LETTERS

Gamete formation without meiosis in *Arabidopsis*

Maruthachalam Ravi[1], Mohan P. A. Marimuthu[1] & Imran Siddiqi[1]

Apomixis, the formation of asexual seeds in plants, leads to populations that are genetically uniform maternal clones. The transfer of apomixis to crop plants holds great promise in plant breeding for fixation of heterozygosity and hybrid vigour because it would allow the propagation of hybrids over successive generations[1,2]. Apomixis involves the production of unreduced (diploid) female gametes that retain the genotype of the parent plant (apomeiosis), followed by parthenogenetic development of the egg cell into an embryo and the formation of functional endosperm[3]. The molecular mechanisms underlying apomixis are unknown. Here we show that mutation of the *Arabidopsis* gene *DYAD/SWITCH1* (*SWI1*)[4,5], a regulator of meiotic chromosome organization, leads to apomeiosis. We found that most fertile ovules in *dyad* plants form seeds that are triploid and that arise from the fertilization of an unreduced female gamete by a haploid male gamete. The unreduced female gametes fully retain parental heterozygosity across the genome, which is characteristic of apomeiosis. Our results show that the alteration of a single gene in a sexual plant can bring about functional apomeiosis, a major component of apomixis.

Apomixis is found in more than 400 species of flowering plants and occurs by three distinct developmental routes[2]. It has been suggested that apomixis results from deregulated expression of the sexual programme[3]; however, the molecular mechanisms controlling apomixis remain undeciphered. One hypothesis is that genes controlling apomixis may be variant alleles of genes that act during normal sexual development[6]. Such genes may be revealed by an analysis of model sexual plants. In *Arabidopsis*, the female gametophyte containing the egg and associated cells develops from one of four haploid megaspores formed by meiotic divisions of a single cell within the ovule, the megasporocyte. *SWI1* is required for sister chromatid cohesion and centromere organization during meiosis. Mutations in *swi1* (Supplementary Fig. 1) cause a single equational division in place of normal female meiosis, followed by arrest in further progression[4,5] (Fig. 1a–d). These defects lead to the production of two diploid cells in place of four haploid megaspores, and failure to form a female gametophyte. The *dyad* allele of *SWI1* causes female specific sterility without affecting pollen development[7]. Although *dyad* plants show sterility, there is variable expressivity and only a few seeds are

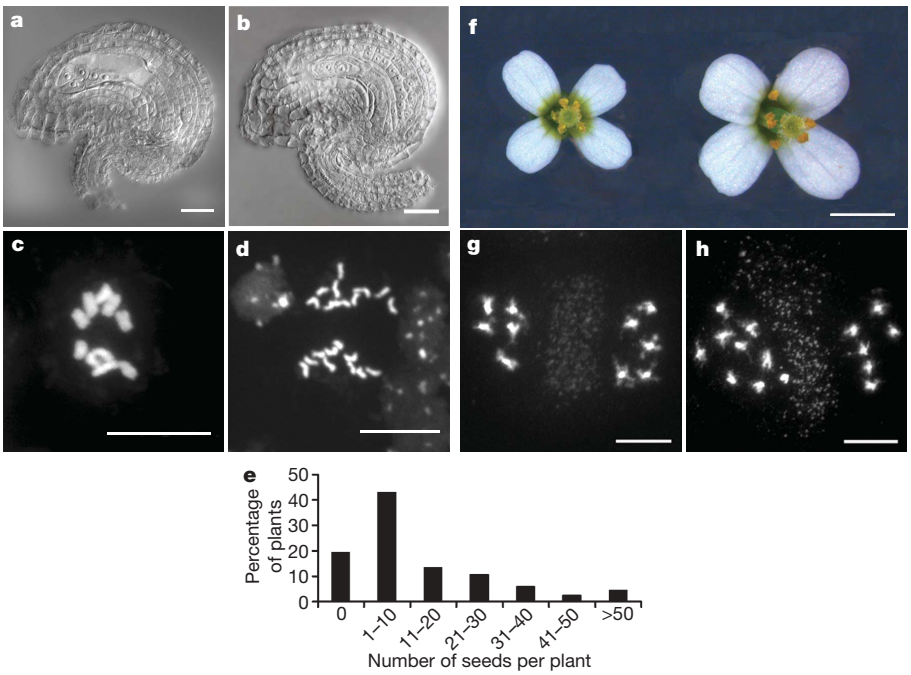

Figure 1 | The *dyad* mutant produces triploid progeny. **a**, Wild-type ovule containing a female gametophyte. **b**, *dyad* ovule showing two arrested cells in place of a female gametophyte. **c**, Wild-type female meiosis anaphase I: reductional (5–5) segregation of chromosomes. **d**, *dyad* female meiosis anaphase I: equational (10–10) separation of chromosomes. **e**, Reduced seed set in *dyad* plants: the mode is 1–10 seeds per plant, whereas wild-type plants produce more than 2,000 seeds. The frequency of functional female gamete formation in *dyad* is about 0.24% (Supplementary Notes). **f**, A flower from progeny of a self-fertilized *dyad* plant (right) is larger than a flower from a *dyad* F_2 segregant (left). **g**, **h**, Male meiocyte at the end of meiosis I from a diploid *dyad* plant showing 5–5 segregation of chromosomes (**g**), and a triploid *dyad* plant showing 9–6 segregation (**h**). Scale bars, 20 μm (**a**, **b**); 10 μm (**c**, **d**, **g**, **h**); 2 mm (**f**).

