Mutation in *Arabidopsis* MOR1 gene impairs endocytosis in stamen filament cells and results in anther indehiscence

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Abstract
Anther dehiscence is a crucial step for pollen grains release and male fertility. Filaments, which transport water, nutrients and hormones to the anthers, are important for anther dehiscence. In this study, we characterized the *Arabidopsis* MICROTUBULE ORGANIZATION 1 (MOR1) gene that involves in the filament functions and plays important roles in anther dehiscence. The *Arabidopsis* microtubule organization 1–1 (mor1-1) mutant exhibited an anther indehiscence phenotype at 24 °C. Such the defect did not occur at a lower temperature (19 °C). Further analysis indicated that both the cortical microtubule (CMT) organization and plasma membrane homeostasis were drastically impaired and disturbed in mor1-1 filament cells under the growth conditions of 24 °C. Transmission electron microscopy and FM4-64 up-take assays showed that endocytosis process in the mor1-1 filament cells were disrupted at 24 °C. Furthermore, the cortical-associated RFP tagged clathrin light chain foci were reduced in the mor1-1 filament cells. These results suggested that the MOR1 mediated CMT organization is important for clathrin-mediated endocytosis in the filament cells, and critical for anther dehiscence in thermosensitivity.

Keywords  *Arabidopsis* · Anther dehiscence · Filaments · Microtubule associated protein · Endocytosis · Cortical microtubule organization

Introduction

The *Arabidopsis* flower consists of four concentric whorls of organs from center to edge: pistil, stamens, petals, and sepals (Smyth et al. 1990). A stamen comprises an anther in which pollen grains are produced, and a stalk-like filament which functions in transport of water, nutrients and phytohormones to the anther and plays important roles in pollination by influencing pollen dispersal (Scott et al. 2004). In particular, at the floral stage 13, the filaments elongate rapidly, and anthers dehisce to release the mature pollen grains onto the stigma for pollination and subsequent fertilization (Sanders et al. 1999; Wilson et al. 2011). Therefore, investigating the molecular mechanisms how the filaments involve in anther dehiscence will provide effective tools for control of male fertility useful for crop hybrid breeding.

Anther dehiscence involves the localized cellular differentiation and degeneration, coupled with changes in the structure and water status of the anther to facilitate complete anther opening to release the pollen grains (Ma 2005; Sanders et al. 2005). Several lines of evidences had showed that the filaments may influence anther dehiscence through the following processes. First, filaments participate in active water transport from anther, which lead to wall dehydration and epidermal cells shrinkage in anther, and then result in anther dehiscence (Keijzer 1987; Ge et al. 2001; Wilson et al. 2011). Second, the filaments are important sites for biosynthesis and transport of jasmonate acids (JAs), a critical signal for the anther dehiscence (Scott et al. 2004; Acosta and Przybyl 2019). However, little has been known about the molecular mechanisms that regulate transport of water, nutrients and phytohormones in the filaments.
Endocytosis, which enable the cells to take up extra-cellular materials and cell surface proteins via vesicle transport, is essential for regulation of signal transduction, maintenance of PM homeostasis (Fan et al. 2015; Shen et al. 2020), so it might play important roles in anther dehiscence. In plants, the microtubule (MT) cytoskeleton is a central player in a multitude of developmental and environmental roles (Ledbetter and Porter 1964; Hussey et al. 2002). Previous studies indicated that plant MT cytoskeleton organization is important for endocytosis, especially clathrin mediated endocytosis (CME). First, clathrin-coated structures that are clustered around cortical microtubules have been observed in field emission scanning electron microscopy (FESEM) micrographs (Fowke et al. 1999). Second, the microtubule inhibitor oryzalin significantly affects the lifetime and mobility of clathrin-coated membranes on the PM in Arabidopsis root cells (Konopka et al. 2008).

A number of microtubule-associated proteins (MAPs) interact with MTs and regulate their dynamics, including the rates at which MTs grow or shrink (Sedbrook and Kaloriti 2008; Hamada 2014). The Arabidopsis MAP protein MOR1/GEM1 (and the tobacco homolog MAP200) belongs to the MAP215 family. The animal and plant MAP215 family members function to increase MT assembly in vitro and in vivo (Kawamura and Wasteneys 2008). In Arabidopsis, the MOR1 localizes to nearly all MT structures, including cortical MT (CMT) array, phragmoplast, spindle and pre-prophase band (Whittington et al. 2001; Twell et al. 2002). Mutants of MOR1 in Arabidopsis and tobacco exhibit the prominent defects in CMT, mitotic spindle, and phragmoplast organization, leading to cytokinesis defects in somatic cells and gametophytes, left-handed twisting of organs, isotropic cell expansion (Whittington et al. 2001; Twell et al. 2002; Eleftheriou et al. 2005). However, the roles of MOR1 in filament function remain unclear, including its roles in anther dehiscence.

