Production of unreduced microspores in Arabidopsis flowers cultivated in culture medium suggests a role of sucrose in facilitating meiotic cytokinesis

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Abstract

Live-cell imaging microscopy has been increasingly applied to plant meiosis research, which largely depends on establishing a healthy explant culture system to ensure that the captured chromosome dynamics approximate the natural characteristics of meiosis. Here, we report that Arabidopsis flowers cultured in a medium (CCM) composed of half-strength Murashige and Skoog basal salts, MES, inositol, sucrose, and agar produce diploid microspores due to the occurrence of meiotic restitution. Cytological studies show that meiotic cells in CCM flowers exhibit adjacent nuclear distribution and incomplete cytokinesis at anaphase II. Immunolocalization of α-tubulin and the microtubule-associated protein MAP65-3 reveals that spindle orientation at metaphase II and radial microtubule array organization at the tetrad stage are disrupted, which explains the production of meiotic restitution microspores. Furthermore, expression of the key transcription factor Aborted Microspores (AMS), which regulates tapetum development and meiotic cytokinesis, is progressively impaired in CCM flowers. Interestingly, increased sucrose supply in the medium promotes AMS expression and partially rescues haploid microspore formation in CCM flowers. Taken together, this study suggests a role for sucrose in promoting plant meiotic cytokinesis and gametophytic ploidy stability.

Full Text

Preamble

Production of Unreduced Microspores in Arabidopsis Flowers Cultivated in Culture Medium Suggests a Role for Sucrose in Facilitating Meiotic Cytokinesis

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Short title: Sucrose facilitates male meiotic cytokinesis

One-sentence summary: Arabidopsis flowers cultivated in culture medium produce unreduced microspores due to disrupted meiotic cytokinesis, which is partially rescued by increased sucrose supply.

Abstract

Live-imaging microscopy has become increasingly important for studying meiosis in plants, relying on the establishment of a robust ex vivo culture system for inflorescences that ensures captured chromosome dynamics reflect natural meiotic processes. Here, we report that *Arabidopsis thaliana* flowers cultivated in a culture medium (CCM) composed of half-strength Murashige and Skoog basal salts, MES, myo-inositol, sucrose, and agar produce diploid microspores through meiotic restitution. Cytological analysis revealed adjacent nuclear distribution and incomplete cytokinesis at late meiosis II in meiocytes from CCM flowers. Immunolocalization of α-tubulin and the microtubule-associated protein MAP65-3 showed disrupted spindle orientation at metaphase II and abnormal organization of radial microtubule arrays at the tetrad stage, explaining the production of meiotically restituted microspores. Moreover, CCM flowers exhibited gradually impaired expression of ABORTED MICROSPORES (AMS), a key transcription factor regulating tapetum development and meiotic cytokinesis. Interestingly, increasing sucrose supply in the culture medium promoted AMS expression and partially rescued haploid microspore formation in CCM flowers. Together, these findings suggest that sucrose plays a role in facilitating meiotic cytokinesis and maintaining gametophytic ploidy stability in plants.

Keywords: Meiosis; unreduced microspore; meiotic restitution; cytokinesis; microtubule; sucrose; tapetum

Introduction

Meiosis is a specialized cell division in which chromosomal DNA replicates once followed by two successive nuclear divisions. Through recombination of homol-

ogous chromosomes, meiosis generates genetic diversity and produces gametes with halved chromosome numbers, which is essential for genome stability across generations (Zickler and Kleckner, 2023). Most angiosperms have undergone whole genome duplication (WGD) or polyploidization during their evolutionary history, which has played a crucial role in speciation and environmental adaptation (Otto, 2007; Ren et al., 2018; Van de Peer et al., 2020). Formation of unreduced gametes through meiotic restitution—a phenomenon defining non-reductional meiotic events—is considered the primary pathway to WGD in flowering plants (Ramsey and Schemske, 1998).

