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The *Arabidopsis* SWR1 Chromatin-Remodeling Complex Is Important for DNA Repair, Somatic Recombination, and Meiosis[W][OPEN]

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All processes requiring interaction with DNA are attuned to occur within the context of the complex chromatin structure. As it does for programmed transcription and replication, this also holds true for unscheduled events, such as repair of DNA damage. Lesions such as double-strand breaks occur randomly; their repair requires that enzyme complexes access DNA at potentially any genomic site. This is achieved by chromatin remodeling factors that can locally slide, evict, or change nucleosomes. Here, we show that the Swi2/Snf2-related (SWR1 complex), known to deposit histone H2A.Z, is also important for DNA repair in *Arabidopsis thaliana*. Mutations in genes for *Arabidopsis* SWR1 complex subunits PHOTOPERIOD-INDEPENDENT EARLY FLOWERING1, ACTIN-RELATED PROTEIN6, and SWR1 COMPLEX6 cause hypersensitivity to various DNA damaging agents. Even without additional genotoxic stress, these mutants show symptoms of DNA damage accumulation. The reduced DNA repair capacity is connected with impaired somatic homologous recombination, in contrast with the hyper-recombinogenic phenotype of yeast SWR1 mutants. This suggests functional diversification between lower and higher eukaryotes. Finally, reduced fertility and irregular gametogenesis in the *Arabidopsis* SWR1 mutants indicate an additional role for the chromatin-remodeling complex during meiosis. These results provide evidence for the importance of *Arabidopsis* SWR1 in somatic DNA repair and during meiosis.

INTRODUCTION

Double-strand breaks (DSBs) are a particularly deleterious type of DNA damage, and their quick and efficient removal is of the utmost importance, as a single unrepaired DSB can be lethal to cells (Bennett et al., 1993). DSB repair is accomplished by two main pathways: nonhomologous end joining (NHEJ) and homologous recombination (HR). NHEJ is achieved by stabilization and religation of broken DNA ends (reviewed in Lieber, 2010), often with a loss or mutation of bases. HR is a more complex and more conservative mechanism in which intact homologous regions are used as a template for repair (reviewed in Heyer et al., 2010). Apart from its role in somatic DSB removal, HR is also fundamental during meiosis for the establishment of reciprocal crossovers between homologous chromosomes (reviewed in Osman et al., 2011). The molecular mechanisms that control DSB signaling and repair by both pathways have been characterized and reviewed extensively (Schuermann et al., 2005; Heyer et al., 2010; Knoll and Puchta, 2011; Waterworth et al., 2011). Nonetheless, surprisingly little is known about how DSB repair is regulated in the context of chromatin, an important aspect since DNA lesions occur within the context of the complex higher order structure of chromatin.

The basic unit of chromatin organization is the nucleosome, which is composed of 146 bp of DNA wrapped around a core histone octamer of two H2A/H2B heterodimers and one H3/H4 heterotetramer. Nucleosome compaction hampers DNA accessibility during various nuclear processes, including DNA repair (Groth et al., 2007). Hence, remodeling activities that change chromatin structure and increase the accessibility of DNA to repair factors are essential for efficient removal of lesions. Various members of the SWItch/Sucrose NonFermentable (SWI2/SNF2) superfamily of chromatin remodelers have been implicated in DSB repair pathways (Bao and Shen, 2007; Hopfner et al., 2012). Members of this family can catalyze the repositioning, eviction, or change in composition of nucleosomes and can change chromatin configuration at DSB sites. In *Arabidopsis thaliana*, mutations in several of the 41 identified SWI2/SNF2 proteins (Knizewski et al., 2008; Clapier and Cairns, 2009) lead to hypersensitivity after DNA damage induction. Furthermore, RADiation sensitive54 (RAD54) and INOsitol auxotrophy 80 (INO80), SWI2/SNF2s with nucleosome repositioning activity, are positive regulators of somatic HR (SHR; Fritsch et al., 2004; Shaked et al., 2006). Nevertheless, other important SWI2/SNFs components have not been analyzed for a potential role in DNA repair.

Based on its functional characterization in yeast and the identification of conserved homologs in *Arabidopsis*, we chose to study the SWR1 complex as a member of the INO80 subfamily of SWI2/SNF2 chromatin remodelers. In yeast, this complex catalyzes the unilateral substitution of H2A/H2B dimers for those containing the H2A.Z histone variant (Kobor et al., 2004; Mizuguchi et al., 2004; Luk et al., 2010). H2A.Z is found at the transcriptional start sites of many genes and is globally required for proper gene expression and stability of chromatin (Lu et al.,

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2009). SWR1 is recruited to DSB sites, where it transiently deposits H2A.Z; however, the role of this process in the context of repair is not well understood. Several reports highlighted the involvement of H2A.Z and SWR-C in NHEJ and in HR in yeast. H2A.Z is recruited early and transiently to DSBs, where it is required for single-stranded DNA formation, probably affecting DNA resection (Kalocsay et al., 2009; Lukas et al., 2011). Recently, works in mammals showed that H2A.Z and p400, the mammalian counterpart of the SWR-C ATPase, are required to create an open chromatin configuration around DSBs and to recruit proteins like RAD51 and BRCA1 involved in HR (Courilleau et al., 2012; Xu et al., 2012). Sequence conservation and experimental evidence suggest that the SWR1 complex exists also in plants (March-Díaz and Reyes, 2009). Three putative *Arabidopsis* SWR1 (At-SWR1) subunits have been identified and studied: PHOTOPERIOD-INDEPENDENT EARLY FLOWERING1 (PIE1), ACTIN-RELATED PROTEIN6 (ARP6), and SWR1 COMPLEX6 (SWC6). Mutations in any of the corresponding genes cause similar morphological and developmental phenotypes, including early flowering, global reduction in size, curly leaves, and reduced fertility (Choi et al., 2005, 2007; Deal et al., 2005, 2007; Lázaro et al., 2008; March-Díaz and Reyes, 2009). Both PIE1 and ARP6 are required for the incorporation of histone variant H2A.Z throughout the genome (Deal et al., 2007), and their loss alters the transcript state of many genes (March-Díaz et al., 2008; Kumar and Wigge, 2010). Remarkably, *pie1* mutants have more severe phenotypes than those of *arp6* or *swc6*, which corresponds to its suggested role as catalytic subunit, as reported for the homolog SWR1 in the yeast complex (Mizuguchi et al., 2004). Beyond an unspecified note about irradiation sensitivity in a *PIE1* knockdown line (Shaked et al., 2006), the connection between the plant SWR1 complex and DNA repair has remained unclear.

In this study, we show that *pie1-3*, *arp6-3*, and *swc6-1* are hypersensitive to DNA damage-inducing agents and display signs of accumulated DNA damage. Double mutant analysis reveals that SWR1 is involved mainly in HR repair pathways. SHR frequencies are decreased strongly in *arp6-3* and *swc6-1* plants with or without external stimulus by DNA damaging agents. Reduced fertility and formation of aberrant meiotic products in At-SWR1 mutants suggest an additional role of At-SWR1 during meiosis. Therefore, in addition to transcriptional regulation via H2A.Z deposition, At-SWR1 is also important for survival and reproduction through its role in somatic DNA repair and regular meiotic progression.