[1]Centre for Cellular and Molecular Biology, Uppal Road, Hyderabad 500007, India.

produced, the modal range being one to ten seeds per plant (Fig. 1e). We found that seeds obtained from a pool of *dyad* plants germinated efficiently (89%; $n = 296$) and grew into plants that showed normal vegetative growth. However, a majority of plants examined (80%; $n = 52$) showed large flowers compared with both the wild type and the *dyad* parent (Fig. 1f). A possible reason for greater floral size was higher ploidy, which results in an increased size of floral structures including pollen grains[8]. Pollen from progeny of *dyad* plants was heterogeneous in size, indicative of differences in ploidy (Supplementary Fig. 2). To determine the ploidy of *dyad* progeny, we performed chromosome counts on 19 randomly chosen plants and found that 17 were triploid (Fig. 1h) and 2 were diploid. Diploid *dyad* plants therefore produce triploid progeny with incomplete penetrance. The formation of triploids in *dyad* does not reflect a background level that is present in the wild type, because we did not detect any triploids among more than 7,000 wild-type plants (Supplementary Notes). We therefore examined the mechanism by which triploids arise.

Triploids can form by fusion of an unreduced ($2n$) gamete with a haploid gamete or by polyspermy. Equational division of the female meiocyte in *dyad*[5] indicated the likelihood of fertilization of unreduced female gametes by haploid male gametes. In this case, the triploid seeds would carry an excess contribution from the maternal genome. Experiments that alter the relative dosage of the maternal and paternal genomes by crosses between diploid and tetraploid plants have shown that an excess maternal genome reduces seed size, whereas an excess paternal genome causes increase in seed size. These findings have been interpreted according to theories of parental conflict or differential dosage[9,10]. To address the origin of triploid seeds in *dyad* we examined seed size (Fig. 2). Seeds produced by *dyad* fell into three categories: shrunken (44%), normal (27%) and large (29%) ($n = 1{,}131$; Fig. 2e). To determine the ploidy of seeds in each category, we performed chromosome counts on all the progeny ($n = 119$) from a set of *dyad* plants. Most shrunken and normal seeds were triploid, whereas most large seeds were diploid (Fig. 2f). To compare seeds from *dyad* with maternal and paternal excess triploid seeds, we performed reciprocal crosses between wild-type diploid and tetraploid *Arabidopsis* and measured seed weight. The seed weight of shrunken seeds from *dyad* (13.7 μg) was similar to that for the maternal excess triploids (13.0 μg) and less than that for the paternal excess triploids, which weighed more than twice as much (32.0 μg). Because the largest class of seeds from *dyad* is shrunken and triploid, our observations suggest that these seeds arise from an excess maternal genome contribution.

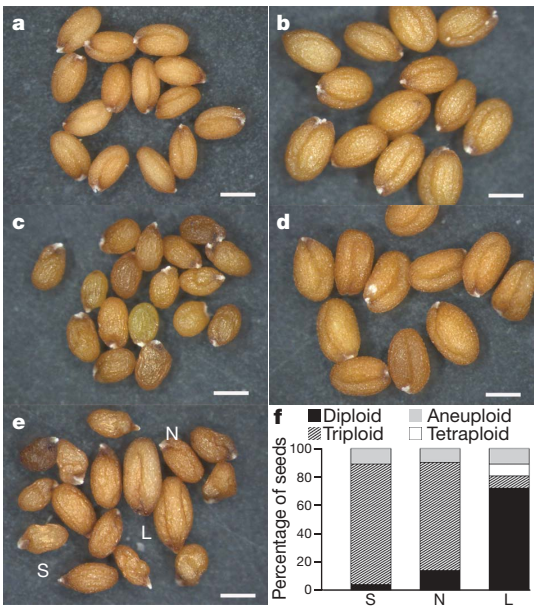

Figure 2 | Triploids arise from a maternal excess contribution. **a**, Wild-type diploid seeds (21.3 μg). **b**, Tetraploid seeds (33 μg). **c**, Maternal excess seeds from a tetraploid female × diploid male cross (13 μg) **d**, Paternal excess seeds from a wild-type diploid female × tetraploid male cross (32 μg). **e**, Heterogeneous seeds obtained from a diploid *dyad* mutant plant: L, large (33.5 μg); N, normal (20.2 μg); S, shrunken (13.7 μg). **f**, Proportion of different ploidy types in progeny of *dyad* mutant plants from each category of seeds. Shrunken (S), $n = 48$; normal (N), $n = 35$; large (L), $n = 36$. Values in parentheses for **a**–**e** indicate single-seed weights. Scale bars, 0.3 mm.

