significant accession X cross direction interaction. The significance of model terms was determined using standard Type 3 analysis and F-ratios (Littel et al., 1996). The contribution of parental genetic effects to triploid variability was also quantified under additional models considering accession as a random term in data split by cross-direction. A proportion of the triploid reproductive variation explained by the single-dose contribution of the natural accessions was calculated as the ratio of the among-accession variance component to the total phenotypic variability in triploid traits. This allowed the determination of the broad-sense heritability although this differs from standard broad-sense heritability given the unequal parental genomic contributions. Similarly, single dose genetic correlations among triploid reproductive characters (in both cross directions) were estimated by the standard Pearson product-moment correlation of triploid line means. The significance of each genetic correlation was determined using a t-test after a Z transformation of the correlation coefficient. Levels of significance for phenotypic and genetic correlations were not adjusted for multiple tests.

## 4.3 Results

### 4.3.1 Variation in the rates of post-zygotic lethality (%A) in selfed *Arabidopsis thaliana* isogenic triploids depends upon the genotype and parent-of-origin

In order to investigate the effects of polyploidy on *Arabidopsis thaliana* post-zygotic lethality, isogenic F1 triploids were generated by inter-ploidy reciprocal crosses in four genetic backgrounds (C24, Col-0, L*er*-0 and Zu). It was found that triploid reproduction was impaired compared to diploids, which have an almost entirely normal seed set, whether selfed or hybrid (**Figure 4.1, Figure 4.5)**. In addition to F2 seeds which appeared to have developed normally and a number of ovules that remained unfertilized, triploid siliques contained varied proportions of aborted F2 seeds. The rates of post-zygotic lethality within F2 seed sets varied depending on the genetic background and parental genome dosage (**Table 4.1, Figure 4.1**). Col-0 2x X 4x paternal excess triploids, which experience severe lethality following inter-ploidy crosses (Dilkes et al., 2008), surprisingly produced the lowest proportion of aborted F2 seeds (6.9%) after self-fertilization. The seed abortion in the reciprocal direction was significantly higher (20.7%, p-value = 0.001). Within the other three genetic backgrounds (C24, L*er*-0 and Zu) no statistically significant difference in seed abortion was found between the reciprocal pairs of F1 triploids indicating no parent-of-origin effects (**Table 4.1**). L*er*-0 reciprocal triploids had the highest F2 seed lethality, exceeding 22% (**Figure 4.1**).

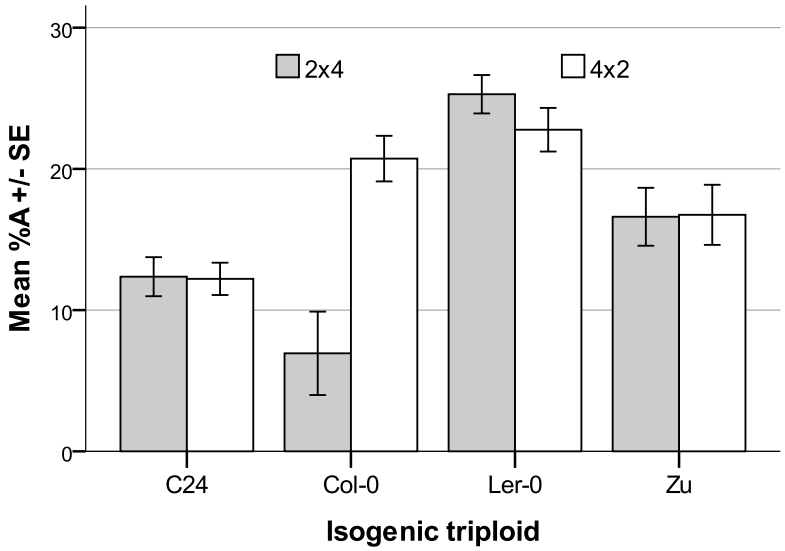



**Figure 4.1**. Post-zygotic lethality in selfed isogenic triploids of *A. thaliana* is determined by cross direction as well as parental genotype. Bars represent mean %A (aborted seeds) in four reciprocal F1 isogenic triploid lines (C24, Col-0, L*er*-0 and Zu). Error bars represent standard error.

| | %A_2 x 4 | %A_4 x 2 | %A_Δ | p-value |
|---|---|---|---|---|
| Col-0 | 6.9 ± 6.6 | 20.7 ± 6.25 | -14 | **0.001*** |
| C24 | 12.4 ± 5.35 | 12.2 ± 4.43 | 0.15 | 0.983 |
| Zu | 16.6 ± 6.49 | 16.7 ± 8.24 | -0.1 | 0.912 |
| L*er*-0 | 25.3 ± 5.26 | 22.8 ± 5.98 | 2.51 | 0.237 |

**Table 4.1.** Aborted seeds (%A) in selfed reciprocal isogenic triploids of *A. thaliana* illustrated by cross direction and parental genotype. Mean values of %A and SD (standard deviation) between pairs of reciprocal triploids were compared using a non-parametric test (Mann-Whitney U) and significant differences are highlighted in bold and marked with an asterisks (p-value < 0.05).

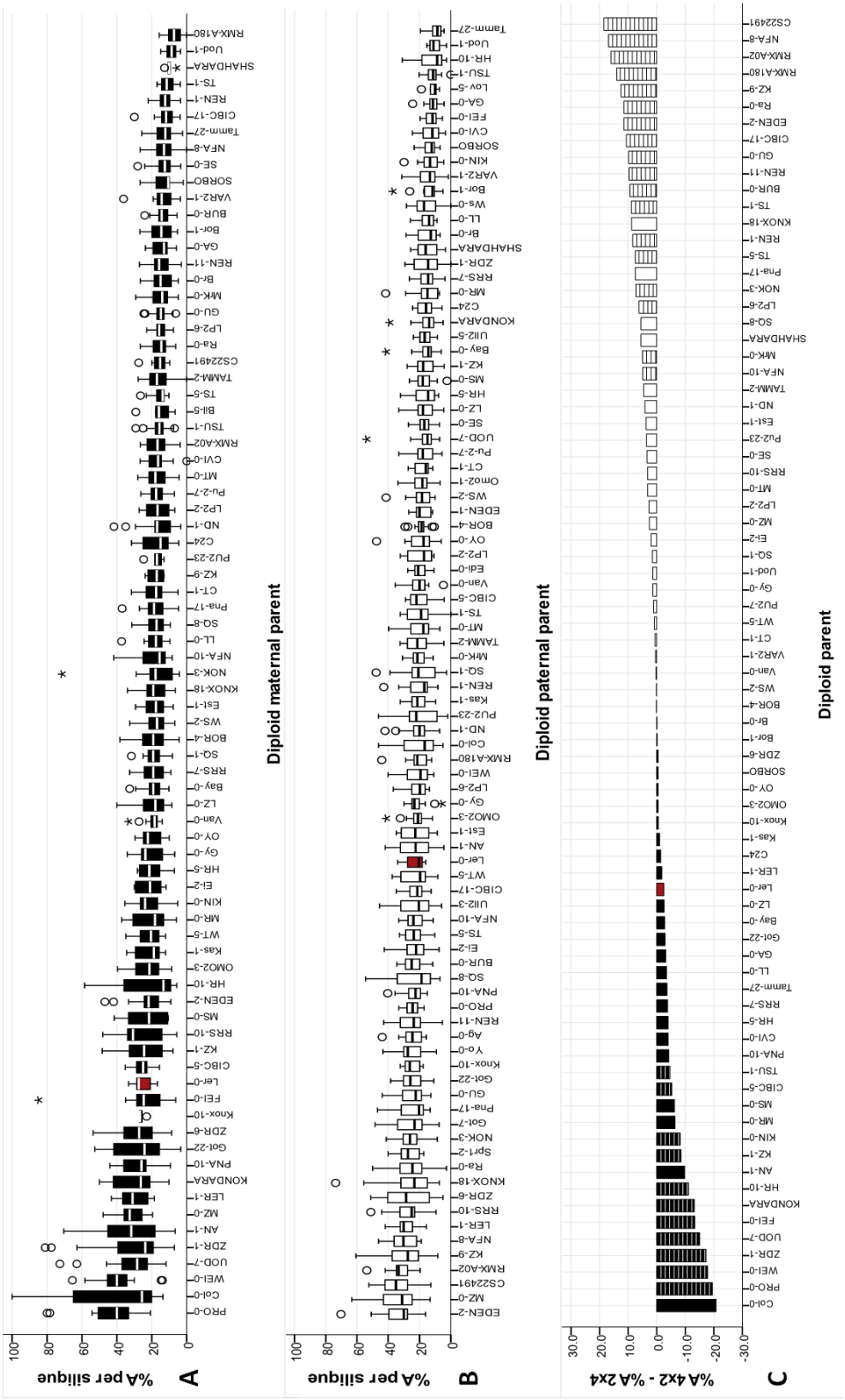
### 4.3.2 F1 hybrid triploids display genetic and epigenetic parent-of-origin effects on seed lethality (%A)

To investigate the effect of hybridity (allelic variation) on the fertility of F1 hybrid triploids, a tetraploid L*er*-0 line was reciprocally crossed to 89 different diploid accessions to generate up to 166 F1 hybrid triploid plants with different genotypes. Relative percentage of seed abortion per triploid line (%A) was calculated as a proportion of aborted seeds to all ovules in the silique (A/∑) (**Table 4.2**, **Figure 4.2**). By comparing these scores, it was possible to demonstrate that, similarly to the analysis of F1 isogenic triploids, the proportion of aborted seeds varied significantly by genotype, that is, between F1 hybrid triploids produced from parents of different diploid accessions (**Table 4.2, Figure 4.3**). Paternal excess triploids produced higher proportion of aborted F2 seed (max.A= 44%, Pro-0) (**Table 4.2**), compared to the triploids generated from the 4x X 2x cross, whose highest post-zygotic lethality was ~10% lower (max.A=34%, EDEN-2, Mz-0, CS22491) (**Table 4.2**). The lowest frequency of post-zygotic seed abortion was similar in hybrid triploids produced in both cross directions (min.A_2x4=7%, RMX-A180 and min.A_4x2=9%, Tamm-27), and in isogenic triploids generated in Col-0 background in the 2x X 4x cross direction (7%) (**Table 4.1, Table 4.2**).


**Figure 4.2.** F1 hybrid triploids of *Arabidopsis thaliana* have many fertility defects compared to their diploid parents. Some of the seeds abort in the triploid silique (A) – brown, shrunken seeds indicated by arrows.

Similarly to F1 isogenic Col-0 triploids, it was found that the rate of seed abortion frequently varied between genetically identical F1 hybrid triploids generated in the two reciprocal cross directions. Such epigenetic parent-of-origin effects on the rate of post-zygotic lethality were statistically significant ($p < 0.05$) in 29 pairs of reciprocal F1 hybrid triploids, whilst the remaining 48 had no significant difference (**Figure 4.3C, Table 4.5**). However, the parent-of-origin difference observed was not always in the same direction: i.e. 18 F1 hybrid triploids produced more aborted seeds as maternal excess 4x X 2x plants (e.g. CS22491), whilst 11 F1 hybrid triploids displayed the opposite trend and produced more aborted seeds in the paternal excess 2x X 4x triploid (e.g. Pro-0) (**Figure 4.3C, Table 4.5**). CS22491 represented one of the most extreme cases of parent-of-origin effects (p-value < 0.001), producing over 34% of aborted F2 seeds formed by 4x X 2x F1 hybrid triploids whilst only 16% of those formed by 2x X 4x F1 triploids (**Table 4.5)**. Pro-0 is an example of a reciprocal extreme effect (p-value < 0.001), where 44% of F2 seeds were aborted in 2x X 4x F1 triploid siliques, while only 24% of F2 seeds were aborted in 4x X 2x F1 hybrid triploids. To author's knowledge, these represent the first evidence of epigenetic parent-of-origin effects controlling seed lethality of selfed F1 hybrid triploid plants.

**Figure 4.3** Post-zygotic lethality in *Arabidopsis thaliana* F1 hybrid triploids is determined by cross direction as well as parental genotype. Box-plots of %A are shown for **A)** 78 paternal excess (2x X 4x) and **B)** 88 maternal excess (4x X 2x) F1 hybrid triploids, each sorted in descending order of seed abortion. **C)** Variation in seed abortion between the 77 pairs of hybrid triploids for which reciprocal crosses were analyzed, showing differences in %A per silique. Accessions showing increased %A in the 2x X 4x cross direction are coloured in black and those showing increased seed abortion in the 4x X 2x cross direction are represented by white bars. Accessions in the centre of the figures are those showing little or no difference in %A between cross directions, and include the isogenic L*er*-0 triploid (highlighted in red colour). Those with statistically significant ($p < 0.05$) differences between cross directions (non-parametric Mann-Whitney Test) are shaded (next page).


A
%A per silique
Diploid maternal parent
B
%A per silique
Diploid paternal parent
C
%A 4x2 - %A 2x4
Diploid parent

| Accession | %A_2x4 | | SD | Accession | %A_4x2 | | SD |
|---|---|---|---|---|---|---|---|
| **PRO-0** | 43.9 | ± | 17.6 | EDEN-2 | 34.5 | ± | 13.1 |
| **Col-0** | 42.1 | ± | 29.9 | MZ-0 | 34.5 | ± | 14.3 |
| **WEI-0** | 39.4 | ± | 13.7 | CS22491 | 34.3 | ± | 11.2 |
| **UOD-7** | 32.9 | ± | 16.9 | RMX-A02 | 32.6 | ± | 8.4 |
| **ZDR-1** | 32.7 | ± | 23.4 | KZ-9 | 30.2 | ± | 14.5 |
| **AN-1** | 32.6 | ± | 18.1 | NFA-8 | 30.1 | ± | 9.4 |
| **MZ-0** | 31.9 | ± | 8.3 | LER-1 | 28.6 | ± | 7.1 |
| **LER-1** | 30.4 | ± | 8.4 | RRS-10 | 28 | ± | 11.6 |
| **KONDARA** | 29.2 | ± | 13.1 | ZDR-6 | 27.6 | ± | 14.9 |
| **PNA-10** | 28.3 | ± | 11.1 | KNOX-18 | 27.4 | ± | 17.9 |
| **ZDR-6** | 28.1 | ± | 11.9 | Ra-0 | 27.1 | ± | 13.2 |
| **Got-22** | 28.1 | ± | 15.4 | Spr1-2 | 27 | ± | 7.5 |
| **Knox-10** | 25.8 | ± | 1.7 | Got-7 | 25.8 | ± | 11.6 |
| **FEI-0** | 25.6 | ± | 18.5 | NOK-3 | 25.8 | ± | 9.1 |
| **Ler-0** | 25.3 | ± | 5.3 | Pna-17 | 25.5 | ± | 11 |
| **CIBC-5** | 25.2 | ± | 5.4 | Knox-10 | 25.2 | ± | 5.3 |
| **KZ-1** | 25.1 | ± | 11.7 | Got-22 | 25.2 | ± | 8.1 |
| **RRS-10** | 24.8 | ± | 13.3 | GU-0 | 25.2 | ± | 9.5 |
| **EDEN-2** | 23 | ± | 10.5 | Yo-0 | 25.2 | ± | 8.4 |
| **MS-0** | 23 | ± | 11.2 | Ag-0 | 24.9 | ± | 7.8 |
| **HR-10** | 22.7 | ± | 17.8 | REN-11 | 24.7 | ± | 10.3 |
| **OMO2-3** | 22.4 | ± | 8.6 | PRO-0 | 24.4 | ± | 5 |
| **Kas-1** | 22.1 | ± | 9.4 | PNA-10 | 24.1 | ± | 7.1 |
| **WT-5** | 22 | ± | 7.8 | SQ-8 | 23.8 | ± | 13.4 |
| **MR-0** | 22 | ± | 10.4 | BUR-0 | 23.7 | ± | 7.4 |
| **KIN-0** | 21.9 | ± | 7.9 | Ei-2 | 23.5 | ± | 10 |
| **Ei-2** | 21.5 | ± | 6.9 | NFA-10 | 23.4 | ± | 6 |
| **HR-5** | 20.9 | ± | 7 | TS-5 | 23.4 | ± | 6.8 |
| **Gy-0** | 20.3 | ± | 9 | Ull2-3 | 23.2 | ± | 12 |
| **OY-0** | 20.1 | ± | 6 | CIBC-17 | 23 | ± | 6.9 |
| **Van-0** | 19.7 | ± | 5 | Ler-0 | 22.8 | ± | 6 |
| **LZ-0** | 19.5 | ± | 8.9 | AN-1 | 22.8 | ± | 12.3 |
| **RRS-7** | 19.2 | ± | 7.2 | WT-5 | 22.8 | ± | 9.4 |
| **Bay-0** | 19.2 | ± | 6.1 | Est-1 | 22.4 | ± | 9.1 |
| **SQ-1** | 19.1 | ± | 5.5 | OMO2-3 | 21.9 | ± | 7.3 |
| **BOR-4** | 19 | ± | 9.1 | Gy-0 | 21.7 | ± | 6.6 |
| **WS-2** | 18.8 | ± | 7.8 | LP2-6 | 21.6 | ± | 7.4 |
| **Est-1** | 18.7 | ± | 5.9 | WEI-0 | 21.6 | ± | 8.6 |
| **KNOX-18** | 18.6 | ± | 7.7 | RMX-A180 | 21.4 | ± | 8.4 |
| **NFA-10** | 18.5 | ± | 9 | Col-0 | 21.3 | ± | 12.6 |
| **NOK-3** | 18.5 | ± | 16.4 | ND-1 | 21.2 | ± | 9.7 |
| **LL-0** | 18.5 | ± | 7 | PU2-23 | 21.1 | ± | 13 |
| **SQ-8** | 18.3 | ± | 6.9 | Kas-1 | 21.1 | ± | 6.8 |
| **Pna-17** | 18 | ± | 8 | REN-1 | 20.7 | ± | 9.5 |
| **CT-1** | 17.9 | ± | 7.6 | SQ-1 | 20.6 | ± | 12.3 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **KZ-9** | 17.7 | ± | 4.5 | TAMM-2 | 20.4 | ± | 9.4 |
| **PU2-23** | 17.5 | ± | 4.5 | MrK-0 | 20.4 | ± | 5.4 |
| **C24** | 17.3 | ± | 8.4 | MT-0 | 20.1 | ± | 9.5 |
| **ND-1** | 17.1 | ± | 11 | TS-1 | 20 | ± | 9.7 |
| **LP2-2** | 17 | ± | 7.1 | CIBC-5 | 19.9 | ± | 6.7 |
| **Pu-2-7** | 16.9 | ± | 5.6 | Van-0 | 19.9 | ± | 7 |
| **CVI-0** | 16.9 | ± | 7.1 | Edi-0 | 19.9 | ± | 4.5 |
| **MT-0** | 16.9 | ± | 6.8 | LP2-2 | 19.7 | ± | 8.3 |
| **RMX-A02** | 16.6 | ± | 6.8 | OY-0 | 19.6 | ± | 10.5 |
| **TSU-1** | 16.3 | ± | 6 | BOR-4 | 19.1 | ± | 5 |
| **Bil-5** | 16 | ± | 8.6 | EDEN-1 | 18.9 | ± | 5.6 |
| **TS-5** | 15.9 | ± | 5.1 | WS-2 | 18.9 | ± | 7.5 |
| **TAMM-2** | 15.8 | ± | 7.5 | Omo2-1 | 18.6 | ± | 6.8 |
| **CS22491** | 15.8 | ± | 4.7 | CT-1 | 18.5 | ± | 5.1 |
| **Ra-0** | 15.6 | ± | 5.4 | Pu-2-7 | 18 | ± | 7.9 |
| **LP2-6** | 15.4 | ± | 4.4 | UOD-7 | 17.8 | ± | 11.1 |
| **GU-0** | 15.4 | ± | 5.4 | SE-0 | 17.1 | ± | 5.1 |
| **MrK-0** | 15.4 | ± | 6.4 | HR-5 | 16.9 | ± | 8.1 |
| **Br-0** | 15.2 | ± | 7.2 | LZ-0 | 16.9 | ± | 8.4 |
| **REN-11** | 15 | ± | 6.3 | MS-0 | 16.8 | ± | 6.6 |
| **GA-0** | 14.8 | ± | 5.3 | KZ-1 | 16.6 | ± | 6.7 |
| **Bor-1** | 14.7 | ± | 7 | Bay-0 | 16.4 | ± | 8.4 |
| **BUR-0** | 14.3 | ± | 4.6 | Ull2-5 | 16.2 | ± | 4.9 |
| **VAR2-1** | 14.1 | ± | 7.7 | KONDARA | 16 | ± | 8.4 |
| **SORBO** | 13.9 | ± | 7 | C24 | 15.9 | ± | 5.4 |
| **SE-0** | 13.7 | ± | 6.8 | MR-0 | 15.6 | ± | 9.5 |
| **NFA-8** | 13.2 | ± | 6.5 | RRS-7 | 15.4 | ± | 6 |
| **Tamm-27** | 13.1 | ± | 6.9 | ZDR-1 | 15.4 | ± | 9.1 |
| **CIBC-17** | 12.4 | ± | 6.5 | SHAHDARA | 15.2 | ± | 6.8 |
| **REN-1** | 12.3 | ± | 4.4 | Br-0 | 15.1 | ± | 7.5 |
| **TS-1** | 11.1 | ± | 4.1 | LL-0 | 15.1 | ± | 5.2 |
| **SHAHDARA** | 9.7 | ± | 2.5 | Ws-0 | 14.8 | ± | 8.3 |
| **Uod-1** | 8.8 | ± | 3.4 | VAR2-1 | 14.5 | ± | 7.2 |
| **RMX-A180** | 7.4 | ± | 4.4 | Bor-1 | 14.5 | ± | 8.1 |
| **EDEN-1** | N.D. | | | KIN-0 | 13.7 | ± | 6.6 |
| **Lov-5** | N.D. | | | SORBO | 13.4 | ± | 4.7 |
| **Spr1-2** | N.D. | | | CVI-0 | 12.9 | ± | 6.3 |
| **Omo2-1** | N.D. | | | FEI-0 | 12.3 | ± | 4.4 |
| **Ull2-5** | N.D. | | | Lov-5 | 11.7 | ± | 4.5 |
| **Ull2-3** | N.D. | | | GA-0 | 11.7 | ± | 4.9 |
| **Got-7** | N.D. | | | HR-10 | 11.6 | ± | 8 |
| **Ws-0** | N.D. | | | TSU-1 | 11.6 | ± | 5 |
| **Yo-0** | N.D. | | | Uod-1 | 10.2 | ± | 3.7 |
| **Ag-0** | N.D. | | | Tamm-27 | 9.5 | ± | 4.3 |
| **Edi-0** | N.D. | | | Bil-5 | N.D. | | |

**Table 4.2.** Siliques produced by *Arabidopsis thaliana* F1 hybrid triploids contain variable percentages of aborted seeds (A). Mean %A were calculated for each F1 hybrid triploid genotype from each reciprocal cross direction and sorted in descending order. Data from isogenic L*er*-0 triploids is included and highlighted in grey. Standard deviation (SD) from the mean is included for all values.

The variable viability of F2 seeds showed strong correlation with genetic variation between the parental accessions. There was a significant heritable variation in %A, and the total number of ovules (potential seed set in F1 hybrid triploid silique, ∑), with broad-sense heritabilities ranging from 0.27 - 0.44, $p < 0.0001$ **(Table 4.3**). Importantly, considerable support for cross direction ($p < 0.1$) and accession x cross direction interaction effects ($p < 0.001$) have been detected (**Table 4.4, Figure 4.4**). Also the pattern of phenotypic and genetic correlation of triploid reproductive defects within and among parental cross directions was investigated. For phenotypic correlations, a similar pattern under both cross directions consisting of negative correlation was found (**Table 4.4, Figure 4.4**). For genetic correlations, again a similar pattern under both cross direction consisting of large negative genetic correlations was observed (**Table 4.4**). Finally, despite similar patterns of genetic correlation within a cross direction, generally low genetic correlations across directions was found (**Table 4.4**). This result is not surprising given the fact that significant accessions x cross direction interaction effects on the %A and ∑ has consistently been observed (Tom Juenger – personal communication).

| Trait | 2 x 4 | 4 x 2 |
|---|---|---|
| **% A** | 0.3 | 0.27 |
| ∑ | 0.35 | 0.44 |

**Table 4.3.** Broad-sense heritabilities of proportions of Aborted seeds (%A), and the total number of ovules per silique (∑) demonstrate a high level of heritability for seed abortion.

| **Phenotypic Correlations** | | | |
|---|---|---|---|
| | 2x4 | ∑ | %A |
| | ∑ | 1 | -0.43 |
| | 4x2 | ∑ | %A |
| | ∑ | 1 | -0.41 |
| **Genetic Correlations** | | | |
| | 2x4 | ∑ | %A |
| | ∑ | 1 | -0.6 |
| | 4x2 | ∑ | %A |
| | ∑ | 1 | -0.61 |
| | **Across Direction** | | |
| | | ∑_2x4 | %A_2x4 |
| | ∑ _4 x2 | 0.21 | -0.13 |
| | %A_4x2 | -0.37 | 0.2 |

**Table 4.4.** Phenotypic and genetic correlations between %A and the total number of ovules (∑) and the effect of parental origin in inter-accession hybrid *Arabidopsis thaliana*.

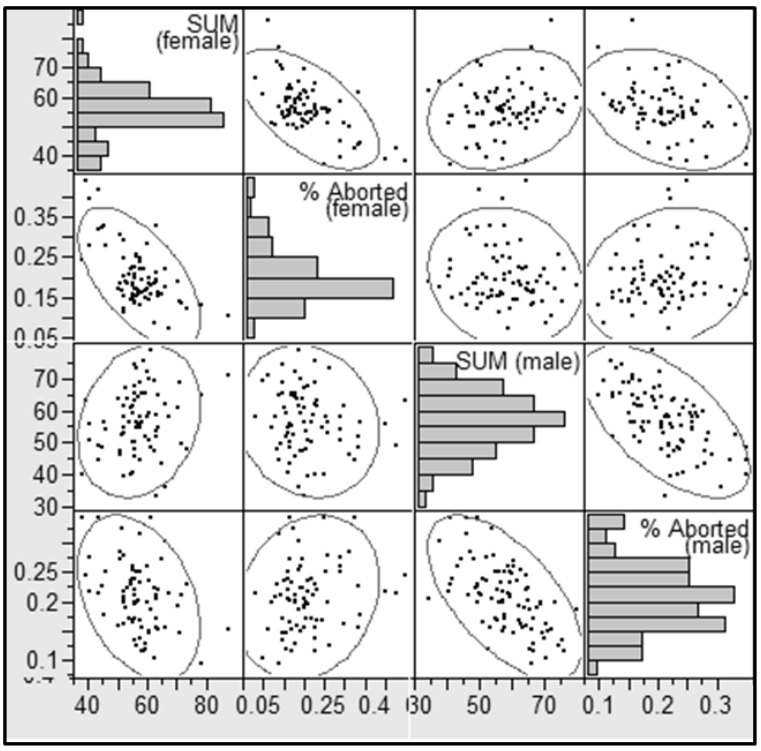



**Figure 4.4.** Diagonal matrix presenting the phenotypic and genetic correlations among the traits, split by cross direction. % Aborted (female) refers to proportion of aborted F2 seeds from selfed 2x X 4x paternal excess triploids, (male) – 4x X 2x maternal excess triploids. Sum (female) refers to sum of ovules (fertilized or not) produced by 2x X 4x paternal excess triploids, (male) - 4x X 2x maternal excess triploids.

| Accession | %A_4x2 | p-value | %A_2x4 | 4x2 - 2x4 |
|---|---|---|---|---|
| **CIBC-17** | **23*** | < 0.001 | 12.4 | 10.6 |
| **CS22491** | **34.3*** | < 0.001 | 15.8 | 18.5 |
| **NFA-8** | **30.1*** | < 0.001 | 13.2 | 16.9 |
| **PRO-0** | 24.4 | < 0.001 | **43.9*** | -19.5 |
| **RMX-A02** | **32.6*** | < 0.001 | 16.6 | 16 |
| **RMX-A180** | **21.4*** | < 0.001 | 7.4 | 14 |
| **BUR-0** | **23.7*** | 0.001 | 14.3 | 9.4 |
| **UOD-7** | 17.8 | 0.001 | **32.9*** | -15.1 |
| **WEI-0** | 21.6 | 0.001 | **39.4*** | -17.8 |
| **REN-11** | **24.7*** | 0.002 | 15 | 9.7 |
| **GU-0** | **25.2*** | 0.003 | 15.4 | 9.8 |
| **Ra-0** | **27.1*** | 0.003 | 15.6 | 11.5 |
| **FEI-0** | 12.3 | 0.004 | **25.6*** | -13.3 |
| **KONDARA** | 16 | 0.004 | **29.2*** | -13.2 |
| **EDEN-2** | **34.5*** | 0.005 | 23 | 11.5 |
| **TS-1** | **20*** | 0.005 | 11.1 | 8.9 |
| **KIN-0** | 13.7 | 0.006 | **21.9*** | -8.2 |
| **REN-1** | **20.7*** | 0.006 | 12.3 | 8.4 |
| **NOK-3** | **25.8*** | 0.008 | 18.5 | 7.3 |
| **TS-5** | **23.4*** | 0.008 | 15.9 | 7.5 |
| **KZ-1** | 16.6 | 0.012 | **25.1*** | -8.5 |
| **KZ-9** | **30.2*** | 0.013 | 17.7 | 12.5 |
| **LP2-6** | **21.6*** | 0.024 | 15.4 | 6.2 |
| **MrK-0** | **20.4*** | 0.024 | 15.4 | 5 |
| **ZDR-1** | 15.4 | 0.024 | **32.7*** | -17.3 |
| **HR-10** | 11.6 | 0.028 | **22.7*** | -11.1 |
| **TSU-1** | 11.6 | 0.031 | **16.3*** | -4.7 |
| **CIBC-5** | 19.9 | 0.033 | **25.2*** | -5.3 |
| **NFA-10** | **23.4*** | 0.034 | 18.5 | 4.9 |
| **Col-0** | 21.3 | 0.051 | 42.1 | -20.8 |
| **SE-0** | 17.1 | 0.068 | 13.7 | 3.4 |
| **CVI-0** | 12.9 | 0.085 | 16.9 | -4 |
| **Pna-17** | 25.5 | 0.089 | 18 | 7.5 |
| **Bay-0** | 16.4 | 0.093 | 19.2 | -2.8 |
| **MR-0** | 15.6 | 0.101 | 22 | -6.4 |
| **GA-0** | 11.7 | 0.12 | 14.8 | -3.1 |
| **ND-1** | 21.2 | 0.12 | 17.1 | 4.1 |
| **Tamm-27** | 9.5 | 0.12 | 13.1 | -3.6 |

| | | | | |
|---|---|---|---|---|
| **AN-1** | 22.8 | 0.13 | 32.6 | -9.8 |
| **TAMM-2** | 20.4 | 0.141 | 15.8 | 4.6 |
| **PNA-10** | 24.1 | 0.146 | 28.3 | -4.2 |
| **HR-5** | 16.9 | 0.164 | 20.9 | -4 |
| **LL-0** | 15.1 | 0.165 | 18.5 | -3.4 |
| **RRS-7** | 15.4 | 0.171 | 19.2 | -3.8 |
| **SHAHDARA** | 15.2 | 0.186 | 9.7 | 5.5 |
| **MS-0** | 16.8 | 0.202 | 23 | -6.2 |
| **KNOX-18** | 27.4 | 0.206 | 18.6 | 8.8 |
| **L*er*-0** | 22.8 | 0.237 | 25.3 | -2.5 |
| **Est-1** | 22.4 | 0.257 | 18.7 | 3.7 |
| **LP2-2** | 19.7 | 0.29 | 17 | 2.7 |
| **Uod-1** | 10.2 | 0.309 | 8.8 | 1.4 |
| **PU2-23** | 21.1 | 0.341 | 17.5 | 3.6 |
| **SQ-8** | 23.8 | 0.342 | 18.3 | 5.5 |
| **LER-1** | 28.6 | 0.481 | 30.4 | -1.8 |
| **MT-0** | 20.1 | 0.494 | 16.9 | 3.2 |
| **LZ-0** | 16.9 | 0.548 | 19.5 | -2.6 |
| **OY-0** | 19.6 | 0.548 | 20.1 | -0.5 |
| **RRS-10** | 28 | 0.633 | 24.8 | 3.2 |
| **C24** | 15.9 | 0.655 | 17.3 | -1.4 |
| **Ei-2** | 23.5 | 0.657 | 21.5 | 2 |
| **Got-22** | 25.2 | 0.694 | 28.1 | -2.9 |
| **Van-0** | 19.9 | 0.771 | 19.7 | 0.2 |
| **MZ-0** | 34.5 | 0.787 | 31.9 | 2.6 |
| **SQ-1** | 20.6 | 0.787 | 19.1 | 1.5 |
| **VAR2-1** | 14.5 | 0.797 | 14.1 | 0.4 |
| **OMO2-3** | 21.9 | 0.821 | 22.4 | -0.5 |
| **Bor-1** | 14.5 | 0.836 | 14.7 | -0.2 |
| **Gy-0** | 21.7 | 0.868 | 20.3 | 1.4 |
| **WT-5** | 22.8 | 0.868 | 22 | 0.8 |
| **ZDR-6** | 27.6 | 0.868 | 28.1 | -0.5 |
| **Knox-10** | 25.2 | 0.896 | 25.8 | -0.6 |
| **Pu-2-7** | 18 | 0.901 | 16.9 | 1.1 |
| **Br-0** | 15.1 | 0.912 | 15.2 | -0.1 |
| **BOR-4** | 19.1 | 0.917 | 19 | 0.1 |
| **Kas-1** | 21.1 | 0.965 | 22.1 | -1 |
| **WS-2** | 18.9 | 0.973 | 18.8 | 0.1 |
| **CT-1** | 18.5 | 0.983 | 17.9 | 0.6 |
| **SORBO** | 13.4 | 0.983 | 13.9 | -0.5 |

**Table 4.5.** Pairs of reciprocal triploids ranked in descending order by the extent of parental impact on triploid fertility as determined by differences in %A in each cross direction. Statistically significant differences (Mann-Whitney U Test) are listed, with those of $p < 0.05$ considered as significant. Triploids with significantly higher %A in given cross direction are highlighted in bold and with an asterisks. A pair of L*er*-0 isogenic triploids is listed among those triploid hybrids with no significant difference in %A.

### 4.3.3 Differences in post-zygotic lethality (%A) in selfed F1 hybrid triploids are not due to hybrid dysgenesis effects in diploids

To confirm that observed post-zygotic lethality in F1 hybrid triploid siliques is specific to genotypic effects seen in the polyploid state, %A was also determined in diploid F1 hybrids. These were derived from reciprocal crosses between diploid L*er*-0 and five accessions: Ga-0 and Fei-0, representing the extremes of the fertility defect (%A F2 seeds) observed in 4x X 2x F1 hybrid triploids; and Ren-1, Wei-0, and Pro-0, representing the most extreme fertility defects (%A F2 seeds) amongst the 2x X 4x F1 hybrid triploids (**Figure 4.3**). The rate of fertility defects was almost entirely ablated when the hybridization occurred without polyploidization. In fact, in all five F1 hybrid diploids only at most 2% of F2 seeds were aborted (**Figure 4.5, Table 4.6**). This indicates that the genotypic and parent-of-origin effects on seed lethality (%A F2 seeds) seen in the F1 hybrid triploids are due to a genome dosage effect rather than being a consequence of hybrid dysgenesis.

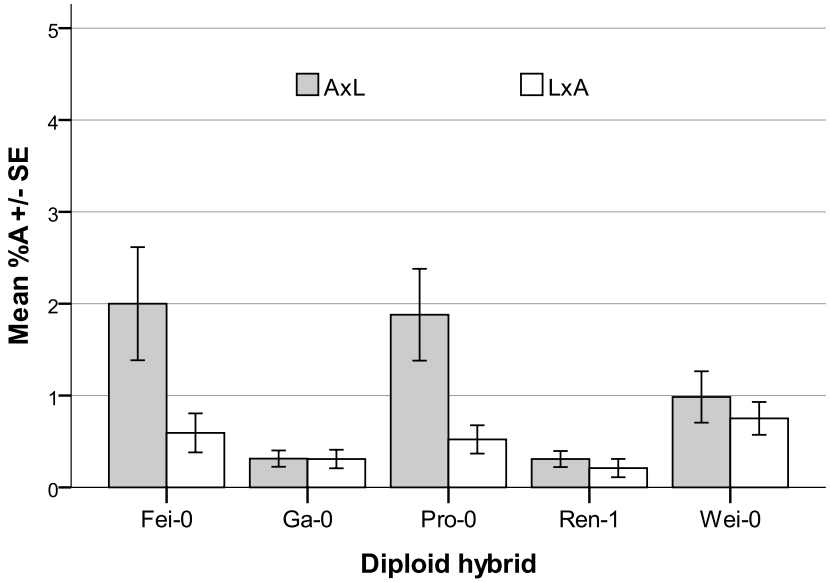



**Figure 4.5** Seed abortion in *Arabidopsis thaliana* F1 hybrid diploids is determined by cross direction as well as parental genotype. Bars represent mean %A produced by selfed F1 hybrid diploids, generated by crosses between diploid (L) L*er*-0 and five different diploid (A) accessions (Fei-0, Ga-0, Pro-0, Ren-1, Wei-0). Error bars represent standard error.

| Accession | %N_AxL | | SD | %N_LxA | | SD | Difference AxL -LxA | p-value |
|---|---|---|---|---|---|---|---|---|
| **Wei-0** | 1 | ± | 1.88 | 0.8 | ± | 1.2 | 0.2 | 0.776 |
| **Ga-0** | 0.3 | ± | 0.6 | 0.3 | ± | 0.68 | 0 | 0.894 |
| **Pro-0** | 1.9 | ± | 0.59 | 0.5 | ± | 0.54 | 1.4 | **0.016*** |
| **Fei-0** | 2 | ± | 3.35 | 0.6 | ± | 1.03 | 1.4 | 0.178 |
| **Ren-1** | 0.3 | ± | 3.37 | 0.2 | ± | 1.16 | 0.1 | 0.499 |

**Table 4.6.** Mean percentage of aborted seeds (%A) per silique in five pairs of reciprocal F1 hybrid diploids. Differences between the mean %A in two cross directions was tested by the Mann-Whitney U test and the significance level was at 0.05.

## 4.4 Discussion

The strength of post-zygotic lethality can vary within species and can lead to reproductive isolation and speciation (Burton and Husband, 2000, Yamauchi et al., 2004). *Arabidopsis thaliana* accession Col-0 experiences particularly severe seed lethality when diploid females are mated to tetraploid males, while L*er*-0 is more permissive (Dilkes et al., 2008). The maternal effect transcription factor *TTG2* has been found to affect the rate of post-zygotic lethality caused by 2x X 4x inter-ploidy crosses involving Col-0 accession (Dilkes et al., 2008). Also, misregulation of imprinted genes such as *MEDEA* has been proposed to cause developmental defects in seeds with unbalanced parental genome contributions (Erilova et al., 2009).

The general little post-zygotic lethality in *Arabidopsis thaliana* inter-ploidy crosses allows surviving F1 triploids to reproduce, and therefore act as triploid bridges giving rise to stable polyploid offspring (Ramsey and Schemske, 1998, Husband, 2004, Henry et al., 2005, Burton and Husband, 2001). Reproductive patterns in isogenic Col-0 and hybrid Col-0/Wa-1 F1 triploids have been investigated by (Henry et al., 2005). These two classes of triploids were found to be fertile and produce significantly more normal seeds as hybrids (75%) than as isogenics (65%), however their reproductive success was impaired compared to their diploid and tetraploid parents (~ 100% normal seeds) (Henry et al., 2005). These data suggest that isogenic Col-0 triploids experience more severe reproductive barriers due to post-zygotic seed lethality than triploid hybrids. This also shows that polyploidy can affect post-zygotic lethality even in the absence of allelic diversity (hybridization) and thus form isolated populations (Soltis et al., 2007).

In this chapter, four isogenic reciprocal triploids (Col-0, C24, L*er*-0, and Zu) have been allowed for self-fertilization in order to investigate the rates of post-zygotic lethality in the F2 seed generation. It has been found that the rates of seed abortion varied depending on the accession and the %A (proportion of aborted F2 seeds in a silique) was ranging between 7 and 25% (**Table 4.1**). Such accession-dependent variation in the frequency of seed abortion has been also found in the former F1 generation, following 2x X 4x inter-ploidy crosses (Dilkes et al., 2008, Scott et al., 1998).

Col-0 triploids originating from a 2x X 4x inter-ploidy cross, known to have the highest frequency of F1 seed lethality (~80%) (Dilkes et al., 2008), have surprisingly been found to produce the lowest frequency of aborted F2 seeds (**Table 4.1, Figure 4.1**). This could be explained by preferential production of diploid female gametes and haploid male gametes (like in the 4x X 2x inter-ploidy cross). However, a significantly increased proportion of F2 seed abortion in the reciprocal Col-0 triploids would act against this hypothesis. In the remaining three genetic backgrounds (C24, L*er*-0, Zu), there was no significant differences in F2 seed failure between reciprocal triploids (**Table 4.1**). The highest degree of post-zygotic seed abortion was observed in L*er*-0 triploids, regardless of the cross direction. Similar frequency of post-zygotic lethality was also observed in F1 2x X 4x L*er*-0 inter-ploidy crosses (Dilkes et al., 2008).

This demonstrates that in *Arabidopsis thaliana,* genetic background affects isogenic triploid reproduction via mechanisms which may vary by cross direction. This also shows that lethality arises due to the parental genome dosage, in the absence of allelic diversity (hybridization).

In this chapter, post-zygotic lethality has been also examined in hybrid triploids incorporating many different genotypes and a strong genetic and heritable link to reproductive defects has been found (**Figure 4.3, Table 4.2, Table 4.3**). Phenotypic negative correlation found between the proportions of %A and sum of all ovules (Σ) suggests that the smaller numbers of ovules produced in the silique, the more seeds are likely to abort post-fertilization. This shows that there is a crosstalk between triploid reproductive pathways controlling ovule development and post-zygotic lethality, such as e.g. maternal effects (**Table 4.4**).

Paternal excess triploids produced higher proportions of aborted F2 seeds (max.A= 44%, Pro-0) (**Table 4.2**), consistently with the higher F1 seed lethality in the 2x X 4x inter-ploidy crosses in many other species (Ramsey and Schemske, 1998). To compare, the highest post-zygotic lethality in the triploids generated from the 4x X 2x cross was about 10% lower (max.A=34%, EDEN-2, Mz-0, CS22491) (**Table 4.2**). The lowest frequency of post-zygotic seed abortion was similar in hybrid triploids produced in both cross directions (min.A_2x4 = 7%, RMX-A180 and min.A_4x2 = 9%, Tamm-27), and in isogenic triploids

generated in Col-0 background in the 2x X 4x cross direction (7%) (**Table 4.1, Table 4.2**). Therefore, the range of F2 seed abortion was found to be greater than indicated by analysis of isogenic F1 triploid plants, as the rates of post-zygotic lethality were either higher or similar to the isogenic F1 triploids (**Table 4.1, Table 4.2**).

Reduction in viability of F2 seed in *Arabidopsis thaliana* triploids can be associated with production of aneuploid gametes spanning a wide range of genome contents (Henry et al., 2005, Ramsey and Schemske, 1998). Fertilization of aneuploid gametes is likely to lead to more severe post-zygotic lethality than inter-ploidy crosses because of a requirement for the balanced dosage of many factors (Birchler et al., 2001). For example, imprinted factors required for endosperm proper development may limit viability of aneuploid endosperm (Vinkenoog et al., 2003). The extent of reproductive defects varies between different triploids in ways which are under genetic control, but remain poorly understood (Henry et al., 2007). The *SDI* locus has been proposed to control aneuploid survival by buffering the effects of dosage imbalance introduced by triploid reproduction (Henry et al., 2007).

It is striking that *Arabidopsis thaliana* triploids, generated by reciprocal crosses, both isogenic and hybrid, frequently differed in post-zygotic lethality (**Table 4.1, Table 4.5**). These parent-of-origin effects caused greater seed abortion in the F1 triploids from the 4x X 2x cross direction or, the 2x X 4x cross direction, and as such define a likely epigenetic effect. Also one pair of reciprocal hybrid diploids (Pro-0) showed a significant difference in the frequency of seed abortion, with Pro-0 X L*er*-0 having more aborted seeds (2%) than L*er*-0 X Pro-0 (0.5%). However, the highest frequency of post-zygotic lethality found in hybrid diploids was only 2% (**Table 4.6, Figure 4.5**). This indicates that the genotypic and parent-of-origin effects on seed lethality seen in the F1 triploids are not a consequence of hybrid dysgenesis.

Genetic basis for post-zygotic lethality in polyploids include dosage sensitivity, maternal effects (sporophytic, cytoplasmic, gametophytic), or/and genomic imprinting (Dilkes and Comai, 2004, Adams et al., 2000, Scott et al., 1998, Erilova et al., 2009). These parental affects often result from challenges encountered by triploids but not seen in other polyploids. It is likely that each of these effects may be involved in observed variation in F2 seed lethality.

## 4.5 Conclusions

In this chapter, a considerable heritable variation in post-zygotic F2 seed lethality has been identified in a panel of F1 triploids (isogenic and hybrid) generated from inter-ploidy reciprocal crosses of different accessions of *Arabidopsis thaliana.* This post-zygotic lethality in F1 triploid siliques is specific to genotypic effects in the polyploid state, and was confirmed not to be a consequence of dysgenesis between diploid accessions.