RESULTS

Arabidopsis SWR1 Is Important for DNA Damage Resistance

To investigate a potential role for At-SWR1 in DNA repair, we performed DNA damage sensitivity assays with mutants lacking different subunits of the complex. We chose the null alleles *pie1-3*, *arp6-3*, and *swc6-1*, which displayed the expected morphological At-SWR1–related phenotypes (Choi et al., 2005, 2007; Deal et al., 2005; Lázaro et al., 2008), including early flowering (see Supplemental Figure 1 online). Populations of 4-d-old seedlings of the wild type and mutants were challenged with γ-irradiation or the radiomimetic drug bleocin (BLEO). Both treatments cause DNA lesions, including damaged nucleotides and single- and double-strand breaks (Plumb et al., 1999; Kandasamy et al., 2009). Accumulation of DNA damage causes arrest of cell division in the apical meristem of young seedlings, which inhibits the development of the first leaves (true leaf assay; Figure 1A). While 10-d-old nontreated (mock) wild-type and mutant seedlings developed one pair of true leaves (see Supplemental Figures 2A and 2B online), treated plants showed no or reduced leaf development. This effect was much more pronounced in *pie1-3*, *arp6-3*, *swc6-1*, and *ku70-2* (a mutant with known sensitivity) than in the wild type (Figures 1B and 1C). The hypersensitivity was still evident in 21-d-old plants, with a clear reduction in size, necrosis, or death of seedlings (see Supplemental Figures 2A and 2B online). Additional true leaf assays were performed using the cross-linking agent mitomycin C (MMC) (Figure 1D) and the DNA synthesis inhibitor hydroxyurea (HU) (Figure 1E). All At-SWR1 mutants showed increased sensitivity to DNA damage compared with the wild type in both treatments, although with some variability between mutants. Finally, we also tested the response to UV-C exposure and observed reduced resistance in *pie1*, *arp6*, and *swc6*, which was evident by high numbers of seedlings with reduced size, chlorotic cotyledons, and death 8 d after treatment (Figure 1F). Consistent with its anticipated catalytic role in the complex, *pie1-3* was the most sensitive mutant in most assays (Figures 1B to 1F).

In summary, all three At-SWR1 mutants are sensitive to a broad spectrum of DNA damaging agents, a first indication of the role of the At-SWR1 complex in DNA repair.

Lack of At-SWR1 Induces Symptoms of DNA Damage Accumulation

To confirm the DNA repair defects in At-SWR1 mutants, we screened for symptoms of accumulated unrepaired DNA lesions. We determined the transcript levels of *RAD51*, *BRCA1*, and *PARP2*, genes that are normally upregulated in the case of DNA damage (Culligan et al., 2006). The expression levels of all three genes were elevated consistently in *pie1*, *arp6*, and *swc6* in comparison to wild-type plants (Figure 2A), again most significantly upon loss of the catalytic subunit in *pie1*, even without DNA damaging treatment. BLEO application can trigger transcription of repair-related genes to the same relative values as in the wild type (see Supplemental Figure 3A online). The At-SWR1 genes themselves are not induced by DNA damaging treatment, and the genes encoding the histone variant H2A.Z are also either unchanged (*HTA9*) or rather reduced due to their cell cycle dependence (Menges et al., 2002) (see Supplemental Figure 3B online).

Another common response to DNA damage is an increase in endopolyploidy. The shift from the regular mitotic cell cycle to the endocycle allows cells to overcome DNA damage checkpoints and compensates for low division rates in cells that have high levels of unrepaired DNA lesions (Adachi et al., 2011). Endopolyploidy levels in the At-SWR1 mutants were determined by flow cytometry using the second pair of true leaves of 28-d-old plants. Nuclei were separated according to DNA content, into populations of 2C (in G0/G1 phase), 4C (having passed one

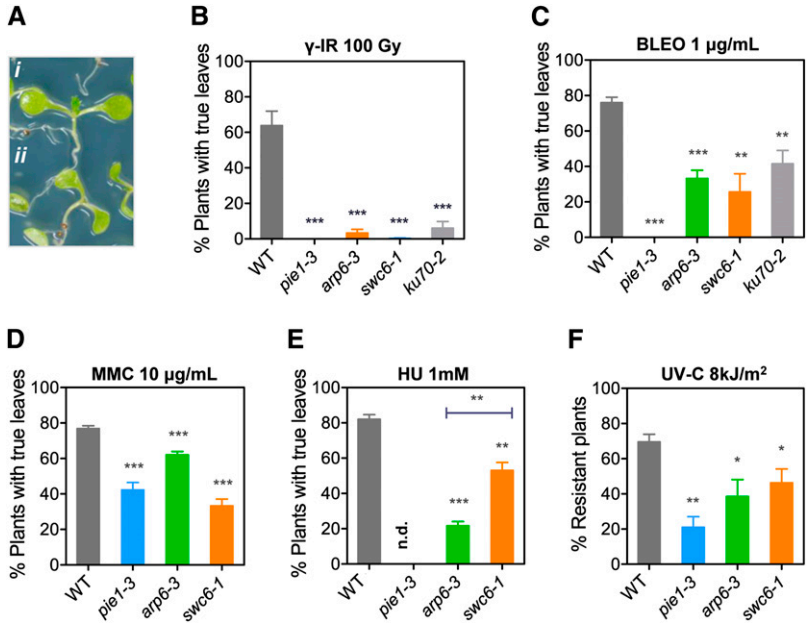

Figure 1. At-SWR1 Mutants Are Hypersensitive to DNA-Damaging Agents.

(A) Phenotypes of 10-d-old seedlings with (i) and without (ii) true leaves.
(B) to **(F)** True leaf assay with seedlings treated with γ-irradiation (γ-IR) **(B)**, BLEO **(C)**, MMC **(D)**, HU **(E)**, or UV light (UV-C) **(F)** at the dosage indicated. The percentage of 10-d-old treated plants with true leaves was calculated in relation to mock populations. The DNA damage-sensitive mutant *ku70-2* was used as a control in **(B)** and **(D)**. Error bars indicate the SE between three or more biological replicates with 50 to 80 seedlings each. Asterisks indicate significant differences according to P values from unpaired *t* tests: ***P < 0.001, **0.001 < P < 0.01, and *0.01 < P < 0.05. n.d., not determined; WT, the wild type.

S phase), 8C, 16C, and 32C (one, two, or three rounds of endoreplication). Less than 10% of wild-type nuclei have C values of more than 4, whereas the fraction of these nuclei is increased in all At-SWR1 mutants. The most pronounced shift in endopolyploidy occurred in *pie1-3*, with 8C as the most prevalent C value (Figure 2B). These increased values are in the same range as those measured after strong DSB induction in the wild type (Adachi et al., 2011) and indicate accumulation of unrepaired lesions.

