Mutation in Arabidopsis MOR1 Gene Impairs Endocytosis in Stamen Filament Cells and Results in Anther Indehiscence

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Research Article

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Abstract

Anther dehiscence is a crucial step for pollen grain 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 in anther dehiscence 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 (TEM) 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 (CLC-RFP) 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.

Key Message

During *Arabidopsis* stamen development, MOR1/MAP215 mediates cortical microtubules organization in filament cells, and then plays an important role in filament cell endocytosis and anther dehiscence.

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 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 crtical signal for the anther dehiscence (Scott et al., 2004; Acosta et al., 2019). However, little has been known about the molecular mechanisms that regulate transport of water, nutrients and phytohormones in the filaments.

Microtubules (MTs) are the basic components of the cytoskeleton in eukaryotic cells and are made up of 13 parallel protofilaments, each of which is composed of α- and β-tubulin unit molecules (Ledbetter and Porter, 1964). In plants, the microtubule (MT) cytoskeleton is a central player in a multitude of developmental and environmental roles ranging from cell division, expansion, hormonal signalling, tropisms, to biotic and abiotic stress (Hussey et al., 2002). 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 et al., 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* (Wasteneys, 2008). In Arabidopsis, the MOR1 localizes to nearly all MT structures, including cortical MT (CMT) array, phragmoplast, spindle and preprophase 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). These results suggest that the MOR1 is important for the plant cellular functions. 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 at 24°C, the *mor1-1* anther could not dehiscence normally, and pollen grains could not disperse effectively. CMT arrays in the *mor1-1* filament cells are disorganized. However, all these defects did not occur when the mutant plants were grown in the condition of 19°C. TEM data and FM4-64 uptake assays showed that at 24°C, endocytosis in the *mor1-1* filament cells were arrested. Furthermore, the cortical-associated clathrin light chain (CLC) foci were dramatically reduced in the *mor1-1* filament epidermal cells. These results suggested that the MOR1-mediated CMT organization is important for clathrin-mediated endocytosis (CME) in the filament cells, which plays a prominent role in filament development and anther dehiscence. Our study also could provide a new insight into the functions of MOR1 on plant male organ development.

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). 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/8h dark.

Phenotyping

For observation of pollen releasing, the stamens from flowers at the floral stage 13 of wild type (WT) and *mor1-1* 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* 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-3000N 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 (Zeiss, www.zeiss.com). The FM4-64 and RFP were excited at 543 nm.

ProMOR1::GUS Transgenic Plants

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 et al., 1993). For GUS histochemical staining, the organs were incubated overnight in a X-Gluc solution (1.9 mM 5-bromo-4-chloro-3-indolyl-β-glucuronide, 0.5 mM $K_3Fe[CN]_6$, 0.5 mM $K_4Fe[CN]_6$, 0.1% Triton X-100, and 50 mM Na-phosphate buffer, pH 7.0) at 37°C.

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. In particular, the mutant siliques were much shorter than those of wild type (Fig. 1A, B), and produced nearly no seeds (Fig. 1C). This result indicated that the moderate temperature of 24°C did not affect the vegetative growth, but drastically reduced fertility in the *mor1-1* mutant. These phenotypes were not observed in *mor1-1* mutants which were grown at 19°C (Fig. S1).

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, D), indicating that the sterile phenotype was associated with a male sterility. 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. 1E, F), indicating that male sterility in *mor1-1* is thermosensitive.

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* 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-* mutation did not affect filament elongation and other floral organs. However, comparison of the anthers from wild type and *mor1-1* showed that the wild type anthers could release their pollen grains on the surface (Fig. 2E), but the *mor1-1* 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). Therefore, we concluded that the male sterility of *mor1-1* was caused by defect in anther indehiscence at 24°C.

Scanning electron microscopy (SEM) was used to further examine the surface of *mor1-1* anther at the floral stage 13 from the plants grown at 24°C. As shown in Fig. 2I, in the wild type flowers, numerous pollen grains could be observed on surface of the dehiscent anther; in contrast, the *mor1-1* anthers were not fully dehiscent, in which, most pollen grains were enclosed in the anther locule (Fig. 2J).

