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Understanding the functions of SMC5/6 complex during the generative development in Arabidopsis

Ph.D. Thesis

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Supervisor: Assoc. prof. Aleš Pečinka, Ph.D.

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Declaration

I hereby declare that I have written the Ph.D. thesis independently under the supervision of Assoc. prof. Aleš Pečinka, Ph.D. using the sources listed in references with no conflict of interest.

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Abstract:

The maintenance of genome stability is a vital issue for all organisms. Structural maintenance of chromosomes 5/6 (SMC5/6) complex is a crucial factor for preserving genome stability. Recently, studies in plants showed the SMC5/6 complex is important for plant fertility. However, the mechanism is little known so far. In this thesis, I aimed to investigate the functions of the SMC5/6 complex during generative development in diploid and autotetraploid plants of Arabidopsis (*Arabidopsis thaliana*).

SMC5/6 complex is one of three SMC complexes, which are highly conserved to regulate chromosome architecture and genome organization in eukaryotes. The SMC5/6 complex is well known for its functions in DNA damage repair. In the first part of this thesis, I showed that loss-of-function mutations in NSE2 subunit of the SMC5/6 complex cause severe meiotic defects. The first defect is chromosome fragmentation observed in meiosis I, suggesting the SMC5/6 complex is partly required for the repair of SPO11-induced DNA double-strand breaks. The second independent defect is the absence of chromosome segregation in the first and/or the second meiotic division, leading to the formation of unreduced male gametes. The unreduced male gametes result in the production of triploid offspring in *nse2* plants. And it may also cause seed abortion as the maternal and paternal genome dosage is disturbed in endosperm. The presence of aborted ovules showed that *nse2* plants indicates any defects in female gametogenesis are maternally lethal.

Polyploidization is a common phenomenon in the evolution of flowering plants. However, our knowledge about the maintenance of polyploid genome stability is still very limited. In the second part of this thesis, I showed the loss-of-function autotetraploid (4x) mutants of SMC5/6 complex enhance fertility defects, cause severe defects in meiosis, and produce hexaploid and aneuploidy progeny, suggesting that the SMC5/6 complex is an important player in the maintenance of tetraploid genome stability. Additional, tetrads with micronuclei were formed in 4x mutant, which were not observed in the case of the diploid (2x) mutant pollen mother cells. Aneuploid offspring were equally caused maternally and paternally in 4x mutant. Rarely, hexaploid plants occurred by unreduced female gametes in 4x *nse2* plants. The absence of aneuploidy offspring and the viable unreduced female gametes in 2x mutants supports they are unique phenotypes of tetraploid plants, indicating the importance of certain molecular regulators may be changed when polyploidization occurs.

In conclusion, our studies uncover a novel SMC5/6 complex function in the maintenance of gametophytic ploidy in both diploid and autotetraploid Arabidopsis. Our work in diploid and autotetraploid Arabidopsis supports that autotetraploid plants have a generally higher frequency of but also higher tolerance for aneuploidy. Moreover, our results emphasize the importance of studying the consequences of mutations in genes regulating the plant fertility in diploid versus polyploid conditions, which may provide the possibility to increase agriculturally important traits as many crop species are polyploidy.

Keywords: SMC5/6 complex, NSE2, meiosis, seed development, polyploidy, genome stability, Arabidopsis

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Abstrakt:

Udržení genomové stability je důležité pro přežití všech organismů. Komplex strukturní údržby chromozomů 5/6 (Structural maintenance of chromosomes, SMC5/6) je klíčovým faktorem pro zachování stability genomu u eukaryot. Nedávné studie na rostlinách ukázaly, že funkce SMC5/6 komplexu jsou důležité pro plodnost rostlin. Jakým způsobem k tomu přispívá však nebylo známo. V této práci jsem se zaměřila na zkoumání funkcí SMC5/6 komplexu během generativního vývoje u diploidních a autotetraploidních rostlin huseníčku rolního (*Arabidopsis thaliana*).

SMC5/6 komplex je jedním ze tří komplexů strukturní údržby chromozomů. Jedná se o vysoce konzervované komplexy, které jsou důležité pro regulaci architektury chromozomů a jejich organizaci u eukaryot. SMC5/6 komplex je dobré známý pro své funkce při opravě poškození DNA. Studie u rostlin nicméně odhalily, že mutace některých podjednotek SMC5/6 komplexu snižují plodnost rostlin. Molekulární mechanismus je však stále záhadou. V této práci jsem pozorovala, že mutace způsobující ztrátu funkce podjednotky NSE2 tohoto komplexu ovlivňují plodnost rostlin. Z mateřské strany se jednalo o častou aborci zárodečného vaku již před oplozením. Studie vývoje pylu odhalila, že rostliny *nse2* snižují jeho životaschopnost. Dále jsem odhalila, že rostliny *nse2* produkují neredukované samčí gamety v důsledku chybějící segregace chromozomů v prvním a/nebo druhém meiotickém dělení. To vedlo k vývoji diploidních pylových zrn a po oplození pak k vývoji abnormálních semen.

Ačkoliv je SMC5/6 komplex zapojen do oprav dvouřetězcových zlomů DNA indukovaných proteinem SPO11v meiotické profázi, zde popsaná přítomnost neredukovaných samčích gamet je nezávislá na aktivitě SPO11. Abnormální semena vzešlá z neredukovaného pylu následně vedla k produkci přibližně 10 % triploidních potomků u *nse2* rostlin.

Polyploidie je častý jev u kvetoucích rostlin. Doposud je známo pouze několik genů kontrolujících stabilitu polyploidního genomuv průběhu redukčního dělení. Zjistili jsme, že ztráta funkce některých podjednotek SMC5/6 komplexu u autotetraploidních (4x) rostlin způsobuje závažné defekty v meióze a vede k produkci tetrád s mikrojádry, které nebyly pozorovány u diploidních (2x) mutantů. Dále bylo detekováno jak hexaploidní, tak aneuploidní potomstvo s extra kopiemi genomu nebo chromozomy pocházejícími od obou rodičů. Vzhledem k tomu, že zmíněné defekty nebyly pozorovány u diploidních mutantů je zřejmé, že se jedná se o znaky spojené s tetraploidií. Celkově naše výsledky zdůrazňují, že mutace SMC5/6 komplexu v polyploidním pozadí způsobují některé unikátní fenotypy, které nebyly nalezeny u diploidních rostlin.

Naše studie tedy odhalují novou komplexní funkci SMC5/6 při udržování gametofytické ploidie u diploidních i autotetraploidních rostlin huseníčku a ukazují, důležitost tohoto komplexu při údržbě stability tetraploidního genomu.

Klíčová slova: SMC5/6 komplex, NSE2, meióza, vývoj semen, polyploidie, stabilita genomu, Arabidopsis

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Contents

1. LITERATURE OVERVIEW

1.1 General principles of chromosome organization in the cell nucleus

The genetic information, encoded by DNA, is compacted into chromatin to fit the cell nucleus (Figure 1). Chromatin is a highly organized complex formed by DNA and proteins. It is a primary component of the nucleus in eukaryotic cells. The fundamental unit of chromatin is the nucleosome, which was first observed as a particle by Don and Ada Olins under the electron microscope (Olins and Olins, 1974). The nucleosome is composed by 147 bp of DNA wrapped around a set of eight histone proteins, known as a histone octamer (Luger et al., 1997). Every histone octamer is formed by two copies of the histone proteins H2A, H2B, H3, and H4 (Kornberg and Lorch, 1999; Pfluger and Wagner, 2007). Further, the repeating nucleosomes are arranged like beads on a string with intervening "linker" DNA to form a 10-nm fiber (Chakravarthy et al., 2005). Linker histones such as H1 and its isoforms are involved in chromatin compaction and sit at the base of the nucleosome near the DNA entry and exit binding to the linker region of the DNA (Zhou et al., 1998). A chain of nucleosomes is arranged in diameter of ~30 nm fiber, forming loops averaging 300 nm per turn (Felsenfeld and Groudine, 2003). Finally, the tight coiling of 250-nm-wide fiber, formed by compressing and folding the 300 nm fibers, produces the chromatid of a chromosome.

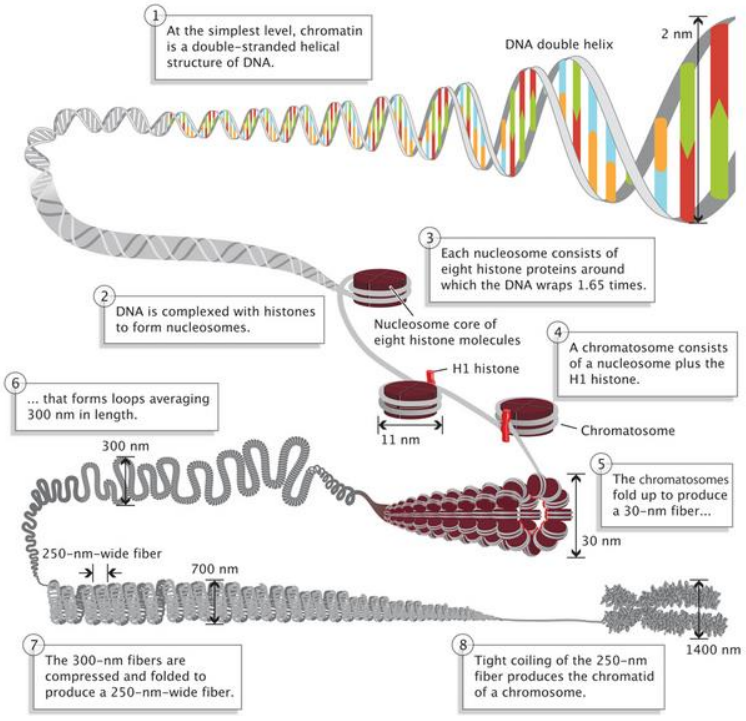

Figure 1: Schematic model of chromatin organization at different levels (Anthony, 2008).

1.2 Structural maintenance of chromosomes (SMC) complexes

The nucleosome structure is a barrier to proteins that regulate DNA metabolic activities, including transcription, replication, recombination, and repair. Different stages of plant development are highly modulated by changes in chromosome organization and dynamics by the action of multiple processes and factors (Casati and Gomez, 2020).

The ring-shaped SMC complexes are the main factors to regulate the chromosome dynamics, structure, and function. The individual SMC complexes are highly conserved from prokaryotes to eukaryotes (Cobbe and Heck, 2000; Hirano, 2006). The SMC protein forms a long intramolecular coiled-coil (Figure 2A). One end of the coiled-coil structure is the ATPase "head" domain, and the other is the "hinge" domain (Hirano, 2006). Two coiled SMC proteins are connected via their "hinge" domains, while their "head" domains are connected by a kleisin protein to form a closed tripartite structure (Figure 2B). In bacteria, the SMC complex consists of the SMC homodimer and one or two non-SMC subunit, modulating multiple chromosome dynamics, like chromosome compaction, segregation, and DNA damage repair (reviewed in Graumann & Knust, 2009; Figure 2B i). There are at least three SMC complexes in eukaryotes containing the SMC heterodimer and multiple non-SMC subunits: Cohesin, Condensin (including Condensin I and Condensin II differing by non-SMC subunits), and SMC5/6 (reviewed in Uhlmann, 2016; Figure 2B ii-v).

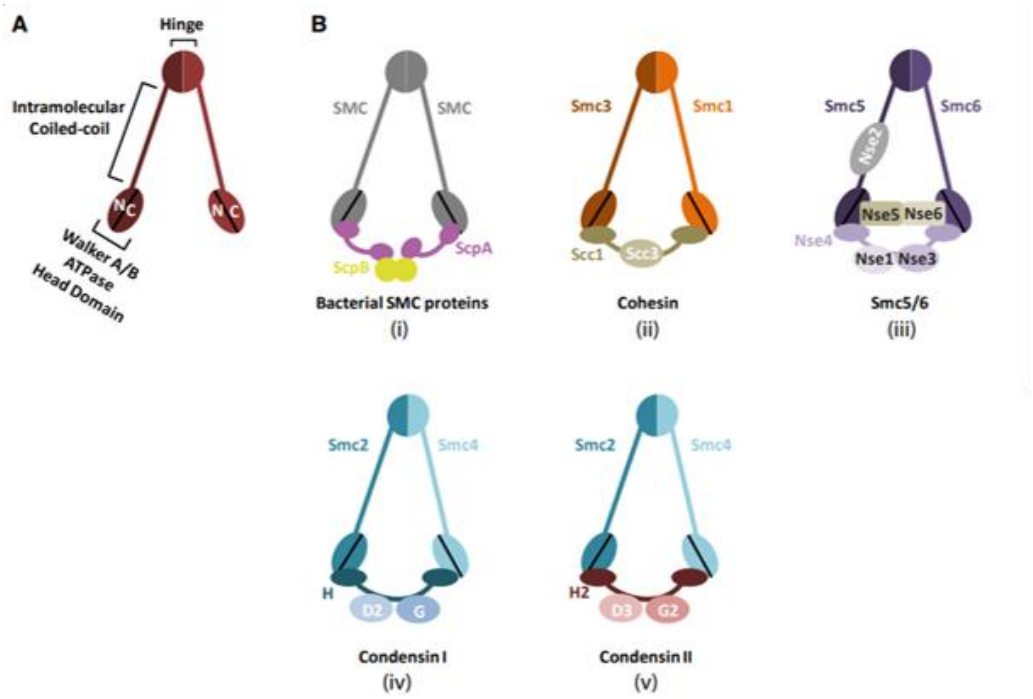

Figure 2. The architectures of the SMC complexes. (A) The general architecture of the SMC dimer. Each SMC protein contains an ATPase head domain and a hinge domain that mediates the dimerization of SMC proteins. (B) The architecture of SMC complexes from bacteria to eukaryotes. Each SMC complex is composed of a specific SMC dimer and several non-SMC subunits. (i) The bacterial SMC complex from *Bacillus subtilis*. ScpA connects the two ATPase heads of the SMC homodimer. (ii) The Cohesion complex. (iii) The SMC5/6 complex. (iv) The Condensin I complex. H, D2 and G stand for CAP-H, CAP-D2, and CAP-G, respectively. (v) The Condensin II complex. H2, D3, and G2 stand for CAP-H2, CAP-D3, and CAP-G2, respectively (Wu and Yu, 2012).

1.2.1 Cohesin

The cohesin complex is crucial for holding replicated sister chromatids together. Via their controlled release, it ensures faithful chromosome segregation during cell division. It is also required for compaction of chromosome, DNA double-strand break repair, and the regulation of gene expression (reviewed in Jeppsson et al., 2014; Uhlmann, 2016). There are four essential subunits in the cohesion complex in eukaryotes: a heterodimer formed by SMC1 and SMC3; two non-SMC proteins, SISTER CHROMATID COHESION 3 (SCC3) and a kleisin protein, either SISTER CHROMATID COHESION 1 (SCC1) or MEIOTIC RECOMBINATION PROTEIN 8 (REC8) (Peters et al., 2008) (Figure 2B ii). The kleisin protein SCC1 is functional during mitosis, and REC8 is its counterpart during meiosis in *Schizosaccharomyces pombe* (*S. pombe*) (Watanabe and Nurse, 1999). There are four SCC1/REC8 orthologs in Arabidopsis: SYNAPTIC 1 (SYN1)/ DETERMINATE, INFERTILE1 (DIF1), SYNAPTIC 2 (SYN2)/ RADIATION-SENSITIVE 21.1 (RAD21.1), SYNAPTIC 3 (SYN3)/ RADIATION-SENSITIVE 21.2 (RAD21.2), and SYNAPTIC 4 (SYN4)/ RADIATION-SENSITIVE 21.3 (RAD21.3) (Yuan et al., 2011). SYN1 is the Arabidopsis REC8 ortholog (Bai et al., 1999). The absence of SYN1 reduces male and female fertility because of severe meiotic defects (Bai et al., 1999; Cai et al., 2003). The expression of SYN2/RAD21.1, SYN3/RAD21.2, and SYN3/RAD21.3 is throughout the plant and they may have unique and overlapping functions (Yuan et al., 2011). There is a single SCC3 ortholog in Arabidopsis. Lack of SCC3 is lethal and may be correlated with mitotic defects (Chelysheva et al., 2005). Arabidopsis contains single SMC1 and SMC3 orthologs. SMC1 and SMC3 are essential for seed development (Lam et al., 2005; Liu et al., 2002; Liu and Meinke, 1998). These studies in Arabidopsis show that the cohesin complex is crucial for plant growth.

1.2.2 Condensin I and II

The condensin complex mediates the proper chromatin condensation and sister chromatid resolution to maintain the chromosomal integrity during cell division (Jeppsson et al., 2014). Most eukaryotic species have two different types of condensin complexes with unique functions, known as Condensin I and II (Hirano, 2012). Condensin I and II share two conserved core proteins, SMC2 and SMC4, forming the heterodimer, but three distinct non-SMC subunits: a kleisin protein and a pair of HEAT repeat-containing proteins (Neuwald and Hirano, 2000; Schleiffer et al., 2003). The heterodimer is connected by the kleisin protein, either CHROMOSOME-ASSOCIATED POLYPEPTIDE H (CAP-H) (Condensin I) or CHROMOSOME-ASSOCIATED POLYPEPTIDE H2 (CAP-H2) (Condensin II). The pair of HEAT repeat-containing proteins are CHROMOSOME-ASSOCIATED POLYPEPTIDE D2 (CAP-D2) and CHROMOSOME-ASSOCIATED POLYPEPTIDE G (CAP-G) (Condensin I) or CHROMOSOME-ASSOCIATED POLYPEPTIDE D3 (CAP-D3) and CHROMOSOME-ASSOCIATED POLYPEPTIDE G2 (CAP-G2) (Condensin II) (Figure 2B iv and v). In mammalian cells, the absence of Condensin I or II causes severe but different defects in chromosome formation, assembly, and architecture, indicating that two condensin complexes have distinct functions in mitotic chromosome organization (Green et al., 2012; Hirota et al., 2004; Ono et al., 2003). Differing from other species, the Arabidopsis genome encodes two SMC2 orthologs, SMC2A and SMC2B, with redundant functions (Siddiqui et al., 2003). The Arabidopsis Condensin I regulates the chromosome assembly and segregation during mitosis. Whereas Condensin II is involved in alleviating DNA damage repair but not require for mitotic division (Sakamoto et al., 2011). Condensin II in Arabidopsis also mediates the repression of a wide range of methylated genes with conditional expression, genome integrity, pollen development, and embryo development (Sakamoto et al., 2011; Schubert et al., 2013; Wang et al., 2017; Municio et al., 2021).

1.2.3 SMC5/6 complex

The SMC5/6 complex is the least characterized of the three main eukaryotic SMC complexes. The functions of the SMC5/6 complex are strongly linked to the maintenance of genome stability (Kegel and Sjögren, 2010; Aragón, 2018; Diaz and Pecinka, 2018). However, recent studies suggest that the SMC5/6 complex is also required for the regulation of gene transcription (Decorsière et al., 2016), maintenance of centromere structure (Gómez et al., 2013), prevention of disease in humans (Payne et al., 2014; Van Der Crabben et al., 2016), or

suppression of immune responses in plants (Yan et al., 2013). The SMC5/6 complex was initially discovered in *S. pombe* (Fousteri and Lehmann, 2000). There are one heterodimer formed by SMC5 and SMC6 and additional six NON-SMC ELEMENTs: NSE1-NSE6 in this complex (Zhao and Blobel, 2005; Sergeant et al., 2005) (Figure 2B iii). The SMC5/6 complex is divided into three specialized functional sub-complexes: NSE2-SMC5-SMC6, NSE1-NSE3-NSE4 and NSE5-NSE6 (Aragón, 2018; Duan et al., 2009b).

1.2.3.1 NSE2-SMC5-SMC6 sub-complex

NSE2-SMC5-SMC6 sub-complex is the most conserved sub-complex containing the SMC5-SMC6 heterodimer. SMC6 is needed to repair the DNA damage which is induced by ultraviolet (UV) and γ-ray in *S. pombe* (Lehmann et al., 1995). Arabidopsis carries two SMC6 homologs: SMC6A and SMC6B (Mengiste et al., 1999; Losada and Hirano, 2005; Watanabe et al., 2009). SMC6B was characterized in 1999, named as MIM (HYPER-SENSITIVE TO MMS, IR-RADIATION AND MITOMYCINC). The Arabidopsis SMC6B is necessary for DNA damage repair and normal frequency of homologous recombination (Mengiste et al., 1999; Watanabe et al., 2009; Hudson et al., 2011; Liu et al., 2015). *smc6a* mutants are viable, but double homozygous *smc6a smc6b* plants are embryo lethal due to their redundant functions in seed and gametophyte development (Watanabe et al., 2009; Liu et al., 2015; Zou et al., 2021). The Arabidopsis SMC5 ensures the seed formation (Watanabe et al., 2009). NSE2 is a Small Ubiquitin-like Modifier (SUMO) E3 ligase. The evidence of NSE2 directly binding to SMC5 was observed from *S. pombe* and *Saccharomyces cerevisiae (S. cerevisiae)* (Sergeant et al., 2005; Duan et al., 2009a). This interaction is conserved in animals and plants (Potts and Yu, 2005; Xu et al., 2013). NSE2 (a.k.a., MMS21, METHANE METHYLSULFONATE SENSITIVE 21; HPY2, HIGH PLOIDY2) was initially identified in a screen for mutants that were sensitive to methyl methane sulfonate (MMS) in *S. cerevisiae* (Prakash & Prakash, 1977). The study in *S. pombe* showed that NSE2 is involved in meiosis progression (Pebernard et al., 2004). *nse2* plants are viable but have strong developmental defects including small growth, short siliques and low fertility (Ishida et al., 2009, 2012; Liu et al., 2014). NSE2 is necessary for cell proliferation regulation in the root, DNA damage repair, and normal gametogenesis (Huang et al., 2009; Liu et al., 2014; Yuan et al., 2014).

1.2.3.2 NSE1-NSE3-NSE4 sub-complex

NSE1 and NSE3 form the other sub-complex with NSE4 in *S. pombe*. This conserved sub-complex bridges the core heterodimer and modulates the DNA binding activity of the SMC5/6 complex (Hudson et al., 2011; Palecek and Gruber, 2015; Zabrady et al.,2016.). The

RING-domain in NSE1 is needed to form this trimer. Mutations in the RING-domain cause DNA damage hypersensitivity and the absence of NSE1 is lethal in *S. pombe* and *S. cerevisiae* (McDonald et al., 2003; Pebernard et al., 2004). The interaction between NSE3 and DNA is required to load or maintain the SMC5/6 complex on chromatin in yeast (Zabrady et al., 2016). NSE3 contains a MAGE (MELANOMA-ASSOCIATED ANTIGEN) family domain. In *S. pombe*, the NSE3 C-terminal domain associates with NSE4 (Hudson et al., 2011). NSE4 is the kleisin protein of the complex. In *S. pombe*, it links SMC5 and NSE1-NSE3-NSE4 sub-complex (Palecek et al., 2006) and in *S. cerevisiae*, it bridges SMC5 and SMC6 heads (Duan et al., 2009b). The Arabidopsis genome carries single NSE1 and NSE3 orthologs and two NSE4 orthologs (NSE4A and NSE4B) (Díaz et al., 2019; Zelkowski et al., 2019). Both NSE1 and NSE3 are required for Arabidopsis embryogenesis and seedling development, and loss-of-function mutations of NSE1 or NSE3 are lethal (Li et al., 2017, 2019). Arabidopsis NSE4A and NSE4B bind to SMC5 and NSE3. Despite an interaction with NSE1 was confirmed by experiments (Díaz et al., 2019), it is likely. NSE4A is essential for DNA damage repair in somatic tissues and plays a role in meiosis and seed development (Díaz et al., 2019; Zelkowski et al., 2019). NSE4B appears to regulate the seed formation (Zelkowski et al., 2019). The study in moss *Physcomitrium patens* revealed that NSE4 is necessary for DNA double-strand break repair, but not for recovery from DNA damage (Holá et al., 2021).

1.2.3.3 NSE5-NSE6 sub-complex

Subunits of the NSE5-NSE6 sub-complex show less conservation across species at the sequence level and also concerning its structural position. In *S. cerevisiae*, the NSE5-NSE6 sub-complex inhibits SMC5/6 ATPase function to facilitate DNA substrate selection, likely by inducing a major rearrangement of the SMC5 and SMC6 head domains (Duan et al., 2009a; Taschner et al., 2021). While in *S. pombe* this sub-complex regulates the function of the SMC5/6 complex in DNA damage repair by binding to the base of the NSE1–NSE3–NSE4 sub-complex (Pebernard et al., 2006). In Arabidopsis, NSE6 was initially characterized as a key immune regulator called SNI1 (SUPPRESSOR OF NPR1-1, INDUCIBLE 1) (Li et al., 1999). Later, it was identified as an interacting factor with SMC5 and SMC6B and with an unknown protein ASAP1 (ARABIDOPSIS SNI1 ASSOCIATED PROTEIN 1) via proteomic experiments (Yan et al., 2013). Based on this, SNI1 and ASAP1 were characterized as the functional counterparts of NSE6 and NSE5 in Arabidopsis despite they are poorly conserved at the sequence level (Yan et al., 2013). The precise structural position of the ASAP1-SNI1 sub-complex is not yet clear in Arabidopsis. However, based on the biochemical pull-down

experiments, it is likely to be associated with the SMC5-SMC6 heterodimer (Yan et al., 2013). Both *asap1* and *sni1* mutants cause root development defects (Yan et al., 2013). SNI1 is involved in DNA damage repair and ensures the normal meiotic recombination by preventing the formation of inappropriate Joint Molecules (JMs) (Yan et al., 2013; Zhu et al., 2021). In contrast, the function of ASAP1 is unknown.

The SMC complexes have high conservation from prokaryotes to eukaryotes, mediating the chromosome dynamics, structure, and function. The studies in plants show that there are common but also unique features to plants as the presence of orthologs genes for diverse subunits in the individual SMC complexes (Figure 3). Hence, further studies about plant SMC complexes will improve our knowledge of the functions of these important complexes.

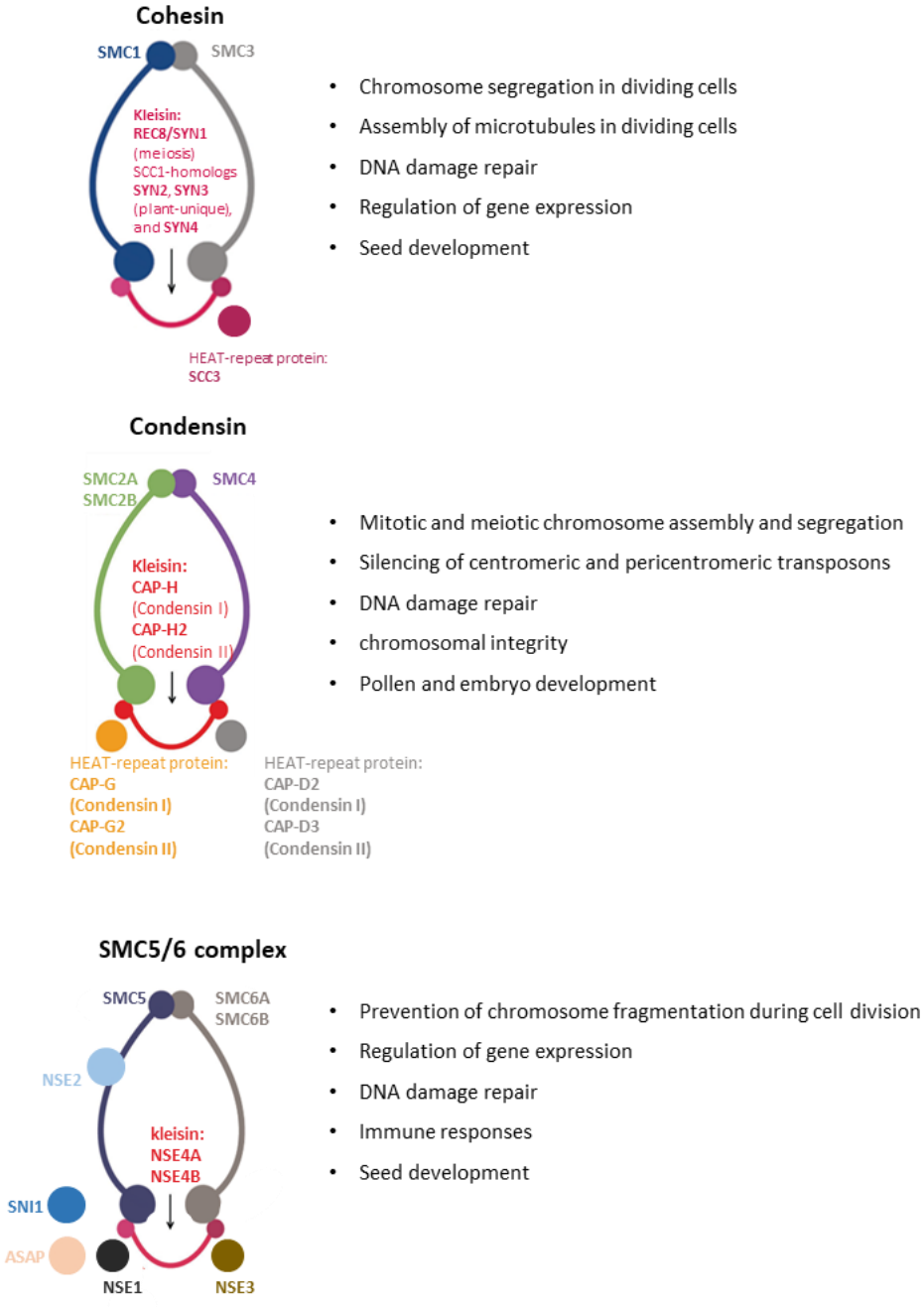

Figure 3. General functions and simplified structures of SMC complexes in *Arabidopsis thaliana.* (A) Cohesin : the heterodimer formed by SMC1 and SMC3, SCC3 and one kleisin, REC8/SYN1 in meiosis; SYN2 and SYN4 in mitosis; Plant-unique SYN3 in meiosis and mitosis. The cohesin complex is involved in chromosome segregation in dividing cells, DNA damage repair, regulation of gene expression and seed development. (B) Condesin : the heterodimer formed by SMC2A or SMC2B and SMC4; CAP-G and CAP-D2 (Condensin I) or CAP-G2 and CAP-D3 (Condensin II). The condensin complexes are involved in chromosome assembly and segregation in dividing cells, silencing of centromeric transposable elements, DNA damage repair, chromosomal integrity, pollen development and embryo development. (C) SMC5 /6 complex: the heterodimer formed by SMC5 and SMC6A or SMC6B; NSE1, NSE2, NSE3, NSE4A or NSE4B, ASAP1 and SNI1. The SMC5/6 complex is involved in the prevention of chromosome fragmentation in dividing cells, regulation of gene expression, DNA damage repair, immune responses and seed development (Modified from Bolaños-Villegas, 2021).

1.3 Reproductive development in plants

The life cycle of plants contains vegetative and reproductive stages. The reproductive development involves the "alternation of generations" between a haploid gametophyte and a diploid sporophyte (Haig and Wilczek, 2006). The sporophyte produces haploid (n) spores through meiosis. Each haploid spore develops into a gametophyte, generating haploid gametes through mitotic divisions. During fertilization, the haploid male gamete fuses with the haploid female one, producing the diploid sporophyte, thus perpetuating the life cycle. The reproduction development is tightly regulated as it results in seed production and a new generation of plant individuals.

1.3.1 Male gametogenesis in plants

In angiosperms, male gametogenesis occurs in the anther. There are two distinct and successive developmental phases: microsporogenesis and microgametogenesis (Figure 4). During microsporogenesis, the diploid sporogenous cells differentiate as microsporocytes (pollen mother cells). Then chromosome number is halved by two rounds of meiotic divisions, producing four haploid microspores (Owen and Makaroff, 1995). Each microspore develops into mature pollen by two rounds of mitotic divisions in microgametogenesis. Mitosis I after meiosis produces a vegetative cell, containing a large haploid nucleus with decondensed chromatin, and a generative cell, containing a small haploid nucleus with condensed chromatin. The generative cell is completely engulfed by the cytoplasm of the vegetative cell (Berger and Twell, 2011). In the plants with trinucleate pollen, including Arabidopsis, two sperm cells are generated in the second mitosis before pollen maturation. In other plants with binucleate pollen, like tomato, two sperm cells are generated in the pollen tube after pollen germination (Ma, 2005).

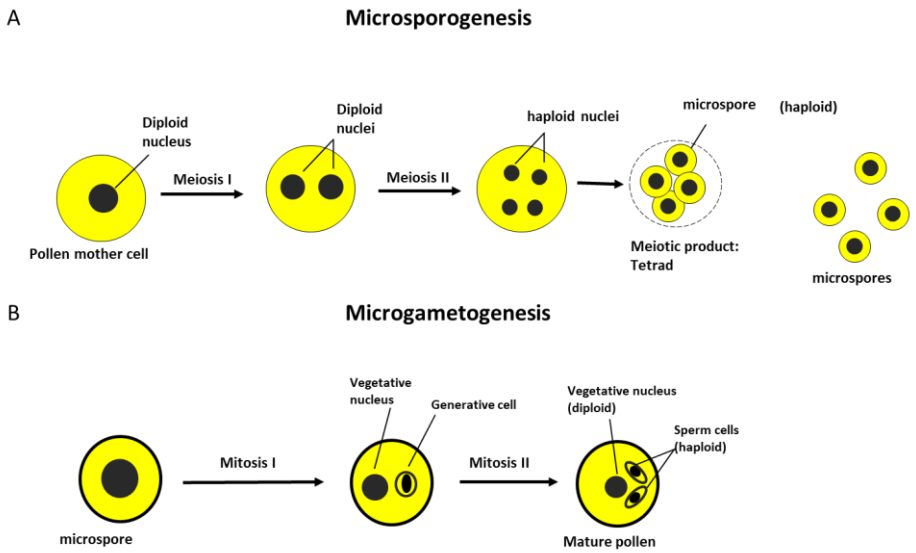

Figure 4. Overview of male gametogenesis in plants. (A) Microsporogenesis. It starts with the meiotic divisions of the pollen mother cell, producing a tetrad of haploid microspores. This tetrad is initially a syncytium enclosed in a particularly thick callose wall. Following meiosis, this wall is degraded, and the four individual haploid microspores are released. (B) Microgametogenesis. The released microspores undergo an asymmetric mitotic division. The first round of mitosis generates two cells: the larger vegetative cell, and the smaller generative cell. The generative cell is engulfed by the vegetative cell. After this, an additional round of mitosis of the generative cell occurs in Arabidopsis, maize, and other plants with trinucleate pollen to form two sperm cells.

Meiosis is one of the most important and complicated steps for the production of haploid gametes. Meiosis halves the chromosome number as DNA is replicated once followed by two rounds of cell divisions (Meiosis I and Meiosis II). The segregation of homologous chromosomes takes place in the first cell division (which is very specific to meiosis), and the segregation of sister chromatids takes place in the second cell division (Figure 5).

The first division relies on the formation of bivalent based on homologous pairing and recombination. The meiosis-specific topoisomerase SPO11 mediates double-strand breaks (DSBs) along the paired homologous chromosomes, initiating meiotic recombination (Villeneuve & Hillers, 2001). There are three SPO11 paralogues in Arabidopsis: SPO11-1, SPO11-2 and SPO11-3. The functions of SPO11-1 and SPO11-2 are non-redundant in meiosis (Hartung et al., 2007). The absence of meiotic DSBs is observed in the *spo11-1* and *spo11-2* plants, which leads to formation of univalent instead of bivalent in the first cell division. As univalent chromosomes segregate randomly, *spo11* plants often produce the unbalanced gametes (Grelon et al., 2001; Hartung et al., 2007). There are at least another five Arabidopsis

proteins involved in meiotic DSB formation by classical genetic screens: PUTATIVE RECOMBINATION INITIATION DEFECT 1 (PRD1), PUTATIVE RECOMBINATION INITIATION DEFECT 2 (PRD2), PUTATIVE RECOMBINATION INITIATION DEFECT 3 (PRD3), ARABIDOPSIS DSB FORMING (DFO), and CENTRAL REGION COMPONENT 1 (CRC1). PRD3 and DFO appear to be plant-specific (Mercier et al., 2015). They all share the same mutant phenotype of univalent chromosome segregation (De Muyt et al., 2007; De Muyt et al., 2009; Zhang et al., 2012; Miao et al., 2013; Seear et al., 2020). The SPO11-induced breaks are repaired by the MRN complex (MRE11/RAD50/NBS1) by creating single-stranded DNA overhangs, which then invade appropriate regions on the homologous chromosomes (Borde, 2007). Two recombination proteins, DISRUPTION OF MEIOTIC CONTROL 1 (DMC1) and RADIATION SENSITIVE 51 (RAD51) are involved in this process (Mercier et al., 2015). In Arabidopsis, mutations of RAD51 and DMC1 show different phenotypes in meiosis: SPO11-dependent chromosome fragmentation, and univalent segregation phenotype, respectively. This supports that the functions of DMC1 and RAD51 are distinct (Couteau et al., 1999; Li et al., 2004; Pradillo et al., 2012). DMC1 is capable of meiotic DSB repair, and RAD51 only plays a supporting role in this process by inhibiting the SMC5/6 complex in meiosis (Da Ines et al., 2013; Chen et al., 2021). Resolution of the DNA recombination intermediates results in crossovers (COs) and non-crossovers. Usually two, but in some cases up to four, COs are on one bivalent in most plant species, ensuring the proper chromosome segregation at anaphase I (Crismani and Mercier, 2012). The interference-dependent pathway is a crucial part of this control, and it needs a group of proteins called ZMM, including ZINC TRANSPORTER 1 PRECURSOR 1 (ZIP1), ZINC TRANSPORTER 1 PRECURSOR 2 (ZIP2), ZINC TRANSPORTER 1 PRECURSOR 3 (ZIP3), ZINC TRANSPORTER 1 PRECURSOR 4 (ZIP4), MUTS HOMOLOG 4 (MSH4), MUTS HOMOLOG 5 (MSH5), and MEIOTIC RECOMBINATION (MER3) in *S. cerevisiae* (Börner et al., 2004). Homologs of these proteins are identified in Arabidopsis and they have similar functions in the formation of COs (Higgins et al., 2004, 2005, 2008; Chen et al., 2005; Mercier et al., 2005; Chelysheva et al., 2007). Besides the ZMM pathway, The formation of COs depends on a group of proteins, including MMS AND UV SENSITIVE 81 (MUS81) and ESSENTIAL MEIOTIC ENDONUCLEASE 1 (EME1) in *S. pombe* (Berchowitz et al., 2007). Studies in yeast and Arabidopsis revealed that the SMC5/6 complex is also involved in resolving the DNA recombination intermediates in the MUS81-dependent pathway (Xaver et al., 2013; Zhu et al., 2021). The physical connection formed by COs is called chiasmata, which keeps bivalents together to ensure proper orientation and segregation of chromosomes during the first meiotic division (Zamariola et al., 2014b).

The segregation of sister chromatids occurs in the second cell division and resembles the dynamics of a mitotic cell division. The cohesin complex at centromeres is retained until anaphase II. In this step, the meiotic-specific protection is needed, and it was first described in *Drosophila*, named SGO (SHUGOSHIN) (Kerrebrock et al., 1995). However, the study in *Oryza sativa* suggested that SGO proteins in plants might be functional in prophase I (Wang et al., 2011). The plant-specific protection protein named PATRONUS 1 (PANS1) has been identified in Arabidopsis (Cromer et al., 2013). The absence of PANS1 causes a premature release of sister chromatid cohesion at metaphase II, leading to unbalanced male gametes and low fertility (Zamariola et al., 2014a). Maintaining the cohesin complex in the pericentromeric region at metaphase II ensures the attachment between the microtubules from opposite poles and the sister chromatid kinetochore. The organelle band is a physical barrier between the two spindles and the disturbed band leads to a random movement of the spindles (Brownfield et al., 2015). PARALLEL SPINDLE 1 (PS1) and JASON have been identified in Arabidopsis contributing to the regulation of spindle orientation in male meiosis. *ps1* and *jason* plants disturb the spindle orientation, leading to unreduced male gametes and triploid offspring (d'Erfurth et al., 2008; De Storme and Geelen, 2011).

The formation of unreduced gametes is the major way towards polyploidy in plants. Although meiosis defects often severely affect meiocyte viability, mutants in several genes produce viable unreduced gametes in Arabidopsis. One of them is CYCLIN A1;2 (CYCA1;2; a.k.a. TAM, TARDY ASYNCHRONOUS MEIOSIS), a member of the cyclin A family. Loss-of-function mutation of CYCA1;2 in Arabidopsis shows that meiocytes complete the first meiotic division but fail to enter meiosis II and thus produce two diploid daughter cells (d'Erfurth et al., 2010; Wang et al., 2010). OMISSION OF SECOND DIVISION 1 (OSD1) is also required for entry into meiosis II (D'Erfurth et al., 2009). Unexpectedly, male meiocytes fail to enter meiosis I in *cyca1;2 osd1* double mutant plants (d'Erfurth et al., 2010). It may be because the absence of CYCA1;2 and OSD1 both reduces the CDK activity sufficiently and thus prevent the male meiocytes entry into meiosis I (Brownfield and Köhler, 2011). The defects in cytokinesis after normal nuclear divisions also produce unreduced gametes. *tetraspore* (*tes*)

mutant identified in Arabidopsis causes disturbed meiotic cytokinesis leading to unreduced male gametes (Yang et al., 2003).

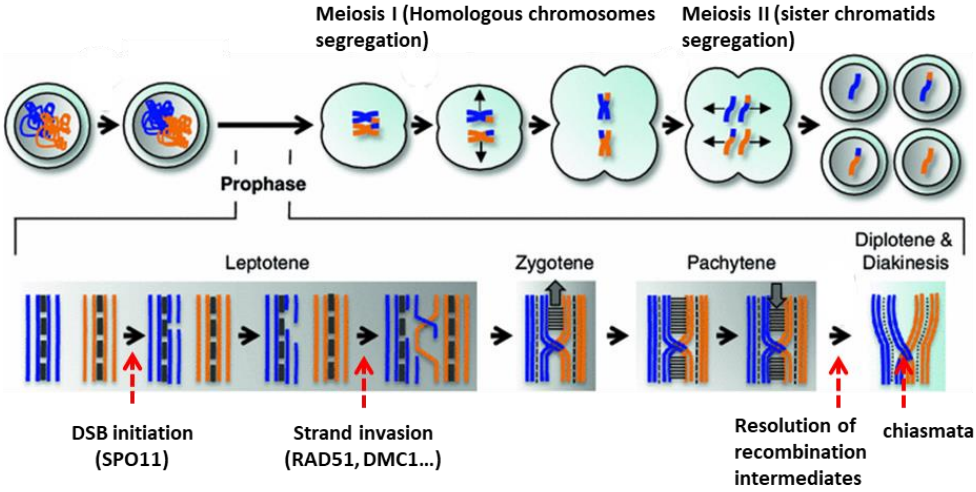

Figure 5. Scheme of male meiosis. Only one pair of homologous chromosomes is shown in orange and blue, with each line representing one chromatid. Chromatids duplicate during the S phase, condense at prophase and segregate at anaphase followed by decondensation. During meiosis I homologous chromosomes segregates. The segregation of sister chromatids occurs at meiosis II. The lower panel highlights different stages of the meiotic prophase. Single blue and orange lines in this panel indicate single DNA strands, and two adjacent lines represent one chromatid. DSBs are initiated by SPO11, then repaired by RAD51, DMC1 and other factors. At the end of the prophase, the chiasmata are formed with the resolution of recombination intermediates to ensure proper orientation and segregation of chromosomes during the first meiotic division (Modified from Wijnker and Schnittger, 2013).

1.3.2 Female gametogenesis in plants

Female gametogenesis in angiosperms occurs in the ovule. Like male gametogenesis, there are two stages: megasporogenesis and megagametogenesis. A cell in the ovule differentiates into a megaspore mother cell (MMC). The MMC produces four haploid megaspores by two rounds of meiosis. In most plants, including Arabidopsis, the female gametophyte is formed in a Polygonum-type developmental pattern: three megaspores degrade and only one functional megaspore remains in each ovule. This whole process is known as megasporogenesis (Caryl et al., 2003). This megaspore nucleus develops into a seven-nucleate structure with three antipodal cells, two synergid cells, one egg cell, and one central cell by three rounds of mitotic divisions. The central cell is homodiploid as it contains two identical haploid nuclei (Drews and Koltunow, 2011). The egg cell is always located at the micropylar end of the ovule, with the two synergid cells sitting side by side, the central cell is in the middle of the ovule with a large vacuole, and the three antipodal cells are at the chalazal end of the ovule (Caryl et

al., 2003). The formation of mature female gametophyte (embryo sac) is completed after these nuclei cellularization and differentiation. This entire process is called megagametogenesis (Tedeschi et al., 2017; Figure 6).

Female gametophyte mutants have been identified by partial or complete sterility (Caryl et al., 2003). The process of male and female meiosis in the flowering plants is similar, thus many mutants causing male meiotic defects also exhibit the impact on the female meiosis (Pagnussat et al., 2005; Drews and Koltunow, 2011). For example, SPO11-1 is necessary for DSB initiation in both male and female meiosis I in Arabidopsis (Caryl et al., 2003). Mutations of CYCA1;2 and OSD1 could produce functional unreduced female gametes. Upon fertilization, most progeny from *cyca1;2* and *osd1* mutants are polyploids (Brownfield and Köhler, 2011). SWITCH1/DYAD (SWI1/DYAD) protein in Arabidopsis is crucial for sister chromatid cohesion in early meiosis I (Mercier et al., 2001). The *dyad* allele is one of the mutations in *SWI1/DYAD* gene and it only presents the defective female meiotic division (Siddiqi et al., 2000). Univalents are observed in *swi1/dyad* mutants during meiosis I. Then sister chromatids segregate equally during meiosis I, resulting in unreduced female gametes.Most of the unreduced female gametes subsequently degenerate and fail to form a female gametophyte in the mutant. A small number of viable female gametes are produced and eventually triploid offspring (Maruthachalam et al., 2008). Mutations of Maize AMEIOTIC 1 (AM1) and rice OsAM also show the defective sister chromatid cohesion, as in *swi1/dyad* (Pawlowski et al., 2009). However, the completely blocked female meiosis in *am1* mutant indicates there is an other checkpoint in maize for entry into the meiosis (Yang et al., 2010).

The development of the embryo sac is essential for seed formation. However, defective female meiosis usually causes abnormal embryo sac development, presenting various embryo sac arrest phenotypes, thus reducing the seed set. But it is not true for male gametophyte mutants because pollen is not usually limiting (Drews and Koltunow, 2011).

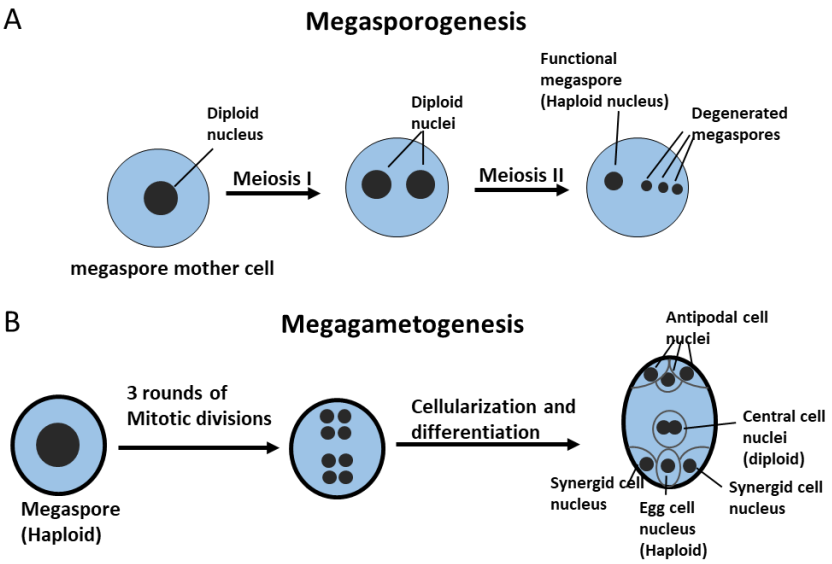

Figure 6. Overview of female gametogenesis (*Polygonum*-type) in plants. (A) Megasporogenesis. The megaspore mother cell undergoes meiosis to produce four haploid megaspores, of which three degenerate, and only one survives – the functional megaspore. (B) Megagametogenesis. The functional megaspore undergoes three consecutive rounds of mitosis, to produce a total of eight haploid nuclei. This is followed by a cellularisation step, whereby these eight nuclei are organized into seven cells: 3 antipodal cells, 2 synergid cells, an egg cell and one central cell. These nuclei cellularize and differentiate to form the mature embryo sac.

1.3.3 Double fertilization and seed development

The formation of male and female gametes is essential for successful fertilization. After the pollen grain adheres to the stigma of the carpel, the vegetative cell grows a tube through the style to reach the ovule. The sperm cells are released from the pollen tube, and the synergid cells degenerate, and the sperm cells arrive at the gamete site, fertilizing the egg cell inside of the ovule (Bleckmann et al., 2014; Figure 7A). After fertilization occurs, each ovule develops into a seed (Hamamura et al., 2011). Two sperm cells from the pollen tube fertilize the egg cell and the central cell in flowering plants. This process is called double fertilization, which is unique in flowering plants. The mature embryo sac structure is vital to ensure successful fertilization. For instance, synergid cells are necessary to guide the pollen tube and break its tip to release the sperm cells (Higashiyama, 2002).