In summary, increased transcript levels of repair-responsive genes and higher endopolyploidy in At-SWR1 mutants indicate a constitutively higher level of DNA damage even without additional treatment and support a possible role for At-SWR1 in DNA repair.

Repair Defects of At-SWR1 Mutants Are Aggravated by Impaired NHEJ

DSB repair can be performed via NHEJ or HR. In order to determine to which pathway At-SWR1 contributes, we combined *arp6-3* and *swc6-1* with either *brca1-1* or *ku70-2*, two mutants affected in SHR or NHEJ, respectively. *pie1-3* was not included in this set of experiments due to its extremely low fertility (see below), which impaired the generation of double mutants. However, double mutants homozygous for either *brca1* or *ku70* and *arp6* or *swc6* were viable and showed *arp6* or *swc6*-like phenotypes, with no additional developmental defects under normal growth conditions. The response of the double mutants to BLEO-induced DNA damage was analyzed with the true leaf assay described above, this time with a reduced concentration of the drug to allow detection of more subtle differences in the response. Increased sensitivity compared with the wild type was observed in all four single mutants, although *brca1-1* was less affected than *ku70-2*, *arp6-3*, and *swc6-1* (Figure 3; see Supplemental Figures 3B and 4 online). The combination of *ku70-2* with At-SWR1 mutations resulted in a drastic increase in sensitivity compared with single mutants, with nearly complete inhibition of true leaf formation within 10 d (Figure 3; see Supplemental Figure 4 online). The response was stronger than the additive effect of both mutations, indicating impairment in different pathways. By contrast, the combination of At-SWR1 mutants with *brca1-1* did not increase the sensitivity beyond the degree of single mutants, suggesting that the mutations are epistatic. These results suggest that At-SWR1 acts independently of NHEJ repair but is connected to, or involved in, the HR pathway.

At-SWR1 Is Involved in SHR

To analyze the role of At-SWR1 in HR more directly, we measured the frequency of SHR events using two different transgenic *Arabidopsis* reporter lines with recombination substrates, DGU.US-1 and IU.GUS-8 (Figure 4A) (Puchta, 1998; Siebert and Puchta, 2002). These lines were designed to distinguish single-strand annealing and synthesis-dependent strand annealing

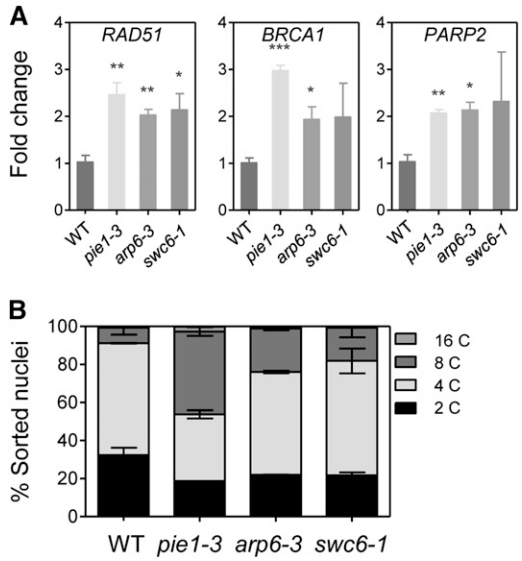

Figure 2. At-SWR1 Mutants Show Symptoms of DNA Damage Accumulation.

(A) Transcript levels of DNA damage–induced genes *RAD51*, *BRCA1*, and *PARP2* in At-SWR1 mutants, without DNA damaging treatment. Values are presented relative to untreated wild-type (WT) Columbia (set as 1). Transcript levels were measured by quantitative RT-PCR and normalized to *UBC28*. The values represent the average of two or more biological replicates. Error bars correspond to the standard deviation of the mean (SD). Asterisks indicate significant differences according to P values from unpaired *t* tests: ***P < 0.001, **0.001 < P < 0.01, and *0.01 < P < 0.05.

(B) Distribution of DNA content in nuclei from the second pair of true leaves of 30-d-old wild-type and At-SWR1 mutant plants determined by flow cytometry. Error bars correspond to the SD in two replicates.

upon DSB repair after induced expression of the endonuclease *Sce*I (Orel et al., 2003). However, they are used here to measure HR without *Sce*I but after different exogenous DNA damage treatments. Both recombination events can be visualized and the frequency of SHR estimated due to restoration of a disrupted β-glucuronidase (GUS) gene and histochemical detection (Orel et al., 2003; Mannuss et al., 2010). We introgressed the reporter constructs into the background of *arp6-3* and *swc6-1* (for the reason noted before, *pie1-3* was omitted) and determined the SHR frequency in plants homozygous for the mutation and for the reporter substrate, either without treatment or following BLEO, MMC, or UV-C treatment.

Untreated wild-type populations have, on average, 2.5 and 0.4 spots/plant in lines DGU.US-1 and IU.GUS-8, respectively, and the numbers of blue sectors per plant increased significantly after induction with DNA damaging agents (Figures 4B to 4D). By contrast, most *arp6-3* and *swc6-1* plants did not show a single recombination event in mock controls, and the average numbers increased only slightly after induction (Figures 4E and 4F). For the DGU.US-1 line, this increase was statistically significant for both mutants, except for UV-C (Figure 4E). For the other line, only *swc6-1* treated with MMC showed a significant increase (Figure 4F). The total number of SHR events in At-SWR1 mutants is reduced with high significance compared with the wild type for both substrates. The degree of SHR reduction in At-SWR1 mutants compared with the wild type was different and ranged from 6- to 220-fold, depending on the line and treatment, and again was more pronounced in the DGU.US-1 line.

Taken together, analysis of SHR frequencies with the reporter lines suggests an important role for At-SWR1 in the repair of DNA damage through SHR.

Reduced Fertility of At-SWR1 Mutants Is Caused by Meiotic Defects

The evidence of a role for At-SWR1 in SHR prompted us to investigate its involvement during meiosis, where HR between homologous chromosomes is fundamental for correct segregation. Smaller flowers, altered petal number, short anthers, shortened and thickened gynoecia and siliques, aborted ovules, and a reduced number of seeds per silique were described previously for mutants in At-SWR1 subunits (March-Díaz and Reyes, 2009). These strong indications for reduced fertility led us to investigate reproduction in At-SWR1 mutants in more detail. In agreement with previous reports, we found a large number of siliques in mutant plants to be short and misshaped (Figure 5A). Seed set in self-pollinated mutant flowers was reduced to ~50% of the wild-type seed set in *arp6-3* and *swc6-1* and more severely (only 15%) in *pie1-3* (Figure 5B). To examine possible female- or male-specific defects, we performed reciprocal crosses between mutants and the wild type, with the result being equally reduced seed numbers, independent of the orientation (Figure 5B).