In this study, we demonstrated that mutation in the Arabidopsis MOR1 could cause thermosensitive anther indehiscent phenotype. The mor1-1 mutant (Whittington et al. 2001) exhibit a male sterility under the growth condition of 24 °C, but did not showed any other developmental defects under the same conditions. Further analysis showed that CMT arrays in the mor1-1 mutant filament cells are disorganized, TEM data and FM4-64 uptake assays showed that endocytosis in the mor1-1 mutant filament cells were arrested. Furthermore, the cortical-associated clathrin light chain (CLC) foci were dramatically reduced in the mor1-1 mutant filament epidermal cells. These results suggested that the MOR1 mediated CMT organization is important for clathrin-mediated endocytosis (CME) in the filament cells, and plays a prominent role in filament development and anther dehiscence.

**Materials and methods**

**Plant material and growth conditions**

All Arabidopsis plants used in this study are of the Col-0 ecotype. The mor1-1 mutant was obtained from Nottingham Arabidopsis Stock Centre (NASC, http://nasc.nott.ac.uk), which carry a point mutation in MOR1 gene and resulted in one amino acid change (L174F) in MOR1 (Whittington et al. 2001). The mCherry-TUA5 and CLC-RFP transgenic lines were kindly provided by Dr. Kezhen Yang (Institute of Botany, Chinese Academy of Sciences, Beijing, China) and Dr. Yiqun Bao (Nanjing Agricultural University, Nanjing, China), respectively. The Plants were grown at 24 °C or 19 °C as described in text, under a cycle of 16 h light/8 h dark.

**Phenotyping**

For observation of pollen releasing, the stamens from flowers at the floral stage 13 of wild type (WT) and mor1-1 mutant were stained with aniline blue solution (0.1%, w/v), and characterized by CSLM. For observation of anther transverse sections, flowers at the floral stage 13 from WT and mor1-1 mutant were fixed with 4% paraformaldehyde in PBS. Five-micrometer paraffin sections were prepared and stained with toluidine blue.

**Scanning and transmission electron microscopy**

For scanning electron microscopy (SEM) observation, stamens were mounted on sample stubs. After dehydration in air for 30 min, the stamens were coated with gold particles (EIKO IB-3). The gold-coated stamen samples were then observed using a HITACHI S-3000 N scanning electron microscope (Hitachi High-Technologies).

To visualize structures of the filament cells by TEM, the anthers from the flowers at the floral stage 13 were cut and fixed in fixing solution containing 5% (v/v) glutaraldehyde, 0.1 M sodium cacodylate, pH 7.2, post-fixed in 1% osmium tetroxide for 1 h, then dehydrated in a series of acetone solutions [25, 50, 75, and 100% (v/v), followed by a resin/acetone dilution series [25, 50, and 75% (v/v)], and embedded in epoxy resin. After polymerization at 65 °C for 18 h, serial cross-sections were prepared and examined by a Hitachi H-7650 transmission electron microscope with a charge-coupled device camera (Hitachi High-Technologies) operating at 80 kV.

**Confocal laser scanning microscopy**

Confocal images of fluorescent proteins or FM4-64 fluorescence signals were collected using an LSM800 system.
The FM4-64 and RFP were excited at 543 nm.

Generation of ProMOR1::GUS transgenic plants and GUS staining

The 1.5-kb PstI–XbaI fragment covering the 5′ upstream region of the MOR1 gene was isolated and subcloned into the binary vector Pcambia 1300221 between PstI and XbaI to make a translational fusion of the MOR1 promoter and β-glucuronidase (GUS) gene. Transformation of Arabidopsis (Col-0) was performed using the vacuum infiltration method (Bechtold and Pelletier 1998). For GUS histochemical staining, the organs were incubated overnight in an X-Gluc solution (1.9 mM 5-bromo-4-chloro-3-indolyl-β-glucuronide, 0.5 mM K₃Fe[CN]₆, 0.5 mM K₄Fe[CN]₆, 0.1% Triton X-100, and 50 mM Na-phosphate buffer, pH 7.0) at 37 °C.