Double fertilization initiates seed formation in flowering plants. The fusion of sperm and egg cells leads to the diploid embryo, and the fusion of sperm cells and central cells leads to triploid endosperm. Embryo and endosperm develop simultaneously. The embryo starts developing by forming an apical-basal axis by asymmetric cell division (Peris et al., 2010). Apical

cells initially develop the embryo proper, which ultimately differentiates into the mature embryo in many eudicots, like Arabidopsis, and embryonic leaves in monocots, like maize (Meinke, 2013). Basal cells develop into suspensor, which supports the growth of the embryo by connecting with maternal tissue. The suspensor degenerates and often disappears in mature seeds (Meinke, 2013). The endosperm starts the development by several cell divisions without cell wall formation (Olsen, 2004). Later, the development turns to cellularization, supporting embryo development (Olsen, 2004). For many eudicots, like Arabidopsis, the endosperm is absorbed during the embryo development and disappears at maturity (Figure 7B). By contrast, in monocots such as maize, the endosperm is observed in mature seeds and supports seedling growth (Meinke, 2013).

Although there is no conclusive evidence that the formation of an embryo depends on the presence of the endosperm (Peris et al., 2010), defective endosperm cellularization negatively impacts embryo growth, indicating that endosperm cellularization is an essential step for seed growth (Hehenberger et al., 2012). The transition from proliferation to cellularization takes place at a defined time point in plants. In Arabidopsis, it starts at around the fifth day after pollination (Adams et al., 2000). However, endosperm cellularization goes abnormal if the parental genome balance is disturbed. The maternal to paternal genome ratio in endosperm after double fertilization is 2:1 (2m:1p) (Adams et al., 2000). Increased maternal genome dosage inhibits the mitosis in the endosperm and reduces the weight of seeds, while additional paternal genome dosage leads to seed abortion due to the delay or complete failure of endosperm cellularization. Due to meiotic defects, this ratio can be disturbed by the unreduced gametes, which often causes seed abortion. Nevertheless, under some conditions, the union of unreduced gametes with reduced gametes leads to triploid offspring, which can form swarms of euploid and aneuploid gametes giving rise to stable tetraploids (Henry et al., 2005). This mechanism has been termed triploid bridge and is considered the main route to forming autopolyploids (Leitch et al., 2004; Mason and Pires, 2015).

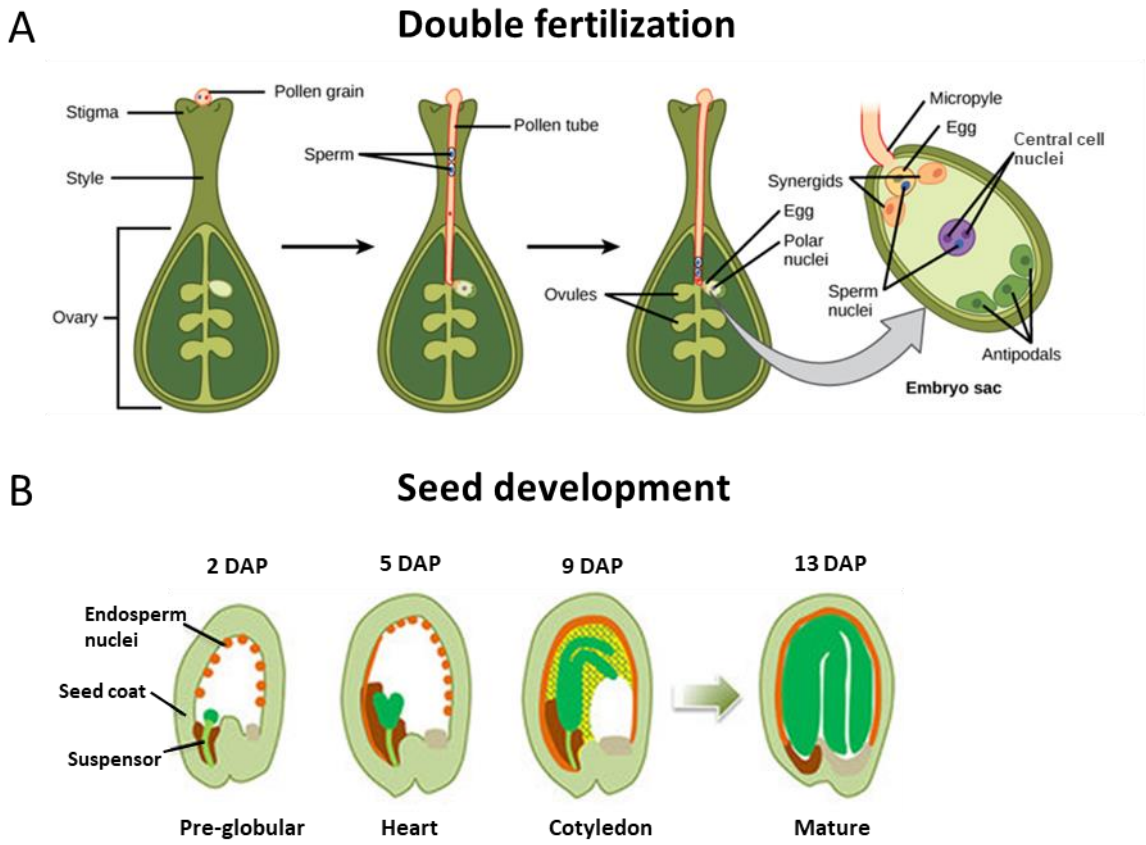

Figure 7. Double fertilization in flowering plants and seed development in eudicots. (A) Double fertilization. The pollen grain adheres to the stigma, the pollen tube grows into the ovule. The sperm cells are released to fertilize the egg cell nucleus and central cell nuclei. (B) Seed development in eudicots. Double fertilization leads to the diploid embryo and triploid endosperm. Embryo goes through per-globular, globular, heart, cotyledon stages developing into mature seed. During this time, endosperm goes through proliferation, cellularization, degeneration and is not present in the mature seed (Modified from Locascio et al., 2014).

1.4 Polyploidy

Polyploidy is a heritable condition of possessing more than two sets of homologous chromosomes. It is especially common in plants and has been and continues to be a major force in plant evolution. The polyploid plants are caused by genome duplication events (Jiao et al., 2011). There are two different groups of polyploidy: autopolyploidy and allopolyploidy. Autoploids are the polyploids containing the chromosomes with the same origin from within-species whole-genome duplication events, while the chromosomes in allopolyploids have the different origin from genome duplication events involving inter-specific hybridization (Comai, 2005; Sattler et al., 2016). Plants can benefit from being polyploid: the buffering effect of gene

redundancy on mutations and, in certain cases the facilitation of reproduction through self-fertilization or asexual means, making better use of heterozygosity and higher tolerance to some stresses, such as nutrient deficiency, drought, water deficit, temperature, pests, and pathogens (Botany et al., 2004; Osborn, 2004; Comai, 2005). On the other hand, the disadvantages of being polyploid are also evident. Polyploidy can change the cellular architecture, cause epigenetic instability and reduce fertility by arising problems in mitosis and meiosis (Comai, 2005). Polyploid plants produce high frequency of aneuploid progeny, leading to partially or completely sterile (Ramsey and Schemske, 2002). For instance, there are 30–40% of offspring from autotetraploid maize are aneuploid, reducing plant fertility (Datura, 1935; Doyle, 1986).

One of the major challenges for polyploids is in meiosis because of the presence of additional set(s) of chromosomes. In newly formed polyploids, multivalents are often observed in metaphase I, which are associated with incorrect segregation and reduced fertility (Lloyd and Bomblies, 2016). One of the adaptive mechanisms to suppress multivalents in autotetraploid *Arabidopsis arenosa* is to limit the crossover rates, therefore the number of multivalent connections is reduced (Pelé et al., 2018). While the molecular mechanism is limited known, it appears to be regulated via the chromosome axes (Hollister, 2015). A well-known example is the *Ph1* (*Pairing homeologous* 1) locus in wheat (Sears, 1976). *Ph1* promotes homologous synapsis through alterations to chromatin state and reduces crossovers on paired homoeologues during meiosis (Greer et al., 2012; Martín et al., 2014). However, the study in tetraploid *Allium porrum* showed that it forms mostly bivalents in metaphase I despite a relatively high crossover (Jones et al., 1996), suggesting another pathways in autopolyploids modulate the chromosome segregation in meiosis. In allopolyploid, because the multiple sets of chromosomes have the the different origin, bivalents are formed between the two most closely related homologs at metaphase I, resulting in independent segregation of subgenomes (Lloyd and Bomblies, 2016). This process is genetically controlled in most, if not all, allopolyploids (Jenczewski and Alix, 2004). Our knowledge about the maintenance of genome stability during meiosis in polyploidy is still very limited although it has been studied for a long time.

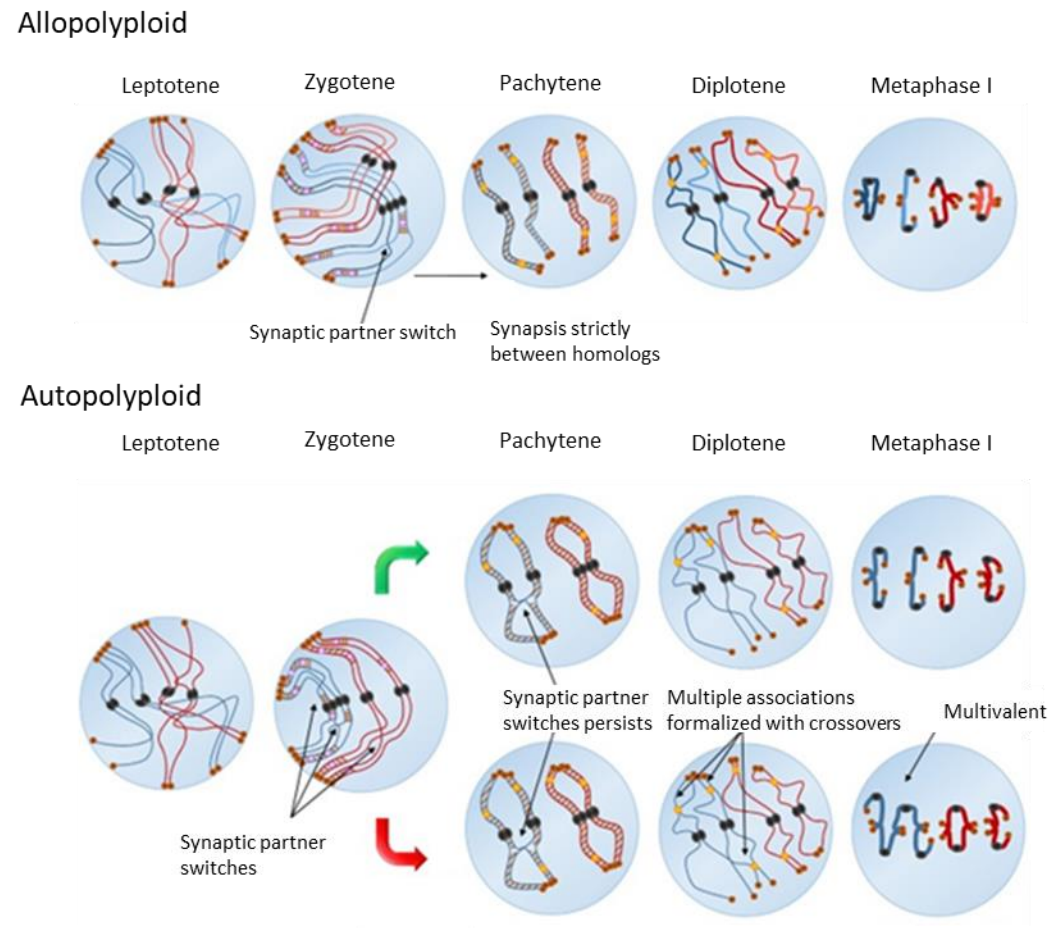

Figure 8. A model of meiotic prophase and metaphase in allo- and autopolyploids. Allopolyploids: In leptotene homologs and homeologous align and associate via telomeres (brown dots) and centromeres (black dots). In zygotene as the synaptonemal complex (SC, dashed lines) begins to form these pre-synaptic associations may develop into synaptic partner switches. In pachytene chromosomes show exclusive homologous synapsis. At metaphase, only bivalent is observed. Autopolyploids: In leptotene, homologs align and associate via telomeres and centromeres. Homologous pairing often involves all four homologs. In zygotene formation of the SC leads to synaptic partner switches between sets of homologous chromosomes. Some synaptic partner switches are corrected through late zygotene early pachytene but many persist. If crossover interference is low (red arrow), the crossover may be designated that reinforce multivalent associations such that multivalents are observed at metaphase I. If crossover interference is high (green arrow) then crossovers may be restricted to one per chromosome, such that only bivalent are observed at metaphase I (Lloyd and Bomblies, 2016).

2. AIMS OF THE THESIS

The principal goal of this thesis is to obtain a deeper understanding of the functions of the SMC 5/6 complex in *Arabidopsis thaliana*. Particular aims are as follows:

2.1 Characterization of the functions of SMC5/6 complex during reproductive development in diploid *Arabidopsis thaliana*

The first aim of the thesis is to understand the functions of the SMC5/6 complex during reproductive development in Arabidopsis. During the reproductive development, the haploid gametes are generated by meiosis and the fertilization gives rise to the diploid sporophty. The SMC5/6 complex is crucial to maintain genome stability. Our previous results uncovered that mutations of the SMC5/6 complex subunit reduced plant fertility (D áz et al., 2019). To promote our knowledge about the functions of the SMC5/6 complex in reproductive development, mutants in several *Arabidopsis thaliana* SMC5/6 complex subunits are used to analyze the phenotypes in reproductive development including the stages of the production of haploid gametes and seed development.

2.2 Characterization of the functions of SMC5/6 complex for tetraploid genome stability in *Arabidopsis thaliana*

Polyploidization is a common phenomenon in the evolution of flowering plants. However, our knowledge about the maintanence of polyploid genome stability is still very limited. The second aim of this thesis is to characterize the functions of the SMC5/6 complex for tetraploid genome stability in *Arabidopsis thaliana*. We produced autotetraploid mutants in several SMC5/6 complex subunits and used them to analyze the phenotypes in vegetative and reproductive development.

3. RESULTS

3.1 Characterization of the functions of SMC5/6 complex during reproductive development in diploid *Arabidopsis thaliana*

The previous study showed that a partial loss-of-function NSE4A mutant reduced plant fertility (D áz et al., 2019). In this thesis, I focused on the characterization of the functions of the SMC5/6 complex during reproductive development mainly using several loss-of-function mutants of SMC5/6 complex subunits. The main results are summarized below.

3.1.1 *nse2* mutations cause paternally-inherited abnormal seed development

To understand the functions of the SMC5/6 complex during reproductive development, mutants in several *Arabidopsis thaliana* SMC5/6 complex subunits were analyzed in this thesis. Homozygous loss-of-function *nse2-1* and *nse2-2* (Ishida et al., 2009) plants are viable and produce dry seeds in various colors, sizes and shapes, including light/dark brown, large/small and regular/shrunken ones (Figure 9A). The analysis of the siliques after thirteen-day pollination (DAP) showed that wild type (WT) plants produced 94.4% normal seeds, 2.8% abnormal seeds, and 2.8% aborted ovules (total seeds = 1424; Figure 9B and Table 1). However, there are only 34.7% and 30.8% healthy looking seeds found in *nse2-1* and *nse2-2* plants, respectively (total seeds = 1343 and 1253, respectively; Figure 9B and Table 1). Instead, 16.0% and 23.4% abnormal seeds, and 49.3% and 45.8% aborted ovules were observed in *nse2-1* and *nse2-2* plants, respectively (total seeds = 1343 and 1253, respectively; Figure 9B and Table 1). Both aborted ovules and abnormal seeds are significantly more produced in *nse2* plants (Fisher's exact test, $P < 0.005$ or lower). To examine the parental contribution to the seed phenotypes, reciprocal crossings between WT and *nse2* plants were performed. Only 2.0% and 3.7% of abnormal seeds were observed when *nse2-1* or *nse2-2* plants were pollinated by WT, respectively. The frequency of abnormal seeds matched the one produced in self-pollinated WT (2.8%, Table 1). However, pollination of WT plants by *nse2-1* or *nse2-2* resulted in 14.4% and 21.5% of abnormal seeds, representing a 7.2- and 5.8-fold increase relative to the reciprocal cross (Figure 9B and Table 1). The results indicated that the seed abnormality in *nse2* plants is paternally-inherited in Arabidopsis.

3.1.2 *nse2* mutations lead to defective embryo sac development

The frequent aborted ovules observed in *nse2* plants indicates severe maternal defects in *nse2* plants (Table 1). Then, I analyzed the *nse2-2* ovules. There are 25.5% (14 out of 55) *nse2-2* ovules with the normal-looking embryo sacs (Figure 10-i), 20% ones with embryo sacs containing three large nuclei (Figure 10-ii), 10.9% (6 out of 55) ones with embryo sacs containing zero nuclei (Figure 10-iii), and 43.6% ones without embryo sac (Figures 10-iv). The abnormal embryo sacs suggested that any defects in female gametogenesis may cause embryo sacs abortion before fertilization in *nse2* plants.

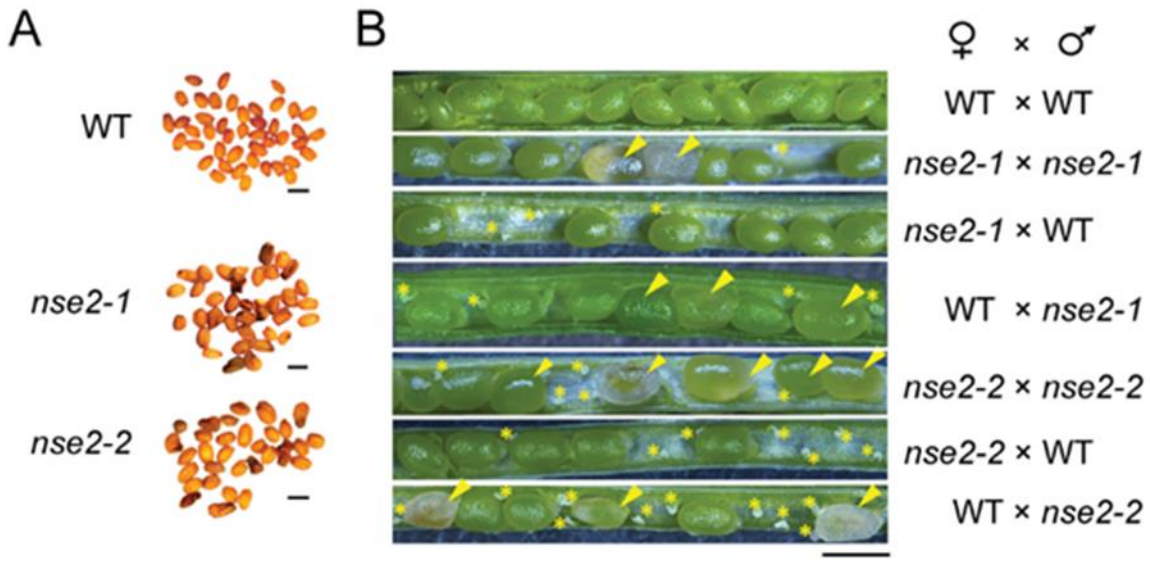

Figure 9: Paternally -inherited abnormal seed development in *nse2* plants. (A) Representative dry seeds of WT, *nse2-1* and *nse2-2*. Seeds from *nse2-1* and *nse2-2* plants represent a mixture of normally sized, large and aborted seeds. Scale bars 1 mm. (B) Dissected siliques 13 days after pollination (DAP). Aborted ovules are marked with yellow asterisks and abnormal seeds (typically larger, white or pale and partially transparent) with yellow arrowheads. Scale bar 400 μm (Yang et al., 2021a).

3.1.3 *nse2* mutations reduce pollen viability and produce diploid sperm nuclei

To explain the paternally-inherited abnormal seed phenotype, we focused on the investigation of pollen development. First, the pollen viability was analyzed with fluorescein diacetate (FDA) staining (Li, 2011). As expected (Liu et al., 2014), the viable pollen were significantly reduced from *nse2-1* plants (63.6%; n = 503; Fisher's exact test, $P < 0.001$ or lower; Table 2) and *nse2-2* plants (52.4%; n = 609; Fisher's exact test, $P < 0.001$ or lower; Table 2) compared to WT plants (83.4%; n = 1504). Meanwhile, we noticed that the sizes of *nse2* pollen are not identical (Figure 11A). Next, we quantified the diploid (2x) WT, 2x *nse2-1*, 2x *nse2-2* and tetraploid (4x) WT pollen area (Figure 11B). *nse2* plants produced two groups of differently

sized pollen. The larger pollen may contain 2C instead of 1C DNA content as in Arabidopsis the pollen area is strongly related to nuclear DNA content (De Storme and Geelen, 2011). To test this hypothesis, we measured the ploidy of *nse2* pollen nuclei with flow cytometry. Arabidopsis pollen contain two 1C sperm cell nuclei and one 2C vegetative cell nucleus. The samples were prepared as described (Borges et al., 2012) to purify sperm cell nuclei then the ploidy was measured by flow cytometry. The almost exclusively 1C nuclei detected in WT plants supported that 2C vegetative nuclei were effectively eliminated under our experimental conditions. With *nse2-1* and *nse2-2* pollen, we obtained both 1C and 2C nuclei (Figure 12A), indicating that *nse2* plants produce not only haploid but also diploid sperm cells.

Table 1. Seed phenotypes of self-pollination and reciprocal crossing between *nse2* and WT plants.

Mother	**Father**	**Events (n)**	**Phenotype (%)**		
			Normal seeds	**Aborted ovules**	**Abnormal seeds**
WT	WT	1424	94.4	2.8	2.8
nse2-1	*nse2-1*	1343	34.7	49.3	16.0
nse2-1	WT	919	37.3	60.7	2.0
WT	*nse2-1*	1369	56.5	29.1	14.4
nse2-2	*nse2-2*	1253	30.8	45.8	23.4
nse2-2	WT	629	38.1	58.2	3.7
WT	*nse2-2*	783	36.9	41.6	21.5

Table 2. The analysis of pollen viability by FDA staining. Test statistic values are the results of Fisher's exact test for mutants compared to WT.

	Replication 1		**Replication 2**		**Replication 3**		**Σ of replicates**		**Test statistic values**
	Viable	Total	Viable	Total	Viable	Total	Viable	Total	
WT	365	412	568	709	314	383	1247	1504	
nse2-1	55	85	132	260	113	158	300	503	0.00001
nse2-2	94	166	90	207	135	236	319	609	< 0.00001

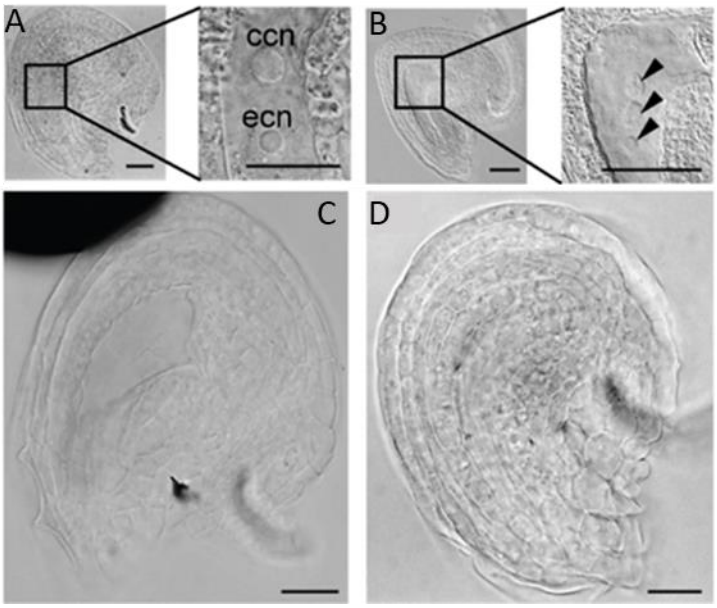

Figure 10. Differential interference contrast micrographs of *nse2-2* cleared ovules. (A) Typical WT embryo sac showing one central cell nucleus (ccn) and one egg cell (ecn) nucleus. (B) Embryo sac with one smaller and two large nuclei. (C) Embryo sac without any nuclei. (D) Ovule without an embryo sac. Scale bars 50 µm (Yang et al., 2021a).

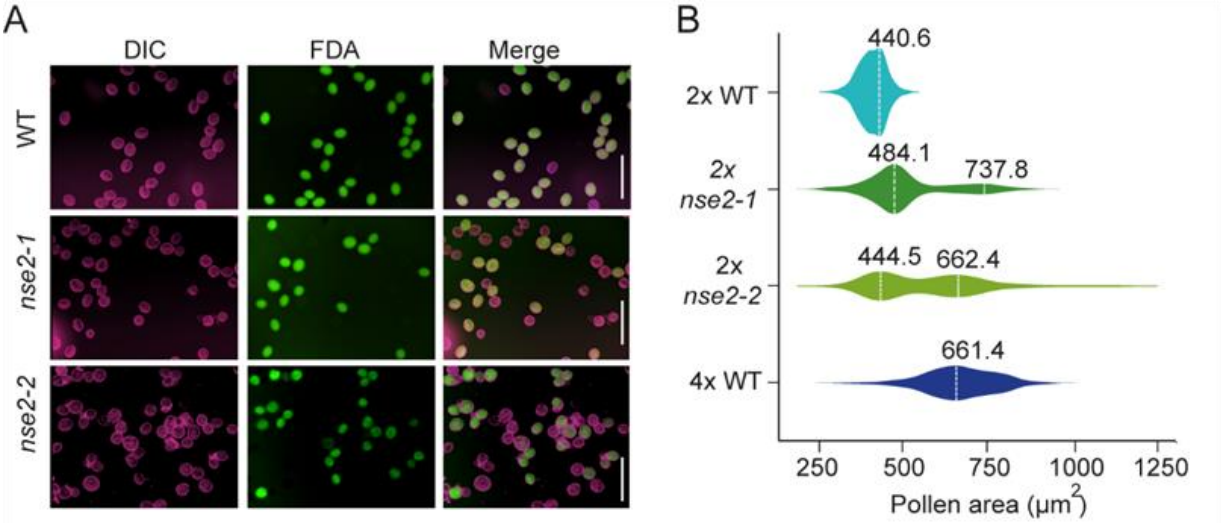

Figure 11. *nse2* plants affect pollen development. (A) Analysis of pollen viability by fluorescein diacetate (FDA) staining in mature pollen of WT, *nse2-1* and *nse2-2* plants. Photographs of pollen were taken using differential interference contrast (DIC, pseudo-colored in magenta) and the FDA signals (pseudocolored in green) were scored with epifluorescence microscopy. Green signals indicate viable pollen. Note uniform sizes of pollen grains produced by WT and different sizes of pollen from *nse2* mutant plants. Scale bars 100 µm. (B) Violin plots showing the area of individual pollen (µm^2) in diploid (2x) and tetraploid (4x) WT and diploid *nse2* plants. The numbers above violins indicate the maximum density of each peak (Yang et al., 2021a).

To test our hypothesis with another method, a double homozygous line of *nse2-1* with the sperm nucleus specific marker line *ProHTR10:HTR10-mRFP* (HTR10-mRFP) (Ingouff et al., 2007) was used to repeat the analysis. Only 1C nuclei population was detected in the WT HTR10-mRFP background. But 1C and 2C nuclei populations were detectd in *nse2-1* HTR10-mRFP pollen (Figure 12B). The nuclei were sorted onto microscopic slides according to their ploidy and inspected under the microscopy. In total, 93.6% (102 out of 109) WT HTR10-mRFP and 85.5% (260 out of 304) *nse2-1* HTR10-mRFP 1C nuclei contained RFP signals (Figure 12C-D). Also 83.1% (172 out 207) *nse2-1* HTR10-mRFP 2C nuclei contained RFP signals (Figure 12C-D). This provided solid evidence that *nse2* plants produce diploid sperm nuclei.

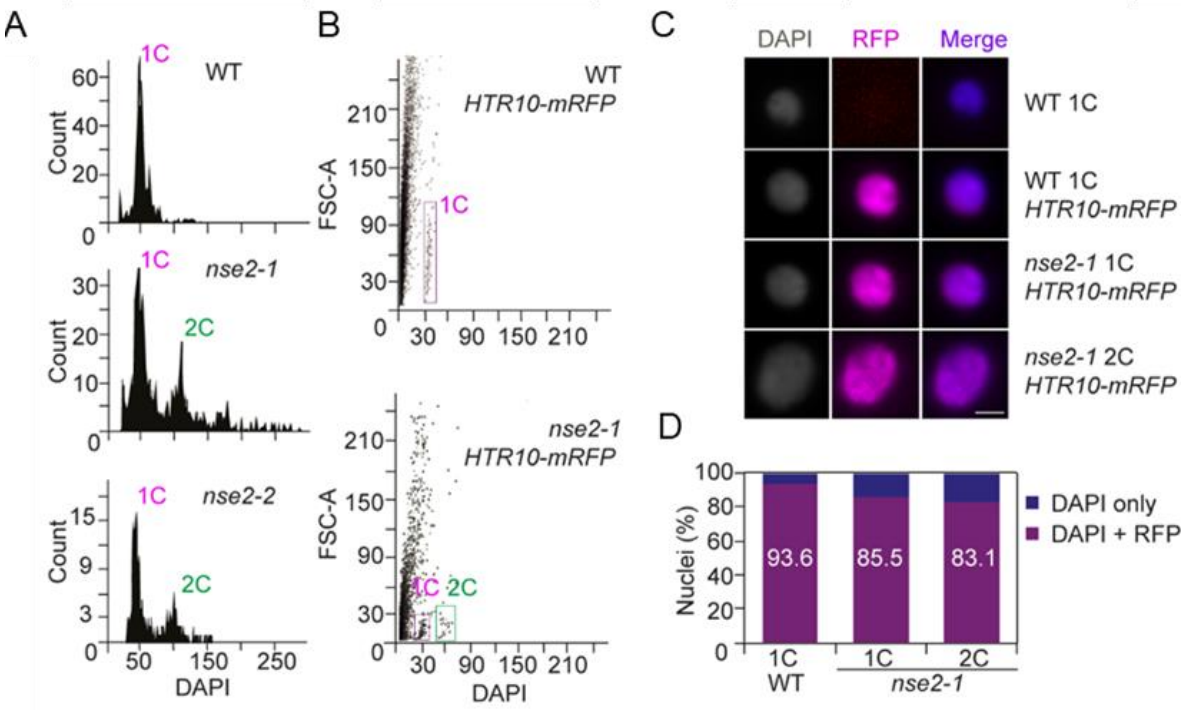

Figure 12. nse2 plants produce diploid sperm cell. (A) Flow cytometric histogram of sperm ploidy in WT, *nse2-1* and *nse2-2*. The x-axis shows relative DAPI intensity and y-axis particle count. (B) Bivariate scatter plot of sperm nuclei from WT and *nse2-1* in *ProHTR10:HTR10-mRFP* (HTR10-mRFP) background. the x-axis shows relative DAPI intensity and the y-axis forward scatter parameter, indicating nuclei size. 1C and 2C nuclei populations are marked and the unmarked signals correspond to debris and organelles. 1C and 2C nuclei were sorted onto slides and used for the experiment shown in (C). (C) Microscopic validation of enriched populations of sperm nuclei sorted based on their DNA content. Mature pollen of WT HTR10-mRFP, *nse2-1* HTR10-mRFP and non-transgenic WT were homogenized to eliminate vegetative nuclei, the remaining 1C and 2C nuclei were flow-sorted separately on microscopic slides and analyzed for RFP signals. Scale bars 1 μm. (D) Quantification of sorted nuclei observed from WT 1C, *nse2-1* 1C and 2C in HTR10-mRFP background (Yang et al., 2021a).

3.1.4 Defective meiosis leads to unreduced microspores in *nse2* plants

To determine the mechanism behind the formation of diploid male gametes, we produced *nse2 qrt1-4* double mutant. The four microspores from one meiosis remain encapsulated in *qrt1* plants (Preuss et al., 1994). The majority of meiotic products from *NSE2 qrt1-4* plants are tetrads (94.6% tetrads, 2.0% triads, 0.7% dyads and 2.7% monads; n = 575; Figure 13A and B). In *nse2-1 qrt1-4*, there were 57.8% tetrads, 14.0% triads, 19.2% dyads and 9.0% monads (n = 1042; Figure 13A and B). And in *nse2-2 qrt1-4*, there were 20.8% tetrads, 17.3% triads, 40.1% dyads and 21.8% monads (n = 479; Figure 13A and B).

The non-tetrad meiotic products observed in *nse2 qrt1* indicated *nse2* plants caused defective meiosis. Therefore, we cooperated with Mónica Pradillo's group at Complutense University of Madrid, Spain to analyze male meiosis in *nse2*. The cytological results showed that two major problems in chromosome behaviors found in *nse2* plants: (i) chromosome fragmentation and (ii) the absence of chromosome segregation (Figure 13C and D).

To explain these phenotypes, we first generated *nse2-2 spo11-1-5* double mutant. The number of meiocytes containing the chromosome fragmentation in *spo11 nse2* was reduced, demonstrating that NSE2 is necessary to repair the joint molecules generated from SPO11-induced DSBs (Table 3). However, the absence of chromosome segregation is mostly independent of chromosome fragmentation because of the presence of dyads in *nse2-2 spo11-1-5* but not in *spo11-1-5* (Table 3). 19.5% (34 out of 174) and 40.6% (26 out of 64) monads were observed in *nse2-1 osd1-3* and *nse2-2 osd1-3* plants, respectively (Figure 14A). The result strongly supported that the chromosome segregation problems in *nse2* plants occur in anaphase I. However, the *nse2* mutants (in *qrt1-4* background) produce also 9% to 21.8% monads (Figure 13A and B), indicating that in about 10%-20% of *nse2* meiocytes, there is a non-reduction in both anaphases I and II. Further, we noticed that homologous chromosomes do not completely move to opposite poles on some situations. In these meiocytes, the aberrant position of chromosome relative to the organelle band was often observed, indicating the organelle band is not functional as a physical barrier. As a consequence, a dyad instead of a tetrad was produced (Figure 13D). The α-tubulin immunolocalization assay showed the abnormal spindle structures in mutant meiocytes. The microtubules had a lower density and were disorganized around the chromosomal fragments and/or displaced sideways to the spindle periphery at anaphase II. At meiosis II, the spindles also showed a reduced number of microtubules and appeared more diffuse in *nse2-2* meiocytes (Figure 14B).

In conclusion, our results show the absence of chromosome segregation leading to unreduced gametes can occur mainly in anaphases I and to minor extent in anaphase II and are hallmarked by the disorganized microtubules and aberrant position of the chromosomes relative to the organelle band (Yang et al., 2021a).

Table 3. Quantification of meiotic defects in PMCs of *nse2-2*, *spo11-1-5*, and *nse2-2 spo11-1-5*.

nse2-2	**Second division**			
	Normal	45	31.3%	144
	Fragmented	22	15.3%	
	Abnormal segregation	49	34.0%	
	Unreduced	28	19.4%	
	Meiotic products			
	Tetrad	291	49.2%	591
	Triad	30	5.1%	
	Dyad	270	45.7%	
spo11-1-5	**Second division**			
	Normal	8	10.7%	75
	Fragmented	0	0.0%	
	Abnormal segregation	67	89.3%	
	Unreduced	0	0.0%	
	Meiotic products			
	Polyad	162	52.8%	307
	Unbalanced Tetrad	62	20.2%	
	Tetrad	37	12.1%	
	Triad	12	3.9%	
	Dyad w micronuclei	31	10.1%	
	Dyad	3	0.9%	
nse2-2 spo11-1-5	**Second division**			
	Normal	3	1.8%	164
	Fragmented	9	5.5%	
	Abnormal segregation	137	83.5%	
	Unreduced	15	9.2%	
	Meiotic products			
	Polyad	77	38.7%	199
	Unbalanced Tetrad	47	23.6%	
	Tetrad	35	17.6%	
	Triad	5	2.5%	
	Dyad w micronuclei	27	13.6%	
	Dyad	8	4.0%	

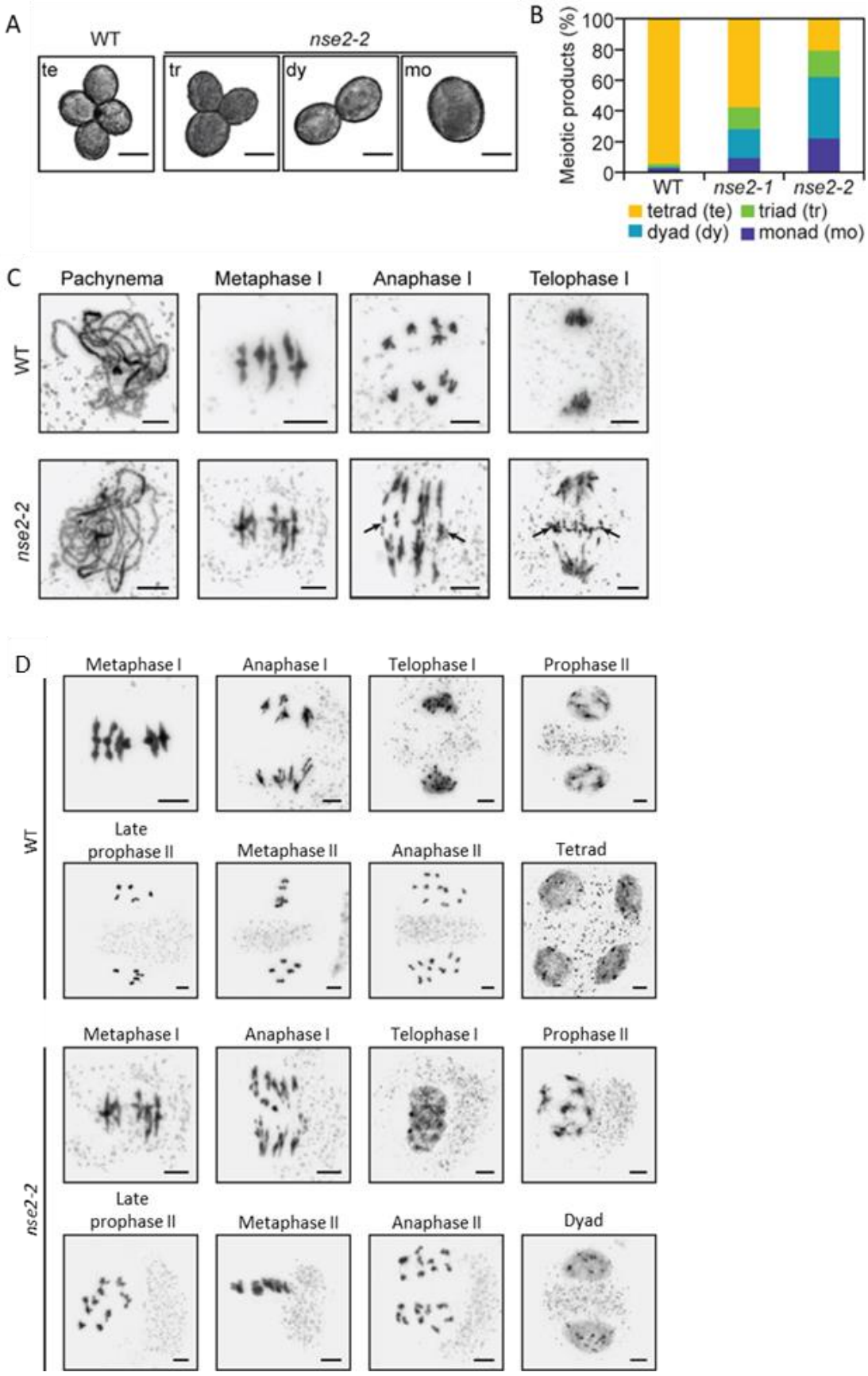
A
WT
nse2-2
te
tr
dy
mo
B
Meiotic products (%)
100
80
60
40
20
0
WT
nse2-1
nse2-2
tetrad (te)
triad (tr)
dyad (dy)
monad (mo)
C
Pachynema
Metaphase I
Anaphase I
Telophase I
WT
nse2-2
D
Metaphase I
Anaphase I
Telophase I
Prophase II
Late prophase II
Metaphase II
Anaphase II
Tetrad
WT
Metaphase I
Anaphase I
Telophase I
Prophase II
Late prophase II
Metaphase II
Anaphase II
Dyad
nse2-2

Figure 13. Defective chromosome behaviors during male meiosis in *nse2-2* plants. (A) Representatives of meiotic products observed in the double homozygous *nse2-2 qrt1-4* background. Scale bar 20 µm. te: tetrad; tr: triad; dy: dyad and mo: monad. (B) Quantification of meiotic products observed from WT, *nse2-1* and *nse2-2* in *qrt1-4* background. (C) First meiotic division. Pachynema - Full synapsis was detected in WT and *nse2-2*. Metaphase I - Five bivalents were observed in *nse2-2*, but chromatin was less condensed than in WT cells, showing constrictions and fragments. Anaphase I - *nse2-2* chromosomes appeared de-condensed and thin thread-like fragments of chromatin were visible spanning the region between all the segregating chromosomes (arrows). Telophase I - A barrier formed by multiple chromosome fragments is apparent between the two groups of segregated chromosomes in the mutant (arrows). Scale bars 5 µm. (D) Representative images exemplifying the formation of a tetrad (WT), and a dyad (*nse2-2*). In metaphase I there are five bivalents in both the control and the mutant. In anaphase I, there are delays in the migration of chromosomes in *nse2-2*, and, as a consequence, in telophase I, the two nuclei that should be observed do not appear separate, and the organelle band is not properly placed be-tween them, unlike in the control. Meiosis proceeds, and in prophase II there is only one nucleus with ten chromosomes, and the organelle band is lateralized (*nse2-2*), while in the control there are two nuclei with five chromosomes, and the organelle band between them. In metaphase II, the ten chromosomes appear on the same equatorial plate in *nse2-2*, while in the WT two equatorial plates are formed, one in each of the nuclei generated. Anaphase II gives rise to four clusters of five chromatids in the control and two clusters of ten chromatids in the mutant. Finally, a tetrad is formed in the control and a dyad in the mutant (in 45.7% of meiotic divisions). Scale bars 5 µm. (Yang et al., 2021a).

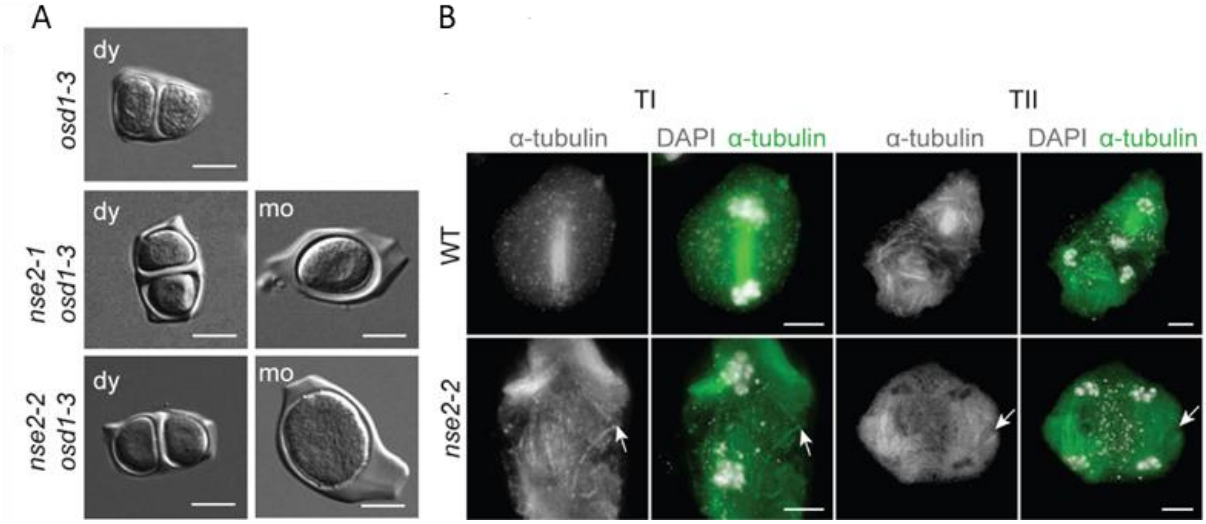

Figure 14. Analysis of male meiosis in *nse2 osd1* double mutant and Immunolocalization of α-tubulin (A) Representative phenotypes of immature meiotic products from *osd1-3* and *nse2 osd1-3* plants. Scale bars 5 µm. (B) Immunolocalization of α-tubulin. Representative images of telophase I (TI) and telophase II (TII) in WT and *nse2-2*. In *nse2-2*, the microtubule bundles are diffuse, have a lower density, and have a disorganized appearance, with some miss-localized microtubules (arrow). Scale bars 5 µm (Yang et al., 2021a).

3.1.5 Mutants in SMC5/6 complex plants produce triploid offspring

The diploid male gametes from *nse2* plants may change the ploidy level of offspring. Hence, the ploidy measurement was performed in the offspring from WT, *nse2-1* and *nse2-2* plants. 7.6% and 8.8% triploid offspring were detected from *nse2-1* and *nse2-2* after self-pollination, respectively (Table 4). Then, the ploidy measurement was performed in F1 generation from reciprocal crossing between WT and *nse2* plants. The triploid offspring were only found when WT plants were pollinated by *nse2* plants (Table 4), suggesting that triploid offspring is caused exclusively by unreduced male gametes. The ploidy measurement was performed in other available mutants in SMC5/6 complex subunits. 9.6% and 68.6% triploid offspring were found from *nse4a-2* and *sni1-3* plants after self-pollination. The reciprocal crossings between WT and *nse4a-2* or *sni1-3* confirmed that all triploids were only caused by the paternal side (Table 4). This result supported that the SMC5/6 complex is involved in the maintenance of gametophytic ploidy in Arabidopsis.

Table 4. Ploidy levels of offspring plants from *nse2* and WT parents and their F1 hybrids. n = number, 2x = diploid, 3x = triploid.

Mother	Father	Sown (n)	Germinated (n)	Germination rate (%)	2x (%)	3x (%)
2x WT	2x WT	113	110	97.3	100	0
2x *nse2-1*	2x *nse2-1*	434	380	87.6	92.4	7.6
2x *nse2-2*	2x *nse2-2*	232	147	63.4	85	8.8
WT 2x	2x *nse2-1*	283	270	95.4	93.3	6.7
WT 2x	2x *nse2-2*	252	174	69.0	94.9	5.2
2x *nse2-1*	2x WT	86	82	95.3	100	0
2x *nse2-2*	2x WT	119	113	95.0	100	0
2x *nse4a-2*	2x *nse4a-2*	300	271	90.3	90.4	9.6
2x WT	2x *nse4a-2*	87	83	95.4	86.7	13.3
2x *nse4a-2*	2x WT	93	93	100	100	0
2x *sni1-3*	2x *sni1-3*	740	137	18.5	31.4	68.6
2x WT	2x *sni1-3*	160	22	13.8	31.8	68.2
2x *sni1-3*	2x WT	98	81	82.7	100	0
2x *smc6b-1*	2x *smc6b-1*	119	103	86.6	100	0
2x *nse4b-1*	2x *nse4b-1*	135	120	88.9	100	0

3.2 Characterization of the functions of SMC5/6 complex for tetraploid genome stability in *Arabidopsis thaliana*

3.2.1 Fertility defects are more severe in tetraploid *nse2-1* and *nse4a-2* plants

Polyploidization is a common phenomenon in the evolution of flowering plants. To understand the functions of SMC5/6 complex in tetraploid Arabidopsis, we first generated autotetraploid Arabidopsis WT and several SMC5/6 complex deficient mutants plants with colchicine treatment. Dry seeds from 4x WT plants were larger than those from 2x WT plants (Figure 15A). Dry seeds from both 2x and 4x mutants had various phenotypes (Figure 15A). Analysis of siliques 13 DAP showed that 84.3% normal seeds, 10.2% aborted ovules and 5.5% abnormal seeds found in 4x WT (total seeds = 899; Figures 15B). Only 11.6% and 51.8% normal seeds were produced by 4x *nse2-1* and 4x *nse4a-2* plants, respectively (total seeds = 739 and 843, respectively; Figures 15B). 4x *nse2-1* and 4x *nse4a-2* plants produced significantly more aborted ovules (72.6% and 31.1% , respectively; Figure 15B) and abnormal seeds (5.8% and 17.1% , respectively; Figure 15B) compared to 4x WT (Fisher's exact test, $P < 0.00001$). Additionally, the aborted ovules and abnormal seeds were significantly increased in the 4x *nse2-1* plants compared to 2x *nse2-1* plants (Fisher's exact test, $P < 0.001$; Table 5). This suggested tetraploidy enhances fertility defects in *nse2-1* plants. However, the frequency of abnormal seeds is not significantly increased in 4x *nse4a-2* compared to the 2x mutant plants (Fisher's exact test, $P = 0.4299$; Table 5). It can be explained by the fact that *nse4a-2* is a partial loss-of-function allele (D ́az et al., 2019).