In flowering plants, defects in multiple meiotic processes can lead to unreduced gamete formation. In Arabidopsis, potato, and horticultural species, dysfunction or down-regulation of spindle regulators JASON or Parallel Spindle 1 (PS1) causes parallel and/or triad-like spindle configurations during meiosis II, resulting in nuclear segregation failure and production of diploid gametes and polyploid offspring (Andreuzza et al., 2015; Clot et al., 2024; d' Erfurth et al., 2008; De Storme and Geelen, 2011; Peloquin et al., 1999; Zhou et al., 2022b). Omission of the meiotic cell cycle, triggered by functional defects in regulators such as Omission of Second Division 1 (OSD1) and Tardy Asynchronous Meiosis (TAM/CYCA1;2) (d' Erfurth et al., 2010; d' Erfurth et al., 2009; Pang et al., 2025; Zhou et al., 2022b), induces meiotically restituted dyads and thus diploid gametes in multiple species. Additionally, irregular cytokinesis interferes with chromosome distribution and can induce unreduced gamete formation (Liu et al., 2021a; Spielman et al., 1997; Takahashi et al., 2010; Yang et al., 2003; Zeng et al., 2011). Beyond genetic lesions, various abiotic stresses—including extreme temperatures and ultraviolet radiation—can induce meiotic restitution in plants (De Storme and Geelen, 2020; Fu et al., 2024; Mai et al., 2019; Zhou et al., 2022a).

Live-imaging microscopy has become increasingly valuable for plant meiosis research because it provides insights into dynamic cellular processes (Prusicki et al., 2021). In this system, the dynamic behavior of meiotic chromosomes and proteins is monitored in living meiocytes within anthers of ex vivo cultured inflorescences (Prusicki et al., 2019). Different culture media are used for live-imaging analysis of meiosis depending on tissue type, experimental purpose, and species, with Murashige and Skoog (MS) salts serving as the basal component with various additives for different microscopy applications (Prusicki et al., 2021). In currently established culture systems, meiotic features—including meiosis duration and recombination rates—recorded via live-imaging are comparable to those obtained from fixed tissues, even under stressful environmental conditions (De Jaeger-Braet et al., 2022; France et al., 2021; Ning et al., 2021; Prusicki et al., 2019; Yang et al., 2022). Additionally, such ex vivo systems provide a convenient and reliable strategy for analyzing meiotic features in response to chemical treatments for biochemical and genetic studies (Yuan et al., 2025). However, the potential impact of cultivating inflorescences in basal culture medium lacking abundant additives—such as vitamins—on meiosis has not been determined, which is relevant for developing reliable culture systems for

different experimental purposes and plant species.

In this study, we report that Arabidopsis (*Arabidopsis thaliana*) flowers cultivated in a basal culture medium produce unreduced microspores due to misorganization of the microtubule cytoskeleton during meiosis II and consequent restituted meiotic division. Moreover, we show that increased sucrose supply partially rescues tapetum development and haploid microspore formation. Together, our findings suggest a positive role for sucrose in facilitating meiotic cytokinesis and maintaining gametophytic ploidy stability in Arabidopsis.

Results

Arabidopsis Flowers Cultivated in Culture Medium Produce Diploid Microspores

To investigate the effect of cultivating inflorescences in culture medium lacking abundant additives on plant meiosis, we excised inflorescences from young flowering Arabidopsis plants and cultivated them in a medium composed of half-strength Murashige and Skoog basal salts, MES (0.05% [w/v]), myo-inositol (0.01% [w/v]), sucrose (1% [w/v]), and agar (0.8% [w/v]). Flowers from inflorescences excised directly from intact plants served as controls. The Arabidopsis *quartet 1* (*qrt1*) mutant, in which released microspores maintain a tetrad configuration (Francis et al., 2006), was used to analyze meiotic products. In control flowers, only normal tetrads consisting of four haploid microspores—indicating normal meiosis—were observed (Fig. 1A [Figure 1: see original paper], B and E). In flowers cultivated in culture medium (CCM) for 40–48 h, approximately 2.4% of pollen mother cells (PMCs) at the unicellular microspore stage exhibited triad configurations (containing one diploid nucleus and two haploid nuclei) or dyads with two equally sized microspores, each showing two nuclei (Fig. 1C–E). These observations suggest that PMCs in Arabidopsis CCM flowers produce unreduced microspores likely resulting from meiotic restitution.

