Chapter 1
Introduction

1.1 Reproduction

Reproduction is one of the most distinctive features of life. It is a process, by which all living organisms ensure survival of their species. There are several ways through which an organism can produce offspring. Reproduction can be classified into two major groups, sexual reproduction and asexual reproduction.

1.2 Asexual reproduction in plants

All plant organs are capable of regenerating into whole plants under the appropriate conditions (a property called totipotency), but most commonly vegetative reproduction via, stems, underground stems, rhizomes, bulbs, corms and tubers is the main source of asexual reproduction in plants. Apomixis is another important route of asexual reproduction in plants. By definition apomixis is the formation of seeds without fertilization. The main advantage of apomixis is that, it can produce clonal seeds. This mode of reproduction is known in more than 400 plant species but not in commercially important food crops. Therefore the transfer of apomixis to crop plants holds great promise as a tool in plant breeding for the fixation of desirable traits like disease resistance, higher yields, and hybrid vigour as it would allow propagation of superior hybrids with desirable traits over successive generations.

1.3 Sexual reproduction in plants

In flowering plants, sexual reproduction occurs in special organs (stamens and ovules) of the flower. The various events during sexual reproduction in plants, such as pollination and fertilization were speculated early in human history from the perspective of practical utilization in agriculture and seed production (Boavida et al., 2005a). Despite this knowledge, the interaction between pollen tube and egg cell remained elusive till Strasburger, first proved the fusion of pollen nuclei with the egg cell in 1884 (Boavida et al., 2005a). Double fertilization was described still later by Sergius Nawashin and Leon Guignard in the beginning of the 20th century (Friedman, 2001). However, the
area of plant reproduction biology has grown significantly ever since the mid 1950s due to the work of many outstanding plant biologists like P. Maheshwari (Maheshwari, 1950).

1.4 Alternation of generations in plants

The life cycle of higher plants consists of an ordered succession of events comprising seed germination, vegetative growth, flowering, sporogenesis, gametogenesis, pollination, fertilization, development of the embryo and seed maturation. The evolutionary success of higher plants relies heavily on the short gametophytic phase, consisting of a few cells. Plants, alternate between this short phase and a relatively longer sporophytic phase during their life cycle. This alternation of gametophytic and sporophytic generations during the plant life cycle is commonly referred to as alternation of generations. This kind of an alternation between a diploid sporophytic phase and a short lived haploid gametophytic phase presents a well suited strategy for successful evolutionary selection and it also brings an opportunity for selection at the haploid level to occur (Ottaviano et al., 1990).

1.5 Arabidopsis as a plant model system

*Arabidopsis thaliana* is a small winter annual species belonging to the family Brassicaceae and grows as a weed in the moderate temperate parts of the world. Arabidopsis was first discovered by Johannes Thal back in the sixteenth century (http://www.arabidopsis.org). Common names for Arabidopsis exist in many languages indicating its widespread occurrence. In English it is known as wall cress or mouse-ear cress. As of now there is no clear proof regarding the exact geographical place of origin of this plant species. Experimental research with Arabidopsis was initiated by Friedrich Laibach in 1907 (Huala et al., 2001). The main attraction of this plant species was its relatively small stature and short life cycle, which makes it a good model system for plant biology (Redei, 1975).

The advantages of this organism for molecular biology studies become more apparent after the small size and simple structure of its nuclear genome
was determined (Meyerowitz and Pruitt, 1985). Arabidopsis has one of the smallest genomes among all flowering plants. The nuclear DNA of Arabidopsis is packed into five chromosomes (n=5, plant is typically diploid). The estimated haploid genome size is approximately 114.5 Mb to 125 Mb with approximately 26,000 protein coding genes (http://www.arabidopsis.org/). Such a small size of the genome facilitates elucidation of gene functions by methods that would be difficult or almost impossible to employ in other plants. However, despite the unusually small size and simple nature of the Arabidopsis genome, the genome arrangement, structure of chromosomes, genetic properties and overall complement of genes in the genome are similar to those observed in other flowering plants. Therefore the information obtained from Arabidopsis can be used to decipher gene function in other plants. Arabidopsis also has the distinction of being the first plant species with a complete sequenced genome (The Arabidopsis Genome Initiative, 2000). Hence even though Arabidopsis is not an economically important plant species for mankind, its value can be determined by the amount of scientific information, which can be gathered by its use in experimental research.

1.6 Sexual reproduction in Arabidopsis

Sexual reproduction in Arabidopsis and other plants occurs towards the end of a long sporophytic phase and leads to the gametophytic generation which culminates in the formation of the zygote, a link to the next round of the sporophytic phase. The early steps upto meiosis of male and female reproductive development are similar in higher plants, however the subsequent stages after meiosis are clearly distinct. In the male lineage all the four meiotic products develop to produce functional pollen grains whereas in the female lineage only one of the four meiotic products survives and develops into a functional megaspore (Scott et al., 2004; Drews et al., 1998) while the other three megaspores undergoes a form of programmed cell death (Yadegari and Drews, 2004). Unlike animals, where differentiation of the primordial germ line occurs early during development (during embryogenesis), the development of
reproductive organs happens very late in plants (Wylie, 1999; Zhao and Garbers, 2002; Meyerowitz, 2002). The different stages of sexual reproduction in Arabidopsis are discussed below.