The F2 seed abortion often significantly differed between reciprocal triploids and as such defined novel parent-of-origin dependent genome dosage effects.

Phenotypic negative correlation found between the proportions of %A and the sum of all ovules (Σ) suggests that there is a crosstalk between triploid reproductive pathways controlling ovule development and post-zygotic lethality.

# CHAPTER 5 – Post-fertilization effects on seed development in *Arabidopsis thaliana* F1 triploids

## 5.1 Introduction

Triploid reproduction is complicated by the fact that three sets of chromosomes must be resolved into two poles during meiosis which often results in non-disjunction of chromatids and chromosomes (Ramsey and Schemske, 1998). This causes uneven chromosome numbers in gametes and subsequent zygotes. Some types of aneuploidy can lead to post-zygotic lethality due to the disrupted balance of dosage sensitive genes and their products in endosperm (Birchler, 1993) (**Chapter 4**). Surviving progeny often display dramatic phenotypical and developmental changes, such as extreme dwarfism or sterility, however in some cases they can be advantageous and even be selected for (**Chapter 3**) (Ramsey and Schemske, 2002, Hughes et al., 2000).

Sensitivity to aneuploidy can differ significantly between species but it can also differ between varieties of the same species, e.g. tomato and barley (Rick and Notani, 1961, Ramage, 1960). This suggests also that different *Arabidopsis thaliana* accessions can have different levels of tolerance to aneuploidy. A *SENSITIVE TO DOSAGE IMBALANCE* (*SDI*) locus affecting aneuploid survival in *Arabidopsis thaliana* triploids was mapped using recombinant inbred populations (RILs) from a Col-0/Wa-1 hybrid triploid (Henry et al., 2005). Ploidy-dependent transmission distortion at this locus suggested a role for *SDI* in buffering the dosage effects in aneuploids (Henry et al., 2007).

Aneuploids are very frequent in polyploid populations and support the role of triploid bridges in neopolyploid formation (Ramsey and Schemske, 1998, Ramsey and Schemske, 2002). Viable aneuploids act as vectors for gene flow between triploid and tetraploid populations as triploid progeny drift towards stable diploid or tetraploid forms, whilst inviable aneuploids may lead to polyploid speciation (Soltis et al., 2004).

Due to the recent advances in genotyping and sequencing technology, genome-wide association (GWA) studies have become a useful approach for studying the genetics of natural variation and traits of agricultural importance (Atwell et al., 2010). Linkage disequilibrium (LD), the basis for GWA studies, is the non-random association of alleles at different loci explaining genetic variation in natural populations. Associations, known as linkage disequilibria, typically exist between very closely linked loci which tend to be

inherited together (as haplotypes) more often than alleles at unlinked loci. LD mapping (GWA) allows identification of sequence variants that correlate with a given phenotype across a large number of populations of unrelated individuals, and thus can be applied to determine the genetic basis of phenotypic variation, such as triploid reproductive rates (Myles et al., 2009, Aranzana et al., 2005, Weigel and Nordborg, 2005).

### 5.1.1 Research Objectives

This chapter is focused on investigating the mechanisms of reproductive success in *Arabidopsis thaliana* reciprocal F1 hybrid triploids. Given the variation in tolerance to ploidy changes and hybridization which can exist between plant species, it was hypothesised that genetic differences between *Arabidopsis thaliana* accessions could alter their reproductive fitness when such genomic changes were induced.

The proportion of normal seed development (%N) was screened in the seed-set of self-pollinated reciprocal F1 hybrid triploids generated through inter-ploidy crosses between a tetraploid L*er*-0 and 89 different diploid accessions. Additionally, %N arising from reproduction of four different reciprocal F1 isogenic triploids and five different reciprocal F1 hybrid diploids was also investigated.

The diploid accessions of *Arabidopsis thaliana* which were used to generate reciprocal F1 hybrid triploids have been characterized for single nucleotide polymorphisms (SNPs) at over 1.4 million genomic positions (Magnus Nordborg lab, unpublished data). This allowed the use of a genome-wide association (GWA) approach to determine the genetic basis of phenotypic variation in F1 hybrid triploid reproduction (%N) through the identification of sequence variants (SNPs) that correlate with %N across different *Arabidopsis thaliana* accessions.

## 5.2 Methodology and Materials

### 5.2.1 Plant material

Diploid plants of 89 wild inbred accessions of the 96 Nordborg *Arabidopsis thaliana* mapping panel (Nordborg et al., 2005) were grown from seeds provided by NASC (CS22564-CS22659). Tetraploid seeds originated from: L*er*-0 Ueli Grossniklaus's lab (originally obtained from Cold Spring Harbor Lab), Col-0 and C24 (from Luca Comai's lab, University of Washington), Zurich (from Ortrun Mittelsten Scheid's lab, GMI Vienna); All tetraploids used were generated by colchicine doubling (Blakeslee, 1922). Seeds were surface sterilized by soaking in 5% v/v sodium hypochlorite; 0.05 % v/v Triton-X solution (5 min), and washed five times with sterile distilled water. Sterilized seeds were individually sown on plates of Murashige and Skoog medium containing 4.6 g $L^{-1}$ MS salts, 30 g $L^{-1}$ sucrose and 8 g $L^{-1}$ agarose (Murashige and Skoog, 1962). Seeded MS plants were incubated in a Percival Tissue Culture Cabinet under a 16:8 hr light: dark (21 °C/18 °C) regime until the fourth true leaf stage (Boyes et al., 2001a). Seedlings were transplanted to individual pots of soil (8 parts Westland multipurpose compost (Dungannon, N. Ireland): 1 part perlite: 1 part vermiculite) and transferred to growth chambers to grow under fluorescent lamps at 200 μmol $m^{-2}$ $s^{-1}$ at 21 °C/18 °C and a 16:8 hr light:dark photoperiod.

### 5.2.2 Reciprocal crosses

Plants were crossed reciprocally by manual emasculation and cross-pollination prior to anthesis under a Leica MZ6 dissecting microscope using Dumostar No. 5 tweezers. Mature siliques were harvested after 7 days. F1 plants were grown in randomized positions to minimize genotype-by-environment (G x E) interactions.

### 5.2.3 Flow cytometry

All F1 triploid plants used in this study were confirmed to be eutriploid by flow cytometry using a PAI PARTEC ploidy analyzer. Fresh leaf tissue (~300 mg) was macerated in a round petri dish with a razor blade for 1 min and incubated in a nuclei extraction buffer (Cystain UV Precise P, Partec) for 5 min and the suspensions were filtered through nylon filters of 30 µm mesh width. Nuclei were labelled with fluorescence dye (Cystain UV Precise P, Partec, according to manufacturer's instructions) and ploidy levels determined by direct comparison with a diploid standard.

### 5.2.4 Reproductive phenotypes

All ploidy-confirmed F1 (hybrid and isogenic) triploids and F1 hybrid diploids were allowed to self–pollinate and nearly-mature siliques were dissected with fine tweezers under a Leica MZ6 microscope. Five siliques were analyzed per F1 plant (for each of three individual F1 hybrid plants) and the silique contents scored for % normal seeds (%N), calculated as a proportion of normal seeds to all possible ovules in the silique (N/∑). Normal seeds (N) were identified as those with a green and plump appearance (Meinke, 1994).

### 5.2.5 Statistical analyses of heritability of triploid reproductive trait (N)

Data for %N and ∑ were analyzed using linear mixed models in PROC Mixed in SAS (Littel et al., 1996) with accession and cross-direction as fixed factors, and including their interactions. In this framework, the contribution of (a) parental genetic variation in triploid traits is indicated by a significant accession term, (b) parent-of-origin effects indicated by a significant direction term, and (c) genetic variation in parent-of-origin effects by significant accession X cross direction interaction. The significance of model terms was determined using standard Type 3 analysis and F-ratios (Littel et al., 1996). The contribution of parental genetic effects to triploid variability was also quantified under additional models considering accession as a random term in data split by cross-direction. Proportion of the triploid reproductive variation explained by the single-dose contribution of the natural accessions was calculated as the ratio of the among-accession variance component to the total phenotypic variability in triploid traits. This allowed the determination of the broad-sense heritability although this differs from the standard broad-sense heritability given the

unequal parental genomic contributions. Similarly, single dose genetic correlations among triploid reproductive characters (in both cross directions) were estimated by the standard Pearson product-moment correlation of triploid line means. The significance of each genetic correlation was determined using a t-test after a Z transformation of the correlation coefficient. Levels of significance for phenotypic and genetic correlations were not adjusted for multiple tests.

### 5.2.6 Single Seed Ploidy Analysis

DNA content of ~90 single seeds per triploid parent were measured in relation to diploid Col-0 control seeds, as described in (Matzk et al., 2001), with modifications specific to *Arabidopsis thaliana* species. Briefly, seeds were homogenized in a 96-deep well plate containing 80µl of nuclei extraction buffer (0.1 M citric acid monohydrate, 0.5% Tween 20, β-mercaptomethanol, pH 2.5) and three metal balls of 3 mm diameter, for 1min. 100µl of DNA staining buffer ($Na_2HPO_4$, 0.4M DAPI, pH 8.5) was added and the suspensions were filtered through a nylon filter of 30 µm mesh width. 80µl of the filtrate and additional 80µl of staining buffer was mixed in a new 96-well plate. Fluorescence intensity of DAPI stained nuclei from individual embryos was measured using a high throughput Ploidy Analyser PAII PARTEC equipped with a multiplex 96-well plate Robby-Well autoloader. Flomax software was used to analyse obtained genome content profiles (PARTEC GmbH).

### 5.2.7 Genome-wide association mapping

A GWA approach was used to find a correlation between the measured variation in triploid reproductive character (%N) and 1.4 million genome-wide SNP polymorphisms determined for the diploid accessions of *Arabidopsis thaliana*. A mixed-model implemented in the program EMMA was used to control for population structure (Atwell et al., 2010, Yu et al., 2005b). Regions of significance were defined as the distance between the nearest adjacent non-significant SNPs flanking each significant SNP ($p < 0.01$). Genes located 120 kb upstream and downstream of significant SNPs were considered as candidate loci for explaining the normal seed phenotype (%N) in F1 hybrid triploids after self-pollination. The genes were identified using TAIR (http://www.arabidopsis.org/).

## 5.3 Results

### 5.3.1 Proportion of normal seeds (%N) set from selfed F1 isogenic triploids of *Arabidopsis thaliana* varies by genotype and displays epigenetic parent-of-origin effects in Col-0

Isogenic F1 triploids of the *Arabidopsis thaliana* Col-0 accession are fertile, however they do not perform as well as their diploid and tetraploid parents in terms of producing normal F2 seeds (Henry et al., 2005). To determine whether the % normal F2 seeds obtained from isogenic F1 triploids of differing genetic background (accession) differs, four reciprocal isogenic F1 triploid lines were generated (Col-0, C24, L*er*-0, and Zu) and allowed to self-fertilize and set F2 seeds. Depending on genetic background and parental genome dosage, the proportions of normal seeds (%N) varied between 17% and 50%, with Col-0 and Zu having the highest levels (**Figure 5.1, Table 5.1**). The paternal excess Col-0 F1 triploids produced significantly more normal F2 seeds than Col-0 reciprocal triploids (%N_2x4 = 50, %N_4x2 = 37, p-value < 0.05) indicating a parent-of-origin effect. Col-0 was the most fertile triploid line (**Figure 5.1)**.Within the other three genetic backgrounds (L*er*-0, C24, Zu), no statistically significant difference in reproductive success was found between the reciprocal pairs of F1 triploids indicating no parent-of-origin effects (**Figure 5.1, Table 5.1**).

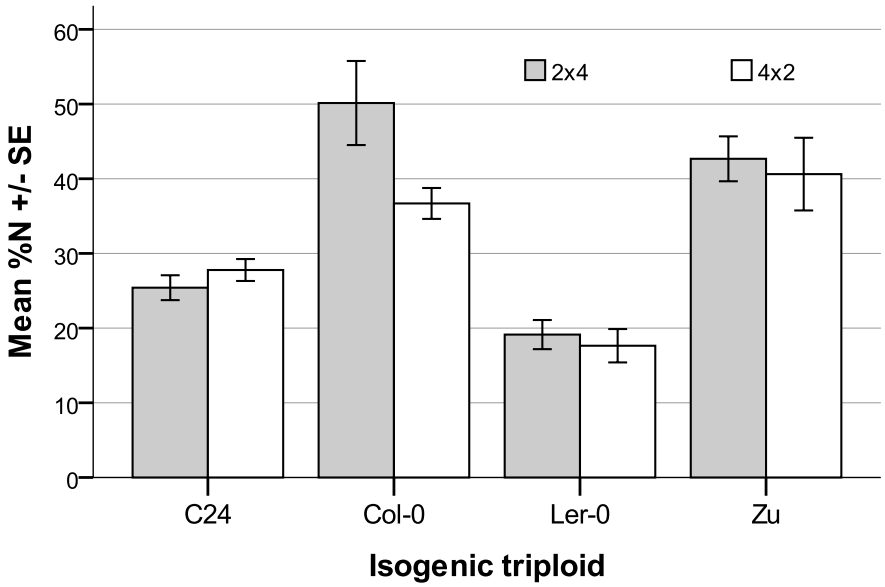



**Figure 5.1**. Reproductive success of *Arabidopsis thaliana* F1 isogenic triploids is determined by cross direction as well as parental genotype. Bars represent mean %N (normal seeds) in four reciprocal F1 isogenic triploid lines (C24, Col-0, L*er*-0 and Zu). Error bars represent standard error (SE). 2 X 4 = selfed paternal excess F1isogenic triploid; 4 X 2 = selfed maternal excess F1 isogenic triploid

| | %N_2 x 4 | %N_4 x 2 | %N_Δ | p-value |
|---|---|---|---|---|
| Col-0 | 50.1 ± 12.6 | 36.7 ± 8 | 13.4 | **0.04*** |
| C24 | 25.4 ± 6.4 | 27.8 ± 5.7 | -2.4 | 0.419 |
| Zu | 42.7 ± 9.5 | 40.6 ± 18.8 | 2.05 | 0.739 |
| L*er*-0 | 19.1 ± 8.6 | 17.6 ± 7.5 | 1.5 | 0.534 |

**Table 5.1.** Normal seeds (%N) in reciprocal F1 isogenic triploids of *Arabidopsis thaliana* illustrated by cross direction and parental genotype. Mean values of %N between pairs of reciprocal triploids were compared using non-parametric test (Mann-Whitney U) and significant differences are highlighted in bold and with an asterisks (p-value < 0.05).

### 5.3.2 Proportion of normal seeds (%N) set from selfed of F1 hybrid triploids depends on the genetic background and displays epigenetic parent-of-origin effects.

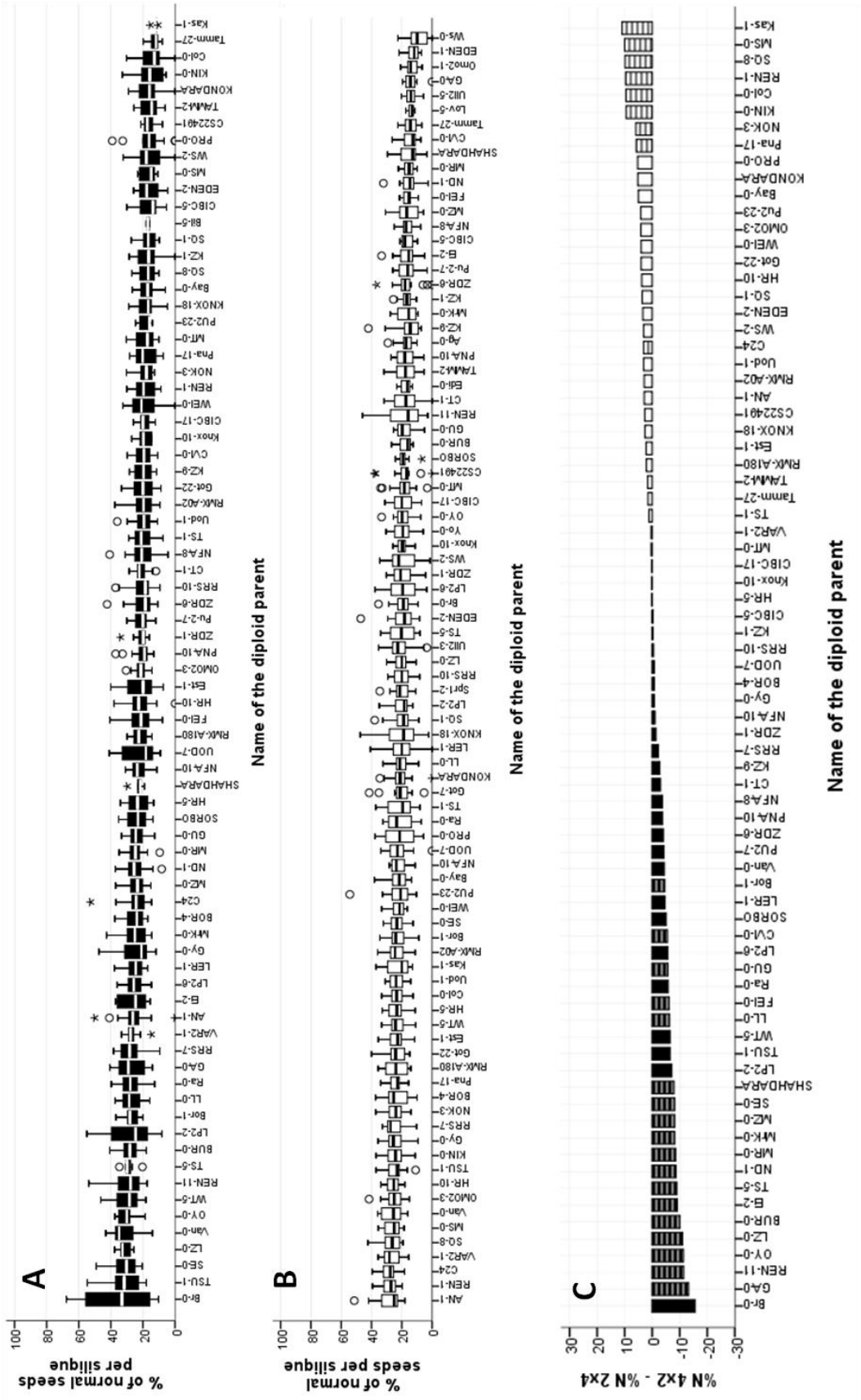
To investigate the effect of hybridity (allelic variation) on the reproductive success of F1 hybrid triploids, a tetraploid L*er*-0 line was reciprocally crossed with 89 different diploid accessions to generate up to 166 different F1 hybrid triploid genotypes. Similar to the analysis of F1 isogenic triploids, all F1 hybrid triploids produced reduced proportions of normal F2 seeds within the silique (**Figure 5.2**). The proportion of normal seeds (%N) varied significantly between F1 hybrid triploids produced from parents of different diploid accessions (**Table 5.2, Figure 5.3**). Depending on the genotype, the F1 hybrid triploids generated in both cross directions displayed rates of viable seeds that were either lower or similar to the isogenic F1 triploids (**Table 5.1**, **Table 5.2**). Hence, *Arabidopsis thaliana* triploids are able to self-fertilize to produce viable seeds in all genetic backgrounds, but do so with variable, reduced rates of success.

Generally, higher rates of normal seeds were produced by 2x X 4x paternal excess triploids, ranging from 12% in Kas-1 to 35% in Br-0. A slightly lower range of viable seeds was observed in 4x X 2x hybrid triploids – 10% in Ws-0 to 29% in An-1 (**Table 5.2**). The trend towards higher %N from F1 triploids from the 2x X 4x cross was consistent with data from the isogenic triploids – the %N range in 2x X 4x triploids was 19 – 50%, and was higher than in 4x X 2x, where it was 17 – 41% (**Table 5.1**).

Consistent with the general trend showing that 2x X 4x triploids produce more viable seeds (higher %N), 18 paternal excess F1 hybrid triploids were found to reproduce significantly better than reciprocal maternal excess F1 hybrid triploids, whilst 9 maternal excess F1 hybrid triploids reproduced significantly better than in paternal excess (**Table 5.3**). The remaining pairs of reciprocal triploids reproduced with the same rates of success regardless of the cross direction (**Figure 5.3C**). Hence, while the reciprocal F1 hybrid triploids generated by some accessions displayed epigenetic parent-of-origin effects, other reciprocal F1 hybrid triploid genotypes did not display any significant epigenetci parent-of-origin effect.


**Figure 5.2.** Siliques produced by selfed inter-accession *Arabidopsis thaliana* triploids show high variability in the seed set. (A) Some of the seeds develop normally. (B) Normal seeds, as set in the silique of the typical non-hybrid diploid accession are shown as a comparison.

**Figure 5.3.** Reproduction of *Arabidopsis thaliana* F1 hybrid triploids is determined by cross direction as well as parental genotype. Box-plots of %N are shown for (A) 78 paternal excess (2x X 4x) triploids and (B) 88 maternal excess (4x X 2x) triploids, each sorted in descending order of reproductive success. The median values, inter-quartile ranges and outliers are displayed. (C) Variation in fertility between the 77 pairs of hybrid triploids showing differences in mean %N per silique between triploids generated in the 4x X 2x and 2x X 4x cross direction. Accessions showing decreased reproduction in the 2x X 4x cross direction (higher %N) are coloured in black and those with improved reproduction (lower %N) are represented by white bars. Accessions in the centre of the figure show little difference in reproductive success between cross directions. Those with statistically significant ($p < 0.05$) differences between cross directions (non-parametric Mann-Whitney Test) are shaded (next page).


A
% of normal seeds per silique
Name of the diploid parent
B
% of normal seeds per silique
Name of the diploid parent
C
%N 4x2 - %N 2x4
Name of the diploid parent

| Accession | %N_2x4 | | SD | Accession | %N_4x2 | | SD |
|---|---|---|---|---|---|---|---|
| **Br-0** | 35.5 | ± | 20.9 | AN-1 | 28.8 | ± | 9.1 |
| **LZ-0** | 31.2 | ± | 3.6 | REN-1 | 28.3 | ± | 5.2 |
| **TSU-1** | 31.2 | ± | 10.2 | C24 | 28 | ± | 5.7 |
| **SE-0** | 31.2 | ± | 7.7 | VAR2-1 | 27.1 | ± | 5.8 |
| **Van-0** | 31 | ± | 8.2 | SQ-8 | 27.1 | ± | 6.5 |
| **OY-0** | 30.9 | ± | 5.2 | Van-0 | 26.6 | ± | 7.1 |
| **WT-5** | 30.2 | ± | 9.3 | MS-0 | 26.6 | ± | 5.9 |
| **REN-11** | 29.7 | ± | 9.6 | OMO2-3 | 25.6 | ± | 6.5 |
| **TS-5** | 29 | ± | 3.7 | HR-10 | 25.5 | ± | 4.9 |
| **BUR-0** | 28.3 | ± | 6.2 | TSU-1 | 24.7 | ± | 6.9 |
| **LP2-2** | 27.6 | ± | 15 | KIN-0 | 24.7 | ± | 7.2 |
| **Bor-1** | 27.5 | ± | 4.9 | RRS-7 | 24.6 | ± | 6.6 |
| **Ra-0** | 27.2 | ± | 7.4 | Gy-0 | 24.6 | ± | 7 |
| **LL-0** | 27.2 | ± | 6.6 | NOK-3 | 24.4 | ± | 6.7 |
| **GA-0** | 27 | ± | 9 | RMX-A180 | 24.3 | ± | 7.7 |
| **RRS-7** | 26.8 | ± | 8.6 | Pna-17 | 24.3 | ± | 5.3 |
| **VAR2-1** | 26.7 | ± | 4.4 | BOR-4 | 24.3 | ± | 8.7 |
| **AN-1** | 25.9 | ± | 11.5 | Got-22 | 24.1 | ± | 7.1 |
| **Ei-2** | 25.8 | ± | 8.2 | Est-1 | 23.8 | ± | 6 |
| **LP2-6** | 25.5 | ± | 6 | WT-5 | 23.7 | ± | 5.8 |
| **LER-1** | 25.5 | ± | 5.4 | HR-5 | 23.4 | ± | 6.6 |
| **Gy-0** | 25.4 | ± | 10.6 | Uod-1 | 23.2 | ± | 5.5 |
| **MrK-0** | 25.3 | ± | 7.9 | Col-0 | 23.2 | ± | 5.9 |
| **BOR-4** | 25.1 | ± | 6.3 | RMX-A02 | 23 | ± | 6.8 |
| **C24** | 24.9 | ± | 8.3 | Bor-1 | 23 | ± | 6.4 |
| **MZ-0** | 24.5 | ± | 6.3 | Kas-1 | 23 | ± | 7.7 |
| **ND-1** | 24.4 | ± | 7.2 | SE-0 | 23 | ± | 5.5 |
| **MR-0** | 24.1 | ± | 6.2 | WEI-0 | 22.9 | ± | 5.3 |
| **GU-0** | 24 | ± | 5.5 | PU2-23 | 22.6 | ± | 9.8 |
| **SORBO** | 23.7 | ± | 6.8 | Bay-0 | 22.4 | ± | 6.5 |
| **HR-5** | 23.2 | ± | 7 | NFA-10 | 22 | ± | 5.4 |
| **NFA-10** | 23 | ± | 5 | UOD-7 | 21.8 | ± | 8.7 |
| **SHAHDARA** | 23 | ± | 4.1 | PRO-0 | 21.7 | ± | 10.4 |
| **UOD-7** | 22.6 | ± | 11.2 | Ra-0 | 21.4 | ± | 8.9 |
| **RMX-A180** | 22.2 | ± | 4.9 | TS-1 | 21.4 | ± | 9.1 |
| **FEI-0** | 22.1 | ± | 9.2 | Got-7 | 21.3 | ± | 8.4 |
| **HR-10** | 21.7 | ± | 9.3 | LER-1 | 20.9 | ± | 10 |
| **Est-1** | 21.6 | ± | 9.2 | LL-0 | 20.9 | ± | 6.3 |
| **OMO2-3** | 21.5 | ± | 4.3 | KONDARA | 20.9 | ± | 8.3 |
| **PNA-10** | 21.3 | ± | 6.5 | KNOX-18 | 20.7 | ± | 12.7 |
| **ZDR-1** | 21.1 | ± | 4.2 | LP2-2 | 20.6 | ± | 7 |
| **Pu-2-7** | 21.1 | ± | 5.6 | SQ-1 | 20.6 | ± | 7.7 |
| **RRS-10** | 20.9 | ± | 7.9 | RRS-10 | 20.4 | ± | 6.2 |
| **ZDR-6** | 20.9 | ± | 8.5 | Spr1-2 | 20.4 | ± | 6.1 |
| **CT-1** | 20.9 | ± | 4.3 | LZ-0 | 20.2 | ± | 5.4 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **NFA-8** | 20.3 | ± | 8 | Ull2-3 | 20.1 | ± | 9.1 |
| **TS-1** | 20.2 | ± | 6.5 | TS-5 | 20.1 | ± | 8.2 |
| **RMX-A02** | 20.1 | ± | 7 | EDEN-2 | 20 | ± | 9.7 |
| **Got-22** | 20.1 | ± | 7.6 | LP2-6 | 19.9 | ± | 11.3 |
| **Uod-1** | 20.1 | ± | 6.8 | Br-0 | 19.9 | ± | 6.5 |
| **KZ-9** | 19.9 | ± | 5.5 | ZDR-1 | 19.7 | ± | 7.8 |
| **CVI-0** | 19.9 | ± | 5.8 | Knox-10 | 19.6 | ± | 3.9 |
| **Knox-10** | 19.4 | ± | 5.3 | Yo-0 | 19.6 | ± | 6.7 |
| **CIBC-17** | 19.3 | ± | 4.3 | OY-0 | 19.6 | ± | 6.3 |
| **L*er*-0** | 19.1 | ± | 7.6 | WS-2 | 19.6 | ± | 9 |
| **WEI-0** | 18.9 | ± | 10 | CIBC-17 | 19.5 | ± | 7.3 |
| **REN-1** | 18.7 | ± | 6.8 | MT-0 | 18.9 | ± | 8.2 |
| **Pna-17** | 18.5 | ± | 7.1 | CS22491 | 18.8 | ± | 9.6 |
| **MT-0** | 18.5 | ± | 6.4 | SORBO | 18.7 | ± | 4 |
| **NOK-3** | 18.5 | ± | 5.1 | REN-11 | 18.3 | ± | 12.3 |
| **PU2-23** | 18.4 | ± | 4.5 | GU-0 | 18.3 | ± | 5.9 |
| **KNOX-18** | 18.1 | ± | 6.9 | BUR-0 | 18.3 | ± | 4.7 |
| **Bay-0** | 17.4 | ± | 5.8 | CT-1 | 17.9 | ± | 9.5 |
| **SQ-8** | 17.3 | ± | 5.4 | Edi-0 | 17.6 | ± | 3.5 |
| **KZ-1** | 17.3 | ± | 8 | L*er*-0 | 17.6 | ± | 8.7 |
| **SQ-1** | 17 | ± | 5.6 | PNA-10 | 17.5 | ± | 6.9 |
| **Bil-5** | 16.8 | ± | 0.9 | TAMM-2 | 17.5 | ± | 7 |
| **CIBC-5** | 16.8 | ± | 7.5 | Ag-0 | 17.3 | ± | 5.1 |
| **EDEN-2** | 16.6 | ± | 6.9 | KZ-9 | 17.2 | ± | 11.3 |
| **MS-0** | 16.6 | ± | 4.6 | MrK-0 | 17.1 | ± | 6.4 |
| **WS-2** | 16.5 | ± | 8.6 | KZ-1 | 17 | ± | 3.7 |
| **PRO-0** | 16.4 | ± | 10.4 | ZDR-6 | 16.9 | ± | 9 |
| **CS22491** | 16.1 | ± | 4.4 | Pu-2-7 | 16.8 | ± | 6.7 |
| **TAMM-2** | 15.9 | ± | 6.2 | Ei-2 | 16.7 | ± | 7.4 |
| **KONDARA** | 15.7 | ± | 8 | NFA-8 | 16.6 | ± | 5.1 |
| **KIN-0** | 15.3 | ± | 8.7 | CIBC-5 | 16.6 | ± | 3.9 |
| **Col-0** | 13.7 | ± | 8.5 | MZ-0 | 16.3 | ± | 7.7 |
| **Tamm-27** | 12.7 | ± | 3.4 | FEI-0 | 16 | ± | 3.7 |
| **Kas-1** | 12.1 | ± | 1.9 | ND-1 | 15.7 | ± | 6.7 |
| **EDEN-1** | N.D. | | | MR-0 | 15.6 | ± | 3.5 |
| **Lov-5** | N.D. | | | SHAHDARA | 15.1 | ± | 7 |
| **Spr1-2** | N.D. | | | CVI-0 | 14.4 | ± | 5.5 |
| **Omo2-1** | N.D. | | | Tamm-27 | 14.2 | ± | 5.2 |
| **Ull2-5** | N.D. | | | Lov-5 | 14 | ± | 2.3 |
| **Ull2-3** | N.D. | | | Ull2-5 | 13.9 | ± | 4.2 |
| **Got-7** | N.D. | | | GA-0 | 13.8 | ± | 4.9 |
| **Ws-0** | N.D. | | | Omo2-1 | 13.5 | ± | 4.1 |
| **Yo-0** | N.D. | | | EDEN-1 | 12.9 | ± | 4.6 |
| **Ag-0** | N.D. | | | Ws-0 | 9.7 | ± | 6.8 |
| **Edi-0** | N.D. | | | Bil-5 | N.D. | | |

**Table 5.2.** Siliques produced by *Arabidopsis thaliana* F1 hybrid triploids contain variable percentages of normal seeds (N). Mean %N were calculated for each F1 hybrid triploid genotype from each reciprocal cross direction and sorted in descending order for each trait. Data from isogenic L*er*-0 triploids is included and highlighted in a grey colour. Standard deviation (SD) from mean is included.

| Accession | %N_4x2 | p-value | %N_2x4 |
|---|---|---|---|
| **BUR-0** | 18.3 | < 0.001 | **28.3*** |
| **GA-0** | 13.8 | < 0.001 | **27*** |
| **LZ-0** | 20.2 | < 0.001 | **31.2*** |
| **MR-0** | 15.6 | < 0.001 | **24.1*** |
| **OY-0** | 19.6 | < 0.001 | **30.9*** |
| **SQ-8** | **27.1*** | < 0.001 | 17.3 |
| **MS-0** | **26.6*** | 0.001 | 16.6 |
| **REN-1** | **28.3*** | 0.001 | 18.7 |
| **Col-0** | **23.2*** | 0.002 | 13.7 |
| **Kas-1** | **23*** | 0.002 | 12.1 |
| **ND-1** | 15.7 | 0.002 | **24.4*** |
| **SE-0** | 23 | 0.004 | **31.2*** |
| **TS-5** | 20.1 | 0.004 | **29*** |
| **REN-11** | 18.3 | 0.005 | **29.7*** |
| **KIN-0** | **24.7*** | 0.006 | 15.3 |
| **Ei-2** | 16.7 | 0.008 | **25.8*** |
| **MrK-0** | 17.1 | 0.009 | **25.3*** |
| **MZ-0** | 16.3 | 0.011 | **24.5*** |
| **NOK-3** | **24.4*** | 0.012 | 18.5 |
| **GU-0** | 18.3 | 0.015 | **24*** |
| **CVI-0** | 14.4 | 0.016 | **19.9*** |
| **LL-0** | 20.9 | 0.021 | **27.2*** |
| **C24** | **28*** | 0.028 | 24.9 |
| **SHAHDARA** | 15.1 | 0.032 | **23*** |
| **FEI-0** | 16 | 0.033 | **22.1*** |
| **Bor-1** | 23 | 0.044 | **27.5*** |
| **Pna-17** | **24.3*** | 0.044 | 18.5 |
| **Bay-0** | 22.4 | 0.049 | 17.4 |
| **WT-5** | 23.7 | 0.051 | 30.2 |
| **Ra-0** | 21.4 | 0.058 | 27.2 |
| **KONDARA** | 20.9 | 0.059 | 15.7 |
| **Van-0** | 26.6 | 0.059 | 31 |
| **OMO2-3** | 25.6 | 0.064 | 21.5 |
| **NFA-8** | 16.6 | 0.069 | 20.3 |
| **SORBO** | 18.7 | 0.071 | 23.7 |
| **TSU-1** | 24.7 | 0.078 | 31.2 |
| **LER-1** | 20.9 | 0.089 | 25.5 |
| **Pu-2-7** | 16.8 | 0.101 | 21.1 |
| **Br-0** | 19.9 | 0.114 | 35.5 |
| **Uod-1** | 23.2 | 0.13 | 20.1 |
| **RMX-A02** | 23 | 0.135 | 20.1 |
| **Got-22** | 24.1 | 0.146 | 20.1 |
| **KZ-9** | 17.2 | 0.174 | 19.9 |
| **LP2-2** | 20.6 | 0.206 | 27.6 |
| **HR-10** | 25.5 | 0.211 | 21.7 |

| | | | |
|---|---|---|---|
| **LP2-6** | 19.9 | 0.223 | 25.5 |
| **PRO-0** | 21.7 | 0.229 | 16.4 |
| **SQ-1** | 20.6 | 0.229 | 17 |
| **WS-2** | 19.6 | 0.237 | 16.5 |
| **PU2-23** | 22.6 | 0.277 | 18.4 |
| **PNA-10** | 17.5 | 0.319 | 21.3 |
| **Est-1** | 23.8 | 0.325 | 21.6 |
| **RRS-7** | 24.6 | 0.372 | 26.8 |
| **EDEN-2** | 20 | 0.395 | 16.6 |
| **WEI-0** | 22.9 | 0.418 | 18.9 |
| **ZDR-6** | 16.9 | 0.443 | 20.9 |
| **RMX-A180** | 24.3 | 0.468 | 22.2 |
| **L*er*-0** | 17.6 | 0.534 | 19.1 |
| **Tamm-27** | 14.2 | 0.534 | 12.7 |
| **CT-1** | 17.9 | 0.561 | 20.9 |
| **TAMM-2** | 17.5 | 0.584 | 15.9 |
| **KNOX-18** | 20.7 | 0.648 | 18.1 |
| **AN-1** | 28.8 | 0.648 | 25.9 |
| **UOD-7** | 21.8 | 0.663 | 22.6 |
| **MT-0** | 18.9 | 0.724 | 18.5 |
| **Knox-10** | 19.6 | 0.76 | 19.4 |
| **NFA-10** | 22 | 0.762 | 23 |
| **VAR2-1** | 27.1 | 0.767 | 26.7 |
| **CS22491** | 18.8 | 0.787 | 16.1 |
| **BOR-4** | 24.3 | 0.803 | 25.1 |
| **TS-1** | 21.4 | 0.836 | 20.2 |
| **KZ-1** | 17 | 0.839 | 17.3 |
| **ZDR-1** | 19.7 | 0.852 | 21.1 |
| **CIBC-5** | 16.6 | 0.906 | 16.8 |
| **HR-5** | 23.4 | 0.917 | 23.2 |
| **CIBC-17** | 19.5 | 0.934 | 19.3 |
| **RRS-10** | 20.4 | 0.983 | 20.9 |
| **Gy-0** | 24.6 | 1 | 25.4 |

**Table 5.3**. Pairs of reciprocal F1 hybrid triploids ranked in descending order according to the significance of the parent-of-origin effect on normal seeds (N). F1 hybrid triploids with significantly higher %N in a given cross direction ($p < 0.05$, Mann-Whitney test) are highlighted in bold with asterisks. The reciprocal pair of L*er*-0 isogenic F1 triploids is listed among the pairs of triploid F1 hybrids which are not significantly different from each other.

The normal (% N) F2 seeds obtained from *Arabidopsis thaliana* F1 hybrid triploids showed strong links to genetic variation between the parental accessions. Mixed models detected significant genetic variation in both %N and the sum of ovules (∑), with broad-sense heritabilities ranging from 0.19 – 0.44, $p < 0.0001$ (**Table 5.4**). In addition, considerable support for cross direction ($p < 0.1$), and accession x cross direction interaction effects ($p < 0.001$) was detected. Also the pattern of correlation of the triploid reproductive characters (%N, ∑) within and among parental cross directions was investigated. For both phenotypic and genetic correlations, there was no relationship between %N and the sum of ovules ∑ (**Table 5.5, Figure 5.4**). Finally, low genetic correlation across cross directions was found (Tom Juenger – personal communication).

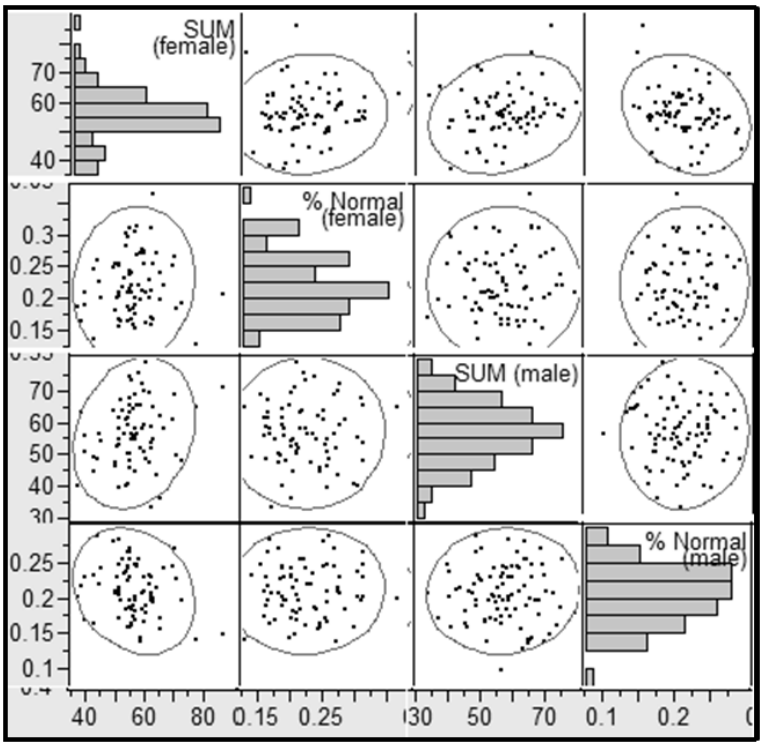



**Figure 5.4.** Diagonal matrix presenting the phenotypic and genetic correlations among the %N and ∑ traits, split by cross direction. % Normal (female) refers to proportion of normal F2 seeds from selfed 2x X 4x paternal excess triploids, (male) – 4x X 2x maternal excess triploids. Sum (female) refers to sum of ovules (fertilized or not) produced by 2x X 4x paternal excess triploids, (male) - 4x X 2x maternal excess triploids.

| Trait | 2 x 4 | 4 x 2 |
|---|---|---|
| **% N** | 0.25 | 0.19 |
| ∑ | 0.35 | 0.44 |

**Table 5.4**. Broad-sense heritabilities of proportions of normal seeds (%N) and the total number of ovules per silique (∑) demonstrating the high level of heritability for normal seed development.

| **Phenotypic Correlations** | | | |
|---|---|---|---|
| | 2x4 | ∑ | %N |
| | ∑ | 1 | -0.01 |
| | 4x2 | ∑ | %N |
| | ∑ | 1 | -0.04 |
| **Genetic Correlations** | | | |
| | 2x4 | ∑ | %N |
| | ∑ | 1 | -0.04 |
| | 4x2 | ∑ | %N |
| | ∑ | 1 | 0.06 |
| | **Across Direction** | | |
| | | ∑_2x4 | %N_2x4 |
| | ∑ _4 x2 | 0.21 | 0.01 |
| | %N_4x2 | -0.22 | 0.04 |

**Table 5.5.** Phenotypic and genetic correlations between %N and the total number of ovules (∑) and the effect of parental origin in inter-accession hybrid *Arabidopsis thaliana*.

### 5.3.3 Low levels of % N F2 seed from selfed F1 hybrid triploids is not due to hybrid dysgenesis

Decreased reproductive success of F1 hybrid triploids could be a consequence of hybrid dysgenesis between L*er*-0 and some accessions (Bomblies et al., 2010). Hence, it was necessary to determine if F1 hybrid diploids displayed any evidence of hybrid dysgenesis effects that could lead to low levels of % N F2 seeds. To test whether the changes in %N F2 seeds is specific to genotypic effects manifest only in the polyploid state, %N was also determined in diploid F1 hybrids. The reciprocal F1 hybrid diploids were derived from reciprocal crosses between diploid L*er*-0 and five accessions: Wei-0, Fei-0, and Ren-1, representing the extremes of % N F2 seed set observed in 4x X 2x F1 hybrid triploids; and Ga-0 and Pro-0, representing the most extreme % N F2 seed set amongst the 2x X 4x F1 hybrid triploids (**Figure 5.3**). In all of the F1 hybrid diploids tested (including reciprocal F1s) there was no evidence of any significant impairment of % N F2 seed set due to hybrid dysgenesis at the diploid level. In all five F1 hybrid diploids at least 91% of ovules were successfully fertilized, and developed into normal F2 seeds (**Figure 5.5, Table 5.6**). This indicates that the genotypic and parent-of-origin effects on the rates of normal seed production (%N F2 seeds) seen in the F1 hybrid triploids is due to a genome dosage effect rather than being a consequence of hybridity.

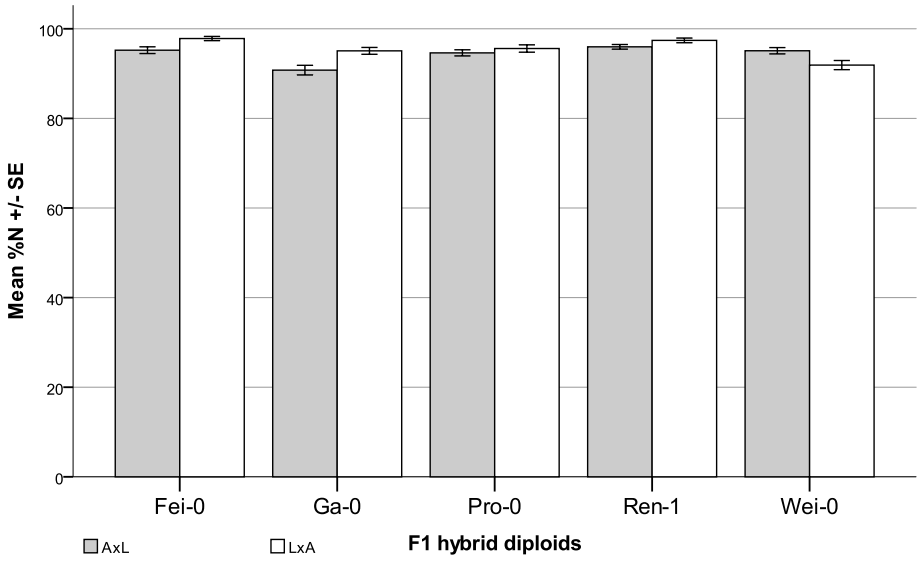



**Figure 5.5.** Reproductive success of *Arabidopsis thaliana* F1 hybrid diploids is determined by cross direction as well as parental genotype. Bars represent mean %N produced by selfed F1 hybrid diploids, generated by crosses between diploid L*er*-0 and five different diploid accessions (Fei-0, Ga-0, Pro-0, Ren-1, Wei-0). Error bars represent standard error (SE).

| Accession | %N_AxL | | SD | %N_LxA | | SD | AxL -LxA | p-value |
|---|---|---|---|---|---|---|---|---|
| **Wei-0** | 95 | ± | 4.75 | 92 | ± | 6.9 | 3.2 | **0.023*** |
| **Ga-0** | 91 | ± | 7.25 | 95 | ± | 5.1 | -4.3 | **0.002*** |
| **Pro-0** | 95 | ± | 3.47 | 96 | ± | 2.9 | -1 | 0.099 |
| **Fei-0** | 95 | ± | 4.61 | 98 | ± | 5.5 | -2.6 | **0.007*** |
| **Ren-1** | 96 | ± | 4.11 | 97 | ± | 2.6 | -1.4 | 0.106 |

**Table 5.6.** Mean percentage of normal seeds (%N) per silique in five pairs of reciprocal F1 hybrid diploids. Differences between mean %N in two cross directions was tested by the Mann-Whitney U test and the significance level was at 0.05.