To test for the contribution of the parents to the abnormal seed phenotype, we performed reciprocal crossings between 4x WT and 4x mutant plants. The results revealed that seed abnomality is mainly paternally-inherited and to a less degree , controlled by maternally, in 4x mutant plants (% of abnormal seeds when mutant plants offer pollen vs ones when WT plants offer pollen: 11.0% vs 6.9% and 15.1% vs 3.5% in 4x *nse2-1* and 4x *nse4a-2*, respectively).

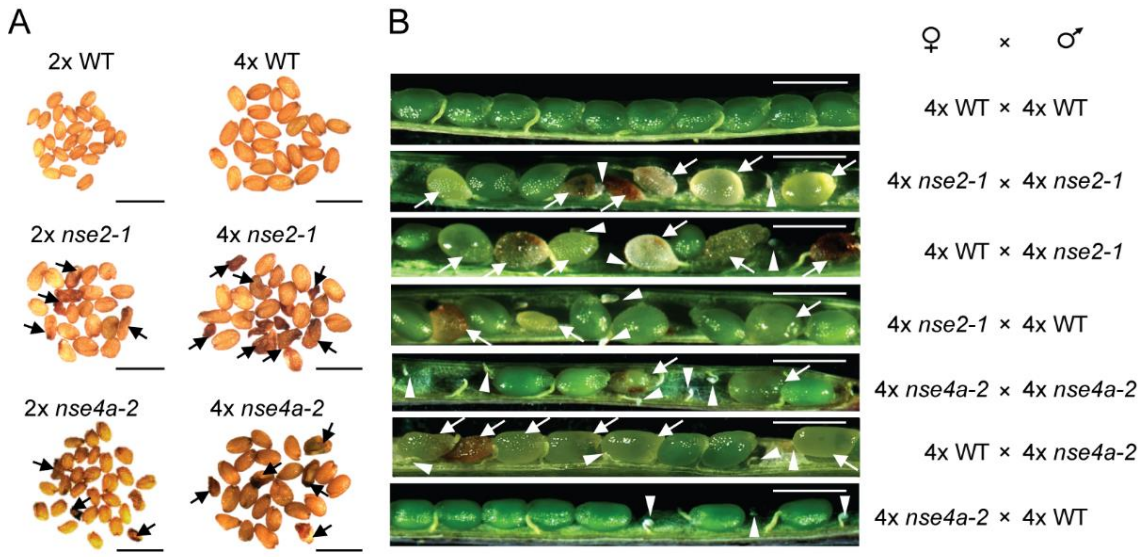

Figure 15. Reduced fertility of tetraploid *nse2-1* and *nse4a-2* plants. A) Representative dry seeds of diploid (2x) and tetraploid (4x) WT, *nse2-1* and *nse4a-2* plants. Arrows indicate examples of shrunken seeds. Scale bars 1 mm. (B) Opened siliques 13 days after pollination (DAP). Aborted ovules are marked with arrowheads and abnormal seeds (typically shrunk, brown/pale or partially transparent) with arrows. Scale bars 1 mm (Yang et al., 2021b).

Table 5. Source data for analysis of seed morphology after self-pollination from diploid (2x) and autotetraploid (4x) *nse2*, *nse4a-2* and WT plants. Test statistic values are the results of Fisher's exact test compared to ones from diploid mutant and WT plants, respectively.

Pheno-type	Genotype	Repli-cate 1	Repli-cate 2	Replicate 3	Sum	Test statis-tic values
Normal seeds	2x WT	692	533	100	1424	0.1194
	4x WT	228	279	251	758	
	2x *nse2-1*	196	105	170	471	0.00001
	4x *nse2-1*	54	10	11	75	
	2x *nse4a-2*	194	230	245	669	0.00001
	4x *nse4a-2*	94	208	135	437	
Aborted ovules	2x WT	10	35	1	46	0.00001
	4x WT	40	21	31	92	
	2x *nse2-1*	267	180	210	657	0.0002
	4x *nse2-1*	122	192	130	444	
	2x *nse4a-2*	34	37	20	91	0.00001
	4x *nse4a-2*	113	62	87	262	
Abnormal seeds	2x WT	38	14	1	53	0.0624
	4x WT	22	17	10	49	
	2x *nse2-1*	87	58	70	215	0.0009
	4x *nse2-1*	64	32	64	160	
	2x *nse4a-2*	78	31	68	177	0.4299
	4x *nse4a-2*	57	49	38	144	

3.2.2 Tetraploid *nse2* leads to defects in both male and female gametophytes

The frequent aborted ovules and abnormal seeds observed in 4x *nse2-1* led us to analyze the development of female and male gametes. Firstly, we analyzed the development of ovules in 2x and 4x *nse2-1* plants. We observed 13.0% ovules with WT-like embryo sacs, 38.9% ovules without embryo sacs, 22.2% ovules with embryo sacs containing zero nucleus and 25.9% ovules with embryo sacs containing three nuclei in 2x *nse2-1* plants (n = 54). 4x *nse2-1* plants (n= 104) produced only 4.8% ovules with WT-like embryo sac (Figure 16A), 72.1% ovules without an embryo sac or without detectable nuclei (Figure 16B and C), 23.1% ovules with embryo sacs containing nuclei with various phenotypes differing from the normal one (Figure 16D – G). This indicates that 4x *nse2-1* plants lead to severe defects in ovules development resulting in female pre-zygotic sterility.

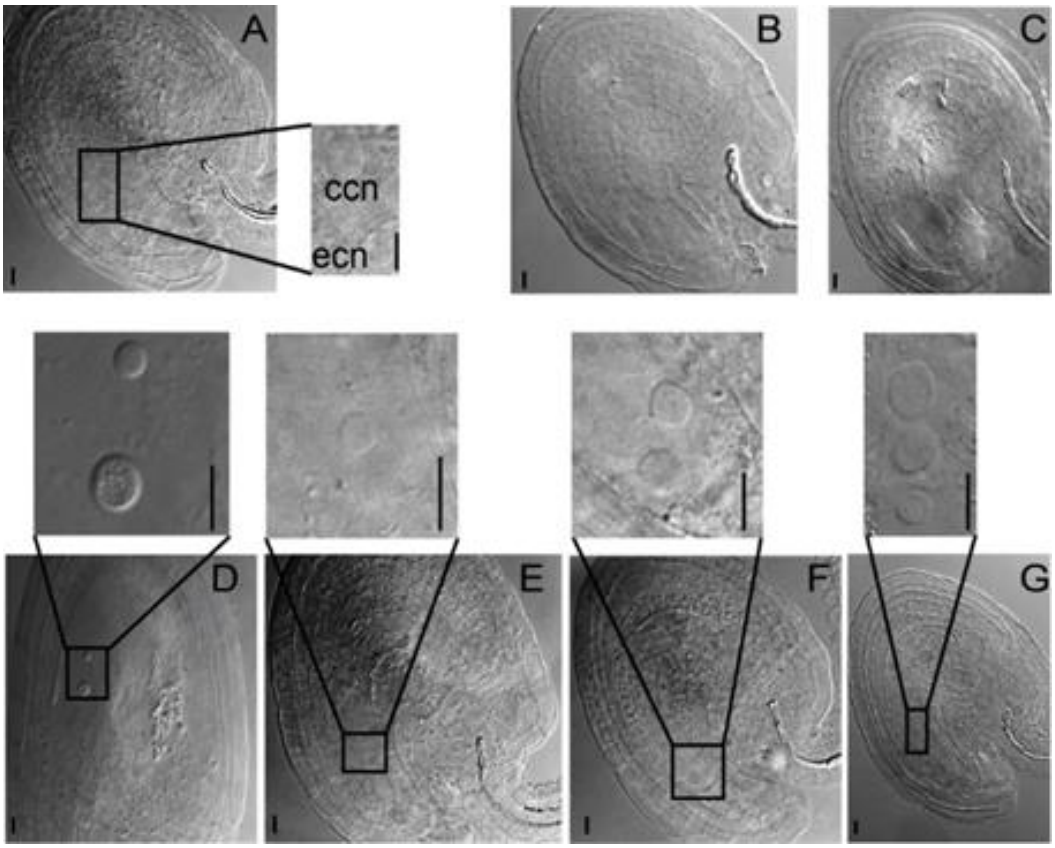

Figure 16. Developmental defects in female gametophyte of tetraploid *nse2-1*. Cleared ovules from 4x *nse2-1* were observed under a differential interference contrast microscope. (A) A typical 4x WT-like embryo sac shows one egg cell nucleus (ecn) and one central cell nucleus (ccn). (B-G) 4x *nse2-1* ovules displaying specific defects: (B) the absence of embryo sac, (C) embryo sac without nuclei, (D) embryo sac with two nuclei at the abnormal position, (E) embryo sac with only one nucleus, (F) embryo sac with two equally size nuclei, and (G) embryo sac with one smaller nucleus and two bigger nuclei. Scale bars 10 μm.(Yang et al., 2021b).

Second, we assessed several male gametophyte phenotypes. The pollen viability was analyzed with FDA staining and was significantly reduced in 4x *nse2-1* plants (% of viable pollen in 4x *nse2-1* plants vs in 4x WT plants: 29.4% vs 62.2%; n = 1275 and 1512, respectively; Fisher's exact test, $P < 0.00001$). The viable pollen were significantly less produced in both 4x WT and *nse2-1* plants compared to 2x WT and 2x *nse2-1* plants (95.3% and 65.0%, respectively; Fisher's exact test, $P < 0.00001$) grown under the same cultivation conditions.

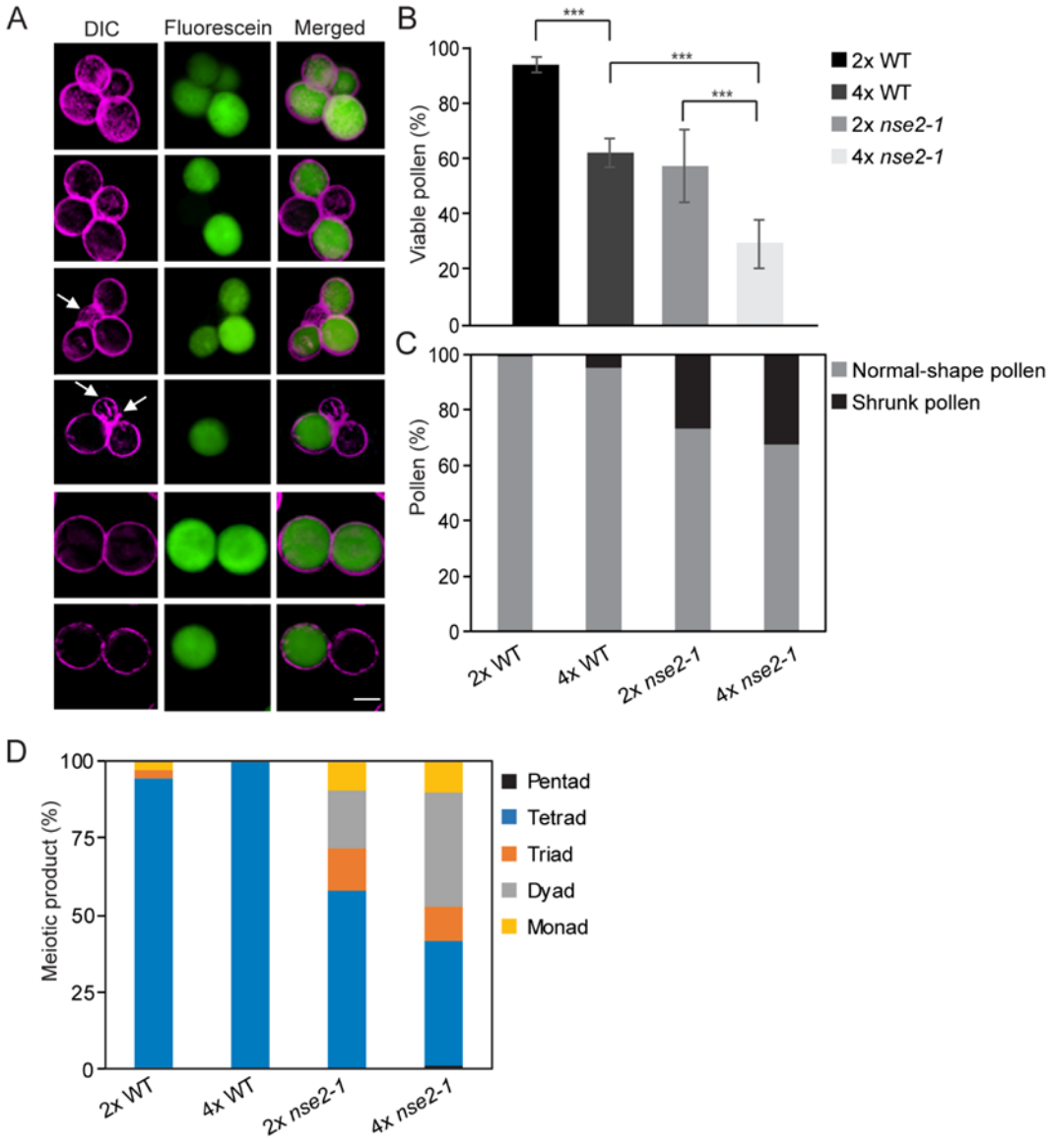

Figure 17. Microspore phenotype of tetraploid (4x) *nse2-1* plants. (A) Representative mature pollen stained by fluorescein diacetate from 4x *nse2-1 qrt1-4* plants. Differential interference contrast (DIC) images were pseudocolored in violet. Viable microspores are indicated by fluorescein signals (green). Shrunk microspores are indicated by arrows. Scale bar 20 μm. (B) Frequencies of viable pollen from 2x WT, 4x WT, 2x *nse2-1* and 4x *nse2-1* (all in *qrt1-4* background). Error bars represent standard deviations among the means of two or three individual plants. Significance in Fisher's exact test: - = $P > 0.05$, *= $P < 0.05$, ** = $P < 0.01$ and *** = $P < 0.001$. (C) Quantification of normal-shape and shrunken pollen observed in 2x WT, 4x WT, 2x *nse2-1* and 4x *nse2-1* in *qrt1-4* background. (D) Quantification of meiotic products with different numbers of microspores as observed in 2x WT, 4x WT, 2x *nse2-1* and 4x *nse2-1* in *qrt1-4* background (Yang et al., 2021b).

The plants we used for pollen viability assay were in *qrt1-4* mutant background, which allowed us to analyze the meiotic products from 4x WT and 4x *nse2-1* plants. In 4x *nse2-1 qrt1-4*, we found 32.5% (414 out of 1275) shrunken microspores (Figure 17A, arrows), which matched the 27% in 2x *nse2-1 qrt1-4* plants (Figure 17C). There were only tetrads in 4x WT *qrt1-4*, indicating that meiosis in 4x WT plants is normal. On the contrary, there were 40.4% tetrads, 11.2% triads, 37.8% dyads, 9.6% monads and rarely even 1.1% pentads observed in 4x *nse2-1 qrt1-4* plants (Figure 17D). This indicated the absence of NSE2 disturbs the meiotic division in tetraploid Arabidopsis.

Male meiosis was analyzed in Mónica Pradillo's group. Compared to the results from 2x mutant, the defects in 4x *nse2-1* plants were more drastic. Meiotic irregularities in meiosis II were detected in 93.0% analyzed meiocytes (40 out of 43), described as : (i) chromosome fragmentation (27.9%), (ii) chromatin bridges (23.3%), (iii) abnormal segregation (13.9%), (iv) meiocytes with several problems including chromosomal bridges and fragments (16.3%), and (v) non-reduced meiocytes (11.6%) (Figure 18). On the contrary, only 68.7% of the meiocytes have the second meiotic division defects in 2x *nse2* plants (Table 3), indicating that tetraploidy enhances meiotic defects in *nse2* plants.

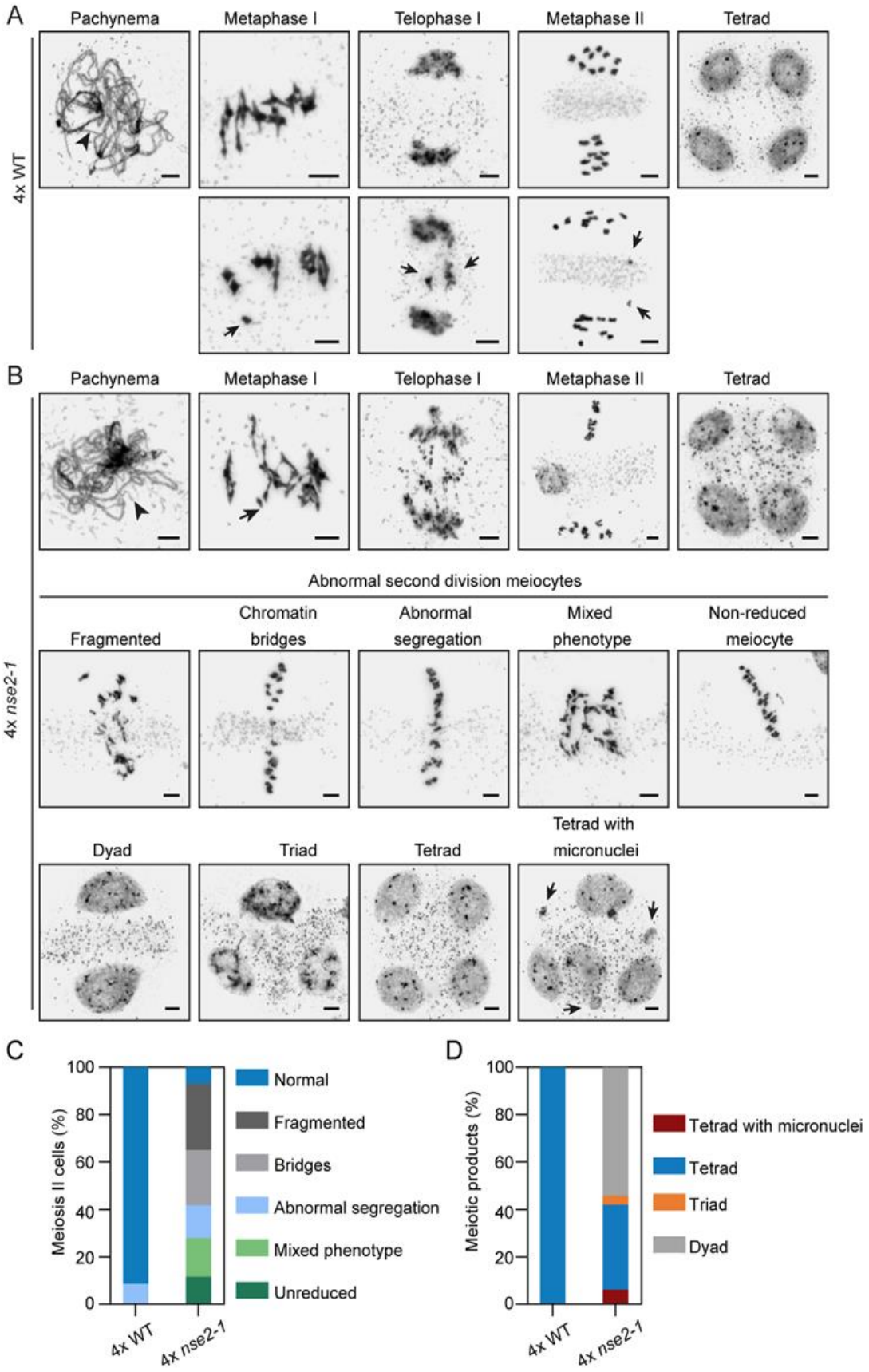
A
Pachynema
Metaphase I
Telophase I
Metaphase II
Tetrad
4x WT
B
Pachynema
Metaphase I
Telophase I
Metaphase II
Tetrad
Abnormal second division meiocytes
Fragmented
Chromatin bridges
Abnormal segregation
Mixed phenotype
Non-reduced meiocyte
4x nse2-1
Dyad
Triad
Tetrad
Tetrad with micronuclei
C
Meiosis II cells (%)
100
80
60
40
20
0
4x WT
4x nse2-1
Normal
Fragmented
Bridges
Abnormal segregation
Mixed phenotype
Unreduced
D
Meiotic products (%)
100
80
60
40
20
0
4x WT
4x nse2-1
Tetrad with micronuclei
Tetrad
Triad
Dyad

Figure 18. Analysis of male meiosis in 4x *nse2-1* plants. (A) Representative images of selected meiotic stages in 4x WT plants. Top row: Unsynapsed regions are detected in pachynema (arrowhead). At metaphase I, the chromosome associations are mostly bivalents and quadrivalents. In most meiocytes, chromosome segregation is correct, both in the first and second meiotic division, resulting in the formation of tetrads with balanced nuclei. Bottom row: Examples of metaphase I with a univalent, telophase I with a delay in chromosome segregation, and a metaphase II with chromatids resulting from the equational segregation of anaphase I univalent. Scale bars 5 μm. (B) Representative images of the phenotype observed in 4x *nse2-1* plants. As well as in WT plants, there were some unsynapsed regions at pachynema (arrowhead). At metaphase I, an increase in the frequency of univalents (arrow) was detected relative to the WT. Complex entanglements were also frequent at this stage. Telophases I displayed a high frequency of chromosome fragments. Meiotic problems were also evident during the second meiotic division in almost all cells analyzed, including chromosome fragmentation, chromatin bridges and/or abnormal chromosome segregation, and non-reduced meiocytes. At the end of the meiotic division, dyads, triads, tetrads, and tetrads with micronuclei were formed (bottom row). Scale bars 5 μm. (C) Quantification of the different phenotypes observed in 4x *nse2-1* plants during second meiotic division. (D) Quantification of division products at the end of meiosis (Yang et al., 2021b).

3.2.2 Tetraploid *nse2-1* and *nse4a-2* plants produce aneuploid offspring

The meiotic defects in 4x *nse2-1* plants may change the ploidy level of their offspring. To test this hypothesis, we measured the ploidy of progeny from 4x *nse2-1* plants. There were 96.7% (117 out of 120) tetraploids and 3.3% (3 out of 120) putative aneuploids found in self-pollinated 4x WT plants (Table 6). The minimal shifts of the flow cytometry peaks were detected in these three putative aneuploid WT plants relative to the known tetraploid control. There were 32.6% tetraploids, 47.8% putative aneuploids and 19.6% hexaploids detected from self-pollinated 4x *nse2-1* plants (n = 92) (Table 6 and Figure 19). 70.5% (31 out of 44) of the aneuploid mutant plants gained chromosomes and 29.5% (13 out of 44) lost chromosomes with the flow cytometry analysis (Table 6). The similar result was observed from the self-pollinated 4x *nse4a-2* plants (Table 6). To confirm the aneuploidy, cytology analysis was used to measure the mitotic chromosomes in the selected candidates (Figure 19C and D). Further, we performed reciprocal crossings between 4x WT and 4x *nse2* plants and followed by ploidy measurement in the F1 plants (Table 6). The result revealed that hexaploidy and aneuploidy offspring were caused by paternally and maternally (Table 6). Similar results were found in 4x *nse4a-2* plants (Table 6). Hence, we concluded that higher-polyploid and aneuploid offspring are produced from both patents in 4x SMC5/6 complex mutants.

Taken together, the results supported SMC5/6 complex is needed to maintain genome stability in autotetraploid Arabidopsis. Additional, our work in diploid and autotetraploid Arabidopsis indicated that autotetraploid plants have a generally higher frequency of but also higher tolerance for aneuploidy.

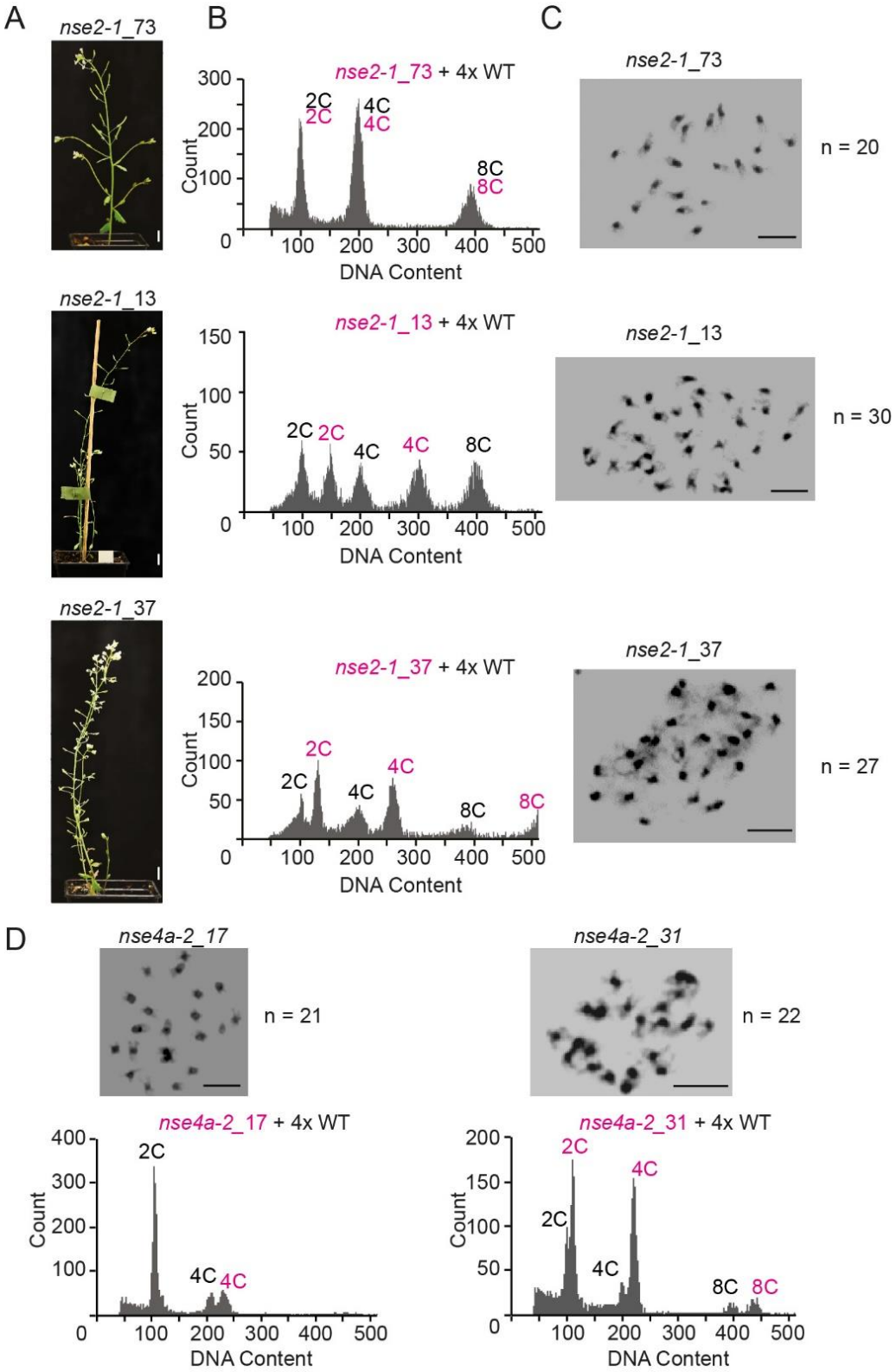

Figure 19. Ploidy determination in offspring of 4x *nse2-1* and 4x *nse4a-2* plants. (A) Phenotypes of selected four-weeks-old plants of *nse2-1*_73, *nse2-1*_13 and *nse2-1*_37. Scale bars 1 cm. (B) Flow-cytometry histograms of plants shown in (A), indicating that *nse2-1*_73 is tetraploid, *nse2-1*_13 is hexaploid and *nse2-1*_37 is aneuploid. For the ploidy measurements, the nuclei suspension was prepared by mixing leaves from tested *nse2-1* plants and 4x WT control. Multiple peaks correspond to nuclei of different C content as indicated. (C) Mitotic figures of *nse2-1*_73, *nse2-1*_13 and *nse2-1*_37 plants. The number of chromosomes is given next to the figures. Scale bars 5 µm. (D) Mitotic metaphase plates and flow-cytometry histograms of the ploidy level of two selected aneuploid *nse4-2* plants. Chromosome numbers are given right of the figures. Scale bars 5 µm. The ploidy levels were measured by preparing the nuclei suspension from a mix of the leaves of the *nse4a-2* candidate plant and 4x WT control. Note that an addition of a single or two chromosomes is clearer visible at 4C peaks (or 8C peaks if visible) (Yang et al., 2021b).

Table 6. Flow cytometry-based ploidy levels of F1 offspring plants from tetraploid (4x) WT, *nse2-1* and *nse4a-2* parents. 4x = tetraploid, 6x = hexaploid. + = DNA gain, - = DNA loss.

Mother	Father	Events (n)	Euploid		Aneuploid		
			4x	6x	Total	+	-
4x WT	4x WT	120	96.7	0.0	3.3	100.0	0.0
4x *nse2-1*	4x *nse2-1*	92	32.6	19.6	47.8	70.5	29.5
4x WT	4x *nse2-1*	163	75.4	8.6	16.0	88.5	11.5
4x *nse2-1*	4x WT	247	84.2	1.2	14.6	69.4	30.6
4x *nse4a-2*	4x *nse4a-2*	196	85.2	6.6	8.2	68.8	31.2
4x WT	4x *nse4a-2*	91	91.2	4.4	4.4	100.0	0.0
4x *nse4a-2*	4x WT	124	93.5	0.0	6.5	75.0	25.0

3.3 Original publications

3.3.1 Defects in meiotic chromosome segregation lead to unreduced male gametes in Arabidopsis SMC5/6 complex mutants

(Appendix I)

3.3.2 SMC5/6 complex is necessary for tetraploid genome stability in Arabidopsis thaliana

(Appendix II)

3.3.1 Defects in meiotic chromosome segregation lead to unreduced male gametes in Arabidopsis SMC5/6 complex mutants

Fen Yang [1], Nadia Fernández-Jiménez[1], Martina Tučková, Jan Vrána, Petr Cápal, Mariana Díaz, Mónica Pradillo, Ales Pecinka

[1]These authors contributed equally to this work.

The Plant Cell

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IF (2020): 11.277

Abstract:

Structural maintenance of chromosomes 5/6 (SMC5/6) complex is a crucial factor for preserving genome stability. Here, we show that mutants in several Arabidopsis thaliana SMC5/6 complex subunits produce triploid offspring. This is caused by a meiotic defect leading to the production of unreduced male gametes. The SMC5/6 complex mutants show an absence of chromosome segregation in the first and/or the second meiotic division and a partially disorganized microtubule network. Importantly, although the SMC5/6 complex is partly required for the repair of SPO11-induced DNA double-strand breaks, the non-reduction described here is independent of it. A frequent abortion of the ovules suggests that, if produced, such defects are maternally lethal. Upon fertilization with unreduced pollen, the unbalanced maternal and paternal genome dosage in endosperm most likely causes seed abortion observed in several SMC5/6 complex mutants. In conclusion, we found a novel SMC5/6 complex function in the maintenance of gametophytic ploidy in Arabidopsis.

3.3.2 SMC5/6 complex is necessary for tetraploid genome stability in *Arabidopsis thaliana*

Fen Yang, Nadia Fernández-Jiménez, Joanna Majka1, Mónica Pradillo, Ales Pecinka

Frontiers in Plant Science

doi: https://doi.org/10.3389/fpls.2021.748252

IF (2020): 5.753

Abstract:

Polyploidization is a common phenomenon in the evolution of flowering plants. However, only a few genes controlling polyploid genome stability, fitness, and reproductive success are known. Here, we studied the effects of loss-of-function mutations in NSE2 and NSE4A subunits of the Structural maintenance of chromosomes 5/6 (SMC5/6) complex in autotetraploid Arabidopsis thaliana plants. The diploid nse2 and nse4a plants show partially reduced fertility and produce about 10% triploid offspring with two paternal and one maternal genome copies. In contrast, the autotetraploid nse2 and nse4a plants were almost sterile and produced hexaploid and aneuploid progeny with the extra genome copies or chromosomes coming from both parents. In addition, tetraploid mutants had more severe meiotic defects, possibly due to the presence of four homologous chromosomes instead of two. Overall, our study suggests that the SMC5/6 complex is an important player in the maintenance of tetraploid genome stability and that autotetraploid Arabidopsis plants have a generally higher frequency of but also higher tolerance for aneuploidy compared to diploids.

3.4 Published abstracts – poster presentations

3.4.1 Understanding functions of STRUCTURAL MAINTENANCE OF CHROMOSOMES 5/6 complex during generative development

(Appendix III)

3.4.2 Analysis of SMC5/6 complex mutant defects during reproductive development in Arabidopsis

(Appendix IV)

3.4.3 Understanding functions of SMC5/6 complex during generative development in Arabidopsis

(Appendix V)

3.4.1 Understanding functions of STRUCTURAL MAINTENANCE OF CHROMOSOMES 5/6 complex during generative development

Fen Yang, Anna Nowicka, Mariana D áz, Ales Pecinka

In: Abstract of the "6th international Meeting Plant Genome Stability and Change"

Gatersleben, Germany, 2018

Abstract:

Flowering plants undergo a series of complex developmental events including production of gametes and seeds during generative development. Double fertilization is the beginning of seed developmental process in flowering plants, which produces the diploid embryo and the triploid nutritive tissue endosperm. Structural maintenance of chromosomes (SMC) 5/6 complex has a crucial function in control of genome stability and DNA damage repair. However, precise molecular mechanisms of SMC5/6 complex functions in plants are unknown. We found that the loss-of-function mutants in a NON-SMC ELEMENT (NSE) subunit of the SMC5/6 complex HPY2 (HIGH PLOIDY2) produces a smaller number of pollen with poor fertility. Mutants in HPY2 and NSE4A, the other NSE subunit, increase the number of aborted seeds. Mutations in both genes lead to delayed embryo development and its arrest latest in torpedo stage, while endosperm does not cellularize and its over-proliferation causes abnormally sized seeds. This suggests that SMC5/6 complex has unknown function in gametogenesis and seed development. We will show molecular and developmental phenotypes and will discuss possible causes of this defect.

3.4.2 Analysis of SMC5/6 complex mutant defects during reproductive development in Arabidopsis

Fen Yang, Nadia Fernández-Jiménez, Martina Tučková, Jan Vrána, Petr Cápal, Mariana Díaz, Mónica Pradillo, Ales Pecinka

In: Abstract of the "Second main INDEPTH meeting"

Prague, Czech Republic, 2019

Abstract:

Structural maintenance of chromosomes 5/6 (SMC5/6) complex has a crucial function in the organization of chromatin, control of genome stability and DNA damage repair. However, precise molecular mechanisms of SMC5/6 complex functions are unknown in plants. We found that mutations in HPY2, E3 SUMO ligase subunit of the SMC5/6 complex, cause uniparentally-inherited abnormal seed development characterized by poor embryo development and liquid endosperm. By searching for the cause of this defect, we noticed that *hpy2* plants have reduced pollen viability and produce pollen of variable sizes. Because large size of plant organs is often associated with higher polyploidy, we measured ploidy of the *hpy2* offspring and found that some plants are triploid. This suggested that SMC5/6 complex has unknown function in gametogenesis and seed development leading to polyploidization of the progeny plants. We will show molecular and developmental phenotypes and will discuss possible causes of these defects.

3.4.3 Understanding functions of SMC5/6 complex during generative development in Arabidopsis

Fen Yang, Nadia Fernández-Jiménez, Martina Tučková, Jan Vrána, Petr Cápal, Mariana Díaz, Mónica Pradillo, Ales Pecinka

In: Abstract of the "6th European Workshop on Plant Chromatin"

Cologne, Germany, 2019

Abstract:

Angiosperms undergo a series of complex developmental transitions including production of haploid gametes and seed development during generative development. Double fertilization starts the seed development in flowering plants. Most seeds contain the diploid embryo and the triploid nutritive tissue endosperm. Structural maintenance of chromosomes (SMC) 5/6 complex has a crucial function in control of genome stability and DNA damage repair. However, the role of SMC5/6 complex in plant development and stress responses is little known. We found that mutations in HPY2, E3 SUMO ligase subunit of the SMC5/6 complex, cause uniparentally-inherited abnormal seed development characterized by poor embryo development and liquid endosperm. By searching for the cause of this defect, we noticed that *hpy2* plants have reduced pollen viability and produce pollen of variable sizes. Because large size of plant organs is often associated with higher polyploidy, we measured ploidy of the *hpy2* offspring and found that some plants are triploid. This suggested that SMC5/6 complex has unknown function in gametogenesis and seed development leading to polyploidization of the progeny plants. We will show molecular and developmental phenotypes and will discuss possible causes of these defects.

3.5 Published abstracts – oral presentations

3.3.1 Analysis of SMC5/6 complex mutant defects during reproductive development in Arabidopsis

3.3.2 Understanding functions of SMC5/6 complex during generative development in Arabidopsis

3.3.3 Defects in meiotic chromosome segregation lead to triploid offspring in Arabidopsis SMC5/6 complex mutants

3.3.4 Defects in meiotic chromosome segregation lead to triploid offspring in Arabidopsis SMC5/6 complex mutants

3.5.1 Analysis of SMC5/6 complex mutant defects during reproductive development in Arabidopsis

Fen Yang, Nadia Fernández-Jiménez, Martina Tučková, Jan Vrána, Mariana Díaz, Mónica Pradillo, Ales Pecinka

In: the "Second main INDEPTH meeting"

Prague, Czech Republic, 2019

Abstract:

Structural maintenance of chromosomes 5/6 (SMC5/6) complex has a crucial function in the organization of chromatin, control of genome stability and DNA damage repair. However, precise molecular mechanisms of SMC5/6 complex functions are unknown in plants. We found that mutations in HPY2, E3 SUMO ligase subunit of the SMC5/6 complex, cause uniparentally-inherited abnormal seed development characterized by poor embryo development and liquid endosperm. By searching for the cause of this defect, we noticed that *hpy2* plants have reduced pollen viability and produce pollen of variable sizes. Because large size of plant organs is often associated with higher polyploidy, we measured ploidy of the *hpy2* offspring and found that some plants are triploid. This suggested that SMC5/6 complex has unknown function in gametogenesis and seed development leading to polyploidization of the progeny plants. We will show molecular and developmental phenotypes and will discuss possible causes of these defects.

3.5.2 Understanding functions of SMC5/6 complex during generative development in Arabidopsis

Fen Yang, Nadia Fernández-Jiménez, Martina Tučková, Jan Vrána, Anna Nowicka, Mariana Díaz, Mónica Pradillo, Ales Pecinka

In: the "6th European Workshop on Plant Chromatin"

Cologne, Germany, 2019

Abstract:

Angiosperms undergo a series of complex developmental transitions including production of haploid gametes and seed development during generative development. Double fertilization starts the seed development in flowering plants. Most seeds contain the diploid embryo and the triploid nutritive tissue endosperm. Structural maintenance of chromosomes (SMC) 5/6 complex has a crucial function in control of genome stability and DNA damage repair. However, the role of SMC5/6 complex in plant development and stress responses is little known. We found that mutations in HPY2, E3 SUMO ligase subunit of the SMC5/6 complex, cause uniparentally-inherited abnormal seed development characterized by poor embryo development and liquid endosperm. By searching for the cause of this defect, we noticed that *hpy2* plants have reduced pollen viability and produce pollen of variable sizes. Because large size of plant organs is often associated with higher polyploidy, we measured ploidy of the hpy2 offspring and found that some plants are triploid. This suggested that SMC5/6 complex has unknown function in gametogenesis and seed development leading to polyploidization of the progeny plants. We will show molecular and developmental phenotypes and will discuss possible causes of these defects.

3.5.3 Defects in meiotic chromosome segregation lead to triploid offspring in Arabidopsis SMC5/6 complex mutants

Fen Yang[1], Nadia Fernández-Jiménez[1], Martina Tučková, Jan Vrána, Petr Cápal, Mariana Díaz, Mónica Pradillo, Ales Pecinka

In: the "COST-INDEPTH final meeting"

Thessaloniki, Greece and online

[1]These authors contributed equally to this work.

Abstract

Maintenance of genome stability is a key issue for all living organisms. Structural maintenance of chromosomes 5/6 (SMC5/6) complex is a crucial factor for preserving genome stability. We detected triploid offspring from several Arabidopsis thaliana loss-of-function SMC5/6 complex mutants, which was caused by meiotic defects leading to unreduced microspores. Our genetic and cytological analysises showed an absence of chromosome segregation in the first and/or the second meiotic division and partially disorganized microtubule network in SMC5/6 complex mutants. Furthermore, this non-reduction is independent on their function in SPO11-induced DNA double-strand breaks repair. After fertilization with an unreduced pollen, the unbalanced maternal and paternal genome dosage in endosperm frequently causes seed abortion observed in several SMC5/6 complex mutants. In summary, our results reveal a novel function of SMC5/6 complex in maintenance of ploidy stability during generations in Arabidopsis thaliana.

3.5.4 Defects in meiotic chromosome segregation lead to triploid offspring in Arabidopsis SMC5/6 complex mutants

Fen Yang[1], Nadia Fernández-Jiménez[1], Martina Tučková, Jan Vrána, Petr Cápal, Mariana Díaz, Mónica Pradillo, Ales Pecinka

In: the "The Czech Plant Nucleus Workshop 2021"

Olomouc, Czech Republic

[1]These authors contributed equally to this work.

Abstract

Keeping genome stability is a key issue for all living organisms. Structural maintenance of chromosomes 5/6 (SMC5/6) in evolutionary conserved protein complex crucial for ensuring genome stability. We found that *Arabidopsis thaliana* loss-of-function SMC5/6 complex mutants generate triploid offspring. This is due to production of unreduced microspores during male meiosis and is associated with partially disorganized microtubule network. The absence of chromosome segregation was observed in both the first and the second meiotic division. Importantly, although the SMC5/6 complex is partly required for the repair of SPO11-induced DNA double-strand breaks, the observed non-reduction was SPO11-independent. The diploid pollen lead to an unbalanced maternal and paternal genome dosage in the endosperm, which is most likely responsible for seed abortion observed in SMC5/6 complex mutants. In summary, we describe a novel function of SMC5/6 complex in the maintenance of gametophytic ploidy in Arabidopsis.

4. CONCLUSION AND DISCUSSION

4.1 SMC5/6 complex is required for the maintenance of gametophytic ploidy in Arabidopsis

SMC5/6 complex is an evolutionary conserved ATP-dependent molecular machine involved in the maintenance of nuclear genome stability (Aragón, 2018). Here, we found that loss-of-function mutants from SMC5/6 complex subunits NSE2, NSE4A and SNI1 cause male meiotic defects, form diploid microspores and generate triploid offspring in Arabidopsis. But these phenotypes are absent in *smc6b-1* which is most likely due to partial functional redundancy of Arabidopsis SMC6 paralogs (Watanabe et al., 2009). It is not possible to analyze the homozygous *smc6a smc6b* plants as they are early embryo lethality (Watanabe et al., 2009). Lack of triploids for *nse4b-1* is because of the fact that NSE4B could not be connected with DNA damage repair (Díaz et al., 2019).

The triploidy defects were traced to male meiosis. Chromosome fragmentation was observed during meiosis I, suggesting the SMC5/6 complex is partly required for the repair of SPO11-induced DNA double-strand breaks. The resembles situation is in budding yeast, where SMC5/6 complex mutants accumulate HR (homologous recombination) intermediates resulting in joint molecules (JMs) (Copsey et al., 2013; Xaver et al., 2013; Menolfi et al., 2015). The meiotic cells containing high chromosome fragmentations are likely to produce non-viable gametes. Hence aneuploid *nse2* offspring were not detected. The second independent defect was characterized by the absence of chromosome segregation mainly during meiosis I, which could lead to the formation of unreduced gametes and eventually the triploid offspring. Further experiments and constructing various higher-order mutants are needed to reveal the underlying mechanism. Analysis of *nse2* ovules showed the presence of abnormal embryo sac structures, suggesting that any failures in female gametogenesis will lead to abortion. The viable unreduced male gametes fuse with haploid female gametes leading to triploid offspring. And it may also cause seed abortion due to the unbalanced maternal and paternal genome dosage in endosperm.

Taken together, we concluded that the SMC5/6 complex acts as a novel diplogamete suppressor in Arabidopsis. In the future, controlled ploidy changes or producing offspring with different chromosome numbers may promote our knowledge about meiosis and endosperm development in plants.

4.2 SMC5/6 complex is necessary for tetraploid genome stability in *Arabidopsis thaliana*

Here, we generated autotetraploid Arabidopsis WT and several SMC5/6 complex deficient mutants plants. 4x mutant causes severe defects in meiosis, reduces the pollen viability and enhances the fertility defects. The analysis of meiosis in 4x mutant pollen mother cells showed that 4x mutant generates tetrads with micronuclei, which were not observed in the 2x mutant pollen mother cells. Many of offspringof 4x mutant are aneuploids, equally caused by maternally and paternally. Rarely, the hexaploid offspring were produced by unreduced female gametes in 4x *nse2* plants. The absence of aneuploidy offspring and the viable unreduced female gametes in 2x *nse2* plants, supports these are unique phenotypes in tetraploid plants, indicating the importance of certain molecular regulators may be changed when polyploidization occurs.

In summary, our results confirmed SMC5/6 complex is important to maintain the tetraploid genome stability in Arabidopsis. Our work in diploid and autotetraploid Arabidopsis supports that autotetraploid plants have a generally higher frequency of but also higher tolerance for aneuploidy.

5. REFERENCES

Adams, S., Vinkenoog, R., Spielman, M., Dickinson, H.G., and Scott, R.J. (2000). Parent-of-origin effects on seed development in Arabidopsis thaliana require DNA methylation. Development **127**: 2493–2502.

Aragón, L. (2018). The Smc5/6 complex: New and old functions of the enigmatic long-distance relative. Annu. Rev. Genet. **52**: 89–107.

Bai, X., Peirson, B.N., Dong, F., Xue, C., and Makaroff, C.A. (1999). Isolation and characterization of SYN1, a RAD21-like gene essential for meiosis in Arabidopsis. Plant Cell **11**: 417–430.

Berchowitz, L.E., Francis, K.E., Bey, A.L., and Copenhaver, G.P. (2007). The role of AtMUS81 in interference-insensitive crossovers in A. thaliana. PLoS Genet. **3**: 1355–1364.

Berger, F. and Twell, D. (2011). Germline specification and function in plants. Annu. Rev. Plant Biol. **62**: 461–484.

Bergerat, A., De Massy, B., Gadelle, D., Varoutas, P.C., Nicolas, A., and Forterre, P. (1997). An atypical topoisomerase II from archaea with implications for meiotic recombination. Nature **386**: 414–417.

Bolaños-Villegas, P. (2021). The Role of Structural Maintenance of Chromosomes Complexes in Meiosis and Genome Maintenance: Translating Biomedical and Model Plant Research Into Crop Breeding Opportunities. Front. Plant Sci. **12**:659558.

Borde, V. (2007). The multiple roles of the Mre11 complex for meiotic recombination. Chromosom. Res. **15**: 551–563.

Börner, G.V., Kleckner, N., and Hunter, N. (2004). Crossover/noncrossover differentiation, synaptonemal complex formation, and regulatory surveillance at the leptotene/zygotene transition of meiosis. Cell **117**: 29–45.

Botany, S., By, P., Society, T.A., and Doi, P.T. (2004). The Role of Chromosomal Change in Plant Evolution by Donald A. Levin. Syst. Bot. **29**: 460–461.

Brownfield, L. and Köhler, C. (2011). Unreduced gamete formation in plants: Mechanisms and prospects. J. Exp. Bot. **62**: 1659–1668.

Brownfield, L., Yi, J., Jiang, H., Minina, E.A., Twell, D., and Köhler, C. (2015). Organelles maintain spindle position in plant meiosis. Nat. Commun. **6**:6492.

Cai, X., Dong, F., Edelmann, R.E., and Makaroff, C.A. (2003). The Arabidopsis SYN1 cohesin protein is required for sister chromatid arm cohesion and homologous chromosome pairing. J. Cell Sci. **116**: 2999–3007.

Caryl, A.P., Jones, G.H., and Franklin, F.C.H. (2003). Dissecting plant meiosis using Arabidopsis thaliana mutants. J. Exp. Bot. **54**: 25–38.

Casati, P. and Gomez, M.S. (2020). Chromatin dynamics during DNA damage and repair in plants: new roles for old players. J. Exp. Bot. **72**: 4119–4131.

Chakravarthy, S., Park, Y.J., Chodaparambil, J., Edayathumangalam, R.S., and Luger, K. (2005). Structure and dynamic properties of nucleosome core particles. FEBS Letters **573**: 895–898.

Chelysheva, L., Diallo, S., Vezon, D., Gendrot, G., Vrielynck, N., Belcram, K., Rocques, N., Márquez-Lema, A., Bhatt, A.M., Horlow, C. and Mercier, R. (2005). AtREC8 and AtSCC3 are essential to the monopolar orientation of the kinetochores during meiosis. J. Cell Sci. **118**: 4621–4632.