To determine parental contributions in the triploids directly, we performed a three-way cross between *dyad*, a marker line ET60 carrying a single copy of a kanamycin resistance gene (Kan^R), and a wild-type strain. We first crossed *dyad* as a female parent with ET60 and obtained five seeds from 360 crosses. The five F_1 plants were analysed for ploidy and also crossed to the wild type. Segregation of Kan^R:Kan^S in the second cross followed a 1:1 ratio diagnostic of a simplex condition (K– –) for the Kan^R marker in the parent, demonstrating that the plants arose from fertilization of an unreduced female gametophyte by a haploid pollen and did not occur as a result of an unreduced male gametophyte or polyspermy (Table 1).

Several mechanisms can give rise to unreduced gametes in sexual plants, but most do not result in the full retention of parental heterozygosity[11]. Complete retention of parental heterozygosity in the unreduced female gametes defines apomeiosis. *dyad*, like other mutants that show unreduced female gamete formation[12–14], is incompletely penetrant and produces both reduced and unreduced gametes. In all mutants that have been examined, loss of parental alleles in the unreduced gametes occurs, to different extents[12,15–17]. To determine whether unreduced female gametes in *dyad* retain parental genotype we examined progeny from *dyad* for loss of parental heterozygosity. We crossed *dyad* in the Columbia genetic background to a wild-type Nossen strain and genotyped F_2 mutant plants for five polymorphic microsatellite markers distributed over four chromosomes. Seeds collected from 52 F_2 plants were grown to give F_3 plants, and each F_3 plant was genotyped for those markers for which the F_2 parent was heterozygous. Out of 262 plants, 52 had lost heterozygosity for at least one marker (Table 2). The ploidy of 41 of these 52 plants was determined; 40/41 were diploid, whereas 1 was hyperdiploid with 13 chromosomes. Loss of heterozygosity therefore occurred almost exclusively in diploids. A marker unlinked to the centromere would be expected to show loss of heterozygosity in 16.7% of triploid plants at normal levels of recombination. Failure to detect loss of heterozygosity in triploids indicates the complete absence of recombination in unreduced gametes and further rules out polyspermy and post-meiotic doubling as mechanisms for their formation. These results clearly show that triploids arise from the fertilization of an apomeiotic female gamete. The proportion of *dyad* progeny arising from apomeiosis is 63%.

Loss of heterozygosity in diploid progeny of *dyad* shows that they form by the sexual union of haploid gametes rather than by the parthenogenesis of a diploid egg cell. Thus, both reduced and

Table 1 | Triploids arise from an unreduced female gamete

Plant no.	Ploidy of ♀ parent	Kan^R:Kan^S	χ^2 (1:1)
1	$3n$	254:236	0.660
2	$3n + 1$	61:52	0.717
3	$3n$	121:132	0.478
4	$3n + 2$	112:94	1.573
5	$3n + 1$	107:86	2.285

Segregation of kanamycin resistance after germination of seeds obtained from the three-way cross (*dyad* ♀ × ET60 (Kan^R) ♂) ♀ × wild-type (Kan^S) ♂. Five seeds from a cross of *dyad* ♀ × ET60 (Kan^R) ♂ were obtained. Two were triploid ($3n = 15$) and three were hypertriploid (16–17 chromosomes). The five plants were crossed to the wild type. A 1:1 segregation for Kan^R:Kan^S indicates that the Kan^R determinant is present in the simplex condition (K– –) in the ♀ parent, which would be the case only if the triploid parent were formed from an unreduced female gamete in the first cross. In each case, $P(\chi^2) > 0.1$ for a 1:1 model and $P(\chi^2) < 0.005$ for a 5:1 model predicted for a duplex condition (KK–). The duplex control cross (KK-) ♀ × wild-type (Kan^S) ♂ segregated 126:31 Kan^R:Kan^S, for which $P(\chi^2) > 0.25$ for a 5:1 model and $P(\chi^2) < 0.005$ for a 1:1 model.

Table 2 | Retention of marker heterozygosity in triploid progenies of *dyad*

Marker	Chromosome number	Centromere distance (cM)	No. of plants	No. of homozygotes*
nga168	2	58	185	22 (12)
nga6	3	37	108	7 (6.5)
nga162	3	29	74	8 (11)
nga1107	4	76	107	11 (10)
nga225	5	56	169	20 (12)

* Numbers in parentheses show the percentage homozygosity observed for a given marker. No homozygous triploids were obtained. A subset of plants ($n = 65$) that had not lost heterozygosity for any of the five markers were analysed and found to be 78% triploids, 11% diploids and 11% aneuploids.

unreduced embryo sacs coexist in the same plant (in two F_3 families analysed, comprising 44 and 22 sibs, there were 17 and 6 diploids, and 23 and 13 triploids, respectively), a feature that is characteristic of facultative apomicts. Apart from producing apomictic and sexual seeds, apomicts are capable of giving rare $3n$ and $4n$ seeds, as well as aneuploids arising from the mis-segregation of chromosomes[18,19]. The range of ploidy in *dyad* progeny (Fig. 2f) therefore resembles that observed in natural apomictic species.