Fig. 1. Arabidopsis flowers cultivated in culture medium produce diploid microspores. (A) Model describing Arabidopsis inflorescence cultivation in culture medium. (B–D) Tetrad (B), triad (C), and balanced dyad (D) at the unicellular microspore stage produced by control or CCM flowers in the *qrt* mutant. (E) Graph showing the rate of unreduced microspores produced by control and CCM flowers. Significance was determined by unpaired t-test; the average rate of unreduced microspores and number of analyzed inflorescences are shown; *** indicates $P < 0.001$; CCM, cultivated in culture medium; MSP, microspore. Scale bar, 10 m.

PMCs in CCM Flowers Show Defects in Meiotic Cytokinesis

To confirm meiotic restitution in PMCs from Arabidopsis CCM flowers, we stained PMCs at the tetrad and microspore stages in the *qrt* mutant with 4 ,6-diamidino-2-phenylindole (DAPI). Tetrad-stage PMCs from control flowers showed separated haploid nuclei in cell corners with visible organelle bands

between them (Fig. 2A [Figure 2: see original paper]). These tetrads developed into unicellular microspores, each containing a single haploid nucleus (Fig. 2C). In CCM flowers, adjacent distribution of two nuclei was observed at the tetrad stage without organelle bands between them, showing a triad configuration (Fig. 2B). These triads produced microspores with two similarly sized nuclei, indicating diploid microspores (Fig. 2D and E). At later developmental stages, triad microspores with thick cell walls that blocked DAPI entry and prevented nuclear staining were observed (Fig. 2F).

The irregular distribution of haploid nuclei suggested unsuccessful cell wall formation, indicating a cytokinesis defect. To investigate this, we stained tetrad-stage PMCs with DAPI and aniline blue, which labels callose—the main component of meiotic cell walls during meiosis and early microspore stages. In normal tetrads, callosic cell walls formed between the four isolated nuclei, preventing fusion and facilitating haploid microspore formation (Fig. 2G). Remarkably, tetrad-stage PMCs in CCM flowers showed failed and/or incomplete assembly of callosic cell walls, leading to adjacent nuclear localization (Fig. 2H–L). These observations reveal a defect in meiotic cytokinesis in PMCs from CCM flowers.

Figure 2. Pollen mother cells in flowers cultivated in culture medium show defects in meiotic cytokinesis. (A and B) DAPI staining of PMCs at the tetrad stage showing normal (A) and adjacent (B) nuclear distribution. (C–F) DAPI-stained unicellular microspores in control flowers (C) showing normal tetrad configuration and in CCM flowers (D–F) showing triad configuration. (G–L) Combined DAPI and aniline blue staining of tetrad-stage PMCs in control (G) and CCM flowers (H–L). Scale bars, 10 m.

PMCs in CCM Flowers Show Irregular Chromosome Distribution at the End of Meiosis II

To further characterize meiotic defects in Arabidopsis CCM flowers, we analyzed chromosome spreads by DAPI staining. In control flowers, meiocytes at pachytene showed full juxtaposition of homologous chromosomes (Fig. 3A [Figure 3: see original paper]), indicating complete homolog pairing and synapsis. Five bivalents were consistently observed at diakinesis and metaphase I (M I) (Fig. 3B and C), confirming completion of meiotic recombination. Homologs separated at anaphase I (A I) and temporarily decondensed at interkinesis before recondensing and aligning at two cell poles at M II through spindle pulling forces (Fig. 3D–F). At telophase II (T II), sister chromatid separation formed four haploid chromosome sets, each developing into a haploid nucleus at the tetrad stage (Fig. 3G and H). In CCM flowers, meiocytes during meiosis I showed no obvious defects (Fig. 3I–M), indicating normal meiotic recombination and homolog separation. However, defective orientation of aligned chromosomes at M II was observed (Fig. 3N and O), suggesting improper organization and/or misorientation of spindles. At T II, adjacent nuclear localization indicative of irregular chromosome distribution was seen (Fig. 3P and Q), leading to production of tetrad meiocytes showing triad configurations (Fig. 3R–T). Thus,

the observed meiotically restituted unreduced microspores likely resulted from defective distribution of chromosome sets and nuclei at the end of meiosis II.