To determine if defect in dehiscence was caused by morphological abnormality of the anther tissues, the transverse sections of wild type and *mor1-1* anthers at floral stage 13 from the plants grown at 24°C were prepared and examined (Fig. 2K, L). Most cell types in the *mor1-1* anthers at the floral stage 13, such as endothecium, epidermis, and connective tissues appeared normal like wild type. In particular, in the anthers at the floral stage 13, degeneration of the tapetum, breakage of the septum, differentiation of the stomium, and development of fibrous bands in the endothecium and connective cells all were normal in *mor1-1* like those in wild type anthers. However, unlike wild type anthers, in which the stomium was

ruptured (Fig. 2K) at the floral stage 13, the stomium in the *mor1-1* anther at the same stages appeared unbroken (Fig. 2L). These results indicated that the anther development process in the *mor1-1* mutant proceeded normally toward dehiscence but were interrupted immediately before stomium breakage, leading to an anther indehiscence.

CMT organization in the filament cells was altered in mor1-1

The promoter activity assays using the histochemical β-glucuronidase (GUS) reporter revealed strong activities of *MOR1* promoter in stamen filaments rather than in anther (Fig. 3A). This implied that the *mor1-1* mutation might impair the function of filament at 24°C, which could be associated with the anther indehiscence phenotype. SEM analysis showed that at the floral stage 13, the wild type filament cell surface showed the normal straight stripes (Fig. 3B). In contrast, unlike wild type, the *mor1-1* filaments exhibited twisty stripes on their surfaces (Fig. 3C), indicating that the *mor1-1* filament cell morphology was impaired at 24°C. This phenotype did not appear when the mutant plants were grown at 19°C (Fig. 3D).

Previous study has shown that the CMT arrays are important for cell morphogenesis (Paredez et al., 2006). Therefore, the CMT organization in the *mor1-1* 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. 3E and 3F, CMT arrays in the WT filament cells were mostly transversely aligned, whereas in *mor1-1* 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* could disrupt CMT organization in filament cells at 24°C (Fig. 3G-H), compared to that the CMT organizations in the *mor1-1* mutant filament cells were normal at 19°C (Fig. 3I-J).

PM homeostasis was disturbed in the mor1-1 filament cells

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. 4A). Some small bubbles, whose diameters are ~ 80 nm were occasionally observed at the PM (Fig. 4B). In contrast, almost no distinguishable organelles were observed in the *mor1-1* filament epidermal cells (n = 18) (Fig. 4C). Many big bubbles, whose diameters are more than 500 nm, were formed via PM invagination (Fig. 4C, 4D), suggesting a severely defective endocytosis in *mor1-1* filament epidermal cells. Compared to epidermal cells, the endodermis cells in the *mor1-1* filament were not severely affected, and the organelles could be clearly distinguished as in wild type (Fig. 4E-H). The big bubbles formed by PM invaginating were also observed (Fig. 4G and 4H), which was not observed in endodermis cells in the wild type filaments (Fig. 4E and 4F). These data suggested that the *mor1-1* mutation might impair endocytosis in the filament cells, resulting in disordering of PM homeostasis. On the other hand, no

vesicles accumulation were observed in *mor1-1* filament cells, indicating that vesicle secretion was not affected by *mor1-1*.

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

To confirm if endocytosis process in the *mor1-1* filament cells was disturbed at 24°C, we further examined the rate of endocytosis with FM4-64 dyes, a widely used fluorescent markers 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 minutes after labeling (Fig. 5A, B), whereas in the *mor1-1* mutant just few FM4-64 uptakes could be detected even 20 minutes after labeling (Fig. 5C, 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* 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* filament epidermal cells, the number of cortical-associated CLC-RFP foci was reduced dramatically (Fig. 5E and 5F). This result suggested that CMT might recruit clathrin subunits to the PM or attach them on the PM.

Discussion

The thermosensitive anther indehiscence in *mor1-1* at 24°C may be caused by disorder of PM homeostasis in the filament cells

In this study, we characterized the roles of *MOR1* gene in Arabidopsis sexual reproduction. The *MOR1* is highly expressed in filament rather than anther. However, loss of MOR1 functions affects not only filament but also anther. The *mor1-1* mutant exhibited a thermosensitive phenotype of altered cell morphology in the filaments and drastic defect in anther dehiscence at 24°C. In particular, the filament cells showed a twisted cell morphology, disorganized CMT arrays and PM homeostasis disorders (Fig. 3 and Fig. 4), while in the *mor1-1* anthers, although all other cell types developed normally. Whereas the stomium was severely defective, leading to an inefficient dehiscence of anther and resulting in a significantly reduced seed set at 24°C. These results implied that the MOR1 is associated with anther dehiscence, which is important for normal pollination, through influencing the cytoskeleton and transport in the filament cells.