A likely origin of the apomeiotic embryo sac in *dyad* is one of the diploid cells formed after equational division of the megasporocyte. To assess the developmental potential of the division products of the megasporocyte, we examined expression of the FM1 marker, which is expressed first in the functional megaspore (but not in degenerating megaspores) as well as in the developing female gametophyte[20]. FM1 expression was detected in *dyad* ovules after megasporocyte division, at a frequency that was comparable to that in the wild type (67% ($n = 106$) and 93% ($n = 150$), respectively). Expression was restricted to the lower (chalazal) cell of the dyad, as is observed for the functional megaspore in the wild type (Fig. 3). It is therefore likely that the apomeiotic embryo sacs arise from this cell, as occurs in diplospory[3]. However, because the frequency of seed formation is low, we cannot exclude alternative mechanisms. The low frequency of functional female gamete formation in *dyad* is likely to be at least partly due to the triggering of checkpoint mechanisms that block progression through the meiotic cell cycle[5].

The discovery of apomeiotic female gamete formation in the *dyad* mutant of *Arabidopsis* demonstrates that alteration of a gene that is directly involved in meiotic chromosome organization can confer apomeiosis. Mapping experiments in the apomictic species *Tripsacum dactyloides* indicate that a marker that is linked to apomixis is syntenic to a region in maize that contains the *AMEIOTIC1* (*AM1*) gene[21]. *AM1* has recently been proposed to be a functional homologue of *SWI1* (ref. 22). Thus, *SWI1* homologues may have a function in apomixis in nature. Whereas a strong *swi1* allele causes both male and female sterility[4], the *dyad* allele, which is predicted to form a truncated protein[5], is of intermediate strength. The phenotype of *dyad* most closely resembles natural apomicts, in that the alteration in sporogenesis leading to apomeiosis is biased towards the female side. Hence, female specificity in this case is due to a partial reduction in activity and not to a complete loss of function. Quantitative rather than binary changes in gene activity may be a wider feature of genetic mechanisms controlling apomixis, as has recently been found for the evolution of autogamy in tomato[23].

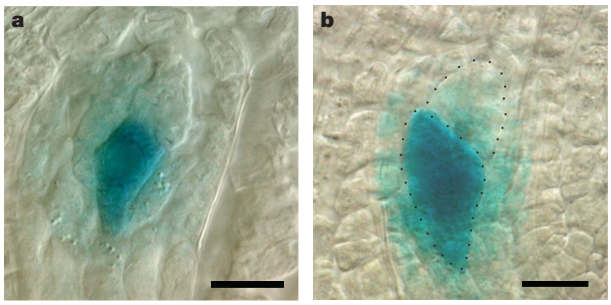

Figure 3 | *dyad* ovules express a functional megaspore marker. **a**, Functional-megaspore-specific GUS expression driven by the FM1 promoter in a wild-type ovule. **b**, FM1-promoter-driven GUS expression specific to the chalazal (lower) cell of the dyad in a *dyad* ovule. Black dots outline the two cells of the dyad for clarity. Scale bars, 10 μm.

Attempts to introgress apomixis from natural apomicts into crop species have failed[2], and efforts to identify apomixis genes in natural apomicts by map-based cloning have been hampered by the finding that apomixis is associated with large genomic regions that are repressed for recombination[24–27]. The difficulties involved in the genetic analysis of natural apomicts have motivated alternative approaches towards understanding and ultimately engineering apomixis by the analysis of genes that control meiosis, gametogenesis and seed development in sexual plants[2]. The *fertilization independent seed* (*FIS*) class genes act as regulators of cell division and proliferation in the female gametophyte and the developing seed, and encode members of the Polycomb group of proteins[3]. *fis* class mutants show spontaneous initiation of endosperm development, and, in *msi1*, spontaneous division of the egg cell[3,28]. However, these structures abort early in development and do not lead to the formation of viable seeds. Here we have shown that mutation of *SWI1* leads to the formation of functional apomeiotic female gametes. Our results demonstrate the occurrence of apomeiosis, a major component of apomixis, by mutation of a single gene whose molecular identity is known. The findings represent a significant step towards the synthesis of apomixis by the manipulation of genes that function in normal sexual development.

METHODS SUMMARY

Plant material and growth conditions. Wild-type *Arabidopsis* strains were Col-0 and No-0. The *dyad* mutant was maintained as an F_2 segregating population in the Col-0 background. The tetraploid *Arabidopsis* strain CS3900, which is quadruplex for Kan^R (KKKK), was obtained from the Arabidopsis Biological Resource Centre (ABRC). The ET60 enhancer trap line contains a single-copy *Ds* transposon[29] in the gene At1g73160. Plants were grown at 21 °C in a growth chamber under a 16-h/8-h light/dark cycle. For growth under sterile conditions, seeds were surface sterilized with ethanol and plated on MS medium supplemented with the appropriate antibiotic.