1.6.1 Flowering and sporogenesis

The induction of flowering in plants is triggered by environmental cues such as light (photoperiod), temperature (vernalization) and nutrient access (Bagnall, 1992). The integration of signals generated from these cues direct the activation of a specific group of genes called meristem identity genes. These genes subsequently specify the floral identity and as a result the shoot apical meristem is converted into an inflorescence meristem (Pineiro and Coupland, 1998). Afterwards by the action of another set of floral identity genes, development of four floral organ types: sepals, petals, stamens (androecium) and carpels (gynoecium) commences (Pineiro and Coupland, 1998). The androecium and gynoecium are the organs in which male and female sporogenesis and gametogenesis take place at a later stage.

Sporogenesis is the process of formation of haploid spores from diploid organisms by a specialized cell division called meiosis. Sporogenesis is generally classified as microsporogenesis (male sporogenesis) and megasporogenesis (female sporogenesis). The process of sporogenesis begins with the differentiation of one or more hypodermal cells in the ovule and anthers. These cells subsequently develop into sporocytes (reviewed by Wilson and Yang, 2004). The sporocytes in the male lineage are called microsporocytes and those in the female lineage are called megasporocytes. There is evidence that, genetically regulated mechanisms determine which cell will become a sporocyte and when the cell cycle is shifted from mitotic to a more specialized meiotic one. Mutants have been reported in both Arabidopsis and maize which are altered in the above mentioned pathways, such as *multiple archesporial cells (mac)*1 in maize and *excess microsporocytes (ems)*1, *sporocyteless (spl)* and *tapetum determinant (tpd)*1 in Arabidopsis (reviewed by Yang and Sundaresan, 2000).
1.6.2 Microsporogenesis

Male sporogenesis occurs in the stamens. Each stamen consists of an anther where pollen development takes place, and a stalk-like filament, which is mainly supportive in function (Ma, 2005). Microspores, which contribute the male gametes, are formed within the anther. A typical anther consists of four microsporangia, where microsporogenesis takes place. At the onset of microsporogenesis some hypodermal cells within the anther primordium become more prominent than the rest. These cells afterwards constitute the archesporial cells. Subsequently, these differentiated archesporial cells start to divide in a plane parallel to the outer wall of the anther lobe, forming a primary parietal cell (PPC) towards the epidermis and primary sporogenous cell (PSC) towards the interior of the anther. The parietal cells undergo a series of divisions to form 2-5 layers of anther wall, which later differentiate into the endothecium, middle layer and the tapetum (reviewed by Ma, 2005). The tapetum performs a nutritional role to the developing pollen grains and is known to be essential for successful male gametogenesis (Koltunow et al., 1990). The primary sporogenous cells either directly or after a few rounds of mitosis (depending on the plant species) function as the microspore mother cell or the pollen mother cell (PMC), which subsequently undergoes meiosis and gives rise to four microspores.

1.6.3 Megasporogenesis

Megasporogenesis occurs within the ovule (Skinner et al., 2004). A typical Arabidopsis gynoecium comprises of approximately 50 ovules and each ovule consists of the following parts: two integuments, the nucellus that encloses the embryo sac, a central chalaza, and the funiculus (Smyth et al., 1990; Sessions, 1997). The ovules are initiated from the sub-epidermal cells of the placenta along the margins of the fused carpels (Arabidopsis has two fused carpels). During megasporogenesis a single hypodermal cell in the nucellus of the ovule develops into the archesporial cell. This archesporial cell becomes more prominent than its surrounding cells mainly because of its large size and denser cytoplasm and a more conspicuous nucleus (Yang et al., 1999a). This specified archesporial cell,
either directly (tenuinucellate type) or after few rounds of mitosis (crassinucellate type) functions as the megaspore mother cell (MMC). The MMC undergoes meiosis to form four haploid megaspores (Maheshwari, 1950).

In 1999, Yang and co-workers identified the SPOROCYTELESS (SPL) gene which affects the initiation of sporogenesis in Arabidopsis (Yang et al., 1999a). This gene was found to be essential for the differentiation of both male and female archesporial cells into respective sporocytes. Subsequently the SPL gene has been cloned and shown to encode a nuclear protein with limited similarity to MADS box transcription factors. Till date SPL remains as the earliest known gene in the sexual reproduction pathway in Arabidopsis.

1.6.4 Male gametogenesis

Male gametogenesis begins in the anther locus or pollen sac and involves a series of events culminating in the production and discharge of pollen grains (McCormick, 1993). It is interesting to note that a large number of genes are expressed during pollen development, perhaps greater number of genes than during any other single event in the plant life cycle (Scott et al., 2004). Male gametogenesis starts after release of the haploid microspores (Ma, 2005). Subsequently the microspores undergo cytoplasmic reorganization mediated by the cytoskeleton (Brown and Lemmon, 1991; Zonia et al., 1999; Ma, 2005). The resulting polarization in the microspore leads to an asymmetric cell division called pollen mitosis I. This results in two cells, a large vegetative cell (VC) and a small generative cell (GC) (Horvitz and Herskowitz, 1992; Twell and Howden, 1998). These two cells have very different cell fates; the larger VC accumulates a dense cytoplasm and does not divide further whereas the generative cell undergoes a round of mitosis (Ma, 2005). Genetic screens in Arabidopsis have revealed several mutants, which are defective in various aspects of male gametogenesis and pollen mitosis I. Examples are sidecar (scp), two-in-one pollen (tio) and gemini pollen 1 (gem1) (Chen and McCormick, 1996; Twell and Howden, 1998; Twell et al., 1998; Park et al., 1998; Twell et al., 2002). After pollen mitosis I, the
GC cytoplasm gets separated from the VC by the formation of a thin callose wall called the intine.