Chelysheva, L., Gendrot, G., Vezon, D., Doutriaux, M.P., Mercier, R., and Grelon, M. (2007). Zip4/Spo22 is required for class I CO formation but not for synapsis completion in Arabidopsis thaliana. PLoS Genet. **3**: 802–813.

Chen, C., Zhang, W., Timofejeva, L., Gerardin, Y., and Ma, H. (2005). The Arabidopsis ROCK-N-ROLLERS gene encodes a homolog of the yeast ATP-dependent DNA helicase MER3 and is required for normal meiotic crossover formation. Plant J. **43**: 321–334.

Chen, H., He, C., Wang, C., and Wang, X. (2021). RAD51 supports DMC1 by inhibiting the SMC5 / 6 complex during meiosis. Plant cell **33**: 2869-2882.

Cobbe, N. and Heck, M.M.S. (2000). Review: SMCs in the world of chromosome biology - From prokaryotes to higher eukaryotes. J. Struct. Biol. **129**: 123–143.

Comai, L. (2005). The advantages and disadvantages of being polyploid. Nat. Rev. Genet. **6**: 836–846.

Couteau, F., Belzile, F., Horlow, C., Grandjean, O., Vezon, D., and Doutriaux, M.P. (1999). Random chromosome segregation without meiotic arrest in both male and female meiocytes of a dmc1 mutant of Arabidopsis. Plant Cell **11**: 1623–1634.

van der Crabben S. N., Hennus M. P., McGregor G. A., Ritter D. I., Nagamani S. C., Wells O. S., Harakalova M., Chinn I. K., Alt A., Vondrova L., Hochstenbach R., van Montfrans J. M., Terheggen-Lagro S. W., van Lieshout S., van Roosmalen M. J., Renkens I., Duran K., Nijman I. J., Kloosterman W. P., Hennekam E., Orange J. S., van Hasselt P. M., Wheeler D. A., Palecek J. J., Lehmann A. R., Oliver A. W., Pearl L. H., Plon S. E., Murray J. M., van Haaften G. (2016). Destabilized SMC5/6 complex leads to chromosome breakage syndrome with severe lung disease. J. Clin. Invest. **126**: 2881–2892.

Crismani W., Mercier R. (2012). What limits meiotic crossovers? Cell Cycle **11**, 3527–3528.

Cromer, L., Jolivet, S., Horlow, C., Chelysheva, L., Heyman, J., De Jaeger, G., Koncz, C., De Veylder, L., and Mercier, R. (2013). Centromeric cohesion is protected twice at meiosis, by SHUGOSHINs at anaphase i and by PATRONUS at interkinesis. Curr. Biol. **23**: 2090–2099.

d'Erfurth, I., Cromer, L., Jolivet, S., Girard, C., Horlow, C., Sun, Y., To, J.P.C., Berchowitz, L.E., Copenhaver, G.P., and Mercier, R. (2010). The CYCLIN-A CYCA1;2/TAM is required for the meiosis I to meiosis II transition and cooperates with OSD1 for the prophase to first meiotic division transition. PLoS Genet. **6**: 1–12.

D'Erfurth, I., Jolivet, S., Froger, N., Catrice, O., Novatchkova, M., and Mercier, R. (2009). Turning meiosis into mitosis. PLoS Biol. **7**: e1000124.

D'Erfurth, I., Jolivet, S., Froger, N., Catrice, O., Novatchkova, M., Simon, M., Jenczewski, E., and Mercier, R. (2008). Mutations in AtPS1 (Arabidopsis thaliana Parallel Spindle 1) lead to the production of diploid pollen grains. PLoS Genet. **4**: 1–9.

Datura, O. (1935). Appearance of tetraploids. Journal of Agricultural Research **50**: 591–606.

Decorsière, A., Mueller, H., Van Breugel, P.C., Abdul, F., Gerossier, L., Beran, R.K., Livingston, C.M., Niu, C., Fletcher, S.P., Hantz, O., and Strubin, M. (2016). Hepatitis B virus X protein identifies the Smc5/6 complex as a host restriction factor.

Nature **531**: 386–389.

Diaz, M. and Pecinka, A. (2018). Scaffolding for repair: Understanding molecular functions of the SMC5/6 complex. Genes **9**: 36.

Díaz, M., Pečinková, P., Nowicka, A., Baroux, C., Sakamoto, T., Gandha, P.Y., Jeřábková, H., Matsunaga, S., Grossniklaus, U., and Pecinka, A. (2019). The SMC5/6 complex subunit NSE4A is involved in DNA damage repair and seed development. Plant Cell **31**: 1579–1597.

Doyle, G.G. (1986). Aneuploidy and inbreeding depression in random mating and self-fertilizing autotetraploid populations. Theor. Appl. Genet. **72**: 799–806.

Drews, G.N. and Koltunow, A.M.. (2011). The Female Gametophyte. Arab. B. **9**: e0155.

Duan, X., Sarangi, P., Liu, X., Rangi, G.K., Zhao, X., and Ye, H. (2009a). Structural and Functional Insights into the Roles of the Mms21 Subunit of the Smc5/6 Complex. Mol. Cell **35**: 657–668.

Duan, X., Yang, Y., Chen, Y.-H., Arenz, J., Rangi, G.K., Zhao, X., and Ye, H. (2009b). Architecture of the Smc5/6 Complex of Saccharomyces cerevisiae Reveals a Unique Interaction between the Nse5-6 Subcomplex and the Hinge Regions of Smc5 and Smc6. J. Biol. Chem. **284**: 8507–8515

Felsenfeld, G. and Groudine, M. (2003). Controlling the double helix. Nature **421**: 448–453.

Fousteri, M.I. and Lehmann, A.R. (2000). A novel SMC protein complex in Schizosaccharomyces pombe contains the Rad18 DNA repair protein. EMBO J. **19**: 1691–1702.

Gadelle, D., Filé, J., Buhler, C., and Forterre, P. (2003). Phylogenomics of type II DNA topoisomerases. BioEssays **25**: 232–242.

Gómez, R., Jordan, P.W., Viera, A., Alsheimer, M., Fukuda, T., Jessberger, R., Llano, E., Pendás, A.M., Handel, M.A., and Suja, J.A. (2013). Dynamic localization of SMC5/6 complex proteins during mammalian meiosis and mitosis suggests functions in distinct chromosome processes. J. Cell Sci. **126**: 4239–4252.

Graumann, P.L. and Knust, T. (2009). Dynamics of the bacterial SMC complex and SMC-

like proteins involved in DNA repair. Chromosom. Res. **17**: 265–275.

Green, L.C. et al. (2012). Contrasting roles of condensin I and condensin II in mitotic chromosome formation. J. Cell Sci. **125**: 1591–1604.

Greer, E., Martín, A.C., Pendle, A., Colas, I., Jones, A.M.E., Moore, G., and Shaw, P. (2012). The Ph1 Locus Suppresses Cdk2-Type Activity during Premeiosis and Meiosis in Wheat. Plant Cell **24**: 152–162.

Grelon, M., Vezon, D., Gendrot, G., and Pelletier, G. (2001). AtSPO11-1 is necessary for efficient meiotic recombination in plants. EMBO J. **20**: 589–600.

Haig, D. and Wilczek, A. (2006). Sexual conflict and the alternation of haploid and diploid generations. Philos. Trans. R. Soc. B Biol. Sci. **361**: 335–343.

Hamamura, Y., Saito, C., Awai, C., Kurihara, D., Miyawaki, A., Nakagawa, T., Kanaoka, M.M., Sasaki, N., Nakano, A., Berger, F., and Higashiyama, T. (2011). Live-cell imaging reveals the dynamics of two sperm cells during double fertilization in Arabidopsis thaliana. Curr. Biol. **21**: 497–502.

Hartung, F., Wurz-Wildersinn, R., Fuchs, J., Schubert, I., Suer, S., and Puchta, H. (2007). The catalytically active tyrosine residues of both SPO11-1 and SPO11-2 are required for meiotic double-strand break induction in Arabidopsis. Plant Cell **19**: 3090–3099.

Hehenberger, E., Kradolfer, D., and Köhler, C. (2012). Endosperm cellularization defines an important developmental transition for embryo development. Development **139**: 2031–2039.

Henry, I.M., Dilkes, B.P., Young, K., Watson, B., Wu, H., and Comai, L. (2005). Aneuploidy and genetic variation in the Arabidopsis thaliana triploid response. Genetics **170**: 1979–1988.

Higashiyama, T. (2002). The synergid cell: attractor and acceptor of the pollen tube for double fertilization. J. Plant Res **115**: 0149–0160.

Higgins, J.D., Armstrong, S.J., Franklin, F.C.H., and Jones, G.H. (2004). The Arabidopsis MutS homolog AtMSH4 functions at an early step in recombination: Evidence for two classes of recombination in Arabidopsis. Genes Dev. **18**: 2557–2570.

Higgins, J.D., Sanchez-Moran, E., Armstrong, S.J., Jones, G.H., and Franklin, F.C.H. (2005). The Arabidopsis synaptonemal complex protein ZYP1 is required for chromosome synapsis and normal fidelity of crossing over. Genes Dev. **19**: 2488–2500.

Higgins, J.D., Vignard, J., Mercier, R., Pugh, A.G., Franklin, F.C.H., and Jones, G.H. (2008). AtMSH5 partners AtMSH4 in the class I meiotic crossover pathway in Arabidopsis thaliana, but is not required for synapsis. Plant J. **55**: 28–39.

Hirano, T. (2006). At the heart of the chromosome: SMC proteins in action. Nat. Rev. Mol. Cell Biol. **7**: 311–322.

Hirano, T. (2012). Condensins: Universal organizers of chromosomes with diverse functions. Genes Dev. **26**: 1659–1678.

Hirota, T., Gerlich, D., Koch, B., Ellenberg, J., and Peters, J.M. (2004). Distinct functions of condensin I and II in mitotic chromosome assembly. J. Cell Sci. **117**: 6435–6445.

Holá, M., Vágnerová, R., and Angelis, K.J. (2021). Kleisin NSE4 of the SMC5 / 6 complex is necessary for DNA double strand break repair , but not for recovery from DNA damage in Physcomitrella (Physcomitrium patens). Plant Mol. Biol. (published online ahead of print).

Hollister, J.D. (2015). Minireview Polyploidy: adaptation to the genomic environment. New Phytol. **205**: 1034–1039.

Huang, L., Yang, S., Zhang, S., Liu, M., Lai, J., Qi, Y., Shi, S., Wang, J., Wang, Y., Xie, Q., and Yang, C. (2009). The Arabidopsis SUMO E3 ligase AtMMS21, a homologue of NSE2/MMS21, regulates cell proliferation in the root. Plant J. **60**: 666–678.

Hudson, J.J.R., Bednarova, K., Kozakova, L., Liao, C., Guerineau, M., Colnaghi, R., Vidot, S., Marek, J., Bathula, S.R., Lehmann, A.R., and Palecek, J. (2011). Interactions between the Nse3 and Nse4 components of the SMC5-6 complex identify evolutionarily conserved interactions between MAGE and EID families. PLoS One **6**: e17270

Da Ines, O., Degroote, F., Goubely, C., Amiard, S., Gallego, M.E., and White, C.I. (2013). Meiotic Recombination in Arabidopsis Is Catalysed by DMC1, with RAD51 Playing a Supporting Role. PLoS Genet. **9**: e1003787.

Ishida, T., Fujiwara, S., Miura, K., Stacey, N., Yoshimura, M., Schneider, K., Adachi,

S., Minamisawa, K., Umeda, M., and Sugimoto, K. (2009). SUMO E3 ligase HIGH PLOIDY2 regulates endocycle onset and meristem maintenance in Arabidopsis. Plant Cell **21**: 2284–2297.

Ishida, T., Yoshimura, M., Miura, K., and Sugimoto, K. (2012). MMS21/HPY2 and SIZ1, Two Arabidopsis SUMO E3 Ligases, Have Distinct Functions in Development. PLoS One **7**: 1–10.

Jenczewski, E. and Alix, K. (2004). From Diploids to Allopolyploids: The Emergence of Efficient Pairing Control Genes in Plants. CRC. Crit. Rev. Plant Sci. **23**: 21–45.

Jeppsson, K., Kanno, T., Shirahige, K., and Sjögren, C. (2014). The maintenance of chromosome structure: Positioning and functioning of SMC complexes. Nat. Rev. Mol. Cell Biol. **15**: 601–614.

Jiao, Y. et al. (2011). Ancestral polyploidy in seed plants and angiosperms. Nature **473**: 97–100.

Jones, G.H., Khazanehdari, K.A., and Ford-Lloyd, B. V (1996). Meiosis in the leek (Allium porrum L.) revisited. II. Metaphase I observations. Heredity **76**: 186–205.

Kegel, A. and Sjögren, C. (2010). The Smc5/6 complex: More than repair? Cold Spring Harb. Symp. Quant. Biol. **75**: 179–187.

Kerrebrock, A.W., Moore, D.P., Wu, J.S., and Orr-Weaver, T.L. (1995). Mei-S332, a drosophila protein required for sister-chromatid cohesion, can localize to meiotic centromere regions. Cell **83**: 247–256.

Kornberg, R.D. and Lorch, Y. (1999). Twenty-five years of the nucleosome, fundamental particle of the eukaryote chromosome. Cell **98**: 285–294.

Lam, W.S., Yang, X., and Makaroff, C.A. (2005). Characterization of Arabidopsis thaliana SMC1 and SMC3: Evidence that AtSMC3 may function beyond chromosomes cohesion. J. Cell Sci. **118**: 3037–3048.

Lehmann, A.R., Walicka, M., Griffiths, D.J., Murray, J.M., Watts, F.Z., McCready, S., and Carr, A.M. (1995). The rad18 gene of Schizosaccharomyces pombe defines a new subgroup of the SMC superfamily involved in DNA repair. Mol. Cell. Biol. **15**: 7067–7080.

Leitch, E. a R., Soltis, D.E., Soltis, P.S., Leitch, I.J., Pires, J.C., and Husband, B.C. (2004). The role of triploid hybrids in the evolutionary dynamics of mixed-ploidy populations. Biological Journal of the Linnean Society **82**: 537–546.

Li, G., Zou, W., Jian, L., Qian, J., Deng, Y., and Zhao, J. (2017). Non-SMC elements 1 and 3 are required for early embryo and seedling development in Arabidopsis. J. Exp. Bot. **68**: 1039–1054.

Li, G., Zou, W., Jian, L., Qian, J., and Zhao, J. (2019). AtNSE1 and AtNSE3 are required for embryo pattern formation and maintenance of cell viability during Arabidopsis embryogenesis. J. Exp. Bot. **70**: 6229–6244.

Li, W., Chen, C., Markmann-Mulisch, U., Timofejeva, L., Schmelzer, E., Ma, H., and Reiss, B. (2004). The Arabidopsis AtRAD51 gene is dispensable for vegetative development but required for meiosis. Proc. Natl. Acad. Sci. U. S. A. **101**: 10596–10601.

Li, X. (2011). Pollen Fertility/viability Assay Using FDA Staining. BIO-PROTOCOL **1**.

Li, X., Zhang, Y., Clarke, J.D., Li, Y., and Dong, X. (1999). Identification and cloning of a negative regulator of systemic acquired resistance, SNI1, through a screen for suppressors of npr1-1. Cell **98**: 329–339.

Liu, C.H., Finke, A., D áz, M., Rozhon, W., Poppenberger, B., Baubec, T., and Pecinka, A. (2015). Repair of DNA damage induced by the cytidine analog zebularine requires ATR and ATM in arabidopsis. Plant Cell **27**: 1788–1800.

Liu, C.M., McElver, J., Tzafrir, I., Joosen, R., Wittich, P., Patton, D., Van Lammeren, A.A.M., and Meinke, D. (2002). Condensin and cohesin knockouts in Arabidopsis exhibit a titan seed phenotype. Plant J. **29**: 405–415.

Liu, C.M. and Meinke, D.W. (1998). The titan mutants of Arabidopsis are disrupted in mitosis and cell cycle control during seed development. Plant J. **16**: 21–31.

Liu, M., Shi, S., Zhang, S., Xu, P., Lai, J., Liu, Y., Yuan, D., Wang, Y., Du, J., and Yang, C. (2014). SUMO E3 ligase AtMMS21 is required for normal meiosis and gametophyte development in Arabidopsis. BMC Plant Biol. **14**: 1–12.

Lloyd, A. and Bomblies, K. (2016). Meiosis in autopolyploid and allopolyploid Arabidopsis. Curr. Opin. Plant Biol. **30**: 116–122.

Locascio, A., Roig-Villanova, I., Bernardi, J., and Varotto, S. (2014). Current perspectives on the hormonal control of seed development in Arabidopsis and maize: A focus on auxin. Front. Plant Sci. **5**: 1–22.

Losada, A. and Hirano, T. (2005). Dynamic molecular linkers of the genome: The first decade of SMC proteins. Genes Dev. **19**: 1269–1287.

Ma, H. (2005). Molecular genetic analyses of microsporogenesis and microgametogenesis in flowering plants. Annu. Rev. Plant Biol. **56**: 393–434.

Martín, A.C., Shaw, P., Phillips, D., Reader, S., and Moore, G. (2014). Licensing MLH1 sites for crossover during meiosis. Nat. Commun. **5**: 1–5.

Mason, A.S. and Pires, J.C. (2015). Unreduced gametes: Meiotic mishap or evolutionary mechanism? Trends Genet. **31**: 5–10.

McDonald, W.H., Pavlova, Y., Yates, J.R., and Boddy, M.N. (2003). Novel Essential DNA Repair Proteins Nse1 and Nse2 Are Subunits of the Fission Yeast Smc5-Smc6 Complex. J. Biol. Chem. **278**: 45460–45467.

Meinke, D.W. (2013). Seed Development, Genetics of. Brenner's Encyclopedia of Genetics **6**: 344–345.

Mengiste, T., Revenkova, E., Bechtold, N., and Paszkowski, J. (1999). An SMC-like protein is required for efficient homologous recombination in arabidopsis. EMBO J. **18**: 4505–4512.

Mercier, R., Jolivet, S., Vezon, D., Huppe, E., Chelysheva, L., Giovanni, M., Nogué, F., Doutriaux, M.P., Horlow, C., Grelon, M., and Mézard, C. (2005). Two meiotic crossover classes cohabit in Arabidopsis: One is dependent on MER3, whereas the other one is not. Curr. Biol. **15**: 692–701.

Mercier, R., Mézard, C., Jenczewski, E., Macaisne, N., and Grelon, M. (2015). The molecular biology of meiosis in plants. Annu. Rev. Plant Biol. **66**: 297–327.

Mercier, R., Vezon, D., Bullier, E., Motamayor, J.C., Sellier, A., Lefèvre, F., Pelletier, G., and Horlow, C. (2001). SWITCH1 (SWI1): a novel protein required for the establishment of sister chromatid cohesion and for bivalent formation at meiosis. Genes Dev. **15**: 1859-1871.

Miao, C., Tang, D., Zhang, H., Wang, M., Li, Y., Tang, S., Yu, H., Gu, M., and Cheng, Z. (2013). Central region component1, a novel synaptonemal complex component, Is essential for meiotic recombination initiation in RiceC. Plant Cell **25**: 2998–3009.

Municio, C. et al. (2021). The Arabidopsis condensin CAP-D subunits arrange interphase chromatin. New Phytol. **230**: 972–987.

De Muyt, A., Pereira, L., Vezon, D., Chelysheva, L., Gendrot, G., Chambon, A., Lainé-Choinard, S., Pelletier, G., Mercier, R., Nogué, F., and Grelon, M. (2009). A high throughput genetic screen identifies new early meiotic recombination functions in Arabidopsis thaliana. PLoS Genet. **5**: e1000654.

De Muyt, A., Vezon, D., Gendrot, G., Gallois, J.L., Stevens, R., and Grelon, M. (2007). AtPRD1 is required for meiotic double strand break formation in Arabidopsis thaliana. EMBO J. **26**: 4126–4137.

Neuwald, A.F. and Hirano, T. (2000). HEAT repeats associated with condensins, cohesins, and other complexes involved in chromosome-related functions. Genome Res. **10**: 1445–1452.

Olins, A.L. and Olins, D.E. (1974). Spheroid Chromatin Units (ν Bodies). Science **183**: 330 LP – 332.

Olsen, O.A. (2004). Nuclear endosperm development in cereals and Arabidopsis thaliana. Plant Cell **16**: 214–227.

Ono, T., Losada, A., Hirano, M., Myers, M.P., Neuwald, A.F., and Hirano, T. (2003). Differential contributions of condensin I and condensin II to mitotic chromosome architecture in vertebrate cells. Cell **115**: 109–121.

Osborn, T.C. (2004). The contribution of polyploidy to variation in Brassica species. Physiol. Plant. **121**: 531–536.

Owen, H.A. and Makaroff, C.A. (1995). Ultrastructure of microsporogenesis and microgametogenesis in Arabidopsis thaliana (L.) Heynh. ecotype Wassilewskija (Brassicaceae). Protoplasma **185**: 7–21.

Pagnussat, G.C., Yu, H.J., Ngo, Q.A., Rajani, S., Mayalagu, S., Johnson, C.S., Capron, A., Xie, L.F., Ye, D., and Sundaresan, V. (2005). Genetic and molecular identification of genes required for female gametophyte development and function in Arabidopsis.

Development **132**: 603–614.

Palecek, J., Vidot, S., Feng, M., Doherty, A.J., and Lehmann, A.R. (2006). The Smc5-Smc6 DNA repair complex: Bridging of the Smc5-Smc6 heads by the kleisin, nse4, and non-kleisin subunits. J. Biol. Chem. **281**: 36952–36959.

Pawlowski, W.P., Wang, C.J.R., Golubovskaya, I.N., Szymaniak, J.M., Shi, L., Hamant, O., Zhu, T., Harper, L., Sheridan, W.F., and Cande, W.Z. (2009). Maize AMEIOTIC1 is essential for multiple early meiotic processes and likely required for the initiation of meiosis. Proc. Natl. Acad. Sci. U. S. A. **106**: 3603–3608.

Payne, F., Colnaghi, R., Rocha, N., Seth, A., Harris, J., Carpenter, G., Bottomley, W. E., Wheeler, E., Wong, S., Saudek, V., Savage, D., O'Rahilly, S., Carel, J. C., Barroso, I., O'Driscoll, M., and Semple, R. (2014). Hypomorphism in human NSMCE2 linked to primordial dwarfism and insulin resistance. J. Clin. Invest. **124**: 4028–4038.

Pebernard, S., McDonald, W.H., Pavlova, Y., John R. Yates, I., and Boddy, M.N. (2004). Nse1, Nse2, and a Novel Subunit of the Smc5-Smc6 Complex, Nse3, Play a Crucial Role in Meiosis. Mol. Biol. Cell. **15**: 4866–4876.

Pebernard, S., Wohlschlegel, J., McDonald, W.H., Yates, J.R., III, and Boddy, M.N. (2006). The Nse5-Nse6 Dimer Mediates DNA Repair Roles of the Smc5-Smc6 Complex. Mol. Cell. Biol. **26**: 1617-1630.

Peris, C.I.L., Rademacher, E.H., and Weijers, D. (2010). Green beginnings - pattern formation in the early plant embryo. Current Topics in Developmental Biology **91**: 1-27.

Peters, J.M., Tedeschi, A., and Schmitz, J. (2008). The cohesin complex and its roles in chromosome biology. Genes Dev. **22**: 3089–3114.

Pfluger, J. and Wagner, D. (2007). Accessibility in Plants. Curr. Opin. Plant Biol. **10**: 645–652.

Potts, P.R. and Yu, H. (2005). Human MMS21/NSE2 Is a SUMO Ligase Required for DNA Repair. Mol. Cell. Biol. **25**: 7021-7032.

Pradillo, M., López, E., Linacero, R., Romero, C., Cuñado, N., Sánchez-Morán, E., and Santos, J.L. (2012). Together yes, but not coupled: New insights into the roles of RAD51 and DMC1 in plant meiotic recombination. Plant J. **69**: 921–933.

Prakash, S. and Prakash, L. (1977). Increased spontaneous mitotic segregation in MMS-sensitive mutants of Saccharomyces cerevisiae. Genetics **87**: 229–236.

Ramsey, J. and Schemske, D.W. (2002). Neopolyploidy in flowering plants. Annu. Rev. Ecol. Syst. **33**: 589–639.

Ravi, M., Marimuthu, M.P.A., and Siddiqi, I. (2008). Gamete formation without meiosis in Arabidopsis. Nature **451**: 1121–1124

Sakamoto, T., Inui, Y.T., Uraguchi, S., Yoshizumi, T., Matsunaga, S., Mastui, M., Umeda, M., Fukui, K., and Fujiwara, T. (2011). Condensin ii alleviates DNA damage and is essential for tolerance of boron overload stress in arabidopsis. Plant Cell **23**: 3533–3546.

Sattler, M.C., Carvalho, C.R., and Clarindo, W.R. (2016). The polyploidy and its key role in plant breeding. Planta **243**: 281–296.

Schleiffer, A., Kaitna, S., Maurer-Stroh, S., Glotzer, M., Nasmyth, K., and Eisenhaber, F. (2003). Kleisins: A superfamily of bacterial and eukaryotic SMC protein partners. Mol. Cell **11**: 571–575.

Schubert, V. (2009). SMC proteins and their multiple functions in higher plants. Cytogenet. Genome Res. **124**: 202–214.

Schubert, V., Lermontova, I., and Schubert, I. (2013). The Arabidopsis CAP-D proteins are required for correct chromatin organisation, growth and fertility. Chromosoma **122**: 517–533.

Sears, E.R. (1976). Genetic control of chromosome pairing in wheat. Annu. Rev. Genet. **10**: 31–51.

Seear, P.J., France, M.G., Gregory, C.L., Heavens, D., Schmickl, R., Yant, L., and Higgins, J.D. (2020). A novel allele of ASY3 is associated with greater meiotic stability in autotetraploid Arabidopsis lyrata. PLOS Genet. **16**: e1008900.

Sergeant, J., Taylor, E., Palecek, J., Fousteri, M., Andrews, E.A., Sweeney, S., Shinagawa, H., Watts, F.Z., and Lehmann, A.R. (2005). Composition and Architecture of the Schizosaccharomyces pombe Rad18 (Smc5-6) Complex . Mol. Cell. Biol. **25**: 172–184.

Siddiqi, I., Ganesh, G., Grossniklaus, U., and Subbiah, V. (2000). The dyad gene is required for progression through female meiosis in Arabidopsis. Development **127**: 197–207.

Siddiqui, N.U., Stronghill, P.E., Dengler, R.E., Hasenkampf, C.A., and Riggs, C.D. (2003). Mutations in Arabidopsis condensin genes disrupt embryogenesis, meristem organization and segregation of homologous chromosomes during meiosis. Development **130**: 3283–3295.

De Storme, N. and Geelen, D. (2011). The arabidopsis mutant jason produces unreduced first division restitution male gametes through a parallel/ fused spindle mechanism in meiosis II. Plant Physiol. **155**: 1403–1415.

Taschner, M., Basquin, J. ́, Steigenberger, B., Schaefer, I., Soh, Y.-M., Basquin, C., Lorentzen, E., Aschle, M.R. ̈, Scheltema, R.A., and Gruber, S. (2021). Nse5/6 inhibits the Smc5/6 ATPase and modulates DNA substrate binding. EMBO J **40**: e107807.

Tedeschi, F., Rizzo, P., Rutten, T., Altschmied, L., and Bäumlein, H. (2017). RWP-RK domain-containing transcription factors control cell differentiation during female gametophyte development in Arabidopsis. New Phytol. **213**: 1909–1924.

Uhlmann, F. (2016). SMC complexes: From DNA to chromosomes. Nat. Rev. Mol. Cell Biol. **17**: 399–412.

Villeneuve, A.M. and Hillers, K.J. (2001). Whence meiosis? Cell **106**: 647–650.

Wang, J., Blevins, T., Podicheti, R., Haag, J.R., Tan, E.H., Wang, F., and Pikaard, C.S. (2017). Mutation of Arabidopsis SMC4 identifies condensin as a corepressor of pericentromeric transposons and conditionally expressed genes. Genes Dev. **31**: 1601–1614.

Wang, M., Tang, D., Wang, K., Shen, Y., Qin, B., Miao, C., Li, M., and Cheng, Z. (2011). OsSGO1 maintains synaptonemal complex stabilization in addition to protecting centromeric cohesion during rice meiosis. Plant J. **67**: 583–594.

Wang, Y., Jha, A.K., Chen, R., Doonan, J.H., and Yang, M. (2010). Polyploidy-associated genomic instability in Arabidopsis thaliana. Genesis **48**: 254–263.

Watanabe, K., Pacher, M., Dukowic, S., Schubert, V., Puchta, H., and Schubert, I.

(2009). The STRUCTURAL MAINTENANCE of CHROMOSOMES 5/6 complex promotes sister chromatid alignment and homologous recombination after DNA damage in Arabidopsis thaliana. Plant Cell **21**: 2688–2699.

Watanabe, Y. and Nurse, P. (1999). Cohesin Rec8 is required for reductional chromosome segregation at meiosis. Nature **400**: 461–464.

Wijnker, E. and Schnittger, A. (2013). Control of the meiotic cell division program in plants. Plant Reprod. **26**: 143–158.

Wu, N. and Yu, H. (2012). The Smc complexes in DNA damage response. Cell Biosci. **2**: 5.

Xaver, M., Huang, L., Chen, D., and Klein, F. (2013). Smc5/6-Mms21 Prevents and Eliminates Inappropriate Recombination Intermediates in Meiosis. PLoS Genet. **9**: e1004067.

Xu, P., Yuan, D., Liu, M., Li, C., Liu, Y., Zhang, S., Yao, N., and Yang, C. (2013). AtMMS21, an SMC5/6 complex subunit, is involved in stem cell niche maintenance and DNA damage responses in Arabidopsis roots. Plant Physiol. **161**: 1755–1768.

Yan, S., Wang, W., Marqués, J., Mohan, R., Saleh, A., Durrant, W.E., Song, J., and Dong, X. (2013). Salicylic acid activates DNA damage responses to potentiate plant immunity. Mol. Cell **52**: 602–610.

Yang, C.Y., Spielman, M., Coles, J.P., Li, Y., Ghelani, S., Bourdon, V., Brown, R.C., Lemmon, B.E., Scott, R.J., and Dickinson, H.G. (2003). TETRASPORE encodes a kinesin required for male meiotic cytokinesis in Arabidopsis. Plant J. **34**: 229–240.

Yang, F., Fernández-Jiménez, N., Tučková, M., Vrána, J., Cápal, P., Díaz, M., Pradillo, M., and Pecinka, A. (2021a). Defects in meiotic chromosome segregation lead to unreduced male gametes in Arabidopsis SMC5/6 complex mutants. Plant Cell **33**: 3104–3119.

Yang, F., Fernández Jiménez, N., Majka, J., Pradillo, M., and Pecinka, A. (2021b). Structural Maintenance of Chromosomes 5/6 Complex Is Necessary for Tetraploid Genome Stability in Arabidopsis thaliana. Front. Plant Sci. **12**: 1–14.

Yang, W.C., Shi, D.Q., and Chen, Y.H. (2010). Female gametophyte development in flowering plants. Annu. Rev. Plant Biol. **61**: 89–108.

Yuan, D., Lai, J., Xu, P., Zhang, S., Zhang, J., Li, C., Wang, Y., Du, J., Liu, Y., and Yang, C. (2014). AtMMS21 regulates DNA damage response and homologous recombination repair in Arabidopsis. DNA Repair **21**: 140–147.

Yuan, L., Yang, X., and A. Makaroff, C. (2011). Plant Cohesins, Common Themes and Unique Roles. Curr. Protein Pept. Sci. **999**: 1–12.

Zabrady, K., Adamus, M., Vondrova, L., Liao, C., Skoupilova, H., Novakova, M., Jurcisinova, L., Alt, A., Oliver, A.W., Lehmann, A.R., and Palecek, J.J. (2016). Chromatin association of the SMC5/6 complex is dependent on binding of its NSE3 subunit to DNA. Nucleic Acids Res. **44**: 1064–1079.

Zamariola, L., De Storme, N., Vannerum, K., Vandepoele, K., Armstrong, S.J., Franklin, F.C.H., and Geelen, D. (2014a). SHUGOSHINs and PATRONUS protect meiotic centromere cohesion in Arabidopsis thaliana. Plant J. **77**: 782–794.

Zamariola, L., Tiang, C.L., De Storme, N., Pawlowski, W., and Geelen, D. (2014b). Chromosome segregation in plant meiosis. Front. Plant Sci. **5**: 1–19.

Zelkowski, M., Zelkowska, K., Conrad, U., Hesse, S., Lermontova, I., Marzec, M., Meister, A., Houben, A., and Schubert, V. (2019). Arabidopsis NSE4 proteins act in somatic nuclei and meiosis to ensure plant viability and fertility. Front. Plant Sci. **10**: 1–20.

Zhang, C., Song, Y., Cheng, Z.H., Wang, Y.X., Zhu, J., Ma, H., Xu, L., and Yang, Z.N. (2012). The Arabidopsis thaliana DSB formation (AtDFO) gene is required for meiotic double-strand break formation. Plant J. **72**: 271–281.

Zhao, X. and Blobel, G. (2005). A SUMO ligase is part of a nuclear multiprotein complex that affects DNA repair and chromosomal organization. Proc. Natl. Acad. Sci. U. S. A. **102**: 4777–4782.

Zhou, Y. B., Gerchman, S. E., Ramakrishnan, V., Travers, A., and Muyldermans, S. (1998). Position and orientation of the globular domain of linker histone H5 on the nucleosome. Nature **395**: 402–405.

Zhu, L., Fernández-jiménez, N., Szymanska-lejman, M., and Pelé, A., Underwood, C.J., Serra, H., Lambing, C., Dluzewska, J., Bieluszewski, T., Pradillo, M., and Ziolkowski,

P.A. (2021). Natural variation identifies SNI1, the SMC5/6 component, as a modifier of meiotic crossover in Arabidopsis. Proc. Natl. Acad. Sci. U. S. A. **118**. 33.

6. LIST OF ABBREVIATIONS

AM1	*AMEIOTIC 1*
ASAP1	*ARABIDOPSIS SNI1 ASSOCIATED PROTEIN 1*
bp	base pairs
CAP-D2	*CHROMOSOME-ASSOCIATED POLYPEPTIDE D2*
CAP-D3	*CHROMOSOME-ASSOCIATED POLYPEPTIDE D3*
CAP-G	*CHROMOSOME-ASSOCIATED POLYPEPTIDE G*
CAP-G2	*CHROMOSOME-ASSOCIATED POLYPEPTIDE G2*
CAP-H	*CHROMOSOME-ASSOCIATED POLYPEPTIDE H*
CAP-H2	*CHROMOSOME-ASSOCIATED POLYPEPTIDE H2*
CCN	central cell nucleus
COs	crossovers
CRC1	*CENTRAL REGION COMPONENT 1*
CYCA1;2	*CYCLIN A1;2*
DAP	day after pollination
DAPI	4′,6-diamidino-2-phenylindole
DFO	*ARABIDOPSIS DSB FORMING*
DIC	differential interference contrast
DIF1	*DETERMINATE, INFERTILE1*
DMC1	*DISRUPTION OF MEIOTIC CONTROL 1*
DNA	deoxyribonucleic acid
DSBs	double-strand breaks
ECN	egg cell nucleus
EME1	*ESSENTIAL MEIOTIC ENDONUCLEASE 1*

FDA	fluorescein diacetate
JMs	joint molecules
HPY2	HIGH PLOIDY2
HR	Homologous recombination
MAGE	MELANOMA-ASSOCIATED ANTIGEN
MER3	*MEIOTIC RECOMBINATION*
MIM	*HYPER-SENSITIVE TO MMS, IR-RADIATION AND MITOMYCINC*
MMC	megaspore mother cell
MMS	methyl methane sulfonate
MMS21	*METHANE METHYLSULFONATE SENSITIVE 21*
MRE11	*MEIOTIC RECOMBINATION 11*
MSH4	*MUTS HOMOLOG 4*
MSH5	*MUTS HOMOLOG 5*
MUS81	*MMS AND UV SENSITIVE 81*
NBS1	*NIJMEGEN BREAKAGE SYNDROME 1*
NSE1	*NON-SMC ELEMENT 1*
NSE2	*NON-SMC ELEMENT 2*
NSE3	*NON-SMC ELEMENT 3*
NSE4	*NON-SMC ELEMENT 4*
NSE5	*NON-SMC ELEMENT 5*
NSE6	*NON-SMC ELEMENT 6*
OSD1	*OMISSION OF SECOND DIVISION 1*
PANS1	*PATRONUS 1*
Ph1	*Pairing homeollogous 1*

PS1	*PARALLEL SPINDLE 1*
PRD1	*PUTATIVE RECOMBINATION INITIATION DEFECT 1*
PRD2	*PUTATIVE RECOMBINATION INITIATION DEFECT 2*
PRD3	*PUTATIVE RECOMBINATION INITIATION DEFECT 3*
QRT1	*QUARTET 1*
RAD21.1	*RADIATION-SENSITIVE 21.1*
RAD21.2	*RADIATION-SENSITIVE 21.2*
RAD21.3	*RADIATION-SENSITIVE 21.3*
REC8	*MEIOTIC RECOMBINATION PROTEIN 8*
SC	synaptonymal complex
SCC1	*SISTER CHROMATIC COHESION 1*
SCC3	*SISTER CHROMATIC COHESION 3*
SGO	*SHUGOSHIN*
SMC	Structural maintenance of chromosomes
SNI1	*SUPPRESSOR OF NPR1-1, INDUCIBLE 1*
SUMO	small ubiquitin-like modifier
SWI1	*SWITCH 1*
SYN1	*SYNAPTIC 1*
SYN2	*SYNAPTIC 2*
SYN3	*SYNAPTIC 3*
TAM	*TARDY ASYNCHRONOUS MEIOSIS*
TES	*TETRASPORE*
UV	ultraviolet
WT	wild-type

ZIP1	*ZINC TRANSPORTER 1 PRECURSOR 1*
ZIP2	*ZINC TRANSPORTER 1 PRECURSOR 2*
ZIP3	*ZINC TRANSPORTER 1 PRECURSOR 3*
ZIP3	*ZINC TRANSPORTER 1 PRECURSOR 4*

7. LIST OF APPENDICES

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Appendix V Understanding functions of SMC5/6 complex during generative development in Arabidopsis

APPENDIX I

Defects in meiotic chromosome segregation lead to unreduced male gametes in Arabidopsis SMC5/6 complex mutants

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Research Article

Defects in meiotic chromosome segregation lead to unreduced male gametes in Arabidopsis SMC5/6 complex mutants

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A.P., M.P. and F.Y. conceived and designed the study. F.Y., N.F.J., M.T. and M.D. performed experiments. J.V. and P.C. calibrated and maintained flow cytometers and performed flow-sorting. A.P., F.Y., M.P. and N.F.J. analyzed data and interpreted the results. A.P., F.Y. and M.P. wrote the paper. All authors read and approved the final manuscript.
The author responsible for distribution of materials integral to the findings presented in this article in accordance with the policy described in the Instructions for Authors (https://academic.oup.com/plcell) is: Ales Pecinka (pecinka@ueb.cas.cz).

Abstract

Structural maintenance of chromosome 5/6 (SMC5/6) complex is a crucial factor for preserving genome stability. Here, we show that mutants for several Arabidopsis (*Arabidopsis thaliana*) SMC5/6 complex subunits produce triploid offspring. This phenotype is caused by a meiotic defect leading to the production of unreduced male gametes. The SMC5/6 complex mutants show an absence of chromosome segregation during the first and/or the second meiotic division, as well as a partially disorganized microtubule network. Importantly, although the SMC5/6 complex is partly required for the repair of SPO11-induced DNA double-strand breaks, the nonreduction described here is SPO11-independent. The measured high rate of ovule abortion suggests that, if produced, such defects are maternally lethal. Upon fertilization with an unreduced pollen, the unbalanced maternal and paternal genome dosage in the endosperm most likely causes seed abortion observed in several SMC5/6 complex mutants. In conclusion, we describe the function of the SMC5/6 complex in the maintenance of gametophytic ploidy in Arabidopsis.

Introduction

Meiosis is the reductional division that prevents chromosome doubling at every sexual generation. Chromosomes undergo homologous recombination (HR) during the first meiotic division, which creates new combinations of alleles. In the second meiotic division, the number of genome copies per nucleus is reduced to one copy. In most flowering plants, including Arabidopsis (*Arabidopsis thaliana*), haploid

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IN A NUTSHELL

Background: Plant sexual reproduction is an intricate affair. First, the number of chromosomes from the diploid plant is reduced by half (in haploid gametes) via meiosis, and later the gametes fuse in the process of double fertilization, which gives rise to seeds containing a diploid embryo and a triploid (meaning three chromosome sets) nourishing tissue, the endosperm. Although we know that this process is strictly regulated, the exact roles and functions of the many molecular factors involved remain unknown. The key factor affecting plant fertility is the Structural Maintenance of Chromosomes (SMC) 5/6 complex, which is involved in safeguarding genome stability.

Question: We wanted to understand why mutants in the SMC5/6 complex often develop abnormal seeds. We studied this question by analyzing male and female reproductive development in the mutants using genetic, molecular, and cytology methods.

Findings: We discovered that SMC5/6 complex mutants produce about 10% offspring with one maternal and two paternal genome copies. Such triploid plants have an unstable genomic constitution. By looking at different reproductive stages, we determined that a specific defect occurs during meiosis in the mutant, when the chromosomes do not divide to the poles. As a consequence, the meiocyte does not reduce its chromosome numbers. Surprisingly, this defect can happen during each of the two meiotic divisions, and aberrant microtubule organization and delayed division may be responsible. Pollination with diploid pollen, originating from unreduced meiocytes, gives rise to triploid embryo and tetraploid endosperm. The extra copy of the paternal genome in the endosperm causes problems with seed development and is responsible for frequent seed abortion.

Next steps: Understanding plant reproductive development is key to unlocking many biotechnological and breeding applications. At this point, chromosome non-reduction in Arabidopsis SMC5/6 complex mutants is still not fully understood at the molecular level. Therefore, further experiments and constructing various higher-order mutants are needed to reveal the underlying mechanism. In the future, controlled ploidy changes or producing offspring with different chromosome numbers may prove valuable to further our understanding of meiosis and endosperm development.

microspores develop into a tricellular microgametophyte (pollen) containing two sperm cells and one vegetative cell (Kawashima and Berger, 2014). In the female sporophyte, three out of the four haploid megaspores die, while the remaining megaspore divides three times into a megagametophyte (embryo sac) consisting of the haploid egg cell, the diploid central cell, and other accessory cells. Seed development starts with double fertilization, whereby the fertilized egg gives rise to the diploid embryo and the fertilized central cell to the triploid endosperm. The endosperm, containing one paternal and two maternal genomes (1p:2m), nourishes and supports embryo growth. However, an unbalanced maternal to paternal genome dosage in the endosperm slows down or even arrests embryo development and compromises seed viability (Scott et al., 1998).

Structural maintenance of chromosomes (SMC) complexes are key factors mediating large-scale chromatin organization in different functional contexts (Jeppsson et al., 2014; Uhlmann, 2016). The cohesin complex is essential for a plethora of processes, including sister chromatid attachment or cis-regulatory loop formation (Nasmyth and Haering, 2009; Rowley and Corces, 2018). The condensin complex compacts chromosomes via an asymmetric loop extrusion (Ganji et al., 2018). The functions of an enigmatic SMC5/6 complex are strongly linked to the maintenance of genome stability (Kegel and Sjögren, 2010; Aragón, 2018; Díaz and Pecinka, 2018). However, recent studies suggest that the SMC5/6 complex is also involved in the regulation of transcription (Decorsière et al., 2016), maintenance of centromere structure (Gómez et al., 2013), suppression of immune responses (Yan et al., 2013), or prevention of disease (Payne et al., 2014; van der Crabben et al., 2016). SMC5, SMC6, and four NON-SMC ELEMENTs 1–4 (NSEs) represent evolutionarily conserved subunits. SMC5 and SMC6 form a heterodimer via their hinge domains, while their head domains are bridged by the NSE1–NSE3–NSE4 subcomplex, which exhibits DNA binding capacity and can open/close the SMC5–SMC6 ring. Furthermore, the SMC5 coiled-coil region serves as a docking platform for the E3 small ubiquitin modifier ligase NSE2 (also named HIGH PLOIDY2 [HPY2] and METHYL METHANE SULFONATE SENSITIVITY 21 [MMS21]). In addition, each major phylogenetic group contains two to three nonconserved subunits implicated in the loading of the SMC5/6 complex onto chromatin: Nse5 and Nse6 together with BRCT-containing protein 1 (Brc1) in fission yeast (*Schizosaccharomyces pombe*) or Regulator of Ty1 Transposition107 (Rtt107) in budding yeast (*Saccharomyces cerevisiae*; Pebernard et al., 2006; Leung et al., 2011) and SMC5–6 complex localization factor proteins 1 and 2 (SLF1 and SLF2) in mammals (Räschle et al., 2015). The putative plant functional homologs of NSE5 and NSE6 are ARABIDOPSIS SNI1-ASSOCIATED PROTEIN 1 and SUPPRESSOR OF NPR1-1, INDUCIBLE 1 (SNI1), respectively (Yan et al., 2013).

Besides an essential role in the maintenance of plant genome stability (Mengiste et al., 1999; Watanabe et al., 2009;

Diaz et al., 2019), the Arabidopsis SMC5/6 complex controls apical meristem growth (Ishida et al., 2009), suppresses precocious flowering (Kwak et al., 2016) and hyper-immune responses (Li et al., 1999; Yan et al., 2013), and ensures normal gamete and seed development (Liu et al., 2014; Diaz et al., 2019; Zelkowski et al., 2019). While some of these functions have been well described at the phenotypic level through mutant analyses, the underlying mechanisms remain largely unknown. Here, we investigated the mechanism of seed abortion observed in several SMC5/6 mutants (Liu et al., 2014; Diaz et al., 2019), and show here that this phenotype is very likely due to paternally induced genome dosage imbalance in the endosperm. Some abnormal seeds escape this block and develop triploid offspring. Importantly, the defects originate as recombination-independent problems in chromosome segregation during meiosis of SMC5/6 complex mutants.

Results

Paternally inherited *nse2* mutations cause abnormal seed development

We used the Arabidopsis *NSE2* loss-of-function mutants (Supplemental Figure S1A) *nse2-1* (Q115*) and *nse2-2* (T-DNA; Ishida et al., 2009). Plants homozygous for either *nse2* allele were viable with developmental abnormalities including reduced height, small siliques, and short roots (Supplemental Figure S1, B–D). Dry seeds from wild-type (WT) plants were light brown and of regular shape, while mutant seeds were variable in color, size, and shape, including light/dark brown, large/small, and regular/shrunken (Figure 1A). Thirteen days after controlled manual pollination (DAP), WT plants produced 94.4% normal seeds, 2.8% abnormal seeds, and 2.8% aborted ovules (plants/siliques/cases, $n = 7/29/1{,}424$; Figure 1B; Supplemental Table S1). In contrast, *nse2-1* and *nse2-2* plants bore, respectively, 34.7% and 30.8% healthy-looking seeds, 16.0% and 23.4% abnormal seeds, and almost 49.3% and 45.8% aborted ovules (plants/siliques/cases, $n = 7/32/1{,}343$ for *nse2-1* and $n = 7/29/1{,}253$ *nse2-2*; Figure 1B; Supplemental Table S1), representing a significant increase in both aborted ovules and abnormal seeds (Fisher's exact test, $P < 0.005$ or lower; Figure 1, C and D; Supplemental Table S2). The abnormal seeds were larger with a glossy surface and watery endosperm (Figure 1B). Embryos were fully developed in WT seeds, but arrested between the torpedo and cotyledon stages in abnormal *nse2* seeds at 13 DAP (Figure 1E). At later stages, all abnormal seeds turned dark brown and shrunk (Figure 1A).