Furthermore, we examined male and female gametophyte development in more detail. Mutant anthers were reduced

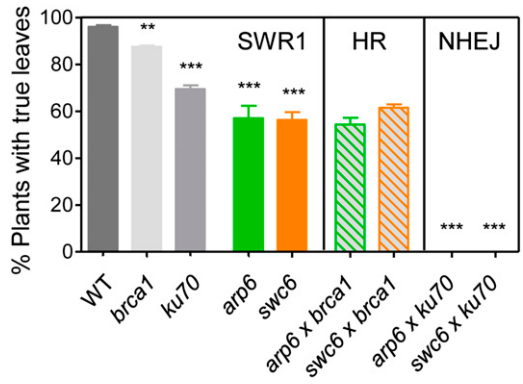

Figure 3. Double Mutant Analysis Indicates a Role for At-SWR1 in HR.

True leaf assay with seedlings of the wild type (WT), *brca1-1* (impaired in HR), *ku70-2* (impaired in NHEJ), *arp6-3* and *swc6-1* (At-SWR1 mutants), and double mutants *arp6 brca*, *arp6 ku70*, *swc6 brca*, and *swc6 ku70* treated with 0.5 μg/mL BLEO. The percentage of 10-d-old treated plants with true leaves was calculated in relation to mock populations. Error bars indicate the SE between two or more biological replicates with 50 to 80 seedlings each. Asterisks in single mutants indicate significant differences from the wild type. Asterisks in double mutants indicate significant differences from either the *arp6-3* or the *swc6-1* single mutant. P values were determined in unpaired *t* tests: ***P < 0.001 and **0.001 < P < 0.01.

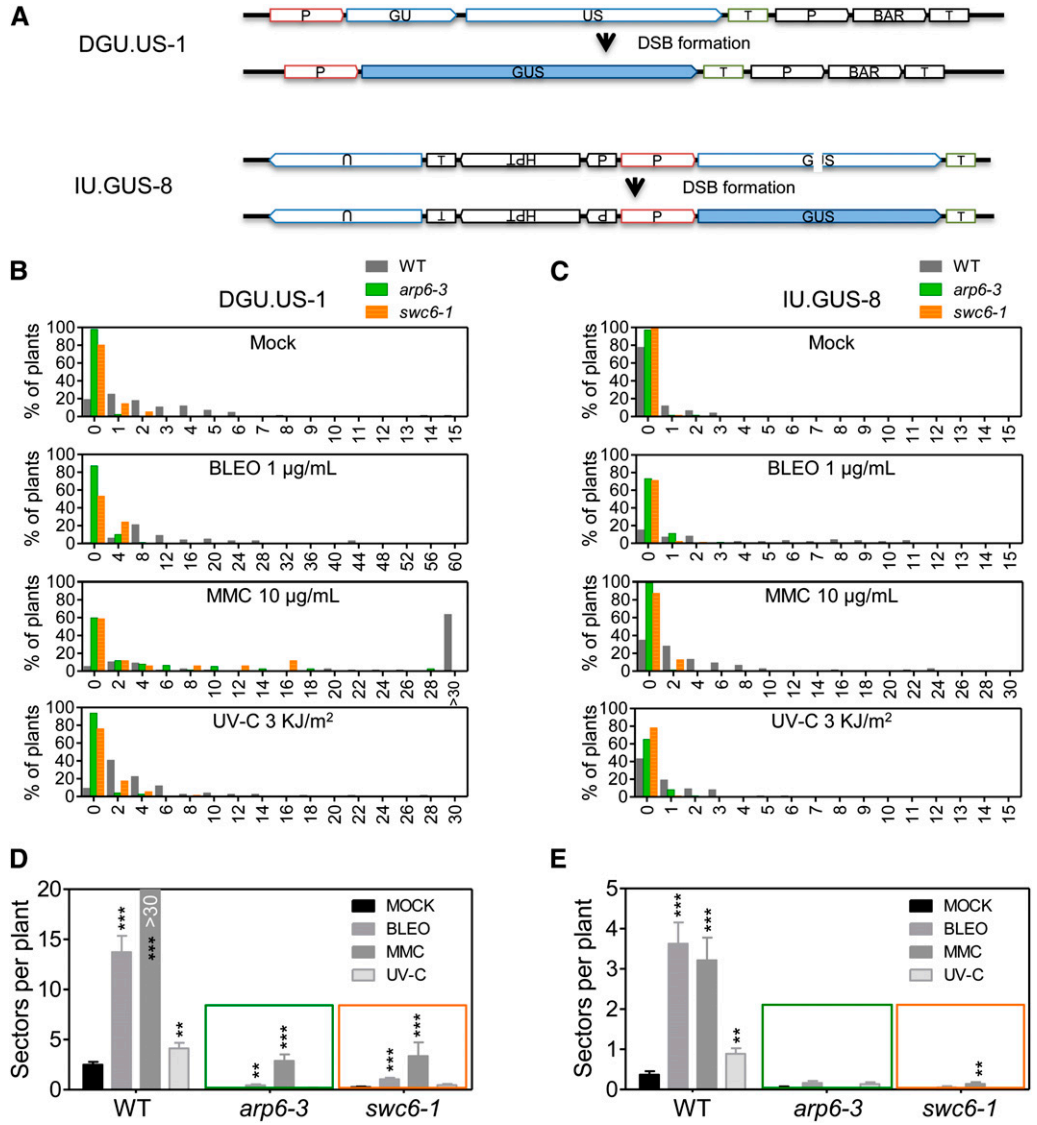

Figure 4. The Frequency of SHR Is Strongly Reduced in the At-SWR1 Mutant Background.

(A) Recombination substrates in SHR reporter lines DGU.US-1 and IU.GUS-8 (Orel et al., 2003). P, 35S promoter from the *Cauliflower mosaic virus*; BAR and HPT, selection markers phosphinothricin acetyltransferase and hygromycin phosphotransferase; T, 35S terminator.
(B) to **(E)** Distribution of seedlings with different numbers of blue spots (**[B]** and **[C]**) and total frequency (**[D]** and **[E]**) in line DGU.US-1 and line IU.GUS-8. Error bars correspond to the SE. Each mutant population was significantly different to its wild-type (WT) counterpart, with a P value < 0.001. Asterisks indicate the significance between treated and mock populations according to P values from unpaired *t* tests: ***P < 0.001 and **0.001 < P < 0.01.

substantially in size compared with the wild type and had the heart-shaped characteristic of immature anthers. Although most mutant anthers contained some viable pollen, as assayed by staining according to Alexander (1969), the number of pollen grains was reduced greatly compared with the wild type, with *pie1-3* being most affected (Figure 5C). Male gametogenesis is thus clearly affected in At-SWR1 mutants.

Examination of embryo sac development revealed that female gametogenesis is also disturbed in these mutants. This was visible by comparing progression of nuclear organization in the developing embryo sac. Upon analysis of 80 to 100 ovules in developmental stages 3-I to 3-VI of megagametogenesis (Schneitz et al., 1995), 33% of *pie1-3* and ~10% of *arp6-3* and *swc6-1* megaspores had clear defects, including the presence of aborted structures and, in the most severe cases, empty embryo sacs (Figure 5D). Thus, mutations in At-SWR1 subunits cause impaired male and female gametophyte development, explaining the reduced fertility.