Fig. 3. PMCs in flowers cultivated in culture medium show adjacent nuclear distribution at the end of meiosis II. (A–T) DAPI-stained meiotic chromosome spreads at pachytene (A and I), diakinesis (B and J), metaphase I (C and K), anaphase I (D and L), interkinesis (E and M), metaphase II (F, N and O), telophase II (G, P and Q), and tetrad (H and R–T) stages in control flowers (A–H) and CCM flowers (I–T). Scale bars, 10 m.

PMCs in CCM Flowers Show Irregular Spindle and Phragmoplast Organization at Meiosis II

To determine whether lesions in meiotic chromosome distribution and cytokinesis in CCM flowers result from microtubular cytoskeleton defects, we analyzed spindle and phragmoplast organization by immunolocalizing tubulin and the microtubule-associated protein MAP65-3 using anti-α-tubulin and anti-GFP antibodies in the pMAP65-3::MAP65-3-GFP reporter line (Sofroni et al., 2020). In meiocytes from both control and CCM flowers, a spindle formed at M I with MAP65-3 co-localizing with microtubules (Fig. 4A [Figure 4: see original paper]). At A I and interkinesis, a phragmoplast structure organized between separated homologs, with MAP65-3 localizing to the middle region of the phragmoplast (Fig. 4B and C). These data confirm that CCM flowers have no defects in meiosis I. In control meiocytes at M II, two spindles organized perpendicularly to drive sister chromatid segregation at T II, with MAP65-3 showing a localization pattern similar to that in meiosis I (Fig. 4D and E). At the tetrad stage, mini-phragmoplasts composed of radial microtubule arrays (RMAs) assembled between isolated haploid nuclei, with MAP65-3 localizing to the center of mini-phragmoplasts (Fig. 4F). In CCM flowers, MAP65-3 localization was unaltered (Fig. 4G–P). However, meiocytes at M II and T II stages displayed triangular spindle and phragmoplast configurations (Fig. 4H–I), indicating irregular orientation. At the tetrad stage, triads showed adjacent nuclear localization and omission of RMAs (Fig. 4J–N). Moreover, in some tetrads with separated haploid nuclei, RMAs and MAP65-3 failed to assemble regularly between nuclei (Fig. 4O and P). These defective microtubule assembly and/or organization patterns could explain the lesions in meiotic chromosome distribution and cytokinesis in CCM flowers.

Fig. 4. Meiocytes in flowers cultivated in culture medium show irregular microtubule organization during meiosis II. (A–P) Immunolocalization of α-tubulin (green) and MAP65-3-GFP (red) in meiocytes at metaphase I (A), anaphase I (B), interkinesis (C), metaphase II (D and G), telophase II (E, H and I), and tetrad (F, J–P) stages showing normal (A–F) or abnormal organization and/or assembly (G–P) in control and CCM flowers. Scale bar, 10 m.

Increased Sucrose Supply Partially Rescues AMS Expression in CCM Flowers

Normal tapetum development is required for faithful RMA organization and meiotic cytokinesis in Arabidopsis (Tidy et al., 2022). The defects in meiotic cell wall formation and RMA organization led us to hypothesize that meiotic restitution in CCM flowers is triggered by attenuated tapetal function and/or development. To test this, we analyzed expression of ABORTED MICROSPORES (AMS), a transcription factor required for tapetum development whose dysfunction causes defective meiotic cytokinesis and meiotic restitution, using live-imaging of CCM flowers from a pAMS::AMS-GFP reporter (Xiong et al., 2016). In control flowers, anthers at the tetrad and unicellular microspore stages showed AMS-GFP expression specifically in the tapetal cell layer (Fig. 5A [Figure 5: see original paper] and B). In tetrad-stage anthers at one day post-cultivation (1 dpc) in medium, no obvious alteration in AMS-GFP expression was detected (Fig. 5C). However, reduced AMS-GFP expression was observed at 2 dpc, and no AMS-GFP was detected at 3 dpc (Fig. 5E and G), indicating impaired AMS expression in CCM flowers.