Real-time RT-PCR

Total RNA was isolated using the RNA prep pure plant kit (Tiangen). The first-strand cDNA was synthesized using oligo(dT)18 as the primer and PrimeScript reverse transcriptase (TaKaRa). Arabidopsis gene Tubulin8 was used as an internal control. Quantitative analysis was performed using an ABI 7500 real-time qPCR system with the SYBR Green Mix (Bio-Rad) and three biological repeats (Tan et al. 2017).

JA treatment

All opened flowers were removed from the inflorescence, and the remaining flower bud clusters were dipped into 500 μM MeJA (Macklin), dissolved in 0.05% aqueous Tween 20, as described in Ishiguro et al. 2001.

Gene accessions numbers

Sequence data from this article can be found in the Arabidopsis Genome Initiative database under accession numbers At2g35630 (MOR1), At2g40060 (CLC) and At5g19780 (TUA5).

Results

The mor1-1 mutant was male-sterile at 24 °C

The mor1-1 mutant plants were normal like wild type when grown in the condition of 19 °C. In contrast, when grown at an extreme temperature of 30 °C, the mor1-1 mutant plants were drastically squat, could not develop any flowers, and eventually die (Whittington et al. 2001). These results implied that the mor1-1 mutant was highly thermosensitive. We found that when the mor1-1 mutant plants were grown at a moderate temperature of 24 °C, they all had normal vegetative development and could produce normal inflorescence, but exhibited an extremely lower fertility (Fig. 1A, B). When the mor1-1 pistils in the pre-emasculated flowers were pollinated with wild type pollen grains, the resulting siliques had normal seed set like wild type (Fig. 1C), indicating that the sterile phenotype was associated with a male sterility. The mor1-1 mutant siliques were much shorter than those of wild type, and produced nearly no seeds (Fig. 1E). This result indicated that the moderate temperature of 24 °C did not affect the vegetative growth, but drastically reduced male fertility in the mor1-1 mutant. These phenotypes were not observed in mor1-1 mutants which were grown at 19 °C (Fig. S1).

To further understand if the male sterile phenotype was thermosensitive, the mor1-1 plants were grown at 24 °C until they had set several siliques, then were moved to 19 °C for 5 days. The result showed that newly set siliques at 19 °C had normal seed set like wild type siliques (Fig. 1F, G), indicating that male sterility in mor1-1 mutant is thermosensitive.

To understand if the thermosensitive male-sterile phenotype of mor1-1 mutant is caused by mutation in MOR1 gene, we generated a construct which contains full length genomic DNA fragments of MOR1 gene (Assigned as gMOR1, including the 727 bp promoter region, 13,534 bp coding region and the 681 bp transcription termination region), and transformed it into mor1-1 mutant. The transformants (gMOR1 mor1-1) showed normal fertility at 24 °C (Fig. 1D, H), indicating that the thermosensitive male-sterile phenotype had been restored by the transgenic MOR1 gene.

The mor1-1 mutant was defective in anther dehiscence at 24 °C

To understand the causes that lead to the male-sterile phenotype in mor1-1 mutant at 24 °C, flowers at the floral stage 13 from the plants grown at 24 °C were examined. The floral organs besides stamens looked normal in the mor1-1 flowers (Fig. 2A, B); and the filament length was similar to that in wild type plants (Fig. 2C, D), indicating that the mor1-1 mutation did not affect filament elongation and other floral organs. However, comparison of the anthers from wild type and mor1-1 mutant showed that the wild type anthers could release their pollen grains on the surface (Fig. 2E), but the mor1-1 mutant anthers with a smooth surface could not release pollen grains efficiently (Fig. 2F), suggesting the mor1-1 mutant was defective in pollen release. Aniline blue staining of whole anthers further confirmed this observation (Fig. 2G, H), and the defects was not observed in gMOR1 mor1-1 plants (Fig. 2I, J). Therefore, we concluded that the
male sterility of mor1-1 mutant was caused by defect in pollen release at 24 °C.

SEM was used to further examine the surface of mor1-1 mutant anther at the floral stage 13 from the plants grown at 24 °C. As shown in Fig. 2K and M, in the wild type and gMOR1 mor1-1 flowers, numerous pollen grains could be observed on surface of the dehiscent anther; in contrast, the mor1-1 mutant anthers were not fully dehiscent, in which, most pollen grains were enclosed in the anther locule (Fig. 2L), these data indicated that anther dehiscence was impaired in mor1-1 mutant at 24 °C.