Analysis of ovule and seed phenotypes. Whole-mount analysis of cleared ovules was as described previously[7]. Ploidy was determined by the analysis of male meiotic chromosomes[30]. Female meiosis was analysed as described previously[5]. Seeds were classified and sorted according to size by observation under a stereomicroscope. Seed weight was estimated by weighing two sets of 100 seeds in a fine microbalance (Sartorius) and calculating the average weight of a single seed.

Microsatellite marker analysis. DNA isolation and simple sequence length polymorphism (SSLP) marker analysis was performed as described previously[5]. The *dyad* (Col-0) and No-0 parental strains were screened for polymorphisms on the basis of known differences between the two ecotypes (www.arabidopsis.org) and a set of five markers was selected.

Generation and analysis of transgenic plants. The *proFM1::uidA* fusion was constructed as described in an earlier study[20]. The construct was introduced into heterozygous *dyad*/+ plants by *Agrobacterium*-mediated transformation *in planta*[5]. Wild-type and *dyad* mutant plants were identified among primary transformants. Staining of ovules in GUS staining solution was as described previously[20], using an incubation time of two days. Images were observed on a Zeiss Axioplan 2 microscope equipped with differential interference contrast optics using 40× or 100× Apochromat objectives, and captured on an Axiocam HRC charge-coupled device camera.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

Received 15 October; accepted 20 December 2007.
Published online 13 February 2008.

1. Koltunow, A. M., Bicknell, R. A. & Chaudhury, A. M. Apomixis: molecular strategies for the generation of genetically identical seeds without fertilization. *Plant Physiol.* **108,** 1345–1352 (1995).
2. Spillane, C., Steimer, A. & Grossniklaus, U. Apomixis in agriculture: the quest for clonal seeds. *Sex. Plant Reprod.* **14,** 179–187 (2001).
3. Koltunow, A. M. & Grossniklaus, U. Apomixis: a developmental perspective. *Annu. Rev. Plant Biol.* **54,** 547–574 (2003).