Faithful tapetum development relies on normal sugar metabolism in flowers (Borghi, 2025; Liu et al., 2021b). We therefore asked whether damaged AMS expression in CCM flowers results from sucrose shortage in anthers. We monitored AMS expression in CCM flowers exposed to elevated sucrose concentrations. In most flowers from 1 to 3 dpc, we observed normal AMS-GFP expression in tetrad-stage anthers, indicating that increased sucrose supply partially rescued AMS expression in the tapetum (Fig. 5I, K and M). Since sucrose breaks down into other sugars to induce downstream cellular responses (Yoon et al., 2021), we tested whether fructose, a sucrose metabolite, has similar effects on AMS expression in CCM flowers. CCM flowers exposed to 1% fructose showed normal AMS expression at 1 dpc but reduced and impaired AMS expression at 2 and 3 dpc, respectively (Fig. 5D, F and H). Under 10% fructose conditions, AMS expression in most flowers at 2 and 3 dpc was recovered (Fig. 5J, L and N). These findings reveal a positive impact of sucrose and its metabolite on AMS expression in anthers.

Fig. 5. Increased sucrose or fructose supply rescues AMS expression in the tapetum. (A and B) AMS-GFP expression in the tapetum of anthers at the tetrad (A) and microspore (B) stages in control flowers. (C–H) AMS-GFP expression in the tapetum of tetrad-stage anthers in flowers cultivated in culture medium containing 1% sucrose (C, E and G) or 1% fructose (D, F and H) for 24 (C and D), 48 (E and F) and 72 h (G and H). (I–N) AMS-GFP expression in the tapetum of tetrad-stage anthers in flowers cultivated in culture medium containing 10% sucrose (I, K and M) and 10% fructose (J, L and N) for 24 (I and J), 48 (K and L) and 72 h (M and N). Scale bar, 50 m.

Increased Sucrose Supply Partially Rescues Haploid Microspore Formation

To test whether increased sucrose supply could complement meiotic cytokinesis defects in CCM flowers, we quantified unreduced microspore rates in CCM flowers exposed to different sucrose concentrations. In control flowers, only tetrads and haploid microspores were observed (Fig. 6A [Figure 6: see original paper], E and I). In flowers cultivated in medium with 1% sucrose, meiocytes showing meiotic restitution at the tetrad stage were observed, with approximately 3.1% unreduced microspores recorded (Fig. 6B–D, F–H and I). A similar rate of unreduced microspores was found in flowers grown in medium without sucrose compared to 1% sucrose-supplied medium (Fig. 6I). Interestingly, flowers cultivated in medium with 10% sucrose yielded a significantly lower rate (~1.3%) of unreduced microspores (Fig. 6I, $P < 0.01$), suggesting partially rescued meiotic cytokinesis and haploid microspore formation.

Fig. 6. Increased sucrose supply in culture medium partially reduces diploid microspore formation. (A–H) Orcein staining of meiocytes at the tetrad stage (A–D) and unicellular microspores (E–H) in control flowers (A and E) and CCM flowers (B–D, F–H) showing tetrad (A and E), triad (B and F), balanced dyad (C and G) or unbalanced dyad (H) configurations. (I) Graph showing the rate of diploid microspores produced by control flowers and flowers in culture medium containing 0%, 1% and 10% sucrose. Significance levels were determined by unpaired t-tests; the average rate of unreduced microspores and number of inflorescences are shown; *** indicates $P < 0.001$; ** indicates $P < 0.01$; ns indicates $P > 0.05$; MSP, microspore. Scale bar, 10 m.