To determine if defect in dehiscence was caused by morphological abnormality of the anther tissues, the transverse sections of wild type, mor1-1 mutant, mor1-1♀ × WT ♂ and gMOR1 mor1-1 silques at 24 °C. F–H A comparison between wild type (F), mor1-1 mutant (G) and gMOR1 mor1-1 (H) plants as they were grown at 24 °C until producing several siliques, and then moved to 19 °C.

MOR1 expressed in stamens in a temperature-independent manner

To understand the function of MOR1 in stamen development, the ProMOR1::GUS transgenic plants were generated to analyze the expression patterns of MOR1. When grown at 19 °C, strong GUS activities were detected in seedlings, roots, and stamens, indicating that MOR1 plays a prominent role in the fast growing cells (Fig. 3A). Similar results were observed when ProMOR1::GUS transgenic plants were grown at 24 °C (Fig. 3A). Real-time PCR analysis indicated that MOR1 expression in seedling and inflorescence is not up-regulated at 24 °C compared to 19 °C (Fig. 3B). These data indicated that expression of MOR1 gene in wild-type is not temperature-dependent.

CMT organization in the filament cells was altered in mor1-1 mutant at 24 °C

As strong GUS activities of MOR1 promoter in filament cells were observed, which could be associated with the
anther indehiscence phenotype at 24 °C. SEM was performed to study the morphology of filament cells. The results showed that at the floral stage 13, the wild type filament cell surface showed the normal straight stripes (Fig. 4A). In contrast, the *mor1-1* mutant filaments exhibited twisty stripes on their surfaces (Fig. 4B), indicating that the *mor1-1* mutant filament cell morphology was...
impaired at 24 °C. This phenotype did not appear when the mutant plants were grown at 19 °C (Fig. 4C).

Previous study has shown that the CMT arrays are important for cell morphogenesis (Paredez et al. 2006). Therefore, the CMT organizations in the mor1-1 mutant filament cells from the plants grown at 24 °C were examined with comparison to the wild type. To do so, a microtubule marker mCherry-TUA5 was introduced into the mor1-1 mutant genome. Then, the CMTs in the mCherry-TUA5 labeled filament cells from flowers at the floral stage 13 were examined. As shown in Fig. 4D and E, CMT arrays in the WT filament cells were mostly transversely aligned, whereas in mor1-1 mutant filament cells, the MT arrays were dramatically reduced, which were linked in network. In addition, some dot-like mCherry signals could be observed as appearing on the MTs bundles. This result indicated that the mor1-1 mutant could disrupt CMT organization in filament cells at 24 °C (Fig. 4F, G). In contrast, the CMT organizations in the mor1-1 mutant filament cells were normal at 19 °C (Fig. 4H, I), indicated that CMT disruption in mor1 mutant filament cells is temperature dependent.

**The mor1-1 mutant filament cells were defective in endocytosis**

To investigate if mor1-1 mutation impaired cell structures in filament at 24 °C, we examined ultrastructures of the filament cells in the flowers at the floral stage 13 from wild type and mor1-1 plants grown at 24 °C using TEM. In wild type filaments, most epidermal cells (n = 16) were highly vacuolated and had the normal organelles, such as mitochondrial, Golgi apparatus in the cytosol (Fig. 5A). Some small bubbles, whose diameters are ~ 80 nm were occasionally observed at the PM (Fig. 5B). In contrast, almost no distinguishable organelles were observed in the mor1-1 mutant filament epidermal cells (n = 18) (Fig. 5C). Many big bubbles, whose diameters are more than 500 nm, were formed via PM invagination (Fig. 5G, H), which was not observed.
in endodermis cells in the wild type filaments (Fig. 5E, F). These data suggested that the mor1-1 mutation might impair endocytosis in the filament cells, resulting in disordering of PM. On the other hand, no vesicles accumulation was observed in mor1-1 filament cells, suggesting that vesicle secretion was not affected by mor1-1 mutation.

To confirm if endocytosis process in the mor1-1 mutant filament cells was disturbed at 24 °C, we further examined the rate of endocytosis with FM4-64 dyes, a widely used fluorescent marker for the endocytotic pathway (Bolte et al. 2004; Fan et al. 2013). In the wild type, FM4-64-labeled fluorescent puncta could be detected in the cytoplasm 5 min after labeling (Fig. 6A, B), whereas in the mor1-1 mutant just few FM4-64 uptakes could be detected even 20 min after labeling (Fig. 6C, D). These data indicated that the endocytosis process was arrested in the mor1-1 mutant.