In Arabidopsis, the GC undergoes a second mitosis (pollen mitosis II) before pollen release, giving rise to two identical sperm cells (Ma, 2005). Following this second pollen mitosis the VC and sperm cells assume a specific structural arrangement commonly known as the male germ unit (Boavida et al., 2005b). Mutants like *male germ unit displaced (mud)* and *germ unit malformed (gum)* reported in Arabidopsis show disturbances in the integrity or positioning of the male germ unit (Lalanne and Twell, 2002). The Arabidopsis homologue of *cdc2*, *CDKA1* was shown to be required for pollen mitosis II (Iwakawa et al., 2006). In the *cdka1* mutant pollen, the GC cell fails to undergo pollen mitosis II resulting in a bicellular pollen consisting of a single vegetative cell and a sperm-like cell. The final stages of male gametogenesis usually coincide with floral anthesis and anther dehiscence culminating in the release of mature pollen grains. The released pollen subsequently finds its female counterpart and accomplishes fertilization. Mature pollen grains are usually released in a dehydrated state and are almost quiescent in metabolism (Heslop-Harrison, 1979; Hoekstra and Bruinsma, 1980). In Arabidopsis, a mutation in the *AtMYB26* gene inhibited anther dehiscence; however the pollen is viable and is able to fertilize when released mechanically, suggesting that downstream events of male gametogenesis are also genetically controlled (Steiner-Lange et al., 2003). Another Arabidopsis gene involved in male gamete production is the *QUARTET1 (QRT1)* (Francis et al., 2006). Detailed characterization of the *qrt1* mutant pollens revealed that, after the completion of male meiosis the four daughter cells remain fused together. Hence in the *qrt1* homozygous plants, pollens grains are released as tetrads (Francis et al., 2006).

1.6.5 Female gametogenesis

Female gametogenesis occurs in the ovules. It begins when the surviving chalazal megaspore undergoes four successive rounds of mitosis to form an eight nucleated embryo sac (Yang and Sundaresan, 2000). Subsequent nuclear
migration and cellularization in the embryo sac lead to the formation of a seven celled female gametophyte. Out of the three cells, which are positioned at the micropylar end of the embryo sac, two become synergids and the remaining one differentiates into the egg cell. This egg cell eventually gets fertilized by one of the two sperm nuclei to form a diploid zygote. The three cells which are positioned at the chalazal end are specified to form antipodal cells. The two polar nuclei which had migrated to the center of the embryo sac fuse together and form a diploid central cell, which upon fertilization by one of the two sperm nuclei (double fertilization) gives rise to the triploid endosperm initial (Chaudhury and Berger, 2001; Higashiyama et al., 2003). A large number of mutants with various abnormalities during female gametogenesis have been isolated in Arabidopsis (Drews et al., 1998; Yadegari and Drews, 2004). These mutants are classified into diverse groups based on their characteristic phenotypes, such as mutants defective in mitosis, vacuole formation, nuclear fusion and migration, cellularization and cell death etc (Schneitz et al., 1997; Feldmann et al., 1997; Drews et al., 1998; Yadegari and Drews, 2004). A schematic representation of both male and female sporogenesis and gametogenesis is given in Figure 1.1.

1.7 Meiosis in Arabidopsis

A remarkable feature of life on earth is genetic diversity, a great deal of which comes from sexual reproduction. Meiosis is a very critical step for sexual reproduction. It is by this specialized cell division that the diploid sporophytic cells are capable of giving rise to haploid gametes (Bennett, 1977). In other words, meiosis provides the trigger for splitting plant life cycle into sporophytic and gametophytic generations. Meiosis involves a single round of DNA replication followed by two rounds of nuclear divisions, meiosis I and II (Hamant et al., 2006). As a result meiosis converts a diploid cell (pollen mother cell or megaspore mother cell) into four haploid daughter cells and also facilitates exchange of genetic material between parental chromosomes (Bhatt et al., 2001). This ensures genetic diversity in the population as each offspring produced will be different from one another and also from their parents.
Figure 1.1 Schematic diagram of male and female sporogenesis and gametogenesis in Arabidopsis.

Illustration of events involved in gamete formation in plants. HC, hypodermal cell; AC, archesporial cell; MMC, megaspore mother cell; PMC, pollen mother cell; ES, embryo sac; FES, functional embryo sac; Ap, antipodal cells; Cn, central nuclei; Ec, egg cell; Sg, synergids; GC, generative cell; VC, vegetative cell; Sn, sperm nuclei.
Meiosis is a very complex phenomenon involving unique processes such as chromosome pairing, synaptonemal complex formation and recombination (Lee and Amon, 2001). In the first meiotic division (meiosis I), homologous chromosomes segregate to opposite poles (reductional division) and during the second meiotic division (meiosis II), the sister chromatids separate from one another (equational division) to produce haploid gametes (Cnudde and Gerats, 2005). A coordinated series of events during meiosis I ensure homologous chromosome pairing and synapsis, which assist in the formation of bivalents (Pawlowski and Cande, 2005). This mechanism of pairing facilitates recombination between non-sister chromatids within each bivalent, resulting in genetic diversity (Bhatt et al., 2001; Consiglio et al., 2003; Lee and Amon, 2001; Pawlowski and Cande, 2005). The complexity of events during meiosis indicates that a large number of genes might participate during meiosis.