Defects in gametogenesis are frequently observed in mutants impaired in meiosis (Li et al., 2004; Siaud et al., 2004; Samach et al., 2011). Therefore, we compared different stages of meiotic progression after 4′,6-diamidino-2-phenylindole (DAPI) staining

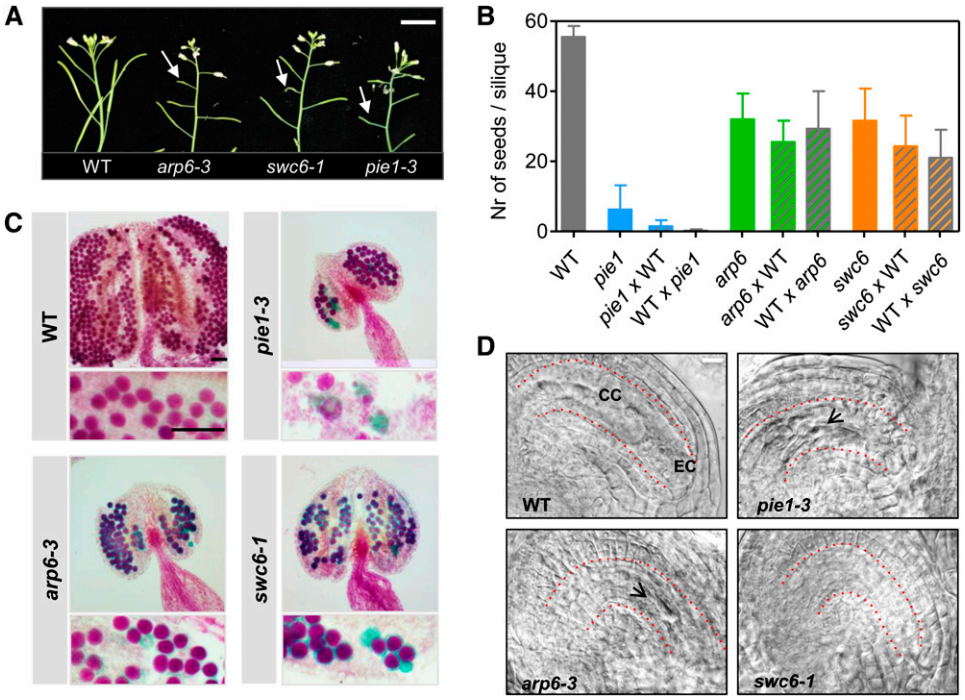

Figure 5. Reduced Fertility of At-SWR1 Mutants Is Due to Defects in Both Male and Female Gametogenesis.

(A) Reduced elongation of siliques in At-SWR1 mutants (arrows). WT, the wild type. Bar = 1 cm.
(B) Average number of seeds per silique in selfed *pie1-3*, *arp6-3*, and *swc6-1* or upon reciprocal crosses with wild-type plants.
(C) Assay for pollen viability by Alexander staining of wild-type and mutant anthers. Bar = 100 μm.
(D) Cytological analysis of megagametogenesis in wild-type and mutant ovules. The central cell (CC) and the egg cell (EC) are present only in wild-type ovules; mutant ovules have aborted structures (arrows) or are empty. Bar = 10 μm.

of wild-type and *pie1-3* pollen mother cells (Figures 6A and 6B). Early stages were indistinguishable between the wild type and mutants but, after the anaphase II stage, *pie1-3* meiocytes frequently displayed abnormalities with single chromatids not being incorporated into the newly forming nuclei of developing microspores (Figures 6C and 6D). All aberrant phenotypes present in *pie1-3* were also observed in *arp6-3* and *swc6-1* pollen mother cells (examples in Figure 6C), although less frequently. The ratio of aberrant meiotic products was high in the mutant background, with 40% in *arp6-3* and *swc6-1*, and 60% in *pie1-3* (Figure 6D). To further investigate the observed meiotic defects, we performed immunolocalization experiments to examine the loading of ASY1 and RAD51, proteins that are involved in axis formation and synapsis of homologous chromosomes and HR, respectively (Armstrong et al., 2002; Li et al., 2004). Both proteins were recruited to chromosomes in *arp6-3* and *swc6-1* male meiocytes to the same extent as in the wild type (see Supplemental Figure 5 online). Indeed, the distribution of ASY1 showed the typical filamentous appearance characteristic of regular axis formation (Armstrong et al., 2002), while the strand exchange protein RAD51 formed numerous foci, indicating a regular number of resected single-stranded DNA ends after DSBs (Mercier et al., 2003). Taken together, this implies that meiosis progresses normally in the absence of At-SWR1 components at least until anaphase II, but regular microspore formation is often compromised.

DISCUSSION

In contrast with the high frequency of extrachromosomal HR between "naked" plasmid molecules (Baur et al., 1990; Puchta and Hohn, 1991), intrachromosomal events observed with recombination reporters integrated into the genome are more rare. The major cause underlying this difference is thought to be the barrier posed by chromatin. Therefore, changes in chromatin structure that increase DNA accessibility are fundamental for correct DNA repair, as they are for transcription and replication. Various chromatin-remodeling activities have been connected with DNA repair, although available reports to date focus on yeast and mammalian components (Lans et al., 2012). Only few components have been implicated in DNA repair-related processes in *Arabidopsis* (Fritsch et al., 2004; Endo et al., 2006; Shaked et al., 2006; Kandasamy et al., 2009; Gao et al., 2012). Our data show that the *Arabidopsis* SWI2/SNF2 family complex SWR1, with a known function in transcriptional regulation and histone variant deposition, has an additional role in facilitating DNA repair. This is evident from genetic, molecular, developmental, and cytological data documenting increased sensitivity to DNA damage, apparent DNA damage symptoms, decreased SHR, and meiotic defects in mutants lacking one of three subunits of the complex. Quantitative differences between the mutants correlate well with the severity of previously reported morphological and molecular phenotypes (Noh and Amasino,