### 5.3.4 Selfed *Arabidopsis thaliana* triploids produce normal (N) F2 seeds with varied DNA content

*Arabidopsis thaliana* single seed flow cytometric analysis (Matzk et al., 2000) has been used to identify ploidy of surviving F2 embryos. The *Arabidopsis thaliana* endosperm tissue is transient and is consumed by the growing embryo during seed development, leaving only a single layer of cells at maturity (Brown et al., 1999). Therefore a strong flow cytometry peak represented embryo DNA content and not any other seed components.

The DNA content of the F2 seed offspring from different F1 hybrid triploids and from L*er*-0 F1 isogenic triploids were compared to determine the extent of DNA content variability in the F2 N seed set from different F1 triploid genotypes. The distribution of ploidy (DNA content) levels in most cases was not as expected from triploid meiosis, as calculated by Henry et al., (2005) (**Figure A**). The DNA content of F2 seeds varied considerably, with most F1 triploids producing F2 seed offspring with a wide range of ploidy levels and others with much more restricted ranges (**Figure 5.6**, **Figure 5.7**). Many ploidy distributions were bimodal, where first peak was usually in around 2.0 – 2.4 genome content class, and the other one in around 3.0 – 3.4 class, consistent with previous observations of Col-0 isogenic and Col-0 X Wa-1 hybrid triploids (**Figure B**) (Henry et al., 2005). However, some of the triploids analysed in this study seemed to preferentially produce offspring only in the lower ploidy class (Pro-0 in 4x X 2x, Knox-10 in 2x X 4x, and Uod-1 in both cross directions). Very few F2 seed progeny with higher genome content (e.g around 3.6 – 4.0) were detected, but some rare instances were observed e.g. EDEN-2 from 2x X 4x cross direction. Some accessions appeared particularly variable in terms of DNA content of F2 seeds. For instance, Lp2-6 produced some normal (N) F2 seeds higher than pentaploid, and other F2 seeds less than diploid when the F2 seeds were derived from the 4x X 2x cross direction (**Figure 5.7**). There were a few histograms that resembled the shape of an expected genome content distribution (**Figure A**). These included Ga-0 in 2x X 4x and Lp2-6 in 4x X 2x cross direction.

There were also differences in the ploidy distribution of the F2 seeds generation produced from some reciprocal F1 triploids, i.e. highlighting possible epigenetic grandparent-of-origin and/or dosage effects which can also be evident in the F2 seed offspring. In Ga-0, for example, the F2 offspring of the 4x X 2x triploids, fell mainly into the 2.2 genome content class, whilst the offspring of the triploid from the reciprocal cross was equally distributed between the 2.2 and 4.4 class (**Figure 5.7)** .

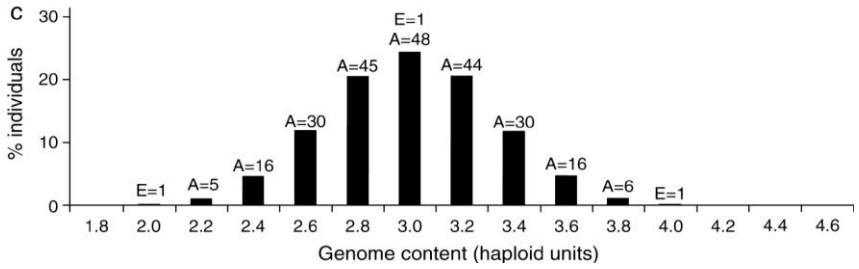



**Figure A.** Expected distribution of genome sizes in the F2 progeny from a triploid. Above each bar are indicated the number of euploid (E) and aneuploid (A) types contained in each genome content class. Image reproduced from (Henry et al., 2005).

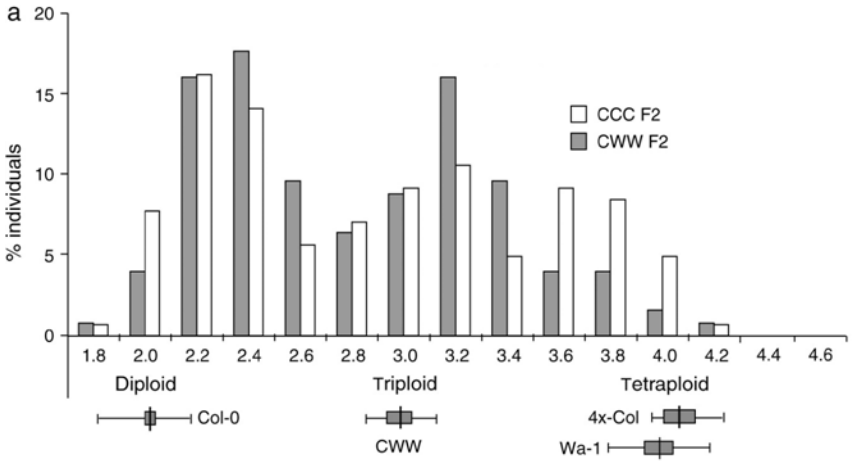



**Figure B.** Distribution of genome contents in the aneuploid swarms produced by the Col-0 isogenic and Col-0 X Wa-1 hybrid triploids. Image reproduced from (Henry et al., 2005).

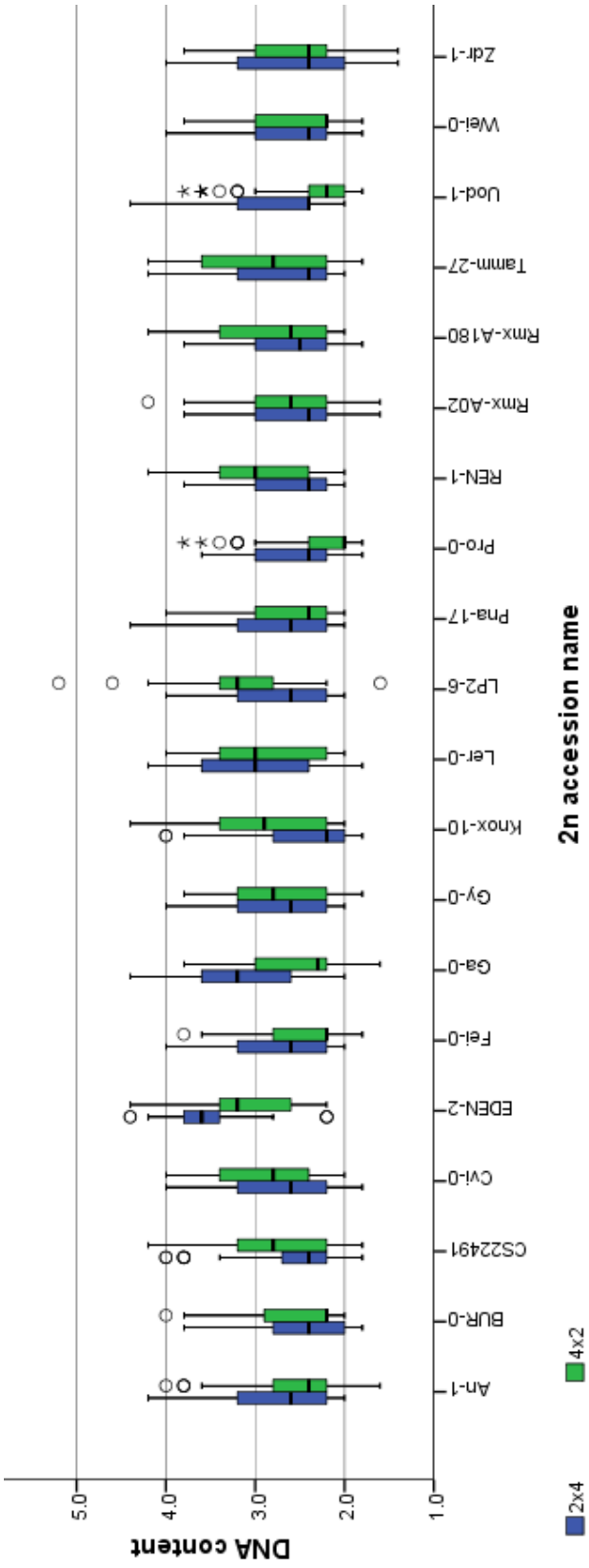
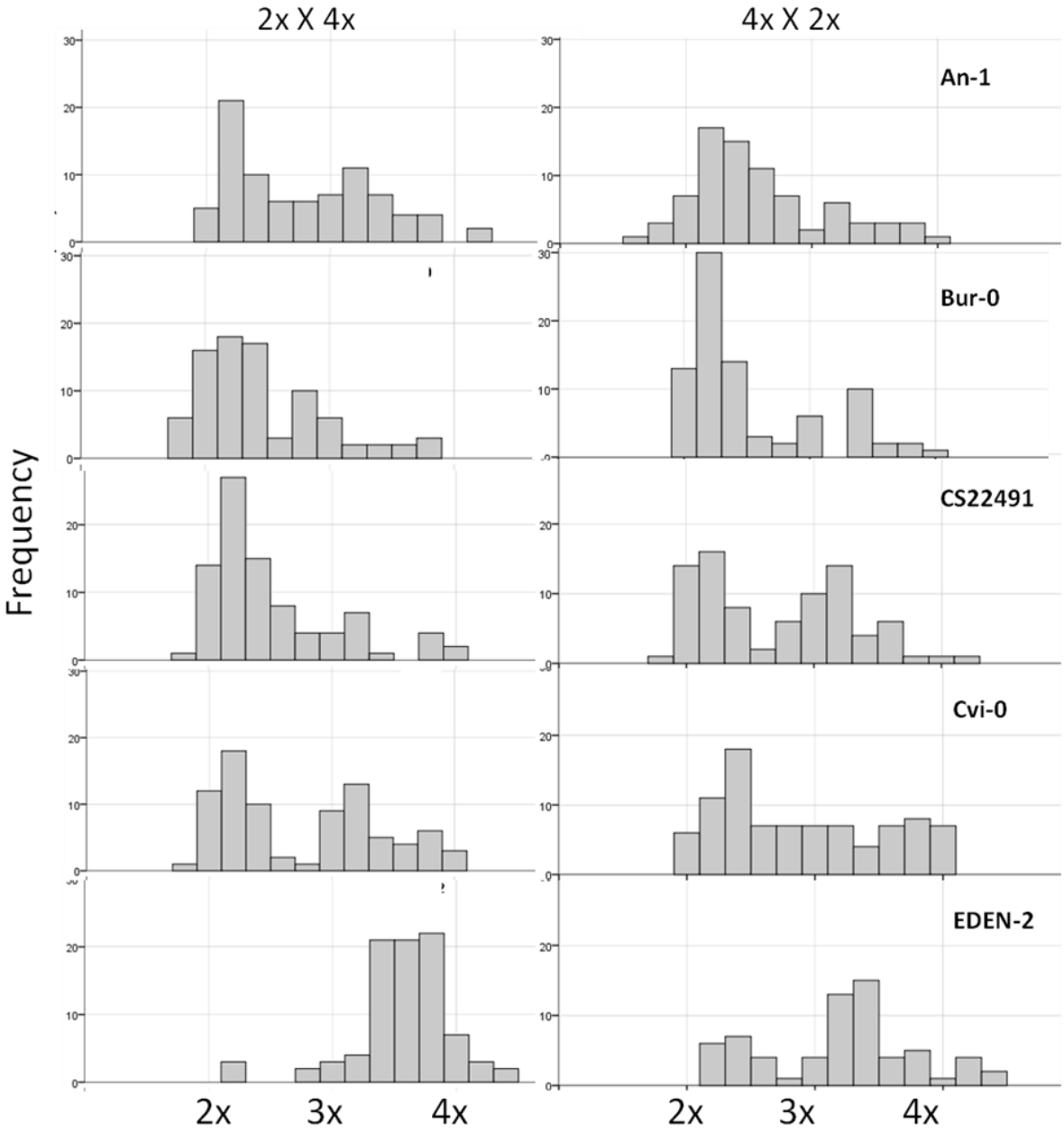
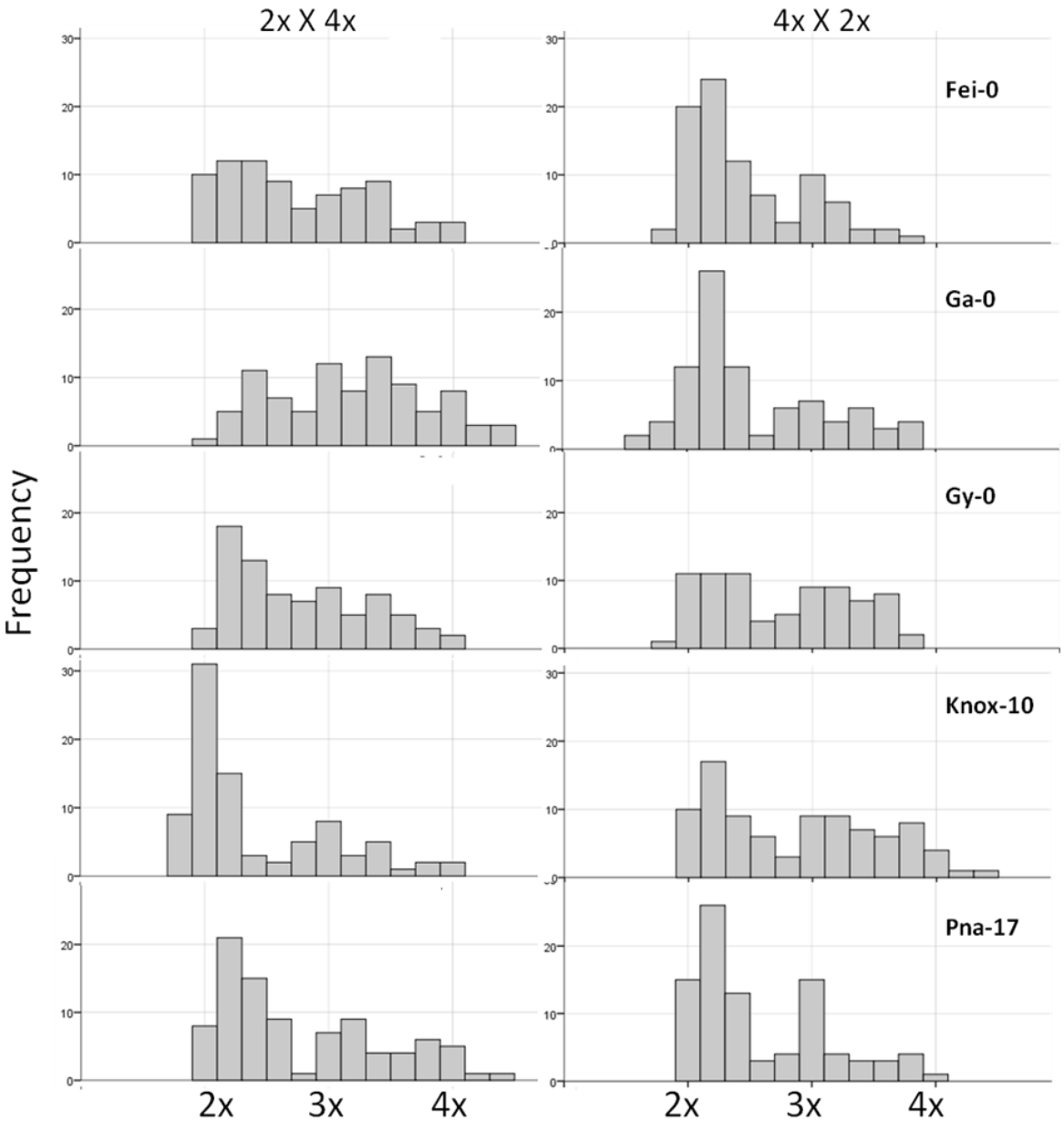
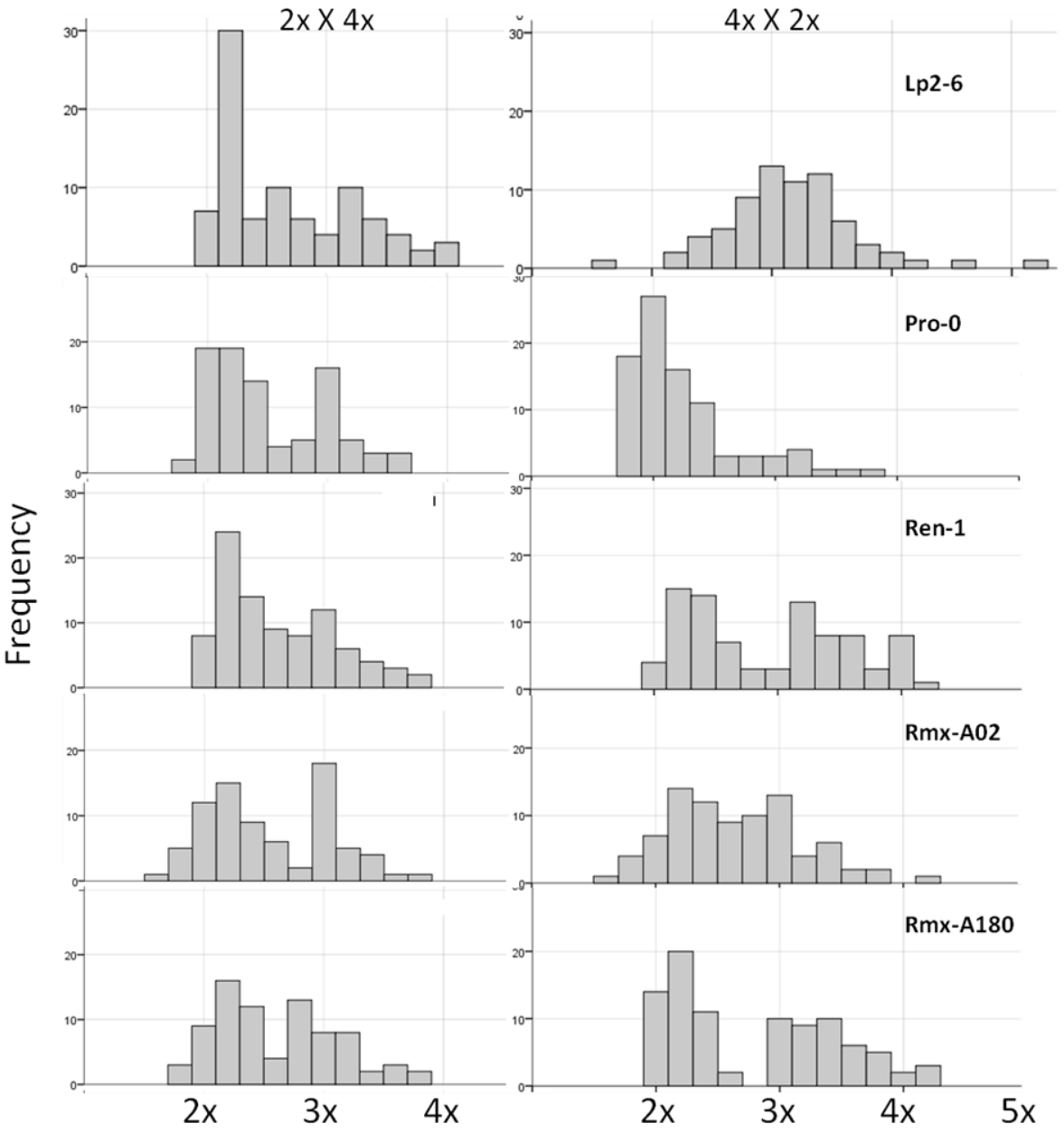



**Figure 5.6.** Variation in DNA content in F2 embryos produced by Arabidopsis triploids. Triploids are listed by the accession name, and demarcated by cross direction – 2x X 4x, paternal excess = blue; 4x X 2x, maternal excess = green. Data is shown as standard box-plots illustrating the interquartile range, median and presence of outliers if any (circles and asterisks).


2x X 4x
4x X 2x
An-1
Bur-0
CS22491
Cvi-0
EDEN-2
Frequency
2x
3x
4x
2x
3x
4x


2x X 4x
4x X 2x
Fei-0
Ga-0
Gy-0
Knox-10
Pna-17
Frequency
0
10
20
30
2x
3x
4x


2x X 4x
4x X 2x
Lp2-6
Pro-0
Ren-1
Rmx-A02
Rmx-A180
Frequency
2x
3x
4x
5x

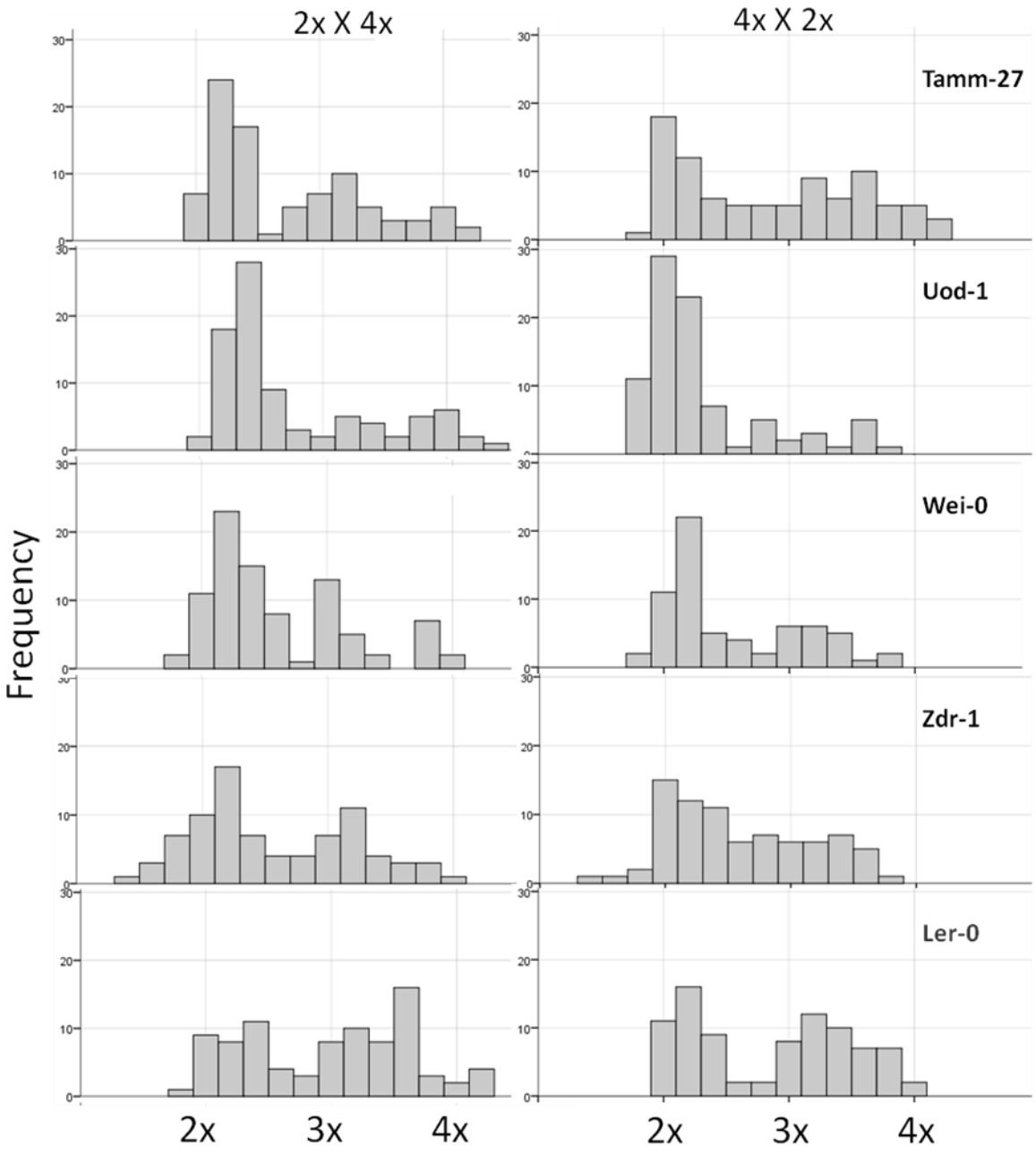



**Figure 5.7.** Distribution of F2 embryo DNA content from 19 pairs of reciprocal F1 hybrid triploids and F1 isogenic L*er*-0 triploids (n = ~90).

### 5.3.5 GWAS identifies two SNPs associated with %N F2 seeds set from selfed *Arabidopsis thaliana* F1 triploids

As analysis of triploid *Arabidopsis thaliana* indicates that reproductive success and ploidy levels associated with viable progeny vary by parental genotype, a GWAS approach was employed to identify loci associated with tolerance of F1 triploid reproduction and parent-of-origin effects. Hypothetically, such loci could be important for influencing the tolerance of *Arabidopsis thaliana* to triploidy, and help to explain the tendency of some F1 triploids to display parent-of-origin effects (**Figure 5.3C**). For this purpose, a Genome-Wide Association (GWA) mapping was employed with the Efficient Mixed-Model Association (EMMA) approach that corrects for population structure, as was successfully used for the identification of genes controlling flowering time and other phenotypes (Atwell et al., 2010, Zhao et al., 2007). While association mapping is most powerful when candidate genes associated with the trait have already been identified (Zhao et al., 2007, Ehrenreich et al., 2009), unbiased GWAS (without candidate genes) can also be employed for gene discovery and hypothesis generation.

In the GWAS approach, it was sought to determine statistical associations between a genome-wide sampling of SNP polymorphism and triploid reproduction traits (e.g. %N, %U, %A, $\sum$) . To identify loci associated with a %N F2 seed set (from selfed F1 triploid) trait, a significance cut-off of $p < 0.01$ for the SNPs was applied. Using this cut-off, genome-wide analyses of peaks associated with the %N trait, indicated many non-significant ($p > 0.01$) peaks across the genome (**Figure 5.8**). However, two highly significant SNPs associated with % N F2 seeds were identified by GWAS. One significant SNP associated with %N in the 4x X 2x cross was found on chromosome 1, and one SNP associated with %N in the 2x X 4x cross was found on chromosome 4 (**Figure 5.8, Table 5.7**). These loci identified by GWAS have been termed *MATERNAL OVERDOSE TRIPLOID* (*MOT*) and *PATERNAL OVERDOSE TRIPLOID* (*POT*) respectively.

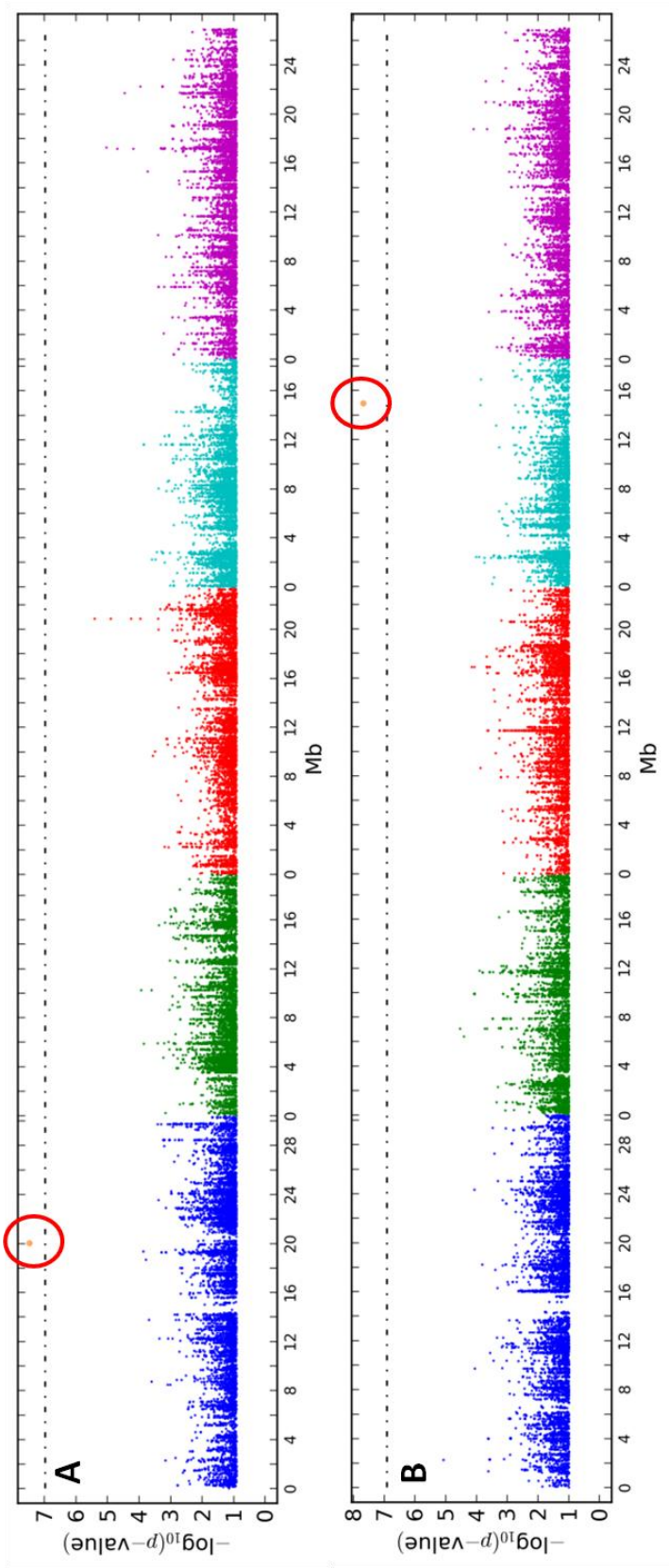



**Figure 5.8.** Loci associated with altered triploid fertility are distributed across the genome and differ by cross direction. Manhattan plots of SNP p-values genome-wide illustrating distribution of SNPs associated with significant variation in % N where diploid mapping accessions were used as (A) the male parent (4x X 2x cross) and (B) the female parent (2x X 4x cross) in crosses to a *Ler*-0 tetraploid line. SNPs were predicted by the mixed model EMMA, and illustrate the distribution of peaks across all five Arabidopsis chromosomes (represented by different colours) with differences between the two cross directions. One peak of significant loci was observed in the 4x X 2x cross direction on chromosome 1, and one peak in the 2x X 4x cross direction on chromosome 4. Dotted lines represent the significance threshold derived from the p-value.

| Chromosomes | Position | p-value | Phenotype | Locus |
|---|---|---|---|---|
| 1 | 20021220 | $<10^{-7}$ | %N_4x2 | *MOT* |
| 4 | 14941541 | $<10^{-8}$ | %N_2x4 | *POT* |

**Table 5.7.** SNPs significantly associated with the production of normal seeds by *Arabidopsis thaliana*F1 hybrid triploids.

To identify candidate genes, that may be genetically linked to the SNP which is associated with the %N F2 seed set, all genes 120 kb upstream and 120 kb downstream of the each significant SNP were identified. Using this approach, the gene list containing possible candidates for explaining the variation in the 2x X 4x paternal excess triploid reproductive success (%N) are listed in **Table 5.8**. They include 73 genes located on the long arm of chromosome 4 in the 240 kb window overlapping the significant SNP in the *POT* locus (position 14941541; **Table 5.7, Figure 5.9**). While it remains challenging to identify causal loci from GWAS studies (and recognising that the causal loci could be in promoter regions or in non-coding RNAs), four of the 73 genes appear to be particularly interesting in terms of their relevance to seed development, and thus more attention is focused on them.

1) The At4g30580 gene is located only 7 kb upstream from the SNP and encodes a plastidic lysophosphatidic acid acyltransferase (LPAAT), critical for chloroplast phosphatidic acid biosynthesis. The null allele is embryo lethal (Kim and Huang, 2004, Yu et al., 2004, Li-Beisson et al., 2010).
2) The adjacent gene – At4g30590 – encodes an early nodulin-like protein 12 (ENODL12) that is anchored in the membrane. It was found to be expressed in the embryo sac (Steffen et al., 2007, Yu et al., 2005a, Johnston et al., 2007).
3) The At4g30860 gene encodes a member of the trxG protein family. This gene contains a SET domain which is known to be involved in modification of histone tails by methylation. Over-expression of this gene results in pleiotropic developmental defects (Przybilski et al., 2005, Springer et al., 2003, Baumbusch et al., 2001, Cartagena et al., 2008).

4) Finally, the At4g30930 gene was identified, which encodes a ribosomal RPL21M protein that is localized to the mitochondrion and is involved in karyogamy during female gametophyte development and fertilization. Mutants display defects in both male and female gametophyte development such as collapsed pollen and female gametophytes with unfused central cells (Portereiko et al., 2006b).

The expression pattern of these selected candidate genes can be seen in **Appendix B**.

In relation to the other significant SNP in the *MOT* locus, seventy four genes were found on the second arm of chromosome 1 in the 240 kb window of the significant SNP (position 20021220) associated with the variation in the reproductive success (%N) of 4x X 2x maternal excess triploids (**Table 5.7, Table 5.9, Figure 5.9)**. The significant SNP itself was located inside a non-coding region (intron) of a gene of unknown function (At1g53640) expressed in a seed and 19 other plant structures. In addition to At1g53640, five genes have been selected as promising candidates for controlling the variation in F1 triploid reproductive success.

1) The At1g53500 gene (*MUM4*) encodes a putative rhamnose synthase, an enzyme required for the synthesis of the pectin, the major component of *Arabidopsis thaliana* mucilage. *MUM4* is involved in seed coat mucilage cell development and mutant analyses suggest that this gene is required for complete mucilage synthesis, cytoplasmic rearrangement and seed coat development (Usadel et al., 2004, Western et al., 2001, Western et al., 2004, Young et al., 2008, Arsovski et al., 2009, Shi et al., 2011).
2) The At1g53650 gene is located 7.8 kb downstream of the significant SNP and it encodes a putative RNA-binding protein containing the PAB2 domain, which facilitates binding to PABC (C-terminal domain of poly(A) binding) proteins (Bravo et al., 2005, Tiwari et al., 2008).
3) The At1g53860 gene is known to encode the remorin family protein and could support a possible link between triploid reproduction and DNA methylation (Penterman et al., 2007).

4) The At1g53690 gene encodes a protein of unknown function that is homologous to At5g41010, which encodes a non-catalytic subunit common to nuclear DNA-dependent RNA polymerases II, IV and V (Ream et al., 2009).
5) The At1g53910 gene encodes a member of the ERF (ethylene response factor) subfamily of the ERF/AP2 transcription factor family (*RAP2.12*). The protein contains one AP2 domain (Okamuro et al., 1997, Kim et al., 2006).

The expression patterns of these selected candidate genes can be seen in **Appendix B**.

Significantly, the *MOT* locus identified by the significant SNP mapped on chromosome 1, lies roughly 850 kb upstream of the marker most closely associated with the *SDI* locus (Henry et al., 2007). The *SDI* locus has been characterized as the locus controlling sensitivity to dosage imbalance; however the actual gene underlying its action has yet to be cloned. Thus, it is possible that the gene(s) responsible for the %N F2 seed trait within the *MOT* locus may be the same gene(s) within the *SDI* locus that is responsible for sensitivity to dosage imbalance (**Table 5.9).**

| AGI | Coordinates | | | Strand | Distance from the SNP (bp) | Description |
|---|---|---|---|---|---|---|
| **AT4G30280** | 14825808 | - | 14827047 | - | -114494 | Encodes a xyloglucan endotransglucosylase/hydrolase with only the endotransglucosylase activity towards xyloglucan and non-detectable endohydrolytic activity. Expressed in the mature or basal regions of both the main and lateral roots, but not in the tip of these roots where cell division occurs. |
| **AT4G30290** | 14828712 | - | 14830016 | - | -111525 | Encodes a xyloglucan endotransglucosylase/hydrolase with only the endotransglucosylase activity towards xyloglucan and non-detectable endohydrolytic activity. Expressed throughout both the main and the lateral root, with intensive expression at the dividing and elongating regions. Is expressed in lateral root primordia but expression ceases after lateral root begins to grow. |
| **AT4G30300** | 14831005 | - | 14831550 | - | -109991 | member of NAP subfamily |
| **AT4G30310** | 14831861 | - | 14835214 | + | -106327 | FGGY family of carbohydrate kinase; FUNCTIONS IN: carbohydrate kinase activity, phosphotransferase activity, alcohol group as acceptor; INVOLVED IN: carbohydrate metabolic process; LOCATED IN: chloroplast |
| **AT4G30320** | 14835833 | - | 14836318 | + | -105223 | CAP (Cysteine-rich secretory proteins, Antigen 5, and Pathogenesis-related 1 protein) superfamily protein |
| **AT4G30330** | 14836548 | - | 14838020 | - | -103521 | Small nuclear ribonucleoprotein family protein |
| **AT4G30340** | 14838291 | - | 14841067 | - | -100474 | Encodes a diacylglycerol kinase. Applying a specific diacylglycerol kinase inhibitor to the growth media resulted in reduced root elongation and plant growth. Gene is expressed throughout the plant but is strongest in flowers and young seedlings. |
| **AT4G30350** | 14847740 | - | 14851143 | + | -90398 | Double Clp-N motif-containing P-loop nucleoside triphosphate hydrolases superfamily protein |
| **AT4G30360** | 14854811 | - | 14858003 | - | -83538 | Member of Cyclic nucleotide |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | | | | gated channel family |
| **AT4G30370** | 14858490 | - | 14859503 | - | -82038 | RING/U-box superfamily protein; FUNCTIONS IN: zinc ion binding; INVOLVED IN: response to chitin |
| **AT4G30380** | 14860486 | - | 14860994 | + | -80547 | Encodes a Plant Natriuretic Peptide (PNP). PNPs are a class of systemically mobile molecules distantly related to expansins; their biological role has remained elusive. |
| **AT4G30390** | 14862009 | - | 14863185 | - | -78356 | Unknown protein |
| **AT4G30400** | 14866946 | - | 14868628 | + | -72913 | RING/U-box superfamily protein; FUNCTIONS IN: zinc ion binding; INVOLVED IN: response to karrikin |
| **AT4G30410** | 14871109 | - | 14872176 | - | -69365 | Sequence-specific DNA binding transcription factors |
| **AT4G30420** | 14877069 | - | 14878914 | + | -62627 | Nodulin MtN21 /EamA-like transporter family protein |
| **AT4G30430** | 14878783 | - | 14880331 | - | -61210 | Member of TETRASPANIN family |
| **AT4G30440** | 14881855 | - | 14883480 | - | -58061 | UDP-D-glucuronate 4-epimerase |
| **AT4G30450** | 14886027 | - | 14886675 | - | -54866 | Glycine-rich protein |
| **AT4G30460** | 14888980 | - | 14889802 | - | -51739 | Glycine-rich protein |
| **AT4G30470** | 14894105 | - | 14896815 | + | -44726 | NAD(P)-binding Rossmann-fold superfamily protein; FUNCTIONS IN: coenzyme binding, binding, cinnamoyl-CoA reductase activity, catalytic activity; INVOLVED IN: lignin biosynthetic process, cellular metabolic process, metabolic process |
| **AT4G30480** | 14897346 | - | 14899121 | + | -42420 | Encodes one of the 36 carboxylate clamp (CC)-tetratricopeptide repeat (TPR) proteins |
| **AT4G30490** | 14899034 | - | 14902144 | - | -39397 | AFG1-like ATPase family protein; FUNCTIONS IN: ATPase activity, ATP binding |
| **AT4G30500** | 14903487 | - | 14904917 | + | -36624 | Protein of unknown function |
| **AT4G30510** | 14905137 | - | 14907592 | - | -33949 | Homolog of yeast autophagy 18 (ATG18) B (G18B) |
| **AT4G30520** | 14908000 | - | 14911307 | - | -30234 | Leucine-rich repeat protein kinase family protein; FUNCTIONS IN: protein serine/threonine kinase activity, protein kinase activity, ATP binding; INVOLVED IN: protein amino acid phosphorylation; LOCATED IN: endomembrane system |

| | | | | | | |
|---|---|---|---|---|---|---|
| **AT4G30530** | 14920534 | - | 14922494 | + | -19047 | Encodes a gamma-glutamyl peptidase, outside the GGT family, that can hydrolyze gamma-glutamyl peptide bonds. |
| **AT4G30540** | 14923327 | - | 14925106 | + | -16435 | Class I glutamine amidotransferase-like superfamily protein; FUNCTIONS IN: catalytic activity |
| **AT4G30550** | 14925410 | - | 14926861 | + | -14680 | Class I glutamine amidotransferase-like superfamily protein; FUNCTIONS IN: catalytic activity, acyltransferase activity; INVOLVED IN: L-methionine biosynthetic process from homoserine via O-succinyl-L-homoserine and cystathionine; LOCATED IN: endomembrane system, cytoplasm |
| **AT4G30560** | 14926834 | - | 14930156 | - | -11385 | Member of Cyclic nucleotide gated channel family |
| **AT4G30570** | 14930677 | - | 14931951 | - | -9590 | Glucose-1-phosphate adenylyltransferase family protein; FUNCTIONS IN: transferase activity, nucleotidyltransferase activity; INVOLVED IN: biosynthetic process |
| **AT4G30580** | 14932334 | - | 14934528 | - | -7013 | Encodes a plastidic lysophosphatidic acid acyltransferase (LPAAT). Is critical for chloroplasts phosphatidic acid biosynthesis. The null allele is embryo lethal. |
| **AT4G30590** | 14935623 | - | 14936562 | - | -4979 | Early nodulin-like protein 12 (ENODL12); FUNCTIONS IN: electron carrier activity, copper ion binding; LOCATED IN: anchored to membrane |
| **AT4G30600** | 14937840 | - | 14941018 | - | -523 | Signal recognition particle receptor alpha subunit family protein; FUNCTIONS IN: nucleoside-triphosphatase activity, signal recognition particle binding, GTP binding, GTPase activity, nucleotide binding; INVOLVED IN: intracellular protein transport, protein targeting, SRP-dependent cotranslational protein targeting to membrane; LOCATED IN: endoplasmic reticulum, signal recognition particle, endoplasmic reticulum targeting |

| | | | | | | |
|---|---|---|---|---|---|---|
| **AT4G30610** | 14944129 | - | 14948605 | + | 2588 | Encodes a secreted glycosylated serine carboxypeptidase with broad substrate preference that is involved in brassinosteroid signalling via BRI1. It is proteolytically processed in vivo by a separate as yet unidentified protease |
| **AT4G30620** | 14948586 | - | 14950072 | - | 7045 | Uncharacterised BCR, YbaB family COG0718; LOCATED IN: chloroplast, chloroplast envelope |
| **AT4G30630** | 14950638 | - | 14952376 | + | 9097 | Unknown protein |
| **AT4G30640** | 14952670 | - | 14953682 | + | 11129 | RNI-like superfamily protein |
| **AT4G30650** | 14954320 | - | 14954968 | + | 12779 | Low temperature and salt responsive protein family; INVOLVED IN: response to salt stress, response to cold, hyperosmotic salinity response, defence response to fungus; LOCATED IN: endomembrane system, integral to membrane |
| **AT4G30660** | 14955373 | - | 14956171 | + | 13832 | Low temperature and salt responsive protein family; INVOLVED IN: response to cold, hyperosmotic salinity response; LOCATED IN: endomembrane system, integral to membrane |
| **AT4G30662** | 14955909 | - | 14956790 | - | 14368 | Unknown protein |
| **AT4G30670** | 14957358 | - | 14957962 | - | 15817 | Putative membrane lipoprotein |
| **AT4G30680** | 14958687 | - | 14960321 | - | 17146 | Initiation factor eIF-4 gamma, MA3; |
| **AT4G30690** | 14960646 | - | 14962466 | + | 19105 | Translation initiation factor 3 protein; FUNCTIONS IN: translation initiation factor activity; INVOLVED IN: translational initiation; LOCATED IN: cell wall, chloroplast |
| **AT4G30700** | 14962493 | - | 14965015 | - | 20952 | Pentatricopeptide repeat (PPR) superfamily protein |
| **AT4G30710** | 14965200 | - | 14969763 | - | 23659 | Family of unknown function (DUF566) |
| **AT4G30720** | 14971906 | - | 14975519 | - | 30365 | Encodes a putative oxidoreductase/electron carrier detected in the chloroplast stroma that is essential to ensure a correct electron flow through the photosynthetic chain and, hence, photosynthesis efficiency and normal growth. Mutations in the Col-0 allele result in pale green pigmentation and defective growth. |

| | | | | | | |
|---|---|---|---|---|---|---|
| **AT4G30730** | 14976418 | - | 14976732 | - | 34877 | Unknown protein |
| **AT4G30740** | 14979246 | - | 14979521 | - | 37705 | Unknown protein |
| **AT4G30750** | 14981186 | - | 14982354 | - | 39645 | Unknown protein |
| **AT4G30760** | 14982522 | - | 14983421 | + | 40981 | Putative endonuclease or glycosyl hydrolase |
| **AT4G30770** | 14985641 | - | 14986872 | - | 44100 | Putative membrane lipoprotein |
| **AT4G30780** | 14990341 | - | 14993069 | - | 48800 | Unknown protein |
| **AT4G30790** | 14993086 | - | 14997785 | - | 51545 | INVOLVED IN: autophagy |
| **AT4G30800** | 15001134 | - | 15002671 | + | 59593 | Nucleic acid-binding, OB-fold-like protein |
| **AT4G30810** | 15003451 | - | 15006212 | + | 61910 | Serine carboxypeptidase-like 29 (scpl29); FUNCTIONS IN: serine-type carboxypeptidase activity; INVOLVED IN: proteolysis; LOCATED IN: vacuole |
| **AT4G30820** | 15006489 | - | 15008546 | + | 64948 | Cyclin-dependent kinase-activating kinase assembly factor-related / CDK-activating kinase assembly factor-related; INVOLVED IN: cell cycle; LOCATED IN: nucleus |
| **AT4G30825** | 15009605 | - | 15012319 | + | 68064 | Tetratricopeptide repeat (TPR)-like superfamily protein; INVOLVED IN: cell cycle; LOCATED IN: nucleus |
| **AT4G30830** | 15015267 | - | 15016977 | - | 73726 | Protein of unknown function |
| **AT4G30840** | 15017615 | - | 15019735 | - | 76074 | Transducin/WD40 repeat-like superfamily protein;LOCATED IN: chloroplast |
| **AT4G30845** | 15019877 | - | 15020509 | + | 78336 | Unknown protein;LOCATED IN: chloroplast |
| **AT4G30850** | 15020182 | - | 15022445 | - | 78641 | Heptahelical transmembrane protein homologous to human adiponectin receptors and progestin receptors |
| **AT4G30860** | 15024472 | - | 15027616 | + | 82931 | Encodes a member of the trxG protein family. Contains a SET domain which is known to be involved in the modification of histone tails by methylation. Interacts physically with AMS, but the implications of this interaction are unknown. Overexpression results in plieotrophic developmental defects. |
| **AT4G30870** | 15028427 | - | 15032634 | + | 86886 | Encodes an Arabidopsis homolog of the endonuclease MSU81. T-DNA insertion lines of AtMSU81 have a deficiency in homologous |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | | | | recombination in somatic cells but only after genotoxic stress. Crosses with a hyperrecombinogenic mutant of the AtRecQ4A helicase resulting in synthetic lethality in the double mutant |
| **AT4G30872** | 15032978 | - | 15033256 | - | 91437 | Other RNA |
| **AT4G30880** | 15035207 | - | 15035843 | + | 93666 | Bifunctional inhibitor/lipid-transfer protein/seed storage 2S albumin superfamily protein; FUNCTIONS IN: lipid binding; INVOLVED IN: lipid transport; LOCATED IN: endomembrane system |
| **AT4G30890** | 15036052 | - | 15039281 | - | 94511 | Encodes a ubiquitin-specific protease |
| **AT4G30900** | 15039432 | - | 15042311 | + | 97891 | DNAse I-like superfamily protein |
| **AT4G30910** | 15042413 | - | 15045315 | - | 100872 | Cytosol aminopeptidase family protein; FUNCTIONS IN: manganese ion binding, metalloexopeptidase activity, aminopeptidase activity; INVOLVED IN: proteolysis, protein metabolic process; LOCATED IN: chloroplast; EXPRESSED IN: guard cell |
| **AT4G30920** | 15046457 | - | 15049373 | - | 104916 | Encodes LAP2, an aminopeptidase playing key roles in senescence, stress response and amino acid turnover |
| **AT4G30930** | 15049824 | - | 15051665 | - | 108283 | Encodes a ribosomal RPL21M protein that is localized to the mitochondrion and is involved in karyogamy during female gametophyte development and fertilization. Mutants display defects in both male and female gametophyte development (i.e. collapsed pollen and female gametophytes with unfused central cells). |
| **AT4G30935** | 15051814 | - | 15054042 | - | 110273 | Member of WRKY Transcription Factor; Group I |
| **AT4G30940** | 15054873 | - | 15056826 | + | 113332 | BTB/POZ domain with WD40/YVTN repeat-like protein; FUNCTIONS IN: voltage-gated potassium channel activity; INVOLVED IN: potassium ion transport; LOCATED IN: voltage-gated potassium channel complex, membrane; EXPRESSED IN: leaf whorl, male gametophyte, flower, pollen |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | | | | tube; EXPRESSED DURING: L mature pollen stage, M germinated pollen stage, 4 anthesis |
| **AT4G30950** | 15056975 | - | 15059794 | - | 115434 | Chloroplastic enzyme responsible for the synthesis of 16:2 and 18:2 fatty acids from galactolipids, sulpholipids and phosphatidylglycerol. Uses ferredoxin as electron donor. Gene mutation resulted in reduced level of unsaturated fatty acids leading to susceptibility to photoinhibition |