The clathrin-mediated endocytosis (CME) is the primary endocytic route into plant cells and starts with the initiation of invagination of clathrin-coated membrane (Konopka et al. 2008; Fan et al. 2015). Therefore, we further examined localization of clathrin light chain fusion RFP protein (CLC-RFP) in the mor1-1 mutant filament cells. CSLM analysis showed that in wild type filament epidermal cells, most CLC-RFP foci were localized to the cell cortex; and just a few foci were localized on intracellular structures (presumably the trans-Golgi network). In the mor1-1 mutant filament epidermal cells, the number of cortical-associated CLC-RFP foci was reduced dramatically (Fig. 6E, F). This result indicated that CMT might recruit clathrin subunits to the PM or attach them on the PM. In mor1-1 mutant (grown at 24 °C) filament cells, this process might be disturbed due to CMT disorganization.

**Exogenously application of MeJA could partially rescue fertility of mor1-1 mutant**

To understand if the male sterile phenotype is involved in JA transporting, biosynthesis or signaling, we applied methyl jasmonate (MeJA) to bud clusters of the mor1-1 mutant at 24 °C. 2 days after treatment, we found that the anthers dehisced at the same time as flower opening in the newly opened flowers (Fig. 7A, B). Pollen grains of these flowers germinated effectively on the pollen germination medium as well as on stigmas, and these flowers bore many seeds (Fig. 7C, D). However, the siliques is shorter than that in wild-type plants (Fig. 7C, D), so we concluded that exogenously application of MeJA could partially rescue fertility of mor1-1 mutant, this result indicated that JA signaling transduction in mor1-1 mutant stamen was not blocked, and JA level might be reduced, which resulted in male sterile phenotype.
To understand if JA treatment rescued the cortical MTs disorganization in *mor1-1* mutant filament cells, cortical MT arrays in JA treated *mor1-1* mutant filament cells were observed, they looks similar to *mor1-1* mutant without JA treatment. The ultrastructures of the JA treated filament cells were identical to that without treatment. All these data indicated that MT disorder and endocytosis defects in filament cells was not rescued by JA treatment. These results indicated that cortical MTs disorganization is not a result of putative JA level reduction in *mor1-1* stamen.
Fig. 6 Endocytosis process was impaired in mor1-1 mutant. A FM4-64 internalization in wild type filament cells. B Quantification of endocytic vesicles in wild type, n = 12. C FM4-64 internalization in mor1-1 filament cells. D Quantification of endocytic vesicles in mor1-1 mutant, n = 12. (E) Distribution of CLC-RFP foci in wild type and mor1-1 filament epidermal cells. (F) Quantification of cortical-associated CLC-RFP foci in wild type and mor1-1 filament epidermal cells, n = 86 in wild type and 71 in mor1-1 mutants. Bars = 10 μm
Discussion

CMT organization in *mor1-1* mutant filament cells was disrupted at 24 °C

Microtubule-associated proteins of the highly conserved XMAP215/Dis1 family promote both microtubule growth and shrinkage, and move with the dynamic microtubule ends. The *Arabidopsis* MAP215 homologue MOR1 promotes rapid growth and shrinkage, and suppresses the pausing of microtubules in vivo (Kawamura and Wasteneys 2008). In this study, we found that *mor1-1* mutant showed a thermosensitive phenotype with altered CMT organization in the filament cells and drastic defect in anther dehiscence at 24 °C. These data indicated that microtubule organization in filament cells is critical for anther dehiscence.

The *mor1-1* plant is a point mutant with one amino acid altered in MOR1 protein (L174 changed to F), this mutation is located in an alpha helical stretch of the fifth HEAT-like repeat in the first of five N-terminal TOG domains (Kawamura and Wasteneys 2008), whereas HEAT repeats in MAP215 are implicated in interaction with α- and β-tubulins (Al-Bassam et al. 2007).

Furthermore, study had showed that L174F mutation increased the affinity of MOR1 TOG12 domains for microtubules in vitro, and then impaired the microtubules dynamic (Lechner et al. 2012), a previous study also showed that the duration of MT plus end pause events in *mor1-1* was increased to about 3 times to wild type when grown at 31 °C.
(Kawamura and Wasteneys 2008). The reduced MT dynamics impaired MTs organization and lead to too many developmental defects in mor1-1 mutant (Whittington et al. 2001). In this study, when mor1-1 plants grown at 24 °C, cortical MTs arrays were disorganized in filament cells, indicated that this condition impaired MTs organization in filament cells.