4. Mercier, R. *et al.* SWITCH1 (SWI1): a novel protein required for the establishment of sister chromatid cohesion and for bivalent formation at meiosis. *Genes Dev.* **15,** 1859–1871 (2001).
5. Agashe, B., Prasad, C. K. & Siddiqi, I. Identification and analysis of *DYAD*: a gene required for meiotic chromosome organisation and female meiotic progression in *Arabidopsis. Development* **129,** 3935–3943 (2002).
6. Mogie, M. A model for the evolution and control of generative apomixis. *Biol. J. Linn. Soc.* **35,** 127–154 (1988).
7. Siddiqi, I., Ganesh, G., Grossniklaus, U. & Subbiah, V. The *dyad* gene is required for progression through female meiosis in *Arabidopsis. Development* **127,** 197–207 (2000).
8. Altmann, T. *et al.* Easy determination of ploidy level in *Arabidopsis thaliana* plants by means of pollen size measurement. *Plant Cell Rep.* **13,** 652–666 (1994).
9. Dilkes, B. P. & Comai, L. A. Differential dosage hypothesis for parental effects in seed development. *Plant Cell* **16,** 3174–3180 (2004).
10. Spielman, M., Vinkenoog, R., Dickinson, H. G. & Scott, R. J. The epigenetic basis of gender in flowering plants and mammals. *Trends Genet.* **17,** 705–711 (2001).
11. Bretagnolle, F. & Thompson, J. D. Gametes with the somatic chromosome number: mechanisms of their formation and role in the evolution of autopolyploid plants. *New Phytol.* **129,** 1–22 (1995).
12. Rhoades, M. M. & Dempsey, E. Induction of chromosome doubling at meiosis by the *elongate* gene in maize. *Genetics* **54,** 505–522 (1966).
13. Finch, R. A. & Bennet, M. D. Action of *triploid inducer* (*tri*) on meiosis in barley (*Hordeum vulgare* L.). *Hereditas* **43,** 87–93 (1979).
14. Curtis, C. A. & Doyle, D. G. Production of aneuploid and diploid eggs by meiotic mutants of maize. *J. Hered.* **83,** 335–341 (1992).
15. Douches, D. S. & Quiros, C. F. Genetic strategies to determine the mode of *2n* egg formation in diploid potatoes. *Euphytica* **38,** 247–260 (1988).
16. Barone, A., Gebhardt, C. & Frusciante, L. Heterozygosity in *2n* gametes of potato evaluated by RFLP markers. *Theor. Appl. Genet.* **91,** 98–104 (1995).
17. Vorsa, L. & Rowland, L. J. Estimation of *2n* megagametophyte heterozygosity in a diploid Blueberry *(Vaccinium darrowi* Camp) clone using RAPDs. *J. Hered.* **8,** 423–426 (1997).
18. Noyes, R. D. Inheritance of apomeiosis (diplospory) in fleabanes (*Erigeron, Asteraceae*). *Heredity* **94,** 193–198 (2005).
19. Savidan, Y. H. Apomixis: genetics and breeding. *Plant Breed. Rev.* **18,** 13–86 (2000).
20. Huanca-Mamani, W., Garcia-Aguilar, M., Leon-Martinez, G., Grossniklaus, U. & Vielle-Calzada, J. P. CHR11, a chromatin-remodeling factor essential for nuclear proliferation during female gametogenesis in *Arabidopsis thaliana. Proc. Natl Acad. Sci. USA* **102,** 17231–17236 (2005).
21. Blakey, C. A., Goldman, S. L. & Dewald, C. L. Apomixis in *Tripsacum*: Comparative mapping of a multigene phenomenon. *Genome* **44,** 222–230 (2001).
22. Hamant, O., Ma, H. & Cande, W. Z. Genetics of meiotic prophase I in plants. *Annu. Rev. Plant Biol.* **57,** 267–302 (2006).
23. Chen, K. Y., Cong, B., Wing, R., Vrebalov, J. & Tanksley, S. D. Changes in regulation of a transcription factor lead to autogamy in cultivated tomatoes. *Science* **318,** 643–645 (2007).
24. Ozias-Akins, P., Roche, D. & Hanna, W. W. Tight clustering and hemizygosity of apomixis-linked molecular markers in *Pennisetum squamulatum* implies genetic control of apospory by a divergent locus that may have no allelic form in sexual genotypes. *Proc. Natl Acad. Sci. USA* **95,** 5127–5132 (1998).
25. Pessino, S. C. *et al.* A genetic map of the apospory-region in *Brachiaria* hybrids: Identification of two markers closely associated with the trait. *Hereditas* **128,** 153–158 (1998).
26. Grimanelli, D. *et al.* Mapping diplosporous apomixis in tetraploid *Tripsacum*: one gene or several genes? *Heredity* **80,** 33–39 (1998).
27. Pupilli, F. *et al.* Comparative mapping reveals partial conservation of synteny at the apomixis locus in *Paspalum spp. Mol. Genet. Genomics* **270,** 539–548 (2004).
28. Guitton, A. E. & Berger, F. Loss of function *MULTICOPY SUPPRESSOR OF IRA1* produces nonviable parthenogenetic embryos in *Arabidopsis. Curr. Biol.* **15,** 750–754 (2005).
29. Sundaresan, V. *et al.* Patterns of gene action in plant development revealed by enhancer trap and gene trap transposable elements. *Genes Dev.* **9,** 1797–1810 (1995).
30. Ross, K. J., Fransz, P. & Jones, G. H. A light microscopic atlas of meiosis in *Arabidopsis thaliana. Chromosome Res.* **4,** 507–516 (1996).

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements We thank V. Vijaybhaskar and V. Subbiah for characterization of the ET60 transposon line; S. Andreuzza, J. Dhawan, S. Mayor, B. Nishal, M. Ramaswami, A. Ray and O. Siddiqi for comments on the manuscript; and the ABRC for seeds. This work was supported by the Council for Scientific and Industrial Research (CSIR; Government of India), and a Centre of Excellence grant from the Department of Biotechnology (to I.S.). M.R. and M.M. were supported by fellowships from the University Grants Commission and the CSIR, respectively.

Author Contributions M.P.M. conducted the experiments on marker gene expression and interpreted the results. M.R. and I.S. planned, and M.R. performed, the remaining experiments and interpreted the results. I.S. wrote the paper with input from M.R.

Author Information Reprints and permissions information is available at www.nature.com/reprints. Correspondence and requests for materials should be addressed to I.S. (imran@ccmb.res.in).

METHODS

Plant growth conditions. Plants were grown in pots under fluorescent lights (7,000 lx at 20 cm) in a plant growth cabinet at 20 °C using a 16-h light/8-h dark cycle. Synthetic medium for growing plants was prepared by mixing equal proportions of peat, perlite and vermiculite (Keltech Energies Ltd.). The medium was drenched with 1 × Murashige–Skoog (MS) solution (4 mM $CaCl_2$, 1.5 mM $MgSO_4$, 18.8 mM KNO_3, 20.6 mM NH_4NO_3, 1.25 mM KH_2PO_4 pH 5.6, 0.05 mM Fe-EDTA, 1 × minor nutrients). Minor nutrients (1,000× stock) consisted of 70 mM H_3BO_3, 14 mM $MnCl_2$, 0.5 mM $CuSO_4$, 1 mM $ZnSO_4$, 0.2 mM $NaMoO_4$, 10 mM NaCl and 0.01 mM $CoCl_2$. The pH of the final MS solution was adjusted to 5.6–5.7. Thereafter the plants were irrigated with distilled water at regular intervals.