To assess the parental contribution to these phenotypes, we performed reciprocal crosses between WT and *nse2* plants (Figure 1, B–D; Supplemental Table S1). *nse2-1* or *nse2-2* plants pollinated by WT produced 2.0% and 3.7% abnormal seeds, respectively, which matched the percentage of 2.8% of abnormal seeds in self-pollinated WT. In contrast, pollination of WT plants with *nse2-1* or *nse2-2* pollen resulted in 14.4% and 21.5% of abnormal seeds, representing a 7.2- and 5.8-fold increase, respectively, relative to the reciprocal cross. This result indicated that the loss of NSE2 function causes paternally inherited aberrant seed development in Arabidopsis. We observed about 60% of aborted ovules in *nse2* plants pollinated by WT, suggesting severe maternal defects in *nse2* mutants as well. Therefore, we took

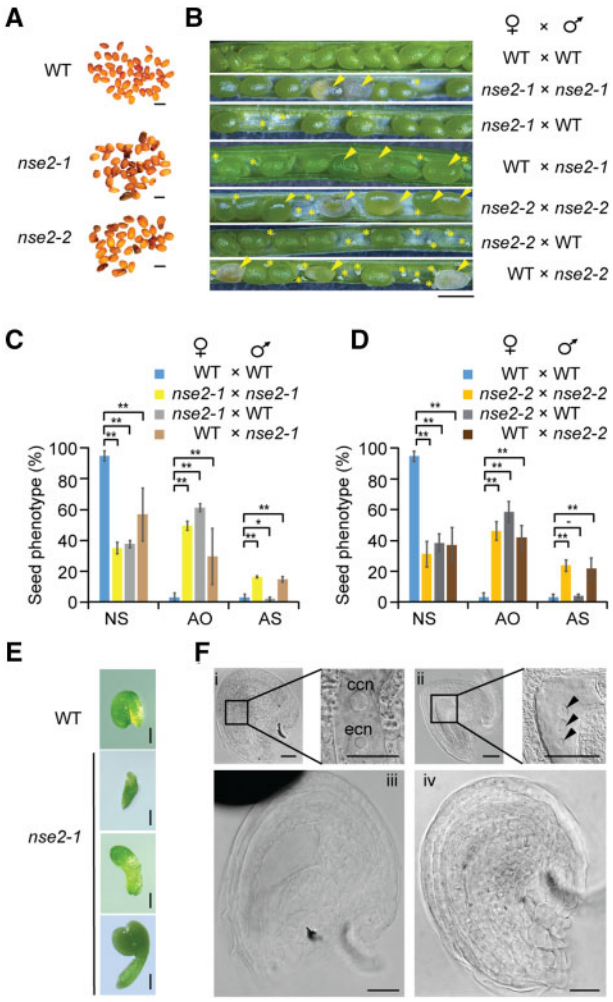

Figure 1 Paternally inherited abnormal seed development in *nse2* plants. A, Representative dry seeds of wild-type Col-0 (WT), *nse2-1*, and *nse2-2*. Note that the seeds from *nse2-1* and *nse2-2* plants represent a mixture of normal-sized, large, and aborted seeds. Scale bars = 1 mm. B, Dissected siliques 13 DAP. Aborted ovules are marked with yellow asterisks and abnormal seeds (typically larger, white or pale and partially transparent) with yellow arrowheads. Scale bar = 400 μm. C, D, Percentage of normal seeds (NS), aborted ovules (AO), and aborted seeds (AS) in manually pollinated WT, *nse2* mutants and their reciprocal crosses. Source values and basic counts are provided in Supplemental Tables S1 and S2. Significance in Fisher's exact test: $^{\cdot}P > 0.05$, $^{*}P < 0.005$, $^{**}P < 0.00001$. E, Representative embryos dissected from WT and *nse2-1* seeds 13 DAP. Scale bars = 200 μm. WT seeds contain a mature embryo, while *nse2* seeds have embryos arrested at the torpedo to cotyledon stages. F, Differential interference contrast micrographs of cleared *nse2-2* ovules. Scale bars = 50 μm. (i), Typical WT embryo sac showing one central cell nucleus (ccn) and one egg cell (ecn) nucleus; (ii), embryo sac with one smaller and two large nuclei; (iii), embryo sac without any nuclei; (iv), ovule without an embryo sac.

a closer look at *nse2-2* embryo sacs ($n = 55$; Figure 1F). We determined that 25.5% of embryo sacs look normal, 20% have three large nuclei, 10.9% have no nuclei, and 43.6% consist of ovules without embryo sac (Figure 1F[i–iv]). These severe defects suggested that either the two polar nuclei do not fuse into a central cell nucleus, that the megagametophytic nuclei degenerates, or that no megagametogenesis takes place. The presence of 45%–50% nondeveloping ovules in *nse2* mutants suggested that the defective embryo sacs abort before fertilization. These experiments showed that the paternally induced *nse2* defects are at least partially transmissible while the maternally induced defects result in substantial gametophytic lethality. Since abnormal seed development was caused paternally, we focused on male gametophyte development.

nse2 plants produce diploid sperm nuclei

To elucidate the paternally induced abnormal seed development, we first analyzed the viability of WT, *nse2-1* and *nse2-2* pollen by fluorescein diacetate (FDA) staining (Figure 2A). FDA staining indicated significantly lower pollen viability in *nse2-1* (63.6%; $n = 503$; Supplemental Table S3; Fisher's exact test, $P < 0.001$ or lower) and *nse2-2* (52.4%; $n = 609$; Supplemental Table S3; Fisher's exact test, $P < 0.001$ or lower) compared to WT (83.4%; $n = 1{,}504$) plants, as expected (Liu et al., 2014). However, we noticed surprisingly variable sizes in *nse2* pollen, in sharp contrast with the uniform size of WT pollen (Figure 2A). We quantified this observation by measuring pollen area using the same data as for the FDA analysis (Figure 2B). The area of WT pollen ranged from 250 to 500 μm^2 with a peak at 440.6 μm^2. The smallest *nse2-1* and *nse2-2* pollen had an area of about 250 μm^2, but the largest pollen grains were ~1,000 μm^2. In addition, the distribution of *nse2* pollen sizes showed two peaks at 484.1 and 737.8 μm^2 for *nse2-1*, and 444.5 and 662.4 μm^2 for *nse2-2*. Hence, *nse2* plants produce two cohorts of differently sized pollen.

Arabidopsis pollen size increases with nuclear DNA content (De Storme and Geelen, 2011). We thus hypothesized that the larger pollen might be polyploid. To test this hypothesis, we generated autotetraploid (4x) WT Arabidopsis by colchicine treatment and measured its pollen size. Indeed, pollen area ranged from 340 to ~1,000 μm^2, with a distribution peak at 661.4 μm^2 ($n = 357$) that perfectly matched the large pollen seen in *nse2-2*, although tetraploid pollen was still smaller than the largest pollen grains of *nse2-1* plants. Next, we used flow cytometry to obtain direct evidence of the ploidy of *nse2* pollen nuclei. For this analysis, it is essential to notice that Arabidopsis haploid pollen nuclei rest in different stages of cell cycle and therefore differ as to their DNA content (De Storme and Geelen, 2011). Sperm cell nuclei have DNA contents of 1C while vegetative cell nuclei have a 2C nuclear DNA content. We collected mature pollen from WT, *nse2-1* and *nse2-2* plants, destroyed vegetative nuclei as described (De Storme and Geelen, 2011), and measured ploidy of the remaining fraction. From WT preparations, we detected almost exclusively 1C nuclei, indicating that our experimental conditions effectively eliminated 2C vegetative nuclei. For *nse2-1* and *nse2-2* preparations, we obtained both 1C and 2C nuclei (Figure 2C), further indicating that *nse2* plants produce not only haploid but also diploid sperm cells.

To confirm this result with another approach, we produced a double homozygous line of *nse2-1* expressing the sperm nucleus-specific marker line *ProHTR10:HTR10-mRFP* (Histone three related [HTR10] fused to monomeric red fluorescent protein [mRFP]; (Ingouff et al., 2007) and repeated the analysis. We only detected 1C nuclei ($n = 427$) in the WT *HTR10-mRFP* background. In contrast, *nse2-1 HTR10-mRFP* pollen presented 68.8% of 1C and 31.2% of 2C nuclei ($n = 245$ and $n = 111$, respectively; Figure 2D). We then sorted nuclei onto microscopic slides according to their ploidy and inspected them using epifluorescence microscopy. In total, 93.6% of WT *HTR10-mRFP* and 85.5% of *nse2-1 HTR10-mRFP* 1C nuclei contained RFP fluorescence ($n = 109$ and $n = 304$, respectively; Figure 2, E and F; Supplemental Figure S2A). Importantly, 83.1% of *nse2-1 HTR10-mRFP* 2C nuclei also showed mRFP fluorescence ($n = 207$; Figure 2E, EF; Supplemental Figure S2A). Together, these results provided solid evidence that *nse2* plants produce diploid sperm nuclei.

Defective meiosis leads to unreduced microspores in *nse2* plants

2C *nse2* sperm nuclei indicated that the defects originated during microsporogenesis. Therefore, we analyzed the progression of meiosis in WT and *nse2-2* pollen mother cells (PMCs). Chromosome spreads stained with 4′,6-diamidino-2-phenylindole (DAPI) revealed no obvious differences between *nse2-2* and WT at prophase I. *nse2-2* PMCs displayed typical thread-like chromosomes at leptonema, homologous chromosome pairing at zygonema, and full synapsis at pachynema (Figure 3A). Chromosomes began to de-synapse and condense at diplonema and five bivalents were visible at diakinesis and metaphase I. However, despite the presence of five aligned bivalents, the appearance of the chromatin was not normal in 72.0% of *nse2-2* metaphase I PMCs (total $n = 93$). The bivalents were more stretched and elongated than in WT, chromosomes progressively lost compactness, and the constrictions in the chromatin began to be visible as segregation took place at anaphase I (Figure 3A). At almost all anaphase I figures (98.4%; $n = 122$), *nse2-2* chromosomes appeared de-condensed and thread-like chromatin fibers or fragments were visible, spanning the region between the segregating chromosomes (Figure 3A).

There is a cycle of de-condensation and re-condensation during the second meiotic division, and the chromosomes show the maximum degree of condensation in metaphase II of PMCs. During prophase II, two sets of five chromosomes are separated by a clear band of numerous organelles and re-condense before metaphase II in WT plants (Brownfield et al., 2015). At anaphase II, sister chromatids segregated to generate a tetrad with four balanced nuclei (Figure 3B). At

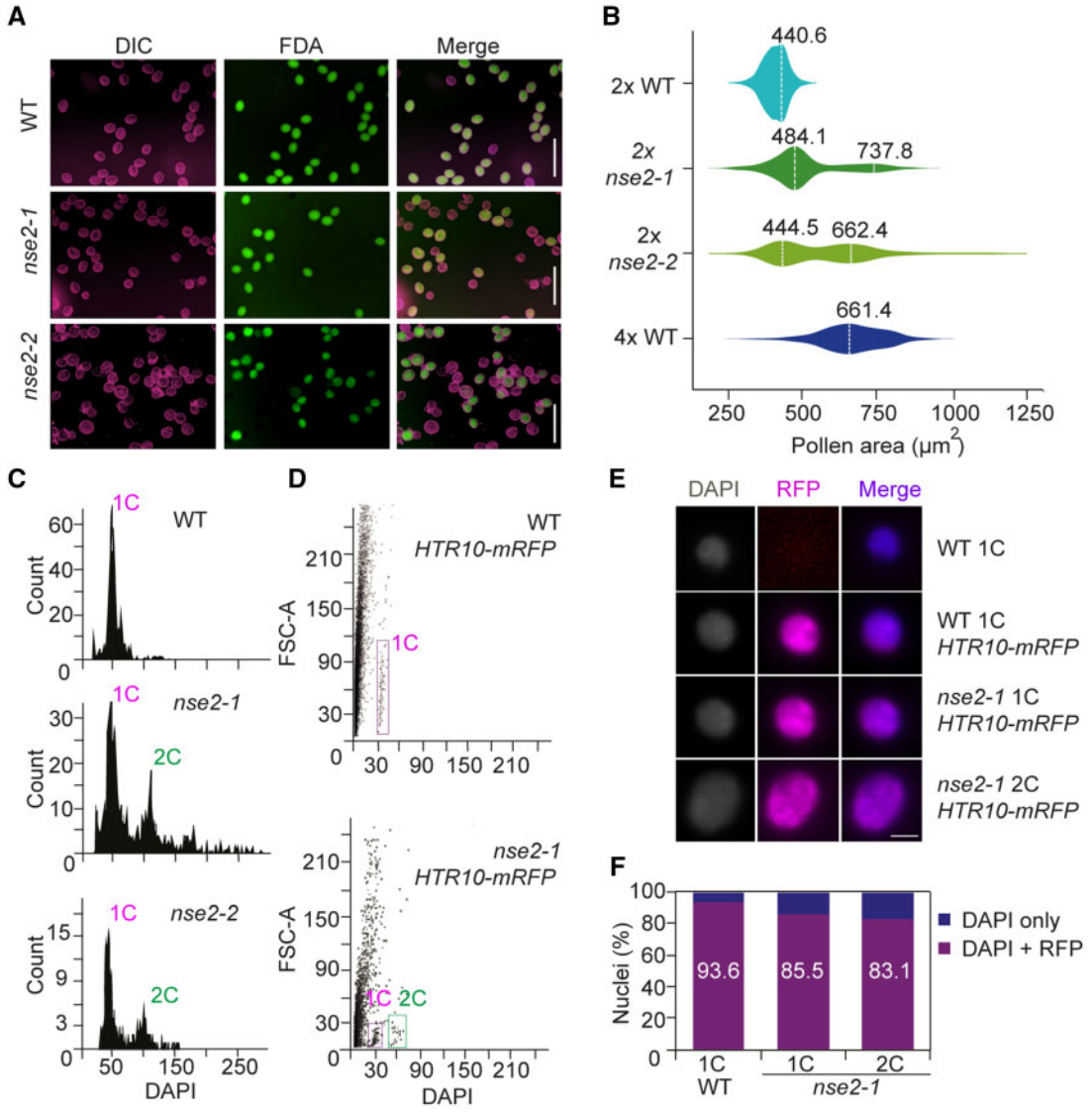

Figure 2 *nse2* plants produce diploid pollen. A, Analysis of pollen viability by FDA staining in mature pollen of WT, *nse2-1*, and *nse2-2* plants. Photographs of pollen were taken using differential interference contrast (DIC, pseudo-colored in magenta) while the FDA signals (pseudocolored in green) were observed by epifluorescence microscopy. Green signals indicate viable pollen. Note uniform sizes of pollen grains produced by WT and different sizes of pollen from *nse2* mutant plants. Scale bars = 100 μm. B, Violin plots showing the area of individual pollen grains (μm^2) in diploid (2×) and tetraploid (4×) WT and diploid *nse2* plants. The numbers above violins indicate the peak area. C, Flow cytometric histogram of sperm ploidy in WT, *nse2-1*, and *nse2-2*. The *x*-axis shows relative DAPI intensity and the *y*-axis particle count. D, Bivariate scatter plot of sperm nuclei from WT and *nse2-1* in *ProHTR10:HTR10-mRFP* (*HTR10-mRFP*) background. The *x*-axis shows relative DAPI intensity; the *y*-axis is the forward scatter parameter, indicating nuclei size. 1C and 2C nuclei populations are marked and the unmarked signals correspond to debris and organelles. 1C and 2C nuclei were sorted onto slides and used for the experiment shown in (E). E, Microscopy validation of enriched populations of sperm nuclei sorted based on their DNA content. Mature pollen of *HTR10-mRFP*, *nse2-1 HTR10-mRFP*, and nontransgenic WT were homogenized to eliminate vegetative nuclei, the remaining 1C and 2C nuclei were flow-sorted separately onto microscope slides and analyzed for RFP signals. Scale bar = 1 μm. F, Quantification of sorted nuclei observed from WT 1C, *nse2-1* 1C, and 2C in the *HTR10-mRFP* background.

prophase II, the frequency of normal-looking cells with two sets of five chromosomes decreased to only 31.3% in *nse2-2* ($n = 144$). The remaining meiocytes showed some of the following problems (Figure 3, B and C). (1) Nuclei containing two sets of homologous chromosomes (nonreduced nuclei). In such PMCs, the organelles were not organized in a defined band and appeared throughout the cytoplasm (19.4%), preventing the formation of two defined nuclei with five chromosomes (Supplemental Figures S3 and S4A). (2) One or more sets of chromosomes displaying chromosome fragmentation (15.3%). And (3) Chromosome bridges and chromatin masses linking them, suggesting unresolved anaphase I defects (34.0%); this last phenotype possibly combined the previous two. As a consequence of such abnormalities, we observed a range of meiotic products ($n = 591$) consisting of tetrads (49.2%), triads (5.1%), and dyads (45.7%) in *nse2-2* plants (Figure 3, B and D). Putative monads were detected but not quantified, as they are hard to distinguish with this method.

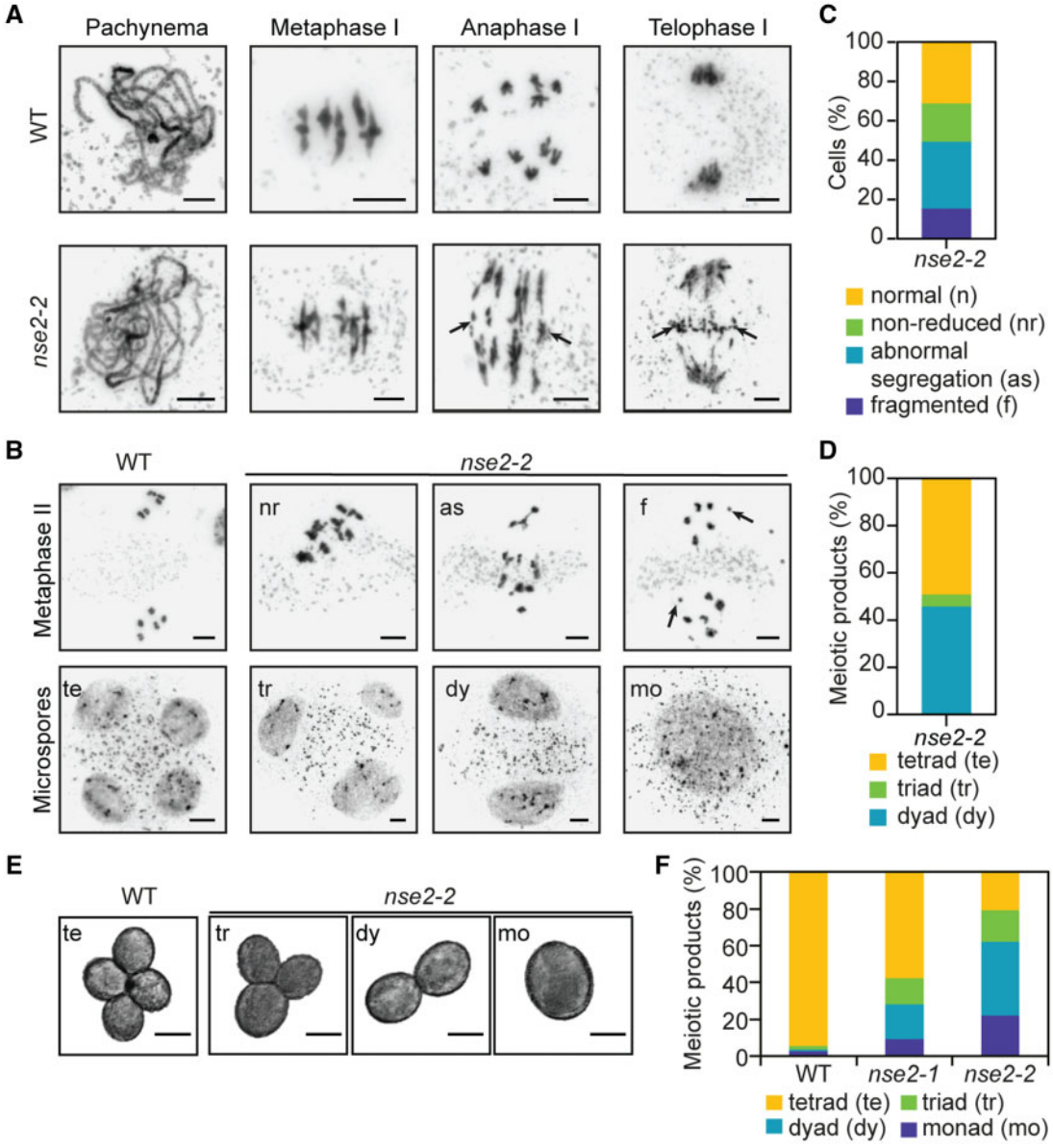

Figure 3 Characterization of male meiosis and meiotic products in *nse2*. A, First meiotic division. Pachynema: Full synapsis was detected in the WT and *nse2-2*. Metaphase I: Five bivalents were observed in *nse2-2*, but chromatin was less condensed than in WT cells, showing constrictions and fragments. Anaphase I: *nse2-2* chromosomes appeared de-condensed and thin thread-like fragments of chromatin were visible spanning the region between all the segregating chromosomes (arrows). Telophase I: A barrier formed by multiple chromosome fragments is apparent between the two groups of segregated chromosomes in the mutant (arrows). Scale bars = 5 μm. B, Representative images of the phenotypes in metaphase II and microspores. Metaphase II: WT figures are followed by nonreduced meiocyte (nr), abnormal chromosome segregation (as), and chromosome fragmentation (f). Fragments are marked with black arrows. Microspores: WT tetrad (te), followed by mutant triad (tr), dyad (dy), and monad (mo). Scale bars = 5 μm. C, Quantification of the different phenotypes observed in *nse2-2* second meiotic division. Only normal meiotic figures were observed in WT cells. D, Quantification of different meiotic products observed in *nse2-2*. E, Examples of meiotic products observed in the double homozygous *nse2-2 qrt1-4* mutant background. Scale bars = 20 μm. F, Quantification of meiotic products observed from the WT, *nse2-1*, and *nse2-2* in the *qrt1-4* background.

Instead, we used *nse2 qrt1* double mutant plants to quantify monads (Figure 3, E and F). We confirmed the chromosome constitution of the dyads (10 chromosomes in each nucleus) by performing immunolocalization to detect CENTROMERIC HISTONE H3 (CENH3; Supplemental Figure S4B).

To quantify meiotic products in an independent manner, we produced *nse2-1 qrt1-4* and *nse2-2 qrt1-4* double mutants. The *qrt1* mutation causes a stable association of the microspores arising from one meiosis (Preuss et al., 1994). The *NSE2 qrt1-4* plants produced on average 3.9 microspores per meiosis (575 microspores/148 meiotic products) and the products included 94.6% tetrads, 2.0% triads, 0.7% dyads, and 2.7% monads (Figure 3F; Supplemental Table S4). We measured an average of 3.2 microspores per

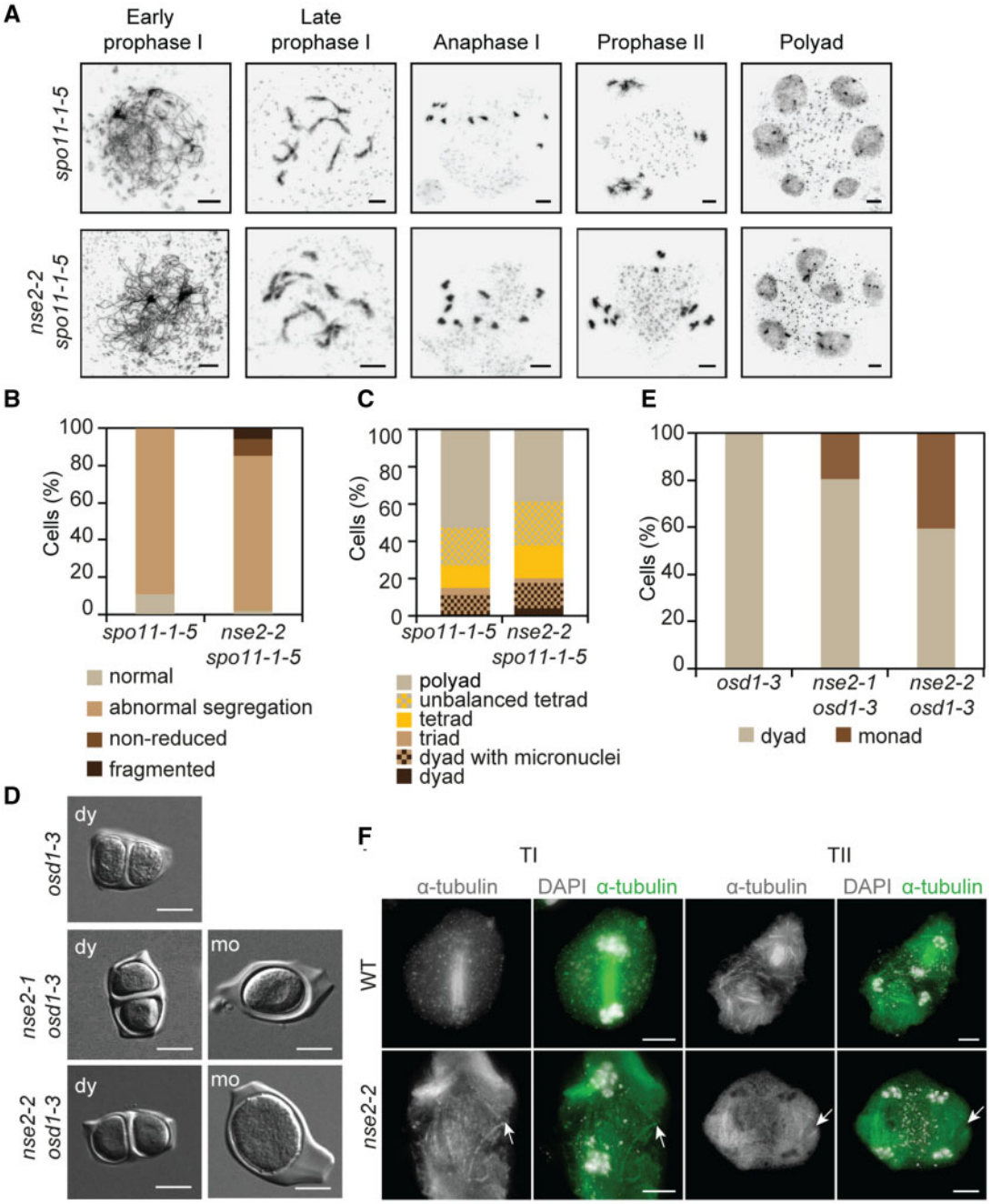

Figure 4 Analysis of male meiosis in *nse2 spo11* and *nse2 osd1* double mutants. A, Meiotic figures in *spo11-1-5* and *nse2-2 spo11-1-5* plants. Prophase I to anaphase I show chromosome univalents in both genotypes. Note the absence of chromosome fragments as observed in *nse2-2 spo11-1-5* anaphase I and telophase I (Figure 3A). Segregation to more than two poles can be observed in prophase II, resulting in formation of polyads. Scale bars = 5 μm. B, Percentage of meiocytes with the specified phenotypes in *spo11-1-5* and *nse2-2 spo11-1-5* plants. Source data are in Supplemental Table S5. C, Percentage of meiotic cells with given number of products in *spo11-1-5* and *nse2-2 spo11-1-5* plants. Source data are in Supplemental Table S9. D, Representative phenotypes of immature meiotic products from *osd1-3* and *nse2 osd1-3* plants. Microspores: dyad (dy) and monad (mo). Scale bars = 5 μm. E, Quantification of dyads and monads produced in *osd1-3* and *nse2 osd1-3* double mutants. F, Immunolocalization of α-tubulin. Representative images of telophase I (TI) and telophase II (TII) in the WT and *nse2-2*. In *nse2-2*, the microtubule bundles are diffuse, have lower density and a disorganized appearance, with some miss-localized microtubules (arrow). Scale bars = 5 μm.

meiosis in *nse2-1 qrt1-4* (1,042 microspores/322 meiotic products) and only 2.4 microspores per meiosis in *nse2-2 qrt1-4* (479 microspores/202 meiotic products). In *nse2-1 qrt1-4*, there were 57.8% tetrads, 14.0% triads, 19.2% dyads, and 9.0% monads (Figure 3F; Supplemental Table S4). Similarly, we observed a higher frequency of nontetrad meiotic products in *nse2-2 qrt1-4* (20.8% tetrads, 17.3% triads, 40.1% dyads, and 21.8% monads). The vast majority of *NSE2 qrt1-4* microspores had a normal shape (99.3%) and was viable (95.3%; Supplemental Table S4). *nse2-1 qrt1-4* showed

26.7% abnormal, small and shrunken microspores and only 65.0% of pollen was viable (Supplemental Figure S2B and Supplemental Table S2). *nse2-2 qrt1-4* showed 26.3% abnormal, small and shrunken microspores and only 40.7% of pollen was viable (Supplemental Table S4).

Altogether, the initial analysis of *nse2* meiosis revealed abnormal progression in some cells (there were 31.3% of apparently normal cells) with the defects originating/emerging from the later stages of meiosis I. Importantly, two major problems in chromosome behavior were observed (1) chromosome fragmentation and (2) defects in chromosome segregation.

Chromosome fragments are the consequence of SPO11-induced DNA breaks in *nse2* plants

To ascertain whether SPO11 function is involved in the chromosome fragmentation phenotype observed at anaphase I in *nse2*, we generated the *spo11-1-5 nse2-2* double mutant. Homozygous *spo11-1-5* plants showed reduced height and partial sterility, phenotypes that became more pronounced in the *spo11-1-5 nse2-2* double mutant (Supplemental Figure S5). Meiosis in the *spo11* single mutant is characterized by the presence of ten univalents at metaphase I (Grelon et al., 2001). In contrast with *nse2*, chromosome fragmentation was almost absent, with all ten univalents detected at metaphase I in the double mutant ($n = 24$; Figure 4A). The suppression of chromosome fragmentation in *spo11 nse2* demonstrated that fragments produced by the absence of NSE2 are caused by the failure to repair joint molecules (JMs) generated from SPO11-induced double-strand breaks (DSBs). To further investigate the HR process in *nse2*, we analyzed RAD51 foci numbers at pachynema and monitored synaptonemal complex formation by detecting its central component ZYP1 (Higgins et al., 2005; Supplemental Figure S6A). Defects in DSB repair during early stages are associated with persistent RAD51 foci on pachytene chromosomes (Wang et al., 2012). The numbers of RAD51 pachytene foci were not altered in *nse2* ($n = 36$ for WT and $n = 38$ for *nse2-2*; two-tailed Mann–Whitney–Wilcoxon test, $P = 0.891$; Supplemental Figure S6B and Supplemental Table S6), supporting the hypothesis that the SMC5/6 complex activity is required after RAD51. We also did not detect a delay in these early prophase I stages according to the quantification of cells with partial synapsis (27.78% in WT, $n = 36$, versus 13.16% in *nse2-2*, $n = 38$; Fisher's exact test, $P = 0.1526$; Supplemental Table S7). This result also demonstrated that there is no increased number of DSBs in the mutant and no delay in recombination, confirming a conserved role for the complex in resolving aberrant JMs (Xaver et al., 2013).

The problems in chromosome segregation thus appeared to be mostly independent of the meiotic recombination defects in *nse2-2*. We noticed differences in the percentage of the different meiotic products in the *nse2-2 spo11-1-5* double mutant relative to either *nse2-2* or *spo11-1-5* plants. In the double mutant, the frequency of dyads was lower than in the *nse2-2* single mutant (4.02% versus 45.69%, Fisher's exact test, $P < 0.0001$; Supplemental Figure S8) and there were polyads (which are not present in *nse2*). These differences may be explained by the presence of univalents at metaphase I. In addition, the *nse2-2 spo11-1-5* double mutant has a higher frequency of dyads (4.02% versus 0.98%), and a lower frequency of polyads (38.69% versus 52.77%) than the *spo11-1-5* single mutant (χ^2 test, $P = 0.0014$; Supplemental Figure S9), revealing that the formation of unreduced gametes in *nse2* is a consequence of problems independent of HR.

Hence, the chromosomal fragmentation phenotype in *nse2* can be explained by the role of SMC5 in the resolution of recombination intermediates but does not clarify how the unreduced gametes are produced.

Unreduced gametes arise via an HR-independent process during the meiosis I and II

To better understand the origin of unreduced gametes, we generated the *nse2 osd1* double mutant. OMISSION OF SECOND DIVISION (OSD1) is a negative regulator of the Anaphase promoting complex/Cyclosome needed for the second meiotic division, and its absence leads to diploid gametes (Cromer et al., 2012). The *nse2* mutant was crossed with heterozygous *osd1* plants and meiosis was analyzed in double homozygous F_2 plants. Homozygous *osd1-3* plants only produced dyads ($n = 199$; Figure 4, D and E). In double homozygous *nse2-1 osd1-3* and *nse2-2 osd1-3* plants, we observed 19.5% and 40.6% monads, respectively ($n = 174$ and $n = 64$, respectively; Figure 4, D and E). The presence of monads in the *nse2 osd1* double mutant provided strong genetic evidence that the chromosome segregation problems in *nse2* plants are due to anaphase I failures. However, it should be noted that *nse2* mutants (in the *qrt1-4* background) produce not only dyads but also 9%–21.8% monads (Figure 3, E and F), indicating that in about 1 out of 10 meioses in *nse2*, there is a nonreduction in both anaphases I and II.

Therefore, the problems in the segregation of homologous chromosomes appeared to be the main foundation of the unreduced gametes in *nse2*. Despite normal-looking five bivalents at metaphase I in *nse2* meiocytes, homologous chromosomes did not complete their migration to opposite poles on some occasions, and they were closer than in WT meiocytes. This observation was strongly associated with the improper position of chromosomes relative to the organelle band, which in these meiocytes is not positioned to function as a physical barrier (Supplemental Figures S3 and S4). As a consequence, instead of being equally divided on either side of the band, all chromosomes appeared only on one side at the end of the first meiotic division (Figure 3B, nr). In the second division, the repetition of this abnormal organization will lead to a monad while the normal progression will produce a dyad instead of tetrad.

To determine whether the chromosome segregation abnormalities are associated with problems in spindle organization, we conducted α-tubulin immunolocalization. In

general, WT and *nse2* PMCs behaved similarly, with microtubules organized as a radial array around each nucleus at prophase I and as polar oriented spindles at metaphase I. However, we observed abnormal spindle structures in mutant meiocytes (Figure 4F). The microtubule bundles had a lower density in *nse2* than in WT at telophase I, which was consistent with the appearance of chromosome organization problems. In addition, microtubules were disorganized around the chromosomal fragments and/or displaced sideways to the spindle periphery. At meiosis II, the spindles also showed a reduced number of microtubules and appeared more diffuse in *nse2-2* meiocytes (Figure 4F).

Based on these results, we conclude that the defects in chromosome segregation leading to unreduced gametes can occur in both anaphases I and II (being more frequent in the former) and are hallmarked by disorganized microtubules in the spindle and an aberrant position of the chromosomes relative to the organelle band.

nse2 plants produce triploid offspring

Unreduced gametes may lead to a polyploid progeny. Therefore, we analyzed ploidy in the progeny of self-pollinated WT and *nse2* plants by flow cytometry. In total, 97.6% WT, 87.6% *nse2-1*, and 63.4% *nse2-2* seeds germinated and grew at least until the cotyledon stage (Table 1 and Figure 5A). All 110 WT plants were diploid. In contrast, 7.6% *nse2-1* plants ($n = 380$) and 8.8% *nse2-2* plants ($n = 147$) were triploid (Table 1 and Figure 5B). We confirmed the triploidy of selected individuals by chromosome counting (Figure 5C). The triploids were sometimes slightly larger or smaller than diploids, but otherwise within the range of *nse2* phenotypes (Figure 5A, arrows). Surprisingly, we detected no *nse2* aneuploids by flow-cytometric ($n = 1{,}166$) or by cytological ($n = 6$) analysis. We also did not identify any tetraploids, suggesting that the increased ploidy is transmitted through only one parental gamete. However, we cannot fully exclude trisomy with whole or fragmented chromosomes.

To corroborate the uniparental induction of triploidy, we tested reciprocal crosses between WT and mutant (Table 1; throughout the paper female × male). Ploidy measurements of the F_1 2x (diploid) *nse2* × 2x WT hybrids revealed only diploids. In contrast, there were 6.7% and 5.2% of triploids among F_1 2x WT × 2x *nse2-1* or 2x WT × 2x *nse2-2* hybrids, respectively. A fusion of haploid maternal (m) and diploid paternal (p) gametes will lead not only to a triploid embryo, but also to tetraploid endosperm with an imbalanced parental dosage of 2m:2p genomes (Scott et al., 1998). Excess of the paternal genome will result in delayed or halted endosperm cellularization, arrested embryo development, and finally reduced or compromised seed viability (Köhler et al., 2012), which all align with the phenotypes of *nse2* mutants (Figure 1A). Consequently, the observed frequencies may be an underestimation because many triploid seeds die. To offer a balanced endosperm environment for diploid pollen, we reciprocally crossed 2x *nse2* plants with 4x WT plants. All crosses between 2x maternal and 4x paternal plants produced only few germinating seeds (2x WT × 4x WT, 5.6%; 2x *nse2-1* × 4x WT, 27.7%; 2x *nse2-2* × 4x WT, 4.2%; Table 1). In contrast, the control cross between 4x WT maternal and 2x WT paternal plants resulted in 72.9% germination and all the plants were triploids (Table 1). The germination rate of seeds derived from the crosses between 4x WT and 2x *nse2-1* or 2x *nse2-2* was over 90% (94.3%, $n = 299$, and 91.9%, $n = 160$, respectively) and hence better than in 4x WT × 2x WT cross (72.7%, $n = 88$). The F_1 hybrids of 4x WT × 2x *nse2-1* ($n = 282$) included 78.4% triploids, 21.3% tetraploids and 0.3% aneuploids. Similarly, the F_1 hybrids of 4x WT × 2x *nse2-2* ($n = 147$) plants were represented by 57.1% triploids, 41.5% tetraploids, and 1.4% aneuploids (Table 1). Hence, the frequency of viable polyploids derived from 2x *nse2* plants can be several-fold increased by crossing with 4x WT mothers, and our results strongly suggest that the triploid *nse2* offspring is caused exclusively by unreduced male gametes (diplogametes).

Mutations in *NSE4A* and *SNI1* also lead to triploid offspring

To test whether polyploidy also occurs in other Arabidopsis SMC5/6 complex mutants, we first checked the dry seeds

Table 1. Ploidy levels of offspring plants from *nse2* and WT parents and their F_1 hybrids. *n* = number, 2x = diploid, 3x = triploid, 4x = tetraploid.

Mother	Father	Sown (*n*)	Germinated (*n*)	Germination rate (%)	2× (%)	3× (%)	4× (%)	Aneuploids (%)
2× WT	2× WT	113	110	97.3	100	0	0	0
2× *nse2-1*	2× *nse2-1*	434	380	87.6	92.4	7.6	0	0
2× *nse2-2*	2× *nse2-2*	232	147	63.4	91.2	8.8	0	0
WT 2×	2× *nse2-1*	283	270	95.4	93.3	6.7	0	0
WT 2×	2× *nse2-2*	252	174	69.0	94.8	5.2	0	0
2× *nse2-1*	2× WT	86	82	95.3	100	0	0	0
2× *nse2-2*	2× WT	119	113	95.0	100	0	0	0
4× WT	2× WT	88	64	72.9	0	100	0	0
4× WT	2× *nse2-1*	299	284	94.3	0	78.4	21.3	0.3
4× WT	2× *nse2-2*	160	147	91.9	0	57.1	41.5	1.4
2× WT	4× WT	179	10	5.6	0	100	0	0
2× *nse2-1*	4× WT	112	31	27.7	0	100	0	0
2× *nse2-2*	4× WT	72	3	4.2	0	100	0	0

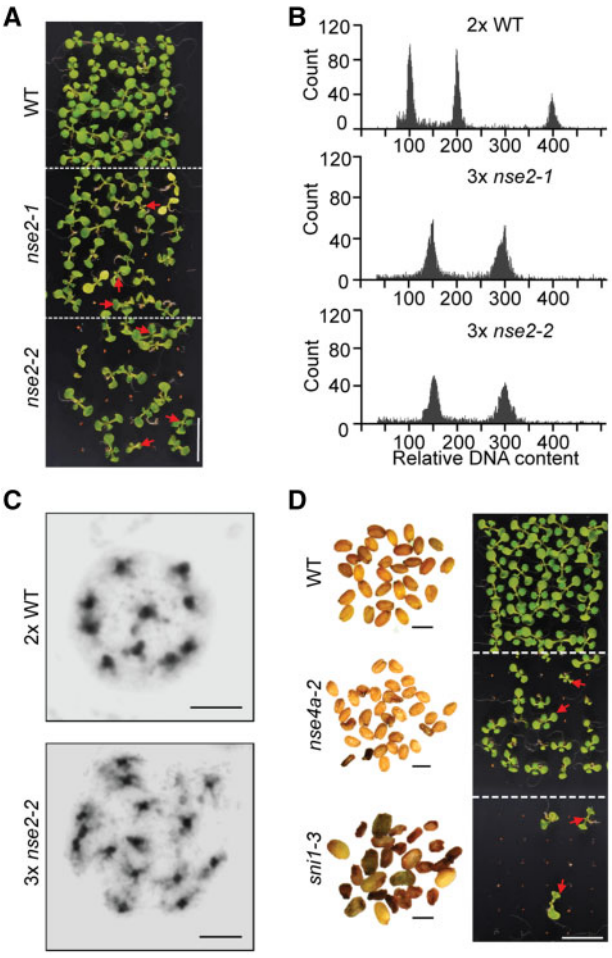

Figure 5 SMC5/6 complex mutants produce triploid offspring. A, Two-week-old in vitro-grown seedlings of the WT, *nse2-1*, and *nse2-2*. Triploid seedlings revealed by flow cytometry are indicated by red arrows. Scale bar = 1 cm. B, Examples of flow cytometry histograms showing representative profiles of peaks from diploid (2×) WT and triploid (3×) *nse2* plants. C, Representative mitotic prophase figures in 2× WT and 3× *nse2-2* plants. Scale bars = 5 µm. D, WT, *nse4a-2* and *sni1-3* mutant phenotypes. Left column: dry seeds. Scale bars = 1 mm. Right column: 2-week-old in vitro-grown seedlings. Triploid seedlings are indicated by red arrows. Scale bar = 1 cm.

from diploid *smc6b-1*, *nse4a-2*, *nse4b-1*, and *sni1-3* plants. The seeds of 2x *smc6b-1* and 2x *nse4b-1* plants were normal (Supplemental Figure S7). However, seeds from 2x *nse4a-2* and 2x *sni1-3* plants showed different sizes and shapes, including dark brown, large, and shrunken seeds (Figure 4D). By flow cytometry-based ploidy analysis, all *smc6b-1* and *nse4b-1* plants ($n = 103$ and 120, respectively; Table 2) were diploid. However, we observed 9.6% triploid *nse4a-2* plants ($n = 271$). As with *nse2*, the triploid plants were sometimes differently sized, but generally within the range of standard *nse4a-2* phenotypes (Figure 5D, right column, arrows). Analysis of the reciprocal crosses between 2x *nse4a-2* and 2x WT revealed that the triploidy is also caused exclusively by the male gamete (Table 2). Since *NSE4A* is expressed from both maternal and paternal gametophytes (Díaz et al., 2019), this result suggests that the defective maternal gametes do not give rise to viable offspring. Homozygous *sni1-3* mutants phenotypically resemble severely affected *nse2* plants, but are almost fully sterile. From homozygous 2x *sni1-3* parent, we obtained 137 offspring plants, of which 68.6% were triploid (Table 2). The reciprocal crossing of 2x WT × 2x *sni1-3* confirmed that all triploids are induced from the paternal side (Table 2).

Discussion

The SMC5/6 complex is an evolutionary conserved ATP-dependent molecular machine involved in the maintenance of nuclear genome stability (Aragón, 2018). Here, we discovered that loss of function in SMC5/6 complex subunits NSE2, NSE4A, and SNI1 lead to defective male meiosis, the formation of diploid microspores and triploid offspring in Arabidopsis. The absence of triploids in *smc6b-1* is most likely due to partial functional redundancy among Arabidopsis *SMC6* paralogs (Watanabe et al., 2009; Yan et al., 2013). Testing homozygous *smc6a smc6b* plants is not possible due to their early embryo lethality (Watanabe et al., 2009; Yan et al., 2013). Lack of triploids for *nse4b-1* is in agreement with the fact that NSE4B does not appear to be connected to DNA damage repair (Diaz et al., 2019).

We traced the origin of the triploidy defects to male meiosis. At the onset of meiosis, *nse2* meiotic figures show normal five bivalents at metaphase I (Figure 3A), indicating the formation of normal crossovers between homologs, ensuring the presence of obligatory chiasma. We further confirmed normal progression of HR and complete synapsis at pachynema (Supplemental Figure S6). From metaphase I onward, we observed two abnormal phenotypes in *nse2* mutants. The first phenotype was characterized by chromosome fragmentation during meiosis I, while the second phenotypes were associated with defects in chromosome segregation mainly during meiosis I. The second phenotype is relevant for the formation of unreduced gametes, but both phenotypes will be discussed and visualized as the working model of SMC5/6 complex action in Arabidopsis reproductive development (Figure 6).

The *nse2* chromosome fragmentation phenotype is associated with frequent bridges, entanglements, and concatenations from metaphase to telophase I (Figure 3A). Here, we confirmed that these problems in meiotic HR are due to the presence of toxic recombination HR intermediates, as evidenced by a drastic reduction in chromosome fragments in the *spo11 nse2* double mutant (Figure 4, A and B). We also demonstrated that these recombination failures are not a consequence of a higher number of DSBs or of a delay in the repair of these DSBs, as revealed by the immunolocalization results of RAD51 in pachynema (Supplemental Figure S6). These observations resemble the situation in budding yeast, where the accumulation of HR intermediates in SMC5/6 complex mutants result in JMs (Copsey et al., 2013; Xaver et al., 2013; Menolfi et al., 2015). Arabidopsis meiotic cells with highly fragmented chromosomes are likely to result in nonviable gametes or may not even progress to

Table 2. Ploidy levels of offspring plants from *nse4a-2*, *sni1-3*, and WT parents and their F_1 hybrids. *n* = number, 2× = diploid, 3× = triploid.

Mother	Father	Sown (*n*)	Germinated (*n*)	Germination rate (%)	2× (%)	3× (%)
2× WT	2× WT	113	110	97.3	100	0
2× *nse4a-2*	2× *nse4a-2*	300	271	90.3	90.4	9.6
2× WT	2× *nse4a-2*	87	83	95.4	86.7	13.3
2× *nse4a-2*	2× WT	93	93	100	100	0
2× *sni1-3*	2× *sni1-3*	740	137	18.5	31.4	68.6
2× WT	2× *sni1-3*	160	22	13.8	31.8	68.2
2× *sni1-3*	2× WT	98	81	82.7	100	0
2× *smc6b-1*	2× *smc6b-1*	119	103	86.6	100	0
2× *nse4b-1*	2× *nse4b-1*	135	120	88.9	100	0

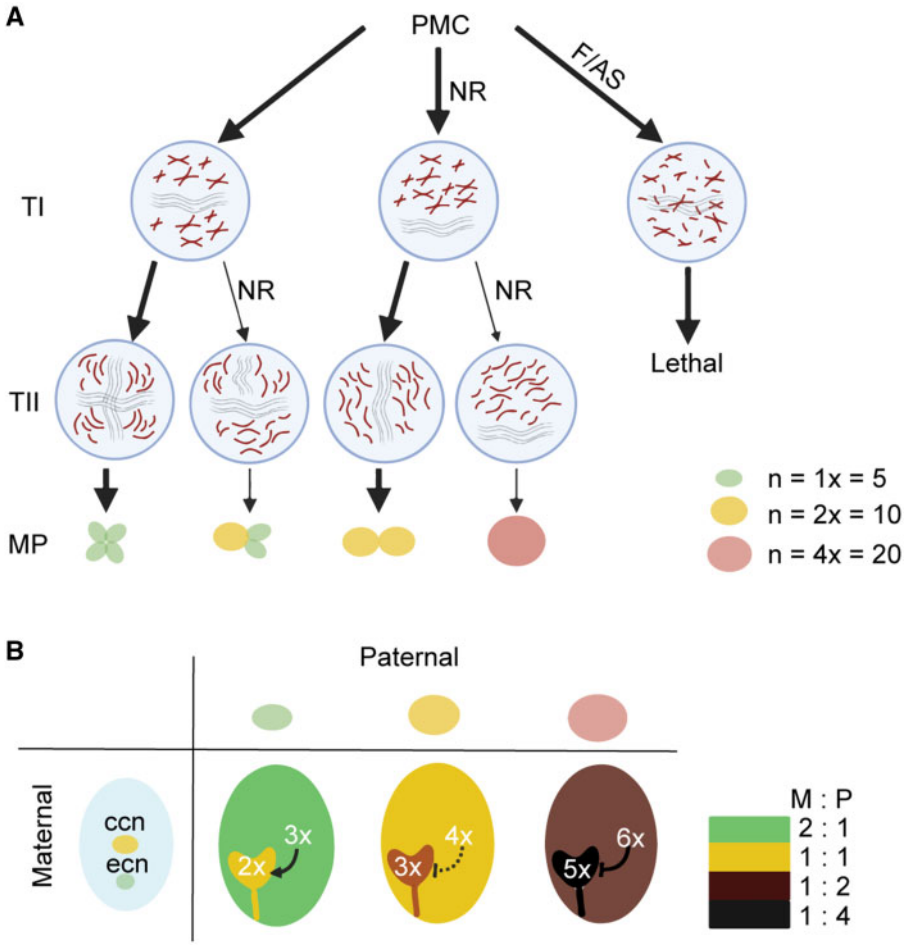

Figure 6 Model for abnormal phenotypes in Arabidopsis SMC5/6 complex mutant meiosis. A, Schematic representations of the PMC, TI and TII and meiotic products. The chains of events are described from left to right. Part of the *nse2* meiocytes goes through two standard reductional divisions corresponding to a tetrad with four haploid microspores (*n* = 1× = 5; green). Occasional nonreduction (NR) in the second division may produce a triad, most of them with one diploid (*n* = 2× = 10; yellow) and two haploid microspores. A more common defect is chromosome nonreduction in the first meiotic division, observed as grouping of all chromosomes on one side of the organelle band in telophase I. This is followed either by a standard second meiotic division and development of a dyad with two diploid microspores or by a defective second meiotic division giving rise to a monad (*n* = 4× = 20; red). The rightmost scenario shows that SPO11 activity in meiotic prophase I may lead to extensive chromosome fragmentation or/and abnormal segregation (F/AS) that requires SMC5/6 for repair. Meiocytes with extensively fragmented chromosomes are not viable and this situation is lethal. B, Effects of aberrant *nse2* meiosis on seed development. Only embryo sacs with haploid egg cell nuclei (ecn) and diploid central cell nuclei (ccn) are viable in *nse2* plants. Depending on the type of microspore, the resulting embryos and endosperm vary in their ploidy. The diploid and the tetraploid microspores lead to endosperm with an unbalanced ratio of the maternal (M) and paternal (P) genome ratio (color scale). This results in the abortion of about 2/3 of the seeds with triploid embryos and the total absence of seeds with a strongly unbalanced hexaploid endosperm.

meiosis II, as suggested by the absence of aneuploid *nse2* offspring. In this sense, the meiotic phenotype of *nse2* is similar (although more drastic) to that described for *nse4a-2* (Zelkowski et al., 2019). *nse2* single mutant plants do not create polyads (Figure 3D). Polyads represented 52.8% of meiotic products in *spo11* and their frequency is reduced to 38.7% in *spo11 nse2* plants (Figure 4, A and C). Therefore, NSE2 acts upstream of SPO11 and partially promotes polyad phenotype of *spo11* plants. In summary, the fragmented chromosomes in *nse2* meiocytes strongly reduce the number of viable gametes but do not explain the occurrence of unreduced gametes and eventually triploid offspring.