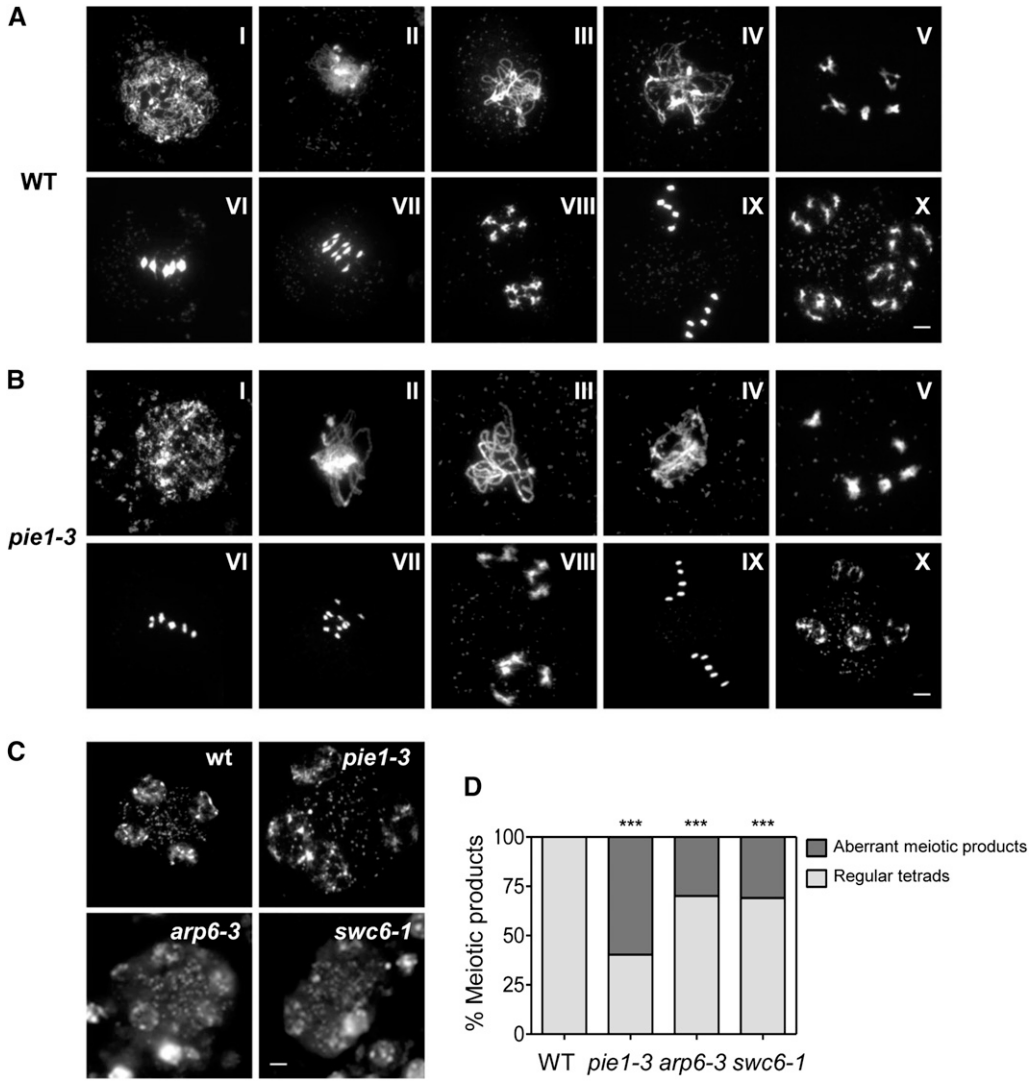

Figure 6. Meiosis Is Perturbed in At-SWR1 Mutants.

(A) and **(B)** Analysis of meiotic progression in DAPI-stained pollen mother cells of the wild type (WT) and *pie1-3*.

(A) Wild-type cells in leptotene (I), zygotene (II), pachytene (III), diplotene (VI), diakinesis (V), metaphase I (VI), anaphase I (VII), interkinesis (VIII), metaphase II (IX), and tetrad stage (X).

(B) *pie1-3* cells in leptotene (I), zygotene (II), pachytene (III), diplotene (VI), diakinesis (V), metaphase I (VI), anaphase I (VII), interkinesis (VIII), metaphase II (IX), and tetrad stage. A regular tetrad is shown (X).

(C) Representative pictures of aberrant meiotic products observed in addition to regular tetrads, in *pie1-3*, *arp6-3*, and *swc6-1* mutant plants. Please note DAPI-stained bodies outside newly forming microspore nuclei.

(D) Quantification of tetrads and aberrant meiotic products in the wild type, *pie1-3*, *arp6-3*, and *swc6-1* in more than 80 samples each.

2003; Choi et al., 2007; Deal et al., 2007), with *pie1* mutants being most affected. PIE1 is the homolog of SWR1, identified to be the catalytic ATPase and giving the name to the whole complex. Therefore, PIE1 is most likely also the central component of the corresponding complex in *Arabidopsis*.

The DNA damaging treatments applied in our experiments induce DNA lesions in different ways: γ-Irradiation and the radiomimetic BLEO induce mainly strand breaks, MMC causes interstrand cross-links, HU inhibits DNA synthesis, and UV-C produces cyclobutane pyrimidine dimers and 6-4 photoproducts (Tuteja et al., 2009). Besides quantitative differences, all three mutants are similarly sensitive to all treatments, suggesting that At-SWR1 is required globally for response to DNA damage induction independent of the type of lesion. Alternatively, the sensitivity phenotypes could originate from secondary DSB induction occurring in all treatments.

Higher endopolyploidy and increased transcript levels of known repair genes in At-SWR1 mutants even without genotoxic treatments are signs of elevated levels of unrepaired DNA damage and indicate that the complex also plays a role in repair

of spontaneous damage and genome stability. The increased sensitivity of At-SWR1 mutants upon combination with NHEJ-defective *ku70*, in contrast with the epistatic interaction with the HR-defective *brca1*, suggests that At-SWR1 functions in conjunction with the HR pathway. This is plausible since HR mechanisms involve, to a larger extent than NHEJ, strand resection and strand invasion, both likely to require (more) nucleosome mobilization. Hence, it is possible that At-SWR1 is involved in HR by facilitating DNA resection. A role for At-SWR1 in HR-related mechanisms is further supported by the severe impairment of these mutants to recombine the GUS gene repeats in two different HR reporter lines. However, two of its functional subunits, *ARP6* and *SWC6*, are not absolutely essential for HR, as a residual number of GUS-positive recombination spots were observed in both mutants. The introgression of the HR reporters into *pie1* mutants, so far hampered by the low fertility of this mutant, could determine if the absence of At-SWR1 catalytic activity would result in complete inhibition of SHR or if other remodeling complexes, for example, INO80 (Fritsch et al., 2004; van Attikum and Gasser, 2005), provide some redundancy for this important process.

The best characterized role of the At-SWR1 complex so far is the deposition of histone variant H2A.Z (March-Díaz and Reyes, 2009; Kumar and Wigge, 2010), but the role of H2A.Z deposition during DNA repair is not clear. Although yeast *htz1* mutations (lacking the histone variant) cause DNA damage–related phenotypes similar to those of SWR1 impairment (Kalocsay et al., 2009; Morillo-Huesca et al., 2010), there is conflicting evidence as to whether, and for how long, H2A.Z associates with DSBs (Papamichos-Chronakis et al., 2006; van Attikum et al., 2007; Kalocsay et al., 2009), and another histone 2 variant, γH2AX, plays a much more prominent role in signaling of DNA damage (Charbonnel et al., 2011; Lukas et al., 2011). Two recent articles gave an insight into the possible role of H2A.Z and SWR-C in DSB repair in mammals. Xu et al. (2012) proposed that the p400-mediated exchange of H2A.Z around the DSBs, in combination with the acetylation of H4, creates an open chromatin state that facilitates further modification of the chromatin and recruitment of repair proteins. Delayed and reduced formation of RAD51 and BRCA1 foci and reduced nucleosome occupancy were observed by Courilleau et al. (2012) in p400 mutants. Moreover, p400 and RAD51 interact physically. The authors speculate that the p400-Rad51 complex is involved in chromatin remodeling on the uncut strand to allow strand invasion.