Plant DNA isolation. Genomic DNA for genotyping and microsatellite marker analysis was isolated as described[31], with modifications. Leaf tissue or inflorescence (100–500 mg) was collected from single plants in 1.5-ml Eppendorf tubes, snap-frozen in liquid nitrogen, and ground to a fine powder with a micro-pestle. Freshly prepared DNA extraction buffer (200 µl; 100 mM Tris-HCl pH 8.0, 50 mM EDTA pH 8.0, 500 mM NaCl, 10 mM 2-mercaptoethanol, 1.4% SDS) was added and the tissue was finely homogenized. An equal volume of 2 × CTAB buffer (2% cetyl trimethyl ammonium bromide, 100 mM Tris-HCl pH 8.0, 20 mM EDTA pH 8.0, 1.4 M NaCl) was added and the mixture was gently vortex-mixed. The mixture was then incubated at 65 °C for 5 min in a shaking water bath. After incubation, the sample was left to cool and an equal volume of 24:1 chloroform:isoamyl alcohol was added, mixed gently, and centrifuged for 10 min at 15,700*g*. The aqueous phase was transferred to a fresh Eppendorf tube and a two-thirds volume of ice-cold propan-2-ol was added to precipitate the DNA. The DNA was pelleted by centrifugation at 15,700*g* and 4 °C for 20 min. The DNA pellet was washed with 70% ethanol, air-dried for 30 min and dissolved in 50 µl of sterile water or TE buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA) containing DNase-free ribonuclease (20 µg ml^{-1}).

Microsatellite (simple sequence repeat) marker analysis. An F_2 segregating population of *dyad* resulting from a *dyad* (Col-0) × wild type (No-0) cross was used for the simple sequence repeat (SSR) marker analysis. The parental strains were screened for polymorphisms on the basis of known differences between the two ecotypes (www.arabidopsis.org), and a set of five polymorphic SSR markers were selected (Table 2). The markers and primer sequences were as follows: nga 162 (nga162F, 5′-CTCTGTCACTCTTTTCCTCTGG-3′; nga162R, 5′-CATGCAATTTGCATCTGAGG-3′), nga225 (nga225F, 5′-TCTCCCCACTAGTTTTGTGTCC-3′; nga225R, 5′-GAAATCCAAATCCCAGAGAGG-3′), nga168 (nga168F, 5′-GAGGACATGTATAGGAGCCTCG-3′; nga168R, 5′-TCGTCTACTGCACTGCCG-3′), nga1107 (nga1107F, 5′-CGACGAATCGACAGAATTAGG-3′; nga1107R: 5′-GCGAAAAAACAAAAAAATCCA-3′) and nga6 (nga6F, 5′-ATGGAGAAGCTTACACTGATC-3′; nga6R, 5′-TGGATTTCTTCCTCTCTTCAC-3′). PCR was performed in PCR buffer (Bioron/Perkin Elmer) containing 2 mM $MgCl_2$, dNTPs (each at 0.2 mM), 1 U of *Taq* DNA polymerase and 5 pmol each of forward and reverse primers. An initial denaturation at 94 °C for 2 min was used followed by 35 cycles of denaturation at 94 °C for 10 s, annealing at 55 °C for 10 s, and extension at 72 °C for 20 s. The PCR products were resolved on an 8% polyacrylamide gel at 150 V for 3 h with 1 × Tris/borate/EDTA buffer, stained with ethidium bromide and captured with a Syngene gel documentation system (Synoptics Inc.).

Chromosome spreads and ploidy analysis. Meiotic chromosome spreads of male and female meiocytes were performed as described[30], with minor modifications. The enzyme digestion mixture contained 0.3% cellulase and 0.3% pectinase (or pectolyase) prepared from 3% stock solutions made in 10 mM citrate pH 4.5, 45% glycerol, and stored at −20 °C. Spreads were stained with 4,6-diamidino-2-phenylindole (1 µg ml^{-1}) and observed on a Zeiss Axioplan 2 microscope using a 365-nm excitation, 420-nm long-pass emission filter and a 100× oil-immersion objective. The images were captured with an Axiocam HRC charge-coupled device camera with the aid of Axiovision software. The captured images were edited with Adobe Photoshop (version 6.0). The ploidy of the *dyad* progeny was determined by chromosome counts on multiple nuclei either from the male meiocytes or from somatic cells of the anther tissue. Well-spread meiotic stages at anaphase I, dyad stage, metaphase II and anaphase II were counted for the determination of chromosome number. Estimation of unreduced pollen was based on the measurement of pollen diameter after Alexander staining with pollen from a tetraploid plant as a control. Seed and flower size differences were examined with a Leica MZ16FA stereomicroscope and the images were captured with a Leica IC3D digital camera system. Images were edited with Adobe Photoshop.