Then, how are the unreduced gametes generated? The most plausible explanation is that in some cells, chromosomes do not separate to two defined poles during meiosis I. The result would be similar to what occurs in a first division restitution (Bretagnolle and Thompson, 1995), but this phenotype does not occur in all *nse2* cells and is the consequence of a more complex process. Diplogametes can originate from the omission of the first and/or the second meiotic division. On the one hand, we confirmed that the unreduced gametes in *nse2* arise mainly from failures during the first meiotic division by analyzing the *osd1 nse2* double mutant (Figure 4, D and E). On the other hand, we also observed about 9%–21.8% of monads in *nse2-1* and *nse2-2* mutants, respectively, suggesting that both meiotic divisions can be affected. Furthermore, *nse2-2* anaphase I chromosomes show reduced chromatin condensation (Supplemental Figure S3). Such phenotype may be related to a loss of chromosome architecture due to the functional interplay between condensin and the SMC5/6 complex, as shown in animals (Hong et al., 2016; Hwang et al., 2017). As a consequence, after the formation of the organelle band (Brownfield et al., 2015), all chromosomes will appear at the same pole during the second meiotic division in some cells (Figure 3B, Supplemental Figure S3). In addition, the absence of two defined poles may occur as a consequence of disrupted organization and orientation of the spindle (Brownfield and Köhler, 2010). In this context, we observed partially abnormal spindle organization in *nse2-2* (Figure 4F). It should be stressed that chromosomes and kinetochores play a more important role in spindle morphogenesis in plants compared to animals due to the absence of centrosomes or proper microtubule organizing centers (Zhang and Dawe, 2011). We speculate that the kinetochores built on de-condensed centromeres might not be fully functional in organizing the spindle. Alternatively, the timing of release of cohesion, spindle elongation and spindle disassembly may be deregulated in *nse2* because of a delayed anaphase I.

We showed that the diplogametes are produced exclusively by the male organs. Analysis of *nse2* ovules revealed the absence of nuclei within embryo sacs and the presence of unfused (presumably polar) nuclei (Figure 1F), suggesting that any failures in female meiosis and/or post-meiotic development will lead to ovule abortion. Hence, megaspores may be more sensitive to the presence of unstable, damaged, incomplete, or excessive genomes. Normally, the number of eggs (and not pollen) determines the number of offspring. Therefore, quality control may be easier to exert through female gametophytic development. From an evolutionary point of view, strictly selected healthy female gametophytes will then be able to recognize less strictly controlled unhealthy male gametes, based on the compromised 2:1 maternal to paternal genome ratio in the endosperm, and will terminate the development of most such seeds (Batista and Köhler, 2020).

The frequency of triploids was around 10% in *nse2* and *nse4a-2* plants and reached up to about 70% in *sni1-3* plants. SNI1 is a DNA binding transcription factor and was originally identified as a suppressor of immune responses in Arabidopsis (Li et al., 1999). SNI1 may be a functional homolog of yeast NSE6 involved in SMC5/6 complex loading to the sites of DNA damage (Yan et al., 2013). Loss of SNI1 may prolong the persistence of specific types of DNA damage and consequently also the frequency of unreduced gametes. There are several other proteins whose loss-of-function mutants produce a high number of diplogametes. TARDY ASYNCHRONOUS MEIOSIS/CYCLIN A1;2 (TAM/CYCA1;2) phosphorylates OSD1. *tam* and *osd1* plants fail to enter meiosis II. Unreduced gametes are produced by both the male and the female meiocytes, as indicated by the offspring ploidy corresponding to about 60% triploids, 25%–40% tetraploid and only 2%–7% diploids (D'Erfurth et al., 2010). Here, we showed a synergistic effect between *nse2* and *osd1* mutations. Mutations in *PARALLEL SPINDLE 1* (*PS1*) cause abnormal spindle orientation in meiosis, which leads to the production of about 60% dyads in male meiocytes and 30% triploid offspring (d'Erfurth et al., 2008). Somewhat similar are the phenotypes of *jason* (*jas*) mutants. *jas* male meiocytes produce a high number of diplogametes and 29%–40% of offspring were triploid (Erilova et al., 2009; De Storme and Geelen, 2011). SMC5/6 mutants differ from the above-described cases by a severe negative effect on female meiosis, absence of 4x offspring (*tam2*) and extensive chromosome fragmentation in some meiocytes. Therefore, we propose that the SMC5/6 complex represents a novel diplogamete suppressor pathway. Further experiments will be directed towards unraveling the regulatory network under which the SMC5/6 complex operates in this process.

Materials and methods

Plant materials and growth conditions

All lines used in this study were in the Columbia-0 (Col-0) background. We used the following mutants: *nse2-1/hpy2-1* (Ishida et al., 2009), *nse2-2/hpy2-2/mms21-2* (SAIL_77_G06), *sni1-3* (SAIL_34_D11), *smc6b-1* (SALK_101968C), *nse4a-2* (GK-768H08), *nse4b-1* (SAIL_296_F02), *spo11-1-5* (SALK_009440), *qrt1-4* (SALK_024104C), and reporter lines *ProHTR10:HTR10m-RFP* (Ingouff et al., 2007). The *osd1-3* mutant was kindly provided by Dr Claudia Köhler; the T-DNA in this mutant is inserted 58 bp after the start codon of the second exon, resulting in a truncated 144-amino acid OSD1

protein (Heyman et al., 2011). Plant zygosity was monitored by PCR (see primers in Supplemental Table S10) and/or mutant phenotype if possible. Double mutants were generated by crossing and selection in the F_2 and F_3 generations. All lines were used as homozygotes unless stated otherwise.

Tetraploid Arabidopsis Col-0 wild-type (4x WT) was generated by submerging 2-week-old in vitro-grown diploid seedlings in 0.1% (w/v) colchicine (Sigma-Aldrich) in the dark at room temperature for 2 h. Subsequently, seedlings were gently washed with copious amounts of tap water, transplanted to soil and grown until maturity. Seeds were collected from individual plants and bigger seeds were selected and propagated into plants for ploidy measurements (see below). One tetraploid individual was self-pollinated to generate 4x WT.

For cultivation in soil, seeds were placed on the surface of soaked soil and stratified for two days at 4°C in the dark before the pots were moved to an air-conditioned chamber with controlled long-day conditions (16-h-light/8-h-dark cycle, 21°C day and 19°C night temperature, 150-µmol photons m^{-2} s^{-1} light intensity provided by Philips fluorescent tube MASTER TL-D 18W/840, catalog NO. ELSZZA0047412). For in vitro growth, Arabidopsis seeds were surface sterilized (70% ethanol with 0.5% Triton X-100 [v/v]) for 10 min and washed three times with sterile water. Air-dried seeds were sown on half-strength Murashige and Skoog agar medium, stratified in the dark for 2 days at 4°C and then cultivated in a climatic chamber (Percival) under 16-h-light/8-h-dark cycle, 21°C day and 19°C night temperature, 150-µmol photons m^{-2} s^{-1} light intensity provided by Philips fluorescent tubes as above.

DNA isolation and PCR

For genotyping of T-DNA mutants, one rosette leaf was mixed with the Phire Plant Direct PCR Kit (Thermo Scientific) dilution buffer and 1 µL was used for PCR according to the kit instructions. Genotyping primers are listed in Supplemental Table S10.

Ploidy measurements and flow cytometry

To minimize any potential selection bias, seeds were collected per silique. The siliques were taken from the central part of the main stem. All seeds per silique were sown and analyzed. To determine somatic ploidy levels, one to two young leaves from a 2-week-old seedling were chopped with a razor blade in 500-µL Otto I solution (0.1 M citric acid, 0.5% [v/v] Tween-20). The suspension of nuclei was filtered through a 50-µm nylon mesh and stained with 1 mL of Otto II solution (0.4 M Na_2HPO_4) containing 2 µg DAPI. Ploidy was analyzed on a Partec PAS I flow cytometer with 2× WT plants used as an external standard. Subsequent *nse2* samples were prepared by simultaneous chopping of equal amounts of tissue from both *nse2* and WT genotypes. In the mutant samples, aneuploid plants would be detected based on the presence of double peaks.

To determine ploidy levels of male gametes, the samples were prepared as described (Borges et al., 2012). The nuclei-enriched pellet was resuspended in 1 mL of sperm extraction buffer (1.3-mM H_3BO_3, 3.6-mM $CaCl_2$, 0.74-mM KH_2PO_4, 438-mM sucrose, 5.83-mM $MgSO_4$, 7-mM MOPS at pH 6) containing 2-µg DAPI. The different nuclei populations were sorted separately on microscope slides by FACSAria (BectonDickinson) flow-sorter. Slides were dried at room temperature for 1 h then mounted with 5-µL Vectashield (Vector Laboratories) and covered with 24 × 40 mm coverslips. The nuclei were then checked under an Olympus IX 83 inverted microscope: at 558/583 nm excitation/emission wavelengths for RFP and at 358/461 nm for DAPI. Images were captured with a HAMAMATSU ORCA-ER digital camera c4742-80 controlled by xCellence rt software (Olympus).

Pollen viability assays and size measurements

For pollen viability analysis, about 1 mL of opened flowers were collected into a 15-mL tube containing 3-mL BK buffer (0.127-mM $Ca(NO_3)_2$, 0.081-mM $MgSO_4$, 0.1-mM KNO_3, 15% (w/v) sucrose, and 10-mM MOPS, pH 7.5), and the tube was vortexed for 5 min to release pollen. Subsequently, the tube was centrifuged (2,600*g*, 5 min), the supernatant was carefully removed and the pollen pellet was carefully resuspended by gentle pipetting in 20-µL FDA buffer mixture (1-µL FDA [Sigma-Aldrich] stock solution [2 mg/mL in acetone] added to 1 mL of BK buffer). The suspension was carefully transferred to a microscope slide and covered with a 24 × 40 mm coverslip. Fluorescein fluorescence was observed after 20 min of staining using an Olympus IX 83 inverted microscope: at 543/620 nm excitation/emission wavelengths and the same region was photographed with differential interference contrast optics to get the number of all pollen grains.

To estimate the diameter of mature pollen, we used the same photos as for FDA analysis. Diameter measurements were done in Fiji/ImageJ on images calibrated using internal standards (Schindelin et al., 2012).

Hoyer's clearing

Clearing of ovules was performed as described (Liu and Meinke, 1998).

Chromosome spreads and analysis of meiosis

Fixations of flower buds, chromosome spreads, and fluorescence in situ hybridization were carried out as described (Sánchez Moran et al., 2001) with minor modifications included in (Martinez-Garcia and Pradillo, 2017). Data for cytological analyses were collected from at least four plants per genotype. The DNA probes used for the analysis were: 45S ribosomal DNA (rDNA) (pTa71 of *Triticum aestivum*; Gerlach and Bedbrook, 1979) and 5S rDNA (pCT4.2; Campell et al., 1992).

Immunolocalization procedures were performed with a spreading technique previously described in Armstrong et al. (2002) in the case of RAD51 detection, and a squash technique as described in Manzanero et al. (2000) for α-tubulin and CENH3 detection. The primary antibodies used were:

anti- α-tubulin (Merck; mouse, 1:50), anti-CENH3 (kindly provided by Dr Andreas Houben; rabbit, 1:500), anti-ZYP1 (kindly provided by Dr Chris Franklin; rat, 1:500), and anti-RAD51 (provided by Dr Chris Franklin; rabbit, 1:500). Secondary antibodies were: FITC-conjugated anti-mouse (Agrisera; 1:100), anti-rabbit Alexa Fluor 555-conjugated (Invitrogen, Molecular Probes; 1:500), anti-rat Alexa Fluor 555-conjugated (Invitrogen, Molecular Probes; 1:500), and anti-rabbit FITC-conjugated (Sigma-Aldrich; 1:50). Preparations were analyzed with an Olympus BX61 epifluorescence microscope and images were captured with an Olympus DP71 digital camera controlled by DP Controller software version 2.2.1.227 (Olympus).

Bioinformatic tools

Microsoft Office Excel 2016, PowerPoint 2016, GraphPad Prism 8.2.1, ImageJ 1.52p, BioRender, Flowing Software 2.5.1, Adobe Photoshop, and Adobe Illustrator were used for graph and image composition.

Accession numbers

Genes described in this article can be found in the TAIR database under the following accession numbers: *NSE2* (At3g15150); *NSE4A* (At1g51130); *NSE4B* (At3g20760); *SNI1* (At4g18470); *SMC6B* (At5g61460); *SPO11-1* (At3g13170); *OSD1* (At3g57860); *QRT1* (At5g55590); *HTR10* (At1g19890).

Supplemental data

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

Supplemental Figure S1. *nse2* affects plant growth.

Supplemental Figure S2. *nse2* affects pollen development.

Supplemental Figure S3. Meiosis progression in WT and *nse2-2*.

Supplemental Figure S4. Characterization of unreduced meiocytes by FISH and CENH3 inmunolocalization.

Supplemental Figure S5. Phenotypes of *spo11-1-5* and *nse2-2 spo11-1-5* plants.

Supplemental Figure S6. Quantification of RAD51 foci on pachytene meiocytes.

Supplemental Figure S7. *nse4b-1* and *smc6b-1* do not affect seed development.

Supplemental Table S1. Seed phenotypes of self-pollination and reciprocal crossing between *nse2* and WT plants.

Supplemental Table S2. Source data for analysis of seed development after self-pollination and reciprocal crossing between nse2 and WT plants.

Supplemental Table S3. Source data for analysis of pollen viability by FDA.

Supplemental Table S4. Quantification of meiotic products.

Supplemental Table S5. Quantification of meiotic defects in PMCs of *nse2-2*, *spo11-1-5*, and *nse2-2 spo11-1-5*.

Supplemental Table S6. Source data for comparison of RAD51 foci between WT and *nse2-2*.

Supplemental Table S7. Source data for comparison of partial synapsis between WT and *nse2-2* cells.

Supplemental Table S8. Source data for comparing meiotic products between *nse2-2* and *nse2-2 spo11-1-5*.

Supplemental Table S9. Source data for comparing meiotic products between *spo11-1-5* and *nse2-2 spo11-1-5*.

Supplemental Table S10. Primers used in this study.

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Conflict of interest statement. The authors declare that they have no conflict of interest.

References

Aragón L (2018) The Smc5/6 complex: new and old functions of the enigmatic long-distance relative. Annu Rev Genet **52:** 89–107

Armstrong SJ, Caryl AP, Jones GH, Franklin FCH (2002) Asy1, a protein required for meiotic chromosome synapsis, localizes to axis-associated chromatin in Arabidopsis and Brassica. J Cell Sci **115:** 3645–3655

Batista RA, Köhler C (2020) Genomic imprinting in plants—revisiting existing models. Genes Dev **34:** 24–36

Borges F, Gardner R, Lopes T, Calarco JP, Boavida LC, Slotkin RK (2012) FACS-based purification of Arabidopsis microspores, sperm cells and vegetative nuclei. Plant Methods **8:** 44

Bretagnolle F, Thompson JD (1995) Gametes with the somatic chromosome number: mechanisms of their formation and role in the evolution of autopolyploid plants. New Phytol **129:** 1–22

Brownfield L, Köhler C (2010) Unreduced gamete formation in plants: mechanisms and prospects. J Exp Bot **62:** 1659–1668

Brownfield L, Yi J, Jiang H, Minina EA, Twell D, Köhler C (2015) Organelles maintain spindle position in plant meiosis. Nat. Commun **6:** 6492

Campell BR, Song Y, Posch TE, Cullis CA, Town CD (1992) Sequence and organization of 5S ribosomal RNA-encoding genes of Arabidopsis thaliana. Gene **112:** 225–228

Copsey A, Tang S, Jordan PW, Blitzblau HG, Newcombe S, Chan AC-H, Newnham L, Li Z, Gray S, Herbert AD, et al. (2013). Smc5/6 coordinates formation and resolution of joint molecules with chromosome morphology to ensure meiotic divisions. PLoS Genet **9:** e1004071

van der Crabben SN, Hennus MP, McGregor GA, Ritter DI, Nagamani SCS, Wells OS, Harakalova M, Chinn IK, Alt A, Vondrova L, et al. (2016) Destabilized SMC5/6 complex leads to chromosome breakage syndrome with severe lung disease. J Clin Invest **126:** 2881–2892

Cromer L, Heyman J, Touati S, Harashima H, Araou E, Girard C, Horlow C, Wassmann K, Schnittger A, De Veylder L, et al. (2012). OSD1 promotes meiotic progression via APC/C inhibition and forms a regulatory network with TDM and CYCA1;2/TAM. PLoS Genet **8:** e1002865–e1002865

D'Erfurth I, Cromer L, Jolivet S, Girard C, Horlow C, Sun Y, To JPC, Berchowitz LE, Copenhaver GP, Mercier R (2010) The cyclin-A CYCA1;2/TAM is required for the meiosis I to meiosis II transition and cooperates with OSD1 for the prophase to first meiotic division transition. PLoS Genet **6:** e1000989–e1000989

d'Erfurth I, Jolivet S, Froger N, Catrice O, Novatchkova M, Simon M, Jenczewski E, Mercier R (2008) Mutations in AtPS1 (Arabidopsis thaliana parallel spindle 1) lead to the production of diploid pollen grains. PLoS Genet **4:** e1000274–e1000274

Decorsière A, Mueller H, Van Breugel PC, Abdul F, Gerossier L, Beran RK, Livingston CM, Niu C, Fletcher SP, Hantz O, et al. (2016). Hepatitis B virus X protein identifies the Smc5/6 complex as a host restriction factor. Nature **531:** 386–389

Díaz M, Pecinka A (2018) Scaffolding for repair: understanding molecular functions of the SMC5/6 complex. Genes (Basel) **9:** 36

Díaz M, Pečinková P, Nowicka A, Baroux C, Sakamoto T, Gandha PY, Jeřábková H, Matsunaga S, Grossniklaus U, Pecinka A (2019) The SMC5/6 complex subunit NSE4A is involved in DNA damage repair and seed development. Plant Cell **31:** 1579–1597

Erilova A, Brownfield L, Exner V, Rosa M, Twell D, Scheid OM, Hennig L, Köhler C (2009) Imprinting of the polycomb group gene MEDEA serves as a ploidy sensor in Arabidopsis. PLOS Genet **5:** e1000663

Ganji M, Shaltiel IA, Bisht S, Kim E, Kalichava A, Haering CH, Dekker C (2018) Real-time imaging of DNA loop extrusion by condensin. Science **360:** 102–105

Gerlach WL, Bedbrook JR (1979) Cloning and characterization of ribosomal RNA genes from wheat and barley. Nucleic Acids Res **7:** 1869–1885

Gómez R, Jordan PW, Viera A, Alsheimer M, Fukuda T, Jessberger R, Llano E, Pendás AM, Handel MA, Suja JA (2013) Dynamic localization of SMC5/6 complex proteins during mammalian meiosis and mitosis suggests functions in distinct chromosome processes. J Cell Sci **126:** 4239–4252

Grelon M, Vezon D, Gendrot G, Pelletier G (2001) AtSPO11-1 is necessary for efficient meiotic recombination in plants. EMBO J **20:** 589–600

Heyman J, Van den Daele H, De Wit K, Boudolf V, Berckmans B, Verkest A, Alvim Kamei CL, De Jaeger G, Koncz C, De Veylder L (2011) Arabidopsis ULTRAVIOLET-B-INSENSITIVE4 maintains cell division activity by temporal inhibition of the anaphase-promoting complex/cyclosome. Plant Cell **23:** 4394–4410

Higgins JD, Sanchez-Moran E, Armstrong SJ, Jones GH, Franklin FCH (2005) The Arabidopsis synaptonemal complex protein ZYP1 is required for chromosome synapsis and normal fidelity of crossing over. Genes Dev **19:** 2488–2500

Hong Y, Sonneville R, Agostinho A, Meier B, Wang B, Blow JJ, Gartner A (2016) The SMC-5/6 complex and the HIM-6 (BLM) helicase synergistically promote meiotic recombination intermediate processing and chromosome maturation during Caenorhabditis elegans meiosis. PLoS Genet **12:** e1005872–e1005872

Hwang G, Sun F, O'Brien M, Eppig JJ, Handel MA, Jordan PW (2017) SMC5/6 is required for the formation of segregation-competent bivalent chromosomes during meiosis I in mouse oocytes. Development **144:** 1648–1660

Ingouff M, Hamamura Y, Gourgues M, Higashiyama T, Berger F (2007) Distinct dynamics of HISTONE3 variants between the two fertilization products in plants. Curr Biol **17:** 1032–1037

Ishida T, Fujiwara S, Miura K, Stacey N, Yoshimura M, Schneider K, Adachi S, Minamisawa K, Umeda M, Sugimoto K (2009) SUMO E3 ligase HIGH PLOIDY2 regulates endocycle onset and meristem maintenance in Arabidopsis. Plant Cell **21:** 2284–2297

Jeppsson K, Kanno T, Shirahige K, Sjögren C (2014) The maintenance of chromosome structure: positioning and functioning of SMC complexes. Nat Rev Mol Cell Biol **15:** 601–614

Kawashima T, Berger F (2014). Epigenetic reprogramming in plant sexual reproduction. Nat Rev Genet **15:** 613–624

Kegel A, Sjögren C (2010) The Smc5/6 complex: more than repair? Cold Spring Harb Symp Quant Biol **75:** 179–187

Köhler C, Wolff P, Spillane C (2012) Epigenetic mechanisms underlying genomic imprinting in plants. Annu Rev Plant Biol **63:** 331–352

Kwak JS, Son GH, Kim SI, Song JT, Seo HS (2016) Arabidopsis HIGH PLOIDY2 Sumoylates and stabilizes flowering locus C through Its E3 ligase activity. Front Plant Sci **7:** 1–9

Leung GP, Lee L, Schmidt TI, Shirahige K, Kobor MS (2011) Rtt107 is required for recruitment of the SMC5/6 complex to DNA double strand breaks. J Biol Chem **286:** 26250–26257

Li X, Zhang Y, Clarke JD, Li Y, Dong X (1999) Identification and cloning of a negative regulator of systemic acquired resistance, SNI1, through a screen for suppressors of npr1-1. Cell **98:** 329–339

Liu CM, Meinke DW (1998) The titan mutants of Arabidopsis are disrupted in mitosis and cell cycle control during seed development. Plant J **16:** 21–31

Liu M, Shi S, Zhang S, Xu P, Lai J, Liu Y, Yuan D, Wang Y, Du J, Yang C (2014) SUMO E3 ligase AtMMS21 is required for normal meiosis and gametophyte development in Arabidopsis. BMC Plant Biol **14:** 1–12

Manzanero S, Arana P, Puertas MJ, Houben A (2000) The chromosomal distribution of phosphorylated histone H3 differs between plants and animals at meiosis. Chromosoma **109:** 308–317

Martinez-Garcia M, Pradillo M (2017) Functional analysis of Arabidopsis ARGONAUTEs in meiosis and DNA repair. *In* A Carbonell, ed, Plant Argonaute Proteins. Humana Press, Totowa, NJ

Mengiste T, Revenkova E, Bechtold N, Paszkowski J (1999) An SMC-like protein is required for efficient homologous recombination in Arabidopsis. EMBO J **18:** 4505–4512

Menolfi D, Delamarre A, Lengronne A, Pasero P, Branzei D (2015) Essential roles of the Smc5/6 complex in replication through natural pausing sites and endogenous DNA damage tolerance. Mol Cell **60:** 835–846

Nasmyth K, Haering CH (2009) Cohesin: its roles and mechanisms. Annu Rev Genet **43:** 525–558

Payne F, Colnaghi R, Rocha N, Seth A, Harris J, Carpenter G, Bottomley WE, Wheeler E, Wong S, Saudek V, et al. (2014). Hypomorphism in human NSMCE2 linked to primordial dwarfism and insulin resistance. J Clin Invest **124:** 4028–4038

Pebernard S, Wohlschlegel J, McDonald WH, Yates JR, Boddy MN (2006) The Nse5-Nse6 dimer mediates DNA repair roles of the Smc5-Smc6 complex. Mol Cell Biol **26:** 3336–3336

Preuss D, Rhee SY, Davis RW (1994) Tetrad analysis possible in Arabidopsis with mutation of the QUARTET (QRT) genes. Science **264:** 1458–1460

Räschle M, Smeenk G, Hansen RK, Temu T, Oka Y, Hein MY, Nagaraj N, Long DT, Walter JC, Hofmann K, et al. (2015) Proteomics reveals dynamic assembly of repair complexes during bypass of DNA cross-links. Science **348:** 1253671

Rowley MJ, Corces VG (2018) Organizational principles of 3D genome architecture. Nat Rev Genet **19:** 789–800

Sánchez Moran E, Armstrong SJ, Santos JL, Franklin FCH, Jones GH (2001) Chiasma formation in Arabidopsis thaliana accession Wassileskija and in two meiotic mutants. Chromosom Res **9:** 121–128

Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T, Preibisch S, Rueden C, Saalfeld S (2012) Fiji: an open-source platform for biological-image analysis. Nat Methods **9:** 676–682

Scott RJ, Spielman M, Bailey J, Dickinson HG (1998) Parent-of-origin effects on seed development in Arabidopsis thaliana. Development **125:** 3329–3341

De Storme N, Geelen D (2011) The Arabidopsis mutant Jason produces unreduced first division restitution male gametes through a parallel/fused spindle mechanism in meiosis II. Plant Physiol **155:** 1403–1415

Uhlmann F (2016) SMC complexes: from DNA to chromosomes. Nat Rev Mol Cell Biol **17:** 399–412

Wang Y, Cheng Z, Huang J, Shi Q, Hong Y, Copenhaver GP, Gong Z, Ma H (2012) The DNA replication factor RFC1 is required for interference-sensitive meiotic crossovers in Arabidopsis thaliana. PLoS Genet **8:** e1003039

Watanabe K, Pacher M, Dukowic S, Schubert V, Puchta H, Schubert I (2009) The STRUCTURAL MAINTENANCE of CHROMOSOMES 5/6 complex promotes sister chromatid alignment and homologous recombination after DNA damage in Arabidopsis thaliana. Plant Cell **21:** 2688–2699

Xaver M, Huang L, Chen D, Klein F (2013) Smc5/6-Mms21 prevents and eliminates inappropriate recombination intermediates in meiosis. PLoS Genet **9:** e1004067

Yan S, Wang W, Marqués J, Mohan R, Saleh A, Durrant WE, Song J, Dong X (2013) Salicylic acid activates DNA damage responses to potentiate plant immunity. Mol Cell **52:** 602–610

Zelkowski M, Zelkowska K, Conrad U, Hesse S, Lermontova I, Marzec M, Meister A, Houben A, Schubert V (2019) Arabidopsis NSE4 proteins act in somatic nuclei and meiosis to ensure plant viability and fertility. Front Plant Sci **10:** 774

Zhang H, Dawe RK (2011) Mechanisms of plant spindle formation. Chromosom Res **19:** 335–344

APPENDIX II

SMC5/6 complex is necessary for tetraploid genome stability in *Arabidopsis thaliana*

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Structural Maintenance of Chromosomes 5/6 Complex Is Necessary for Tetraploid Genome Stability in *Arabidopsis thaliana*

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Polyploidization is a common phenomenon in the evolution of flowering plants. However, only a few genes controlling polyploid genome stability, fitness, and reproductive success are known. Here, we studied the effects of loss-of-function mutations in NSE2 and NSE4A subunits of the Structural Maintenance of Chromosomes 5/6 (SMC5/6) complex in autotetraploid *Arabidopsis thaliana* plants. The diploid *nse2* and *nse4a* plants show partially reduced fertility and produce about 10% triploid offspring with two paternal and one maternal genome copies. In contrast, the autotetraploid *nse2* and *nse4a* plants were almost sterile and produced hexaploid and aneuploid progeny with the extra genome copies or chromosomes coming from both parents. In addition, tetraploid mutants had more severe meiotic defects, possibly due to the presence of four homologous chromosomes instead of two. Overall, our study suggests that the SMC5/6 complex is an important player in the maintenance of tetraploid genome stability and that autotetraploid Arabidopsis plants have a generally higher frequency of but also higher tolerance for aneuploidy compared to diploids.

Keywords: SMC5/6 complex, polyploidy, seed development, meiosis, *NSE2*, genome stability

INTRODUCTION

Maintenance of genome stability is essential for ensuring plant growth, fertility, and proper genomic constitution of the offspring (Roy, 2014; Hu et al., 2016). The family of Structural Maintenance of Chromosomes (SMC) complexes includes ATP-dependent molecular machines with a unique ability to process chromosome-scale DNA molecules (Uhlmann, 2016). The SMC5/6 complex is an evolutionarily conserved member of the SMC family that is involved in DNA damage repair, DNA replication, and cell divisions (Kegel and Sjögren, 2010; Aragón, 2018). The core part of the complex consists of SMC5 and SMC6 protein heterodimer, where the subunits are attached via their hinge domains. SMC6 has two partially functionally redundant paralogs in Arabidopsis. Both play roles under ambient conditions, but only SMC6B takes place in DNA damage repair (Watanabe et al., 2009; Yan et al., 2013; Zou et al., 2021). Opposite to the hinge domains are the head domains,

where the NON-SMC ELEMENT (NSE) NSE1-NSE3-NSE4 subcomplex bridges the SMC heterodimer. Here, the kleisin type protein NSE4 closes the SMC ring by interacting with both SMC5 and SMC6 subunits (Palecek and Gruber, 2015). The NSE1 and NSE3 subunits regulate the conformation of NSE4. While *NSE1* and *NSE3* are single-copy genes in Arabidopsis, there are two NSE4 paralogs (NSE4A and NSE4B) that show distinct expression patterns and functions (Díaz et al., 2019). The NSE2 subunit is attached to the coiled-coil region of SMC5 and is one of the two E3 SUMO ligases in Arabidopsis (Ishida et al., 2012). Additionally, plant-specific SMC5/6 subunits ARABIDOPSIS SNI1 ASSOCIATED PROTEIN 1 (ASAP1) and SUPPRESSOR OF NPR1-1; INDUCIBLE 1 (SNI1) have been described (Yan et al., 2013). ASAP1 and SNI1 were proposed to be functionally homologous to the yeast SMC5/6 complex chromatin-loader subunits NSE5 and NSE6.

SMC5/6 complex controls multiple biological processes in plants. There is solid evidence that the SMC5/6 complex is important for the repair of specific types of DNA damage in Arabidopsis (Mengiste et al., 1999; Watanabe et al., 2009; Yuan et al., 2014; Díaz et al., 2019). This may be mainly due to its essential role in homologous recombination (HR) where the loss of function from SMC6B results in reduced HR levels (Mengiste et al., 1999; Watanabe et al., 2009). NSE2 function is not essential for Arabidopsis survival, but the plants are strongly affected in their vegetative and generative development including poor growth of roots, earlier flowering, reduced height, and decreased fertility (Huang et al., 2009; Ishida et al., 2009; Xu et al., 2013; Liu et al., 2014; Kwak et al., 2016). Recently, several studies pointed toward the importance of the SMC5/6 complex during plant sexual reproduction, including meiosis, pollen viability, and seed development (Liu et al., 2014; Díaz et al., 2019; Yang et al., 2021; Zou et al., 2021). NSE2, NSE4, and SNI1 were found to play an important role in meiosis. NSE4A was localized to the synaptonemal complex and the mutants showed chromosome fragmentation and frequent meiotic irregularities (Zelkowski et al., 2019). At least part of this trait seems due to the role of SMC5/6 in the regulation of meiotic recombination. Here, RAD51 directly suppresses the SMC5/6 complex to promote DMC1-based recombination (Chen et al., 2021). Another role of the SMC5/6 complex is to secure the development of properly reduced haploid gametes in meiotic recombination independent manner (Yang et al., 2021). The NSE2, NSE4A, and SNI1 mutants show recombination-independent problems in chromosome segregation and produce unreduced microspores. Fertilization with diploid pollen leads to abnormal seed development in these mutants. This is most likely due to an unbalanced parental dosage with two maternal and two paternal genome copies in the endosperm (Jullien and Berger, 2010). An excess of paternal genetic information leads to seed overgrowth and the absence of cellularization, which frequently results in seed abortion (Köhler et al., 2012). Some of such abnormal seeds still survive and produce polyploid (triploid) offspring.

Polyploidization, i.e., whole genome duplication, is a common phenomenon in higher plants, and both autopolyploids and allopolyploids often occur in nature. Polyploidization plays a significant role in the evolution of Angiosperms as the major mechanism providing raw material for gene sub- and neofunctionalization (Van De Peer et al., 2009). However, newly established polyploids can experience genomic shock represented by changes at genomic, chromosome, and gene levels (reviewed in Comai, 2005). This includes genome downsizing, structural chromosome rearrangements, amplification and/or reactivation of repetitive elements, modifications of the gene expression patterns, and rapid sequence changes in multigene families, such as rDNAs. Polyploidization often leads to altered morphology compared to the ancestral lines and in autotetraploid occasionally also to developmental abnormalities and/or reduced fertility. One of the major challenges in tetraploids is thought to be the more complex meiosis due to the presence of the four nearly identical (homologous chromosomes, autopolyploids) or similar (homeologous chromosomes, allopolyploids) copies of chromosomes. In both types of polyploids, natural selection should favor strategies to control pairing preferences that result in disomic inheritance and proper segregation of genetic material during meiosis. This is true in Arabidopsis, where auto- and allotetraploids show strict homologous chromosome pairing (Pecinka et al., 2011). The elimination of certain sequences, chromosome rearrangements, and dysploidy seem to contribute to the meiotic cytological diploidization (Mandáková and Lysak, 2018). Since it is a long process, intermediate situations with different chromosomes showing different rates of bivalent formation (tetrasomic inheritance for some chromosomes and disomic inheritance for others) are possible (Santos et al., 2003). Recently, it has been found that particular alleles of the meiotic chromosome pairing genes *ASY1* and *ASY3* lead to a reduced number of quadrivalents compared to bivalents in tetraploid *Arabidopsis arenosa* (Morgan et al., 2020; Seear et al., 2020), indicating an evolutionary selection toward specific tetraploid meiotic phenotypes.

Despite these findings, it remains largely unknown whether tetraploid mutants of meiotic genes show diploid-like or new phenotypes. This may contribute to a better understanding of their role in meiosis. Here, we analyzed the consequences of polyploidy in the SMC5/6 complex mutants and show that autotetraploid plants of two SMC5/6 complex mutants *nse2* and *nse4a* display several characteristics that differ from their diploid cytotypes.

MATERIALS AND METHODS

Plant Materials and Growth Conditions

All strains used in this study were in Columbia-0 (Col-0) background. We used following mutants (diploid): *nse2-1/hpy2-1*, *nse4a-2* (GK-768H08), and *qrt1-4* (SALK_024104C). *nse2-1* is an ethyl methanesulfonate (EMS) mutant allele that was isolated in the laboratory of Prof. Keiko Sugimoto, RIKEN Center for Sustainable Resource Science, Japan. Other T-DNA insertion mutants were collected from the Salk Institute Genomic Analysis Laboratory (SiGnAL[1]; Alonso et al., 2003),

[1]http://signal.salk.edu/cgi-bin/tdnaexpress

and provided by the Nottingham Arabidopsis Stock Centre (NASC). Genotyping of T-DNA mutant was performed by PCR with a combination of three primers, T-DNA specific primers: LBb1.3 (5′-ATTTTGCCGATTTCGGAAC-3′) for *qrt1-4*; o84747_m (5′-ATAATAACGCTGCGGACATCTAC-3′) for *nse4a-2*, and two specific primers for the corresponding gene: LPNSE4A-2 (5′-GCTCAACAGGCGGTCATTTG-3′) and RPNSE4A-2 (5′-ACAAAAGCCACTTAACTGCTACA-3′); LPQRT1-4 (5′-TCTCTTCCCAGAAAAGGCTTC-3′) and RPQ RT1-4 (5′-CGTGGGTCTCAAGAATCTTTG-3′); *nse2-1* plants were selected based on the mutant features (Ishida et al., 2009). Double mutants were generated by crossing and selection in F_2 and F_3 generations. All lines were used as homozygotes unless stated otherwise.

Data related to diploid controls were published in a separate study (Yang et al., 2021). Both diploid and tetraploid plants were cultivated under the same growth conditions. Tetraploid *A. thaliana* plants were generated by submerging 2 weeks old *in vitro* grown diploid plants in 0.1% (w/v) colchicine (Sigma-Aldrich) in dark at room temperature for 1 h. Subsequently, plants were gently washed with copious amounts of tap water, transplanted to soil, and grown until maturity. Seeds were collected from individual plants, 20–30 biggest seeds were manually selected and propagated into plants for ploidy measurements (see below).

For *in vitro* growth, Arabidopsis seeds were surface sterilized (70% ethanol with 0.5% TritonX-100 v/v) for 10 min and washed three times with sterile water. Dried seeds were sown on 0.5 × Murashige and Skoog (MS) agar medium, stratified in dark for 2 days at 4°C and then cultivated in a climatic chamber (Percival) under 16 h light/8 h dark cycle, 21°C day and 19°C night temperature. For cultivation in soil, 2-week-old diploid or tetraploid seedlings were transplanted to the moist soil after ploidy measurements, then the pots were moved to an air-conditioned chamber with controlled long-day conditions (16 h light/8 h dark cycle, 21°C day and 19°C night temperature, 150 μmol photons m^{-2} s^{-1} light intensity provided by white-light tubes).

Ploidy Measurements and Flow Cytometry

For tetraploid selection, plants grown from the big seeds produced by colchicine-treated plants were used. To determine the somatic ploidy levels, 1–2 young leaves were chopped with a razor blade in 500 μL Otto I solution (0.1M citric acid, 0.5% Tween 20 v/v). The nuclear suspension was filtered through 50 μm nylon mesh and stained with 1 mL of Otto II solution (0.4M $Na_2HPO_4{\cdot}12H_2O$) containing 2 μg DAPI (4′,6-diamidino-2-phenylindole). The ploidy was analyzed on a Partec PAS I flow cytometer with diploid WT plants used as an external standard. For the offspring ploidy measurements, seeds were collected per silique and all seeds per silique were sown (this avoids selection bias occurring when seed are collected per whole plant and the shrunk seeds are typically lighter and less round – thus often coming late during standard sowing procedures) and analyzed as described above.

Hoyer's Clearing

Flowers with green or white closed anthers were manually emasculated. Two days later, ovules were dissected and cleared by Hoyer's solution as described (Liu and Meinke, 1998) with modifications. Dissolve 25 g Arabic gum in 25 mL distilled water in a glass beaker by heating to 60°C and stirring with a magnetic stirrer for about 1 h under a fume hood. Add 100 g chloral hydrate and keep dissolving until the solution will be clear and have an amber color. Subsequently add 10 mL glycerol, mix and keep the solution in dark at room temperature. Dissect ovules on a clear microscopic slide, add 20 μl Hoyer's solution and mount with a 24 × 40 mm coverslip without applying a pressure. The slides were kept at 4°C overnight (or longer) and examined with an inverted microscope Olympus IX 83 using differential interference contrast (DIC) optics.

Pollen Viability Assays

Fluorescein diacetate (FDA)-buffer mixture was prepared as described: 1 μL FDA (Sigma-Aldrich) stock solution (2 mg/mL in acetone) was added to 1 mL of BK buffer [0.127 mM Ca(NO3)2·4H2O, 0.081 mM MgSO4·7H2O, 0.1 mM KNO3, 15% Sucrose w/v and 10 mM MOPS, pH 7.5]. 20 μL FDA-buffer mixture was dripped to a microscopic slide then one opened flower was dropped into the FDA-buffer mixture and covered with 24 × 40 mm coverslip carefully. Data for pollen viability analysis were collected from at least three plants per genotype. The fluorescein fluorescence was observed after 20 min of staining using an inverted microscope Olympus IX 83: at 543/620 nm excitation/emission wavelengths and the same region was photographed with DIC optics to get the number of all pollen grains. ImageJ was used to merge the DIC and fluorescein channels.

Cytological Experiments

The fixation of flower buds and chromosome spreads were carried out as described (Sánchez Moran et al., 2001), including minor modifications to adapt the protocol for the study of autopolyploids (Parra-Nunez et al., 2020). Data for cytological analyses were collected from at least three plants per genotype. Meiocytes were analyzed with an Olympus BX-61 epifluorescent microscope and images were captured with an Olympus DP-71 digital camera (Olympus, Germany). Using x100 magnification oil immersion objective resulted in an ultra-high image resolution of 4080 × 3072 and 46.40 pixels/μm. Manual mode was selected to allow the preferred image brightness to be set by clicking and dragging the slider positioned in the exposure time. The images were captured in grayscale and edited in Adobe Photoshop.

For mitotic chromosome number counting, fresh inflorescences were fixed in ethanol: chloroform: acetic acid (6:3:1) solution overnight at room temperature then enzymatically digested in 0.3% (w/v) cellulase Onozuka R-10 (Serva, Germany, catalog no. 1641903), cytohelicase from *Helix pomatia* (Sigma-Aldrich, St. Louis, catalog no. C8274) and pectolyase from *Aspergillus japonicus* (Sigma-Aldrich, St. Louis, catalog no. P3026) for 3 h at 37°C. After the enzymatic digestion, single flower buds were dissected and chopped

in 60% acetic acid and slides were placed on the heating block for 2 min at 50°C. Then, cells on slides were fixed in Carnoy's fixative. Chromosomes were counterstained with DAPI 1.5 μg/mL (Vector Laboratories, United States). All slides were examined with Axio Imager Z.2 Zeiss microscope (Zeiss, Germany) equipped with Cool Cube 1 camera (Metasystems, Germany). We used ×60 and ×100 objectives and filter for DAPI (emission spectrum '405 nm). Scale bars were adjusted to the objective that was applied. Image processing was carried out using ISIS software 5.4.7 (Metasystems, Germany) and Adobe Photoshop software (CS5).

Software

Microsoft Office Excel 2016, PowerPoint 2016, GraphPad Prism 8.2.1, ImageJ 1.52p, Adobe Photoshop CS5 and Illustrator were used for graph and image composition.

RESULTS

Tetraploidy Enhances Fertility Defects in *nse2-1* and *nse4a-2* Plants

The diploid (2x) Arabidopsis *nse2* mutant plants have a significantly reduced fertility (Liu et al., 2014; Yang et al., 2021). To analyze the dosage-dependent role of *NSE2*, we produced autotetraploid wild-type (4x WT) and *nse2-1* (4x *nse2-1*) plants (Yang et al., 2021). With these lines, we noticed that 4x *nse2-1* plants had a reduced root length in the juvenile stage and a lower plant height at the adult stage compared with 4x WT (**Figures 1A,B**). The same differences were observed also between 2x *nse2-1* and 2x WT plants (**Figures 1A,B**), but both 4x WT and 4x *nse2-1* plants were bigger and had longer roots. In contrast, the siliques from 4x *nse2-1* were thicker but shorter than those of 2x *nse2*-1, possibly indicating that the autotetraploidy increases the seed size but enhances fertility defects of *nse2-1*, respectively (**Figure 1C**).

To further explore this observation, we analyzed the seed traits of 4x WT and mutant plants. We included also tetraploid *nse4a-2* (4x *nse4a-2*) because *NSE4A* is another subunit of SMC5/6 complex whose loss-of-function plants have fertility defects (Díaz et al., 2019). Dry seeds from 4x WT plants were larger than those from diploid WT (2x WT), but both were regular in shape and had a normal light brown color (**Figure 2A**). In contrast, both 2x and 4x mutants produced seeds with a variable shape, size, and color, including very large or little seeds, shrunk, and colored from normal to dark brown (**Figure 2A**). Analysis of siliques 13 days after self-pollination (DAP) revealed that 4x WT produced 84.3% normal seeds, 10.2% aborted ovules and 5.5% abnormal seeds (plants/siliques/seeds = 3/15/899, **Figures 2B,C** and **Table 1**). Equally old 4x *nse2-1* and 4x *nse4a-2* plants showed 11.6 and 51.8% normal seeds, 72.6 and 31.1% aborted ovules, and 15.8 and 17.1% abnormal seeds (plants/siliques/seeds = 3/15/739 and 3/15/843, respectively; **Figures 2B–D** and **Table 1**). Hence, the frequencies of both aborted ovules and abnormal seeds were significantly increased in 4x mutants compared to 4x WT (Fisher's exact test, $P < 0.00001$; **Figures 2C,D** and **Supplementary Table 1**). In addition, the comparison of the traits in 4x mutants relative to the 2x mutants (data from Yang et al., 2021) revealed that the ovule abortion and seed abnormality were statistically significantly more pronounced in the 4x *nse2-1* compared to the 2x mutant plants (Fisher's exact test, $P < 0.001$, **Supplementary Table 2**). This suggested ploidy-dependent fertility defects in tetraploid *nse2-1* mutants. However, no significant difference in the frequency of abnormal seeds was found for the 4x and 2x *nse4a-2* plants (Fisher's exact test, $P = 0.4299$; **Supplementary Table 2**). It may be because *nse4a-2* is a partial loss-of-function allele (Díaz et al., 2019).

To test for the contribution of the parents to the abnormal seeds, we performed reciprocal crosses between 4x WT and 4x mutant plants. When 4x WT plants were fertilized by either 4x *nse2-1* or 4x *nse4a*-2, we observed 11.0 and 15.1% abnormal seeds 13 DAP (plants/siliques/seeds = 3/15/691 and 3/14/802, respectively; **Figures 2B–D** and **Table 1**). On contrary, when 4x WT was used to pollinate 4x *nse2-1* or 4x *nse4a-2,* there were only 6.9 and 3.5% abnormal seeds found (plants/siliques/seeds = 3/15/607 and 3/15/722, respectively). This generally matched 5.5% such seeds in self-pollinated 4x WT (plants/siliques/seeds = 3/15/899; **Figures 2B–D** and **Table 1**) and was significantly less than in the above mentioned reciprocal crosses (Fisher's exact test, $P < 0.05$; **Supplementary Table 1**). This suggests that the abnormal seed development is caused predominantly paternally, and to a minor extent also maternally, in 4x *nse2-1* and 4x *nse4a-2* mutants.

Tetraploid *nse2* Leads to Defects in Both Male and Female Gametophytes

Previously, we showed that 2x *nse2-2* mutations cause ovule lethal defects (Yang et al., 2021). In contrast, the genetic material of the diploid microspores was at least partially transmissible and resulted in abnormal seed development. Here, we analyzed the female and male gametophyte development in the context of 4x *nse2-1* mutant plants.