Despite its central role in sexual reproduction, meiosis and the governing molecular mechanisms are not yet completely understood in plants even though meiosis has long been a subject of research especially in maize (Creighton and McClintock, 1931). More recently, Arabidopsis has become a choice model plant for studying different aspects of meiosis because of its unique characteristics many of which are listed earlier in this Chapter. The availability of a large number of T-DNA and transposon insertion lines also aids in meiotic research in Arabidopsis (Consiglio et al., 2003; Alonso et al., 2003). This collectively with improved immunocytoological techniques has made Arabidopsis a suitable model for studying various aspects of meiosis such as meiotic chromosome behaviour and organization (Ross et al., 1996; Armstrong and Jones, 2001). The identification and characterization of genes that regulate critical steps in meiosis have immense potential in the field of plant breeding and genetic improvement.

An appropriate strategy to investigate different steps of meiosis would be isolation of mutants that are defective in various stages of meiosis (Mercier et al., 2001a; Caryl et al., 2003). Two different strategies have been widely employed to achieve this goal, forward and reverse genetic approaches (Mercier et al., 2001a; Consiglio et al., 2003; Caryl et al., 2003).
1.7.1 Forward genetic approach

This approach is based on screening of mutant lines, which are affected in different aspects of meiosis and subsequent cloning of these genes (Mercier et al., 2001a; Consiglio et al., 2003). In the forward genetic approach, visual screening for sterile plants is performed. Both chemical (Ethyl Methanesulphonate or EMS, Nitrosomethyl-urea, etc) and insertional (T-DNA and transposon) mutagenesis have been employed to generate screening populations (Caryl et al., 2003; Hamant et al., 2006).

1.7.2 Reverse genetic approach

In this method, an insertion (T-DNA or transposon) or other modes of gene disruption (RNA interference or antisense) are created in candidate genes, which are known to perform specific function in other organisms. Subsequently the associated phenotypes are analyzed. Studies in budding and fission yeast act as resources for a candidate gene approach in Arabidopsis (Hamant et al., 2006). Moreover advances in the field of bioinformatics have enhanced the efficiency of identifying and analyzing true candidate genes.

1.7.3 RNA interference technology

The mechanism of RNA interference (RNAi) involves the use of double strand (ds) RNA to alter the normal expression level of a particular gene or a group of related genes. This phenomenon was first discovered in Petunia by Jorgensen (Napoli et al., 1990; Jorgensen, 1990). Subsequently many studies revealed that, RNAi is an evolutionarily conserved mechanism and it helps in protecting the organism from invading genetic parasites such as viruses and mobile genetic elements (Yu and Kumar, 2003). More recent reports suggest that this mechanism is also involved in the endogenous regulation of gene expression (Schellander et al., 2007; Chapman and Carrington, 2007). Since its discovery RNAi has evolved as one of the most powerful techniques available to carry out genetic manipulations in a number of organisms including plants. RNAi evokes its effect either by interfering with the stability of the target mRNA by inducing its
degradation, or triggering chromatin remodeling in the target gene locus leading to a repression of transcription (Bernstein et al., 2001; Yu and Kumar, 2003).

The mechanism of RNAi can be broadly divided into two steps. The first step called initiation consists of processes such as homology based detection of the target mRNA, formation of double stranded RNA (dsRNA), recruitment of a unique RNA endonuclease called Dicer to the dsRNA and generation of short interfering RNAs (siRNAs) of 21 to 26 nucleotides (fragments of the target mRNA). The second step is known as the effector stage where the siRNAs generated in the first step will be recruited to a multi protein nuclease complex called RISC (RNA-Induced Silencing Complex), which then triggers target mRNA degradation (Tuschl et al., 1999; Elbashir et al., 2001; Yu and Kumar, 2003). In Arabidopsis, two kinds of siRNAs are produced, the long siRNAs (24-26 nucleotides in length) and the short siRNAs (21-22 nucleotides). The long siRNAs are mainly involved in the systemic transmission of the silencing signal whereas the short siRNAs are involved in the target mRNA degradation (Yu and Kumar, 2003). A schematic description of the RNAi mechanism is depicted in Figure 1.2

In plants especially Arabidopsis, RNAi is a commonly employed technique to dissect out gene functions, which are otherwise difficult to explore. This technique has been further optimized for use in plants by Dr. Peter Waterhouse and his lab (Waterhouse and Helliwell, 2003; Helliwell and Waterhouse, 2003). RNAi appears to be more powerful (ten times stronger) than antisense technology and is heritable and dominant over generations (Kerschen et al., 2004).
Figure 1.2 Diagrammatic representation of RNAi mechanism

A simplistic view of siRNA mediated gene silencing. RISC stands for RNA-induced silencing complex. Transgene or virus can trigger the formation of dsRNA. dsRNA, double stranded RNA; siRNA, short interfering RNA.
1.8 Meiotic mutants in Arabidopsis

In the last few decades, a range of meiotic mutants affecting different stages of meiosis have been reported in Arabidopsis shedding light into the intricacies of this complex process (Mercier et al., 2001a; Hamant et al., 2006). For ease of understanding, these meiotic mutants have been categorized into various subclasses based on the critical step in meiosis in which it is perturbed.