Discussion

In this study, we demonstrated that Arabidopsis flowers cultivated in culture medium produce unreduced microspores through meiotic restitution caused by defective meiotic cytokinesis. Further cytological analysis revealed altered orientation of spindles and phragmoplasts during meiosis II, resulting in irregular formation and/or organization of RMAs at the tetrad stage in CCM PMCs. The altered spindle and phragmoplast orientation at meiosis II in CCM flowers may result from interfered expression and/or function of spindle regulators JASON and/or AtPS1 (De Storme and Geelen, 2011), which have been proposed to mediate microtubule organization responses to environmental stimuli (Cabout et al., 2017; Fu et al., 2024). However, tetrads showing normal nuclear distribution but failed RMA assembly and MAP65-3 localization between separated nuclei (Fig. 4P) suggest that formation and/or composition of organelle bands—which act as physical barriers between metaphase II spindles to ensure correct chromosome distribution (Brownfield et al., 2015)—may be attenuated independently of impacted JASON function (Gasser et al., 1988; Koç and De Storme, 2022).

Meiotic restitution and microtubule organization defects have not been reported in previous studies that cultured Arabidopsis inflorescences in growth medium

for live-imaging microscopy (Prusicki et al., 2019; Sofroni et al., 2020; Valuchova et al., 2022; Wijnker et al., 2019; Yang et al., 2020; Yang et al., 2022; Yuan et al., 2025). The culture medium used here contained only myo-inositol but lacked other vitamins—such as nicotinic acid, pyridoxine hydrochloride, glycine, and thiamine hydrochloride—that play important roles in metabolic regulation, including energy metabolism, amino acid synthesis, oxidation-reduction reactions, stress response, and cell development in plants (Berglund et al., 2017; Schnellbaecher et al., 2019; Sultana et al., 2019). We therefore speculate that the observed meiotic defects are induced by cellular lesions resulting from lack of chemicals necessary for meiocyte development (Prusicki et al., 2021). Thus, culture medium components and possibly their dosages should be carefully tested and optimized when establishing ex vivo culture systems for plant meiosis studies. On the other hand, the average frequency of unreduced microspores in CCM flowers is quite low (less than 3%), which may make it difficult to capture lesions under fast-moving, dynamic intracellular conditions. Given the complexity of chromosome dynamics during meiosis, particularly during recombination, minor differences may exist between meiotic features captured in ex vivo cultured flowers and those under natural conditions.

Normal tapetum development at early flower stages is required for successful RMA organization and proper assembly and configuration of cytokinetic cell walls during meiosis in Arabidopsis (Liu et al., 2017; Tidy et al., 2022). Defects in callosic cell walls together with impaired AMS expression in anthers suggest that meiotic restitution in CCM flowers could be caused by damaged tapetal development and function, which subsequently attenuates expression of meiotic genes or metabolism of enzymes and components for organelle band formation and cell wall assembly in meiocytes (Biswas and Chaudhuri, 2024; Lei and Liu, 2020; Muro et al., 2025; Wei and Ma, 2023). Whether and how the tapetum regulates expression and/or function of JASON, AtPS1, or other microtubule regulators requires further investigation. We found that increased sucrose concentration in culture medium promoted AMS expression and haploid microspore formation, possibly through compensated energy supply or activation of signaling pathways mediated by sucrose and/or its metabolites in anthers (Borghi, 2025; Liu et al., 2021b; Wang et al., 2022; Yoon et al., 2021).

Notably, the rate of unreduced microspores varied considerably among individual CCM inflorescences (Fig. 6I), possibly reflecting differences in inherent sucrose abundance. In multiple plant species, regulatory modules controlling tapetum development and meiosis progression are tightly associated with factors in sugar metabolism and signaling (Lei and Liu, 2020; Liu et al., 2021b; Sun et al., 2025; Wang et al., 2023). Additionally, in rice, TDR Interacting Protein 2 (TIP2), a basic helix-loop-helix protein required for tapetum development, regulates expression of carbohydrate-active glycosyltransferases and glycosyl hydrolases in the tapetum, and its dysfunction causes arrested meiosis progression (Fu et al., 2014; Wang et al., 2025). These findings suggest that the tapetum may influence meiotic cytokinesis fidelity by regulating sugar metabolism. We propose that the partial rescue of microsporogenesis by increased sucrose supply

reflects recovered tapetal function.