First, we inspected the female gametophyte development (**Table 1**). In crosses where 4x *nse2-1* was used as a mother, we found 42.5% aborted ovules. This suggests that 4x *nse2* has a pre-zygotic maternal dysfunction. To determine a possible source of this defect, we analyzed the morphology of 54 embryo sacs in 2x and 104 in 4x *nse2-1* plants, respectively. In 2x *nse2-1*, there were 13.0% (7 out of 54) WT-like embryo sacs, 38.9% (21 out of 54) ovules without embryo sacs, 22.2% (12 out of 54) embryo sacs without nucleus and 25.9% embryo sacs with three nuclei (14 out of 54). In 4x *nse2-1*, only 4.8% of ovules (5 out of 104) carried WT-like embryo sacs (with a smaller egg cell nucleus and larger central cell nucleus positioned closer and more distant to the micropylar pole, respectively) and the majority of the ovules (95.2%; 99 out of 104) showed diverse defects (**Figure 3**). In total, 72.1% (75 out of 104) ovules fully lacked an embryo sac or it was without detectable nuclei (**Figures 3B,C**). In 23.1% (24 out of 104) of the ovules, embryo sacs contained nuclei, but they deviated from the WT parameters. There was only one nucleus, three nuclei, or occasionally also two nuclei that were abnormally positioned (**Figures 3D–G**). This suggests that tetraploidy in combination with *nse2-1* mutation results in severe female pre-zygotic sterility,

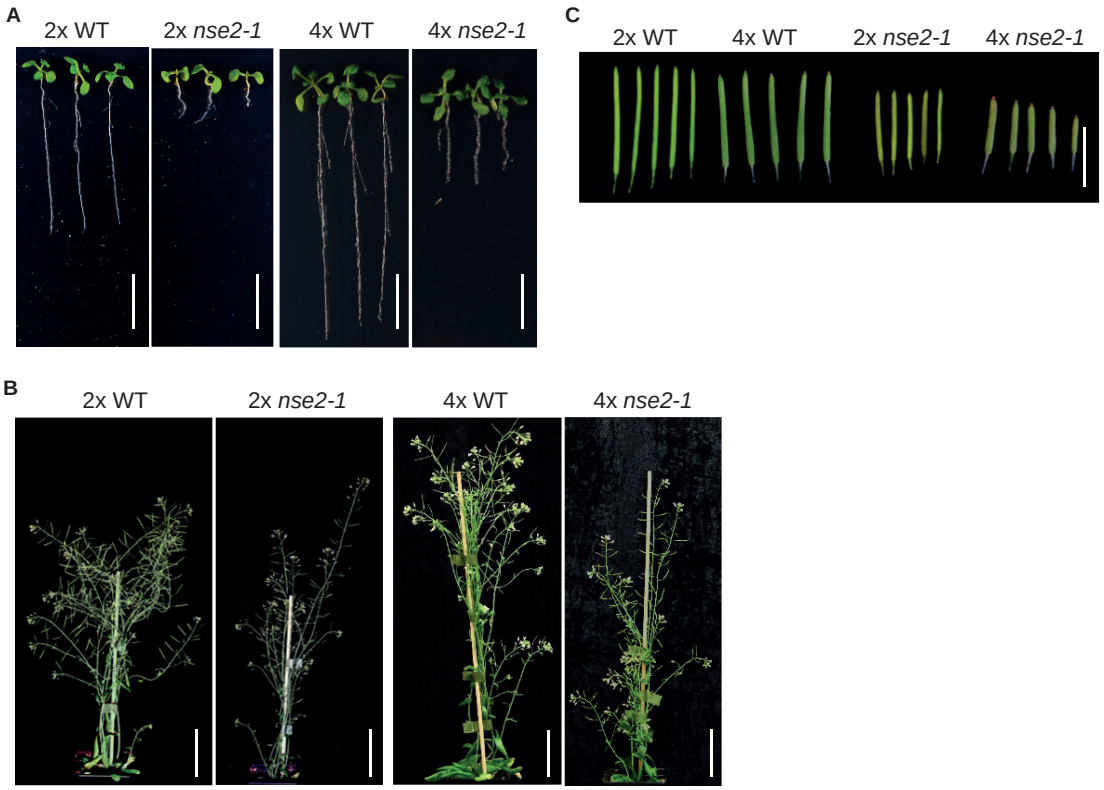

FIGURE 1 | Developmental phenotype of diploid (2x) and tetraploid (4x) WT and *nse2-1* plants. **(A)** The phenotype of 2-weeks-old *in vitro* grown seedlings. Scale bar = 1 cm. **(B)** The whole plant phenotype of 5-week-old plants. Scale bars = 5 cm. **(C)** Representative siliques of the analyzed genotypes. Scale bar = 1 cm.

but at least some of the embryo sacs with abnormal nuclei can be fertilized.

Second, we assessed several male gametophyte traits. Using fluorescein diacetate (FDA) assay, we quantified the microspore viability. This revealed 29.4% (375 out of 1275) viable pollen in 4x *nse2-1* which was significantly less than 62.2% (941 out of 1512, Fisher's exact test, $P < 0.00001$) of such pollen in 4x WT plants (**Figures 4A,B**). It has to be noted that both 4x WT and *nse2-1* had also significantly less viable pollen compared to 2x WT and 2x *nse2-1* (95.3 and 65.0%, respectively; based on published data of Yang et al. (2021); Fisher's exact test, $P < 0.00001$) grown under the same cultivation conditions (**Supplementary Table 3**).

The 4x WT and 4x *nse2-1* genotypes used in this study were produced in the *qrt1-4* mutant background and were representing 4x WT *qrt1-4* single and 4x *nse2-1 qrt1-4* double mutants. The *qrt1* mutations cause a stable association of the microspores arising from one meiosis which allows scoring for a constitution of the male meiotic products and also for abnormally developed (small and shrunk) microspores (Preuss et al., 1994). In 4x *nse2-1 qrt1-4*, we found 32.5% (414 out of 1275) shrunk microspores (**Figure 4A**, arrows), which is similar with the 27% in 2x *nse2-1 qrt1-4* plants (**Figure 4C**). However, both 4x and 2x WT *qrt1-4* plants showed much lower frequencies (5.0 and 0.7%, **Figure 4C**) of shrunken microspores in our experiments (75 out of 1512 and 4 out of 575, respectively; **Figure 4A**, arrows; **Supplementary Table 4**). This suggests that 4x WT plants have a seven-fold higher frequency of pollen abortion compared to the 2x WT and the pollen abortion rate remained similarly high in the mutants irrespective of their ploidy. Finally, we scored how many microspores were produced from one meiotic division (irrespective of their viability and shape). All meiotic products were tetrads in 4x WT *qrt1-4*, indicating that these plants undergo normal reductional division. On contrary, 4x *nse2-1* plants produced less than half (40.4%) of microspores in tetrads (**Figure 4D**). The remaining meiotic products were monads (9.6%), dyads (37.8%), triads (11.2%), and rarely even pentads (1.1%). This suggests abnormal meiosis in 4x *nse2* with the possible absence of the reductional divisions (monads to triads) or multipolar spindle (pentads).

Taken together, our results showed that 4x *nse2* plants produce a high number of abnormal male and female gametes.

Defective Male Meiosis Leads to Unreduced and Aneuploid Microspores in 4x *nse2-1* Plants

Meiotic progression in 2x *nse2* pollen mother cells takes place normally at prophase I. During metaphase I five bivalents are formed, but they are more stretched and elongated than in 2x WT. At anaphase I, chromosome fragments are present in most cells. During second meiotic division different problems are revealed such as non-reduced nuclei, extensive

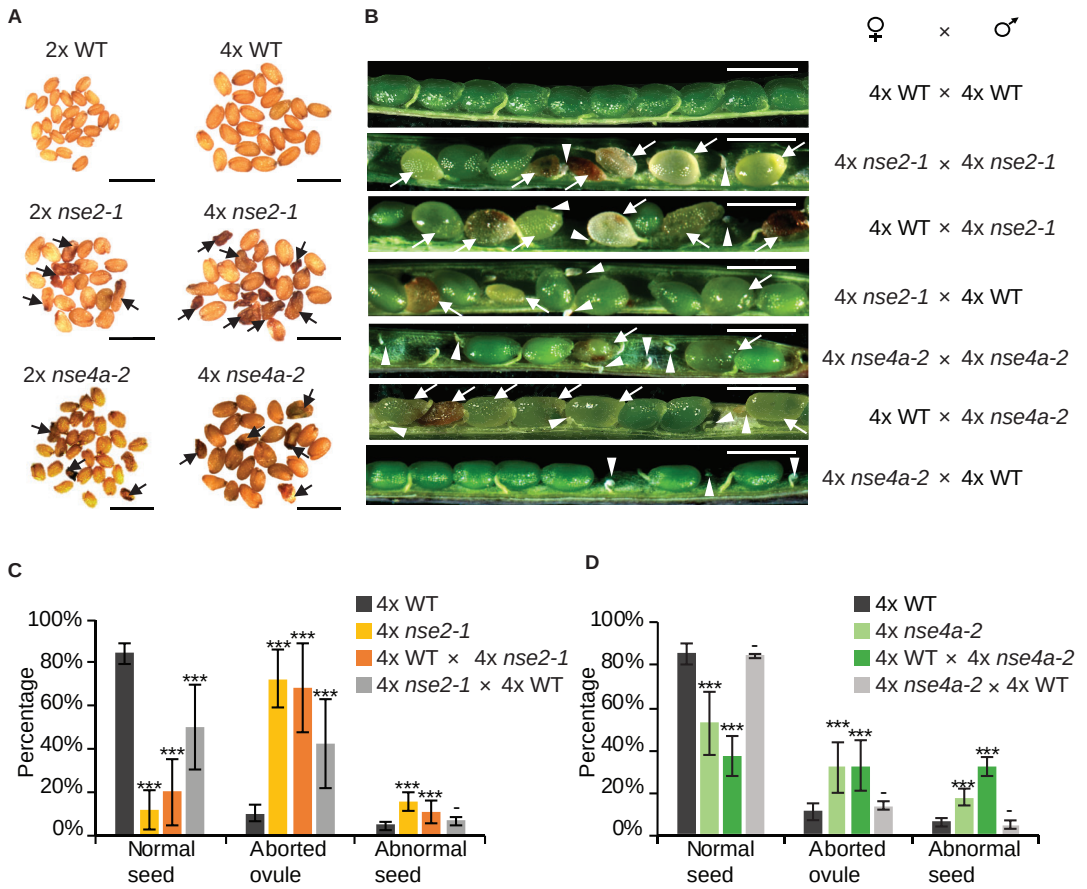

FIGURE 2 | Reduced fertility of tetraploid *nse2-1* and *nse4a-2* plants. **(A)** Representative dry seeds of diploid (2x) and tetraploid (4x) WT, *nse2-1*, and *nse4a-2* plants. Arrows indicate examples of shrunken seeds. Scale bars = 1 mm. **(B)** Opened siliques 13 days after pollination (DAP). Aborted ovules are marked with arrowheads and abnormal seeds (typically shrunk, brown/pale, or partially transparent) with arrows. Scale bar = 1 mm. **(C,D)** Frequencies of normal seeds (NS), aborted ovules (AO) and abnormal seeds (AS) in manually pollinated 4x WT, 4x *nse2-1*, and 4x *nse4a-2* plants and their F1 reciprocal crosses. Error bars represent standard deviation among the means of three individual plants. Significance in Fisher's exact test relative to 4x WT in the given group (NS, AO, AS): – = $P > 0.05$, * = $P < 0.05$, ** = $P < 0.01$, and *** = $P < 0.001$. Source values and basic counts are provided in **Supplementary Table 1**.

chromosome fragmentation, and chromosome bridges, among others (Yang et al., 2021).

Similar to the situation in 2x *nse2*, we found no apparent differences between 4x *nse2-1* and 4x WT plants at prophase I. In both genetic backgrounds, we observed nearly complete synapsis at pachynema with some unsynapsed regions due to the presence of synaptic partner switches produced by multivalent associations involving three or even four chromosomes (**Figures 5A,B**, arrowheads). At metaphase I, the different multivalent associations in tetraploids depend on the pattern of CO formation among the four homologous chromosomes. In 4x WT plants, we observed bivalents and quadrivalents, but occasionally also trivalents and univalents (**Figure 5A**, arrowheads). In 4x *nse2-1* plants, we did not detect apparent differences in chromosome associations from WT, with bivalent and quadrivalent associations also being the majority (**Figure 5B**). Nevertheless, the frequency of cells with univalents was twice (14.81%, 4 out of 27) that of the WT (7.30%, 6 out of 82). Chromatin did not appear normal either, due to the frequent presence of constrictions and even fragments, which was similar to the observations in 2x *nse2* plants (Yang et al., 2021). During anaphase I and telophase I, chromosomal fragmentation increased, spanning the region between the segregating chromosomes, being evidenced in all 45 cells analyzed (**Figure 5B**). During these stages, we did not observe fragmentation in 4x WT plants in any case and we only detected chromosome laggards in one of the cells analyzed (6.25%, 1 out of 16) (**Figure 5A**, bottom row, arrows).

In the second meiotic division, the defects in 4x *nse2-1* plants were more drastic than in the first meiotic division, as a result of an accumulation of errors (**Figures 5B,C**). Meiotic irregularities were detected in almost all analyzed cells (93.0%, 40 out of 43), namely: (i) chromosome fragmentation (27.9%), (ii) chromatin bridges (23.3%), (iii) abnormal segregation (13.9%), (iv) meiocytes with several problems including chromosomal bridges and fragments (16.3%), and (v) non-reduced meiocytes (11.6%). In contrary, only 68.75% of the meiocytes have second meiotic division defect in 2x *nse2* pollen mother cells (Yang et al.,

TABLE 1 | Seed phenotype of self-pollinated and reciprocally crossed between tetraploid (4x) WT, *nse2-1*, and *nse4a-2* plants.

Mother	Father	Events (n)	Trait (%)		
			Normal seeds	Aborted ovules	Abnormal seeds
4x WT	4x WT	899	84.3	10.2	5.5
4x *nse2-1*	4x *nse2-1*	739	11.6	72.5	15.8
4x WT	4x *nse2-1*	691	20.3	68.7	11.0
4x *nse2-1*	4x WT	607	50.6	42.5	6.9
4x *nse4a-2*	4x *nse4a-2*	843	51.8	31.1	17.1
4x WT	4x *nse4a-2*	802	53.2	31.7	15.1
4x *nse4a-2*	4x WT	722	83.4	13.2	3.5

TABLE 2 | Flow cytometry-based ploidy levels of F1 offspring plants from tetraploid (4x) WT, *nse2-1*, and *nse4a-2* parents.

Genotype		Germination rate (%)	Events (n)	Ploidy (%)				
Mother	Father			Euploid		Aneuploid		
				4x	6x	Total	+	−
4x WT	4x WT	72.7%	120	96.7	0.0	3.3	100.0	0.0
4x *nse2-1*	4x *nse2-1*	48.7%	92	32.6	19.6	47.8	70.5	29.5
4x WT	4x *nse2-1*	66.3%	163	75.4	8.6	16.0	88.5	11.5
4x *nse2-1*	4x WT	84.9%	247	84.2	1.2	14.6	69.4	30.6
4x *nse4a-2*	4x *nse4a-2*	89.1%	196	85.2	6.6	8.2	68.8	31.2
4x WT	4x *nse4a-2*	55.8%	91	91.2	4.4	4.4	100.0	0.0
4x *nse4a-2*	4x WT	88.6%	124	93.5	0.0	6.5	75.0	25.0

4x = tetraploid, 6x = hexaploid. + = DNA gain, − = DNA loss.

2021). In 4x WT plants, the irregularities were also detected in a low percentage of meiocytes (8.6%, 13 out of 150) during the second meiotic division (**Figure 5C**). On contrary to 4x *nse2-1*, chromosomal bridges or fragments were never observed in 4x WT plants. However, they displayed incorrect segregation of one or two chromosomes (possibly due to the formation of univalents and multivalents in metaphase I).

At the end of the meiotic division, only tetrads (n = 134) with apparently balanced nuclei were observed in 4x WT plants (**Figures 5A,D**), although we cannot exclude occasional aneuploidies (as a consequence of improper segregation during the second meiotic division). These aneuploidies would only affect one or two chromosomes. In 4x *nse2-1* plants, we observed dyads (54.3%, 44 out of 81), triads (3.7%, 3 out of 81), tetrads (35.8%, 29 out of 81), and even tetrads with micronuclei (6.17%, 5 out of 81) (**Figures 5B,D**). In 2x *nse2* plants the tetrads represented 49.2% and no micronuclei were observed (Yang et al., 2021). In summary, analysis of meiosis in 4x *nse2-1* plants revealed that the defects during later stages of meiosis were enhanced compared to 2x *nse2-1* plants.

Tetraploid *nse2-1* and *nse4a-2* Plants Produce Aneuploid Offspring

The spectrum of meiotic defects in 4x *nse2-1* plants prompted us to analyze the ploidy levels of their progeny by flow cytometry. Among the offspring from self-pollinated 4x WT plants (n = 120), we found 96.7% tetraploids (117 out of 120) and 3.3% (3 out of 120) putative aneuploids (**Table 2**). The three putative aneuploid WT plants showed minimal shifts of the flow cytometry peaks relative to the known tetraploid control. This is in contrast with an earlier study which showed that natural and synthetic 4x WT Arabidopsis plants produced about 30% aneuploid progeny (Henry et al., 2006). Since Henry and colleagues used more sensitive detection methods and also pointed to a lower sensitivity of flow cytometry to detect single chromosome addition/loss genotypes, our frequencies are most likely an underestimation.

Among 92 plants derived from self-pollinated 4x *nse2-1*, we found 32.6% tetraploids (30 out of 92), 47.8% putative aneuploids (44 out of 92), and 19.6% hexaploids (18 out of 92) (**Table 2** and **Figure 6**). The hexaploids were most likely a product of a fusion of one reduced and one unreduced gamete. The flow cytometry analysis of the aneuploid mutant plants indicated that 70.5% (31 out of 44) gained and 29.5% (13 out of 44) lost one or more chromosomes (**Table 2**). In the self-pollinated 4x *nse4a-2* (n = 196), we found 85.2% tetraploids (167 out of 196), 8.2% putative aneuploids (16 out of 196), and 6.6% hexaploids (13 out of 196) (**Table 2**). Similar to 4x *nse2-1*, about two-thirds (68.7%) of the putative aneuploids gained and one-third (31.3%) lost one or more chromosomes (**Table 2**).

To confirm the aneuploidy, we performed cytology analysis on the selected candidates that appeared to have either extra or missing chromosomes (**Figure 5C**). To address the parental contribution to the ploidy changes in the progeny, we performed reciprocal crosses between 4x WT and 4x *nse2* plants and analyzed the ploidy of the resulting plants (**Table 2**). The

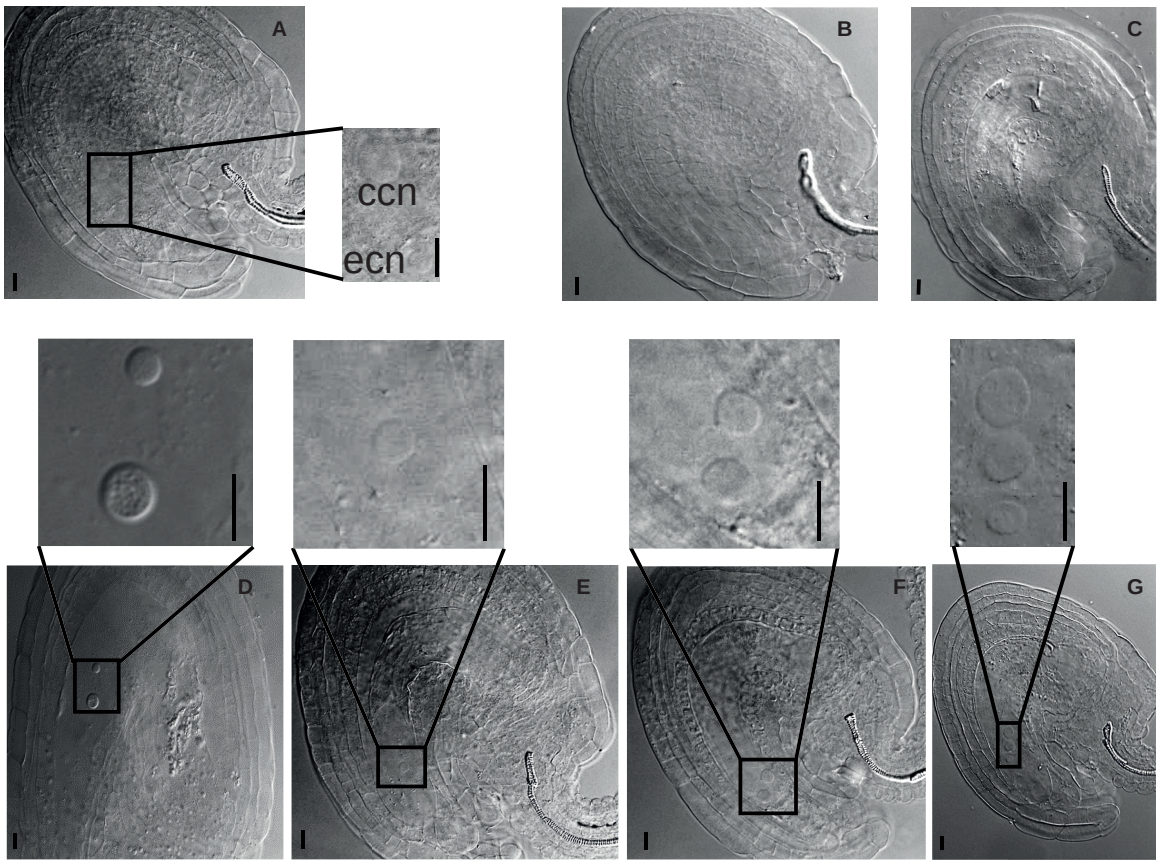

FIGURE 3 | Developmental defects in female gametophyte of tetraploid *nse2-1*. Cleared ovules from 4x *nse2-1* were observed under a differential interference contrast microscope. The nuclei are marked with dashed circles and arrows. Scale bars = 10 μm. **(A)** A typical 4x WT-like embryo sac showing one egg cell nucleus (ecn) and one central cell nucleus (ccn). **(B–G)** 4x *nse2-1* ovules displaying specific defects: **(B)** absence of embryo sac and **(C)** embryo sacs **(C)** without nuclei, **(D)** with two nuclei at the abnormal position, **(E)** only one nucleus, **(F)** two equally size nuclei, and **(G)** one smaller nucleus and two bigger nuclei.

hexaploidy was caused 7.2-fold more frequently by the unreduced paternal over the maternal 4x *nse2-1* gametes (8.6 versus 1.2%, respectively; **Table 2**). In contrast, the aneuploidy was caused equally by the paternal (15.3%) and the maternal (14.2%) 4x *nse2-1* gametes (**Table 2**). We found similar trends for the induction of hexaploidy and aneuploidy in the progeny of 4x *nse4a-2*. There were 4.0% hexaploids in 4x WT × 4x *nse4a-2* crosses versus 0% hexaploids in reciprocal crossing direction (**Table 2**). The aneuploid offspring arose almost equally from both paternal and maternal gametes (4.4 and 6.5%, respectively; **Table 2**).

Hence, the 4x SMC5/6 complex mutants produce higher-polyploid and also aneuploid offspring from both parents.

DISCUSSION

Here, we analyzed the effects of polyploidy on the genome stability and reproductive success in the background of autotetraploid Arabidopsis SMC5/6 complex deficient mutants. Most of our experiments focused on *NSE2*, which encodes an important, but in Arabidopsis non-essential, E3 SUMO ligase subunit of the SMC5/6 complex (Ishida et al., 2009). For a subset of experiments, we analyzed also *NSE4A*, as the kleisin subunit of the SMC5/6 complex that is active in both somatic and reproductive tissues and is essential for plant survival (Díaz et al., 2019). The strong loss-of-function alleles of *NSE4A* are lethal in Arabidopsis and the *nse4a-2* allele used here is a partial loss-of-function mutant. This is in agreement with our observations that the defects of both mutants are similar, but those of *nse4a-2* plants are generally weaker.

The SMC5/6 complex has multiple functions during meiosis. It is required for the repair of SPO11-induced DNA double-strand breaks and its absence produces a severe chromosome fragmentation due to the presence of entanglements and concatenations generated as a consequence of an accumulation of joint molecules (JM) (Copsey et al., 2013; Xaver et al., 2013; Menolfi et al., 2015). A recent study from Arabidopsis also suggested that RAD51 restrains the SMC5/6 complex from inhibiting the activity of meiotic recombinase DMC1 (Chen et al., 2021). In addition, *nse2* mutants generate diplogametes (Yang et al., 2021). The non-reduced nuclei result from cells with an abnormal spindle organization in which organelles are not organized in a defined band after telophase I. Interestingly, this trait is recombination-independent. In this study, we analyzed

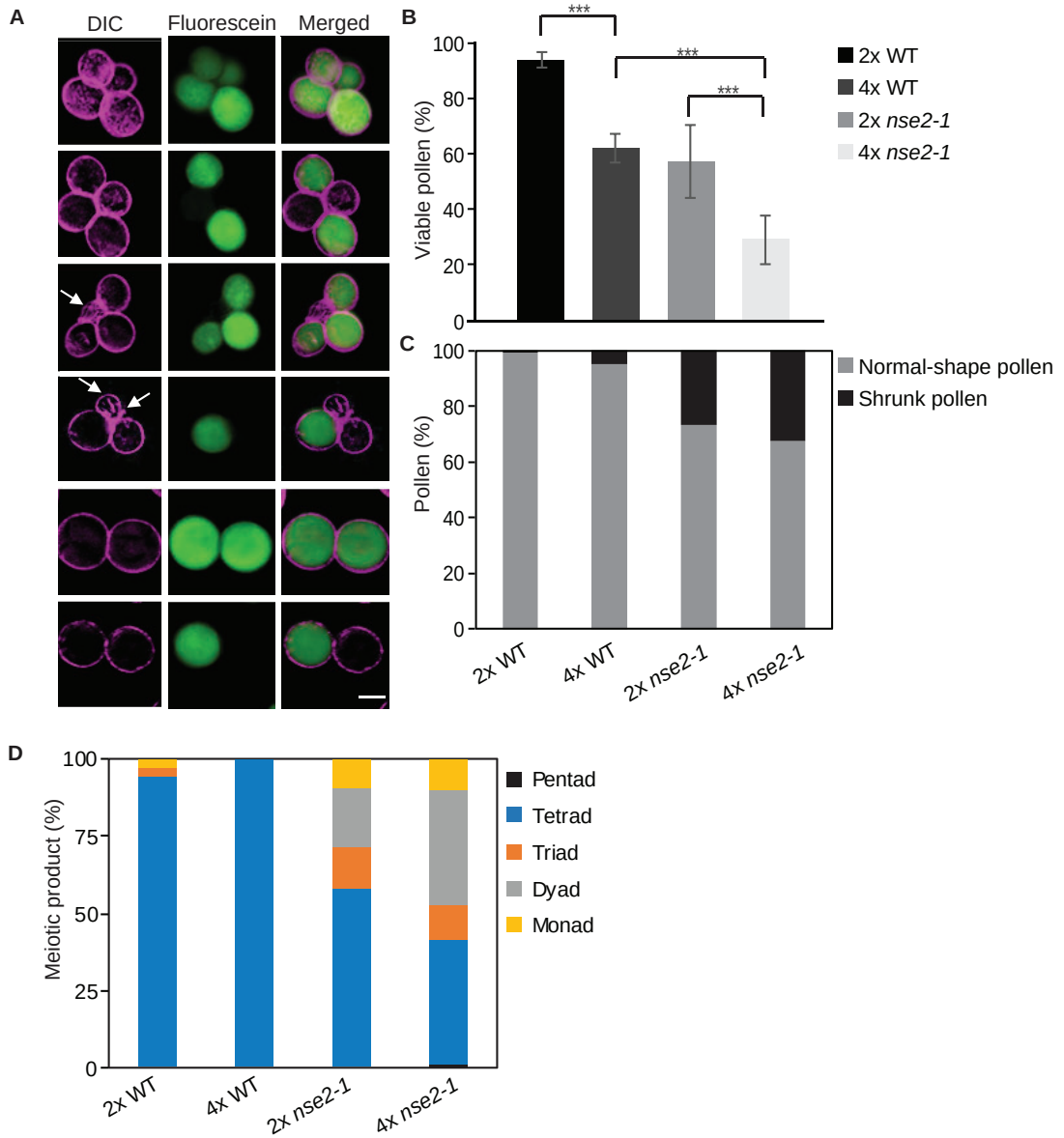

FIGURE 4 | Microspore phenotype of tetraploid (4x) *nse2-1* plants. **(A)** Representative mature pollen stained by fluorescein diacetate from 4x *nse2-1 qrt1-4* plants. Differential interference contrast (DIC) images were pseudocolored in violet. Viable microspores are indicated by fluorescein signals (green). Shrunk microspores are indicated by arrows. Scale bar = 20 μm. **(B)** Frequencies of viable pollen from 2x WT, 4x WT, 2x *nse2-1*, and 4x *nse2-1* (all in *qrt1-4* background). Error bars represent standard deviations among the means of two or three individual plants. Significance in Fisher's exact test: – = $P > 0.05$, * = $P < 0.05$, ** = $P < 0.01$, and *** = $P < 0.001$. Source values and basic counts are provided in **Supplementary Table 3**. **(C)** Quantification of normal-shape and shrunken pollen observed in 2x WT, 4x WT, 2x *nse2-1*, and 4x *nse2-1* in *qrt1-4* background. **(D)** Quantification of meiotic products with different numbers of microspores as observed in 2x WT, 4x WT, 2x *nse2-1*, and 4x *nse2-1* in *qrt1-4* background.

the consequences of polyploidy in the SMC5/6 complex mutants to find whether the duplication of the entire genome will buffer or enhance the defects present in the diploid mutant and whether there will be new features compared to WT plants. We observed a higher frequency of univalents in 4x *nse2* compared to the 4x WT plants. However, in the diploid mutant, five bivalents are invariably formed, as in the diploid control (Yang et al., 2021). The defects of the tetraploid mutant were more drastic in the second meiotic division. A higher percentage of meiocytes with abnormalities was observed with respect to the diploid mutant (93 *vs.* 69%, see also Yang et al., 2021). This could be explained by the role of the SMC5/6 complex in the HR process (Chen et al., 2021), which may be more important in the autotetraploid context as suggested by our data. The accumulation of JMs would be higher in a situation in which the chances of finding homologous sequences to recombine are

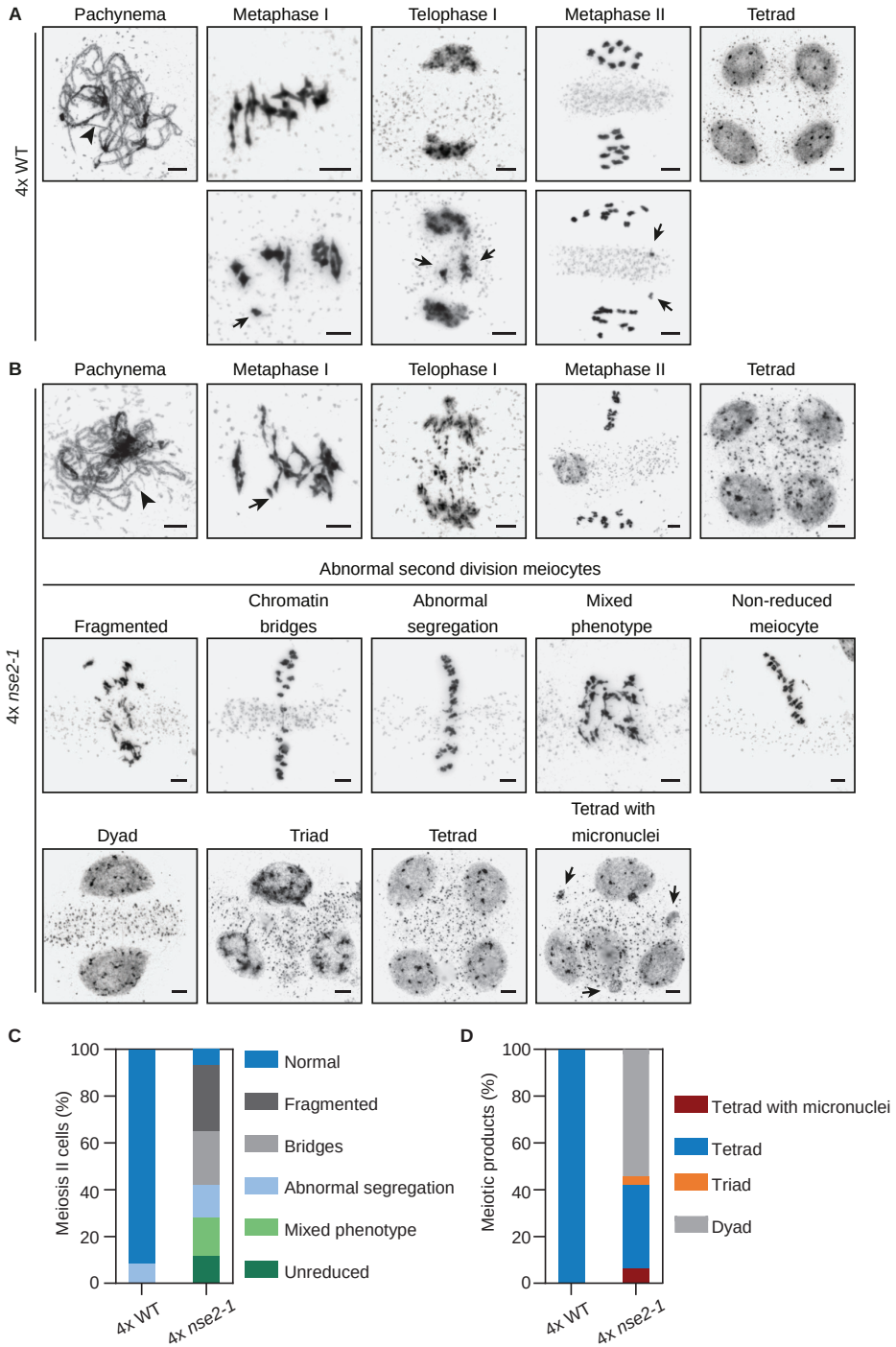

FIGURE 5 | Analysis of male meiosis in 4x *nse2-1* plants. **(A)** Representative images of selected meiotic stages in 4x WT plants. Top row: Unsynapsed regions are detected in pachynema (arrowhead). At metaphase I, the chromosome associations are mostly bivalents and quadrivalents. In most meiocytes, chromosome segregation is correct, both in the first and second meiotic division, resulting in the formation of tetrads with balanced nuclei. Bottom row: Examples of metaphase I with a univalent, telophase I with a delay in chromosome segregation, and a metaphase II with chromatids resulting from the equational segregation of anaphase I univalent. **(B)** Representative images of the phenotype observed in 4x *nse2-1* plants. As well as in WT plants, there were some unsynapsed regions at pachynema (arrowhead). At metaphase I, an increase in the frequency of univalents (arrow) was detected with respect to the WT. Complex entanglements were also frequent at this stage. Telophases I displayed a high frequency of chromosome fragments. Meiotic problems were also evident during the second meiotic division in almost all cells analyzed, including chromosome fragmentation, chromatin bridges and/or abnormal chromosome segregation, and non-reduced meiocytes. At the end of the meiotic division, dyads, triads, tetrads, and tetrads with micronuclei were formed (bottom row). See the text for more details. Scale bars = 5 μm. **(C)** Quantification of the different phenotype observed in 4x *nse2-1* plants during second meiotic division. **(D)** Quantification of division products at the end of meiosis. Note: The data for 2x WT and 2x *nse2* plants were published previously (Yang et al., 2021; **Figures 3**, **4**).

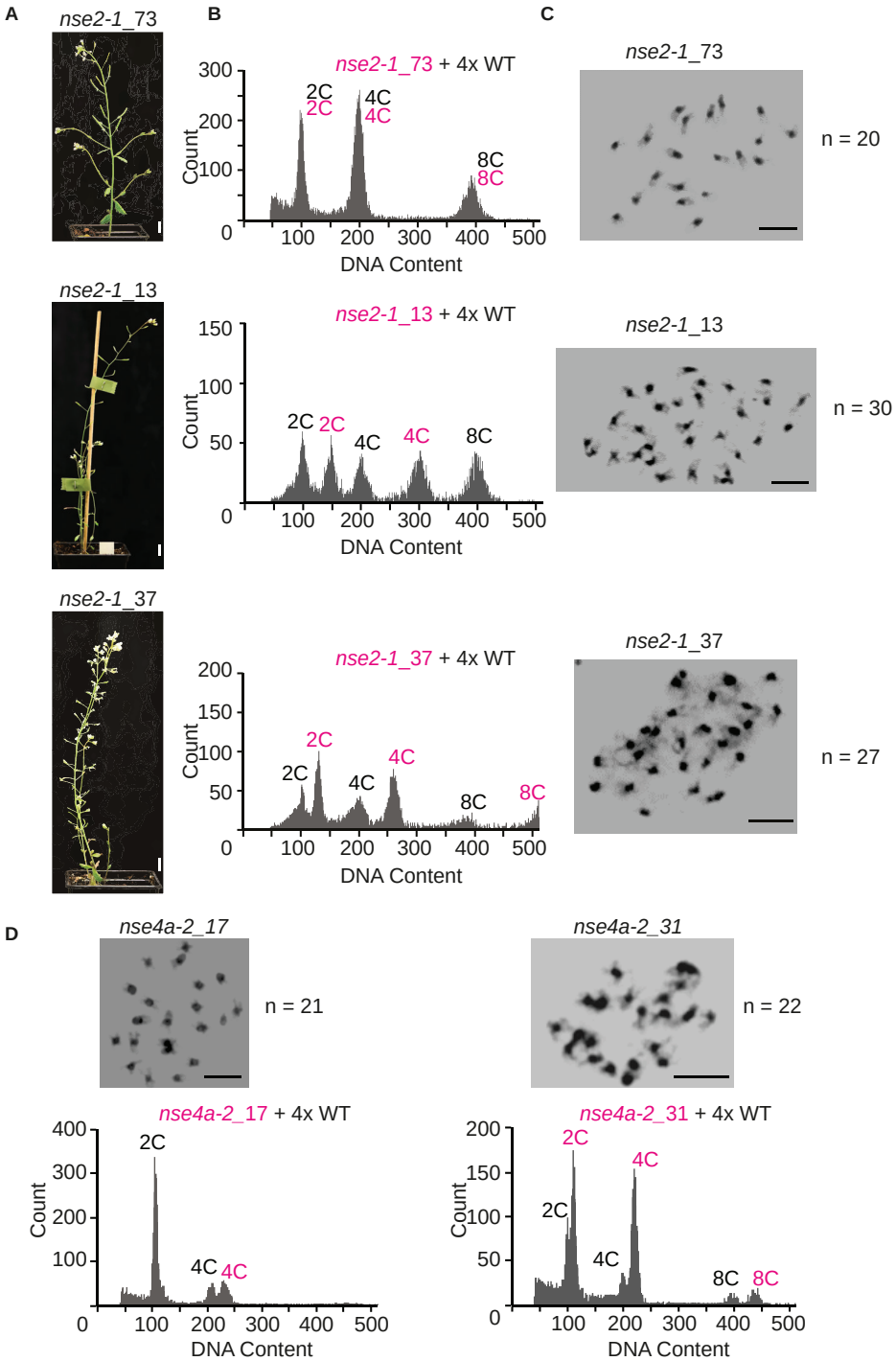

FIGURE 6 | Flow-cytometry ploidy determination in offspring of 4x *nse2-1* and 4x *nse4a-2* plants. **(A)** Phenotype of selected 4-weeks-old plants of *nse2-1_73*, *nse2-1_13*, and *nse2-1_37*. Scale bars = 1 cm. **(B)** Flow-cytometry histograms of plants shown in panel **(A)**, indicating that *nse2-1_73* is tetraploid, *nse2-1_13* hexaploid, and *nse2-1_37* aneuploid. For the ploidy measurements, the nuclei suspension was prepared by mixing leaves from tested *nse2-1* plants and 4x WT control. Multiple peaks correspond to nuclei of different C content as indicated. **(C)** Mitotic figures of *nse2-1_73*, *nse2-1_13*, and *nse2-1_37* plants. The number of chromosomes is given next to the figures. Scale bars = 5 μm. **(D)** Mitotic metaphase plates and flow-cytometry histograms of the ploidy level of two selected aneuploid *nse4-2* plants. Chromosome numbers are given right of the figures. Scale bars = 5 μm. The ploidy levels were measured by preparing the nuclei suspension from a mix of the leaves the *nse4a-2* candidate plant and 4x WT control. Note that an addition of a single or two chromosomes is clearer visible at 4C peaks (or 8C peaks if visible).

increased (Voorrips and Maliepaard, 2012). On the other hand, in 4x *nse2* plants we detected a lower percentage (nearly half) of second division meiocytes displaying all chromosomes on one side of the organelle band (non-reduced) compared to 2x *nse2* plants (11.63 *vs.* 19.40%, see also Yang et al., 2021). Although the percentage of dyads was similar in the tetraploid and the diploid *nse2-1* plants, a lower percentage of tetrads was detected in 4x *nse2* plants. In the 4x mutant, we also observed tetrads with micronuclei, which were not formed in the case of the 2x mutant. As mentioned above, non-reduced meiocytes appear as a consequence of recombination-independent problems generated by the absence of the SMC5/6 complex. In our recent study focusing on the meiosis of 2x *nse2* plants, we showed that this may be related to the organization of the spindle, to the interaction of the kinetochores and the spindle or to delays in chromosome segregation (Yang et al., 2021). In this context, the improper localization of a defined organelle band prevents the formation of two defined nuclei with five chromosomes each (2x *nse2*) or ten chromosomes each (4x *nse2*). The fact that the frequency of non-reduced meiocytes is lower in the 4x relative to the 2x mutant can be explained by the increase in the number of chromosomes. The location of all twenty chromosomes in a single nucleus is less likely than the location of the ten chromosomes. This would also explain why there are more aneuploidies in the tetraploid mutant and a greater reduction in fertility.

The meiotic irregularities in SMC5/6 complex mutants have also profound effects on the seed development and offspring genomic constitution. The 4x *nse2* plants are almost sterile, with less than 10% normal seeds, compared to about 35% such seeds in the 2x mutant. This is due to a strongly affected ovule development leaving only about 30% of ovules capable of seed development in 4x *nse2-1*. Many of the developing seeds are aneuploid, represented mostly by addition of one or two chromosomes. Importantly, the aneuploidy was caused equally from both maternal and paternal sides. Rarely, also unreduced female gametes of tetraploid *nse2* plants gave rise to the hexaploid offspring. These are tetraploidy-associated characters because we observed neither the aneuploidy offspring nor the viable unreduced female gametes in 2x *nse2* plants.

The analysis of polyploids makes it possible to explore some aspects of meiosis more in depth compared to diploids, since in a polyploid condition the chances of pairing and finding homologous sequences to recombine increase. Altogether, our results highlight that the mutations in the SMC5/6 complex cause partially common, but also some unique characters when comparing the phenotypes of diploid and the tetraploid plants. A similar situation has been described in other mutants, for example, those affecting suppressors of recombination like *FANCONI ANEMIA COMPLEMENTATION GROUP M* (*FANCM*). The *fancm* mutants produce a significant increase in HR in diploid plants (Crismani et al., 2012; Li et al., 2021). However, silencing FANCM in tetraploid plants has less or no effect on recombination (Blary et al., 2018; Raz et al., 2021). This indicates that due to the specificities of tetraploid meiosis, the importance of certain molecular factors and complexes may increase or decrease. In summary, the described defects highlight the importance of studying the consequences of mutations in genes affecting meiosis and reproductive development in diploid versus polyploid conditions, especially in the crop species, where polyploids could provide the potential to increase agriculturally important traits.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

AP, FY, and MP designed the project. FY performed plant phenotypic characterization, crosses, analysis of pollen, and ploidy measurements. NF prepared and analyzed meiocytes. JM counted mitotic chromosome numbers. AP and FY wrote the manuscript with help of other authors. All authors approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2021.748252/full#supplementary-material

REFERENCES

Alonso, J. M., Stepanova, A. N., Leisse, T. J., Kim, C. J., Chen, H., Shinn, P., et al. (2003). Genome-wide insertional mutagenesis of Arabidopsis thaliana. *Science.* 301, 653–657. doi: 10.1126/science.1086391

Aragón, L. (2018). The Smc5/6 complex: new and old functions of the enigmatic long-distance relative. *Annu. Rev. Genet.* 52, 89–107. doi: 10.1146/annurev-genet-120417-031353

Blary, A., Gonzalo, A., Eber, F., Bérard, A., Bergès, H., Bessoltane, N., et al. (2018). FANCM limits meiotic crossovers in brassica crops. *Front. Plant Sci.* 9:368. doi: 10.3389/fpls.2018.00368

Chen, H., He, C., Wang, C., Wang, X., Ruan, F., Yan, J., et al. (2021). RAD51 supports DMC1 by inhibiting the SMC5/6 complex during meiosis. *Plant Cell* 33, 2869–2882. doi: 10.1093/plcell/koab136

Comai, L. (2005). The advantages and disadvantages of being polyploid. *Nat. Rev. Genet.* 6, 836–846. doi: 10.1038/nrg1711

Copsey, A., Tang, S., Jordan, P. W., Blitzblau, H. G., Newcombe, S., Chan, A. C., et al. (2013). Smc5/6 coordinates formation and resolution of joint molecules with chromosome morphology to ensure meiotic divisions. *PLoS Genet.* 9:e1004071. doi: 10.1371/journal.pgen.1004071

Crismani, W., Girard, C., Froger, N., Pradillo, M., Santos, J. L., Chelysheva, L., et al. (2012). FANCM limits meiotic crossovers. *Science* 336, 1588–1590. doi: 10.1126/science.1220381

Díaz, M., Pečinková, P., Nowicka, A., Baroux, C., Sakamoto, T., Gandha, P. Y., et al. (2019). The SMC5/6 complex subunit NSE4A is involved in DNA damage repair and seed development. *Plant Cell* 31, 1579–1597. doi: 10.1105/tpc.18.00043

Henry, I. M., Dilkes, B. P., and Comai, L. (2006). Molecular karyotyping and aneuploidy detection in *Arabidopsis thaliana* using quantitative fluorescent polymerase chain reaction. *Plant J.* 48, 307–319. doi: 10.1111/j.1365-313X.2006.02871.x

Hu, Z., Cools, T., and De Veylder, L. (2016). Mechanisms used by plants to cope with DNA damage. *Annu. Rev. Plant Biol.* 67, 439–462. doi: 10.1146/annurev-arplant-043015-111902

Huang, L., Yang, S., Zhang, S., Liu, M., Lai, J., Qi, Y., et al. (2009). The Arabidopsis SUMO E3 ligase AtMMS21, a homologue of NSE2/MMS21, regulates cell proliferation in the root. *Plant J.* 60, 666–678. doi: 10.1111/j.1365-313X.2009.03992.x

Ishida, T., Fujiwara, S., Miura, K., Stacey, N., Yoshimura, M., Schneider, K., et al. (2009). SUMO E3 ligase HIGH PLOIDY2 regulates endocycle onset and meristem maintenance in Arabidopsis. *Plant Cell* 21, 2284–2297. doi: 10.1105/tpc.109.068072

Ishida, T., Yoshimura, M., Miura, K., and Sugimoto, K. (2012). MMS21/HPY2 and SIZ1, Two Arabidopsis SUMO E3 Ligases, Have Distinct Functions in Development. *PLoS One* 7:e46897. doi: 10.1371/journal.pone.0046897

Jullien, P. E., and Berger, F. (2010). Parental Genome Dosage Imbalance Deregulates Imprinting in Arabidopsis. *PLoS Genet.* 6:e1000885. doi: 10.1371/journal.pgen.1000885

Kegel, A., and Sjögren, C. (2010). The Smc5 / 6 Complex: more than repair? *Cold Spring Harb. Symp. Quant. Biol.* 75, 179–187. doi: 10.1101/sqb.2010.75.047

Köhler, C., Wolff, P., and Spillane, C. (2012). Epigenetic mechanisms underlying genomic imprinting in plants. *Annu. Rev. Plant Biol.* 63, 331–352. doi: 10.1146/annurev-arplant-042811-105514

Kwak, J. S., Son, G. H., Kim, S. I., Song, J. T., and Seo, H. S. (2016). Arabidopsis HIGH PLOIDY2 Sumoylates and stabilizes flowering locus C through Its E3 ligase activity. *Front. Plant Sci.* 7:530. doi: 10.3389/fpls.2016.00530

Li, X., Yu, M., Bolaños-Villegas, P., Zhang, J., Ni, D., Ma, H., et al. (2021). Fanconi anemia ortholog FANCM regulates meiotic crossover distribution in plants. *Plant Physiol.* 186, 344–360. doi: 10.1093/plphys/kiab061

Liu, C. M., and Meinke, D. W. (1998). The titan mutants of Arabidopsis are disrupted in mitosis and cell cycle control during seed development. *Plant J.* 16, 21–31. doi: 10.1046/j.1365-313x.1998.00268.x

Liu, M., Shi, S., Zhang, S., Xu, P., Lai, J., Liu, Y., et al. (2014). SUMO E3 ligase AtMMS21 is required for normal meiosis and gametophyte development in Arabidopsis. *BMC Plant Biol.* 14:153. doi: 10.1186/1471-2229-14-153

Mandáková, T., and Lysak, M. A. (2018). Post-polyploid diploidization and diversification through dysploid changes. *Curr. Opin. Plant Biol.* 42, 55–65. doi: 10.1016/j.pbi.2018.03.001

Mengiste, T., Revenkova, E., Bechtold, N., and Paszkowski, J. (1999). An SMC-like protein is required for efficient homologous recombination in arabidopsis. *EMBO J.* 18, 4505–4512. doi: 10.1093/emboj/18.16.4505

Menolfi, D., Delamarre, A., Lengronne, A., Pasero, P., and Branzei, D. (2015). Essential Roles of the Smc5/6 Complex in replication through natural pausing sites and endogenous DNA damage tolerance. *Mol. Cell* 60, 835–846. doi: 10.1016/j.molcel.2015.10.023

Morgan, C., Zhang, H., Henry, C. E., Franklin, F. C. H., and Bomblies, K. (2020). Derived alleles of two axis proteins affect meiotic traits in autotetraploid Arabidopsis arenosa. *Proc. Natl. Acad. Sci. U. S. A.* 117, 8980–8988. doi: 10.1073/pnas.1919459117