1.8.1 Sister chromatid cohesion and chromosome condensation mutants

Following DNA replication in S-phase, cohesion is established between sister chromatids and is found to be essential for the successful completion of meiosis (Nasmyth, 2001). A multi subunit protein apparatus called the cohesin complex act as the central player responsible for the maintenance of sister chromatid cohesion during cell cycle progression upto metaphase (Nasmyth and Hearing, 2005). Proper chromosome condensation is also known to be critical for normal homolog segregation at anaphase I (Strunnikov, 2003). Mutations in two well characterized Arabidopsis genes, SWITCH1 (Mercier et al., 2001b; Siddiqi et al., 2000) and AtSYN1/AtREC8 (Bai et al., 1999; Bhatt et al., 1999) impair the cohesion establishment between sister chromatids. The SWITCH1 gene encodes a 639 amino acid protein, which shows limited homology to other known proteins. Several alleles of SWITCH1 are available (Ravi et al., 2008). Interestingly these different alleles have variable phenotypes during male meiosis (for example in swi1-2; male meiosis was severely disrupted leading to the precocious separation of 20 sister chromatids before the end of meiosis I but in dyad (allele of switch1), male meiotic progression appears to be normal). However, they show a similar mutant phenotype during female meiosis (ten univalents during metaphase I, which then segregate evenly into daughter cells) (Agashe et al., 2002; Mercier et al., 2001b). This kind of division in dyad is similar to a mitotic division rather than the first division of meiosis. Subsequently however, it has been demonstrated that dyad female meiocytes do indeed enter meiosis (Agashe et al., 2002). Based on the defects in male meiosis, it was proposed that the SWITCH1 protein might play a role in the establishment of sister chromatids
cohesion (Mercier et al., 2001a). The SWITCH1 protein has also been shown to be nuclear localized during prophase and is absent during leptotene (Mercier et al., 2001b).

Disruption of the AtREC8 gene impairs both male and female fertility in Arabidopsis (Bai et al., 1999; Bhatt et al., 1999). The defects can be seen as early as leptotene, where the chromosomes fail to condense normally leading to a highly fuzzy and disorganized appearance of chromosomes in the mutant meiocytes (Bai et al., 1999). Chromosomes in the mutant exhibited extensive chromosome fragmentation by anaphase I (Bhatt et al., 1999). Various stages of meiosis II were also disrupted in the homozygous mutant plants leading to polyads and invivable division products (Bai et al., 1999). The AtREC8 gene has been cloned and found to encode the Arabidopsis homologue of yeast rec8, which is essential for the assembly of the cohesin complex (Stoop-Myer and Amon, 1999). Further studies on AtREC8 revealed that, it plays an essential role in maintaining chromosome arm and centromere cohesion during later stages of meiosis I (Cai et al., 2003). More recently a weak allele of another cohesin associated gene called AtSCC3 (Atscc3-1) was shown to cause impaired sister chromatid cohesion during meiosis I in Arabidopsis (Chelysheva et al., 2005).

1.8.2 Recombination and DNA repair mutants

In Arabidopsis, meiotic recombination is initiated by double strand break formation (DSBs) by AtSPO11-1, in one of the two participating non-sister chromatids (Grelon et al., 2001). AtSPO11-1 protein is a member of the class of type II-like topoisomerases. AtSPO11-1 generates DSBs via a transesterase mechanism with the assistance of a set of other genes (Keeney, 2001). In addition to its role in double strand break formation, AtSPO11-1 is also involved in chromosome synapsis (Grelon et al., 2001). There are at least two potential paralogs of this gene in Arabidopsis, and recently one of them, AtSPO11-2 was also reported to share the same function during meiosis (Hartung et al., 2007). Both Atspo11-1 and Atspo11-2 mutants are found to affect male and female meiosis leading to reduced fertility in the homozygous mutant plants (Grelon et
al., 2001; Hartung et al., 2007). Other genes in Arabidopsis, which are known to be important for meiotic recombination and related processes are \textit{AtDMC1} and \textit{AtRAD51} (Doutriaux et al., 1998; Hamant et al., 2006). \textit{AtDMC1} is meiotic specific whereas \textit{AtRAD51} seems to have additional roles during mitosis as well (Hamant et al., 2006). \textit{AtDMC1} was cloned and found to encode a 344 amino acid protein (Klimyuk and Jones, 1997). The \textit{Atdmc1} mutant has been subsequently shown to have meiotic abnormalities (univalent formation) leading to reduced male and female fertility (Couteau, 1999). \textit{AtRAD51} is found to be required for both recombination and chromosome pairing (Li et al., 2004). Three other RAD51 paralogs have been described in Arabidopsis so far, RAD51B, RAD51C and XRCC2 (Bleuyard et al., 2005).