Besides the anther indehiscence phenotype, no other obvious development defects were found in mor1-1 mutant. In fact, MOR1 gene is expressed in many organs other than stamens (Fig. 3A, B). So it arises a question that why only stamens showed obvious defects at 24 °C in mor1-1 mutant? We hypothesized that filament cells should be hypersensitive to MTs organization defects at flower stage 12 and 13, because at these stages, stamen filaments extend very fast (Smyth et al. 1990), and highly ordered MTs dynamics might be critical for filament cell morphogenesis and function. Other organs, such as root, might have slightly abnormal cortical MTs structures and endocytosis defects, but does not show obvious defects in growth at 24 °C.

**Defects in CMT organization impaired endocytosis in mor1-1 filament cells**

The enlarged bubbles formed by PM invagination in mor1-1 mutant filament cells suggested a defect in endocytosis, and the FM4-64 uptake assays confirmed this speculation. Clathrin-mediated endocytosis is the primary endocytic route into cells through which extracellular materials are packaged into clathrin-coated vesicles (CCVs) and taken up into cells. Bubbles formed by PM invagination in mor1-1 mutant filament cells suggested a defect in CCVs membrane fission, which is catalyzed by the dynamin-related proteins (DRPs) (Verma et al. 2006; Collings et al. 2008). In yeast and mammalians, DRPs are MT binding proteins (Vallee et al. 1993). In Arabidopsis, studies had showed that microtubule inhibitor oryzalin significantly affects the lifetime and mobility of DRPs (Collings et al. 2008), these data suggested that CMTs are important for anchoring DRPs on PM. Our data showed that PM localized CCV was reduced in filament cells supported this hypothesis. Nevertheless, further more studies are required to address whether DRP1 attaches to CMT arrays and whether the mor1-1 mutation impair localization of DRP1. Our results suggested that CMT bundles are critical for endocytosis vesicle fission, providing a novel insight into the function of MTs in endocytosis.

**Endocytosis defects in mor1-1 filament cells might reduce JA level in anther**

Previous studies showed that filaments are the major sites for JAs biosynthesis and transporting (Acosta and Przybyl 2019), which are critical for anther dehiscence. For an example, **DEFECTIVE IN ANther DEHISCENCE1 (DADI)** gene, which encodes a lipase involved in initiation of JA synthesis, is exclusively expressed in stamen filament shortly before the onset of stamen maturation (Ishiguro et al. 2001). Furthermore, the **JAT1** gene, which encodes an ABCG-type JA transporter are also highly expressed in filament cell (Li et al. 2017). In this study, we observed CME defects in mor1-1 filament cells, and exogenously application of MeJA partially rescued fertility of mor1-1 mutant. Based on these observations, we hypothesis that CME defects might impaired JA biosynthesis and/or transport, and then resulted in JA reduction in anther, which leaded to anther indehiscence, however, this hypothesis need more evidence.

On the other hand, since MOR1 is a conserved MAP for MTs organization, mutation in MOR1 should impaired multiple cellular processes in addition to endocytosis in filament cells, other possibilities that caused anther indehiscence phenotype in mor1-1 plant are exist. First, in addition to transporting JA, the filaments also transport nutrition and water, in particularly, transporting water from the anther is important for dehiscence (Wilson et al. 2011), mor1-1 mutation might impair this process. Second, a recent study in rice suggested that JA can be synthesized in the anther locally (Li et al. 2021), mor1-1 mutation might impair JA biosynthesis and/or transport in anther directly, and then resulted in anther dehiscence.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s10725-021-00777-7.

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**Declarations**

**Conflict of interest** Authors declare they have no conflict of interest.

**References**

Acosta IF, Przybyl M (2019) Jasmonate signaling during Arabidopsis stamen maturation. Plant Cell Physiol 60(12):2648–2659. https://doi.org/10.1093/pcp/pcz201

Al-Bassam J, Larsen NA, Hyman AA, Harrison SC (2007) Crystal structure of a TOG domain: conserved features of XMAP215/Dis1-family TOG domains and implications for tubulin binding. Structure 15(3):355–362. https://doi.org/10.1016/j.str.2007.01.012

Bechtold N, Pelletier G (1998) In planta Agrobacterium mediated transformation of adult Arabidopsis thaliana plants by vacuum infiltration. In: Martinez-Zapater JM, Salinas J (eds) Arabidopsis protocols. Humana Press, Totowa, pp 259–266. https://doi.org/10.1385/0-89603-391-0:259