Previous studies showed that filaments are the major sites for JAs biosynthesis and transporting (Acosta et al., 2019), which are critical for anther dehiscence. For an example, *DEFECTIVE IN ANTHER DEHISCENCE1* (*DAD1*) 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 demonstrated that exogenous application to the flowering *mor1-1* plants grown at 24°C with Me-JA could partially rescued the male sterility (Fig. S2), suggesting that JA biosynthesis and/or transporting was affected in the *mor1-1* stamen. Therefore, the defective anther dehiscence might be caused by the inefficiency of JA resulted from the defects in cytoskeleton and transport at 24°C. On one hand, PM localization of the JA transporter might be disturbed in the *mor1-1* filament cells, which then impaired JA transporting. On the other hand, there might be also a possibility that JA biosynthesis was affected in the *mor1-1* filament cells. However, we cannot rule out the possibility that *mor1-1* might impair the anther stomium cell structure directly, and then disturb anther dehiscence at 24°C. Nevertheless, more investigations are required to address how the MOR1 is involved in anther dehiscence.

The male sterility is useful for crop hybrid breeding. In this study, we demonstrated that loss of MOR1 function could create a recessive phenotype of thermosensitive male sterility in Arabidopsis. When the *mor1-1* homozygous mutant plants were grown at 24 °C, they were almost male sterile, but their vegetative growth was not affected. On the other hand, the *mor1-1* homozygous mutant plants could grow completely normal when grown at a lower temperature of 19 °C. These characteristics have a high potential to be used in crop hybrid breeding.

Function of MOR1 in endocytosis

Endocytosis, which enable the cells to take up extracellular materials and cell surface proteins via vesicle transport, is essential for regulation of signal transduction, maintenance of PM homeostasis and transport of the important molecules from outside the cell to the appropriate compartment in the cytoplasm. Several lines of evidences showed that microtubules are involved in plant endocytosis. 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). Third, an observation showed a ubiquitous microtubule-endosome association and microtubule-regulated PIN abundance on the PM in Arabidopsis (Ambrose et al., 2013). In this study, EM data suggested that the PM homeostasis disorder results from endocytosis defects, and the FM4-64 uptake analysis confirmed that endocytosis is impaired in the *mor1-1* filament cells. The enlarged bubbles formed by PM invagination in *mor1-1* filament cells suggested a defect in endocytosis vesicles membrane fission, which is catalyzed by the dynamin-related proteins (DRPs)(Verma et al., 2006; Collings et al., 2008).We further found that *mor1-1* impaired CMT arrays in filament cells, this defect might impair the localization and/or dynamics of DRP1 proteins to clathrin-coated vesicles, and then impaired the endocytosis vesicles detach. Nevertheless, further more studies are required to address whether DRP1 attaches to CMT arrays and whether the *mor1-1* impair localization of DRP1. Our results suggested that CMT bundles are critical for endocytosis vesicles fission, providing a novel insight into the function of MTs in endocytosis.

Function of MOR1 in vacuole morphogenesis

Vacuole is the most prominent organelle in maturely-differentiated plant cells. Previous studies showed that MTs play important roles in plant vacuole morphogenesis and dynamics. For example, depolymerization of MTs by oryzalin treatment dramatically affected vacuole morphology and motility in *Physcomitrella patens*. Furthermore, tight interactions between microtubules and vacuolar membranes were observed by dual observation of microtubules and vacuolar membranes (Oda et al., 2009).

The function of MOR1 in vacuole morphogenesis is unknown. As shown in Fig. 4A, the central vacuoles were formed in wild type but not found in *mor1-1* filament epidermal cells at 24°C (Fig. 4B). Since defects in endocytosis were observed in *mor1-1* filament cells, we proposed that this phenotype resulted from defects in endocytosis. However, this study cannot rule out the possibility that *mor1-1* might impair vacuole biogenesis directly by affecting some MT systems in the cell. In the future, we will investigate the function of MOR1 in vacuole biogenesis in the root, which have different vacuolated cells and more suitable for address this question.

Abbreviations

CLC, clathrin light chain; CME, clathrin mediated endocytosis; CMT, cortical microtubule; CSLM, confocal laser scanning microscopy; EMT, endoplasmic microtubule; GUS, β-glucuronidase; JA, Jasmonic acid; MOR1, Microtubule Organization 1; NASC, Nottingham Arabidopsis stock centre; PM, Plasma membrane; TEM, transmission electron microscopy; SEM, scanning electron microscopy; MT, microtubule;

Declarations

Author contribution statement:

Xiaolei Liu performed experiments, Xiaoyun Tan designed experiments and wrote manuscript.