The Arabidopsis \textit{Rad50} homologue has also been identified. Mutant plants homozygous for \textit{Atrad50} were reported to be sterile (Gallego et al., 2001). Although a detailed account of mutant meiosis is unavailable, it is probable that \textit{AtRAD50} has a role in the DNA repair pathway because these mutants are hypersensitive to various DNA damaging agents (Gallego et al., 2001). Over the last few years, several other Arabidopsis proteins involved in various aspects of DNA double strand break repair have been isolated and studied. This list includes Arabidopsis homologues of yeast recombination and DNA repair proteins such as Rad51, MRE11, NBS1, MND1, XRCC3 etc (Hamant et al., 2006). These genes and other characterized Arabidopsis meiotic mutants, which disrupt DNA DSBs repair like \textit{brca2}, are listed in Table 1.1.
Table 1.1 Other known meiotic mutants in Arabidopsis

<table>
<thead>
<tr>
<th>No.</th>
<th>Gene &amp; locus No.</th>
<th>Sterility</th>
<th>Function</th>
<th>Mol. Nat. of mutation</th>
<th>References</th>
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<td>DSBs repair</td>
<td>T-DNA</td>
<td>Panoli et al., 2006</td>
</tr>
<tr>
<td>16</td>
<td>AESP/At4g22970</td>
<td>Male &amp; female</td>
<td>Cohesin removal</td>
<td>RNAi</td>
<td>Liu et al., 2006</td>
</tr>
<tr>
<td></td>
<td><strong>PARTING DANCERS</strong>/At3g12410</td>
<td>Reduced fertility</td>
<td>Chiasmata formation</td>
<td>T-DNA</td>
<td>Wijeratne et al., 2006</td>
</tr>
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<tr>
<td>18</td>
<td>AtNBS1/At3g02680</td>
<td>Fertile</td>
<td>DSBs repair, DNA damage signaling and recombination</td>
<td>T-DNA</td>
<td>Waterworth et al., 2007</td>
</tr>
<tr>
<td>19</td>
<td>AtPRD1/At4g14180</td>
<td>Reduced fertility</td>
<td>Meiotic recombination, DSBs repair</td>
<td>T-DNA</td>
<td>DeMuyt et al., 2007</td>
</tr>
<tr>
<td>20</td>
<td>AtMUS81/At4g30870</td>
<td>Decrease in pollen viability</td>
<td>DNA damage repair, recombination</td>
<td>T-DNA</td>
<td>Berchowitz et al., 2007</td>
</tr>
<tr>
<td>21</td>
<td>AtZIP4/At5g48390</td>
<td>Reduced fertility</td>
<td>Class I cross over formation</td>
<td>T-DNA</td>
<td>Chelysheva et al., 2007</td>
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<td>22</td>
<td>AtCOM1/At3g52115</td>
<td>Male &amp; female</td>
<td>DSBs repair</td>
<td>T-DNA</td>
<td>Uanschou et al., 2007</td>
</tr>
<tr>
<td>23</td>
<td>AtBRD1/At4g21070</td>
<td>Fertile</td>
<td>Chromosomal stability, DNA repair</td>
<td>T-DNA</td>
<td>Reidt et al., 2006</td>
</tr>
<tr>
<td>24</td>
<td>AtMLH3/At4g35520</td>
<td>Reduced fertility</td>
<td>Mismatch repair, meiotic crossover formation</td>
<td>T-DNA</td>
<td>Jackson et al., 2006</td>
</tr>
<tr>
<td>25</td>
<td>AtMSH2/At3g18524</td>
<td>Fertile</td>
<td>Mismatch repair, Anti-recombination effects</td>
<td>T-DNA &amp; RNAi</td>
<td>Emmanuel et al., 2006</td>
</tr>
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</table>
1.8.3 Chromosome pairing and synapsis mutants

One of the unique characteristics of meiosis I is the juxtaposition and pairing of homologous chromosomes which in turn paves way for their synapsis (Jones et al., 2003). The term chromosome pairing is generally used to describe various interactions or associations between homologous chromosomes. Usually these interactions are transient in nature and precede synapsis. Chromosome pairing uses DNA homology search to identify homologs, by a mechanism which is still not clearly understood (Wilson et al., 2005). Many studies have shown that pairing usually starts with telomere clustering in different species including Arabidopsis. Following pairing, homologs form synaptonemal complexes (SC) along the length of the chromosomes. The SC is an evolutionary conserved proteinaceous structure that forms between homologs (Osman et al., 2006; Jones et al., 2003). One of the main rationales behind chromosome synapsis and bivalent formation is to ensure proper chromosome segregation during anaphase I. Several Arabidopsis mutants have been isolated that show defects in diverse aspects of chromosome pairing, synapsis and SC formation (Jones et al., 2003; Hamant et al., 2006). Based on mutant phenotypes, these mutants are classified into two subclasses; asynaptic mutants and desynaptic mutants.

1.8.4 Asynaptic mutants

In this class of synaptic mutants, the chromosome synapsis never takes place. A good example is asy1, which is an Arabidopsis homologue of yeast hop1 (Sanchez-Moran et al., 2007; Armstrong et al., 2002). The asy1 mutant was reported to cause both male and female sterility. Subsequent cytological analysis revealed that in homozygous asy1 mutants, there is a total failure of homologous synapsis during prophase I. The ASY1 gene has been cloned and encodes a protein of 596 amino acids. ASY1 was found to be expressed in both meiotic as well as non meiotic tissues in RT-PCR analysis (Armstrong et al., 2002). Immunolocalization studies further demonstrated that ASY1 protein is essential for homologous juxtaposition during early prophase I. The impaired status of homologous chromosome pairing in the asy1 mutant meiocytes leads to severe
defects in the assembly of the synaptonemal complex (Armstrong et al., 2002). Recent reports suggest that ASY1 is involved in AtDMC1 dependent recombination as well during meiosis in Arabidopsis (Sanchez-Moran et al., 2007). Another Arabidopsis mutant compromised in chromosome pairing and bivalent formation is ahp2 (Schommer et al., 2003). It appears that AHP2 represents the Arabidopsis homologue of yeast meu13. Details of other asynaptic mutants reported in Arabidopsis are given in Table 1.1.