**Table 5.8.** Genes located 120 kb upstream (-) and 120 kb downstream (+) of a SNP significantly associated with the production of normal F2 seeds by *Arabidopsis thaliana* triploids generated from 2x X 4x inter-ploidy crosses.

| AGI | Coordinates | | | Strand | Distance from the SNP (bp) | Description |
|---|---|---|---|---|---|---|
| **AT1G53345** | 19902495 | - | 19903700 | + | -117520 | Unknown protein, EXPRESSED DURING: 4 anthesis, F mature embryo stage |
| **AT1G53350** | 19903899 | - | 19907515 | + | -113705 | Disease resistance protein (CC-NBS-LRR class) family; FUNCTIONS IN: ATP binding; INVOLVED IN: apoptosis, defence response |
| **AT1G53360** | 19907517 | - | 19908548 | + | -112672 | F-box associated ubiquitination effector family protein |
| **AT1G53366** | 19909840 | - | 19909977 | - | -111243 | Unknown protein |
| **AT1G53370** | 19910516 | - | 19911646 | + | -109574 | F-box and associated interaction domains-containing protein |
| **AT1G53380** | 19913076 | - | 19916517 | - | -104703 | Plant protein of unknown function (DUF641) |
| **AT1G53390** | 19918047 | - | 19923807 | + | -97413 | Non-intrinsic ABC protein 12 , P-loop containing nucleoside triphosphate hydrolases superfamily protein; FUNCTIONS IN: ATPase activity, coupled to transmembrane movement of substances; LOCATED IN: plasma membrane |
| **AT1G53400** | 19924752 | - | 19926631 | + | -94589 | Ubiquitin domain-containing protein; INVOLVED IN: N-terminal protein myristoylation |
| **AT1G53410** | 19925839 | - | 19925910 | - | -95310 | Pre-tRNA; tRNA-Cys (anticodon: GCA) |
| **AT1G53420** | 19926626 | - | 19931494 | - | -89726 | Leucine-rich repeat transmembrane protein kinase; FUNCTIONS IN: protein serine/threonine kinase activity, kinase activity, ATP binding; INVOLVED IN: transmembrane receptor protein tyrosine kinase signalling pathway, protein amino acid phosphorylation |
| **AT1G53430** | 19935163 | - | 19941088 | + | -80132 | Leucine-rich repeat transmembrane protein kinase; FUNCTIONS IN: kinase activity; INVOLVED IN: protein amino acid phosphorylation |
| **AT1G53440** | 19945959 | - | 19951562 | + | -69658 | Leucine-rich repeat transmembrane protein kinase; FUNCTIONS IN: kinase activity; INVOLVED IN: protein amino acid phosphorylation |
| **AT1G53450** | 19951006 | - | 19954115 | - | -67105 | Unknown protein |
| **AT1G53460** | 19954490 | - | 19956632 | - | -64588 | Ran BP2/NZF zinc finger-like superfamily protein |

| | | | | | | |
|---|---|---|---|---|---|---|
| **AT1G53470** | 19958587 | - | 19961578 | + | -59642 | Mechanosensitive channel of small conductance-like 4 (MSL4); INVOLVED IN: transmembrane transport |
| **AT1G53480** | 19963047 | - | 19964712 | - | -56508 | Encodes MRD1 (mto 1 responding down). Down-regulated in mto1-1 mutant that over-accumulates soluble methionine. |
| **AT1G53490** | 19963267 | - | 19966952 | + | -54268 | RING/U-box superfamily protein |
| **AT1G53500** | 19966944 | - | 19969757 | - | -51463 | Encodes a putative NDP-L-rhamnose synthase, an enzyme required for the synthesis of the pectin rhamnogalacturonan I, the major component of Arabidopsis mucilage. Gene is involved in seed coat mucilage cell development. Mutant analyses suggest that MUM4 is required for complete mucilage synthesis, cytoplasmic rearrangement and seed coat development. |
| **AT1G53510** | 19970664 | - | 19974393 | - | -46827 | Member of MAP Kinase |
| **AT1G53520** | 19976415 | - | 19977955 | - | -43265 | Chalcone-flavanone isomerase family protein; FUNCTIONS IN: chalcone isomerase activity, intramolecular lyase activity; INVOLVED IN: cellular amino acid derivative biosynthetic process, flavonoid biosynthetic process; LOCATED IN: chloroplast stroma, chloroplast; |
| **AT1G53530** | 19978146 | - | 19980084 | + | -41136 | Peptidase S24/S26A/S26B/S26C family protein; FUNCTIONS IN: serine-type peptidase activity, peptidase activity; INVOLVED IN: proteolysis |
| **AT1G53540** | 19980462 | - | 19981109 | + | -40111 | HSP20-like chaperones superfamily protein, heat shock protein 17.4 |
| **AT1G53541** | 19981385 | - | 19981495 | - | -39725 | Unknown protein |
| **AT1G53542** | 19981628 | - | 19981813 | + | -39407 | Unknown protein |
| **AT1G53543** | 19981969 | - | 19982124 | + | -39096 | Unknown protein |
| **AT1G53550** | 19983411 | - | 19984637 | + | -36583 | F-box family protein |
| **AT1G53560** | 19984742 | - | 19985983 | - | -35237 | Ribosomal protein L18ae family |
| **AT1G53570** | 19987191 | - | 19991135 | + | -30085 | MEK kinase (MAP3Ka) |
| **AT1G53580** | 19991275 | - | 19993334 | - | -27886 | Mononuclear Fe(II)-containing member of the b-lactamase fold superfamily. ETHE1 is homodimeric in solution, exhibits low-level esterase activity, and specifically binds a single Fe(II) |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | | | | atom in the active site. |
| **AT1G53590** | 19996315 | - | 20000265 | + | -20955 | Calcium-dependent lipid-binding (CaLB domain) family protein |
| **AT1G53600** | 20001263 | - | 20003416 | + | -17804 | Tetratricopeptide repeat (TPR)-like superfamily protein |
| **AT1G53610** | 20008996 | - | 20009320 | - | -11900 | Unknown protein |
| **AT1G53620** | 20011429 | - | 20011925 | - | -9295 | Unknown protein |
| **AT1G53625** | 20014410 | - | 20014923 | - | -6297 | Unknown protein |
| **AT1G53633** | 20017389 | - | 20017932 | - | -3288 | Unknown protein |
| **AT1G53635** | 20019293 | - | 20019799 | - | -1421 | Unknown protein |
| **AT1G53640** | 20020885 | - | 20025461 | - | 0 | Unknown protein |
| **AT1G53645** | 20026147 | - | 20028635 | - | 4927 | Hydroxyproline-rich glycoprotein family protein |
| **AT1G53650** | 20029029 | - | 20031566 | - | 7809 | RNA-binding protein, putative, similar to RNA-binding protein GB:AAA86641 .Contains PAB2 domain which facilitates binding to PABC proteins. |
| **AT1G53660** | 20034032 | - | 20035946 | + | 12812 | Nucleotide/sugar transporter family protein |
| **AT1G53670** | 20036641 | - | 20038219 | + | 15421 | Methionine sulfoxide reductase B 1 (MSRB1); FUNCTIONS IN: peptide-methionine-(S)-S-oxide reductase activity; INVOLVED IN: response to oxidative stress, N-terminal protein myristoylation; LOCATED IN: chloroplast stroma, chloroplast |
| **AT1G53680** | 20038358 | - | 20039117 | + | 17138 | Encodes glutathione transferase belonging to the tau class of GSTs |
| **AT1G53683** | 20039463 | - | 20039616 | - | 18243 | Encodes a microRNA that targets several HAP2 family members |
| **AT1G53687** | 20041518 | - | 20041734 | + | 20298 | Encodes a microRNA that targets several HAP2 family members |
| **AT1G53690** | 20042640 | - | 20043344 | - | 21420 | Protein of unknown function that is homologous to At5g41010, which encodes a non-catalytic subunit common to nuclear DNA-dependent RNA polymerases II, IV and V |
| **AT1G53700** | 20048586 | - | 20050115 | + | 27366 | The WAG1 and its homolog, WAG2 each encodes a protein-serine/threonine kinase that are nearly 70% identical to PsPK3 protein. All three together with CsPK3 belong to PsPK3-type kinases. |

| | | | | | | |
|---|---|---|---|---|---|---|
| **AT1G53705** | 20050475 | - | 20051451 | - | 29255 | Aminoacyl-tRNA ligases; nucleotide binding; ATP binding; FUNCTIONS IN: nucleotide binding, aminoacyl-tRNA ligase activity, ATP binding; INVOLVED IN: translation, tRNA aminoacylation for protein translation; LOCATED IN: endomembrane system, cytoplasm |
| **AT1G53708** | 20052393 | - | 20053195 | + | 31173 | ROTUNDIFOLIA like 9 (RTFL9) |
| **AT1G53710** | 20053161 | - | 20056396 | - | 31941 | Calcineurin-like metallo-phosphoesterase superfamily protein; FUNCTIONS IN: hydrolase activity, protein serine/threonine phosphatase activity |
| **AT1G53720** | 20056533 | - | 20059638 | + | 35313 | Encodes a cyclophilin, member of a family of modular proteins consisting of a peptidyl-prolyl cis– trans isomerase (PPIase) domain, followed by an RNA recognition motif (RRM), and a C-terminal domain enriched in charged amino acids. Interacts with SCL33/SR33 and with a majority of Arabidopsis SR proteins and the largest subunit of RNA polymerase II. Localizes to the nucleus, but it does not significantly co-localize with SR proteins in nuclear speckles. |
| **AT1G53730** | 20061701 | - | 20065713 | + | 40481 | STRUBBELIG-receptor family 6 (SRF6); FUNCTIONS IN: protein serine/threonine kinase activity, protein kinase activity, ATP binding; INVOLVED IN: transmembrane receptor protein tyrosine kinase signaling pathway, protein amino acid phosphorylation |
| **AT1G53750** | 20065714 | - | 20068466 | - | 44494 | 26S proteasome AAA-ATPase subunit RPT1a (RPT1a) mRNA |
| **AT1G53760** | 20068968 | - | 20071096 | - | 47748 | Unknown protein; LOCATED IN: mitochondrion, plasma membrane, plastid, membrane |
| **AT1G53770** | 20071311 | - | 20073758 | - | 50091 | O-fucosyltransferase family protein |
| **AT1G53780** | 20073996 | - | 20078228 | - | 52776 | Peptidyl-prolyl cis-trans isomerases; hydrolases; nucleoside-triphosphatases; ATP binding; nucleotide binding; ATPases; INVOLVED IN: protein folding, protein catabolic process |

| | | | | | | |
|---|---|---|---|---|---|---|
| **AT1G53785** | 20077798 | - | 20079002 | - | 56578 | Unknown protein; LOCATED IN: mitochondrion |
| **AT1G53800** | 20081699 | - | 20084500 | + | 60479 | Unknown protein |
| **AT1G53801** | 20083211 | - | 20084173 | - | 61991 | Potential natural antisense gene, locus overlaps with AT1G53800 |
| **AT1G53815** | 20090249 | - | 20090962 | - | 69029 | F-box family protein |
| **AT1G53820** | 20091491 | - | 20092423 | + | 70271 | RING/U-box superfamily protein; FUNCTIONS IN: zinc ion binding |
| **AT1G53830** | 20098522 | - | 20100889 | + | 77302 | Encodes a pectin methylesterase |
| **AT1G53840** | 20101445 | - | 20103667 | + | 80225 | Encodes a pectin methylesterase |
| **AT1G53850** | 20103953 | - | 20106015 | - | 82733 | Encodes alpha5 subunit of 20s proteosome involved in protein degradation and RNA degradation |
| **AT1G53860** | 20107107 | - | 20109511 | - | 85887 | Remorin family protein |
| **AT1G53870** | 20112262 | - | 20113522 | + | 91042 | Protein of unknown function (DUF567) |
| **AT1G53880** | 20115069 | - | 20118485 | + | 93849 | Eukaryotic translation initiation factor 2B (eIF-2B) family protein; FUNCTIONS IN: GTP binding, translation initiation factor activity; INVOLVED IN: translational initiation, cellular metabolic process |
| **AT1G53885** | 20119723 | - | 20120458 | + | 98503 | Protein of unknown function (DUF581) |
| **AT1G53887** | 20122426 | - | 20122866 | - | 101206 | Unknown protein |
| **AT1G53890** | 20124827 | - | 20126109 | + | 103607 | Protein of unknown function (DUF567) |
| **AT1G53900** | 20127634 | - | 20131050 | + | 106414 | Eukaryotic translation initiation factor 2B (eIF-2B) family protein; FUNCTIONS IN: GTP binding, translation initiation factor activity; INVOLVED IN: translational initiation, cellular metabolic process |
| **AT1G53903** | 20132288 | - | 20133017 | + | 111068 | Protein of unknown function (DUF581) |
| **AT1G53910** | 20135112 | - | 20136970 | + | 113892 | Encodes a member of the ERF (ethylene response factor) subfamily B-2 of ERF/AP2 transcription factor family (RAP2.12). The protein contains one AP2 domain. |
| **AT1G53920** | 20137712 | - | 20139637 | + | 116492 | Contains lipase signature motif and GDSL domain |
| **AT1G53930** | 20140411 | - | 20140887 | - | 119191 | Ubiquitin-like superfamily protein |

**Table 5.9.** Genes located 120 kb upstream (-) and 120 kb downstream (+) of a SNP significantly associated with the production of normal F2 seeds by *Arabidopsis thaliana* F1 triploids generated from 4x X 2x inter-ploidy crosses.

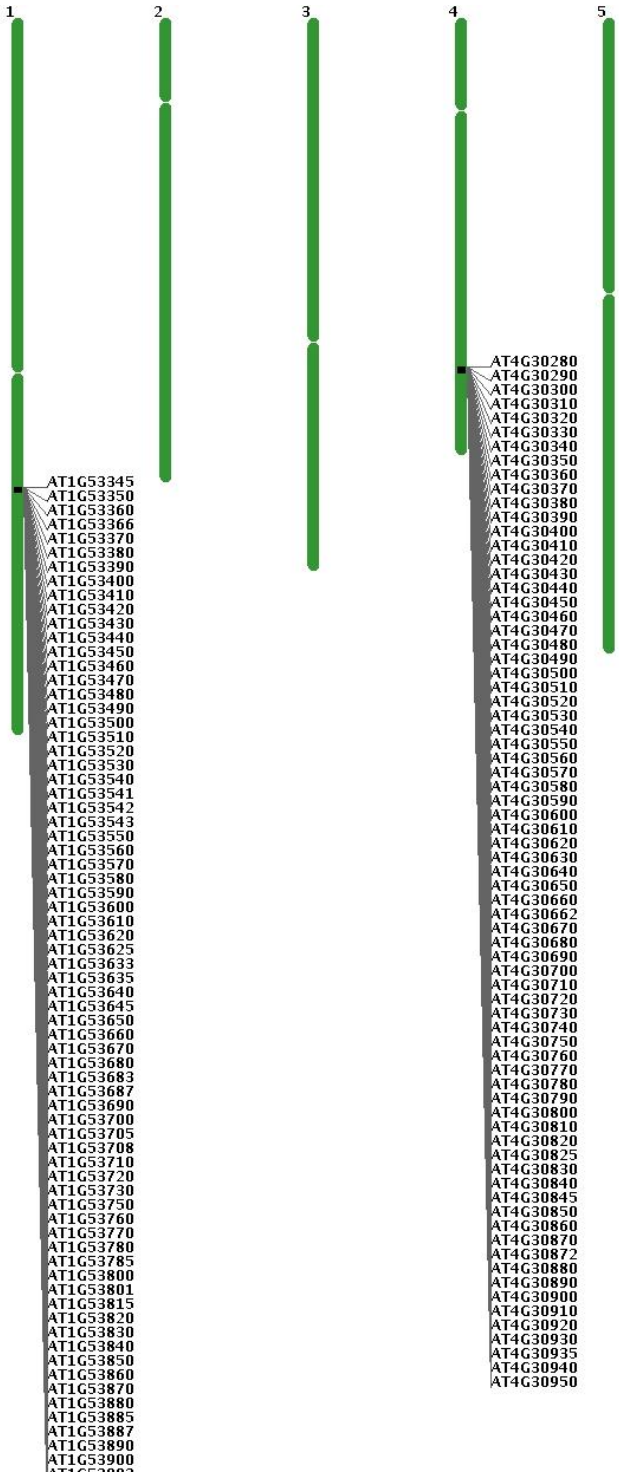



**Figure 5.9.** Genes located 120 kb upstream and 120 kb downstream of the SNPs significantly associated with the production of normal seeds by *Arabidopsis thaliana* F1 triploids generated from 4x X 2x (Chromosome 1) and 2x X 4x (Chromosome 4) inter-ploidy crosses.

## 5.4 Discussion

Isogenic F1 triploids generated from the Col-0 accession displayed different % N F2 seed characteristics to isogenic F1 triploids from the other three accessions (L*er*-0, C24, Zu). These were (1) highest % N F2 seed from F1 isogenic Col-0 plants and (2) parent-of-origin effects on % N F2 seed-set between paternal excess versus maternal excess F1 isogenic triploids (**Figure 5.1, Table 5.1**). Previously, 2x X 4x inter-ploidy Col-0 crosses have been shown to result in low levels of normal (N) F1 isogenic triploid seeds due to extreme lethality (~80% aborted) observed in F1 triploid seeds. In contrast, L*er*-0 inter-ploidy 2x X 4x crosses produced around 80% of viable (N) F1 seeds (Dilkes et al., 2008). In this study, the % of normal (N) F2 seeds obtained from surviving isogenic F1 triploids of Col-0 and L*er*-0 also displayed differences. The surviving Col-0 F1 triploids yielded the highest % of normal (N) F2 seeds, while the surviving L*er*-0 F1 triploids yielded the lowest % of normal (N) F2 seeds (**Figure 5.1**). The observations of Dilkes et al, (2008) suggest that there is a block to F1 seed development from isogenic inter-ploidy crosses of *Arabidopsis thaliana* which differs between accessions (i.e. the block to F1 seed development displays a higher penetrance in the Col-0 inter-ploidy cross). It may be possible that F1 triploid seeds having survived the more extreme block in the Col-0 background have a higher propensity to yield normal F2 seeds when selfed (**Figure 5.1, Table 5.1**).

The reproductive performance of triploids has been studied in diploids of the Col-0 accession crossed with colchicine-doubled Col-0, and Wa-1 natural tetraploids. They were shown to be fertile with CWW hybrid triploids producing significantly more plump (normal) seeds than the CCC isogenic triploid (Henry et al., 2005). In this study, analysis of reproduction in four isogenic triploids (Col-0, C24, L*er*-0, and Zu) revealed that between 17% and 50% of all ovules in a stigma gave rise to normal seeds (**Table 5.1**). The remaining ovules were either left unfertilized or aborted due to post-zygotic lethality. In contrast to Henry et al, (2005) who only studied one isogenic (Col-0) and one hybrid (Col-0/Wa-1) F1 triploid line, hybrid F1 triploids used in this study appeared to experience stronger reduction in normal seed production than isogenic triploids, with %N ranging from 10 to 35%. However, compared to the F1 L*er*-0 isogenic triploids, which produced approximately 18% of normal F2 seeds, F1 hybrid triploids showed both improved and

decreased reproductive performance (N% F2 seeds) depending on the diploid accession used as the other parent (**Table 5.2, Table 5.4, Figure 5.3**).

As decreased reproductive success of F1 hybrid triploids could be a consequence of hybrid dysgenesis between L*er*-0 and some other accessions (Bomblies et al., 2010), it was determined that F1 hybrid diploids displayed no such evidence and they produced over 91% of normal seeds regardless of the cross direction (**Table 5.5**).

This study demonstrates that the fertility (i.e. % N F2 seeds) of inter-accession F1 hybrid triploids can vary significantly between F1 hybrid triploids via some genetic (between accessions) and epigenetic effects (between reciprocal F1 triploids) (**Table 5.3**). To the author's knowledge this represents the first evidence of epigenetic parent-of-origin effects controlling normal F2 seed development from selfed F1 hybrid triploid plants.

Both isogenic (Col-0) and hybrid (Col-0 X Wa-1) triploids have been previously shown to produce a swarm of viable aneuploid F2 offspring after they were allowed to self-pollinate (Henry et al., 2005). The F2 progeny spanned a wide range of DNA content, including diploids, triploids, tetraploids and aneuploids consisting of complex karyotypes of intermediate genome content. The distribution of the F2 isogenic family consisted of more individuals in higher genome content class (3.6 - 4.2), while more F2 hybrid progenies fell in the lower 3.2 – 3.4 class (**Figure B**). The genome content distributions of F2 progeny deviated from the expected theoretical distribution (random chromosome segregation and absence of selection, **Figure A**). Higher than expected frequency of euploid progeny observed in both cases, suggested that there was a strong selection against aneuploidy or aneuploid gamete formation (Henry et al., 2005). This occurred under the control of a single major-effect locus (*SDI*) and demonstrated that *Arabidopsis thaliana* is a species in which triploids can indeed act as bridges between populations with different stable ploidies (Henry et al., 2007).

In this chapter, the ploidy distribution in samples of ~90 seeds produced by 19 pairs of reciprocal hybrid triploids and one pair of isogenic L*er*-0 triploids was determined. It was demonstrated that genome content distributions varied between triploids with different levels of reproductive success. A full model of this process for *Arabidopsis thaliana* would incorporate both the tendency to euploidy seen over generations (Henry et al., 2005), but also the existence of occasional more drastically altered individuals, for example the existence of F2 seeds as large as pentaploid or apparently lower than diploid (**Figure 5.7**). Variation in triploid reproductive success may depend upon the tendency of gametes to experience reduced ploidy levels caused by meiotic defects. Distribution of aneuploidy types in triploid offspring varies between genotypes (**Figure 5.6, Figure 5.7**), and may therefore have differing effects on plant fertility during the evolution of a lineage. This tendency to genome loss or retention displays genetic variation in the response to polyploidy and supports the well-established link between polyploidy and speciation (Soltis and Soltis, 2009). Aneuploid syndrome has a strong phenotypic effect in the next generation, indicating roles in triploid reproduction for a combination of maternal effects, signalling between the parental plants, and, potentially, elements of epigenetic inheritance. This tendency of aneuploids and polyploids to differ transgressively from their parents has been well-marked in hybrids (Rapp et al., 2009) and in heterosis (Chen, 2010) and is particularly notable in invasive species (Ainouche et al., 2009, Blair and Hufbauer, 2010). Polyploidy and aneuploidy are known to induce variation of epigenetic regulation and epigenetic silencing may result from dosage imbalance (Matzke et al., 1999, Pikaard, 2001, Mittelsten Scheid et al., 2003, Mittelsten Scheid et al., 1996). An *SDI* locus could possibly be linked to a specific dosage-sensitive gene or encode a regulator mediating a genome-wide epigenetic response to dosage imbalance (Henry et al., 2007).

A GWA study was used in order to find a correlation between the measured variation in triploid reproductive character (%N) and genome-wide SNP polymorphisms determined for the diploid accessions (Nordborg et al., 2005). *Arabidopsis thaliana* populations are heavily structured which causes the possibility of increased false-positive results (Zhao et al., 2007). Statistical methods used to control population structure include a mixed-model approach (EMMA) which has been proved to perform well in *Arabidopsis thaliana* (Yu et

al., 2005b, Atwell et al., 2010, Kang et al., 2008a). Little evidence for population structure in p-values led to the conclusion that false-positives generated from population structure had little confounding effect on this study.

Even though the power of this analysis was limited due to the relatively low sample size used (88 4x X 2x triploids and 78 2x X 4x triploids), two SNPs significantly associated with the production of viable seeds by reciprocal hybrid triploids have been identified (**Figure 5.8**). Different SNPs associated with the %N trait in triploids generated from reciprocal inter-ploidy crosses suggest that parent-of-origin dosage effects can have trans-generational effects on reproduction. This shows that the locus *MOT* associated with the SNP on chromosome 1 controls the 4x X 2x Maternal Overdose Triploid reproduction but does not have any significant impact on the 2x X 4x paternal overdose triploid reproductive success. Reciprocally, a locus *POT* controlling F2 seed viability in the 2x X 4x Paternal Overdose Triploids associated with the SNP on chromosome 4 does not significantly impact the 4x X 2x maternal overdose triploid reproduction. This observation justified the selection of most promising candidate genes based on the link between triploid reproduction and epigenetic regulation, maternal/paternal effects, dosage sensitivity or genomic imprinting, etc (**Table 5.8, Table 5.9**).

Five candidate genes explaining the variation in reproductive success of 4x X 2x F1 maternal excess triploids are located on the bottom arm of chromosome 1 **(Figure 5.9**). They have been selected among 74 associated genes spanning a 120 kb range up- and downstream of the significant SNP. They include a gene (At1g53500) involved in the seed coat mucilage and a (At1g53910) ERF transcription factor containing a AP2 domain (potential maternal effects through the control of integuments and seed coat), a gene (At1g53650) encoding RNA-binding protein containing a PAB2 domain, interacting with the known *MPC* imprinted gene (*MATERNALLY EXPRESSED PAB C-TERMINAL*), and At1g53860 and At1g53690 genes which may be involved in gene expression control via DNA (de)methylation and DNA-dependent RNA polymerase II, IV and V activity.

1) Fertilization of the angiosperm ovule initiates differentiation of the maternal sporophytic tissues of integuments to form the seed coat. The seed coat layers can undergo a number of specializations that aid embryo nutrition, seed dispersal, germination, and seed longevity. Seed coat epidermis also produce a hydrophilic polysaccharide slime, known as mucilage, which promotes the seed hydration and germination, prevents the gas exchange, and helps in attachment to soil substrates and animal vectors (Arsovski et al., 2009). **At1g53500** – *MUM4* encodes a UDP-L-rhamnose synthase (RHM2) required for the production of the primary mucilage pectin RG I (Usadel et al., 2004, Western et al., 2004). Other genes known to affect seed coat mucilage include: *TRANSPARENT TESTA GLABRA1* (*TTG1*), *TTG2*, *GLABRA2* (*GL2*), *APETALA2* (*AP2*), *ABERRANT TESTA SHAPE*, and *ABSCISIC ACID DEFICIENT1* (Western et al., 2001). Expression studies suggest that *MUM4* is developmentally regulated in the seed coat by *AP2, TTG1*, and *GL2* (Western et al., 2004).
2) In *Arabidopsis thaliana*, the homeotic gene APETALA2 (AP2) has been shown to control processes during flower development such as the establishment of flower meristem identity, regulation of floral organogenesis and the temporal and spatial regulation of flower homeotic gene activity. Genetic studies have shown that AP2 is also required for normal ovule and seed development. **At1g53910** – ERF/AP2 (ethylene response factor) transcription factor belongs to the RAP2 (related to AP2) family of DNA-binding protein because it contains an AP2 domain (Okamuro et al., 1997). Another ERF/AP2 gene which regulates ovule development and floral organ growth is *ANT* (*AINTEGUMENTA).* ERF-like genes appear to be involved in responses to biotic and environmental stress, although their precise functions are largely unknown (Kim et al., 2006).
*3)* The *MATERNALLY EXPRESSED PAB C-TERMINAL (MPC)* imprinted gene encodes the C-terminal domain of poly(A) binding proteins (PABC) (Tiwari et al., 2008). Imprinting in plants has dramatic consequences on seed development, affecting seed size, morphogenesis, and viability (Gehring et al., 2004). Reduction of MPC expression results in seed abortion, decreased seed size, and embryo and endosperm abnormalities. Poly(A) binding proteins (PABPs) are known to play

roles in mRNA stability and translation by binding the poly(A) tails of mRNAs through N-terminal RNA recognition motifs (Bravo et al., 2005). The **At1g53650** gene encodes a RNA-binding protein containing a PAB2 domain which facilitates binding to PABC. The PABC domain is an independent region that mediates protein–protein interactions, thus the **At1g53650** gene product can interact with *MPC.*

4) In *Arabidopsis thaliana*, cytosine methylation is important for genomic imprinting and genome defence against transposable elements, therefore most DNA methylation is located at transposon-rich heterochromatic regions (Chan et al., 2005). DNA methylation is a stable epigenetic mark, but active demethylation mediated by the *DEMETER (DME)* or *ROS1* has been observed in *Arabidopsis thaliana*. Demethylation by *DME* establishes genomic imprinting in the *Arabidopsis thaliana* endosperm by targeting genes such as: *MEDEA (MEA), FWA*, and *FIS2*. In vegetative tissue, the default state for these genes is methylated however, in the reproductive central cell, *DME* excises a 5-methylcytosine at *MEA*, *FWA* and *FIS2,* establishing hypomethylated, transcriptionally active alleles of these genes. A *DME* demethylation functions to protect endogenous genes from potentially deleterious methylation (Penterman et al., 2007). **At1g53860 –** encodes a remorin family protein that was identified as one of the 179 discrete loci throughout the genome that undergo *DME* DNA demethylation. Those loci represented sites where two opposing pathways, DNA methylation and demethylation, converge (Penterman et al., 2007).
5) RNA polymerases, Pol I – V, are enzymes that are central to nuclear gene expression each of which is composed of between 12 and 17 subunits. They play non-redundant roles in siRNA-directed DNA methylation and gene silencing in plants (Ream et al., 2009). **At1g53690** encodes a protein of unknown function; however the gene is homologous to **At5g41010** which is known to express a non-catalytic subunit (NRPB12) of nuclear DNA-dependent RNA polymerases. Pol IV and Pol V share numerous small subunits with Pol II, including NRPB12 family subunits, and the loss-of-function mutations in this subunit cause female gametophyte lethality. Interestingly, defective male microspores develop into

mature gametophytes that fertilize wild-type female gametophytes, and transmit the mutant genes to the next generation (Onodera et al., 2008).

All five of these genes represent an attractive system for explaining the variation in the 4x X 2x F1 maternal excess triploid reproduction. There is also a possibility that some of the other genes listed among the 74 genes spanning the significant SNP have a casual effect, although their involvement in reproductive pathways is less obvious (**Table 5.9**). Moreover, some of the candidate genes discussed above could be potentially responsible for the *SDI* locus action as they lie in the close vicinity of its rough position.

Seventy three genes on chromosome 4 were significantly associated with the variation observed in the F1 2x X 4x paternal excess triploid reproductive success (**Figure 5.9, Table 5.8**). Four of those genes have been selected as particularly interesting candidates due to their known involvement in reproductive pathways.

1) Phosphatidic acid is a key intermediate for chloroplast membrane lipid biosynthesis. **At4g30580** gene encodes plastidic lysophosphatidic acid acyltransferase (*LPAAT*), a critical enzyme for chloroplasts phosphatidic acid biosynthesis. Although the heterozygous mutant has no apparent phenotype, the embryos representing the homozygous *lpaat* mutant die at an early stage during embryogenesis (Kim and Huang, 2004). Apparently, plastidic *LPAAT* is essential for embryo development in *Arabidopsis thaliana* during the transition from the globular to the heart stage when chloroplasts begin to form (Yu et al., 2004).
2) The adjacent gene - **At4g30590** encodes an early nodulin (ENOD)-like protein 12, which localizes to the plasma membrane. It contains a plastocyanin-like copper-binding domain and its biological function is to carry electrons. This gene was found to be expressed in the female gametophyte and thus is believed to play a role during embryo sac development (Yu et al., 2005a). A sieve element-specific *ENODL* was reported to determine reproductive potential in *Arabidopsis thaliana* (Khan et al., 2007).
3) Plant SET domain proteins are known to be involved in the epigenetic control of gene expression through specific methylation of lysine residues on histone H3 and

histone H4 (Springer et al., 2003). The majority of the *Arabidopsis thaliana* SET domain proteins were shown to participate in the floral development pathway and were also expressed in seeds (Baumbusch et al., 2001). Histone methyltransferase activity in *Arabidopsis thaliana* has been well described in the PcG proteins, such as *MEDEA*, *CURLY LEAF* and *SWINGER* (Makarevich et al., 2006, Grossniklaus et al., 1998, Köhler et al., 2003). The **At4g30860** gene (*SDG4*) encodes a member of the trxG protein family containing a SET domain and a PHD-finger. *SDG4* is a chromatin-associated protein that functions in the maintenance of methylated histone H3 levels in the mature pollen grain and *sdg4* mutant results in the reduced pollen tube length, aborted seeds, and unfertilized ovules. The possible function of *SDG4* is gene regulation in the vegetative nucleus by methylation of lysines 4 and 36 of the histone H3 in the mature pollen (Cartagena et al., 2008).

4) Finally, the **At4g30930** gene - *NUCLEAR FUSION DEFECTIVE1* - encodes a mitochondrial 50S ribosomal subunit L21 - RPL21M that is involved in karyogamy (nuclear fusion). In flowering plants, karyogamy occurs twice during double fertilization and once during female gametophyte development when the two polar nuclei fuse to form the diploid central cell nucleus. The *nfd1* mutants display defects in both male and female gametophyte development i.e. collapsed pollen grains and female gametophytes with central cells that fail to undergo fusion (Portereiko et al., 2006b).

Collectively, these four genes are strong candidates for the control of F1 2x X 4x paternal excess triploid reproduction (%N F2 seeds), due to their actions in the female gametophyte or during embryogenesis. Additionally, two examples of potential paternal control over successful triploid reproduction through pollen methylation are demonstrated.

## 5.5 Conclusions

*Arabidopsis thaliana* triploids were shown to be fertile and reproduce with varied rates of success which is under both genetic and epigenetic parent-of-origin control. *Arabidopsis thaliana* can therefore be used to study the phenomenon of triploidy, which is important for understanding plant speciation as well as stability and parent-of-origin effects during polyploid crop breeding.

Phenotypic and GWA analyses of triploid reproduction shed an important light on the success of *Arabidopsis thaliana* triploidy. A total number of 149 genes have been identified as associated with the variation observed in the reciprocal triploid reproduction. Ten of those genes have been selected as the most promising initial candidates for controlling successful seed development through mechanisms including maternal and paternal effects, genomic imprinting, differential DNA methylation, and RNA mediated gene silencing. One example of paternally controlled gene regulation by histone methylation in pollen has also been discussed.

Overall, the GWAS approach has allowed the identification of the *MOT* and *POT* loci on chromosomes 1 and 4 which are strong candidates for harbouring genes controlling reproductive success (i.e. extent of F2 seed-set) of F1 triploid plants. The location of the *MOT* locus suggests that it may overlap with the *SDI* locus previously identified by RIL mapping as a locus associated with genome dosage sensitivity.

# CHAPTER 6 - Summary and future directions

In this thesis it was sought to gain a better insight into triploid flowering plant reproduction, focusing on isogenic and hybrid triploids of *Arabidopsis thaliana*. Triploids have not been widely used as model systems for studying complex reproductive traits as they are much more challenging than diploids in terms of genetics. However, triploidy introduces parental genome dosage imbalance in the seed offspring, resulting in specific phenotypes not observed in plants with parentally balanced ploidy levels. Therefore research focused specifically on triploids will broaden our currently limited understanding of reproductive issues encountered by many important triploid species, which includes triploid crop species.

**Chapter 1** consists of a literature review on the use of *Arabidopsis thaliana* as a model for understanding reproduction, seed development, hybridisation and polyploidy effects.

In **Chapter 2**, triploid F1 seeds were shown to display transgressive seed size phenotypes depending on the parent-of-origin. Both isogenic and hybrid paternal genome excess F1 triploids resulted in best-parent heterosis (BPH) while the reciprocal maternal excess triploids exhibited worst-parent heterosis (WPH) (subtractive heterosis). It was discovered that vigorous offspring can be produced in many different genetic backgrounds (accessions) and that the level of heterosis varies depending on the genotype of the parents of the F1 triploid seeds. Surprisingly, hybrid triploids did not always display higher levels of heterosis than isogenic triploids, as might be expected from the increased heterozygosity in the former triploids. In fact, some isogenic triploids had higher levels of BPH or lower levels of WPH than some hybrid triploids. A negative correlation was found between BPH in paternal excess F1 triploids and WPH in corresponding maternal excess F1 triploids, suggesting a parent-of-origin effect on F1 triploid seed size heterosis. However, a few exceptional examples were found that displayed F1 triploid seed size heterosis in only one cross direction (Col-0, Pro-0, Ei-2, and Hr-5). Therefore, these accessions will be investigated in follow-up studies to determine the sources of variation in parent-of-origin specific mechanisms controlling F1 triploid seed size heterosis.

Triploid plants experience severe reproductive challenges, mainly due to complications during meiosis which lead to the production of aneuploid gametes. This explains the many triploid species that are sterile and which have to reproduce by vegetative propagation or other alternative pathways (e.g. apomixis). In **Chapter 3**, isogenic and hybrid F1 triploids were shown to be sub-fertile at the pre-fertilisation stage, where they reproduced with reduced rates of success due to unfertilised ovules. The proportions of F1 triploid ovules that were left unfertilized (%U) varied depending on the genetic background (accession) and involved a trade-off with the ovule numbers (Σ) in the gynoecium. Significant differences in %U and Σ were found between pairs of reciprocal F1 triploids indicating parent-of-origin dependent genome dosage effects controlling triploid fertility and ovule number. Surprisingly, the higher proportions of the unfertilized ovules (and the total ovule number) were not always found in the same cross direction, as certain F1 triploids appeared to be more fertile with maternal genome excess F1 triploids (2m:1p) while others were more fertile in the paternal genome excess F1 triploids (1m:2p). It was hypothesised that the variation in F1 triploid fertility might be related to the rate of inviable, aneuploid gamete production. However no correlation between rates of pollen abortion and %U was found, therefore it was concluded that the %U likely occurs due to aberrant ovule development. Despite strong heritable variation for %U (unfertilised ovules), no loci statistically associated with %U in reciprocal selfed F1 triploids were detected using the GWAS approach.

In follow-on studies it would be desirable to investigate the viability of both female and male gametes produced by selfed F1 triploids, using more sophisticated methods than Alexander pollen viability test. For example, determining differences in pollen karyotypes produced by reciprocal triploids could explain parent-of-origin effects observed in certain reciprocal triploids. Also, it would be desirable to visualise germinating pollen tubes inside the stigma and determine if viable pollen is able to fertilize available ovules. Finally, screening for ovule defects could be performed, although interpreting developmental aberrations might be challenging due to the female gametophyte (embryo sac) within each ovule being genetically different due to triploid meiosis.

In **Chapter 4**, the same suite of F1 triploid lines was used to investigate post-fertilisation lethality within the F2 seed sets. Similar to the %U and Σ from the previous study, the proportions of ovules that were fertilized by viable pollen but subsequently aborted as F2 seeds (%A) varied among both isogenic and hybrid triploids. Also, the F2 seed abortion rate often differed significantly between reciprocal F1 triploids and highlighted novel parent-of-origin dependent genome dosage effects. The negative correlation between %A and Σ indicates that some common maternal effect might control both reproductive traits, i.e. decreased numbers of ovules will result in higher rates of post-reproduction F2 seed abortion. Despite strong heritable variation for %A F2 (aborted seeds), no loci statistically associated with %A in reciprocal selfed F1 triploids were detected using the GWAS approach.

In **Chapter 5**, strong heritable variation for %N F2 (normal seeds) trait was again found among both isogenic and hybrid triploids. It was shown that although triploid F1 reproduction is impaired compared to diploids, the surviving F2 normal seed progeny comprises a wide range of DNA content. This supports the proposed role for triploids as evolutionary bridges between plant populations of different ploidy levels. It was found that reciprocal F1 triploids displayed parent-of-origin effects on %N, with some F1 triploids producing more normal seeds when they had an excess of the maternal genome and others with an excess of the paternal genome.