Analysis of the role of H2A.Z deposition during DNA repair in plants is hampered by the existence of three *Arabidopsis* genes encoding this variant: *HTA8*, *HTA9*, and *HTA11* (Yi et al., 2006). Single mutants do not have a phenotype, but *hta9 hta11* double mutants and RNA interference knockout lines directed against transcripts from all three genes have lower levels of H2A.Z and mild molecular phenotypes resembling those of At-SWR1 mutants (Choi et al., 2007; March-Díaz et al., 2008). After confirming a downregulation to 50% of the wild-type level for the most highly expressed H2A.Z-encoding transcripts in the RNA interference line, we analyzed the sensitivity of these plants to BLEO, without evidence for a difference to the wild type (see Supplemental Figure 6 online). This might indicate that the availability of H2A.Z does not limit the repair function of the SWR1 complex, but a more detailed analysis is required with plants lacking the histone variant completely.

Despite several analogies, there is a major difference between yeast and plants in the impact of SWR1. Yeast *swr1* and *arp6* mutations, and also the lack of H2A.Z in *htz1* mutants, lead to increased frequencies of SHR (Kawashima et al., 2007; Morillo-Huesca et al., 2010), but HR is impaired in At-SWR1 mutants. Such differences in the molecular functions of homologous components between higher and lower eukaryotes are not unique: Mutations of yeast protein genes involved in DNA replication cause decreased HR, but they have the opposite effect in *Arabidopsis* (Schuermann et al., 2009; Yin et al., 2009; J. Liu et al., 2010a; Q. Liu et al., 2010b). Differences in the role of SWR1 may arise from the preference of recombination pathways (the main pathway is HR in yeast and NHEJ in higher eukaryotes), different interacting proteins, or different chromatin organization. For example, it is possible that deposition of H2A.Z proximal to DSB facilitates histone loss, as occurs at transcription start sites during transcriptional activation, where H2A.Z-containing nucleosomes are quickly evicted. Alternatively, knowing that yeast H2A.Z localizes to subtelomeric regions where it poses a barrier to heterochromatin spread (Shia et al., 2006), it is possible that localization of the histone variant at DSBs counteracts heterochromatinization during the repair process. In plants, this would also be consistent with the antagonistic nature of H2A.Z and DNA methylation, a hallmark of heterochromatic regions (Zilberman et al., 2008).

Mutants affected in SHR often have meiotic defects due to mechanistic similarities between somatic and meiotic recombination. Nevertheless, aberrant meiosis was not described for mutations in the genes encoding INO80 or RAD54, both positive regulators of SHR (Fritsch et al., 2004; Shaked et al., 2006). The reduced fertility of At-SWR1 mutants was assumed to be caused by impaired pollination due to morphological defects of the female reproductive organs (Deal et al., 2005). However, our data suggest that the substantial abnormalities during male and female meiosis in all three At-SWR1 mutants are a major reason for reduced seed set. A plausible explanation could have been that the absence of At-SWR1 leads to defects in the HR pathway responsible for crossover formation. By contrast, our data indicate that DSBs are formed, processed, and repaired in a regular manner and that the meiotic defect occurs after anaphase II. This implies further that the SWR-C complex is required only after DNA resection and HR in plants, while p400 in mammals seems to be required for the recruitment of RAD51 (Courilleau et al., 2012). We speculate that, after anaphase II, massive chromatin remodeling and decondensation are required to trigger the formation of microspore nuclei. Lack of PIE1 (or other components of the SWR-C complex) may hamper this process and some chromosomes may lag behind. This idea is in agreement with the dense DAPI-stained bodies, frequently observed outside of newly formed microspore nuclei, in aberrant meiotic products.

In conclusion, adding to its role in transcriptional regulation via H2A.Z deposition, At-SWR1 also plays vital and multiple roles in regulating somatic DNA repair and meiotic progression. It is so far the only chromatin-remodeling complex in plants to combine these functions, thereby exerting substantial genetic and epigenetic regulation that contributes decisively to the maintenance of

genome stability. As pointed out before (Meagher et al., 2010), it is surprising that plants with mutations in genes encoding the subunits of such a central complex are viable, once more highlighting the plasticity of plants and their utility as model organisms to study conserved processes.

METHODS

Plant Material and Growth Conditions

Seeds were cold treated at +4°C for 5 to 7 d and then surface sterilized with 5% sodium hypochlorite and 0.05% Tween 80 for 6 min, washed, and air-dried overnight. Sterilized seeds were sown on germination medium (GM) (Masson and Paszkowski, 1992) and grown in growth chambers under 16-h-light/8-h-dark cycles at 21°C. Plants for crossings, fertility analysis and propagation were grown on soil in growth chambers under 16-h-light/8-h-dark cycles at 21°C.

T-DNA Insertion Mutants

All T-DNA insertion mutants were in the Columbia background, and seeds were obtained from the Nottingham Arabidopsis Stock Centre. We used the following alleles: *arp6-3* (previously *esd1-10*; Martin-Trillo et al., 2006; WiscDS_Los289_29), *pie1-3* (SAIL_78_C11), *swc6-1* (SAIL_1142_C03), *ku70-2* (SALK_123114C), and *brca1-1* (SALK_01473C). Sequences of primers used for genotyping are listed in Supplemental Table 1 online.

Crosses

Plants genotyped for the traits to be introgressed were crossed by hand-pollination after emasculation of the maternal flowers. The presence of the desired reporters or mutational T-DNA insertions was confirmed in F1 hybrids, and these plants were propagated. F2 populations of plants (an average of 30 per F2 population) were screened for individuals homozygous for the presence of both traits of interest, and F3 or F4 seeds were used for further experiments.

Isolation of DNA and PCR

Leaves (~100 mg) from young plants, grown in soil, were collected, shock-frozen in liquid nitrogen, and homogenized to a fine powder with glass beads in a bead mill (Retsch). Homogenized plant tissue was used subsequently to extract DNA with a Phytopure kit (Amersham). The DNA obtained was diluted 1:50, and 1 μL was used in a 20-μL PCR amplification (Fermentas 5′ PCR kit).