Palecek, J. J., and Gruber, S. (2015). Kite Proteins: a superfamily of SMC/Kleisin partners conserved across bacteria, archaea, and eukaryotes. *Structure* 23, 2183–2190. doi: 10.1016/j.str.2015.10.004

Parra-Nunez, P., Pradillo, M., and Santos, J. L. (2020). "How to Perform an Accurate Analysis of Metaphase I Chromosome Configurations in Autopolyploids of *Arabidopsis thaliana*," in *Plant Meiosis: Methods and Protocols*, eds M. Pradillo and S. Heckmann (New York: Springer), 25–36. doi: 10.1007/978-1-4939-9818-0_3

Pecinka, A., Fang, W., Rehmsmeier, M., Levy, A. A., and Mittelsten Scheid, O. (2011). Polyploidization increases meiotic recombination frequency in Arabidopsis. *BMC Biol.* 9:24. doi: 10.1186/1741-7007-9-24

Preuss, D., Rhee, S. Y., and Davis, R. W. (1994). Tetrad analysis possible in Arabidopsis with mutation of the QUARTET (QRT) genes. *Science* 264, 1458–1460. doi: 10.1126/science.8197459

Raz, A., Dahan-Meir, T., Melamed-Bessudo, C., Leshkowitz, D., and Levy, A. A. (2021). Redistribution of meiotic crossovers along wheat chromosomes by virus-induced gene silencing. *Front. Plant Sci.* 11:635139. doi: 10.3389/fpls.2020.635139

Roy, S. (2014). Maintenance of genome stability in plants: repairing DNA double strand breaks and chromatin structure stability. *Front. Plant Sci.* 5:487. doi: 10.3389/fpls.2014.00487

Sánchez Moran, E., Armstrong, S. J., Santos, J. L., Franklin, F. C. H., and Jones, G. H. (2001). Chiasma formation in *Arabidopsis thaliana* accession Wassileskija and in two meiotic mutants. *Chromosome Res.* 9, 121–128. doi: 10.1023/A:1009278902994

Santos, J. L., Alfaro, D., Armstrong, S. J., Franklin, F. C. H., and Jones, G. H. (2003). Partial diploidization of meiosis in autotetraploid *Arabidopsis thaliana*. *Genetics* 165, 1533–1540. doi: 10.1093/genetics/165.3.1533

Seear, P. J., France, M. G., Gregory, C. L., Heavens, D., Schmickl, R., Yant, L., et al. (2020). A novel allele of ASY3 is associated with greater meiotic stability in autotetraploid Arabidopsis lyrata. *PLoS Genet.* 16:e1008900. doi: 10.1371/journal.pgen.1008900

Uhlmann, F. (2016). SMC complexes: from DNA to chromosomes. *Nat. Rev. Mol. Cell Biol.* 17, 399–412. doi: 10.1038/nrm.2016.30

Van De Peer, Y., Fawcett, J. A., Proost, S., Sterck, L., and Vandepoele, K. (2009). The flowering world: a tale of duplications. *Trends Plant Sci.* 14, 680–688. doi: 10.1016/j.tplants.2009.09.001

Voorrips, R. E., and Maliepaard, C. A. (2012). The simulation of meiosis in diploid and tetraploid organisms using various genetic models. *BMC Bioinformatics* 13:248. doi: 10.1186/1471-2105-13-248

Watanabe, K., Pacher, M., Dukowic, S., Schubert, V., Puchta, H., and Schubert, I. (2009). The Structural maintenance of chromosomes 5/6 complex promotes sister chromatid alignment and homologous recombination after DNA damage in *Arabidopsis thaliana*. *Plant Cell* 21, 2688–2699. doi: 10.1105/tpc.108.060525

Xaver, M., Huang, L., Chen, D., and Klein, F. (2013). Smc5/6-Mms21 prevents and eliminates inappropriate recombination intermediates in meiosis. *PLoS Genet.* 9:e1004067. doi: 10.1371/journal.pgen.1004067

Xu, P., Yuan, D., Liu, M., Li, C., Liu, Y., Zhang, S., et al. (2013). AtMMS21, an SMC5/6 complex subunit, is involved in stem cell niche maintenance and DNA damage responses in Arabidopsis roots. *Plant Physiol.* 161, 1755–1768. doi: 10.1104/pp.112.208942

Yan, S., Wang, W., Marqués, J., Mohan, R., Saleh, A., Durrant, W. E., et al. (2013). Salicylic acid activates DNA damage responses to potentiate plant immunity. *Mol. Cell* 52, 602–610. doi: 10.1016/j.molcel.2013.09.019

Yang, F., Fernández-Jiménez, N., Tučková, M., Vrána, J., Cápal, P., Díaz, M., et al. (2021). Defects in meiotic chromosome segregation lead to unreduced male gametes in Arabidopsis SMC5/6 complex mutants. *Plant Cell* 33, 3104–3119. doi: 10.1093/plcell/koab178

Yuan, D., Lai, J., Xu, P., Zhang, S., Zhang, J., Li, C., et al. (2014). AtMMS21 regulates DNA damage response and homologous recombination repair in Arabidopsis. *DNA Repair (Amst).* 21, 140–147. doi: 10.1016/j.dnarep.2014.04.006

Zelkowski, M., Zelkowska, K., Conrad, U., Hesse, S., Lermontova, I., Marzec, M., et al. (2019). Arabidopsis NSE4 proteins act in somatic nuclei and meiosis to ensure plant viability and fertility. *Front. Plant Sci.* 10:774. doi: 10.3389/fpls.2019.00774

Zou, W., Li, G., Jian, L., Qian, J., Liu, Y., and Zhao, J. (2021). Arabidopsis SMC6A and SMC6B have redundant function in seed and gametophyte development. *J. Exp. Bot.* 72, 4871–4887. doi: 10.1093/jxb/erab181

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APPENDIX III

Understanding functions of STRUCTURAL MAINTENANCE OF CHROMOSOMES 5/6 complex during generative development

Fen Yang, Anna Nowicka, Mariana D áz, Ales Pecinka

In: Abstract of the “6th international Meeting Plant Genome Stability and Change”

Gatersleben, Germany, 2018

Understanding functions of STRUCTURAL MAINTENANCE OF CHROMOSOMES 5/6 complex during generative development

Fen Yang[1,2], Mariana Diaz[1,2], Anna Nowicka[1,2], Ales Pecinka[1,2]

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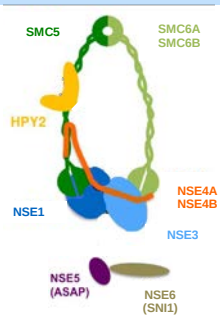
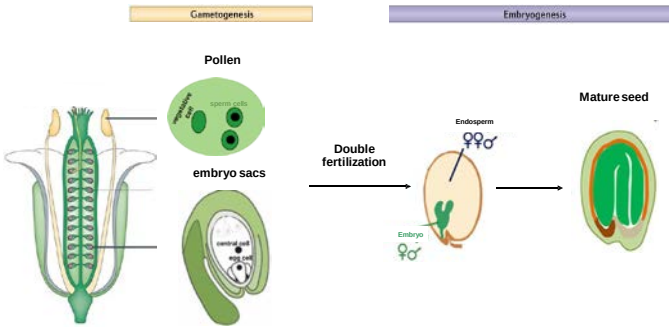
2 Max Planck Institute for Plant Breeding Research, Carl-von-Linne-Weg 10, Cologne, 50829, Germany

Introduction

Flowering plants undergo a series of complex developmental transitions including production of gametes and seeds during generative development. Double fertilization is the beginning of seed developmental process in flowering plants, which produces the diploid embryo and the triploid nutritive tissue endosperm. Structural maintenance of chromosomes (SMC) 5/6 complex has a crucial function in control of genome stability and DNA damage repair. However, precise molecular mechanisms of SMC5/6 complex functions in plants are unknown. This project will study the function of SMC5/6 complex during gametogenesis development in *Arabidopsis*.



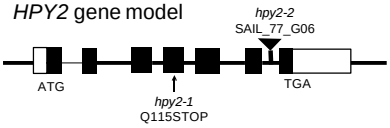
SMC5/6 complex



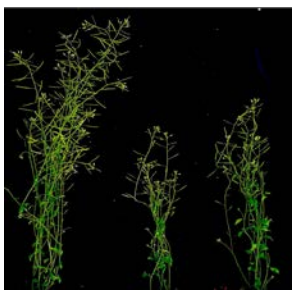
Results

Seed abortion is increased in *hpy2*

- *HPY2* gene model

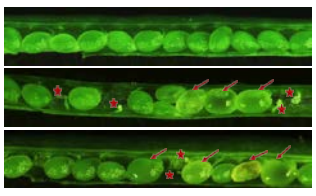


- 6-week-old plants



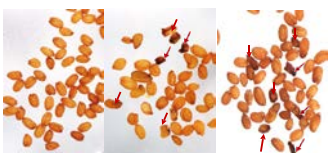
Col-0 *hpy2-1* *hpy2-2*

- Seed development



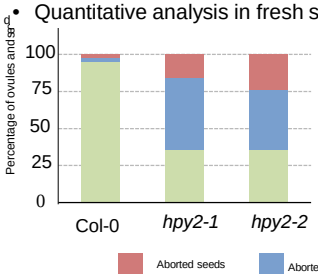
Col-0
hpy2-1
hpy2-2

Red stars indicate aborted ovules and red arrows indicate aborted seeds.

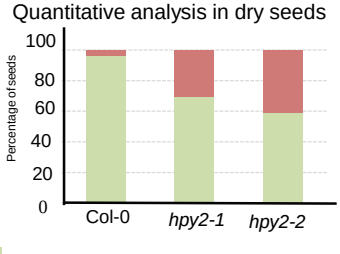


Col-0 *hpy2-1* *hpy2-2*

- Quantitative analysis in fresh seeds

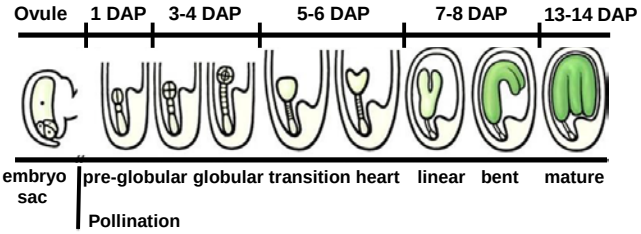


- Quantitative analysis in dry seeds

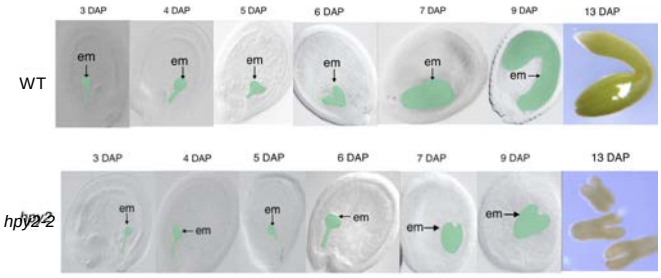


Embryo is arrested in *hpy2*

- Embryo development model in *Arabidopsis*



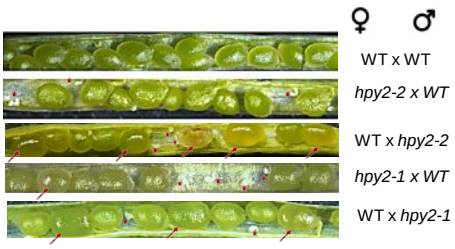
- Embryo development model in WT and *hpy2-2*



DAP: days after pollination, em: embryo.

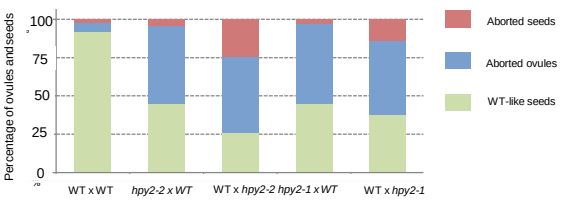
Preferential paternal inheritance of aborted seeds in *hpy2*

- Seed development in reciprocal crosses between *hpy2* and WT



Red stars indicate aborted ovules and red arrows indicate aborted seeds.

- Quantitative analysis in fresh seeds



Conclusion

SMC5/6 complex has important function in gametogenesis and seed development.

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CHINA SCHOLARSHIP COUNCIL
UNIVERZITA PALACKÉHO V OLOMOUCI

APPENDIX IV

Analysis of SMC5/6 complex mutant defects during reproductive development in Arabidopsis

Fen Yang, Nadia Fernández-Jiménez, Martina Tučková, Jan Vrána, Petr Cápal, Mariana Díaz, Mónica Pradillo, Ales Pecinka

In: Abstract of the "Second main INDEPTH meeting"

Prague, Czech Republic, 2019

Understanding functions of STRUCTURAL MAINTENANCE OF CHROMOSOMES 5/6 complex during generative development

Fen Yang[1,2,3], Nadia Fernandez-Lopez[4], Martina Tuckova[1], Anna Nowicka[1], Mariana Diaz[1,2], Monica Pradillo[4], Ales Pecinka[1]

1 Institute of Experimental Botany of AS CZ, Centre of the Region Haná for Biotechnological and Agricultural Research, Šlechtitelů 31, 783 71 Olomouc - Holice, Czech Republic
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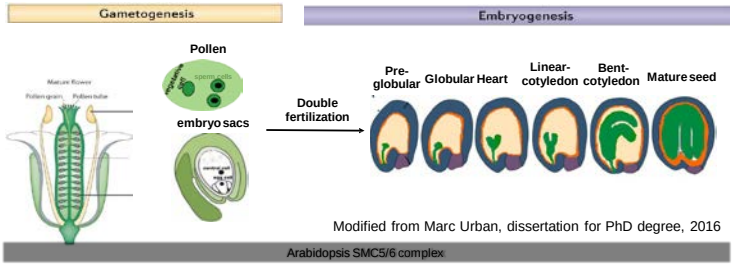
4 Universidad Complutense de Madrid, C/ José Antonio Novais, 12, Ciudad Universitaria, 28040 Madrid, Spain

INTRODUCTION

Angiosperms undergo a series of complex developmental transitions including production of haploid gametes and seeds development during generative development. Double fertilization starts the seed development in flowering plants. Most seeds contain the diploid embryo and the triploid nutritive tissue endosperm. Structural maintenance of chromosomes (SMC) 5/6 complex has a crucial function in control of genome stability and DNA damage repair. However, the role of SMC5/6 complex in plant development and stress responses is little known. This project will study the function of SMC5/6 complex during generative development in *Arabidopsis*.



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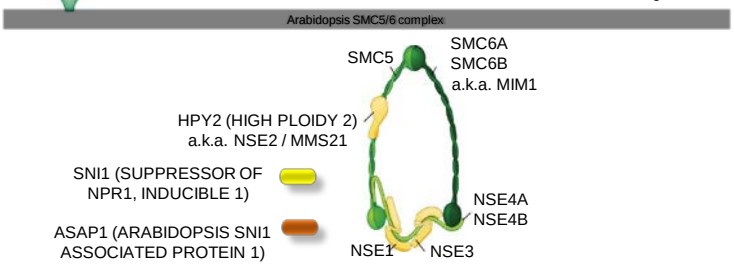
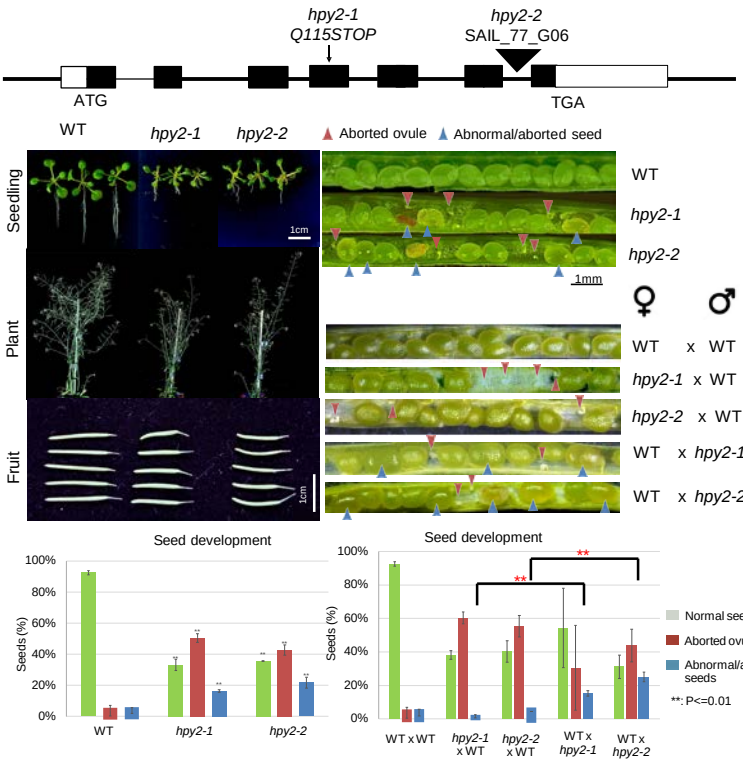


Modified from Haering & Gruber, Cell, 2016

hpy2 causes paternally inherited abnormal seed development

- *HPY2* (HIGH PLOIDY2): Small Ubiquitin-like Modifier (SUMO) E3 ligase



hpy2 impacts pollen development

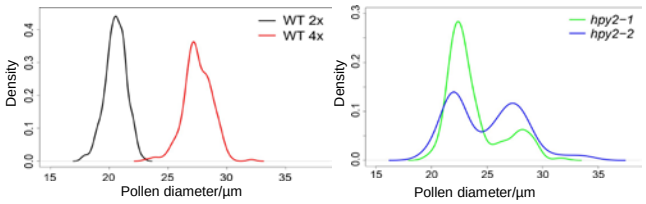
- Viability of pollen is reduced in *hpy2* plants

WT
Viability = 83.4%

hpy2-1
Viability = 63.6%

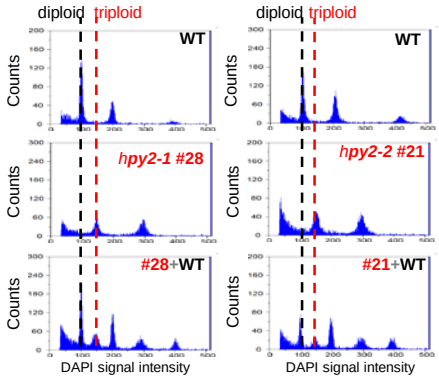
hpy2-2
Viability =52.4%

- *hpy2* produces normal size and bigger pollens



hpy2 produces triploid offspring

- Ploidy measurement by flow cytometry



Mother	Father	Germination	Offspring plants		
			N	2x	3x
WT	WT	97.12%	80	100%	0
hpy2-1	*hpy2-1*	84.86%	183	91.80%	8.20%
hpy2-2	*hpy2-2*	54.47%	93	89.04%	10.96%

Summary

SMC5/6 complex controls the ploidy level of gametes, and it is important for seed development.

APPENDIX V

Understanding functions of SMC5/6 complex during generative development in Arabidopsis

Fen Yang, Nadia Fernández-Jiménez, Martina Tučková, Jan Vrána, Petr Cápal, Mariana Díaz, Mónica Pradillo, Ales Pecinka

In: Abstract of the "6th European Workshop on Plant Chromatin"

Cologne, Germany, 2019

Understanding functions SMC5/6 complex during generative development

Fen Yang[1,2,3], Nadia Fernandez-Lopez[4], Martina Tuckova[1], Anna Nowicka[1], Mariana Diaz[1,2], Monica Pradillo[4], Ales Pecinka[1,2]

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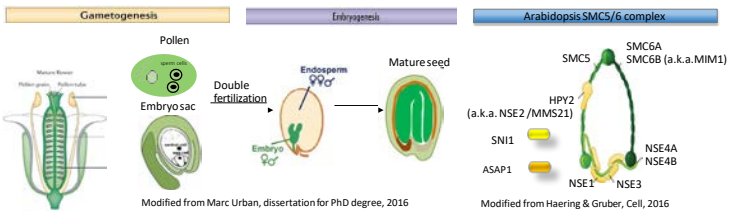

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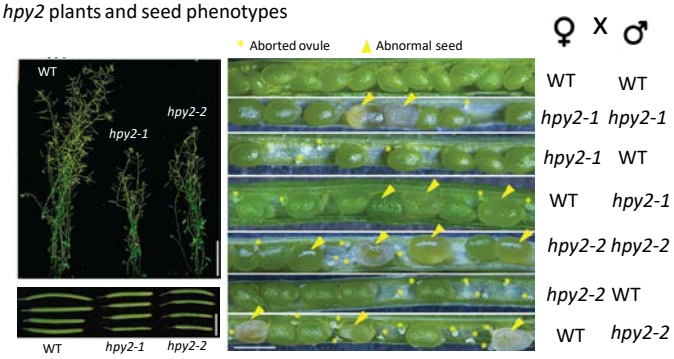
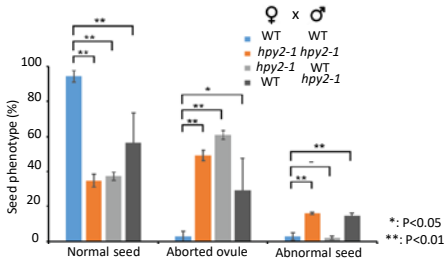
hpy2 causes paternally inherited abnormal seed development

- *HPY2* (*HIGH PLOIDY2*): Small Ubiquitin-like Modifier (SUMO) E3 ligase

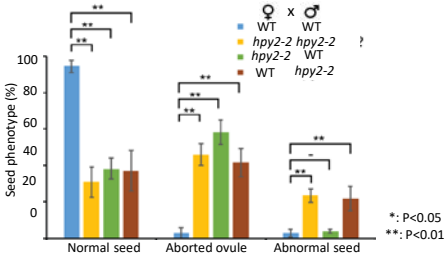
- *hpy2* plants and seed phenotypes



Seed development in *hpy2-1*

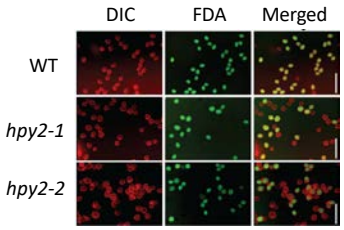


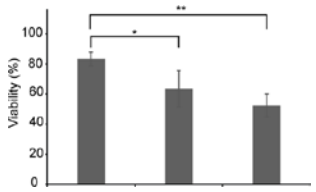
Seed development in *hpy2-2*



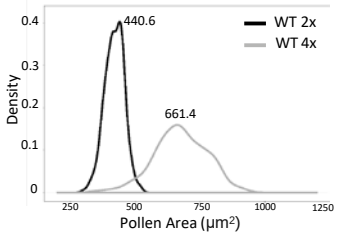
hpy2 impacts pollen development

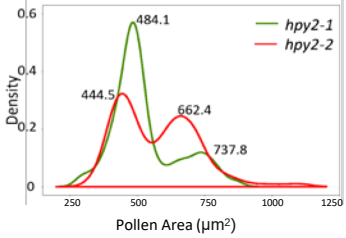
- Viability of pollen is reduced in *hpy2*





- *hpy2* produces two classes of pollen

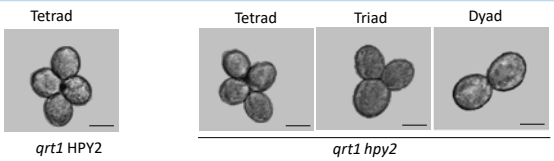


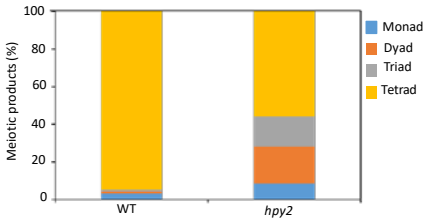


hpy2 produces triploid offspring

♀	♂	Germination rate	Offspring plants N	2x	3x
WT	WT	97.7%	110	100%	0
hpy2-1	*hpy2-1*	87.8%	380	92.4%	7.6%
hpy2-2	*hpy2-2*	62.5%	253	85.0%	15.0%
hpy2-1	WT	95.3%	43	100%	0
WT	*hpy2-1*	95.4%	270	93.3%	6.7%
hpy2-2	WT	95.0%	93	100%	0
WT	*hpy2-2*	62.0%	78	94.9%	5.1%

hpy2 produces dyads and triads as male meiosis outcomes





Summary

SMC5/6 complex controls the ploidy level of male gametes, and it is important for normal chromosome numbers in offspring.

Department of Cell Biology and Genetics

and

Institute of Experimental Botany of the Czech Academy of Sciences

Centre of Plant Structural and Functional Genomics

Fen Yang

Understanding the functions of SMC5/6 complex during the generative development in Arabidopsis

P1527 – Molecular and Cellular Biology

Summary of Ph.D. Thesis

Olomouc 2021

Ph.D. thesis was carried out at the Department of Cell Biology and Genetics, Faculty of Science, Palacký University Olomouc, in years 2017–2021.

Candidate: **M.Sc. Fen Yang**

Supervisor: **Assoc. prof. Aleš Pečinka, Ph.D.**

Reviewers: .....

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The evaluation of the Ph.D. thesis was written by .....
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The summary of the Ph.D. thesis was sent for distribution on .....

The oral defence will take place on ..... in front of the commission for the Ph.D. study of the program Molecular and Cell Biology in .....

The Ph.D. thesis is available in the Library of the biological departments of the Faculty of Science of Palacký University Olomouc, Šlechtitelů 31, Olomouc-Holice.

Prof. RNDr. Zdeněk Dvořák, DrDC. *et* Ph.D.
Chairman of the Commission for the Ph.D.
thesis of the study program Molecular and Cell Biology
Department of Cell Biology and Genetics, Faculty of Science
Palacký University Olomouc

Contents

1. Introduction

The life cycle of plants includes vegetative and reproductive stages. The reproductive development involves the "alternation of generations" between a haploid gametophyte and a diploid sporophyte (Haig and Wilczek, 2006). The sporophyte produces haploid (n) spores through meiosis. Each spore develops into a gametophyte, generating haploid gametes through mitosis. During fertilization, the fusion of two haploid gametes gives rise to the diploid sporophyte, thus perpetuating the cycle. The reproductive development is tightly regulated as it leads to seed production and a new generation of plant individuals.

The maintenance of genome stability is a vital issue for all organisms. Structural maintenance of chromosomes 5/6 (SMC5/6) complex is a crucial factor for preserving genome stability (Aragón, 2018). SMC5/6 complex is one of three eukaryotic SMC complexes (the other two are the cohesin and the condensin), which are highly conserved to regulate chromosome architecture and genome organization. It consists of a heterodimer formed by SMC5 and SMC6 proteins and additional six NON-SMC ELEMENTs (NSE1 to NSE6) in yeast (Sergeant et al., 2005; Zhao and Blobel, 2005). The SMC5/6 complex is well known for its functions in DNA damage repair (reviewed in Kegel and Sjögren, 2010; Aragón, 2018; Diaz and Pecinka, 2018). Recently, studies in plants revealed that the mutations of SMC5/6 complex subunits reduced plant fertility (Ishida et al., 2012; Liu et al., 2014; Díaz et al., 2019; Li et al., 2019; Zhu et al., 2021). However, the affected mechanisms are still not well understood. In the first part of this thesis, I showed that loss-of-function mutations in NSE2 subunit of the SMC5/6 complex produce unreduced male gametes due to an absence of chromosome segregation in the first and/or the second meiotic division. Although the SMC5/6 complex is partly required to repair the SPO11-induced DNA double-strand breaks, the newly identified non-reduction phenotype is independent of it. The unreduced male gametes lead to the production of triploid offspring in *nse2* plants. And it may also cause seed abortion as the maternal and paternal genome dosage is disturbed in endosperm. The presence of aborted ovules showed that *nse2* plants indicates any defects in female gametogenesis are maternally lethal.

Polyploidy is a heritable condition of possessing more than two sets of homologous chromosomes. It is very common in plants. One of the major challenges for polyploids is the meiotic divisions due to the presence of additional set(s) of chromosomes. In newly formed

autopolyploids and some allopolyploids, multivalents are often observed in metaphase I, resulting in incorrect segregation and low fertility (Lloyd and Bomblies, 2016). However, our knowledge about the maintenance of polyploid genome stability is limited. In the second part of this thesis, I showed that our results confirmed that SMC5/6 complex is needed for the maintenance of autopolyploid genome stability in Arabidiopsis. Moreover, the results show that autotetraploid mutants in SMC5/6 complex subunits display several unique phenotypes that differ from their diploid counterparts.

2. Aims of the thesis

The principal goal of this thesis is to obtain a deeper understanding of the functions of the SMC5/6 complex in plants using the model system of *Arabidopsis thaliana*. Particular aims are as follows:

2.1 Characterization of the functions of SMC5/6 complex during reproductive development in the diploid *Arabidopsis thaliana*

The first aim of the thesis is to understand the functions of the SMC5/6 complex during reproductive development. Our previous results uncovered that mutations of the SMC5/6 complex subunit reduced plant fertility (D áz et al., 2019). To obtain the potential mechanisms behind, mutants in several *Arabidopsis thaliana* SMC5/6 complex subunits are used to analyze the phenotypes in reproductive development including the stages of the production of haploid gametes and seed development with multiple genetic, molecular and cytological methods.

2.2 Characterization of the functions of SMC5/6 complex for maintenance of tetraploid genome stability in *Arabidopsis thaliana*

Polyploidization is a common phenomenon in the evolution of flowering plants. However, our knowledge about the maintenance of polyploid genome stability is very limited. The second aim of this thesis is to characterize the functions of the SMC5/6 complex for tetraploid genome stability in *Arabidopsis thaliana*. We produced autotetraploid mutants in several SMC5/6 complex subunits and used them to analyze the phenotypes in different developmental stages including the stages of vegetative and reproductive development.

3. Material and methods

Plant materials and growth conditions

All strains used in this study were in Columbia-0 (Col-0) background. We used following mutants: *nse2-1/hpy2-1* (Ishida et al., 2012), *nse2-2/hpy2-2/mms21-2* (SAIL_77_G06), *sni1-3* (SAIL_34_D11), *smc6b-1* (SALK_101968C), *nse4a-2* (GK-768H08), *nse4b-1* (SAIL_296_F02), *spo11-1-5* (SALK_009440), *qrt1-4* (SALK_024104C), *osd1-3* (kindly offered by Dr. Claudia Köhler) (Heyman et al., 2011), and reporter lines *ProHTR10:HTR10m-RFP* (Ingouff et al., 2007).

Tetraploid Arabidopsis was generated by submerging two weeks old in vitro grown diploid plants in 0.1% (w/v) colchicine (Sigma-Aldrich) in dark at room temperature for 2 hrs. After removing the colchicine, washing plants gently with copious amounts of tap water. Then, plants were transplanted to soil and grown until maturity. Seeds were collected from individual plants and bigger seeds were chosen and propagated into plants for ploidy measurements (see below).

For cultivation in soil, plants grow in an air-conditioned chamber with controlled long-day conditions (16h light / 8h dark cycle, 21°C day and 19°C night temperature, 150 µmol photons m^{-2} s^{-1} light intensity provided by white-light tubes). For in vitro growth, Arabidopsis seeds were surface sterilized (70% ethanol with 0.5% TritonX-100 v/v) for 10 min and washed three times with sterile water. Dried seeds were sown on 0.5x MS agar medium (Murashige and Skoog), stratified in dark for two days at 4℃ and then grown in a climatic chamber (Percival) under the same condition as described above.

Pollen viability assays and size measurements

For pollen viability analysis, the FDA staining was performed as described (Li, 2011). The fluorescein fluorescence was observed after 20 min of staining using an inverted microscope Olympus IX 83 and the same region was photographed with DIC optics to get the number of all pollen grains. Images were captured with a HAMAMATSU ORCA-ER digital camera c4742-80 controlled by xcellence rt software (Olympus).

To estimate the mature pollen diameter, we used the same photos as for FDA assay analysis. Diameter measurements were done in Fiji/ImageJ on images calibrated using internal standards.

Hoyer's clearing

Clearing of ovules was performed as described (Liu and Meinke, 1998).

Ploidy measurements and flow cytometry

The ploidy measurement was performed as described (Doležel et al., 1992) with minor modification. To determine the somatic ploidy levels in diploid plants, the nuclei suspension was prepared by chopping of equal leaf tissues from mutant and WT. To determine the somatic ploidy levels in tetraploid plants, the nuclei suspension was prepared by chopping of equal leaf tissues from mutant or WT and 4x WT samples.

To determine ploidy levels of male gametes, the samples were prepared as described (Borges et al., 2012). The different nuclei populations were sorted based on the DNA content on the slides by FACSAria (Becton Dickinson) flow-sorter. Slides were dried at room temperature for 1h then mounted with 5µL Vectashield (Vector Laboratories) and covered with 24 x 40mm coverslip. Then the nuclei were checked under the inverted microscope Olympus IX 83. Images were captured with a HAMAMATSU ORCA-ER digital camera c4742-80 controlled by xcellence rt software (Olympus).

Chromosome spreading and analysis of meiosis (Performed by M.Sc. Nadia Fernández-Jiménez)

Fixations of flower buds, chromosome spreads, and fluorescence in situ hybridization (FISH) were carried out as described (Sánchez Moran et al., 2001) with minor modifications included in (Martinez-Garcia and Pradillo, 2017). Data for cytological analyses were collected from at least three plants per genotype.

Immunolocalization procedures were performed with a spreading technique previously described in (Armstrong et al., 2002). The antibodies used were: anti- α-tubulin (the primary antibody; Merck; mouse, 1:50), and FITC-conjugated anti-mouse (the secondary antibody; Agrisera; 1:100). Slides were analyzed with an Olympus BX61 epifluorescent microscope. Images were captured with an Olympus DP71 digital camera controlled by DP Controller software version 2.2.1.227 (Olympus)

Mitotic chromosome number counting (Performed by Dr. Joanna Majka)

Fresh inflorescences were fixed in ethanol: chloroform: acetic acid (6:3:1) solution overnight at room temperature and the slides were prepared as describe (Yang et al., 2021b). All slides were examined with Axio Imager Z.2 Zeiss microscope (Zeiss, Germany) equipped with Cool Cube 1 camera. We used x60 and x100 objectives and filter for DAPI (emission spectrum 405 nm). Image processing was carried out using ISIS software 5.4.7 and Adobe Photoshop software (CS5).

4. Summary of results

4.1 Characterization of the functions of SMC5/6 complex during reproductive development in diploid *Arabidopsis thaliana*

SMC5/6 complex is an evolutionary conserved ATP-dependent molecular machine involved in the maintenance of nuclear genome stability (Aragón, 2018). Here, we found that loss-of-function mutants in SMC5/6 complex subunits NSE2, NSE4A and SNI1 cause male meiotic defects, form diploid microspores and generate triploid offspring in Arabidopsis

Triploidy defects are traced to male meiosis. Chromosome fragmentation was observed during meiosis I, suggesting the SMC5/6 complex is partly required for the repair of SPO11-induced DNA double-strand breaks. The resembles situation is in budding yeast, where SMC5/6 complex mutants cause the accumulation of HR intermediates resulting in joint molecules (JMs) (Copsey et al., 2013; Xaver et al., 2013; Menolfi et al., 2015). The meiotic cells containing high chromosome fragmentations are likely to produce non-viable gametes or may fail to enter meiosis II. Hence aneuploid *nse2* offspring were not detected. The second defect was characterized by the absence of chromosome segregation mainly during meiosis I, which could lead to the formation of unreduced gametes and eventually the triploid offspring. Further experiments are required to investigate the SMC5/6 complex is involved in which regulatory network in this process. The presence of abnormal embryo sac structures from *nse2* ovules suggested that any defects in female gametogenesis will lead to maternally lethal. The viable unreduced male gametes fuse with haploid female gametes leading to triploid offspring. And it may also cause seed abortion due to the unbalanced maternal and paternal genome dosage in endosperm after fertilization with unreduced pollen.

We concluded that the SMC5/6 complex acts as an important diplogamete suppressor in Arabidopsis. This work has been published in the Plant Cell (Yang et al., 2021a).

4.2 SMC5/6 complex is necessary for tetraploid genome stability in *Arabidopsis thaliana*

Here, we generated autotetraploid Arabidopsis WT and several SMC5/6 complex deficient mutant plants. The 4x mutans had severe defects in meiosis, reduced pollen viability and enhanced fertility defects. The analysis of meiosis in 4x mutant pollen mother cells showed that 4x mutants generate tetrads with micronuclei, which were not observed in the 2x mutant pollen mother cells. Many of the offspring of 4x mutant are aneuploids, equally caused maternally and paternally. Rarely, hexaploid plants occurred by unreduced female gametes in 4x *nse2* plants. The absence of aneuploid offspring in 2x *nse2* plants supports these are unique

phenotypes of tetraploid plants, and the importance of certain molecular regulators may be changed when polyploidization occurs.

In summary, the SMC5/6 complex is important to maintain the tetraploid genome stability in Arabidopsis. Our work in diploid and autotetraploid Arabidopsis supports that autotetraploid plants have a generally higher frequency but also higher tolerance for aneuploidy. This work has been published in Frontiers in Plant Science (Yang et al., 2021b).

5. Summary

The maintenance of genome stability is a vital issue for all organisms. Structural maintenance of chromosomes 5/6 (SMC5/6) complex is a crucial factor for preserving genome stability. Recently, studies in plants showed the SMC5/6 complex is important for plant fertility. However, the mechanism is little known so far. This thesis aimed to investigate the functions of the SMC5/6 complex during generative development in diploid and autotetraploid plants of Arabidopsis (*Arabidopsis thaliana*) with multiple molecular biology methods, including the flow-cytometry ploidy measurement, immunostaining, and chromosome spreading method.

The SMC5/6 complex is an evolutionary conserved ATP-dependent molecular machine involved in the maintenance of nuclear genome stability (Aragón, 2018). Here, we found that mutations in SMC5/6 complex subunits cause severe male meiotic defects: chromosome fragmentation and the absence of chromosome segregation. The absence of chromosome segregation, being recognized by the disorganized microtubules in the spindle and aberrant position of the chromosomes relative to the organelle band, occurs mainly in anaphase I and rarely in anaphase II, leading to the production of unreduced gametes. The unreduced gametes result in triploid progeny after fertilization and also may cause seed abortion as the maternal and paternal genome dosage is disturbed in the endosperm. The chromosome fragmentation phenotype supports that the SMC5/6 complex is necessary for the SPO11-induced double-strand breaks (DSBs) repair. However, the formation of unreduced gametes is independent of it. The presence of aborted ovules in mutant plants indicates any defects in female gametogenesis are maternally lethal. Taken together, we found that the SMC5/6 complex acts as an important player in the maintenance of gametophytic ploidy in Arabidopsis.

Polyploidization is a common phenomenon in the evolution of flowering plants. To understand the functions of the SMC5/6 complex in tetraploid Arabidopsis, we first generated tetraploid (4x) Arabidopsis WT and several SMC5/6 complex deficient mutants plants. 4x mutant plants enhance the plant fertility defects by increasing the aborted ovules and abnormal seeds.4x mutant plants also cause more severe male meiotic defects compared to 2x ones, which lead to unreduced and aneuploid microspores. Hexaploid and aneuploid progeny were equally caused maternally and paternally, suggesting that autotetraploid Arabidopsis plants have a generally higher frequency of but also higher tolerance for aneuploidy compared to diploids. In conclusion, we found that the SMC5/6 complex is needed for the maintenance of tetraploid genome stability in Arabidopsis.

In summary, our work improves the knowledge on the maintenance of genome stability in Arabidopsis. Moreover, our results emphasize the importance of studying the consequences of mutations in genes regulating plant fertility in diploid versus polyploid conditions, which may facilitate agriculturally important traits as many crop species are polyploid.

6. Reference

Aragón, L. (2018). The Smc5/6 complex: New and old functions of the enigmatic long-distance relative. Annu. Rev. Genet. **52**: 89–107.

Armstrong, S.J., Caryl, A.P., Jones, G.H., and Franklin, F.C.H. (2002). Asy1, a protein required for meiotic chromosome synapsis, localizes to axis-associated chromatin in Arabidopsis and Brassica. J. Cell Sci. **115**: 3645–3655.

Diaz, M. and Pecinka, A. (2018). Scaffolding for repair: Understanding molecular functions of the SMC5/6 complex. Genes **9**: 36.

Díaz, M., Pečinková, P., Nowicka, A., Baroux, C., Sakamoto, T., Gandha, P.Y., Jeřábková, H., Matsunaga, S., Grossniklaus, U., and Pecinka, A. (2019). The SMC5/6 complex subunit NSE4A is involved in DNA damage repair and seed development. Plant Cell **31**: 1579–1597.

Doležel, J., Sgorbati, S., and Lucretti, S. (1992). Comparison of three DNA fluorochromes for flow cytometric estimation of nuclear DNA content in plants. Physiol. Plant. **85**: 625–631.

Haig, D. and Wilczek, A. (2006). Sexual conflict and the alternation of haploid and diploid generations. Philos. Trans. R. Soc. B Biol. Sci. **361**: 335–343.

Ishida, T., Yoshimura, M., Miura, K., and Sugimoto, K. (2012). MMS21/HPY2 and SIZ1, Two Arabidopsis SUMO E3 Ligases, Have Distinct Functions in Development. PLoS One **7**: 1–10.

Kegel, A. and Sjögren, C. (2010). The Smc5/6 complex: More than repair? Cold Spring Harb. Symp. Quant. Biol. **75**: 179–187.

Li, G., Zou, W., Jian, L., Qian, J., and Zhao, J. (2019). AtNSE1 and AtNSE3 are required for embryo pattern formation and maintenance of cell viability during Arabidopsis embryogenesis. J. Exp. Bot. **70**: 6229–6244.

Li, X. (2011). Pollen Fertility/viability Assay Using FDA Staining. BIO-PROTOCOL **1**.

Liu, M., Shi, S., Zhang, S., Xu, P., Lai, J., Liu, Y., Yuan, D., Wang, Y., Du, J., and Yang, C. (2014). SUMO E3 ligase AtMMS21 is required for normal meiosis and gametophyte development in Arabidopsis. BMC Plant Biol. **14**: 1–12.

Martinez-Garcia, M. and Pradillo, M. (2017). Functional Analysis of Arabidopsis ARGONAUTEs in Meiosis and DNA Repair. In Plant Argonaute Proteins. Methods Mol Biol. **1640**:145-158.

Sánchez Moran, E., Armstrong, S.J., Santos, J.L., Franklin, F.C.H., and Jones, G.H.

(2001). Chiasma formation in Arabidopsis thaliana accession Wassileskija and in two meiotic mutants. Chromosom. Res. **9**: 121–128.

Sergeant, J., Taylor, E., Palecek, J., Fousteri, M., Andrews, E.A., Sweeney, S., Shinagawa, H., Watts, F.Z., and Lehmann, A.R. (2005). Composition and Architecture of the Schizosaccharomyces pombe Rad18 (Smc5-6) Complex . Mol. Cell. Biol. **25**: 172–184.

Yang, F., Fernández-Jiménez, N., Tučková, M., Vrána, J., Cápal, P., Díaz, M., Pradillo, M., and Pecinka, A. (2021a). Defects in meiotic chromosome segregation lead to unreduced male gametes in Arabidopsis SMC5/6 complex mutants. Plant Cell **33**: 3104–3119.

Yang, F., Fernández Jiménez, N., Majka, J., Pradillo, M., and Pecinka, A. (2021b). Structural Maintenance of Chromosomes 5/6 Complex Is Necessary for Tetraploid Genome Stability in Arabidopsis thaliana. Front. Plant Sci. **12**: 1–14.

Zhao, X. and Blobel, G. (2005). A SUMO ligase is part of a nuclear multiprotein complex that affects DNA repair and chromosomal organization. Proc. Natl. Acad. Sci. U. S. A. **102**: 4777–4782.

Zhu, L., Fernández-jiménez, N., Szymanska-lejman, M., and Pelé, A., Underwood, C.J., Serra, H., Lambing, C., Dluzewska, J., Bieluszewski, T., Pradillo, M., and Ziolkowski, P.A. (2021). Natural variation identifies SNI1, the SMC5/6 component, as a modifier of meiotic crossover in Arabidopsis. Proc. Natl. Acad. Sci. U. S. A. 118. 33.

7. List of author's publications

7.1 Original publications

Fen Yang, Nadia Fernández-Jiménez, Martina Tučková, Jan Vrána, Petr Cápal, Mariana Díaz, Mónica Pradillo, Ales Pecinka. 2021. Defects in meiotic chromosome segregation lead to unreduced male gametes in Arabidopsis SMC5/6 complex mutants, The Plant Cell, Volume 33, Issue 9, September 2021, Pages 3104–3119, https://doi.org/10.1093/plcell/koab178.

Yang F, Fernández Jiménez N, Majka J, Pradillo M and Pecinka A. 2021. Structural Maintenance of Chromosomes 5/6 Complex Is Necessary for Tetraploid Genome Stability in Arabidopsis thaliana. Front. Plant Sci. 12:748252. doi: 10.3389/fpls.2021.748252.

7.2 Published abstracts of posters

Fen Yang, Anna Nowicka, Mariana Díaz, Ales Pecinka: Understanding functions of STRUCTURAL MAINTENANCE OF CHROMOSOMES 5/6 complex during generative development. In: Proceedings of the "6th international Meeting Plant Genome Stability and Change". Gatersleben, Germany, 2018.

Fen Yang, Nadia Fernández-Jiménez, Martina Tučková, Jan Vrána, Petr Cápal, Mariana Díaz, Mónica Pradillo, Ales Pecinka: Analysis of SMC5/6 complex mutant defects during reproductive development in Arabidopsis. In: Proceedings of the "Second main INDEPTH meeting". Prague, Czech Republic, 2019.

Fen Yang, Nadia Fernández-Jiménez, Martina Tučková, Jan Vrána, Petr Cápal, Mariana Díaz, Mónica Pradillo, Ales Pecinka: Understanding functions of SMC5/6 complex during generative development in Arabidopsis. In: Proceedings of the "6th European Workshop on Plant Chromatin". Cologne, Germany, 2019.

8. Souhrn

Stability genomu je důležitá pro všechny organismy. Komplex Strukturní údržby chromozomů 5/6 (SMC5/6) je zásadním faktorem pro zachování stability genomu. Nedávné studie na rostlinách ukázaly, že SMC5/6 komplex je důležitý pro úspěšný reprodukční vývoj a tvorbu semen pomocí neznámého mechanismu. Cílem této práce bylo prozkoumat funkce SMC5/6 komplexu během generativního vývoje u diploidních a autotetraploidních rostlin huseníčku rolního (*Arabidopsis thaliana*) pomocí metod molekulární biologie, včetně měření ploidie průtokovou cytometrií, imunobarvení a cytologie.

SMC5/6 komplex je evolučně konzervovaný molekulární stroj závislý na ATP a zapojený do údržby stability jaderného genomu (Aragón, 2018). Během své práce jsem zjistila, že mutace v podjednotkách SMC5/6 komplexu způsobují závažné defekty samčí meiózy: fragmentaci a absenci segregace chromozomů. Absence segregace chromozomů se vyskytuje hlavně v anafázi I a zřídka v anafázi II mutantních rostlin a vede k produkci neredukovaných gamet. Neredukované gamety narušují poměr rodičovských genomů v endospermu semene což vede jejich časté aborci. V některých případech semena přežívají a tvoří triploidní potomstvo. Fenotyp fragmentace chromozomů podporuje, že komplex SMC5/6 je nezbytný pro opravu dvouřetězcových zlomů (DSB) indukovaných SPO11. Tvorba neredukovaných gamet je však na ní nezávislá. Přítomnost abortovaných zárodečných vaků v mutantních rostlinách naznačuje, že defekty v samičí gametogenezi jsou letální. V souhrnu jsme zjistili, že komplex SMC5/6 působí jako důležitý hráč při udržování gametofytické ploidie u Arabidopsis.

Polyploidizace je běžný jev v evoluci kvetoucích rostlin. Abychom porozuměli funkcím komplexu SMC5/6 u tetraploidů, tak jsme vytvořili tetraploidní (4x) huseníček rolní jakož I několik mutant SMC5/6 komplexu. U tetraploidních mutantních rostlin docházelo je zvýšené frekvenci defektů v plodnosti, vyššímu počtu abortovaných zárodečných vaků a abnormálních semen. Tetraploidní rostliny vykazovaly také závažnější samčí meiotické defekty než diploiní rostliny, včetně produkce aneuploidních mikrospor. Jak paternální tak i maternální gamety vedly k tvorbě hexaploidního a aneuploidního potomstva, což naznačuje, že autotetraploidní rostliny mají vyšší frekvenci neredukovaných gamet, ale také vyšší toleranci k aneuploidii ve srovnání s diploidy. Závěrem jsme zjistili, že komplex SMC5/6 je potřebný pro udržení stability tetraploidního genomu u huseníčku rolního.

V souhrnu, má práce rozšiřuje znalosti o údržbě stability genomu u huseníčku. Naše výsledky navíc zdůrazňují důležitost studia v důsledků mutací v genech regulujících fertilitu

rostlin v diploidních a polyploidních podmínkách. To může v budoucnu přispět k novým poznatkům v oblasti šlchtění rostlin, protože mnoho druhů plodin je polyploidní.