Bolte S, Talbot C, Boute Y, Catrice O, Read ND, Satiat-Jeunemaitre B (2004) FM-dyes as experimental probes for dissecting vesicle
trafficking in living plant cells. J Microsc 214(2):159–173. https://doi.org/10.1111/j.1365-2818.2003.01348.x

Collings DA, Geibbie LK, Howles PA, Hurley UA, Birch RJ, Cork AH, Hocart CH, Airoli T, Williamson RE (2008) Arabidopsis dynamin-like protein DRP1A: a null mutant with widespread defects in endocytosis, cellulose synthesis, cytokinesis, and cell expansion. J Exp Bot 59(2):361–376. https://doi.org/10.1093/jxb/erm324

Elleftheriou EP, Baskin TI, Hepler PK (2005) Aberrant cell plate formation in the Arabidopsis thaliana microtubule organization 1 mutant. Plant Cell Physiol 46(4):671–675. https://doi.org/10.1093/pcp/pci068

Fan L, Hsu Y, Zhang L, Song K, Ding Z, Botella MA, Wang H, Lin J (2013) Dynamic analysis of Arabidopsis AP2 sigma subunit reveals a key role in clathrin-mediated endocytosis and plant development. Development 140(18):3826–3837. https://doi.org/10.1242/dev.095711

Fan L, Li R, Pan J, Ding Z, Lin J (2015) Endocytosis and its regulation in plants. Trends Plant Sci 20(6):388–397. https://doi.org/10.1016/j.tplants.2015.03.014

Fowke L, Dibbayawan T, Schwartz O, Harper J, Overall R (1999) Combined immunofluorescence and field emission scanning electron microscope study of plasma membrane-associated organelles in highly vacuolated suspensor cells of white spruce somatic embryos. Cell Biol Int 23(6):389–397. https://doi.org/10.1006/cbir.1999.0368

Ge YX, Angenent GC, Dahlhaus E, Franken J, Peters J, Wullens GJ, Twell D, Park SK, Hawkins TJ, Hasenbein NG, Sugimoto K, Creemers-Molenaar J (2001) Partial silencing of the NEC1 gene results in early opening of anthers in Petunia hybrida. Mol Genet Genomics 265(3):414–423. https://doi.org/10.1007/s004380100449

Hamada T (2014) Microtubule organization and microtubule-associated proteins in plants. Int Rev Cell Mol Biol 312:1–52. https://doi.org/10.1016/B978-0-12-800178-3.00001-4

Hussey PJ, Hawkins TJ, Igarashi H, Kaloriti D, Smertenko A (2002) The plant cytoskeleton: recent advances in the study of the plant microtubule-associated proteins MAP-65, MAP-190 and the Xenopus MAP215-like protein, MOR1. Plant Mol Biol 50(6):915–924

Ishiguro S, Kawai-Oda A, Ueda J, Nishida I, Okada K (2001) The DEFECTIVE IN ANTHER DEHISCENCE1 gene encodes a homologue of Xenopus MAP215, promotes rapid growth and degeneration of cells that play a major role in tobacco anther dehiscence. Sex Plant Reprod 17(5):219–241. https://doi.org/10.1007/s0047900105158

Jorgensen BO, Baskin TI, Hepler PK, de Vries J, Smertenko A, Hocart CH, Arioli T, Williamson RE (2008) MOR1/GEM1 has an essential role in the plant-specific cytokinetic phragmoplast. Nat Cell Biol 4(9):711–714. https://doi.org/10.1038/ncb1544

Kawamura E, Wasteneys GO (2008) MOR1, the Arabidopsis thaliana homologue of Xenopus MAP215, promotes rapid growth and shrinkage, and suppresses the pausing of microtubules in vivo. J Cell Sci 121(24):4114–4123. https://doi.org/10.1242/jcs.039065

Keizer CJ (1987) The processes of anther dehiscence and pollen dispersal. I. The opening mechanism of longitudinally dehiscing anthers. New Phytol 105(3):487–498. https://doi.org/10.1111/j.1469-8137.1987.tb00886.x

Konopka CA, Backues SK, Bednarek SY (2008) Dynamics of Arabidopsis dynamin-related protein 1C and a clathrin light chain at the plasma membrane. Plant Cell 20(5):1363–1380. https://doi.org/10.1105/tpc.108.059428