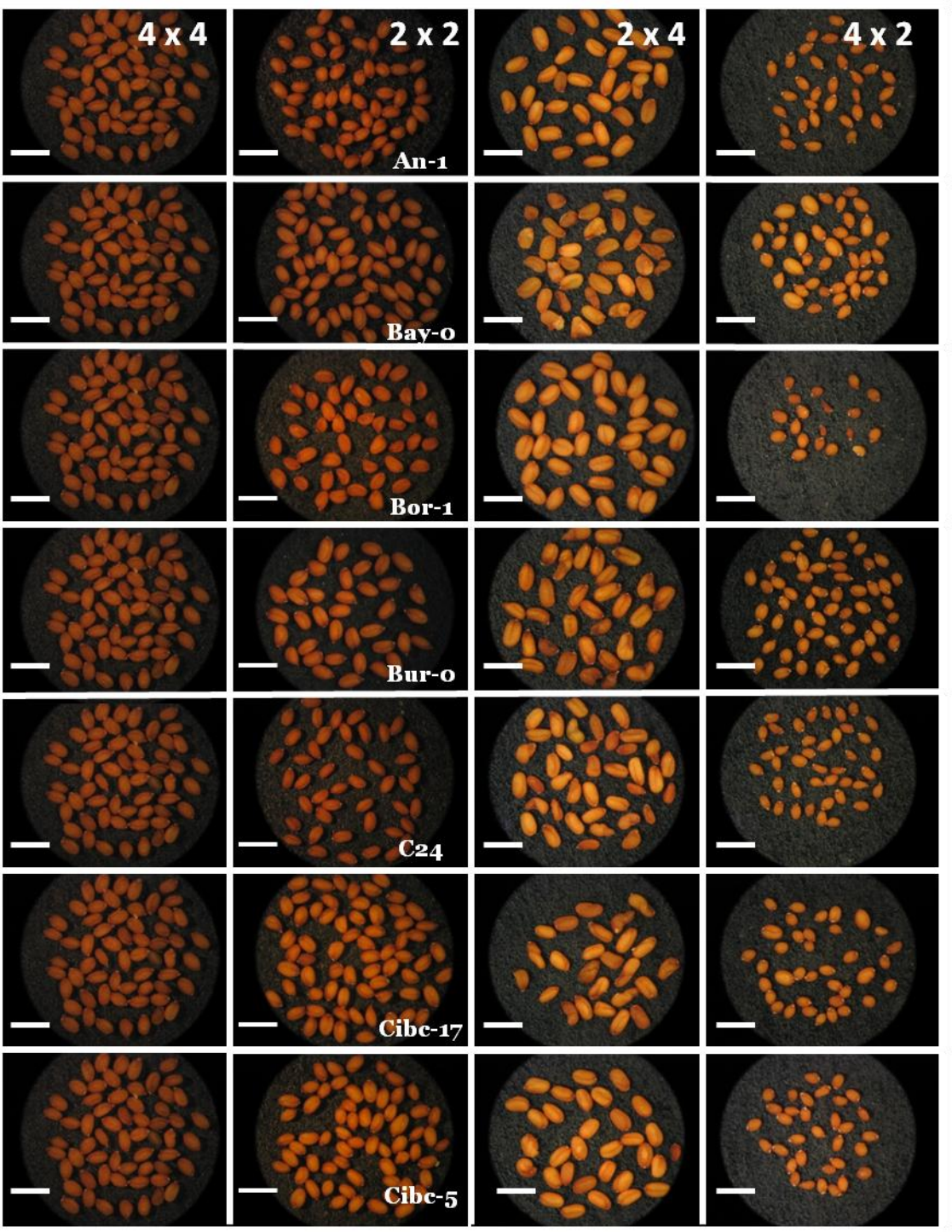
The differences in %N between different F1 triploid genotypes could suggest that there is a differential tolerance to aneuploid gamete production between different F1 triploid parents. Lower levels of DNA content among the F2 progeny of certain triploid lines suggest that those F1 triploids preferentially produce haploid-like viable gametes. F2 offspring with higher ploidy levels suggest that the F1 triploid parent was producing predominantly diploid-like gametes or even was likely to produce higher frequencies of unreduced triploid viable gametes (this would explain the case of a pentaploid individual detected among the offspring of one of the triploid parents). Moreover, the reciprocal F1 triploids of some genotypes also produced F2 normal seed progeny that significantly differed in their DNA

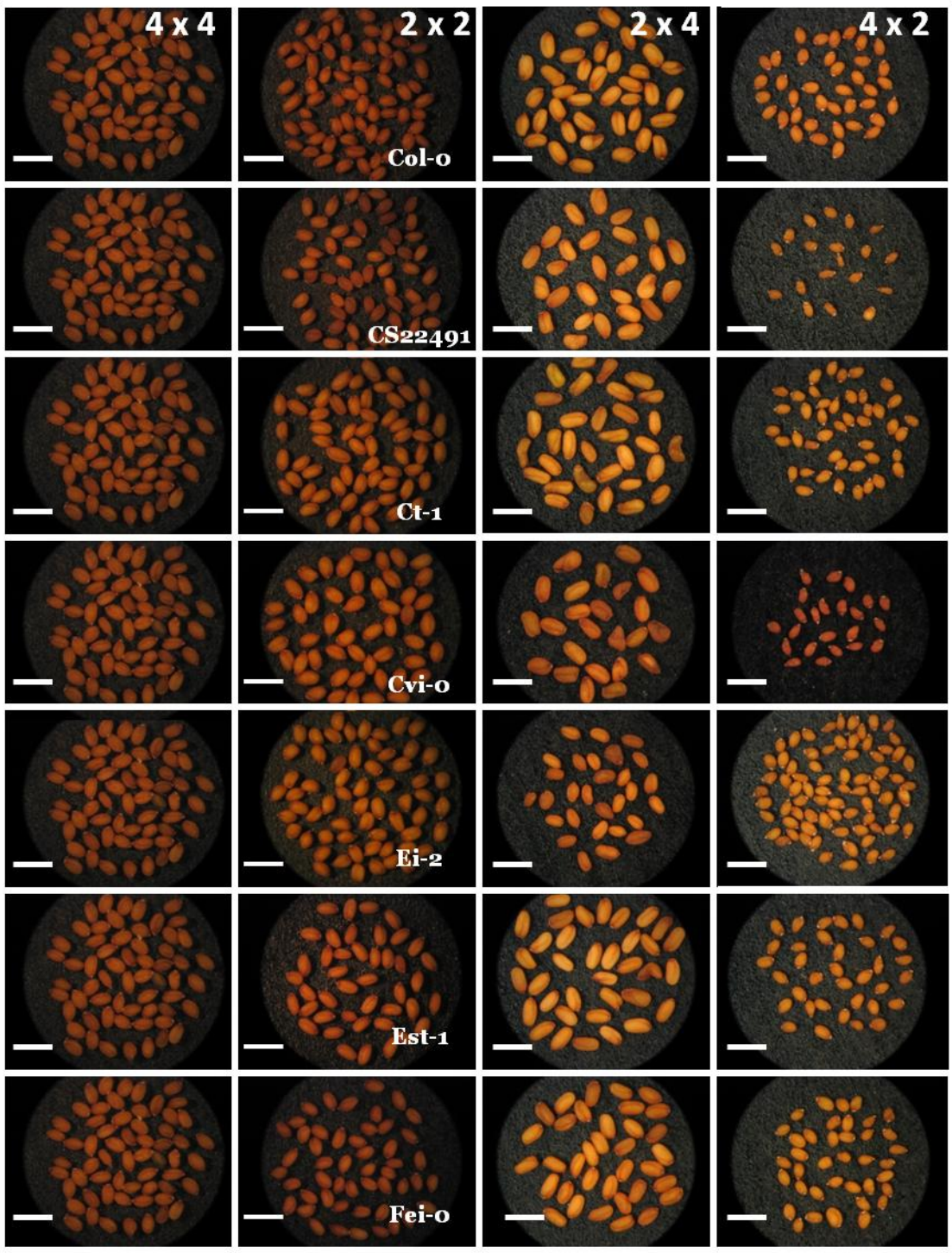
content distribution, highlighting an epigenetic parent of origin effect on chromosome transmission between reciprocal F1 hybrid triploids.

Finally, the GWA approach allowed the identification of two candidate loci involved in the phenotypic variation in %N among F2 seeds produced by 2x X 4x paternal overdose F1 triploids – *POT*, and 4x X 2x maternal overdose F1 triploids - *MOT*. In the 240 kb window spanning both the *POT* and *MOT* loci, 149 protein-coding candidate genes have been identified. Among those, ten genes were identified as strong candidates given their obvious involvement in the processes taking place in the male/female gametophyte, or in the developing seeds. Some of these candidates are linked to epigenetic processes including DNA methylation, histone modification, and siRNA mediated gene silencing. These genes will be subjected to further analyses to provide evidence and mechanistic explanations for the control of parent-of-origin dependent variation in triploid F1 hybrid reproductive success.

# List of Appendices

## Appendix A

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2 x 4
4 x 2
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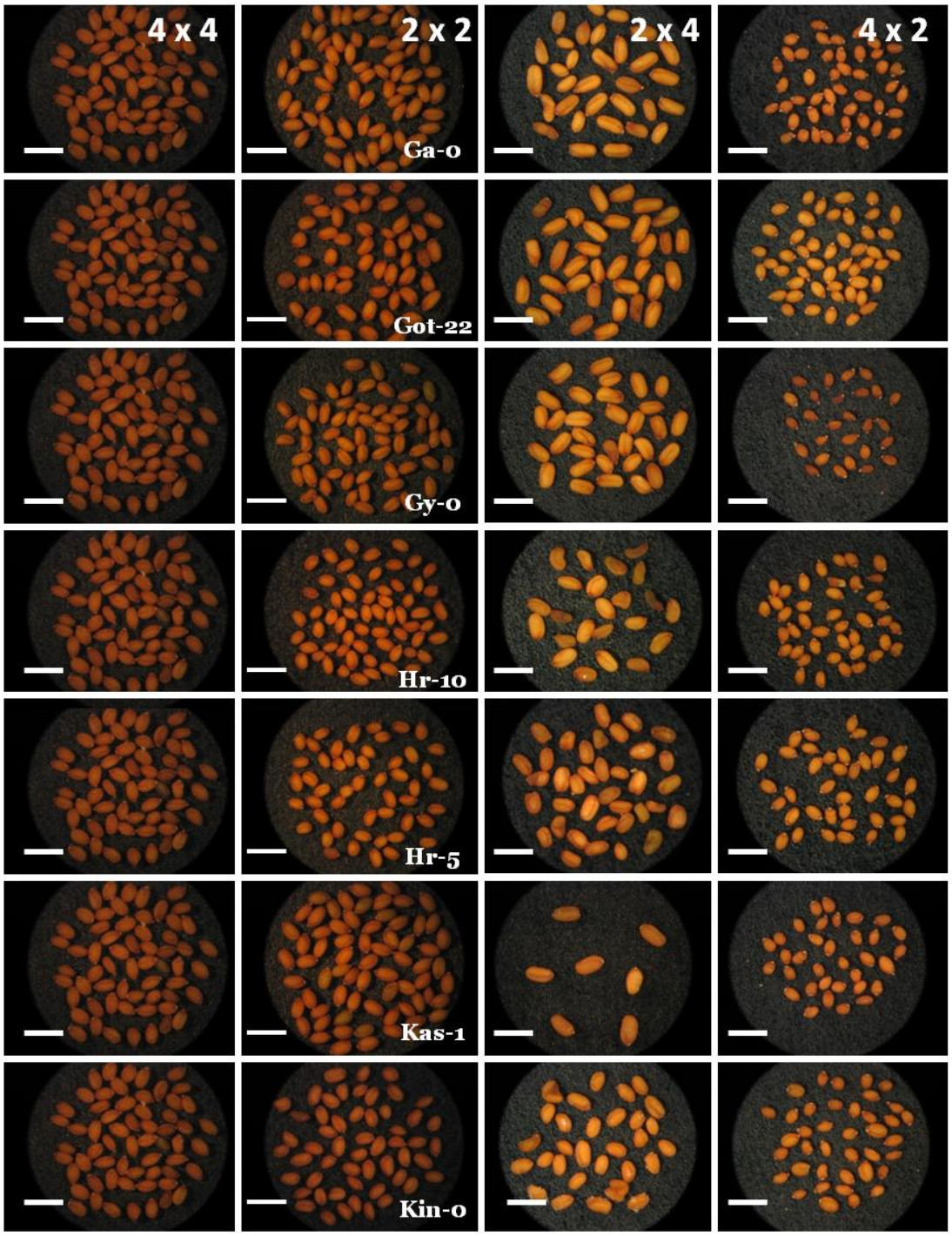
CS22491
Ct-1
Cvi-0
Ei-2
Est-1
Fei-0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2 x 4
4 x 2
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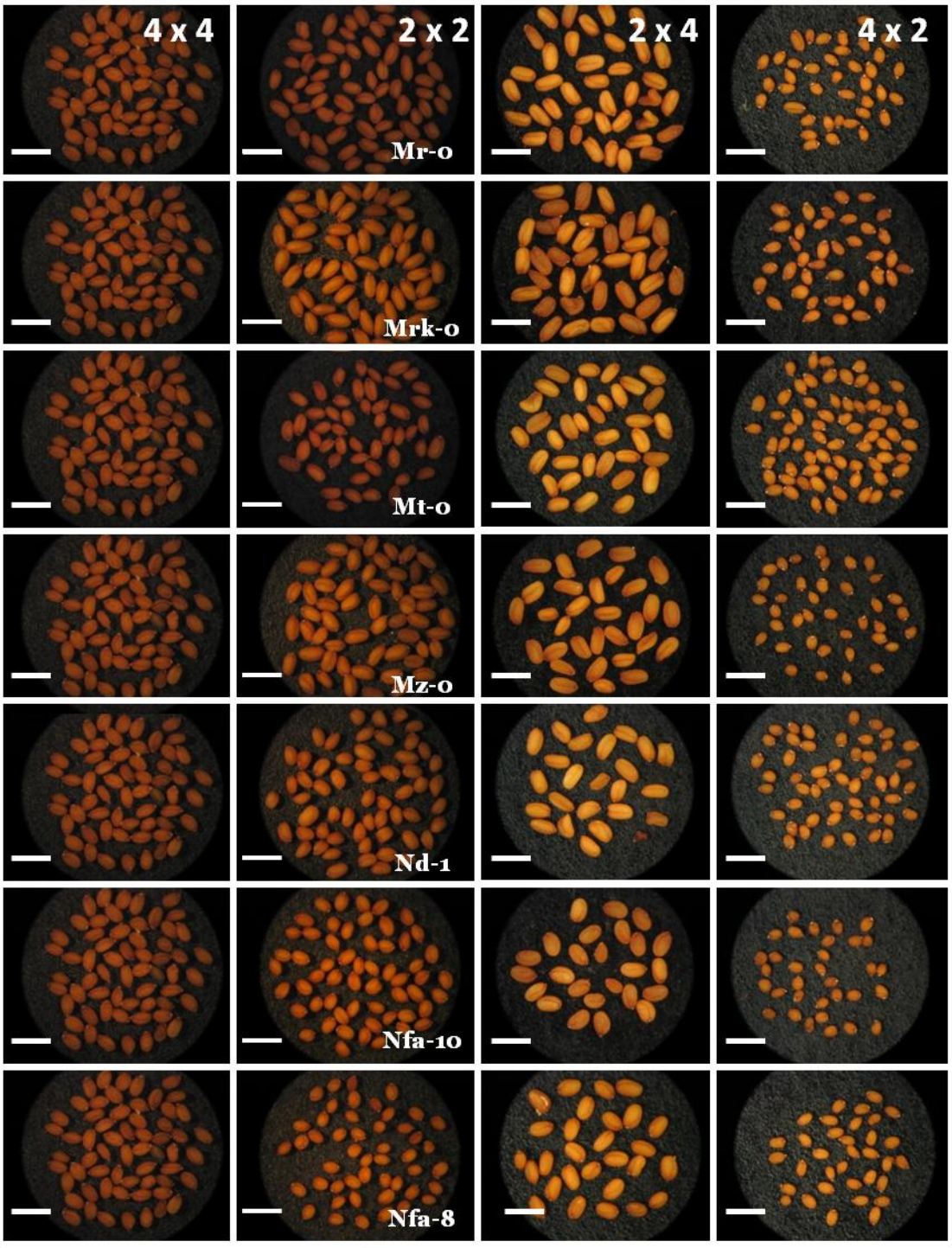
Got-22
Gy-0
Hr-10
Hr-5
Kas-1
Kin-0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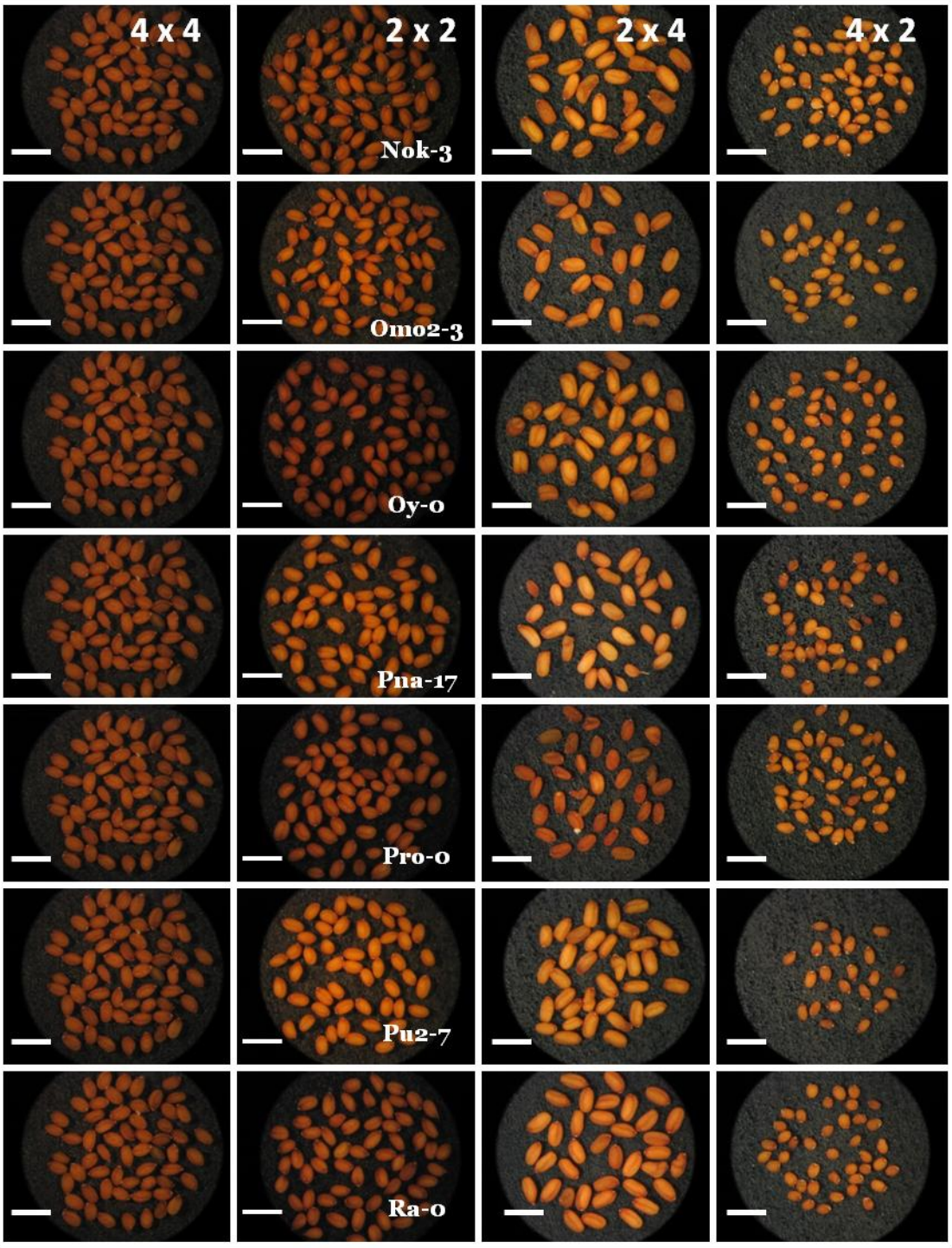
2 x 4
4 x 2
Mr-o
Mrk-o
Mt-o
Mz-o
Nd-1
Nfa-10
Nfa-8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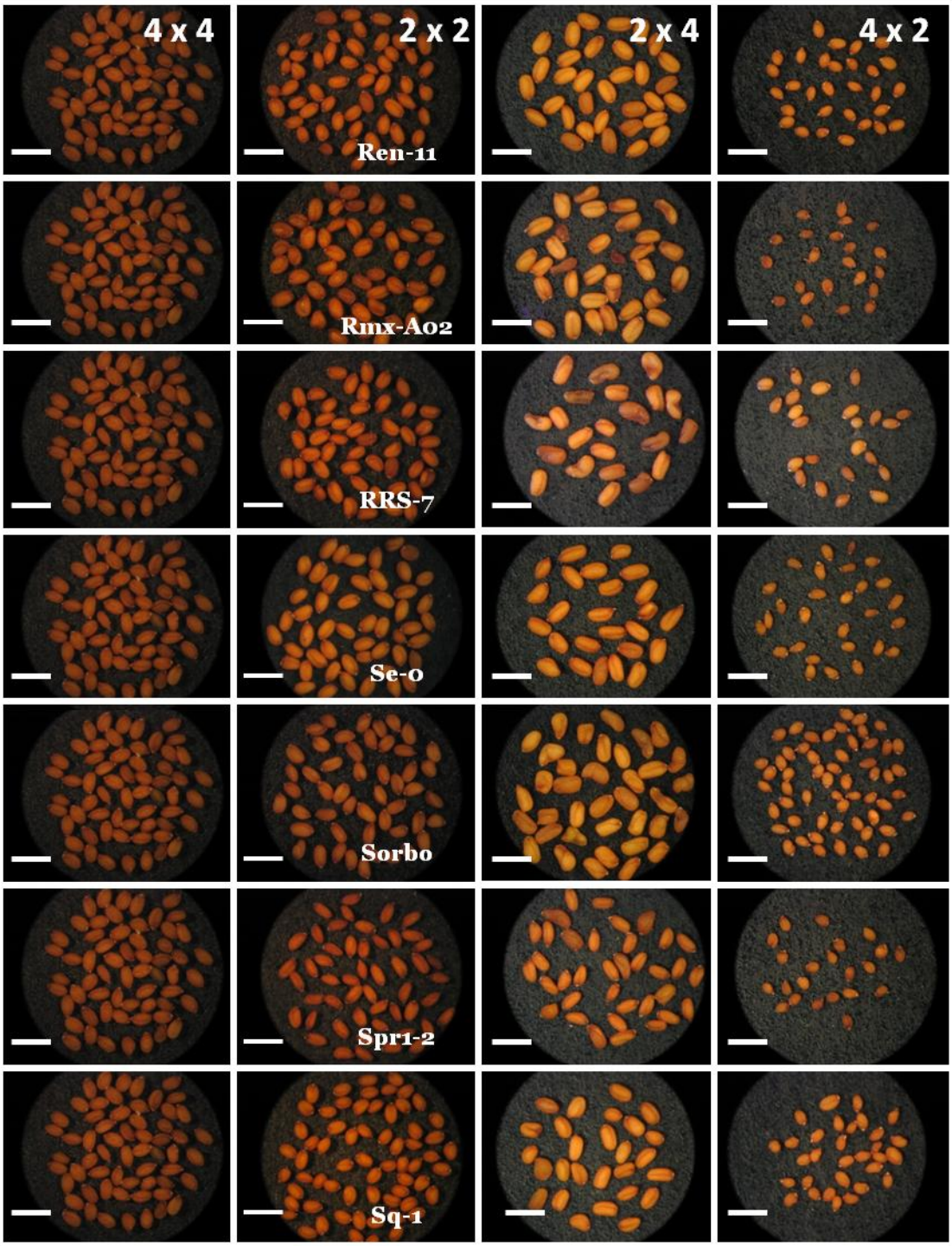
2 x 2
2 x 4
4 x 2
Nok-3
Omo2-3
Oy-o
Pna-17
Pro-o
Pu2-7
Ra-o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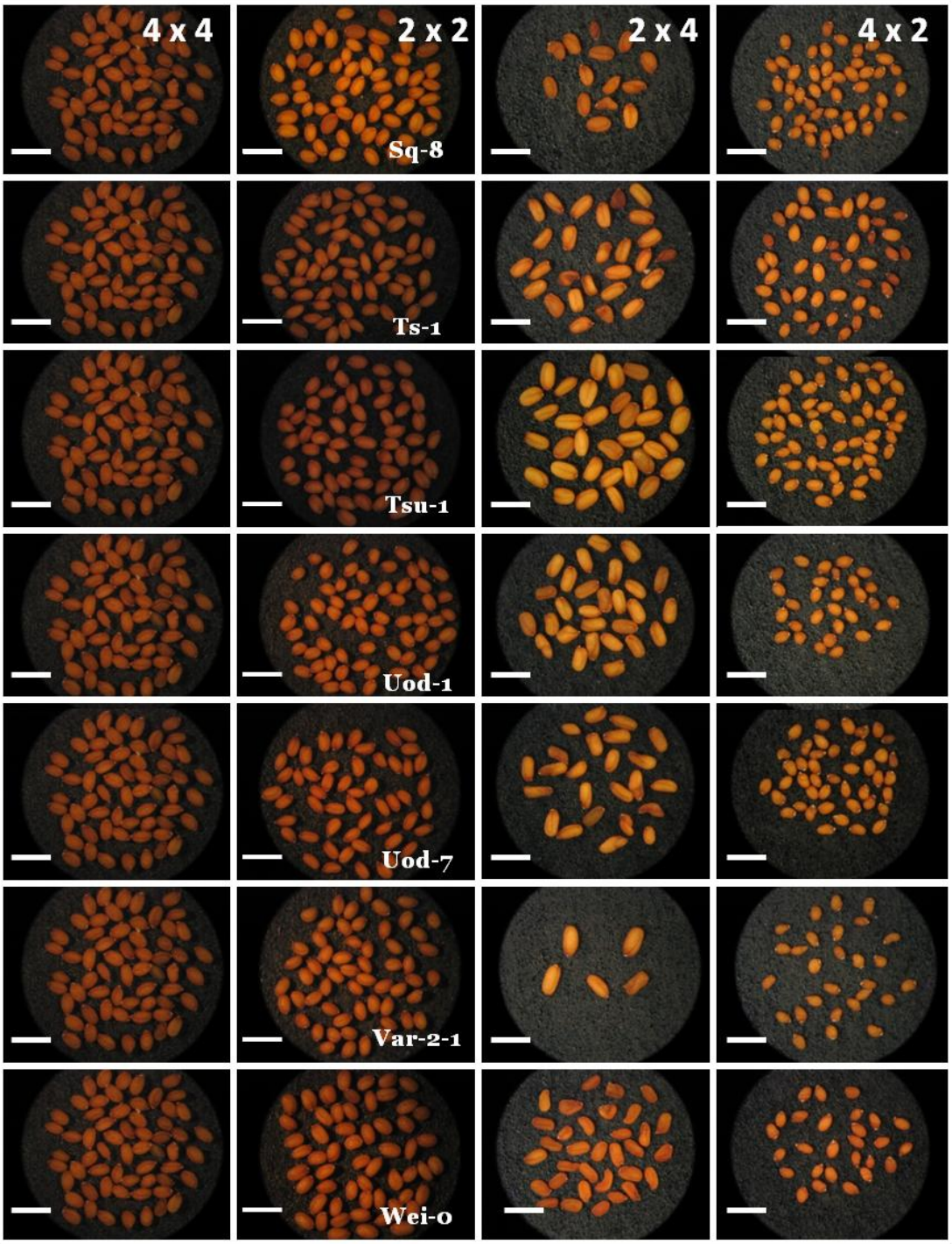
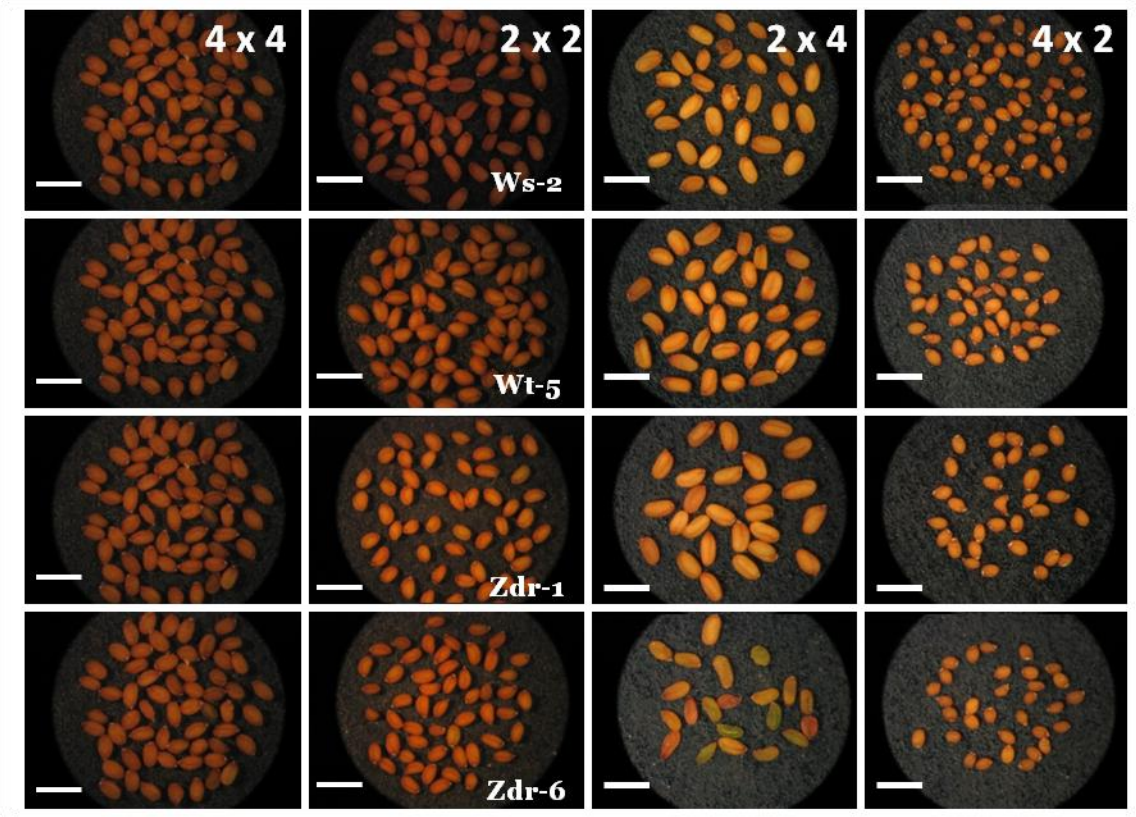
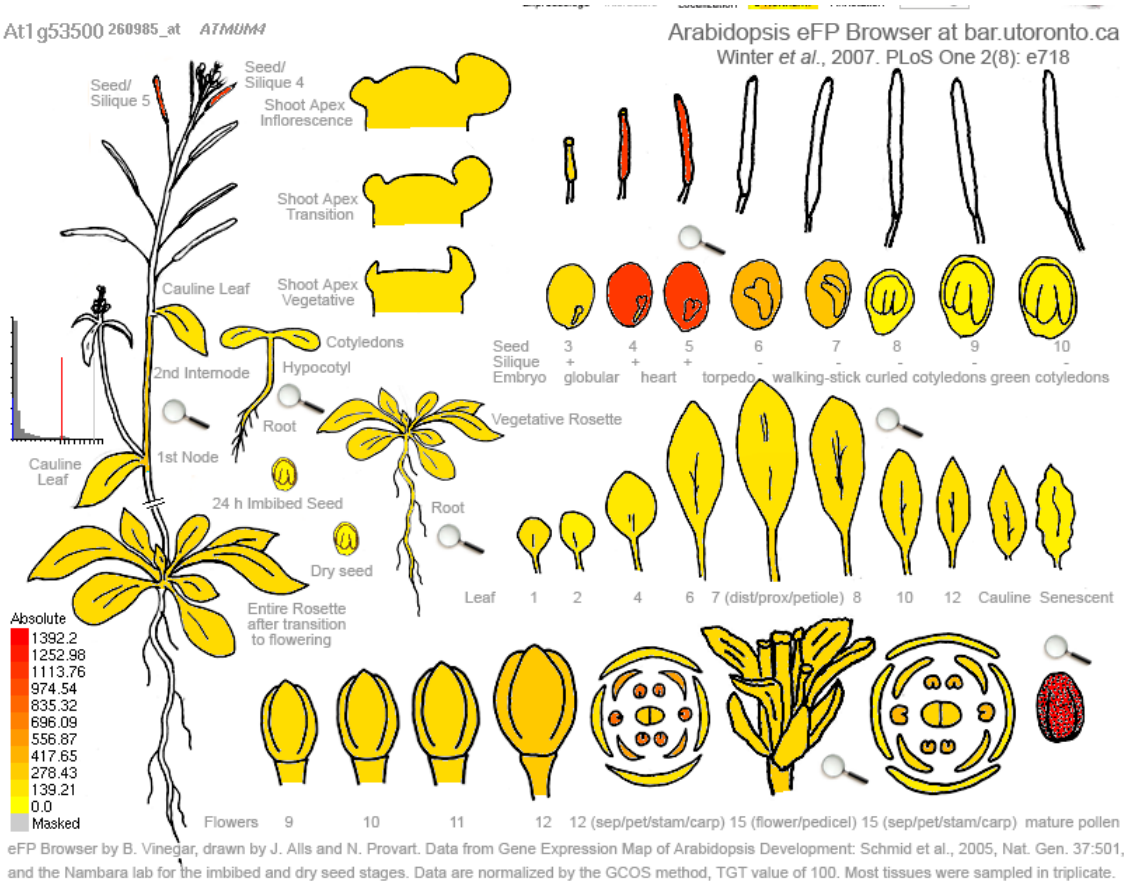
2 x 4
4 x 2
Ren-11
Rmx-Ao2
RRS-7
Se-o
Sorbo
Spr1-2
Sq-1


4 x 4
2 x 2
2 x 4
4 x 2
Sq-8
Ts-1
Tsu-1
Uod-1
Uod-7
Var-2-1
Wei-o




**Appendix A.** Images of 4 x 4 L*er*-0 tetraploid parent, 2 x 2 diploid accessions, and corresponding 2 x 4 paternal excess and 4 x 2 maternal excess F1 hybrid triploid seeds.

## Appendix B

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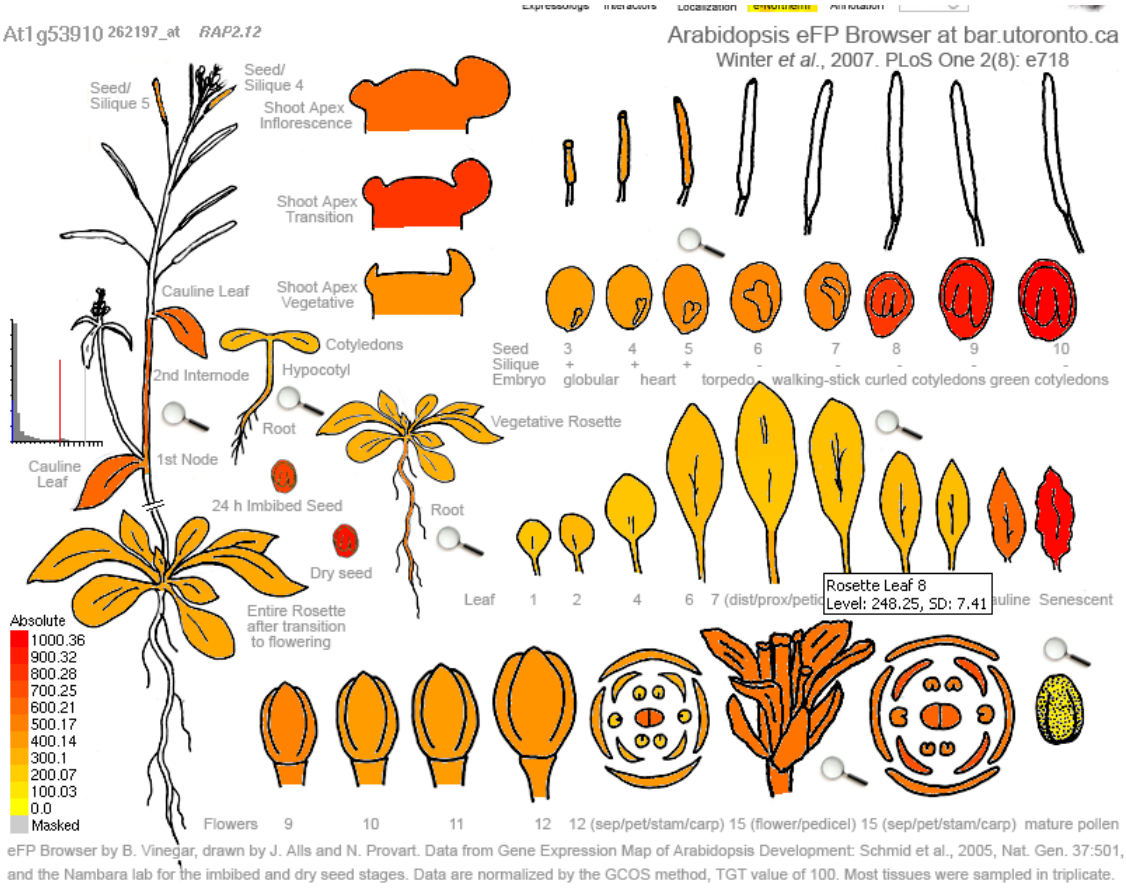
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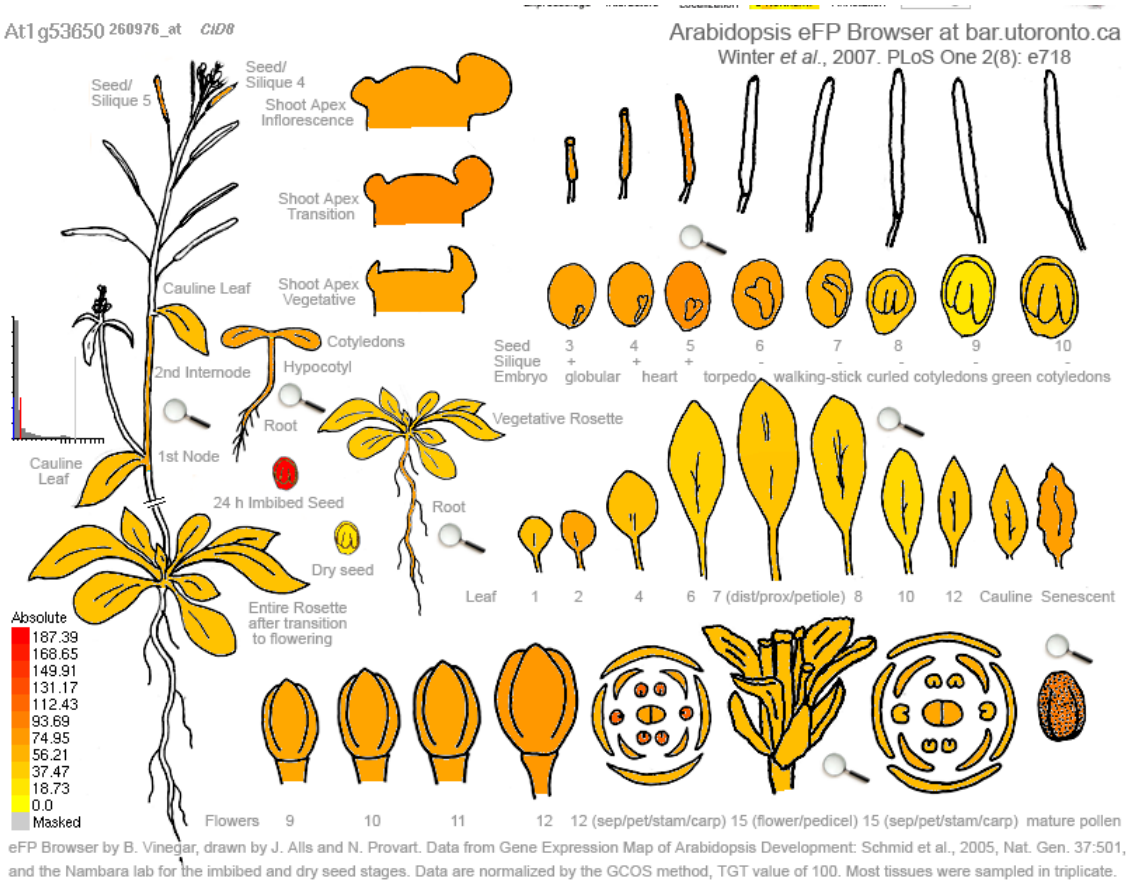
Arabidopsis eFP Browser at bar.utoronto.ca
Winter et al., 2007. PLoS One 2(8): e718
Seed/ Silique 5
Seed/ Silique 4
Shoot Apex Inflorescence
Shoot Apex Transition
Shoot Apex Vegetative
Cauline Leaf
2nd Internode
Cotyledons
Hypocotyl
Root
1st Node
Cauline Leaf
24 h Imbibed Seed
Dry seed
Entire Rosette after transition to flowering
Vegetative Rosette
Root
Seed 3 4 5 6 7 8 9 10
Silique + + + - - - - -
Embryo globular heart torpedo walking-stick curled cotyledons green cotyledons
Leaf 1 2 4 6 7 (dist/prox/petio
Rosette Leaf 8
Level: 248.25, SD: 7.41
uline Senescent
Absolute
1000.36
900.32
800.28
700.25
600.21
500.17
400.14
300.1
200.07
100.03
0.0
Masked
Flowers 9 10 11 12 12 (sep/pet/stam/carp) 15 (flower/pedicel) 15 (sep/pet/stam/carp) mature pollen
eFP Browser by B. Vinegar, drawn by J. Alls and N. Provart. Data from Gene Expression Map of Arabidopsis Development: Schmid et al., 2005, Nat. Gen. 37:501, and the Nambara lab for the imbibed and dry seed stages. Data are normalized by the GCOS method, TGT value of 100. Most tissues were sampled in triplicate.

**At1g53910**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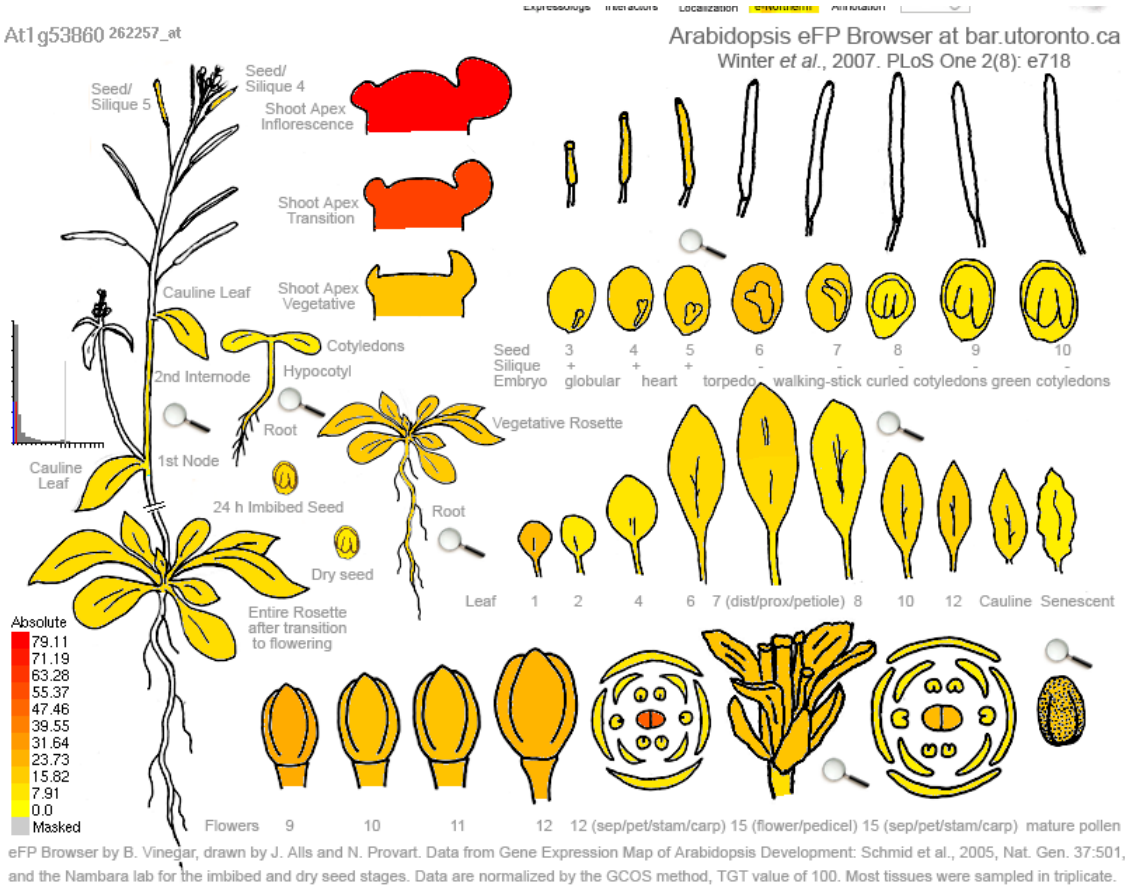
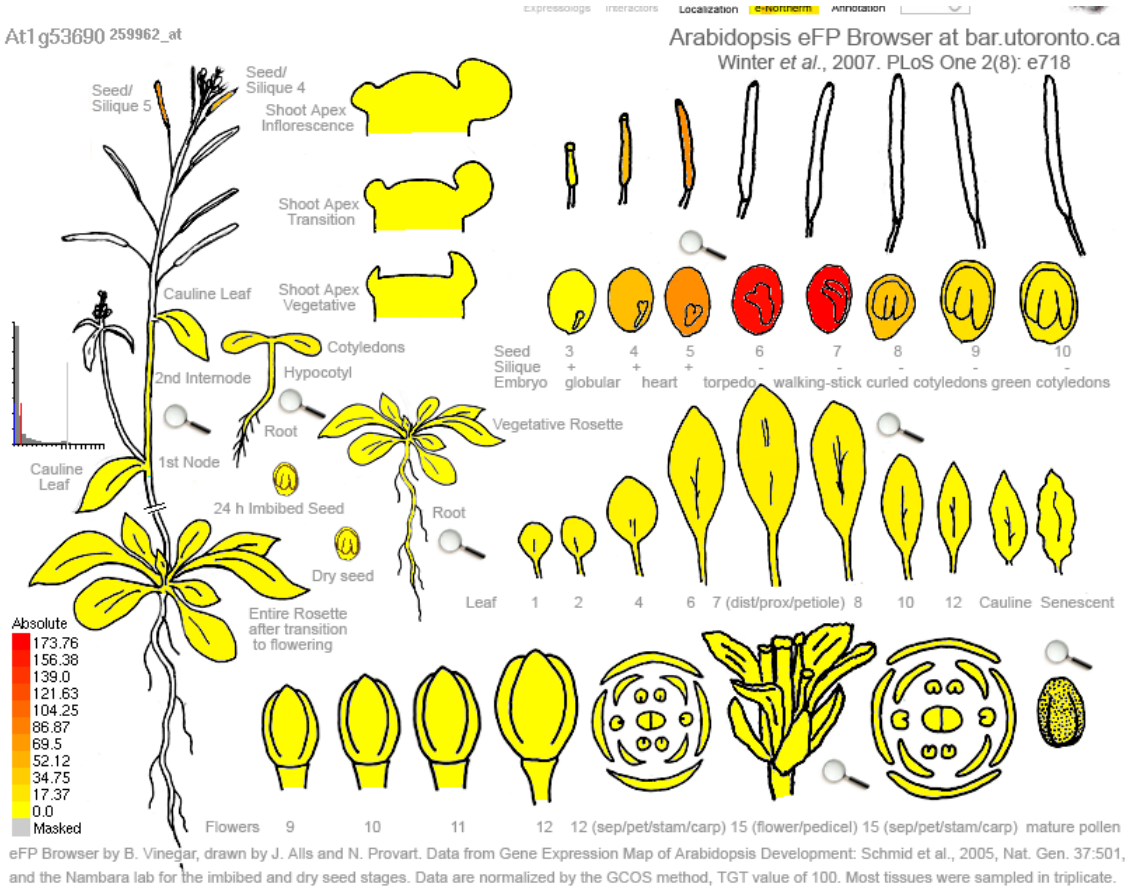
Arabidopsis eFP Browser at bar.utoronto.ca
Winter et al., 2007. PLoS One 2(8): e718
Seed/ Silique 5
Seed/ Silique 4
Shoot Apex Inflorescence
Shoot Apex Transition
Shoot Apex Vegetative
Cauline Leaf
2nd Internode
Cotyledons
Hypocotyl
Root
1st Node
Cauline Leaf
24 h Imbibed Seed
Dry seed
Entire Rosette after transition to flowering
Vegetative Rosette
Root
Seed 3 4 5 6 7 8 9 10
Silique + + + - - - - -
Embryo globular heart torpedo walking-stick curled cotyledons green cotyledons
Leaf 1 2 4 6 7 (dist/prox/petiole) 8 10 12 Cauline Senescent
Absolute
187.39
168.65
149.91
131.17
112.43
93.69
74.95
56.21
37.47
18.73
0.0
Masked
Flowers 9 10 11 12 12 (sep/pet/stam/carp) 15 (flower/pedicel) 15 (sep/pet/stam/carp) mature pollen
eFP Browser by B. Vinegar, drawn by J. Alls and N. Provart. Data from Gene Expression Map of Arabidopsis Development: Schmid et al., 2005, Nat. Gen. 37:501, and the Nambara lab for the imbibed and dry seed stages. Data are normalized by the GCOS method, TGT value of 100. Most tissues were sampled in triplicate.