Overall, our study reveals a role for sucrose in facilitating tapetum development and meiotic cytokinesis. Moreover, cultivating flowers in medium with modified components—including sugars to ensure proper anther development—could potentially be developed as a strategy for inducing unreduced gametes in polyploid breeding programs.

Material and Methods

Plant Materials and Growth Conditions

This study used Arabidopsis thaliana mutant *quartet* (*qrt*) (Francis et al., 2006), the pMAP65-3::GFP-MAP65-3 (Sofroni et al., 2020) and pAMS::AMS-GFP (Xiong et al., 2016) reporters. Seeds were germinated in soil for 6–8 days, and seedlings were transferred to soil and cultivated in growth chambers under 16 h day/8 h night, 20°C, and 50% humidity. For flower cultivation, young inflorescences were excised with their tips embedded in medium. A suitable amount of distilled water was added to the medium to prevent inflorescence desiccation.

Preparation of Culture Medium

Culture medium was prepared by dissolving basal MS salts (0.5×), MES (0.05% [w/v]), myo-inositol (0.01% [w/v]), sucrose (1% [w/v]), and agar (0.8% [w/v]) in distilled water, adjusted to pH 5.7.

Cytological Analysis of Meiocytes and Microspores

Orcein staining, DAPI staining, and aniline blue staining of meiocytes were performed as previously described (Fu et al., 2024). Microspore quantification was performed at 48 h post-cultivation.

Preparation of Chromosome Spreads

Young Arabidopsis inflorescences were fixed in precooled Carnoy's fixative for at least 24 h. Meiotic-stage flower buds were washed twice with distilled water and once with citrate buffer (10 mM, pH 4.5), then incubated in digestion enzyme mixture (0.3% pectolyase and 0.3% cellulase in citrate buffer, 10 mM, pH 4.5) at 37°C for 2.5 h. Digested flower buds were washed once in distilled water and macerated on a glass slide. Two aliquots of 60% acetic acid were added, and the slide was dried on a hotplate at 45°C for 2 min. The slide was washed with ice-cold Carnoy' s fixative and air-dried. DAPI (5 g/mL) diluted in antifade mounting medium was added, and the coverslip was mounted and sealed with nail polish.

Immunolocalization of Microtubules and MAP65-3 Protein

Immunolocalization assays were performed as described (Liu et al., 2017; Wang et al., 2014). Anti-α-tubulin (Lei et al., 2020) and anti-GFP (Zhao et al., 2023) antibodies were diluted 1:500 and 1:300, respectively. Secondary antibodies were described previously (Lei et al., 2020; Zhao et al., 2023).

Live-Imaging of Reporters

To analyze AMS expression, anthers from flowering pAMS::AMS-GFP reporters were isolated and placed on a glass slide with a drop of distilled water, then mounted with a coverslip and examined under an inverted fluorescence microscope. Anther developmental stages were determined by examining released PMCs or microspores.

Microscopy

Fluorescence microscopy was performed using an Olympus IX83 inverted fluorescence microscope equipped with an X-Cite lamp and Prime BSI camera. Bifluorescent images and Z-stacks were processed using ImageJ.

Statistical Analysis

Significance analysis was performed using unpaired t-tests with GRAPHPAD PRISM (v.8), with significance set at $P < 0.05$. Error bars indicate SD.

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Data Availability Statement

Data supporting this study' s findings are available from the corresponding author (B.L.) upon reasonable request.

CRediT Authorship Contribution Statement

H.F., Y.C., X.C., H.H., and J.W. contributed to investigation; C.W. and Z.R. contributed to data analysis; B.L. conceived the project, analyzed data, and wrote and edited the manuscript. All authors read and approved the manuscript prior to submission.

Declaration of Competing Interest

The authors declare no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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