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Compliance with ethical standards

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

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Figures

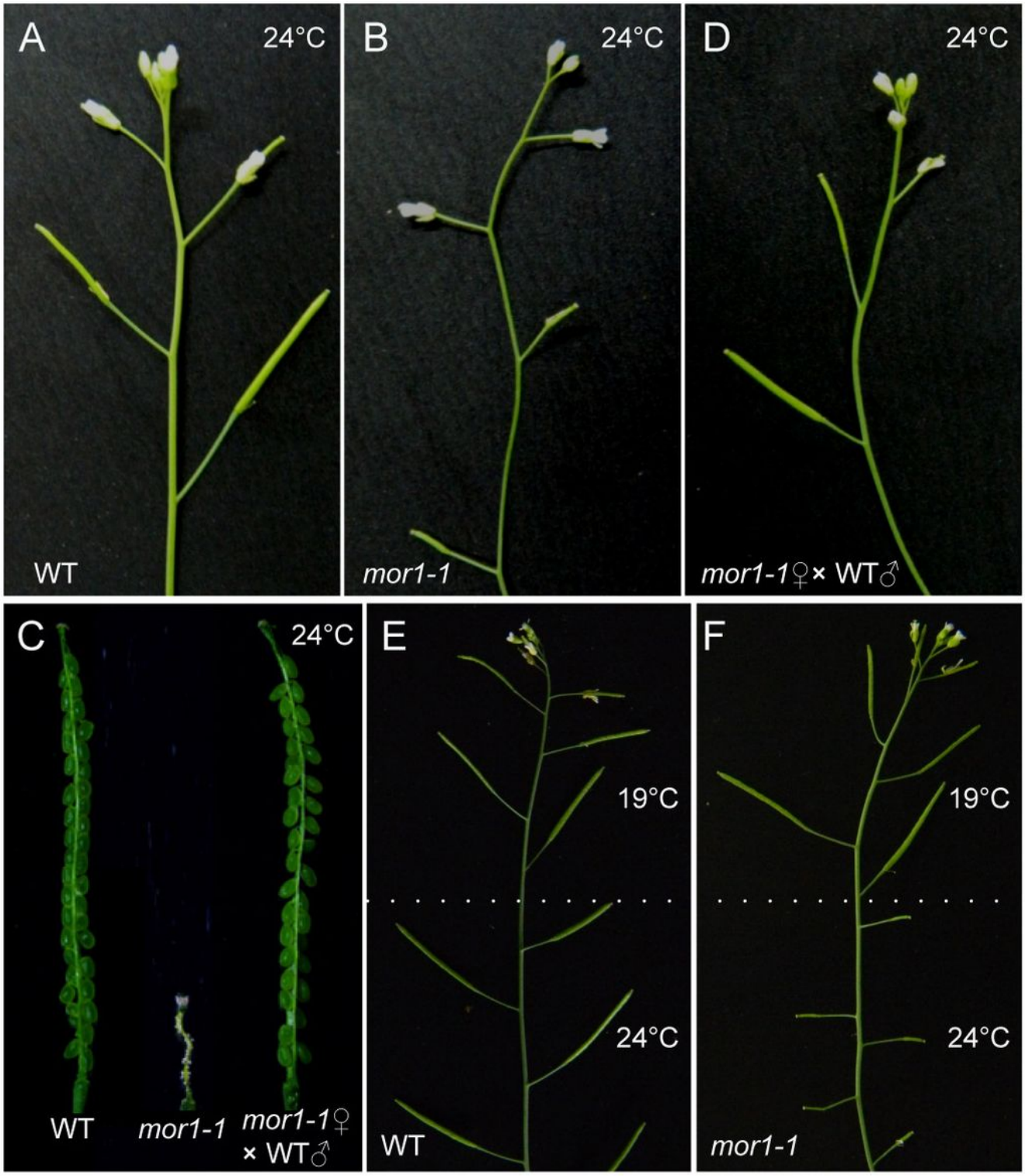

Figure 1

The mor1-1 mutant exhibited a thermosensitive male sterile phenotype (A) A wild type plant with full fertility at 24°C; (B) A mor1-1 with reduced fertility indicated by the shorter siliques at 24°C; (C) A mor1-1 mutant plant showing the normal siliques at 24°C after pollinated with WT pollen. (D) Normal seed sets in wild type and mor1-1 siliques at 19°C. (E) Seed sets in wild type, mor1-1, and mor1-1♀× WT ♂ siliques at

24°C. (F-G) A comparison between wild type (F) and mor1-1 (G) plants as they were grown at 24°C until producing several siliques, and then moved to 19°C.