1.8.5 Desynaptic mutants

In this class of synaptic mutants, chromosome synapsis occurs normally during prophase I, but it is lost prematurely. An example for this group is dsy1 (Ross et al., 1997). The dsy1 mutant causes reduced fertility and affects both male and female meiosis. In these mutant meiocytes the initiation of homologous pairing and synapsis happens normally but by early diplotene stage, the homologous chromosomes appear to lose their association. These mutant meiocytes are defective in maintaining bivalents through the formation and maintenance of chiasmata (Ross et al., 1997). This observation suggests a defect either in chiasmata formation or in their retention in these mutant meiocytes. Usually one observes a mix of reductional and equational division at anaphase I in dsy1 meiocytes leading to daughter cells containing unbalanced amounts of DNA (Caryl et al., 2003). dsy10 (switch1 allele) is another example of a functionally characterized desynaptic mutant in Arabidopsis. This mutant causes asynchronous meiotic development both within and between anthers (Peirson et al., 1996). Upto pachytene stage, the dsy10 mutant meiocytes proceeds normally, however at diplotene stage and onwards the homologous chromosomes appear to separate precociously giving rise to ten univalents instead of the normal five bivalents.

1.8.6 Chromosome segregation mutants

This group represents another subclass of meiotic mutants in Arabidopsis, which are compromised in various aspects of chromosome segregation during
meiosis at anaphase I and II. The main defect observed in this class of mutants is in the function or regulation of the meiotic spindle causing defects in chromosome separation and segregation (Hamant et al., 2006). The *ATK1* gene is one of the best characterized examples for this category of meiotic mutants in Arabidopsis (Chen et al., 2002). Homozygous *atk1* mutant plants exhibited reduced male fertility. Meiosis is normal in this mutant upto metaphase I but the chromosomes fail to align subsequently at the metaphase plate. By anaphase I, many chromosomes appeared as laggards and few completely failed to segregate (Chen et al., 2002). The homolog separation is also asynchronous in the *atk1* mutant and the organelle distribution also seems to be affected. Detailed cytological analysis with *atk1* mutant revealed that meiotic spindle is impaired in the mutant meiocytes as the spindle appeared diffused and unfocused (Chen et al., 2002). Based on these findings, it has been proposed that *ATK1* gene might be required for the spindle morphogenesis in Arabidopsis. The *ATK1* gene has been cloned and is found to be expressed specifically in dividing cells. *ATK1* gene codes for a protein of 793 amino acids and shows homology to a microtubule motor protein Kinesin (Chen et al., 2002). Other genes having similar functions to *ATK1* are also known in Arabidopsis such as *ATK5* and *ASK1* (Chen et al., 2002; Yang et al., 1999b). The *ask1* mutant is also male sterile and female fertile and appears to have non-meiotic functions as well. *ASK1* gene codes for a homolog of the SKP1 protein, which is an essential component of SCF (Skp1-cullin-F-box) complex (Farrás et al., 2001; Hamant et al., 2006). The SCF complex is known to be involved in regulating diverse events during development such as cell-cycle regulation, signal transduction and transcription through selective proteolysis of other regulatory proteins (Lechner et al., 2006).

1.8.7 Cell cycle regulation, meiotic progression and cytokinesis mutants

Meiotic mutants that are compromised in aspects of cell cycle regulation are categorized in this subclass. The best example is the *TAM1* gene. *tam1* mutant results in asynchronous male meiosis in the mutant anthers (Magnard et al., 2001). Overall *tam1* mutation slows down meiotic progression and the delay
appears to be during the G2/M transition (Magnard et al., 2001). \textit{tam1} mutant was also reported to be impaired in processes like chromosomes segregation, chromosome condensation and cytokinesis. Another Arabidopsis mutant impaired in cell cycle regulation is \textit{ms5/tdm1}. In the \textit{tdm1} mutant, initially the microsporocytes appeared normal but soon they degenerated (Chaudhury et al., 1994). Subsequent studies revealed that, the \textit{tdm1} pollen mother cells undergo two rounds of normal meiotic division, however at the end of the second division the cell continues its division cycle and attempts a third division without any additional DNA synthesis (Ross et al., 1997). The \textit{TDM1} gene encodes a 434 amino acid protein that is unique and is reported to be expressed in both meiotic and non meiotic tissues (Glover et al., 1998). The Arabidopsis \textit{MMD/DUET} gene represents another meiotic gene compromised in cell cycle regulation. Detailed analysis of \textit{MMD/DUET} gene indicated that it may be a critical regulator of meiotic progression (Reddy et al., 2003; Yang et al., 2003). An RNAi mediated knock down of the Arabidopsis homologue of the yeast cell-cycle gene \textit{CDC45} was also reported to cause defects during meiotic progression (Stevens et al., 2004). Based on analysis of \textit{CD45-RNAi} lines in Arabidopsis, it seems to be involved in controlling premeiotic DNA replication, similar to its function in yeast (Lei and Tyé, 2001). The \textit{STUD/TETRASPORE (STD/TES)} gene in Arabidopsis is implicated to function in cytokinesis during male meiosis, as in the mutant, cytokinesis (during male meiosis) is blocked (Hulskamp et al., 1997; Spielman et al., 1997). Other Arabidopsis meiotic mutants which are defective in various aspects of cell cycle regulation are listed in Table 1.1.