Lechner B, Rashbrooke MC, Collings DA, Eng RC, Kawamura E, Whittington AT, Wasteneys GO (2012) The N-terminal TOG domain of Arabidopsis MOR1 modulates affinity for microtubule polymers. J Cell Sci 125(Pt 20):4812–4821. https://doi.org/10.1242/jcs.107045

Ledbetter MC, Porter KR (1964) Morphology of microtubules of plant cell. Science 144(3620):872–874. https://doi.org/10.1126/science.144.3620.872

Li Q, Zheng J, Li S, Huang G, Skilling SJ, Wang L, Li L, Li M, Yuan L, Liu P (2017) Transporter-mediated nuclear entry of jasmonoyl-isoleucine is essential for jasmonate signaling. Mol Plant 10(5):695–708. https://doi.org/10.1016/j.molp.2017.01.010

Li S, Cao L, Chen X, Liu Y, Persson S, Hu J, Chen M, Chen Z, Zhang D, Yuan Z (2021) A synthetic biosensor for mapping dynamic responses and spatio-temporal distribution of jasmonate in rice. Plant Biotechnol J. https://doi.org/10.1111/pbi.13718

Ma H (2005) Molecular genetic analyses of microsporogenesis and microgametogenesis in flowering plants. Annu Rev Plant Biol 56:393–434. https://doi.org/10.1146/annurev.arplant.55.031903.141717

Paredes AR, Somerville CR, Ehhardt DW (2006) Visualization of cellulose synthase demonstrates functional association with microtubules. Science 312(5779):1491–1495. https://doi.org/10.1126/science.1126551

Sanders PM, Bui AQ, Weterings K, McIntire KN, Hsu Y-C, Lee PY, Truong MT, Beals TP, Goldberg RB (1999) Anther developmental defects in Arabidopsis thaliana male-sterile mutants. Sex Plant Reprod 11(6):297–322. https://doi.org/10.1007/s0047900105158

Sanders PM, Bui AQ, Le BH, Goldberg RB (2005) Differentiation and degeneration of cells that play a major role in tobacco anther dehiscence. Sex Plant Reprod 17(5):219–241. https://doi.org/10.1007/s004970010231-y

Scott RJ, Spielman M, Dickinson HG (2004) Stamen structure and function. Plant Cell 16(Suppl):S46–60. https://doi.org/10.1105/tpc.017012

Sedbrook JC, Kaloriti D (2008) Microtubules, MAPs and plant directional cell expansion. Trends Plant Sci 13(6):303–310. https://doi.org/10.1016/j.tplants.2008.04.002

Shen J, Wang X, Jiang L (2020) Exocytosis, endocytosis and membrane recycling in plant cells. eLS. Wiley, Hoboken, pp 490–499. https://doi.org/10.1002/9780470015902.a0029126

Smyth DR, Bowman JL, Meyerowitz EM (1990) Early flower development in Arabidopsis. Plant Cell 2(8):755–767. https://doi.org/10.1105/tpc.2.8.755

Tan X, Wei J, Li B, Wang M, Bao Y (2017) AtVps11 is essential for vacuole biogenesis in embryo and participates in pollen tube growth in Arabidopsis. Biochem Biophys Res Commun 491(3):794–799. https://doi.org/10.1016/j.bbrc.2017.07.059

Twell D, Park SK, Hawkins TJ, Schubert D, Schmidt R, Smertenko A, Hussey PJ (2002) MOR1/GEM1 has an essential role in the plant-specific cytokinetic phragmoplast. Nat Cell Biol 4(9):711–714. https://doi.org/10.1038/ncb1544

Vallee RB, Herskovits JS, Burgess CC (1993) Dynamin, a microtubule-activated GTPase involved in endocytosis. Springer, Berlin, Heidelberg

Verma DPS, Hong Z, Menzel D (2006) Dynamin-related proteins in plant endocytosis. In: Samaj J, Baluška F, Menzel D (eds) Plant endocytosis. Springer, Berlin, Heidelberg, pp 217–232. https://doi.org/10.1007/7089_013

Whittington AT, Vugrek O, Wei KJ, Hasenbein NG, Sugimoto K, Rashbrooke MC, Wasteneys GO (2001) MOR1 is essential for organizing cortical microtubules in plants. Nature 411(6837):610–613. https://doi.org/10.1038/35079128

Wilson ZA, Song J, Taylor B, Yang C (2011) The final split: the regulation of anther dehiscence. J Exp Bot 62(5):1633–1649. https://doi.org/10.1093/jxb/er104

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