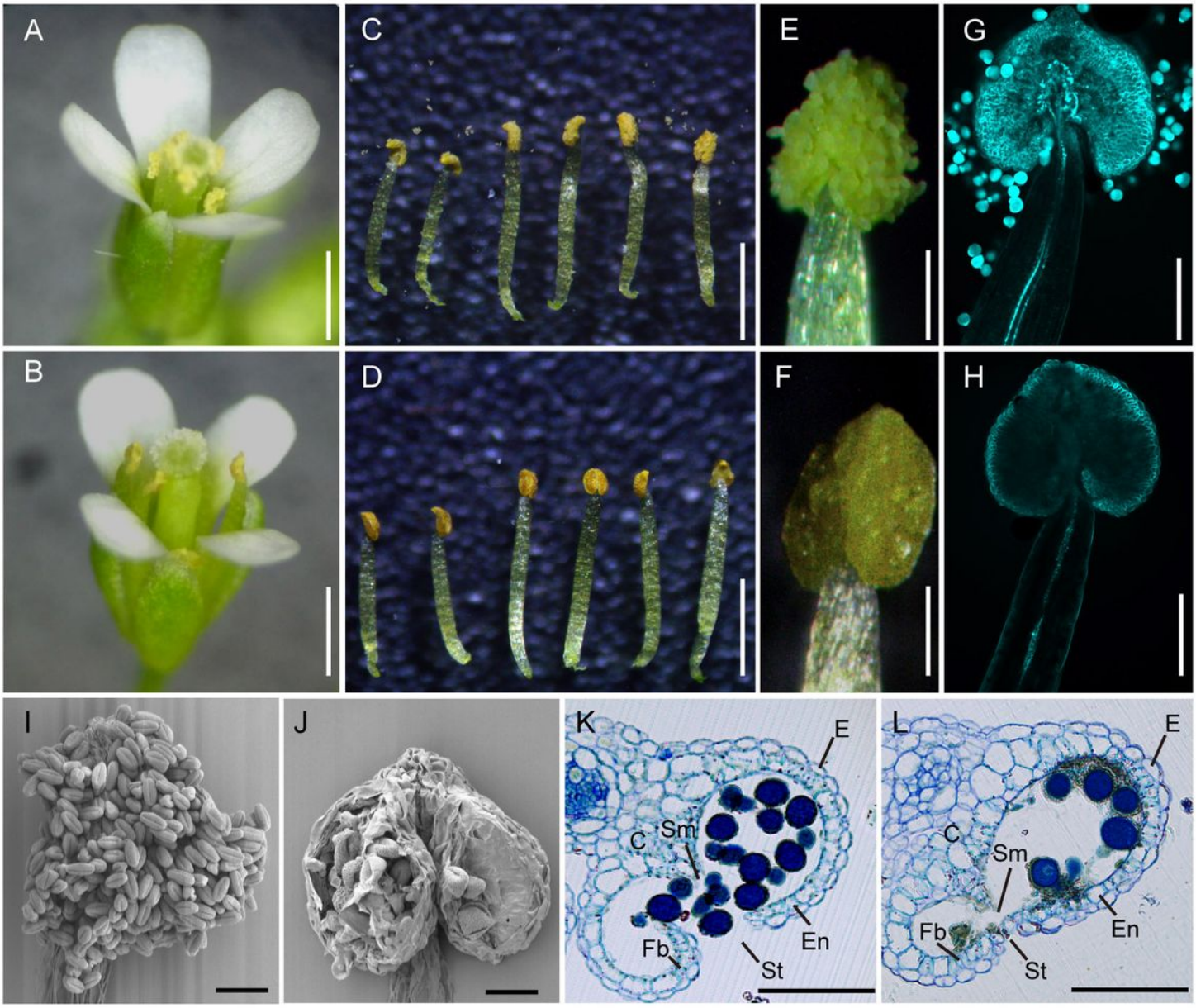

Figure 2

The mor1-1 was defective in anther dehiscence at 24°C (A) and (B) wild type (A) and mor1-1 (B) flowers with normal floral organs. Bars = 1 mm. (C) and (D) Stamens from wild type (C) and mor1-1 (D) flowers. Bars = 1 mm. (E) and (F) Anthers from wild type (E) and mor1-1 (F) flowers. Bars = 100 μm. (G) and (H) Aniline blue staining revealed that pollen grains were released from wild type (G) but not mor1-1 (H) flowers. Bars = 100 μm. (I) and (J) SEM images of wild type (I) and mor1-1 (J) stamens. Bars = 50 μm. (K) and (L) Transverse sections of wild type (K) and mor1-1 (L) anthers. Epidermis (E), endothecium (En), connective (C), fibrous bands (Fb) and septum (Sm) appear to be normal, but the stomium (St) was not opened in mor1-1 anthers. Bars = 50 μm.