Alexander staining. Anthers that were about to dehisce were dissected in a drop of Alexander staining solution[32] (10 ml of 95% ethanol, 10 mg Malachite green (1 ml of 1% solution in 95% alcohol), 50 ml of distilled water, 25 ml of glycerol, 5 g of phenol, 5 g of chloral hydrate 50 mg of acid fuchsin (5 ml of 1% solution in water), 5 mg of Orange G (0.5 ml of 1% solution in water) and 2 ml of acetic acid; the pH of the staining solution was adjusted to 2.3). The anthers were then transferred to a fresh slide containing a drop of Alexander stain. A coverslip was placed on the anthers and gently pressed so that the staining solution penetrated the anthers. The slides were then incubated at 37 °C for 6 h. Images were observed under bright field (10× objective) and captured with a Zeiss Axioplan 2 microscope.

Three-way cross analysis. The homozygosity of the transposon insertion in the marker line ET60 was verified with primer combinations specific for the wild-type allele (ET60F, 5′-CGTTGGTCTTTTATTGACTTCTAGCTACGAC-3′; ET60R, 5′-ACATCCTCGAATTCAAAACTACGAG-3′) and for the insertion allele (Ds5-2 (5′-CGTTCCGTTTTCGTTTTTTACC-3′) and ET60R). The segregation for kanamycin resistance and susceptibility in the three-way cross progenies ((*dyad*♀ × ET60♂)♀ × wild-type♂) was determined 15 days after germination on kanamycin selection medium (MS medium plus 50 µg ml^{-1} kanamycin and 2% sucrose). For the duplex control cross, the tetraploid line CS3900 containing a kanamycin resistance gene in quadruplex condition (KKKK) was crossed to wild-type diploid Col-0. The resulting triploid plants (KK^-) were then backcrossed as female to wild-type diploid Col-0 as a male parent. The seeds were germinated on kanamycin selection medium to determine the segregation ratio for kanamycin resistance.

Generation of maternal and paternal excess triploid seeds. To make maternal excess triploid seeds, the tetraploid line CS3900 was crossed as a female parent to wild-type diploid Col-0 as a male parent. A reciprocal cross between the same parents gave rise to paternal excess triploid seeds.

Construction of a *proFM1::uidA* fusion. The promoter region of At4g12250 as described in a previous study[20] was isolated by PCR amplification with the Eppendorf TripleMaster PCR system with primers EpiF (5′-GTA**AAGCTT**-GCTAACATACTAGCATGTATCCAC-3′) and EpiR (5′-AATA**GGATCC**-GGTGGAACTTTATCGGTTT-3′), which carry restriction sites (in bold) for *Hin*dIII and *Bam*HI, respectively. The amplified fragment was initially cloned into the pGEM-T vector (Promega) in accordance with the manufacturer's instructions and subsequently into the pBI101.2 binary vector followed by transformation into heterozygous *dyad* plants by vacuum infiltration.

Plant transformation. *Agrobacterium*-mediated transformation *in planta* was performed as described[33], with minor modifications. *Arabidopsis* plants were grown in pots covered with a nylon mesh. After the plants had flowered, the pots were inverted into a suspension of *Agrobacterium* in the infiltration medium (0.5 × MS solution containing 2% sucrose, 0.05 µm benzylaminopurine and 0.2% Silwet). The entire setup was then transferred to a closed cabinet connected to a vacuum unit and subjected to 500–600-mm Hg vacuum for 15–20 min. Excess infiltration medium was drained off, and the pots were placed horizontally in a plastic tray and covered with Saran wrap to maintain humidity. The tray was transferred to the growth chamber and left for one or two days until the plants recovered. After recovery, the plants were kept upright and grown to maturity.

GUS assays. Wild-type and *dyad* T_1 transformants were identified on the basis of silique elongation and further confirmed by the ovule phenotype. Pistils from both wild-type and *dyad* plants at different stages were dissected to expose the ovules and incubated in GUS staining solution (10 mM EDTA, 0.1% Triton X-100, 2 mM potassium ferricyanide, 2 mM potassium ferrocyanide, 100 µg ml^{-1} chloramphenicol, 1 mg ml^{-1} 5-bromo-4-chloro-3-indolyl-β-D-glucuronic acid cyclohexylammonium salt (Biosynth), 50 mM sodium phosphate buffer pH 7.0) for up to two days at 37 °C. The stained tissues were mounted on slides with coverslips in clearing solution (20% glycerol, 20% lactic acid) and observed on a Zeiss Axioplan 2 microscope under differential interference contrast optics with 40× and 100× oil-immersion objectives.

31. Dellaporta, S. L., Wood, J. & Hicks, J. B. A plant DNA minpreparation: version II. *Plant Mol. Biol. Rep.* **1**, 19–21 (1983).
32. Alexander, M. P. Differential staining of aborted and nonaborted pollen. *Stain Technol.* **44**, 117–122 (1969).
33. Bechtold, N., Ellis, J. & Pelletier, G. *In planta Agrobacterium*-mediated gene transfer by infiltration of adult *Arabidopsis thaliana* plants. *C. R. Acad. Sci. Life Sci.* **316**, 1194–1199 (1993).