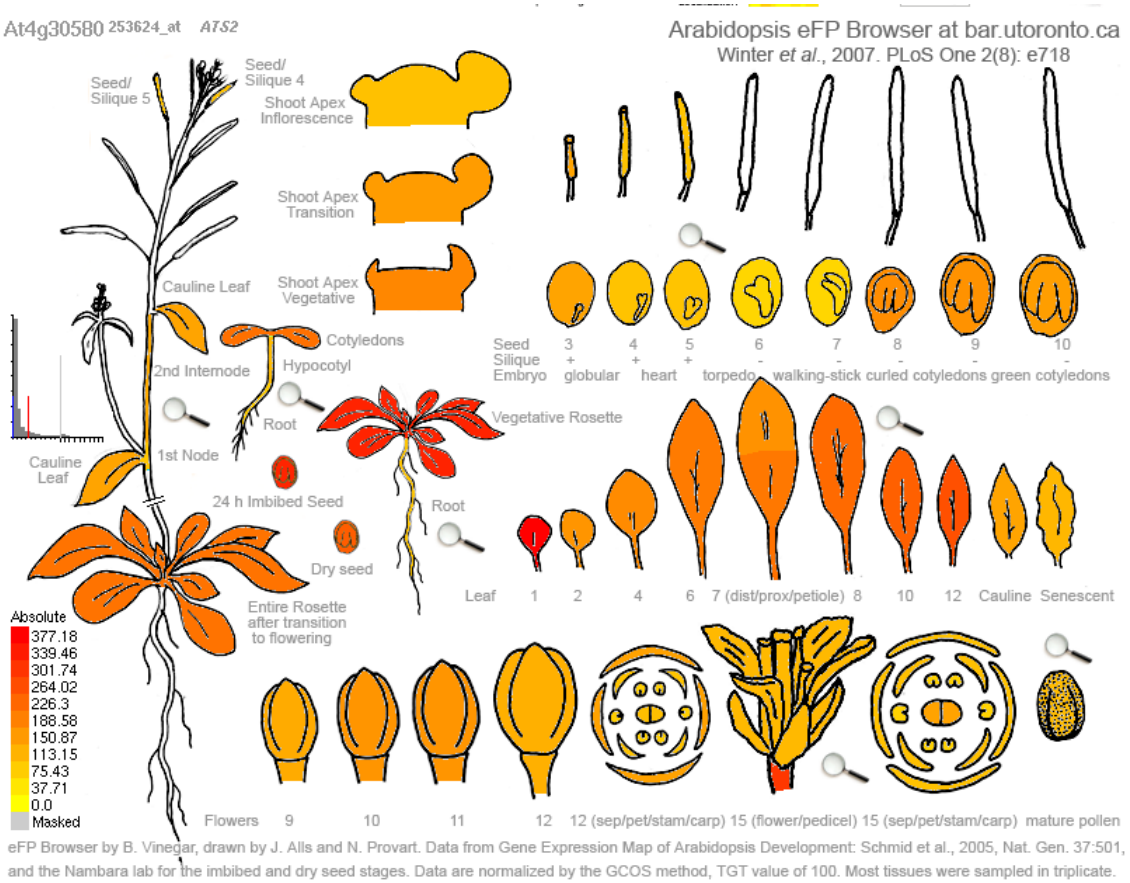
**At1g53650**


At1g53860 262257_at
Arabidopsis eFP Browser at bar.utoronto.ca
Winter et al., 2007. PLoS One 2(8): e718
Seed/ Silique 5
Seed/ Silique 4
Shoot Apex Inflorescence
Shoot Apex Transition
Shoot Apex Vegetative
Cauline Leaf
Cotyledons
Hypocotyl
2nd Internode
Root
1st Node
Cauline Leaf
24 h Imbibed Seed
Dry seed
Entire Rosette after transition to flowering
Vegetative Rosette
Root
Seed 3 4 5 6 7 8 9 10
Silique + + + - - - - -
Embryo globular heart torpedo walking-stick curled cotyledons green cotyledons
Leaf 1 2 4 6 7 (dist/prox/petiole) 8 10 12 Cauline Senescent
Absolute
79.11
71.19
63.28
55.37
47.46
39.55
31.64
23.73
15.82
7.91
0.0
Masked
Flowers 9 10 11 12 12 (sep/pet/stam/carp) 15 (flower/pedicel) 15 (sep/pet/stam/carp) mature pollen
eFP Browser by B. Vinegar, drawn by J. Alls and N. Provart. Data from Gene Expression Map of Arabidopsis Development: Schmid et al., 2005, Nat. Gen. 37:501, and the Nambara lab for the imbibed and dry seed stages. Data are normalized by the GCOS method, TGT value of 100. Most tissues were sampled in triplicate.

**At1g53860**


At1g53690 259962_at
Arabidopsis eFP Browser at bar.utoronto.ca
Winter et al., 2007. PLoS One 2(8): e718
Seed/ Silique 5
Seed/ Silique 4
Shoot Apex Inflorescence
Shoot Apex Transition
Shoot Apex Vegetative
Cauline Leaf
Cotyledons
Hypocotyl
2nd Internode
Root
1st Node
Cauline Leaf
24 h Imbibed Seed
Dry seed
Entire Rosette after transition to flowering
Vegetative Rosette
Root
Seed 3 4 5 6 7 8 9 10
Silique + + + - - - - -
Embryo globular heart torpedo walking-stick curled cotyledons green cotyledons
Leaf 1 2 4 6 7 (dist/prox/petiole) 8 10 12 Cauline Senescent
Absolute
173.76
156.38
139.0
121.63
104.25
86.87
69.5
52.12
34.75
17.37
0.0
Masked
Flowers 9 10 11 12 12 (sep/pet/stam/carp) 15 (flower/pedicel) 15 (sep/pet/stam/carp) mature pollen
eFP Browser by B. Vinegar, drawn by J. Alls and N. Provart. Data from Gene Expression Map of Arabidopsis Development: Schmid et al., 2005, Nat. Gen. 37:501, and the Nambara lab for the imbibed and dry seed stages. Data are normalized by the GCOS method, TGT value of 100. Most tissues were sampled in triplicate.

**At1g53690**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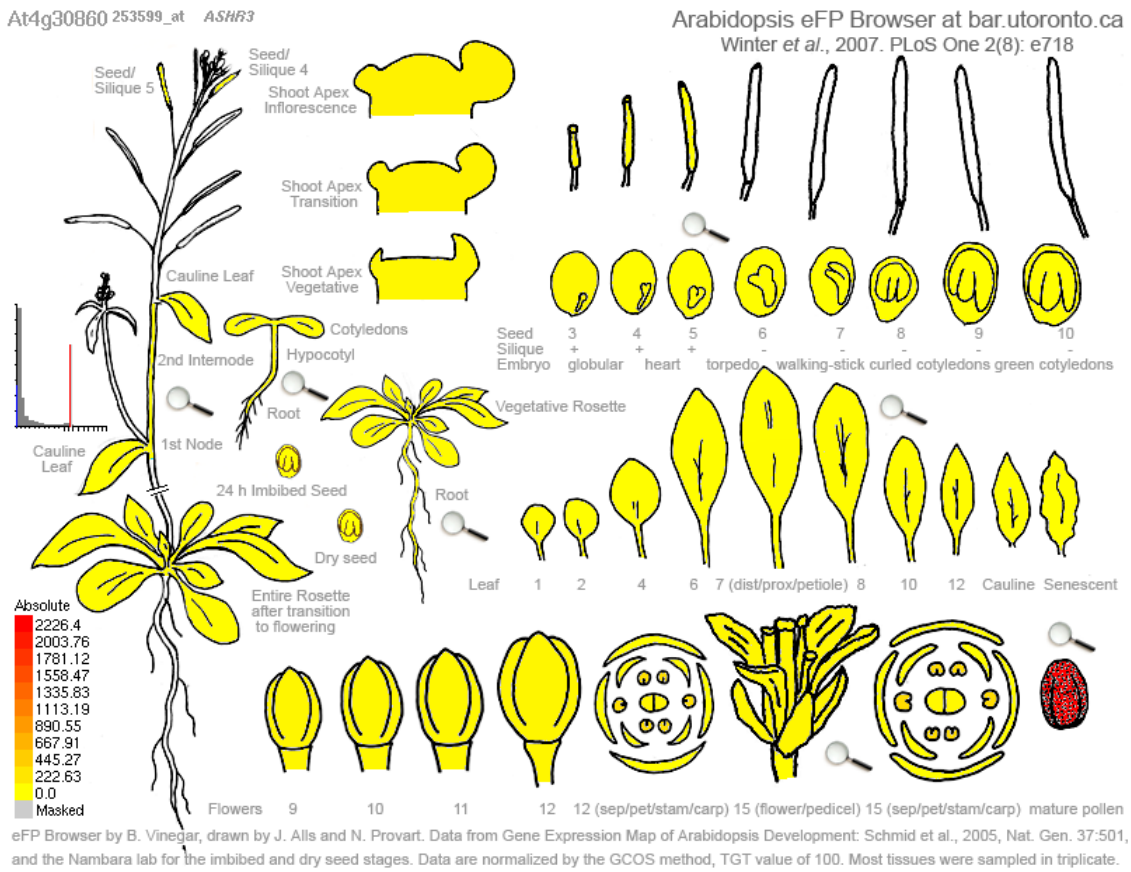
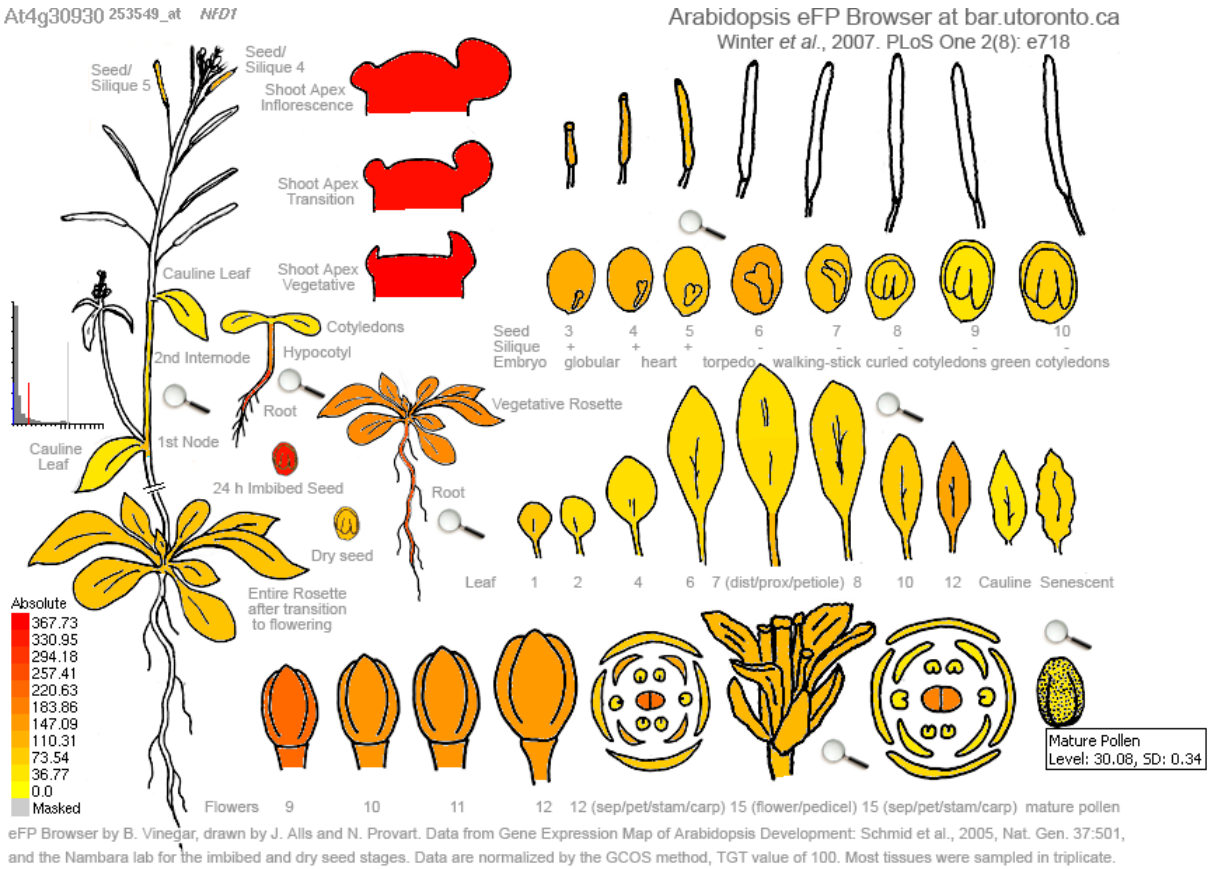
Arabidopsis eFP Browser at bar.utoronto.ca
Winter et al., 2007. PLoS One 2(8): e718
Seed/ Silique 5
Seed/ Silique 4
Shoot Apex Inflorescence
Shoot Apex Transition
Shoot Apex Vegetative
Cauline Leaf
Cotyledons
Hypocotyl
2nd Internode
Root
1st Node
Cauline Leaf
24 h Imbibed Seed
Dry seed
Entire Rosette after transition to flowering
Vegetative Rosette
Root
Seed 3 4 5 6 7 8 9 10
Silique + + + - - - - -
Embryo globular heart torpedo walking-stick curled cotyledons green cotyledons
Leaf 1 2 4 6 7 (dist/prox/petiole) 8 10 12 Cauline Senescent
Absolute
377.18
339.46
301.74
264.02
226.3
188.58
150.87
113.15
75.43
37.71
0.0
Masked
Flowers 9 10 11 12 12 (sep/pet/stam/carp) 15 (flower/pedicel) 15 (sep/pet/stam/carp) mature pollen
eFP Browser by B. Vinegar, drawn by J. Alls and N. Provart. Data from Gene Expression Map of Arabidopsis Development: Schmid et al., 2005, Nat. Gen. 37:501, and the Nambara lab for the imbibed and dry seed stages. Data are normalized by the GCOS method, TGT value of 100. Most tissues were sampled in triplicate.

**At4g30580**


At4g30860 253599_at ASHR3
Arabidopsis eFP Browser at bar.utoronto.ca
Winter et al., 2007. PLoS One 2(8): e718
Seed/ Silique 5
Seed/ Silique 4
Shoot Apex Inflorescence
Shoot Apex Transition
Shoot Apex Vegetative
Cauline Leaf
2nd Internode
Cotyledons
Hypocotyl
Root
1st Node
Cauline Leaf
24 h Imbibed Seed
Dry seed
Entire Rosette after transition to flowering
Vegetative Rosette
Root
Seed 3 4 5 6 7 8 9 10
Silique + + + - - - - -
Embryo globular heart torpedo walking-stick curled cotyledons green cotyledons
Leaf 1 2 4 6 7 (dist/prox/petiole) 8 10 12 Cauline Senescent
Absolute
2226.4
2003.76
1781.12
1558.47
1335.83
1113.19
890.55
667.91
445.27
222.63
0.0
Masked
Flowers 9 10 11 12 12 (sep/pet/stam/carp) 15 (flower/pedicel) 15 (sep/pet/stam/carp) mature pollen
eFP Browser by B. Vinegar, drawn by J. Alls and N. Provart. Data from Gene Expression Map of Arabidopsis Development: Schmid et al., 2005, Nat. Gen. 37:501,
and the Nambara lab for the imbibed and dry seed stages. Data are normalized by the GCOS method, TGT value of 100. Most tissues were sampled in triplicate.

**At4g30860**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Arabidopsis eFP Browser at bar.utoronto.ca
Winter et al., 2007. PLoS One 2(8): e718
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Seed/ Silique 4
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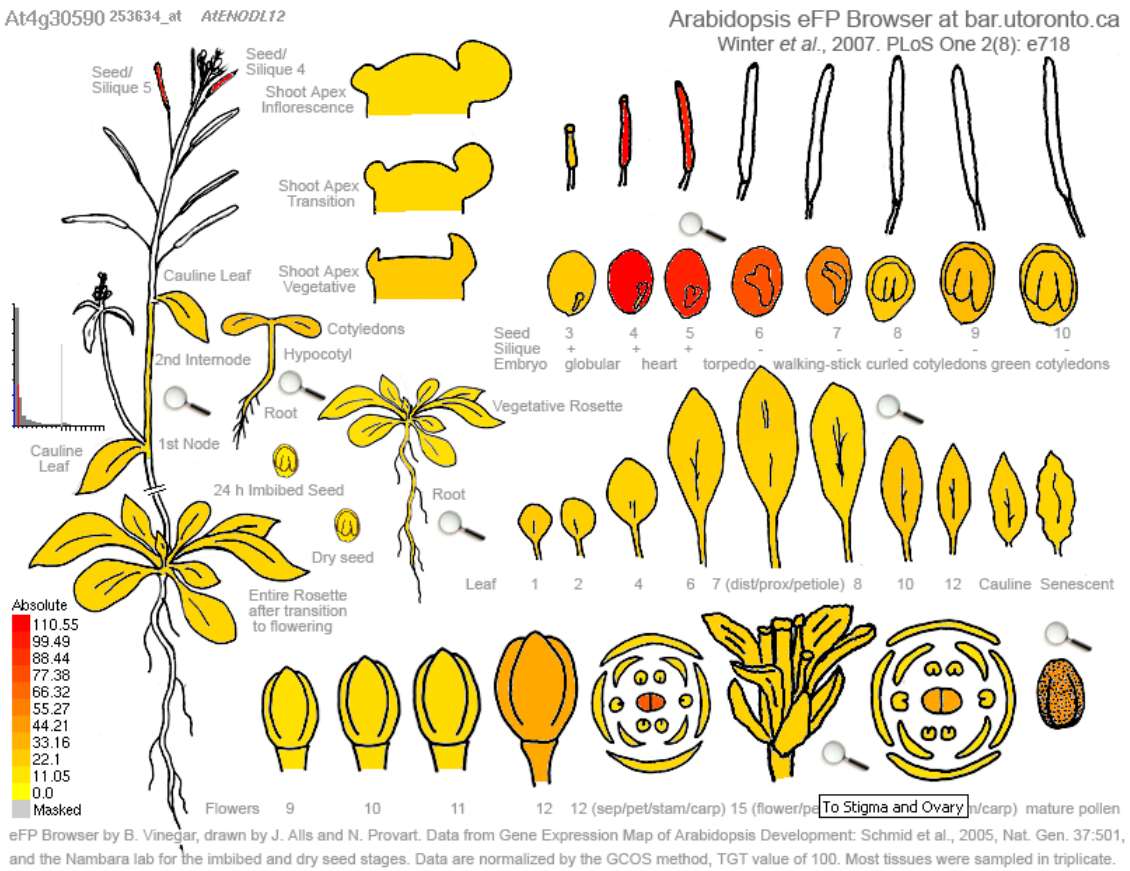
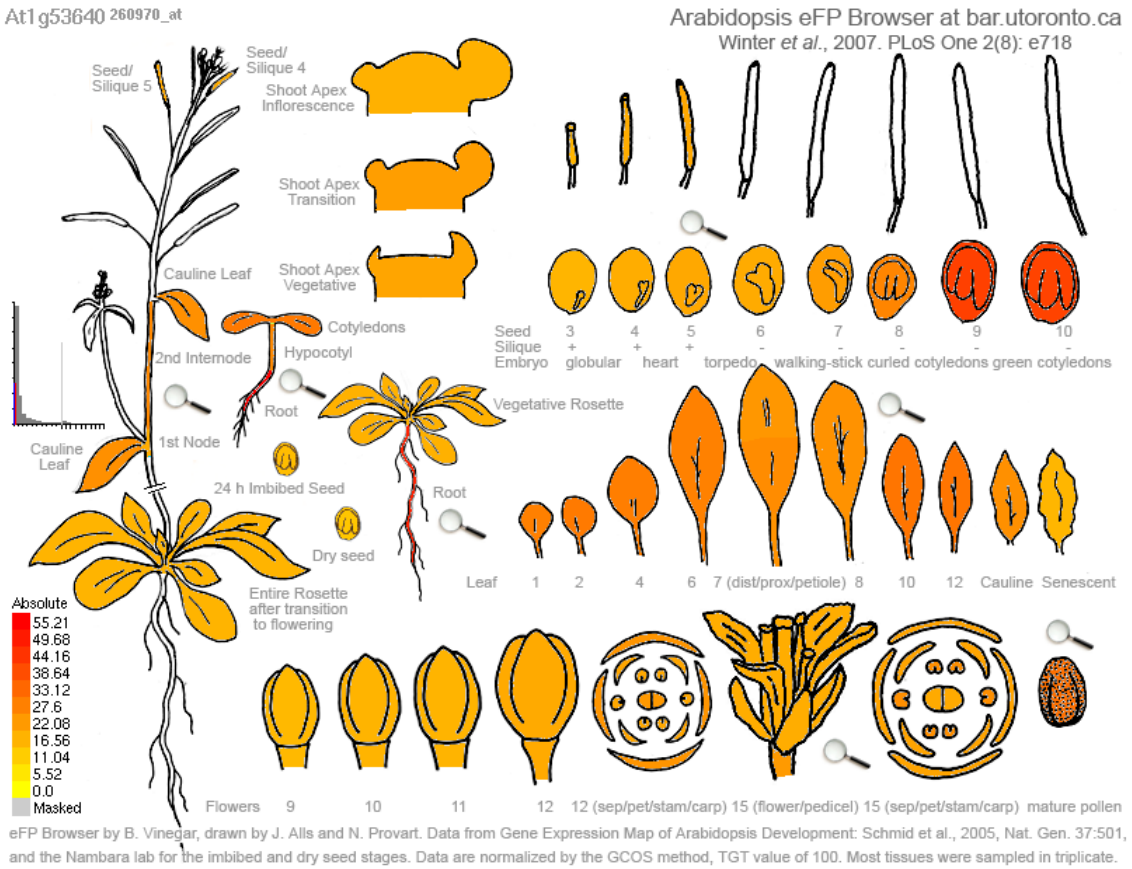
Shoot Apex Transition
Shoot Apex Vegetative
Cauline Leaf
Cotyledons
Hypocotyl
2nd Internode
Root
1st Node
Cauline Leaf
24 h Imbibed Seed
Dry seed
Entire Rosette after transition to flowering
Vegetative Rosette
Root
Seed 3 4 5 6 7 8 9 10
Silique + + + - - - - -
Embryo globular heart torpedo walking-stick curled cotyledons green cotyledons
Leaf 1 2 4 6 7 (dist/prox/petiole) 8 10 12 Cauline Senescent
Absolute
367.73
330.95
294.18
257.41
220.63
183.86
147.09
110.31
73.54
36.77
0.0
Masked
Flowers 9 10 11 12 12 (sep/pet/stam/carp) 15 (flower/pedicel) 15 (sep/pet/stam/carp) mature pollen
Mature Pollen
Level: 30.08, SD: 0.34
eFP Browser by B. Vinegar, drawn by J. Alls and N. Provart. Data from Gene Expression Map of Arabidopsis Development: Schmid et al., 2005, Nat. Gen. 37:501,
and the Nambara lab for the imbibed and dry seed stages. Data are normalized by the GCOS method, TGT value of 100. Most tissues were sampled in triplicate.

**At4g30930**


At4g30590 253634_at AtENODL12
Arabidopsis eFP Browser at bar.utoronto.ca
Winter et al., 2007. PLoS One 2(8): e718
Seed/ Silique 5
Seed/ Silique 4
Shoot Apex Inflorescence
Shoot Apex Transition
Shoot Apex Vegetative
Cauline Leaf
Cotyledons
Hypocotyl
2nd Internode
Root
1st Node
Cauline Leaf
24 h Imbibed Seed
Dry seed
Entire Rosette after transition to flowering
Vegetative Rosette
Root
Seed 3 4 5 6 7 8 9 10
Silique + + + - - - - -
Embryo globular heart torpedo walking-stick curled cotyledons green cotyledons
Leaf 1 2 4 6 7 (dist/prox/petiole) 8 10 12 Cauline Senescent
Absolute
110.55
99.49
88.44
77.38
66.32
55.27
44.21
33.16
22.1
11.05
0.0
Masked
Flowers 9 10 11 12 12 (sep/pet/stam/carp) 15 (flower/pe To Stigma and Ovary n/carp) mature pollen
eFP Browser by B. Vinegar, drawn by J. Alls and N. Provart. Data from Gene Expression Map of Arabidopsis Development: Schmid et al., 2005, Nat. Gen. 37:501,
and the Nambara lab for the imbibed and dry seed stages. Data are normalized by the GCOS method, TGT value of 100. Most tissues were sampled in triplicate.

**At4g30590**




**At1g53640**

**Appendix B** – Expression patterns of candidate genes http://bar.utoronto.ca/efp/cgi-bin/efpWeb.cgi

# Refernces


ADAMS, K. L., CRONN, R., PERCIFIELD, R. & WENDEL, J. F. (2003) Genes duplicated by polyploidy show unequal contributions to the transcriptome and organ-specific reciprocal silencing. *PNAS,* 100**,** 4649.

ADAMS, K. L., PERCIFIELD, R. & WENDEL, J. F. (2004) Organ-specific silencing of duplicated genes in a newly synthesized cotton allotetraploid. *Genetics,* 168**,** 2217.

ADAMS, K. L. & WENDEL, J. F. (2005a) Novel patterns of gene expression in polyploid plants. *TRENDS in Genetics,* 21**,** 539-543.

ADAMS, K. L. & WENDEL, J. F. (2005b) Polyploidy and genome evolution in plants. *Current opinion in plant biology,* 8**,** 135-141.

ADAMS, S., VINKENOOG, R., SPIELMAN, M., DICKINSON, H. G. & SCOTT, R. J. (2000) Parent-of-origin effects on seed development in Arabidopsis thaliana require DNA methylation. *Development,* 127**,** 2493.

ADAMSKI, N. M., ANASTASIOU, E., ERIKSSON, S., O'NEILL, C. M. & LENHARD, M. (2009) Local maternal control of seed size by KLUH/CYP78A5-dependent growth signaling. *PNAS,* 106**,** 20115.

AINOUCHE, M., FORTUNE, P., SALMON, A., PARISOD, C., GRANDBASTIEN, M. A., FUKUNAGA, K., RICOU, M. & MISSET, M. T. (2009) Hybridization, polyploidy and invasion: lessons from Spartina (Poaceae). *Biological invasions,* 11**,** 1159-1173.

ALEXANDER, M. (1969) Differential staining of aborted and nonaborted pollen. *Biotechnic & Histochemistry,* 44**,** 117-122.

ALONSO-BLANCO, C., AARTS, M. G. M., BENTSINK, L., KEURENTJES, J. J. B., REYMOND, M., VREUGDENHIL, D. & KOORNNEEF, M. (2009) What has natural variation taught us about plant development, physiology, and adaptation? *The Plant Cell Online,* 21**,** 1877.

ALONSO-BLANCO, C., BENTSINK, L., HANHART, C. J., BLANKESTIJN-DE VRIES, H. & KOORNNEEF, M. (2003) Analysis of natural allelic variation at seed dormancy loci of Arabidopsis thaliana. *Genetics,* 164**,** 711.

ALONSO-BLANCO, C., BLANKESTIJN-DE VRIES, H., HANHART, C. J. & KOORNNEEF, M. (1999) Natural allelic variation at seed size loci in relation to other life history traits of Arabidopsis thaliana. *PNAS,* 96**,** 4710.

ALONSO-BLANCO, C., EL-ASSAL, S. E. D., COUPLAND, G. & KOORNNEEF, M. (1998a) Analysis of natural allelic variation at flowering time loci in the Landsberg erecta and Cape Verde Islands ecotypes of Arabidopsis thaliana. *Genetics,* 149**,** 749.

ALONSO-BLANCO, C. & KOORNNEEF, M. (2000) Naturally occurring variation in Arabidopsis: an underexploited resource for plant genetics. *Trends in plant science,* 5**,** 22-29.

ALONSO-BLANCO, C., MENDEZ-VIGO, B. & KOORNNEEF, M. (2005) From phenotypic to molecular polymorphisms involved in naturally occurring variation of plant development. *Int. J. Dev. Biol.,* 49**,** 717.

ALONSO-BLANCO, C., PEETERS, A. J. M., KOORNNEEF, M., LISTER, C., DEAN, C., VAN DEN BOSCH, N., POT, J. & KUIPER, M. T. R. (1998b) Development of an AFLP based linkage map of Ler, Col and Cvi Arabidopsis thaliana ecotypes and construction of a Ler/Cvi recombinant inbred line population. *The Plant Journal,* 14**,** 259-271.

ALTMANN, T., DAMM, B., FROMMER, W. B., MARTIN, T., MORRIS, P. C., SCHWEIZER, D., WILLMITZER, L. & SCHMIDT, R. (1994) Easy determination of ploidy level in Arabidopsis thaliana plants by means of pollen size measurement. *Plant Cell Reports,* 13**,** 652-656.

ANDERSSON, S. (1990) Paternal effects on seed size in a population of Crepis tectorum (Asteraceae). *Oikos***,** 3-8.

ARABIDOPSIS, G. I. (2000) Analysis of the genome sequence of the flowering plant Arabidopsis thaliana. *Nature,* 408**,** 796.

ARANZANA, M. J., KIM, S., ZHAO, K., BAKKER, E., HORTON, M., JAKOB, K., LISTER, C., MOLITOR, J., SHINDO, C. & TANG, C. (2005) Genome-wide association mapping in Arabidopsis identifies previously known flowering time and pathogen resistance genes. *PLoS Genetics,* 1**,** e60.

ARSOVSKI, A. A., VILLOTA, M. M., ROWLAND, O., SUBRAMANIAM, R. & WESTERN, T. L. (2009) MUM ENHANCERS are important for seed coat mucilage production and mucilage secretory cell differentiation in Arabidopsis thaliana. *Journal of experimental botany,* 60**,** 2601.

ATWELL, S., HUANG, Y. S., VILHJÁLMSSON, B. J., WILLEMS, G., HORTON, M., LI, Y., MENG, D., PLATT, A., TARONE, A. M. & HU, T. T. (2010) Genome-wide association study of 107 phenotypes in Arabidopsis thaliana inbred lines. *Nature,* 465**,** 627-631.

AUGER, D. L., GRAY, A. D., REAM, T. S., KATO, A., COE, E. H. & BIRCHLER, J. A. (2005) Nonadditive gene expression in diploid and triploid hybrids of maize. *Genetics,* 169**,** 389.

AUTRAN, D., BAROUX, C., RAISSIG, M. T., LENORMAND, T., WITTIG, M., GROB, S., STEIMER, A., BARANN, M., KLOSTERMEIER, U. C. & LEBLANC, O. (2011) Maternal epigenetic pathways control parental contributions to Arabidopsis early embryogenesis. *Cell,* 145**,** 707-19.

AW, S. J., HAMAMURA, Y., CHEN, Z., SCHNITTGER, A. & BERGER, F. (2010) Sperm entry is sufficient to trigger division of the central cell but the paternal genome is required for endosperm development in Arabidopsis. *Development,* 137**,** 2683-2690.

BALASUBRAMANIAN, S., SCHWARTZ, C., SINGH, A., WARTHMANN, N., KIM, M. C., MALOOF, J. N., LOUDET, O., TRAINER, G. T., DABI, T. & BOREVITZ, J. O. (2009) QTL mapping in new Arabidopsis thaliana advanced intercross-recombinant inbred lines. *PLoS One,* 4**,** e4318.

BANAEI MOGHADDAM, A. M., FUCHS, J., CZAUDERNA, T., HOUBEN, A. & METTE, M. F. (2010) Intraspecific hybrids of Arabidopsis thaliana revealed no gross alterations in endopolyploidy, DNA methylation, histone modifications and transcript levels. *TAG THEORETICAL AND APPLIED GENETICS,* 120**,** 215-226.

BAROUX, C., PECINKA, A., FUCHS, J., SCHUBERT, I. & GROSSNIKLAUS, U. (2007) The triploid endosperm genome of Arabidopsis adopts a peculiar, parental-dosage-dependent chromatin organization. *The Plant Cell Online,* 19**,** 1782-1794.

BAROUX, C., SPILLANE, C. & GROSSNIKLAUS, U. (2002) Genomic imprinting during seed development. *Advances in genetics,* 46**,** 165-214.

BARTEE, L., MALAGNAC, F. & BENDER, J. (2001) Arabidopsis cmt3 chromomethylase mutations block non-CG methylation and silencing of an endogenous gene. *Genes & development,* 15**,** 1753.

BARTH, S., BUSIMI, A., UTZ, H. F. & MELCHINGER, A. (2003) Heterosis for biomass yield and related traits in five hybrids of Arabidopsis thaliana L. Heynh. *Heredity,* 91**,** 36-42.

BAUMBUSCH, L. O., THORSTENSEN, T., KRAUSS, V., FISCHER, A., NAUMANN, K., ASSALKHOU, R., SCHULZ, I., REUTER, G. & AALEN, R. B. (2001) The Arabidopsis thaliana genome contains at least 29 active genes encoding SET domain proteins that can be assigned to four evolutionarily conserved classes. *Nucleic Acids Research,* 29**,** 4319-4333.

BAYER, M., NAWY, T., GIGLIONE, C., GALLI, M., MEINNEL, T. & LUKOWITZ, W. (2009) Paternal control of embryonic patterning in Arabidopsis thaliana. *Science,* 323**,** 1485.

BENDER, J. (2004) DNA methylation and epigenetics. *Annual Review of Plant Biology,* 55**,** 41-68.

BENFEY, P. N. & MITCHELL-OLDS, T. (2008) From genotype to phenotype: systems biology meets natural variation. *Science,* 320**,** 495.

BENFEY, T. J. (1999) The physiology and behavior of triploid fishes. *Reviews in Fisheries Science,* 7**,** 39-67.

BENTSINK, L., JOWETT, J., HANHART, C. J. & KOORNNEEF, M. (2006) Cloning of DOG1, a quantitative trait locus controlling seed dormancy in Arabidopsis. *Proceedings of the National Academy of Sciences,* 103**,** 17042.

BENTSINK, L., YUAN, K., KOORNNEEF, M. & VREUGDENHIL, D. (2003) The genetics of phytate and phosphate accumulation in seeds and leaves of Arabidopsis thaliana, using natural variation. *TAG THEORETICAL AND APPLIED GENETICS,* 106**,** 1234-1243.

BERGER, F. (1999) Endosperm development. *Current opinion in plant biology,* 2**,** 28-32.

BERGER, F. (2003) Endosperm: the crossroad of seed development. *Current opinion in plant biology,* 6**,** 42-50.

BERGER, F. (2008) Double-fertilization, from myths to reality. *Sexual Plant Reproduction,* 21**,** 3-5.

BICKNELL, R. (1997) Isolation of a diploid, apomictic plant of Hieracium aurantiacum. *Sexual Plant Reproduction,* 10**,** 168-172.

BIKARD, D., PATEL, D., LE METTÉ, C., GIORGI, V., CAMILLERI, C., BENNETT, M. J. & LOUDET, O. (2009) Divergent evolution of duplicate genes leads to genetic incompatibilities within A. thaliana. *Science,* 323**,** 623.

BIRCHLER, J. A. (1993) Dosage analysis of maize endosperm development. *Annual review of genetics,* 27**,** 181-204.

BIRCHLER, J. A., AUGER, D. L. & RIDDLE, N. C. (2003) In search of the molecular basis of heterosis. *The Plant Cell Online,* 15**,** 2236.

BIRCHLER, J. A., BHADRA, U., BHADRA, M. P. & AUGER, D. L. (2001) Dosage-dependent gene regulation in multicellular eukaryotes: implications for dosage compensation, aneuploid syndromes, and quantitative traits. *Developmental biology,* 234**,** 275-288.

BIRCHLER, J. A., RIDDLE, N. C., AUGER, D. L. & VEITIA, R. A. (2005) Dosage balance in gene regulation: biological implications. *Trends in Genetics,* 21**,** 219-226.

BIRCHLER, J. A., YAO, H. & CHUDALAYANDI, S. (2006) Unraveling the genetic basis of hybrid vigor. *PNAS,* 103**,** 12957.

BLAIR, A. C. & HUFBAUER, R. A. (2010) Hybridization and invasion: one of North America's most devastating invasive plants shows evidence for a history of interspecific hybridization. *Evolutionary Applications,* 3**,** 40-51.

BLAKESLEE, A. F. (1922) Variations in Datura due to changes in chromosome number. *The American Naturalist,* 56**,** 16-31.

BLAKESLEE, A. F. (1941) Effect of induced polyploidy in plants. *The American Naturalist,* 75**,** 117-135.

BLANC, G. & WOLFE, K. H. (2004) Functional divergence of duplicated genes formed by polyploidy during Arabidopsis evolution. *The Plant Cell Online,* 16**,** 1679.

BOGART, J. P. (1980) Evolutionary significance of polyploidy in amphibians and reptiles. *Polyploidy: Biological Relevance***,** 341-378.

BOMBLIES, K., LEMPE, J., EPPLE, P., WARTHMANN, N., LANZ, C., DANGL, J. L. & WEIGEL, D. (2007) Autoimmune response as a mechanism for a Dobzhansky-Muller-type incompatibility syndrome in plants. *PLoS biology,* 5**,** e236.

BOMBLIES, K. & WEIGEL, D. (2007) Hybrid necrosis: autoimmunity as a potential gene-flow barrier in plant species. *Nature Reviews Genetics,* 8**,** 382-393.

BOMBLIES, K., YANT, L., LAITINEN, R. A., KIM, S. T., HOLLISTER, J. D., WARTHMANN, N., FITZ, J. & WEIGEL, D. (2010) Local-scale patterns of genetic variability, outcrossing, and spatial structure in natural stands of Arabidopsis thaliana. *PLoS genetics,* 6**,** e1000890.

BOREVITZ, J. O., HAZEN, S. P., MICHAEL, T. P., MORRIS, G. P., BAXTER, I. R., HU, T. T., CHEN, H., WERNER, J. D., NORDBORG, M. & SALT, D. E. (2007) Genome-wide patterns of single-feature polymorphism in Arabidopsis thaliana. *Proceedings of the National Academy of Sciences,* 104**,** 12057.

BOREVITZ, J. O. & NORDBORG, M. (2003) The impact of genomics on the study of natural variation in Arabidopsis. *Plant physiology,* 132**,** 718.

BOUTELIER, K., WEIJERS, D., QUINT, A. & OFFSIAGA, R. (2001) BABY BOOM technology: engineering adventitious embryony.

BOYES, D. C., ZAYED, A. M., ASCENZI, R., MCCASKILL, A. J., HOFFMAN, N. E., DAVIS, K. R. & GORLACH, J. (2001a) Growth stage-based phenotypic analysis of Arabidopsis: a model for high throughput functional genomics in plants. *The Plant Cell Online,* 13**,** 1499.

BOYES, D. C., ZAYED, A. M., ASCENZI, R., MCCASKILL, A. J., HOFFMAN, N. E., DAVIS, K. R. & GÖRLACH, J. (2001b) Growth stage–based phenotypic analysis of Arabidopsis: A model for high throughput functional genomics in plants. *The Plant Cell Online,* 13**,** 1499.

BRAVO, J., AGUILAR-HENONIN, L., OLMEDO, G. & GUZMAN, P. (2005) Four distinct classes of proteins as interaction partners of the PABC domain of Arabidopsis thaliana Poly (A)-binding proteins. *Molecular Genetics and Genomics,* 272**,** 651-665.

BRIDEAU, N. J., FLORES, H. A., WANG, J., MAHESHWARI, S., WANG, X. & BARBASH, D. A. (2006) Two Dobzhansky-Muller genes interact to cause hybrid lethality in Drosophila. *Science,* 314**,** 1292-1295.

BROWN, R., LEMMON, B. E., NGUYEN, H. & OLSEN, O. A. (1999) Development of endosperm in Arabidopsis thaliana. *Sexual Plant Reproduction,* 12**,** 32-42.

BURD, M., ASHMAN, T. L., CAMPBELL, D. R., DUDASH, M. R., JOHNSTON, M. O., KNIGHT, T. M., MAZER, S. J., MITCHELL, R. J., STEETS, J. A. & VAMOSI, J. C. (2009) Ovule number per flower in a world of unpredictable pollination. *American Journal of Botany,* 96**,** 1159.

BURTON, T. L. & HUSBAND, B. C. (2000) Fitness differences among diploids, tetraploids, and their triploid progeny in Chamerion angustifolium: mechanisms of inviability and implications for polyploid evolution. *Evolution,* 54**,** 1182-1191.

BURTON, T. L. & HUSBAND, B. C. (2001) Fecundity and offspring ploidy in matings among diploid, triploid and tetraploid Chamerion angustifolium (Onagraceae): consequences for tetraploid establishment. *Heredity,* 87**,** 573-582.

BUSHELL, C., SPIELMAN, M. & SCOTT, R. J. (2003) The basis of natural and artificial postzygotic hybridization barriers in Arabidopsis species. *The Plant Cell Online,* 15**,** 1430-1442.

CANALES, C., BHATT, A. M., SCOTT, R. & DICKINSON, H. (2002) EXS, a putative LRR receptor kinase, regulates male germline cell number and tapetal identity and promotes seed development in Arabidopsis. *Current Biology,* 12**,** 1718-1727.

CAO, J., SCHNEEBERGER, K., OSSOWSKI, S., GÜNTHER, T., BENDER, S., FITZ, J., KOENIG, D., LANZ, C., STEGLE, O. & LIPPERT, C. (2011) Whole-genome sequencing of multiple Arabidopsis thaliana populations. *Nature genetics,* 43**,** 956-963.

CAO, X. & JACOBSEN, S. E. (2002) Locus-specific control of asymmetric and CpNpG methylation by the DRM and CMT3 methyltransferase genes. *PNAS,* 99**,** 16491.

CARMAN, J. C. (2001) The gene effect: genome collisions and apomixis. CIMMYT.

CARMAN, J. G. (1997) Asynchronous expression of duplicate genes in angiosperms may cause apomixis, bispory, tetraspory, and polyembryony. *Biological Journal of the Linnean Society,* 61**,** 51-94.