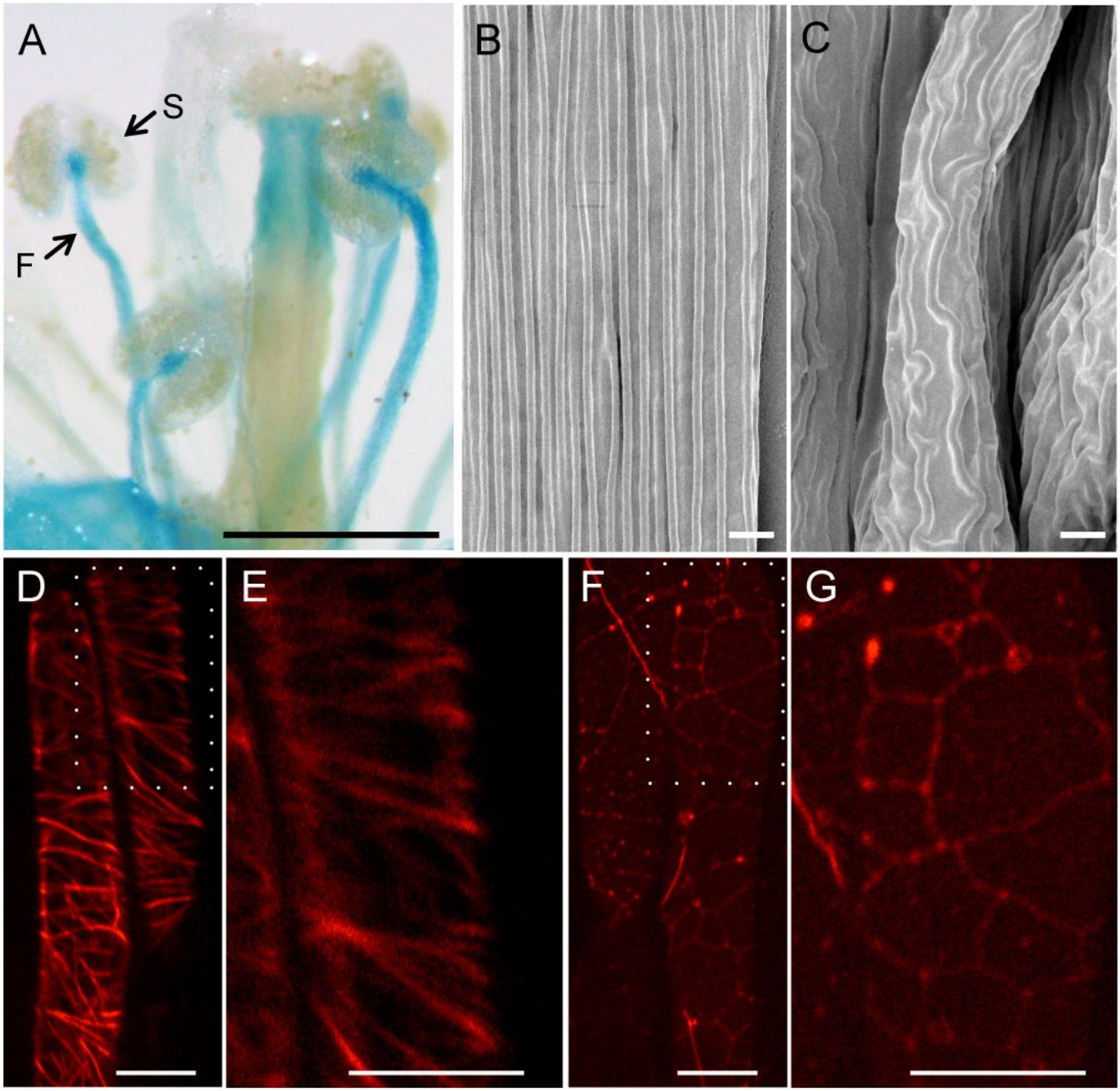

Figure 3

The mor1-1 impaired cortical microtubule organization in filament cells at 24°C. (A) A ProMOR1::GUS transgenic flower, showing GUS stains in filament. F, filament; S, stamen. Bar = 1mm. (B) and (C) SEM images of wild type (B) and mor1-1 (C) filaments at 24°C. (D) SEM images of mor1-1 filaments at 19°C. Bars = 1 μm in (B) to (D). (E) and (F) Cortical microtubules in wild type filament cells at 24°C. (G) and (H) Cortical microtubules in mor1-1 filament cells at 24°C. (I) and (J) Cortical microtubules in mor1-1 filament cells at 19°C. Bars = 10 μm in (E) to (J).