Sensitivity Assays

For all sensitivity assays, seeds were plated on GM medium, stratified in the dark at +4°C for 2 to 4 d, and then transferred to growth chambers with 16-h-light/8-h-dark cycles at 21°C. Four-day-old seedlings were treated with γ-irradiation, BLEO, or MMC (Duchefa) and analyzed at day 10 for development of true leaves. For γ-irradiation, a pulse of 100 Gy (Co-60-gamma source Gamma-cell 220; Nordion International), with a dose rate of 27 to 34 $Gy \cdot min^{-1}$ applied, and plants returned to standard growth conditions until analysis. For BLEO and MMC treatment, seedlings were transferred to liquid GM medium, either without or with a drug (doses ranging from 0.25 to 2 μg/mL of BLEO or 10 to 40 μg/mL of mitomycin). After 9 d, they were washed extensively with drug-free liquid GM and transferred back to solid GM plates, allowed to recover for 1 d, and then analyzed. For application of HU (Sigma-Aldrich), seeds were plated directly on solid GM plates, without or with 0.5 to 2 mM of the chemical, and grown at standard conditions until analysis. For the UV-C assay, seedlings were treated at day 4 of growth with 8 kJ/m^2 UV-C (Stratalinker 2400) and grown under standard conditions until day 12, when they were scored for UV sensitivity phenotypes. For the root elongation assay with HU, seeds were plated on GM containing 1 mM of the chemical. Plates were grown vertically, and total root elongation was measured at day 7. For root elongation under UV-C, vertically grown 4-d-old seedlings were treated with 3 kJ/m^2 UV-C (Stratalinker 2400) and then transferred to standard conditions in the growth chamber. The difference between root growth at days 4 and 7 was measured.

Analysis of DNA Damage-Related Transcription

Seedlings were grown for 14 d on GM medium and transferred to Petri dishes containing liquid GM with 50 μg/mL of BLEO. After 1 h, seedlings were washed extensively with liquid GM and material collected at the time points indicated after removal of the drug. RNA was prepared using the RNeasy plant mini kit (Qiagen) and treated with 50 units of DNase I (Fermentas) for 30 min. cDNA was produced using Revert Aid H Minus, M-MuLV RT, and random hexamer primers (Fermentas) according to the manufacturer's instructions. Real-time quantitative PCR was performed according to Pecinka et al. (2009) on an iQ5 light cycler (Bio-Rad) using the 26 SensiMix Plus SYBR kit and fluorescein kit (Peqlab). All results were normalized to the reference gene *UBC28*. Sequences of primers are listed in Supplemental Table 2 online.

HR Frequency

To quantify the frequency of SHR, seedlings were plated in GM medium and grown until day 7 under standard conditions. For SHR induction with BLEO and MMC, 10 mL of drug-free liquid GM, or GM with 0.1 μg/mL or 10 μg/mL, respectively, was applied to the plates. Plants were grown until day 12, when the GUS assay was performed. For UV-C assays, plants were also treated at day 7 as described and grown under standard conditions until day 12, when assayed. The histochemical GUS staining was performed according to Pecinka et al. (2009).

Measuring DNA Content with Flow Cytometry

The second pair of true leaves of 28-d-old *Arabidopsis* plants was chopped with a sharp razor, and the nuclei stained with propidium iodate according to the manufacturer's protocol (CyStain UV Precise P; PARTEC). The distribution of DNA content was measured for an average of 4000 nuclei in a flow cytometer (BD FACSCanto), and analysis was performed with FACSDiva software.

Analysis of Gametophytic Development

Alexander staining for pollen viability was performed as described (Alexander, 1969). To analyze female gametogenesis, flower buds (bigger than 1mm) were fixed in ethanol/acetic acid (3:1 volume) at room temperature for 1 to 4 h. The solution was then replaced with 100% ethanol and the material incubated for 30 min, a procedure repeated twice. A last incubation of 30 min in 70% ethanol preceded a 1-h (minimum) treatment with a chloral hydrate mixture (8 g of chloral hydrate, 1 mL of glycerol, and 2 mL of water). The material was stored at room temperature until analysis. Pistils were transferred to slides with a drop of chloral hydrate, covered with a cover slip, and tapped gently to release the ovules. Preparations were observed with differential interference contrast microscopy (Axiovert 200M; Zeiss).

Analysis of Meiotic Chromosomes

For chromosome spreads, inflorescences were harvested and fixed in freshly made ethanol/acetic acid (3:1 volume) at −20°C overnight. This solution was replaced once and buds kept at −20°C until analysis. Fixed

flower buds (0.3 to 0.8 mm) were washed in 1× PBS buffer three times for 5 min and equilibrated in citric acid buffer (10 mM sodium citrate, pH 4.8) for 5 min. Flower buds were then incubated with 3% cytohelicase, 3% pectolyase, and 3% cellulase in citric acid buffer for 90 min at 37°C and washed three times in 1× PBS. Squashes were made in 45% acetic acid, slides frozen in liquid nitrogen, and the cover slip removed quickly. Slides were air dried vertically and counterstained with DAPI (1 μg/mL in Vectashield; Vector Laboratories). Preparations were analyzed using a Zeiss Axioplan 2 epifluorescence microscope. Images were acquired with MetaVue (Universal Imaging) and processed with Image J (NIH).

Spreads of pollen mother cells for immunocytology and detection of proteins were performed according to Armstrong et al. (2002), with modifications described in Kurzbauer et al. (2012). Primary antibodies were used as follows: αASY1 raised in rabbit (diluted 1:500 in blocking buffer [PBS + 0.1% Triton + 1% BSA]; Armstrong et al., 2002) and αRAD51 raised in rat (1:500; Kurzbauer et al., 2012). Secondary antibodies were used as follows: Goat-anti-rabbit conjugated to fluorescein isothiocyanate (1:300; Sigma-Aldrich) and goat-anti-rat conjugated to Cy3 (1:300; Chemicon). Slides were examined by fluorescence microscopy using a Zeiss Axioplan microscope, where Z-stacks with 100-nm intervals were acquired with MetaMorph software. AutoQuant software was used for deconvolution of Z-stacks, which are presented as projections done with HeliconFocus software. Overlays were made with Adobe Photoshop CS4.

Accession Numbers

Accession numbers for genes used for RT-PCR are listed in Supplemental Table 2 online.

Supplemental Data

The following materials are available in the online version of this article.

Supplemental Figure 1. Molecular and Morphological Characterization of Novel T-DNA Insertion Mutants.

Supplemental Figure 2. Examples of Plant Phenotypes after DNA Damaging Treatments.

Supplemental Figure 3. Analysis of Transcriptional Response to Bleocin Treatment.

Supplemental Figure 4. Examples of Plant Phenotypes of Single and Double Mutants after Bleocin Treatment.

Supplemental Figure 5. ASY1 Localization and RAD51 Foci in the Wild Type and *swc6* and *arp6* Mutants.

Supplemental Figure 6. Analysis of DNA Damage Sensitivity upon Reduced H2A.Z Expression.

Supplemental Table 1. Sequences of Primers Used to Genotype T-DNA Insertion Lines.

Supplemental Table 2. Sequences of Primers Used in qPCR Analysis of Transcript Levels.

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AUTHOR CONTRIBUTIONS

M.R. performed the majority of the experiments and analyzed the data. M.V.H. performed the immunostaining and analyzed part of the meiotic chromosomes. M.R. and O.M.S. designed the research. M.R., R.A.C., P.S., and O.M.S. wrote the article.

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