CARPUTO, D., BARONE, A., CARDI, T., SEBASTIANO, A., FRUSCIANTE, L. & PELOQUIN, S. J. (1997) Endosperm balance number manipulation for direct in vivo germplasm introgression to potato from a sexually isolated relative (Solanum commersonii Dun.). *Proceedings of the National Academy of Sciences,* 94**,** 12013.

CARTAGENA, J. A., MATSUNAGA, S., SEKI, M., KURIHARA, D., YOKOYAMA, M., SHINOZAKI, K., FUJIMOTO, S., AZUMI, Y., UCHIYAMA, S. & FUKUI, K. (2008) The Arabidopsis SDG4 contributes to the regulation of pollen tube growth by methylation of histone H3 lysines 4 and 36 in mature pollen. *Developmental biology,* 315**,** 355-368.

CERVERA, M. T., RUIZ-GARCIA, L. & MARTINEZ-ZAPATER, J. (2002) Analysis of DNA methylation in Arabidopsis thaliana based on methylation-sensitive AFLP markers. *MOLECULAR GENETICS AND GENOMICS,* 268**,** 543-552.

CHAN, S. W. L., HENDERSON, I. R. & JACOBSEN, S. E. (2005) Gardening the genome: DNA methylation in Arabidopsis thaliana. *Nature Reviews Genetics,* 6**,** 351.

CHANDLER, V. L. & STAM, M. (2004) Chromatin conversations: mechanisms and implications of paramutation. *Nature Reviews Genetics,* 5**,** 532-544.

CHARLESWORTH, B. & CHARLESWORTH, D. (1999) The genetic basis of inbreeding depression. *Genetical research,* 74**,** 329-340.

CHAUDHURY, A. M. & BERGER, F. (2001) Maternal control of seed development. *Seminars in cell & developmental biology*. Elsevier.

CHAUDHURY, A. M., KOLTUNOW, A., PAYNE, T., LUO, M., TUCKER, M. R., DENNIS, E. & PEACOCK, W. (2001) Control of early seed development. *Annual review of cell and developmental biology,* 17**,** 677-699.

CHAUDHURY, A. M., MING, L., MILLER, C., CRAIG, S., DENNIS, E. S. & PEACOCK, W. J. (1997) Fertilization-independent seed development in Arabidopsis thaliana. *PNAS,* 94**,** 4223.

CHEN, Z. J. (2007) Genetic and epigenetic mechanisms for gene expression and phenotypic variation in plant polyploids. *Annual review of plant biology,* 58**,** 377.

CHEN, Z. J. (2010) Molecular mechanisms of polyploidy and hybrid vigor. *Trends in plant science,* 15**,** 57-71.

CHEN, Z. J., COMAI, L. & PIKAARD, C. S. (1998) Gene dosage and stochastic effects determine the severity and direction of uniparental ribosomal RNA gene silencing (nucleolar dominance) in Arabidopsis allopolyploids. *PNAS,* 95**,** 14891.

CHEN, Z. J. & NI, Z. (2006) Mechanisms of genomic rearrangements and gene expression changes in plant polyploids. *Bioessays,* 28**,** 240-252.

CHEN, Z. J. & PIKAARD, C. S. (1997) Transcriptional analysis of nucleolar dominance in polyploid plants: biased expression/silencing of progenitor rRNA genes is developmentally regulated in Brassica. *PNAS,* 94**,** 3442.

CHEN, Z. J., WANG, J., TIAN, L., LEE, H. S., WANG, J. J., CHEN, M., LEE, J. J., JOSEFSSON, C., MADLUNG, A. & WATSON, B. (2004) The development of an Arabidopsis model system for genome-wide analysis of polyploidy effects. *Biological Journal of the Linnean Society,* 82**,** 689.

CHENG, W. H., TALIERCIO, E. W. & CHOUREY, P. S. (1996) The Miniature1 seed locus of maize encodes a cell wall invertase required for normal development of endosperm and maternal cells in the pedicel. *The Plant Cell Online,* 8**,** 971.

CHOI, Y., GEHRING, M., JOHNSON, L., HANNON, M., HARADA, J. J., GOLDBERG, R. B., JACOBSEN, S. E. & FISCHER, R. L. (2002) DEMETER, a DNA glycosylase domain protein, is required for endosperm gene imprinting and seed viability in Arabidopsis. *Cell,* 110**,** 33-42.

CHORY, J., ECKER, J. R., BRIGGS, S., CABOCHE, M., CORUZZI, G. M., COOK, D., DANGL, J., GRANT, S., GUERINOT, M. L. & HENIKOFF, S. (2000) National Science Foundation-Sponsored Workshop Report:"The 2010 Project" functional genomics and the virtual plant. A blueprint for understanding how plants are built and how to improve them. *Plant Physiology,* 123**,** 423.

CHRISTENSEN, C. A., KING, E. J., JORDAN, J. R. & DREWS, G. N. (1997) Megagametogenesis in Arabidopsis wild type and the Gf mutant. *Sexual plant reproduction,* 10**,** 49-64.

CLARK, R. M., SCHWEIKERT, G., TOOMAJIAN, C., OSSOWSKI, S., ZELLER, G., SHINN, P., WARTHMANN, N., HU, T. T., FU, G. & HINDS, D. A. (2007) Common sequence polymorphisms shaping genetic diversity in Arabidopsis thaliana. *Science,* 317**,** 338.

CLARKE, C. A. (1984) Hybrids and hybridity. *Journal of the Royal Society of Medicine,* 77**,** 821.

CLARKE, J. H. & DEAN, C. (1994) Mapping FRI, a locus controlling flowering time and vernalization response in Arabidopsis thaliana. *Molecular and General Genetics MGG,* 242**,** 81-89.

COIMBRA, S., ALMEIDA, J., JUNQUEIRA, V., COSTA, M. L. & PEREIRA, L. G. (2007) Arabinogalactan proteins as molecular markers in Arabidopsis thaliana sexual reproduction. *Journal of Experimental Botany,* 58**,** 4027-4035.

COMAI, L. (2000) Genetic and epigenetic interactions in allopolyploid plants. *Plant molecular biology,* 43**,** 387-399.

COMAI, L. (2005) The advantages and disadvantages of being polyploid. *Nature Reviews Genetics,* 6**,** 836-846.

COMAI, L., MADLUNG, A., JOSEFSSON, C. & TYAGI, A. (2003) Do the different parental 'heteromes' cause genomic shock in newly formed allopolyploids? *Philosophical Transactions of the Royal Society B* 358**,** 1149.

COMAI, L., TYAGI, A. P., WINTER, K., HOLMES-DAVIS, R., REYNOLDS, S. H., STEVENS, Y. & BYERS, B. (2000) Phenotypic instability and rapid gene silencing in newly formed Arabidopsis allotetraploids. *The Plant Cell,* 12**,** 1551.

COSTA, L. M., GUTIERREZ-MARCOS, J. F. & DICKINSON, H. G. (2004) More than a yolk: the short life and complex times of the plant endosperm. *Trends in plant science,* 9**,** 507-514.

CROW, J. F. (1998) 90 years ago: The beginning of hybrid maize. *Genetics,* 148**,** 923-928.

D'ERFURTH, I., JOLIVET, S., FROGER, N., CATRICE, O., NOVATCHKOVA, M. & MERCIER, R. (2009) Turning meiosis into mitosis. *PLoS biology,* 7**,** e1000124.

DARWIN, C. (1876) *The effects of cross and self fertilisation in the vegetable kingdom*.

DE JONG, T. J., HERMANS, C. M. & VAN DER VEEN VAN WIJK, K. (2009) Paternal effects on seed mass in Arabidopsis thaliana. *Plant Biology*.

DE JONG, T. J. & SCOTT, R. J. (2007) Parental conflict does not necessarily lead to the evolution of imprinting. *Trends in plant science,* 12**,** 439-443.

DEBEAUJON, I., LÉON-KLOOSTERZIEL, K. M. & KOORNNEEF, M. (2000) Influence of the testa on seed dormancy, germination, and longevity in Arabidopsis. *Plant physiology,* 122**,** 403.

DEMIDOV, D., HOUBEN, A. & SCHUBERT, I. (2006) Chromosomal histone modification patterns-from conservation to diversity. *Trends in plant science,* 11**,** 199-208.

DESLANDES, L., PILEUR, F., LIAUBET, L., CAMUT, S., CAN, C., WILLIAMS, K., HOLUB, E., BEYNON, J., ARLAT, M. & MARCO, Y. (1998) Genetic characterization of RRS1, a recessive locus in Arabidopsis thaliana that confers resistance to the bacterial soilborne pathogen Ralstonia solanacearum. *Molecular Plant-Microbe Interactions,* 11**,** 659-667.

DEVLIN, B. & ROEDER, K. (1999) Genomic control for association studies. *Biometrics,* 55**,** 997-1004.

DIEPENBROCK, W. (2000) Yield analysis of winter oilseed rape (Brassica napus L.): a review. *Field Crops Research,* 67**,** 35-49.

DILKES, B. P. & COMAI, L. (2004) A differential dosage hypothesis for parental effects in seed development. *The Plant Cell Online,* 16**,** 3174.

DILKES, B. P., SPIELMAN, M., WEIZBAUER, R., WATSON, B., BURKART-WACO, D., SCOTT, R. J. & COMAI, L. (2008) The maternally expressed WRKY transcription factor TTG2 controls lethality in interploidy crosses of Arabidopsis. *PLoS biology,* 6**,** e308.

DIVI, U. K. & KRISHNA, P. (2009) Brassinosteroid: a biotechnological target for enhancing crop yield and stress tolerance. *New biotechnology,* 26**,** 131-136.

DOBZHANSKY, T. G. (1937) *Genetics and the Origin of Species*, Columbia Univ Pr.

DOGANLAR, S., FRARY, A. & TANKSLEY, S. D. (2000) The genetic basis of seed-weight variation: tomato as a model system. *Theoretical and Applied Genetics,* 100**,** 1267-1273.

DORWEILER, J., STEC, A., KERMICLE, J. & DOEBLEY, J. (1993) Teosinte glume architecture 1: a genetic locus controlling a key step in maize evolution. *Science,* 262**,** 233.

DOWLING, T. E. & SECOR, C. L. (1997) The role of hybridization and introgression in the diversification of animals. *Annual Review of Ecology and Systematics***,** 593-619.

DRESSELHAUS, T. (2006) Cell-cell communication during double fertilization. *Current opinion in plant biology,* 9**,** 41-47.

DRESSELHAUS, T., CARMAN, J. G. & SAVIDAN, Y. (2001) Genetic engineering of apomixis in sexual crops: A critical assessment of the apomixis technology.

DRESSELHAUS, T. & COLOMBO, L. (2001) 2nd International Apomixis Conference (APO2001) at Como, Italy, 24-28 April 2001. *Sexual Plant Reproduction,* 14**,** 245-251.

DREWS, G. N., LEE, D. & CHRISTENSEN, C. A. (1998) Genetic analysis of female gametophyte development and function. *The Plant Cell Online,* 10**,** 5-18.

DUDAREWICZ, L., HOLZGREVE, W., JEZIOROWSKA, A., JAKUBOWSKI, L. & ZIMMERMANN, B. (2005) Molecular methods for rapid detection of aneuploidy. *J Appl Genet,* 46**,** 207-215.

EGLI, D. B. (1998) *Seed biology and the yield of grain crops,* CAB international.

EHRENREICH, I. M., HANZAWA, Y., CHOU, L., ROE, J. L., KOVER, P. X. & PURUGGANAN, M. D. (2009) Candidate gene association mapping of Arabidopsis flowering time. *Genetics,* 183**,** 325.

ERILOVA, A., BROWNFIELD, L., EXNER, V., ROSA, M., TWELL, D., SCHEID, O. M., HENNIG, L. & KÖHLER, C. (2009) Imprinting of the polycomb group gene MEDEA serves as a ploidy sensor in Arabidopsis. *PLoS Genetics,* 5**,** e1000663.

ERNST, A. (1918) *Bastardierung als Ursache der Apogamie im Pflanzenreich*, G. Fischer.

FAURE, J. E., ROTMAN, N., FORTUNE, P. & DUMAS, C. (2002) Fertilization in Arabidopsis thaliana wild type: developmental stages and time course. *The Plant Journal,* 30**,** 481-488.

FINNEGAN, E. J. & KOVAC, K. A. (2000) Plant DNA methyltransferases. *Plant molecular biology,* 43**,** 189-201.

FINNEGAN, E. J., PEACOCK, W. J. & DENNIS, E. S. (1996) Reduced DNA methylation in Arabidopsis thaliana results in abnormal plant development. *PNAS,* 93**,** 8449.

FISHMAN, L. & WILLIS, J. H. (2001) EVIDENCE FOR DOBZHANSKY MULLER INCOMPATIBILITES CONTRIBUTING TO THE STERILITY OF HYBRIDS BETWEEN MIMULUS GUTTATUS AND M. NASUTUS. *Evolution,* 55**,** 1932-1942.

FITZGERALD, J., LUO, M., CHAUDHURY, A. & BERGER, F. (2008) DNA methylation causes predominant maternal controls of plant embryo growth. *PLoS One,* 3**,** e2298.

FRANSZ, P. F. & DE JONG, J. H. (2002) Chromatin dynamics in plants. *Current opinion in plant biology,* 5**,** 560-567.

FRARY, A., NESBITT, T. C., FRARY, A., GRANDILLO, S., KNAAP, E., CONG, B., LIU, J., MELLER, J., ELBER, R. & ALPERT, K. B. (2000) fw2. 2: a quantitative trait locus key to the evolution of tomato fruit size. *Science,* 289**,** 85.

FREELING, M. & THOMAS, B. C. (2006) Gene-balanced duplications, like tetraploidy, provide predictable drive to increase morphological complexity. *Genome research,* 16**,** 805.

FU, J., KEURENTJES, J. J. B., BOUWMEESTER, H., AMERICA, T., VERSTAPPEN, F. W. A., WARD, J. L., BEALE, M. H., DE VOS, R. C. H., DIJKSTRA, M. & SCHELTEMA, R. A. (2009) System-wide molecular evidence for phenotypic buffering in Arabidopsis. *Nature genetics,* 41**,** 166-167.

FUNDYGA, R. E., KUYKENDALL, R. J., LEE-YANG, W. & LOTT, T. J. (2004) Evidence for aneuploidy and recombination in the human commensal yeast Candida parapsilosis. *Infection, Genetics and Evolution,* 4**,** 37-43.

GALLOWAY, L. F., ETTERSON, J. R. & MCGLOTHLIN, J. W. (2009) Contribution of direct and maternal genetic effects to life history evolution. *New Phytologist,* 183**,** 826-838.

GARCIA, D., FITZ GERALD, J. N. & BERGER, F. (2005) Maternal control of integument cell elongation and zygotic control of endosperm growth are coordinated to determine seed size in Arabidopsis. *The Plant Cell Online,* 17**,** 52.

GARCIA, D., SAINGERY, V., CHAMBRIER, P., MAYER, U., JÜRGENS, G. & BERGER, F. (2003) Arabidopsis haiku mutants reveal new controls of seed size by endosperm. *Plant Physiology,* 131**,** 1661.

GARNIER GÉRÉ, P. H., NACIRI GRAVEN, Y., BOUGRIER, S., MAGOULAS, A., HERAL, M., KOTOULAS, G., HAWKINS, A. & GÉRARD, A. (2002) Influences of triploidy, parentage and genetic diversity on growth of the Pacific oyster Crassostrea gigas reared in contrasting natural environments. *Molecular Ecology,* 11**,** 1499-1514.

GEHRING, M., BUBB, K. L. & HENIKOFF, S. (2009) Extensive demethylation of repetitive elements during seed development underlies gene imprinting. *Science,* 324**,** 1447.

GEHRING, M., CHOI, Y. & FISCHER, R. L. (2004) Imprinting and seed development. *The Plant Cell Online,* 16**,** S203.

GONG, Z., MORALES-RUIZ, T., ARIZA, R. R., ROLDÁN-ARJONA, T., DAVID, L. & ZHU, J. K. (2002) ROS1, a repressor of transcriptional gene silencing in Arabidopsis, encodes a DNA glycosylase/lyase. *Cell,* 111**,** 803-814.

GREENWAY, C. A. & HARDER, L. D. (2007) Variation in ovule and seed size and associated size–number trade-offs in angiosperms. *American Journal of Botany,* 94**,** 840.

GREGORY, T. R. (2005) *The evolution of the genome,* Academic Press.

GRIMANELLI, D., LEBLANC, O., PEROTTI, E. & GROSSNIKLAUS, U. (2001) Developmental genetics of gametophytic apomixis. *Trends in Genetics,* 17**,** 597-604.

GROSS-HARDT, R., LENHARD, M. & LAUX, T. (2002) WUSCHEL signaling functions in interregional communication during Arabidopsis ovule development. *Genes & development,* 16**,** 1129-1138.

GROSSNIKLAUS, U. (2001) From sexuality to apomixis: molecular and genetic approaches. CIMMYT.

GROSSNIKLAUS, U., NOGLER, G. A. & VAN DIJK, P. J. (2001a) How to avoid sex: The genetic control of gametophytic apomixis. *The Plant Cell Online,* 13**,** 1491-1498.

GROSSNIKLAUS, U. & SCHNEITZ, K. (1998) The molecular and genetic basis of ovule and megagametophyte development. *Seminars in cell & developmental biology,* 9**,** 227-238.

GROSSNIKLAUS, U., SPILLANE, C., PAGE, D. R. & KOEHLER, C. (2001b) Genomic imprinting and seed development: endosperm formation with and without sex. *Current Opinion in Plant Biology,* 4**,** 21-27.

GROSSNIKLAUS, U., VIELLE-CALZADA, J. P., HOEPPNER, M. A. & GAGLIANO, W. B. (1998) Maternal control of embryogenesis by MEDEA, a polycomb group gene in Arabidopsis. *Science,* 280**,** 446.

GUEDES-PINTO, H., DARVEY, N. L. & CARNIDE, V. P. (1996) *Triticale: today and tomorrow,* Kluwer Academic Pub.

GUO, M., DAVIS, D. & BIRCHLER, J. A. (1996) Dosage effects on gene expression in a maize ploidy series. *Genetics,* 142**,** 1349.

GUO, M., RUPE, M. A., YANG, X., CRASTA, O., ZINSELMEIER, C., SMITH, O. S. & BOWEN, B. (2006) Genome-wide transcript analysis of maize hybrids: allelic additive gene expression and yield heterosis. *TAG THEORETICAL AND APPLIED GENETICS,* 113**,** 831-845.

HA, M., LU, J., TIAN, L., RAMACHANDRAN, V., KASSCHAU, K. D., CHAPMAN, E. J., CARRINGTON, J. C., CHEN, X., WANG, X. J. & CHEN, Z. J. (2009) Small RNAs serve as a genetic buffer against genomic shock in Arabidopsis interspecific hybrids and allopolyploids. *Proceedings of the National Academy of Sciences,* 106**,** 17835-17840.

HABASHY, A., TESTA, G., MOSCONI, P., CACCIA, R., MAZZUCATO, A., SANTANGE-LO, E. & SORESSI, G. (2004) Parthenocarpy restores fruitfulness in sterile triploid (3x) tomatoes artificially obtained by crossing 4x x 2x somaclones. *Journal of horticultural science & biotechnology,* 79**,** 322-328.

HAGENBLAD, J., TANG, C., MOLITOR, J., WERNER, J., ZHAO, K., ZHENG, H., MARJORAM, P., WEIGEL, D. & NORDBORG, M. (2004) Haplotype structure and phenotypic associations in the chromosomal regions surrounding two Arabidopsis thaliana flowering time loci. *Genetics,* 168**,** 1627.

HAIG, D. (2000) The kinship theory of genomic imprinting. *Annual Review of Ecology and Systematics*, 9-32.

HAIG, D. & WESTOBY, M. (1989) Parent-specific gene expression and the triploid endosperm. *The American Naturalist*, 134, 147-155.

HAIG, D. & WESTOBY, M. (1991) Genomic imprinting in endosperm: its effect on seed development in crosses between species, and between different ploidies of the same species, and its implications for the evolution of apomixis. *Philosophical Transactions of the Royal Society B*, 333, 1.

HANNAH, M. A., WIESE, D., FREUND, S., FIEHN, O., HEYER, A. G. & HINCHA, D. K. (2006) Natural genetic variation of freezing tolerance in Arabidopsis. *Plant Physiology* 142, 98.

HARDENACK, S., YE, D., SAEDLER, H. & GRANT, S. (1994) Comparison of MADS box gene expression in developing male and female flowers of the dioecious plant white campion. *The Plant Cell Online*, 6, 1775.

HE, G., ZHU, X., ELLING, A. A., CHEN, L., WANG, X., GUO, L., LIANG, M., HE, H., ZHANG, H. & CHEN, F. (2010) Global epigenetic and transcriptional trends among two rice subspecies and their reciprocal hybrids. *The Plant Cell Online*, 22, 17-33.

HEGARTY, M. J., JONES, J. M., WILSON, I. D., BARKER, G. L., COGHILL, J. A., SANCHEZ BARACALDO, P., LIU, G., BUGGS, R. J. A., ABBOTT, R. J. & EDWARDS, K. J. (2005) Development of anonymous cDNA microarrays to study changes to the Senecio floral transcriptome during hybrid speciation. *Molecular Ecology*, 14, 2493-2510.

HENIKOFF, S. & COMAI, L. (1998) A DNA methyltransferase homolog with a chromodomain exists in multiple polymorphic forms in Arabidopsis. *Genetics*, 149, 307.

HENRY, I. M., DILKES, B. P. & COMAI, L. (2006) Molecular karyotyping and aneuploidy detection in Arabidopsis thaliana using quantitative fluorescent polymerase chain reaction. *The Plant Journal*, 48, 307-319.

HENRY, I. M., DILKES, B. P. & COMAI, L. (2007) Genetic basis for dosage sensitivity in Arabidopsis thaliana. *PLoS genetics*, 3, e70.

HENRY, I. M., DILKES, B. P., MILLER, E. S., BURKART-WACO, D. & COMAI, L. (2010) Phenotypic consequences of aneuploidy in Arabidopsis thaliana. *Genetics*, 186, 1231.

HENRY, I. M., DILKES, B. P., TYAGI, A. P., LIN, H. Y. & COMAI, L. (2009) Dosage and parent-of-origin effects shaping aneuploid swarms in A. thaliana. *Heredity*, 103, 458-468.

HENRY, I. M., DILKES, B. P., YOUNG, K., WATSON, B., WU, H. & COMAI, L. (2005) Aneuploidy and genetic variation in the Arabidopsis thaliana triploid response. *Genetics*, 170, 1979.

HIGASHIYAMA, T., YABE, S., SASAKI, N., NISHIMURA, Y., MIYAGISHIMA, S., KUROIWA, H. & KUROIWA, T. (2001) Pollen tube attraction by the synergid cell. *Science*, 293, 1480-1483.

HILU, K. W. (1993) Polyploidy and the evolution of domesticated plants. *American journal of botany*, 1494-1499.

HIROCHIKA, H., OKAMOTO, H. & KAKUTANI, T. (2000) Silencing of retrotransposons in Arabidopsis and reactivation by the ddm1 mutation. *The Plant Cell Online*, 12, 357.

HOFFMANN, M. H. (2002) Biogeography of Arabidopsis thaliana (L.) Heynh.(Brassicaceae). *Journal of Biogeography*, 29, 125-134.

HOUSE, C., ROTH, C., HUNT, J. & KOVER, P. X. (2010) Paternal effects in Arabidopsis indicate that offspring can influence their own size. *Proceedings of the Royal Society B* 277, 2885.

HSIEH, T. F., HAKIM, O., OHAD, N. & FISCHER, R. L. (2003) From flour to flower: how Polycomb group proteins influence multiple aspects of plant development. *Trends in plant science*, 8, 439-445.

HUETTEL, B., KREIL, D. P., MATZKE, M. & MATZKE, A. J. M. (2008) Effects of aneuploidy on genome structure, expression, and interphase organization in Arabidopsis thaliana. *PLoS genetics*, 4, e1000226.

HUGHES, T. R., ROBERTS, C. J., DAI, H., JONES, A. R., MEYER, M. R., SLADE, D., BURCHARD, J., DOW, S., WARD, T. R. & KIDD, M. J. (2000) Widespread aneuploidy revealed by DNA microarray expression profiling. *Nature genetics*, 25, 333-337.

HUH, J. H., BAUER, M. J., HSIEH, T. F. & FISCHER, R. (2007) Endosperm gene imprinting and seed development. *Current Opinion in Genetics & Development*, 17, 480-485.

HUSBAND, B. C. (2004) The role of triploid hybrids in the evolutionary dynamics of mixed-ploidy populations. *Biological Journal of the Linnean Society*, 82, 537-546.

HYTEN, D. L., PANTALONE, V. R., SAMS, C. E., SAXTON, A. M., LANDAU-ELLIS, D., STEFANIAK, T. R. & SCHMIDT, M. E. (2004) Seed quality QTL in a prominent soybean population. *TAG THEORETICAL AND APPLIED GENETICS,* 109**,** 552-561.

JACKSON, J. P., LINDROTH, A. M., CAO, X. & JACOBSEN, S. E. (2002) Control of CpNpG DNA methylation by the KRYPTONITE histone H3 methyltransferase. *Nature,* 416**,** 556-560.

JAHNKE, S., SARHOLZ, B., THIEMANN, A., KÜHR, V., GUTIÉRREZ-MARCOS, J. F., GEIGER, H. H., PIEPHO, H. P. & SCHOLTEN, S. (2010) Heterosis in early seed development: a comparative study of F1 embryo and endosperm tissues 6 days after fertilization. *TAG THEORETICAL AND APPLIED GENETICS,* 120**,** 389-400.

JANSEN, R. C., OOIJEN, J. W., STAM, P., LISTER, C. & DEAN, C. (1995) Genotype-by-environment interaction in genetic mapping of multiple quantitative trait loci. *TAG THEORETICAL AND APPLIED GENETICS,* 91**,** 33-37.

JEAN FINNEGAN, E. & DENNIS, E. S. (1993) Isolation and identification by sequence homology of a putative cytosine methyltransferase from Arabidopsis thaliana. *Nucleic acids research,* 21**,** 2383.

JIANG, C. X., WRIGHT, R. J., EL-ZIK, K. M. & PATERSON, A. H. (1998) Polyploid formation created unique avenues for response to selection in Gossypium (cotton). *PNAS,* 95**,** 4419.

JOFUKU, K. D., OMIDYAR, P. K., GEE, Z. & OKAMURO, J. K. (2005) Control of seed mass and seed yield by the floral homeotic gene APETALA2. *PNAS,* 102**,** 3117.

JOHANSON, U., WEST, J., LISTER, C., MICHAELS, S., AMASINO, R. & DEAN, C. (2000) Molecular analysis of FRIGIDA, a major determinant of natural variation in Arabidopsis flowering time. *Science,* 290**,** 344.

JOHNSON, C. S., KOLEVSKI, B. & SMYTH, D. R. (2002a) TRANSPARENT TESTA GLABRA2, a trichome and seed coat development gene of Arabidopsis, encodes a WRKY transcription factor. *The Plant Cell Online,* 14**,** 1359.

JOHNSON, L. M., CAO, X. & JACOBSEN, S. E. (2002b) Interplay between Two Epigenetic Marks:: DNA Methylation and Histone H3 Lysine 9 Methylation. *Current Biology,* 12**,** 1360-1367.

JOHNSSON, H. (1942) Cytological studies of triploid progenies of Populus tremula. *Hereditas,* 28**,** 306-312.

JOHNSTON, A. J., MEIER, P., GHEYSELINCK, J., WUEST, S. E. J., FEDERER, M., SCHLAGENHAUF, E., BECKER, J. D. & GROSSNIKLAUS, U. (2007) Genetic subtraction profiling identifies genes essential for Arabidopsis reproduction and reveals interaction between the female gametophyte and the maternal sporophyte. *Genome biology,* 8**,** R204.

JOHNSTON, S. & HANNEMAN JR, R. (1995) The genetics of triploid formation and its relationship to endosperm balance number in potato. *Genome,* 38**,** 60-67.

JUENGER, T. E., MCKAY, J. K., HAUSMANN, N., KEURENTJES, J. J. B., SEN, S., STOWE, K. A., DAWSON, T. E., SIMMS, E. L. & RICHARDS, J. H. (2005) Identification and characterization of QTL underlying whole plant physiology in Arabidopsis thaliana: 13C, stomatal conductance and transpiration efficiency. *Plant, Cell & Environment,* 28**,** 697-708.

JULLIEN, P. E., KATZ, A., OLIVA, M., OHAD, N. & BERGER, F. (2006a) Polycomb group complexes self-regulate imprinting of the Polycomb group gene MEDEA in Arabidopsis. *Current biology,* 16**,** 486-492.

JULLIEN, P. E., KINOSHITA, T., OHAD, N. & BERGER, F. (2006b) Maintenance of DNA methylation during the Arabidopsis life cycle is essential for parental imprinting. *The Plant Cell Online,* 18**,** 1360.

KAKUTANI, T., JEDDELOH, J. A., FLOWERS, S. K., MUNAKATA, K. & RICHARDS, E. J. (1996) Developmental abnormalities and epimutations associated with DNA hypomethylation mutations. *PNAS,* 93**,** 12406.

KANG, H. M., ZAITLEN, N. A., WADE, C. M., KIRBY, A., HECKERMAN, D., DALY, M. J. & ESKIN, E. (2008a) Efficient control of population structure in model organism association mapping. *Genetics,* 178**,** 1709.

KANG, I. H., STEFFEN, J. G., PORTEREIKO, M. F., LLOYD, A. & DREWS, G. N. (2008b) The AGL62 MADS domain protein regulates cellularization during endosperm development in Arabidopsis. *The Plant Cell,* 20**,** 635.

KANTAMA, L., LAMBERT, Y., HU, H., DE JONG, H., DE VRIES, S. C. & RUSSINOVA, E. (2006) Use of the SSLP-based method for detection of rare apomictic events in a sexual AtSERK1 transgenic Arabidopsis population. *Sexual Plant Reproduction,* 19**,** 73-82.

KASHKUSH, K., FELDMAN, M. & LEVY, A. A. (2002) Transcriptional activation of retrotransposons alters the expression of adjacent genes in wheat. *Nature genetics,* 33**,** 102-106.

KEHOE, D. M., VILLAND, P. & SOMERVILLE, S. (1999) DNA microarrays for studies of higher plants and other photosynthetic organisms. *Trends in plant science,* 4**,** 38-41.

KELLY, M. G. & LEVIN, D. A. (2000) Directional selection on initial flowering date in Phlox drummondii (Polemoniaceae). *American Journal of Botany,* 87**,** 382.

KEURENTJES, J. J. B., BENTSINK, L., ALONSO-BLANCO, C., HANHART, C. J., BLANKESTIJN-DE VRIES, H., EFFGEN, S., VREUGDENHIL, D. & KOORNNEEF, M. (2007a) Development of a near-isogenic line population of Arabidopsis thaliana and comparison of mapping power with a recombinant inbred line population. *Genetics,* 175**,** 891.

KEURENTJES, J. J. B., FU, J., TERPSTRA, I. R., GARCIA, J. M., VAN DEN ACKERVEKEN, G., SNOEK, L. B., PEETERS, A. J. M., VREUGDENHIL, D., KOORNNEEF, M. & JANSEN, R. C. (2007b) Regulatory network construction in Arabidopsis by using genome-wide gene expression quantitative trait loci. *Proceedings of the National Academy of Sciences,* 104**,** 1708.

KHAN, J. A., WANG, Q., SJÖLUND, R. D., SCHULZ, A. & THOMPSON, G. A. (2007) An early nodulin-like protein accumulates in the sieve element plasma membrane of Arabidopsis. *Plant physiology,* 143**,** 1576-1589.

KHUSH, G. S. (1973) Cytogenetics of aneuploids. *Academic Press*.

KIM, H. U. & HUANG, A. H. C. (2004) Plastid lysophosphatidyl acyltransferase is essential for embryo development in Arabidopsis. *Plant physiology,* 134**,** 1206-1216.

KIM, S., PLAGNOL, V., HU, T. T., TOOMAJIAN, C., CLARK, R. M., OSSOWSKI, S., ECKER, J. R., WEIGEL, D. & NORDBORG, M. (2007) Recombination and linkage disequilibrium in Arabidopsis thaliana. *Nature genetics,* 39**,** 1151-1155.

KIM, S., SOLTIS, P. S., WALL, K. & SOLTIS, D. E. (2006) Phylogeny and domain evolution in the APETALA2-like gene family. *Molecular biology and evolution,* 23**,** 107-120.

KINOSHITA, T. (2007) Reproductive barrier and genomic imprinting in the endosperm of flowering plants. *Genes & genetic systems,* 82**,** 177-186.

KINOSHITA, T., MIURA, A., CHOI, Y., KINOSHITA, Y., CAO, X., JACOBSEN, S. E., FISCHER, R. L. & KAKUTANI, T. (2004) One-way control of FWA imprinting in Arabidopsis endosperm by DNA methylation. *Science,* 303**,** 521.

KINOSHITA, T., YADEGARI, R., HARADA, J. J., GOLDBERG, R. B. & FISCHER, R. L. (1999) Imprinting of the MEDEA polycomb gene in the Arabidopsis endosperm. *The Plant Cell,* 11**,** 1945.

KIYOSUE, T., OHAD, N., YADEGARI, R., HANNON, M., DINNENY, J., WELLS, D., KATZ, A., MARGOSSIAN, L., HARADA, J. J. & GOLDBERG, R. B. (1999) Control of fertilization-independent endosperm development by the MEDEA polycomb gene in Arabidopsis. *PNAS,* 96**,** 4186.

KLIEBENSTEIN, D. (2009) Quantitative genomics: analyzing intraspecific variation using global gene expression polymorphisms or eQTLs. *Plant Biology,* 60**,** 93.

KOBAYASHI, Y. & WEIGEL, D. (2007) Move on up, it's time for change—mobile signals controlling photoperiod-dependent flowering. *Genes & development,* 21**,** 2371.

KÖHLER, C., HENNIG, L., SPILLANE, C., PIEN, S., GRUISSEM, W. & GROSSNIKLAUS, U. (2003) The Polycomb-group protein MEDEA regulates seed development by controlling expression of the MADS-box gene PHERES1. *Genes & Development,* 17**,** 1540.

KÖHLER, C., MITTELSTEN SCHEID, O. & ERILOVA, A. (2010) The impact of the triploid block on the origin and evolution of polyploid plants. *Trends in Genetics,* 26**,** 142-148.

KÖHLER, C., PAGE, D. R., GAGLIARDINI, V. & GROSSNIKLAUS, U. (2004) The Arabidopsis thaliana MEDEA Polycomb group protein controls expression of PHERES1 by parental imprinting. *Nature genetics,* 37**,** 28-30.

KÖHLER, C., PAGE, D. R., GAGLIARDINI, V. & GROSSNIKLAUS, U. (2005) The Arabidopsis thaliana MEDEA Polycomb group protein controls expression of PHERES1 by parental imprinting. *Nature genetics,* 37**,** 28-30.

KOLTUNOW, A. M. (1993) Apomixis: embryo sacs and embryos formed without meiosis or fertilization in ovules. *The Plant Cell,* 5**,** 1425.

KOLTUNOW, A. M. & GROSSNIKLAUS, U. (2003) Apomixis: a developmental perspective. *Annual Review of Plant Biology,* 54**,** 547-574.

KOORNNEEF, M., ALONSO-BLANCO, C., PEETERS, A. J. M. & SOPPE, W. (1998) Genetic control of flowering time in Arabidopsis. *Annual Review of Plant Physiology and Plant Molecular Biology,* 49**,** 345-370.

KOORNNEEF, M., ALONSO-BLANCO, C. & VREUGDENHIL, D. (2004) Naturally occurring genetic variation in Arabidopsis thaliana. *Annual Review of Plant Biology,* 55**,** 141-172.

KOORNNEEF, M. & MEINKE, D. (2010) The development of Arabidopsis as a model plant. *The Plant Journal,* 61**,** 909-921.

KOORNNEEF, M. & VEEN, J. H. (1983) Trisomics inArabidopsis thaliana and the location of linkage groups. *Genetica* 61**,** 41-46.

KORBECKA, G., KLINKHAMER, P. G. L. & VRIELING, K. (2002) Selective embryo abortion hypothesis revisited-a molecular approach. *Plant Biology,* 4**,** 298-310.

KOUZARIDES, T. (2007) Chromatin modifications and their function. *Cell,* 128**,** 693-705.

KRANNITZ, P. G., AARSSEN, L. W. & DOW, J. M. (1991) The effect of genetically based differences in seed size on seedling survival in Arabidopsis thaliana (Brassicaceae). *American journal of botany***,** 446-450.

KRIZEK, B. A. (1999) Ectopic expression of AINTEGUMENTA in Arabidopsis plants results in increased growth of floral organs. *Developmental Genetics,* 25**,** 224-236.

KUNKEL, B. N. (1996) A useful weed put to work: genetic analysis of disease resistance in Arabidopsis thaliana. *Trends in Genetics,* 12**,** 63-69.

LEBLANC, O., POINTE, C. & HERNANDEZ, M. (2002) Cell cycle progression during endosperm development in Zea mays depends on parental dosage effects. *The Plant Journal,* 32**,** 1057-1066.

LEE, H. S. & CHEN, Z. J. (2001) Protein-coding genes are epigenetically regulated in Arabidopsis polyploids. *PNAS,* 98**,** 6753.

LEITCH, I. J. & BENNETT, M. D. (1997) Polyploidy in angiosperms. *Trends in Plant Science,* 2**,** 470-476.

LEMONTEY, C., MOUSSET-DÉCLAS, C., MUNIER-JOLAIN, N. & BOUTIN, J. P. (2000) Maternal genotype influences pea seed size by controlling both mitotic activity during early embryogenesis and final endoreduplication level/cotyledon cell size in mature seed. *Journal of experimental botany,* 51**,** 167.

LEONELLI, S. (2007) Arabidopsis, the botanical Drosophila: from mouse cress to model organism. *Endeavour,* 31**,** 34-38.

LEVAN, A. (1942) The effect of chromosomal variation in sugar beets. *Hereditas,* 28**,** 345-399.

LI-BEISSON, Y., SHORROSH, B., BEISSON, F., ANDERSSON, M. X., ARONDEL, V., BATES, P. D., BAUD, S., BIRD, D., DEBONO, A. & DURRETT, T. P. (2010) Acyl-lipid metabolism. *The Arabidopsis Book,* 8**,** 1-65.

LI, Y., ZHENG, L., CORKE, F., SMITH, C. & BEVAN, M. W. (2008) Control of final seed and organ size by the DA1 gene family in Arabidopsis thaliana. *Genes & Development,* 22**,** 1331.

LI, Z. K., LUO, L. J., MEI, H. W., WANG, D. L., SHU, Q. Y., TABIEN, R., ZHONG, D. B., YING, C. S., STANSEL, J. W. & KHUSH, G. S. (2001) Overdominant epistatic loci are the primary genetic basis of inbreeding depression and heterosis in rice. I. Biomass and grain yield. *Genetics,* 158**,** 1737.

LIN, B. Y. (1982) Association of endosperm reduction with parental imprinting in maize. *Genetics,* 100**,** 475.

LIN, B. Y. (1984) Ploidy barrier to endosperm development in maize. *Genetics,* 107**,** 103.

LINDROTH, A. M., CAO, X., JACKSON, J. P., ZILBERMAN, D., MCCALLUM, C. M., HENIKOFF, S. & JACOBSEN, S. E. (2001) Requirement of CHROMOMETHYLASE3 for maintenance of CpXpG methylation. *Science,* 292**,** 2077.

LIPPMAN, Z. B. & ZAMIR, D. (2007) Heterosis: revisiting the magic. *Trends in genetics,* 23**,** 60-66.

LITTEL, R. C., MILLIKEN, G. A., STROUP, W. W. & WOLFINGER, R. D. (1996) *SAS sysem for mixed models,,* Cary, NC: SAS Institute.

LORA, J., HORMAZA, J. I., HERRERO, M. & GASSER, C. S. (2011) Seedless fruits and the disruption of a conserved genetic pathway in angiosperm ovule development. *Proceedings of the National Academy of Sciences,* 108**,** 5461.

LOTAN, T., OHTO, M., YEE, K. M., WEST, M. A. L., LO, R., KWONG, R. W., YAMAGISHI, K., FISCHER, R. L., GOLDBERG, R. B. & HARADA, J. J. (1998) Arabidopsis LEAFY COTYLEDON1 Is Sufficient to Induce Embryo Development in Vegetative Cells. *Cell,* 93**,** 1195-1205.

LUND, A. H. & VAN LOHUIZEN, M. (2004) Polycomb complexes and silencing mechanisms. *Current opinion in cell biology,* 16**,** 239-246.

LUO, M., BILODEAU, P., DENNIS, E. S., PEACOCK, W. J. & CHAUDHURY, A. (2000) Expression and parent-of-origin effects for FIS2, MEA, and FIE in the endosperm and embryo of developing Arabidopsis seeds. *PNAS,* 97**,** 10637.

LUO, M., BILODEAU, P., KOLTUNOW, A., DENNIS, E. S., PEACOCK, W. J. & CHAUDHURY, A. M. (1999) Genes controlling fertilization-independent seed development in Arabidopsis thaliana. *PNAS,* 96**,** 296.

LUO, M., DENNIS, E. S., BERGER, F., PEACOCK, W. J. & CHAUDHURY, A. (2005) MINISEED3 (MINI3), a WRKY family gene, and HAIKU2 (IKU2), a leucine-rich repeat (LRR) KINASE gene, are regulators of seed size in Arabidopsis. *PNAS,* 102**,** 17531.

MABLE, B. K. (2004) 'Why polyploidy is rarer in animals than in plants': myths and mechanisms. *Biological Journal of the Linnean Society,* 82**,** 453-466.

MACKIEWICZ, H., MALEPSZY, S., SARREB, D. & NARKIEWICZ, M. (1998) Triploids in cucumber. pt. 2: Characterization of embryo rescue plants. *Gartenbauwissenschaft,* 63.

MADLUNG, A., MASUELLI, R. W., WATSON, B., REYNOLDS, S. H., DAVISON, J. & COMAI, L. (2002) Remodeling of DNA methylation and phenotypic and transcriptional changes in synthetic Arabidopsis allotetraploids. *Plant Physiology,* 129**,** 733.

MADLUNG, A., TYAGI, A. P., WATSON, B., JIANG, H., KAGOCHI, T., DOERGE, R. W., MARTIENSSEN, R. & COMAI, L. (2005) Genomic changes in synthetic Arabidopsis polyploids. *The Plant Journal,* 41**,** 221-230.

MAKAREVICH, G., LEROY, O., AKINCI, U., SCHUBERT, D., CLARENZ, O., GOODRICH, J., GROSSNIKLAUS, U. & KÖHLER, C. (2006) Different Polycomb group complexes regulate common target genes in Arabidopsis. *EMBO reports,* 7**,** 947-952.

MAKAREVITCH, I., STUPAR, R. M., INIGUEZ, A. L., HAUN, W. J., BARBAZUK, W. B., KAEPPLER, S. M. & SPRINGER, N. M. (2007) Natural variation for alleles under epigenetic control by the maize chromomethylase zmet2. *Genetics,* 177**,** 749-760.

MANNING, P., HOUSTON, K. & EVANS, T. (2009) Shifts in seed size across experimental nitrogen enrichment and plant density gradients. *Basic and Applied Ecology,* 10**,** 300-308.

MARKS, G. (1966) The origin and significance of intraspecific polyploidy: experimental evidence from Solanum chacoense. *Evolution***,** 552-557.

MARSHALL, D. L. & ELLSTRAND, N. C. (1988) Effective mate choice in wild radish: evidence for selective seed abortion and its mechanism. *American Naturalist***,** 739-756.

MARSHALL, D. L. & WHITTAKER, K. L. (1989) Effects of pollen donor identity on offspring quality in wild radish, Raphanus sativus. *American journal of botany***,** 1081-1088.

MARTIENSSEN, R. A. (2010) Heterochromatin, small RNA and post-fertilization dysgenesis in allopolyploid and interploid hybrids of Arabidopsis. *New Phytologist,* 186**,** 46-53.

MARTIENSSEN, R. A. & COLOT, V. (2001) DNA methylation and epigenetic inheritance in plants and filamentous fungi. *Science,* 293**,** 1070.

MATZK, F., MEISTER, A., BRUTOVSKA, R. & SCHUBERT, I. (2001) Reconstruction of reproductive diversity in Hypericum perforatum L. opens novel strategies to manage apomixis. *The Plant Journal,* 26**,** 275-282.

MATZK, F., MEISTER, A. & SCHUBERT, I. (2000) An efficient screen for reproductive pathways using mature seeds of monocots and dicots. *The Plant Journal,* 21**,** 97-108.

MATZKE, M., KANNO, T., DAXINGER, L., HUETTEL, B. & MATZKE, A. J. M. (2009) RNA-mediated chromatin-based silencing in plants. *Current Opinion in Cell Biology,* 21**,** 367-376.

MATZKE, M., SCHEID, O. M. & MATZKE, A. (1999) Rapid structural and epigenetic changes in polyploid and aneuploid genomes. *Bioessays,* 21**,** 761-767.

MATZKE, M. A., FLORIAN METTE, M., KANNO, T. & MATZKE, A. J. M. (2003) Does the intrinsic instability of aneuploid genomes have a causal role in cancer? *Trends in Genetics,* 19**,** 253-256.