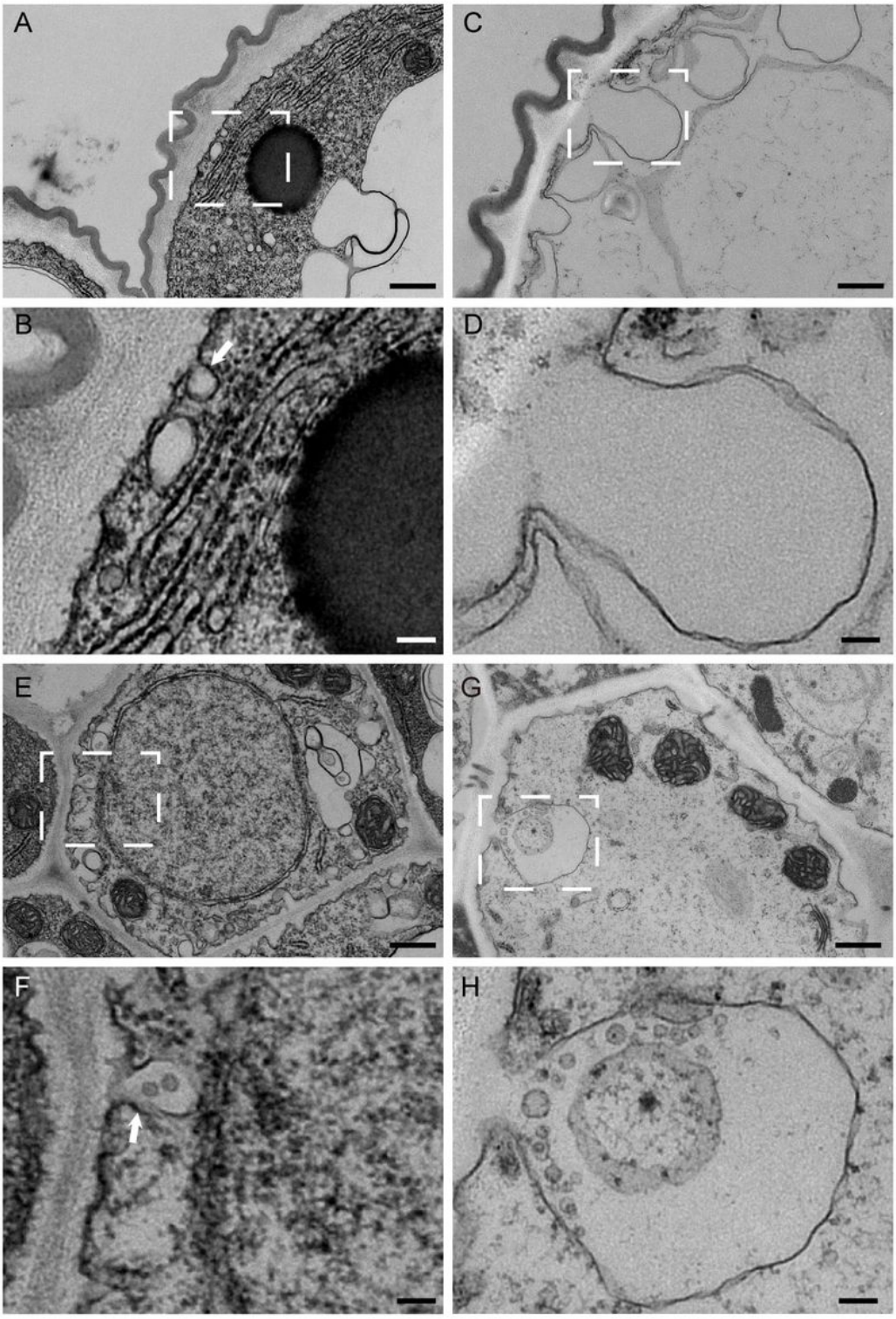

Figure 4

PM homeostasis was disturbed in the mor1-1 filament cells. (A) and (B) TEM images of wild type (A) and mor1-1 (B) filament epidermal cells, notice that many big bubbles formed by PM invagination in (B). Bars = 500 nm. (C) and (D) Magnified images of (A) and (B) respectively. Arrow in (C) indicated a putative budding endocytosis vesicle. Bars = 100 nm. (E) and (F) TEM images of wild type (E) and mor1-1 (F) filament endodermis cells, a big bubble formed by PM invagination in (F). Bars = 500 nm. (G) and (H)

Magnified images of (E) and (F) respectively. Arrow in (G) indicated a putative budding endocytosis vesicle. Bars = 100 nm.