### 1.9 Meiotic chromosome organization in Arabidopsis

Organization generally represents well defined function/performance and is not just demonstration of elegance. Likewise an apt organization of chromosomes is critical for ensuring successful completion of meiosis. The term meiotic chromosome organization represents meiotic chromosome structure and architecture. This includes aspects of chromosome cohesion, condensation, homologous chromosome pairing and synapsis, formation of recombination.
nodoles, bivalent formation, chiasmata and kinetochores (Strunnikov, 1998; Wyman and Kanaar, 2002; Haering and Nasmyth, 2005; Hamant et al., 2006). It is known that diverse aspects of chromatin organization are critical for normal progression of meiosis and successful gamete production (Dawe, 1998). The structure and organization of chromosomes largely depends on the associated proteins such as the structural maintenance of chromosomes (SMC) proteins (Haering et al., 2002; Haering and Nasmyth, 2005). The SMC proteins can be broadly classified into, condensin complex (consisting of SMC2 and SMC4), which help to organize the chromatin threads during cell division, and cohesin (consisting of SMC1 and SMC3), which is involved in maintaining sister chromatid cohesion. Other SMC members like SMC5 and SMC6 are involved in the organization of DNA ends and DNA repair (Strunnikov, 1998; Haering and Nasmyth, 2005). A more detailed description of cohesin class of SMC proteins is provided in Chapter three.

The condensin complex is well conserved in Arabidopsis. Like in other organisms, Arabidopsis also has two condensin complexes, condensin complex I and condensin complex II (Fujimoto et al., 2005). Both these complexes have their core formed by SMC class of proteins (SMC2 and SMC4). The main difference observed between these complexes, is in the non-SMC subunit identity (Ono et al., 2003). Arabidopsis condensin complex I consists of the following non-SMC partners: AtCAPD2, AtCAPH and AtCAPG whereas complex II comprises of AtCAPD3, AtCAPH2 and AtCAPG2 (Fujimoto et al., 2005). All these genes encode unique proteins with limited similarity to other plant proteins. Almost all of these genes are yet to be functionally characterized in plants. Localization studies in tobacco cultured cells using GFP-tagged AtCAPH2 and AtCAPH has shown that, they localized at mitotic chromosomes from prometaphase to telophase (Fujimoto et al., 2005). This observation is consistent with their predicted role in chromosome organization in Arabidopsis. Arabidopsis has two homologues for SMC2: AtCAP-E1 and AtCAP-E2 and studies have shown that double mutants of Atcap-E1 and Atcap-E2 causes embryo lethality and an antisense mediated gene silencing approach revealed defects in meiosis
Arabidopsis has a single homologue of AtSMC4 and recently a disruption in this gene was reported to cause gametophytic lethality, revealing its essential role in plant development (Siddiqui et al., 2006).

1.10 Objectives of the current study

Meiosis is a fascinating mechanism, which creates genetic diversity and possesses immense biotechnological potential for the improvement of agriculture and food production. With the availability and flexibility of tools and facile genetic manipulations, Arabidopsis represents the best model system for dissecting the complexities of meiosis in plants. Moreover, research and development in meiosis in Arabidopsis will in turn aid in the improvement of our understanding of meiosis and meiotic genes in economically important crop plants. This has an important bearing on agriculture and plant breeding programmes for designing superior crop plants, which would be able to satisfy the ever expanding needs of mankind. Despite these facts and predictions, meiosis as a whole and different meiotic genes and their likely functions remain a poorly explored area. This is particularly true regarding genes that influence diverse aspects of chromatin organization, where our knowledge is still in its infancy. Hence, I fixed my objectives to isolate and functionally characterize meiotic genes in Arabidopsis, which are likely to play pivotal role during plant meiosis. I believe that the current study will shed light into the unknown territories of plant meiosis.

I chose a reverse genetic approach, compiling both RNA interference (RNAi) technology and T-DNA insertion mutagenesis to achieve the objectives. In this study, I attempt to functionally characterize the Arabidopsis Mei2 like (AML) gene family and a putative Arabidopsis adherin homologue AtSCC2. The AML gene family comprises of five genes, which are putative homologues of yeast mei2, a gene implicated in premeiotic DNA synthesis and regulating the entry of cells from mitosis to meiosis I (Shimoda et al., 1987). AtSCC2 represents a putative adherin class of protein in Arabidopsis. Adherins are known to be required for various aspects of chromosome dynamics in yeast and other organisms (Dorsett, 2004). I have described the functional characterization of
these important Arabidopsis genes and their implications in the following chapters. I believe that these findings on the AML gene family and AtSCC2 will contribute towards better understanding of meiosis in plants.