MAURICIO, R. (2001) Mapping quantitative trait loci in plants: uses and caveats for evolutionary biology. *Nature Reviews Genetics,* 2**,** 370-381.

MCCLINTOCK, B. (1929) A cytological and genetical study of triploid maize. *Genetics,* 14**,** 180.

MCCLINTOCK, B. (1984) The significance of responses of the genome to challenge. *Science,* 226**,** 792-801.

MCKAY, J. K., RICHARDS, J. H. & MITCHELL-OLDS, T. (2003) Genetics of drought adaptation in Arabidopsis thaliana: I. Pleiotropy contributes to genetic correlations among ecological traits. *Molecular Ecology,* 12**,** 1137-1151.

MCKEOWN, P., LAOUIELLE-DUPRAT, S., PRINS, P., WOLFF, P., SCHMID, M., DONOGHUE, M., FORT, A., DUSZYNSKA, D., COMTE, A. & LAO, N. (2011) Identification of imprinted genes subject to parent-of-origin specific expression in Arabidopsis thaliana seeds. *BMC Plant Biology,* 11**,** 113.

MEINKE, D. W. (1994) Seed development in Arabidopsis thaliana. *Arabidopsis,* 27**,** 253–295.

MEINKE, D. W., CHERRY, J. M., DEAN, C., ROUNSLEY, S. D. & KOORNNEEF, M. (1998) Arabidopsis thaliana: a model plant for genome analysis. *Science,* 282**,** 662.

MEYER, R. C., KUSTERER, B., LISEC, J., STEINFATH, M., BECHER, M., SCHARR, H., MELCHINGER, A. E., SELBIG, J., SCHURR, U. & WILLMITZER, L. (2010) QTL analysis of early stage heterosis for biomass in Arabidopsis. *TAG THEORETICAL AND APPLIED GENETICS,* 120**,** 227-237.

MEYER, R. C., TÖRJÉK, O., BECHER, M. & ALTMANN, T. (2004) Heterosis of biomass production in Arabidopsis. Establishment during early development. *Plant physiology,* 134**,** 1813.

MICHAELS, S. D. & AMASINO, R. M. (2000) Memories of winter: vernalization and the competence to flower. *Plant, Cell & Environment,* 23**,** 1145-1153.

MILBORROW, B. (1998) A biochemical mechanism for hybrid vigour. *Journal of experimental botany,* 49**,** 1063.

MILLER, M. E. & CHOUREY, P. S. (1992) The maize invertase-deficient miniature-1 seed mutation is associated with aberrant pedicel and endosperm development. *The Plant Cell Online,* 4**,** 297.

MITCHELL-OLDS, T. (1996) Genetic constraints on life-history evolution: quantitative-trait loci influencing growth and flowering in Arabidopsis thaliana. *Evolution,* 50**,** 140-145.

MITCHELL-OLDS, T. & PEDERSEN, D. (1998) The molecular basis of quantitative genetic variation in central and secondary metabolism in Arabidopsis. *Genetics,* 149**,** 739.

MITCHELL-OLDS, T. & SCHMITT, J. (2006) Genetic mechanisms and evolutionary significance of natural variation in Arabidopsis. *Nature,* 441**,** 947-952.

MITCHELL-OLDS, T., WILLIS, J. H. & GOLDSTEIN, D. B. (2007) Which evolutionary processes influence natural genetic variation for phenotypic traits? *Nature Reviews Genetics,* 8**,** 845-856.

MITTELSTEN SCHEID, O., JAKOVLEVA, L., AFSAR, K., MALUSZYNSKA, J. & PASZKOWSKI, J. (1996) A change of ploidy can modify epigenetic silencing. *Proceedings of the National Academy of Sciences,* 93**,** 7114.

MITTELSTEN SCHEID, O. M., AFSAR, K. & PASZKOWSKI, J. (2003) Formation of stable epialleles and their paramutation-like interaction in tetraploid Arabidopsis thaliana. *Nature genetics,* 34**,** 450-454.

MITTELSTEN SCHEID, O. M., PROBST, A. V., AFSAR, K. & PASZKOWSKI, J. (2002) Two regulatory levels of transcriptional gene silencing in Arabidopsis. *Proceedings of the National Academy of Sciences,* 99**,** 13659.

MIURA, A., YONEBAYASHI, S., WATANABE, K., TOYAMA, T., SHIMADA, H. & KAKUTANI, T. (2001) Mobilization of transposons by a mutation abolishing full DNA methylation in Arabidopsis. *Nature,* 411**,** 212-214.

MIZUKAMI, Y. & FISCHER, R. L. (2000) Plant organ size control: AINTEGUMENTA regulates growth and cell numbers during organogenesis. *Proceedings of the National Academy of Sciences,* 97**,** 942.

MOSHER, R. A. & MELNYK, C. W. (2010) siRNAs and DNA methylation: seedy epigenetics. *Trends in plant science,* 15**,** 204-210.

MOSHER, R. A., MELNYK, C. W., KELLY, K. A., DUNN, R. M., STUDHOLME, D. J. & BAULCOMBE, D. C. (2009) Uniparental expression of PolIV-dependent siRNAs in developing endosperm of Arabidopsis. *Nature,* 460**,** 283-286.

MOUSSEAU, T. A. & FOX, C. W. (1998) The adaptive significance of maternal effects. *Trends in Ecology & Evolution,* 13**,** 403-407.

MÜLLER, B. & SHEEN, J. (2007) Arabidopsis cytokinin signaling pathway. *Science's STKE,* 2007.

MULLER, H. J. (1925) Why polyploidy is rarer in animals than in plants. *The American Naturalist,* 59**,** 346-353.

MULLER, H. J. (1942) Isolating mechanisms, evolution and temperature. *Genetics,* 6**,** 125.

MURASHIGE, T. & SKOOG, F. (1962) A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia plantarum,* 15**,** 473-497.

MURPHY, A. & TAIZ, L. (1995) A new vertical mesh transfer technique for metal-tolerance studies in Arabidopsis (ecotypic variation and copper-sensitive mutants). *Plant Physiology,* 108**,** 29.

MYLES, S., PEIFFER, J., BROWN, P. J., ERSOZ, E. S., ZHANG, Z., COSTICH, D. E. & BUCKLER, E. S. (2009) Association mapping: critical considerations shift from genotyping to experimental design. *The Plant Cell Online,* 21**,** 2194.

NESI, N., DEBEAUJON, I., JOND, C., STEWART, A. J., JENKINS, G. I., CABOCHE, M. & LEPINIEC, L. (2002) The TRANSPARENT TESTA16 locus encodes the ARABIDOPSIS BSISTER MADS domain protein and is required for proper development and pigmentation of the seed coat. *The Plant Cell,* 14**,** 2463.

NI, Z., KIM, E. D., HA, M., LACKEY, E., LIU, J., ZHANG, Y., SUN, Q. & CHEN, Z. J. (2008) Altered circadian rhythms regulate growth vigour in hybrids and allopolyploids. *Nature,* 457**,** 327-331.

NOGLER, G. A. (1982) How to obtain diploid apomictic Ranunculus auricomus plants not found in the wild state.

NOGLER, G. A. (1984) Gametophytic apomixis. *Embryology of Angiosperms***,** 475-518.

NORDBORG, M. (2000) Linkage disequilibrium, gene trees and selfing: an ancestral recombination graph with partial self-fertilization. *Genetics,* 154**,** 923.

NORDBORG, M. & BERGELSON, J. (1999) The effect of seed and rosette cold treatment on germination and flowering time in some Arabidopsis thaliana (Brassicaceae) ecotypes. *American Journal of Botany,* 86**,** 470.

NORDBORG, M., BOREVITZ, J. O., BERGELSON, J., BERRY, C. C., CHORY, J., HAGENBLAD, J., KREITMAN, M., MALOOF, J. N., NOYES, T. & OEFNER, P. J. (2002) The extent of linkage disequilibrium in Arabidopsis thaliana. *nature genetics,* 30**,** 190-193.

NORDBORG, M., HU, T. T., ISHINO, Y., JHAVERI, J., TOOMAJIAN, C., ZHENG, H., BAKKER, E., CALABRESE, P., GLADSTONE, J. & GOYAL, R. (2005) The pattern of polymorphism in Arabidopsis thaliana. *PLoS Biology,* 3**,** e196.

NORDBORG, M. & WEIGEL, D. (2008) Next-generation genetics in plants. *Nature,* 456**,** 720-723.

NOWACK, M. K., SHIRZADI, R., DISSMEYER, N., DOLF, A., ENDL, E., GRINI, P. E. & SCHNITTGER, A. (2007) Bypassing genomic imprinting allows seed development. *Nature,* 447**,** 312.

NOYES, R. D. & RIESEBERG, L. H. (2000) Two independent loci control agamospermy (apomixis) in the triploid flowering plant Erigeron annuus. *Genetics,* 155**,** 379.

NYBOM, H. (1988) Apomixis versus sexuality in blackberries (Rubus subgen. Rubus, Rosaceae). Springer.

O'NEILL, R. J. W., O'NEILL, M. J. & GRAVES, J. A. M. (1998) Undermethylation associated with retroelement activation and chromosome remodelling in an interspecific mammalian hybrid. *Nature,* 393**,** 68-72.

OHAD, N., MARGOSSIAN, L., HSU, Y. C., WILLIAMS, C., REPETTI, P. & FISCHER, R. L. (1996) A mutation that allows endosperm development without fertilization. *Proceedings of the National Academy of Sciences,* 93**,** 5319.

OHAD, N., YADEGARI, R., MARGOSSIAN, L., HANNON, M., MICHAELI, D., HARADA, J. J., GOLDBERG, R. B. & FISCHER, R. L. (1999) Mutations in FIE, a WD polycomb group gene, allow endosperm development without fertilization. *The Plant Cell,* 11**,** 407.

OHTO, M., FISCHER, R. L., GOLDBERG, R. B., NAKAMURA, K. & HARADA, J. J. (2005) Control of seed mass by APETALA2. *PNAS,* 102**,** 3123.

OKAMURO, J. K., CASTER, B., VILLARROEL, R., VAN MONTAGU, M. & JOFUKU, K. D. (1997) The AP2 domain of APETALA2 defines a large new family of DNA binding proteins in Arabidopsis. *Proceedings of the National Academy of Sciences,* 94**,** 7076.

OLMEDO-MONFIL, V., DURÁN-FIGUEROA, N., ARTEAGA-VÁZQUEZ, M., DEMESA-ARÉVALO, E., AUTRAN, D., GRIMANELLI, D., SLOTKIN, R. K., MARTIENSSEN, R. A. & VIELLE-CALZADA, J. P. (2010) Control of female gamete formation by a small RNA pathway in Arabidopsis. *Nature,* 464**,** 628-632.

OLSEN, O. A. (2004) Nuclear endosperm development in cereals and Arabidopsis thaliana. *The Plant Cell Online,* 16**,** S214.

ONODERA, Y., HAAG, J. R., REAM, T., NUNES, P. C., PONTES, O. & PIKAARD, C. S. (2005) Plant nuclear RNA polymerase IV mediates siRNA and DNA methylation-dependent heterochromatin formation. *Cell,* 120**,** 613-622.

ONODERA, Y., NAKAGAWA, K., HAAG, J. R., PIKAARD, D., MIKAMI, T., REAM, T., ITO, Y. & PIKAARD, C. S. (2008) Sex-biased lethality or transmission of defective transcription machinery in Arabidopsis. *Genetics,* 180**,** 207.

ORTIZ, R. & VUYLSTEKE, D. (2004) Improved polyploid musa germplasm developed through ploidy manipulations. *African Crop Science Journal,* 5.

OSBORN, T. C., CHRIS PIRES, J., BIRCHLER, J. A., AUGER, D. L., JEFFERY CHEN, Z., LEE, H. S., COMAI, L., MADLUNG, A., DOERGE, R. W. & COLOT, V. (2003) Understanding mechanisms of novel gene expression in polyploids. *Trends in genetics,* 19**,** 141-147.

OTTO, S. P. (2007) The evolutionary consequences of polyploidy. *Cell,* 131**,** 452-462.

OTTO, S. P. & WHITTON, J. (2000) Polyploid incidence and evolution. *Annual review of genetics,* 34**,** 401-437.

OZIAS-AKINS, P. & VAN DIJK, P. J. (2007) Mendelian genetics of apomixis in plants. *Annu. Rev. Genet.,* 41.

PAGE, D. R. & GROSSNIKLAUS, U. (2002) The art and design of genetic screens: Arabidopsis thaliana. *Nature Reviews Genetics,* 3**,** 124-136.

PASZKOWSKI, J. & GROSSNIKLAUS, U. (2011) Selected aspects of transgenerational epigenetic inheritance and resetting in plants. *Current opinion in plant biology*.

PASZKOWSKI, J. & WHITHAM, S. A. (2001) Gene silencing and DNA methylation processes. *Current Opinion in Plant Biology,* 4**,** 123-129.

PECINKA, A., FANG, W., REHMSMEIER, M., LEVY, A. & SCHEID, O. M. (2011) Polyploidization increases meiotic recombination frequency in Arabidopsis. *BMC Biology,* 9**,** 24.

PENTERMAN, J., ZILBERMAN, D., HUH, J. H., BALLINGER, T., HENIKOFF, S. & FISCHER, R. L. (2007) DNA demethylation in the Arabidopsis genome. *Proceedings of the National Academy of Sciences,* 104**,** 6752.

PIKAARD, C. S. (2001) Genomic change and gene silencing in polyploids. *Trends in genetics,* 17**,** 675-677.

PLAGNOL, V., PADHUKASAHASRAM, B., WALL, J. D., MARJORAM, P. & NORDBORG, M. (2006) Relative influences of crossing over and gene conversion on the pattern of linkage disequilibrium in Arabidopsis thaliana. *Genetics,* 172**,** 2441.

PONTES, O., NEVES, N., SILVA, M., LEWIS, M. S., MADLUNG, A., COMAI, L., VIEGAS, W. & PIKAARD, C. S. (2004) Chromosomal locus rearrangements are a rapid response to formation of the allotetraploid Arabidopsis suecica genome. *PNAS,* 101**,** 18240.

PORTEREIKO, M. F., LLOYD, A., STEFFEN, J. G., PUNWANI, J. A., OTSUGA, D. & DREWS, G. N. (2006a) AGL80 is required for central cell and endosperm development in Arabidopsis. *The Plant Cell,* 18**,** 1862.

PORTEREIKO, M. F., SANDAKLIE-NIKOLOVA, L., LLOYD, A., DEVER, C. A., OTSUGA, D. & DREWS, G. N. (2006b) NUCLEAR FUSION DEFECTIVE1 encodes the Arabidopsis RPL21M protein and is required for karyogamy during female gametophyte development and fertilization. *Plant physiology,* 141**,** 957-965.

PRASAD, K., ZHANG, X., TOBÓN, E. & AMBROSE, B. A. (2010) The Arabidopsis B sister MADS box protein, GORDITA, represses fruit growth and contributes to integument development. *The Plant Journal,* 62**,** 203-214.

PRICE, R. A., PALMER, J. D. & AL-SHEHBAZ, I. A. (1994) *Systematic relationships of Arabidopsis: a molecular and morphological perspective*, Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

PRITCHARD, J. K., STEPHENS, M., ROSENBERG, N. A. & DONNELLY, P. (2000) Association mapping in structured populations. *The American Journal of Human Genetics,* 67**,** 170-181.

PRZYBILSKI, R., GRÄF, S., LESCOUTE, A., NELLEN, W., WESTHOF, E., STEGER, G. & HAMMANN, C. (2005) Functional hammerhead ribozymes naturally encoded in the genome of Arabidopsis thaliana. *The Plant Cell Online,* 17**,** 1877-1885.

QUARIN, C. L., ESPINOZA, F., MARTINEZ, E. J., PESSINO, S. C. & BOVO, O. A. (2001) A rise of ploidy level induces the expression of apomixis in Paspalum notatum. *Sexual Plant Reproduction,* 13**,** 243-249.

QUEITSCH, C., SANGSTER, T. A. & LINDQUIST, S. (2002) Hsp90 as a capacitor of phenotypic variation. *Nature,* 417**,** 618-624.

RAFALSKI, J. A. (2002) Novel genetic mapping tools in plants: SNPs and LD-based approaches. *Plant Science,* 162**,** 329-333.

RAMAGE, R. (1960) Trisomics from interchange heterozygotes in barley. *Agron. J,* 52**,** 156-159.

RAMSEY, J. & SCHEMSKE, D. W. (1998) Pathways, mechanisms, and rates of polyploid formation in flowering plants. *Annual Review of Ecology and Systematics***,** 467-501.

RAMSEY, J. & SCHEMSKE, D. W. (2002) Neopolyploidy in flowering plants. *Annual Review of Ecology and Systematics*, 589-639.

RAPP, R., UDALL, J. & WENDEL, J. (2009) Genomic expression dominance in allopolyploids. *BMC Biology*, 7, 18.

RAVI, M., MARIMUTHU, M. P. A. & SIDDIQI, I. (2008) Gamete formation without meiosis in Arabidopsis. *Nature*, 451, 1121-1124.

REAM, T. S., HAAG, J. R., WIERZBICKI, A. T., NICORA, C. D., NORBECK, A. D., ZHU, J. K., HAGEN, G., GUILFOYLE, T. J., PASA-TOLIC, L. & PIKAARD, C. S. (2009) Subunit compositions of the RNA-silencing enzymes Pol IV and Pol V reveal their origins as specialized forms of RNA polymerase II. *Molecular cell*, 33, 192-203.

RÉDEI, G. P. (1970) Arabidopsis thaliana (L.) Heynh. A review of the genetics and biology. *Heredity*, 20.

RHEE, S. Y., BEAVIS, W., BERARDINI, T. Z., CHEN, G., DIXON, D., DOYLE, A., GARCIA-HERNANDEZ, M., HUALA, E., LANDER, G. & MONTOYA, M. (2003) The Arabidopsis Information Resource (TAIR): a model organism database providing a centralized, curated gateway to Arabidopsis biology, research materials and community. *Nucleic acids research*, 31, 224.

RICHARDS, D. E., KING, K. E., AIT-ALI, T. & HARBERD, N. P. (2001) How gibberellin regulates plant growth and development: a molecular genetic analysis of gibberellin signaling. *Annual Review of Plant Physiology and Plant Molecular Biology*, 52, 67-88.

RICK, C. M. & BARTON, D. W. (1954) Cytological and genetical identification of the primary trisomics of the tomato. *Genetics*, 39, 640.

RICK, C. M. & NOTANI, N. K. (1961) The tolerance of extra chromosomes by primitive tomatoes. *Genetics*, 46, 1231.

RICKERT, A. M., BALLVORA, A., MATZNER, U., KLEMM, M. & GEBHARDT, C. (2005) Quantitative genotyping of single nucleotide polymorphisms by allele specific oligonucleotide hybridization on DNA microarrays. *Biotechnology and Applied Biochemistry*, 42, 93-96.

RICKERT, A. M., PREMSTALLER, A., GEBHARDT, C. & OEFNER, P. J. (2002) Genotyping of SNPs in a Polyploid Genome by Pyrosequencing™. *BioTechniques*, 32, 592-603.

RIDDLE, N. C. & BIRCHLER, J. A. (2003) Effects of reunited diverged regulatory hierarchies in allopolyploids and species hybrids. *Trends in Genetics*, 19, 597-600.

RIDDLE, N. C., JIANG, H., AN, L., DOERGE, R. W. & BIRCHLER, J. A. (2010) Gene expression analysis at the intersection of ploidy and hybridity in maize. *TAG THEORETICAL AND APPLIED GENETICS*, 120, 341-353.

RIDDLE, N. C., KATO, A. & BIRCHLER, J. A. (2006) Genetic variation for the response to ploidy change in Zea mays L. *TAG THEORETICAL AND APPLIED GENETICS*, 114, 101-111.

RIDDLE, N. C. & RICHARDS, E. J. (2002) The control of natural variation in cytosine methylation in Arabidopsis. *Genetics*, 162, 355.

RIESEBERG, L. H., ARCHER, M. A. & WAYNE, R. K. (1999) Transgressive segregation, adaptation and speciation. *Heredity*, 83, 363-372.

ROACH, D. A. & WULFF, R. D. (1987) Maternal effects in plants. *Annual Review of Ecology and Systematics*, 209-235.

ROHDE, P., HINCHA, D. K. & HEYER, A. G. (2004) Heterosis in the freezing tolerance of crosses between two Arabidopsis thaliana accessions (Columbia 0 and C24) that show differences in non acclimated and acclimated freezing tolerance. *The Plant Journal*, 38, 790-799.

RONEMUS, M. J., GALBIATI, M., TICKNOR, C., CHEN, J. & DELLAPORTA, S. L. (1996) Demethylation-induced developmental pleiotropy in Arabidopsis. *Science*, 273, 654.

ROUNSLEY, S. (2003) Sharing the wealth. The mechanics of a data release from industry. *Plant Physiology*, 133, 438.

ROUNSLEY, S. D. & LAST, R. L. (2010) Shotguns and SNPs: how fast and cheap sequencing is revolutionizing plant biology. *The Plant Journal*, 61, 922-927.

ROWAN, H., ROBERT, D., SAMANTHA, B. & RICHARD, M. (2011) Rapid analysis of seed size in Arabidopsis for mutant and QTL discovery. *Plant Methods*, 7.

SADRAS, V. O. (2007) Evolutionary aspects of the trade-off between seed size and number in crops. *Field Crops Research*, 100, 125-138.

SANCHEZ-MORAN, E., ARMSTRONG, S. J., SANTOS, J. L., FRANKLIN, F. C. H. & JONES, G. H. (2002) Variation in chiasma frequency among eight accessions of Arabidopsis thaliana. *Genetics*, 162, 1415.

SANKARANARAYANAN, K. (1979) 1979 The role of non-disjunction in aneuploidy in man. An overview. *Mutation Research,* 61**,** 1-28.

SATINA, S., BLAKESLEE, A. F. & AVERY, A. G. (1938) Chromosome behavior in triploid Datura. III. The seed. *American Journal of Botany***,** 595-602.

SAVIDAN, Y. (2001) GAMETOPHYTIC APOMIXIS. *Current trends in the embryology of angiosperms***,** 419.

SAX, K. (1923) The association of size differences with seed-coat pattern and pigmentation in Phaseolus vulgaris. *Genetics,* 8**,** 552.

SAZE, H., SCHEID, O. M. & PASZKOWSKI, J. (2003) Maintenance of CpG methylation is essential for epigenetic inheritance during plant gametogenesis. *Nature genetics,* 34**,** 65-69.

SCHIFF, C. L., WILSON, I. W. & SOMERVILLE, S. C. (2001) Polygenic powdery mildew disease resistance in Arabidopsis thaliana: quantitative trait analysis of the accession Warschau 1. *Plant pathology,* 50**,** 690-701.

SCHMID, K. J., SÖRENSEN, T. R., STRACKE, R., TÖRJÉK, O., ALTMANN, T., MITCHELL-OLDS, T. & WEISSHAAR, B. (2003) Large-scale identification and analysis of genome-wide single-nucleotide polymorphisms for mapping in Arabidopsis thaliana. *Genome research,* 13**,** 1250.

SCHMIDT, E., GUZZO, F., TOONEN, M. & DE VRIES, S. (1997) A leucine-rich repeat containing receptor-like kinase marks somatic plant cells competent to form embryos. *Development,* 124**,** 2049-2062.

SCHOLL, R. L., MAY, S. T. & WARE, D. H. (2000) Seed and molecular resources for Arabidopsis. *Plant Physiology,* 124**,** 1477.

SCHRANZ, M. E., DOBES, C., KOCH, M. A. & MITCHELL-OLDS, T. (2005) Sexual reproduction, hybridization, apomixis, and polyploidization in the genus Boechera (Brassicaceae). *American Journal of Botany,* 92**,** 1797-1810.

SCHRUFF, M. C., SPIELMAN, M., TIWARI, S., ADAMS, S., FENBY, N. & SCOTT, R. J. (2006) The AUXIN RESPONSE FACTOR 2 gene of Arabidopsis links auxin signalling, cell division, and the size of seeds and other organs. *Development* 133**,** 251.

SCOTT, R. J., ARMSTRONG, S. J., DOUGHTY, J. & SPIELMAN, M. (2008) Double fertilization in Arabidopsis thaliana involves a polyspermy block on the egg but not the central cell. *Molecular Plant,* 1**,** 611.

SCOTT, R. J., SPIELMAN, M., BAILEY, J. & DICKINSON, H. G. (1998) Parent-of-origin effects on seed development in Arabidopsis thaliana. *Development,* 125**,** 3329.

SHAH, K., GADELLA JR, T. W. J., VAN ERP, H., HECHT, V. & DE VRIES, S. C. (2001) Subcellular localization and oligomerization of the Arabidopsis thaliana somatic embryogenesis receptor kinase 1 protein. *Journal of molecular biology,* 309**,** 641-655.

SHAKED, H., KASHKUSH, K., OZKAN, H., FELDMAN, M. & LEVY, A. A. (2001) Sequence elimination and cytosine methylation are rapid and reproducible responses of the genome to wide hybridization and allopolyploidy in wheat. *The Plant Cell,* 13**,** 1749.

SHARBEL, T. F., HAUBOLD, B. & MITCHELL OLDS, T. (2000) Genetic isolation by distance in Arabidopsis thaliana: biogeography and postglacial colonization of Europe. *Molecular Ecology,* 9**,** 2109-2118.

SHARBEL, T. F. & MITCHELL-OLDS, T. (2001) Recurrent polyploid origins and chloroplast phylogeography in the Arabis holboellii complex (Brassicaceae). *Heredity,* 87**,** 59-68.

SHATTUCK-EIDENS, D. M., BELL, R. N., NEUHAUSEN, S. L. & HELENTJARIS, T. (1990) DNA sequence variation within maize and melon: observations from polymerase chain reaction amplification and direct sequencing. *Genetics,* 126**,** 207.

SHI, L., KATAVIC, V., YU, Y., KUNST, L. & HAUGHN, G. (2011) Arabidopsis glabra2 mutant seeds deficient in mucilage biosynthesis produce more oil. *The Plant Journal*.

SHINDO, C., LISTER, C., CREVILLEN, P., NORDBORG, M. & DEAN, C. (2006) Variation in the epigenetic silencing of FLC contributes to natural variation in Arabidopsis vernalization response. *Genes & Development,* 20**,** 3079-3083.

SHIU, P. K. T., RAJU, N. B., ZICKLER, D. & METZENBERG, R. L. (2001) Meiotic silencing by unpaired DNA. *Cell,* 107**,** 905-916.

SHOMURA, A., IZAWA, T., EBANA, K., EBITANI, T., KANEGAE, H., KONISHI, S. & YANO, M. (2008) Deletion in a gene associated with grain size increased yields during rice domestication. *Nature genetics,* 40**,** 1023-1028.

SHULL, G. H. (1908) The composition of a field of maize. Am Genetic Assoc.

SIDDIQI, I., MARIMUTHU, M. & RAVI, M. (2009) Molecular approaches for the fixation of plant hybrid vigor. *Biotechnology journal,* 4**,** 342-347.

SILVERTOWN, J. (1989) The paradox of seed size and adaptation. *Trends in Ecology & Evolution,* 4**,** 24-26.

SIMON, W. L. C., HENDERSON, I. R. & JACOBSEN, S. E. (2005) Gardening the genome: DNA methylation in Arabidopsis thaliana. *Nature Reviews Genetics,* 6**,** 351-360.

SINGER, T., YORDAN, C. & MARTIENSSEN, R. A. (2001) Robertson's Mutator transposons in A. thaliana are regulated by the chromatin-remodeling gene Decrease in DNA Methylation (DDM1). *Genes & development,* 15**,** 591.

SLOTKIN, R. K. & MARTIENSSEN, R. (2007) Transposable elements and the epigenetic regulation of the genome. *Nature Reviews Genetics,* 8**,** 272-285.

SOLTIS, D. E. & SOLTIS, P. S. (1995) The dynamic nature of polyploid genomes. *PNAS,* 92**,** 8089.

SOLTIS, D. E., SOLTIS, P. S., SCHEMSKE, D. W., HANCOCK, J. F., THOMPSON, J. N., HUSBAND, B. C. & JUDD, W. S. (2007) Autopolyploidy in angiosperms: have we grossly underestimated the number of species? *Taxon,* 56**,** 13-30.

SOLTIS, D. E., SOLTIS, P. S. & TATE, J. A. (2004) Advances in the study of polyploidy since plant speciation. *New Phytologist,* 161**,** 173-191.

SOLTIS, P. S. & SOLTIS, D. E. (2009) The role of hybridization in plant speciation. *Annual Review of Plant Biology,* 60**,** 561-588.

SOMERVILLE, C. & KOORNNEEF, M. (2002) A fortunate choice: the history of Arabidopsis as a model plant. *Nature Reviews Genetics,* 3**,** 883-889.

SONG, X. J., HUANG, W., SHI, M., ZHU, M. Z. & LIN, H. X. (2007) A QTL for rice grain width and weight encodes a previously unknown RING-type E3 ubiquitin ligase. *Nature genetics,* 39**,** 623-630.

SOPPE, W. J. J., JASENCAKOVA, Z., HOUBEN, A., KAKUTANI, T., MEISTER, A., HUANG, M. S., JACOBSEN, S. E., SCHUBERT, I. & FRANSZ, P. F. (2002) DNA methylation controls histone H3 lysine 9 methylation and heterochromatin assembly in Arabidopsis. *The EMBO Journal,* 21**,** 6549-6559.

SPIELMAN, M., VINKENOOG, R., DICKINSON, H. G. & SCOTT, R. J. (2001) The epigenetic basis of gender in flowering plants and mammals. *TRENDS in Genetics,* 17**,** 705-711.

SPIELMAN, M., VINKENOOG, R. & SCOTT, R. J. (2003) Genetic mechanisms of apomixis. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences,* 358**,** 1095-1103.

SPILLANE, C., CURTIS, M. D. & GROSSNIKLAUS, U. (2004) Apomixis technology development—virgin births in farmers' fields? *Nature biotechnology,* 22**,** 687-691.

SPILLANE, C., STEIMER, A. & GROSSNIKLAUS, U. (2001a) Apomixis in agriculture: the quest for clonal seeds. *Sexual Plant Reproduction,* 14**,** 179-187.

SPILLANE, C., VIELLE-CALZADA, J. P. & GROSSNIKLAUS, U. (2001b) APO2001: A sexy apomixer in como. *The Plant Cell Online,* 13**,** 1480-1491.

SPRINGER, N. M., NAPOLI, C. A., SELINGER, D. A., PANDEY, R., CONE, K. C., CHANDLER, V. L., KAEPPLER, H. F. & KAEPPLER, S. M. (2003) Comparative analysis of SET domain proteins in maize and Arabidopsis reveals multiple duplications preceding the divergence of monocots and dicots. *Plant physiology,* 132**,** 907-925.

SPRINGER, N. M. & STUPAR, R. M. (2007) Allelic variation and heterosis in maize: How do two halves make more than a whole? *Genome research,* 17**,** 264.

SPRUNCK, S. & DRESSELHAUS, T. (2009) Gamete interactions during double fertilization in flowering plants. *Zellbiologie aktuell*.

STAM, M. & MITTELSTEN SCHEID, O. (2005) Paramutation: an encounter leaving a lasting impression. *Trends in plant science,* 10**,** 283-290.

STANTON, M. L. (1984) Seed variation in wild radish: effect of seed size on components of seedling and adult fitness. *Ecology***,** 1105-1112.

STEFFEN, J. G., KANG, I. H., MACFARLANE, J. & DREWS, G. N. (2007) Identification of genes expressed in the Arabidopsis female gametophyte. *The Plant Journal,* 51**,** 281-292.

STEFFEN, J. G., KANG, I. H., PORTEREIKO, M. F., LLOYD, A. & DREWS, G. N. (2008) AGL61 interacts with AGL80 and is required for central cell development in Arabidopsis. *Plant Physiology,* 148**,** 259.

STÖCK, M., LAMATSCH, D. K., STEINLEIN, C., EPPLEN, J. T., GROSSE, W. R., HOCK, R., KLAPPERSTÜCK, T., LAMPERT, K. P., SCHEER, U. & SCHMID, M. (2002) A bisexually reproducing all-triploid vertebrate. *Nature genetics*, 30**,** 325-328.

STOKES, D., MORGAN, C., O'NEILL, C. & BANCROFT, I. (2007) Evaluating the utility of Arabidopsis thaliana as a model for understanding heterosis in hybrid crops. *Euphytica*, 156**,** 157-171.

STUPAR, R. M., HERMANSON, P. J. & SPRINGER, N. M. (2007) Nonadditive expression and parent-of-origin effects identified by microarray and allele-specific expression profiling of maize endosperm. *Plant physiology*, 145**,** 411.

SUN, X., SHANTHARAJ, D., KANG, X. & NI, M. (2010) Transcriptional and hormonal signaling control of Arabidopsis seed development. *Current opinion in plant biology*.

SUNDARESAN, V. (2005) Control of seed size in plants. *Proceedings of the National Academy of Sciences of the United States of America*, 102**,** 17887.

TARIQ, M. & PASZKOWSKI, J. (2004) DNA and histone methylation in plants. *TRENDS in Genetics*, 20**,** 244-251.

TIWARI, S., SCHULZ, R., IKEDA, Y., DYTHAM, L., BRAVO, J., MATHERS, L., SPIELMAN, M., GUZMÁN, P., OAKEY, R. J. & KINOSHITA, T. (2008) MATERNALLY EXPRESSED PAB C-TERMINAL, a novel imprinted gene in Arabidopsis, encodes the conserved C-terminal domain of polyadenylate binding proteins. *The Plant Cell Online*, 20**,** 2387-2398.

TIWARI, S., SPIELMAN, M., SCHULZ, R., OAKEY, R., KELSEY, G., SALAZAR, A., ZHANG, K., PENNELL, R. & SCOTT, R. (2010) Transcriptional profiles underlying parent-of-origin effects in seeds of Arabidopsis thaliana. *BMC Plant Biology*, 10**,** 72.

TODESCO, M., BALASUBRAMANIAN, S., HU, T. T., TRAW, M. B., HORTON, M., EPPLE, P., KUHNS, C., SURESHKUMAR, S., SCHWARTZ, C. & LANZ, C. (2010) Natural allelic variation underlying a major fitness trade-off in Arabidopsis thaliana. *Nature*, 465**,** 632-636.

TON, J., PIETERSE, C. M. J. & VAN LOON, L. C. (1999) Identification of a locus in Arabidopsis controlling both the expression of rhizobacteria-mediated induced systemic resistance (ISR) and basal resistance against Pseudomonas syringae pv. tomato. *Molecular plant-microbe interactions*, 12**,** 911-918.

UNGRU, A., NOWACK, M. K., REYMOND, M., SHIRZADI, R., KUMAR, M., BIEWERS, S., GRINI, P. E. & SCHNITTGER, A. (2008) Natural variation in the degree of autonomous endosperm formation reveals independence and constraints of embryo growth during seed development in Arabidopsis thaliana. *Genetics*, 179**,** 829.

USADEL, B., KUSCHINSKY, A. M., ROSSO, M. G., ECKERMANN, N. & PAULY, M. (2004) RHM2 is involved in mucilage pectin synthesis and is required for the development of the seed coat in Arabidopsis. *Plant physiology*, 134**,** 286.

VAN DIJK, P. & VAN DAMME, J. (2000) Apomixis technology and the paradox of sex. Elsevier.

VAN DIJK, P. J. & BAKX-SCHOTMAN, J. M. (2004) Formation of unreduced megaspores (diplospory) in apomictic dandelions (Taraxacum officinale, sl) is controlled by a sex-specific dominant locus. *Genetics*, 166**,** 483.

VAUGHN, M. W., TANURDŽIĆ, M., LIPPMAN, Z., JIANG, H., CARRASQUILLO, R., RABINOWICZ, P. D., DEDHIA, N., MCCOMBIE, W. R., AGIER, N. & BULSKI, A. (2007) Epigenetic natural variation in Arabidopsis thaliana. *PLoS biology*, 5**,** e174.

VEITIA, R. A. (2005) Gene dosage balance: deletions, duplications and dominance. *The Plant Cell Online*, 21**,** 33.

VERDUIJN, M. H., VAN DIJK, P. J. & VAN DAMME, J. M. M. (2004) The role of tetraploids in the sexual–asexual cycle in dandelions (Taraxacum). *Heredity*, 93**,** 390-398.

VIELLE-CALZADA, J. P., BASKAR, R. & GROSSNIKLAUS, U. (1994) Delayed activation of the paternal genome during seed development. *Nature*, 265**,** 666-669.

VIELLE-CALZADA, J. P., BASKAR, R. & GROSSNIKLAUS, U. (2000) Delayed activation of the paternal genome during seed development. *Nature*, 404**,** 91-94.

VIELLE-CALZADA, J. P., THOMAS, J., SPILLANE, C., COLUCCIO, A., HOEPPNER, M. A. & GROSSNIKLAUS, U. (1999) Maintenance of genomic imprinting at the Arabidopsis medea locus requires zygotic DDM1 activity. *Genes & Development*, 13**,** 2971.

VINKENOOG, R., BUSHELL, C., SPIELMAN, M., ADAMS, S., DICKINSON, H. G. & SCOTT, R. J. (2003) Genomic imprinting and endosperm development in flowering plants. *Molecular Biotechnology*, 25**,** 149-184.

VINKENOOG, R., SPIELMAN, M., ADAMS, S., FISCHER, R. L., DICKINSON, H. G. & SCOTT, R. J. (2000) Hypomethylation promotes autonomous endosperm development and rescues postfertilization lethality in fie mutants. *The Plant Cell* 12**,** 2271.

VRANA, P. B., FOSSELLA, J. A., MATTESON, P., DEL RIO, T., O'NEILL, M. J. & TILGHMAN, S. M. (2000) Genetic and epigenetic incompatibilities underlie hybrid dysgenesis in Peromyscus. *Nature genetics,* 25**,** 120-124.

WANG, A., GARCIA, D., ZHANG, H., FENG, K., CHAUDHURY, A., BERGER, F., PEACOCK, W. J., DENNIS, E. S. & LUO, M. (2010) The VQ motif protein IKU1 regulates endosperm growth and seed size in Arabidopsis. *The Plant Journal,* 63**,** 670-679.

WANG, J., TIAN, L., LEE, H. S., WEI, N. E., JIANG, H., WATSON, B., MADLUNG, A., OSBORN, T. C., DOERGE, R. W. & COMAI, L. (2006) Genomewide nonadditive gene regulation in Arabidopsis allotetraploids. *Genetics,* 172**,** 507.

WANG, J., TIAN, L., MADLUNG, A., LEE, H. S., CHEN, M., LEE, J. J., WATSON, B., KAGOCHI, T., COMAI, L. & CHEN, Z. J. (2004) Stochastic and epigenetic changes of gene expression in Arabidopsis polyploids. *Genetics,* 167**,** 1961.

WARMKE, H. E. & BLAKESLEE, A. F. (1940) The establishment of a 4n dioecious race in Melandrium. *American Journal of Botany***,** 751-762.

WARTHMANN, N., FITZ, J. & WEIGEL, D. (2007) MSQT for choosing SNP assays from multiple DNA alignments. *Bioinformatics,* 23**,** 2784.

WEIGEL, D. & MOTT, R. (2009) The 1001 genomes project for Arabidopsis thaliana. *Genome Biol,* 10**,** 107.

WEIGEL, D. & NORDBORG, M. (2005) Natural variation in Arabidopsis. How do we find the causal genes? *Plant physiology,* 138**,** 567.

WEISS, H. & MALUSZYNSKA, J. (2001) Chromosomal rearrangement in autotetraploid plants of Arabidopsis thaliana. *Hereditas,* 133**,** 255-261.

WENDEL, J. F. (2000) Genome evolution in polyploids. *Plant Molecular Biology,* 42**,** 225-249.

WESTERN, T. L., BURN, J., TAN, W. L., SKINNER, D. J., MARTIN-MCCAFFREY, L., MOFFATT, B. A. & HAUGHN, G. W. (2001) Isolation and characterization of mutants defective in seed coat mucilage secretory cell development in Arabidopsis. *Plant physiology,* 127**,** 998-1011.

WESTERN, T. L., YOUNG, D. S., DEAN, G. H., TAN, W. L., SAMUELS, A. L. & HAUGHN, G. W. (2004) MUCILAGE-MODIFIED4 encodes a putative pectin biosynthetic enzyme developmentally regulated by APETALA2, TRANSPARENT TESTA GLABRA1, and GLABRA2 in the Arabidopsis seed coat. *Plant physiology,* 134**,** 296-306.

WESTOBY, M., FALSTER, D. S., MOLES, A. T., VESK, P. A. & WRIGHT, I. J. (2002) Plant ecological strategies: some leading dimensions of variation between species. *Annual Review of Ecology and Systematics***,** 125-159.

WIERZBICKI, A. T., REAM, T. S., HAAG, J. R. & PIKAARD, C. S. (2009) RNA polymerase V transcription guides ARGONAUTE4 to chromatin. *Nature genetics,* 41**,** 630-634.

WINGE, Ö. (1917) The chromosomes. *Comptes Rendus des Travaux du Laboratoire Carlsberg* 13**,** 131-275.

XIAO, J., LI, J., YUAN, L. & TANKSLEY, S. D. (1995) Dominance is the major genetic basis of heterosis in rice as revealed by QTL analysis using molecular markers. *Genetics,* 140**,** 745.

XIAO, W., BROWN, R. C., LEMMON, B. E., HARADA, J. J., GOLDBERG, R. B. & FISCHER, R. L. (2006) Regulation of seed size by hypomethylation of maternal and paternal genomes. *Plant Physiology,* 142**,** 1160.

XIAO, W., GEHRING, M., CHOI, Y., MARGOSSIAN, L., PU, H., HARADA, J. J., GOLDBERG, R. B., PENNELL, R. I. & FISCHER, R. L. (2003) Imprinting of the MEA Polycomb gene is controlled by antagonism between MET1 methyltransferase and DME glycosylase. *Developmental cell,* 5**,** 891–901.

YAMAUCHI, A., HOSOKAWA, A., NAGATA, H. & SHIMODA, M. (2004) Triploid bridge and role of parthenogenesis in the evolution of autopolyploidy. *The American Naturalist,* 164**,** 101-112.

YANG, W. C. & SUNDARESAN, V. (2000) Genetics of gametophyte biogenesis in Arabidopsis. *Current opinion in plant biology,* 3**,** 53-57.

YOKTHONGWATTANA, C., BUCHER, E., ČAIKOVSKI, M., VAILLANT, I., NICOLET, J., SCHEID, O. M. & PASZKOWSKI, J. (2009) MOM1 and Pol-IV/V interactions regulate the intensity and specificity of transcriptional gene silencing. *The EMBO Journal,* 29**,** 340-351.

YOUNG, R. E., MCFARLANE, H. E., HAHN, M. G., WESTERN, T. L., HAUGHN, G. W. & SAMUELS, A. L. (2008) Analysis of the Golgi apparatus in Arabidopsis seed coat cells during polarized secretion of pectin-rich mucilage. *The Plant Cell Online,* 20**,** 1623-1638.

YU, B., WAKAO, S., FAN, J. & BENNING, C. (2004) Loss of plastidic lysophosphatidic acid acyltransferase causes embryo-lethality in Arabidopsis. *Plant and cell physiology,* 45**,** 503.

YU, H. J., HOGAN, P. & SUNDARESAN, V. (2005a) Analysis of the female gametophyte transcriptome of Arabidopsis by comparative expression profiling. *Plant physiology,* 139**,** 1853-1869.

YU, J., HOLLAND, J. B., MCMULLEN, M. D. & BUCKLER, E. S. (2008) Genetic design and statistical power of nested association mapping in maize. *Genetics,* 178**,** 539.

YU, J., PRESSOIR, G., BRIGGS, W. H., BI, I. V., YAMASAKI, M., DOEBLEY, J. F., MCMULLEN, M. D., GAUT, B. S., NIELSEN, D. M. & HOLLAND, J. B. (2005b) A unified mixed-model method for association mapping that accounts for multiple levels of relatedness. *Nature genetics,* 38**,** 203-208.

YU, S. B., LI, J. X., XU, C. G., TAN, Y. F., GAO, Y. J., LI, X. H., ZHANG, Q. & MAROOF, M. A. (1997) Importance of epistasis as the genetic basis of heterosis in an elite rice hybrid. *PNAS,* 94**,** 9226.

ZHANG, M., KIMATU, J. N., XU, K. & LIU, B. (2010) DNA cytosine methylation in plant development. *Journal of Genetics and Genomics,* 37**,** 1-12.

ZHANG, X., SHIU, S., CAL, A. & BOREVITZ, J. O. (2008) Global analysis of genetic, epigenetic and transcriptional polymorphisms in Arabidopsis thaliana using whole genome tiling arrays. *PLoS Genetics,* 4**,** e1000032.

ZHAO, K., ARANZANA, M. J., KIM, S., LISTER, C., SHINDO, C., TANG, C., TOOMAJIAN, C., ZHENG, H., DEAN, C. & MARJORAM, P. (2007) An Arabidopsis example of association mapping in structured samples. *PLoS Genet,* 3**,** e4.

ZHOU, Y., ZHANG, X., KANG, X., ZHAO, X., ZHANG, X. & NI, M. (2009) SHORT HYPOCOTYL UNDER BLUE1 associates with MINISEED3 and HAIKU2 promoters in vivo to regulate Arabidopsis seed development. *The Plant Cell,* 21**,** 106.