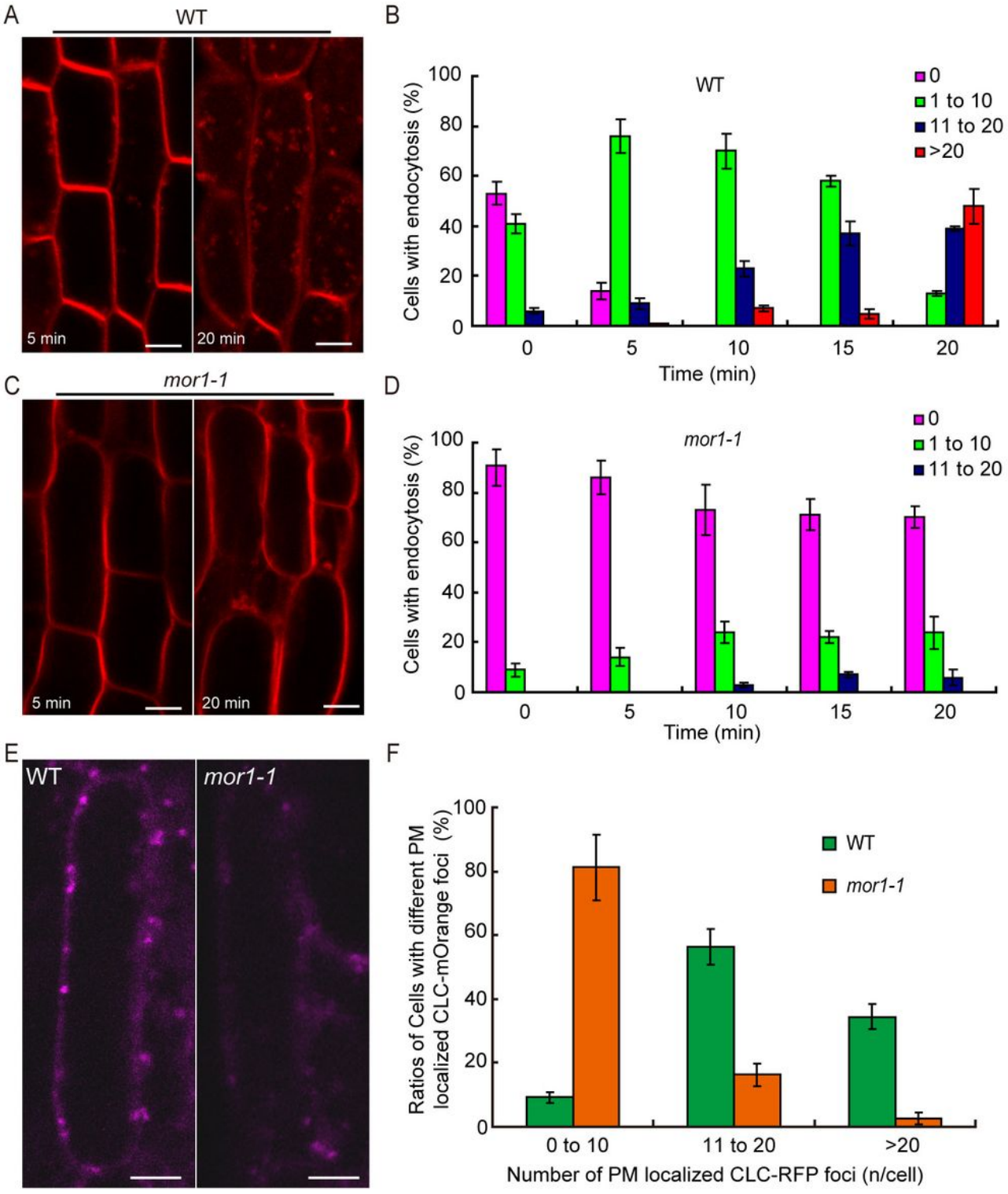

Figure 5

Endocytosis of FM4-64 is reduced in the mor1-1 filament cells (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.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- FigS1.tif
- FigS2.tif
- supplementaldata.doc