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MAP65 proteins influence crop traits

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The plant cytoskeleton is composed of microtubules and actin microfilaments that play instrumental roles in critical processes throughout the cell cycle, including cytokinesis and interphase cell expansion. To facilitate these processes, cytoskeletal polymers are organized into arrays decorated with numerous proteins that modulate array organization and function. Plant genomes contain multiple *Microtubule Associated Protein 65* (MAP65) genes, and only a subset of MAP65's have been functionally characterized. MAP65 proteins bind and crosslink adjacent microtubules in mitotic, cytokinetic, and interphase arrays in plant cells. The nomenclature used to name different MAP65 genes and subgroups is inconsistent between plant species, which hinders evaluation of MAP65 genes across different species. This review compares and clarifies the MAP65 naming systems to aid the transfer of information across plant species. Proteins in the MAP65–3 group may have a conserved cellular role in organizing the phragmoplast array needed for cytokinesis. Phragmoplasts are disorganized and cytokinesis is incomplete in *Arabidopsis thaliana*, *Cucumis sativus*, *Oryza sativa*, and *Zea mays map65–3* loss-of-function mutants. However, the phenotypic severity of *map65–3* alleles differs among species. For example, *OsMAP65–3* is necessary for rice seedling growth and survival, while cucumber plants lacking *CsMAP65–3* appear wildtype but are less susceptible to *Meloidogyne incognita* infection. MAP65–1 proteins are involved in morphogenesis, cold tolerance, and resistance to specific pathogens in *Arabidopsis thaliana*, *Glycine max*, and *Solanum lycopersicon*. Future studies in model and agricultural plants that better define MAP65 subgroups and evaluate the function of MAP65 proteins in multiple processes could be useful to inform the development of new crop strains.

KEYWORDS

cytokinesis, microtubules, phragmoplast, soybean, MAP65–1, MAP65–3

Introduction

MAP65 proteins are found in all eukaryotes and are involved in the organization and function of microtubules arrays throughout the cell cycle (Sasabe and Machida, 2006; Walczak and Shaw, 2010; She et al., 2019). In M-phase, MAP65 proteins play important roles in the assembly and function of the microtubule spindle (Loiodice et al., 2005; Duellberg et al., 2013; Hamada, 2014). Specifically, MAP65 proteins form dimers that

crosslink antiparallel microtubules in the central spindle and midzone which are needed to separate chromosomes and daughter nuclei during mitosis and cytokinesis in animal and yeast cells (Walczak and Shaw, 2010).

Plant genomes encode multiple MAP65 genes that cluster into subgroups based upon sequence similarities and phylogenetic analyses (Smertenko et al., 2008). Different research groups have defined and named the subgroups independently (Smertenko et al., 2008; Jia et al., 2024; Liang et al., 2025; Zhang et al., 2025), and this mini review compares and clarifies these naming systems. Each MAP65 sequence contains a conserved N-terminal region, multiple phosphorylation sites, and at least one C-terminal microtubule binding domain (Smertenko et al., 2008). Despite sequence similarities, significant differences exist in subcellular localization among the plant MAP65 proteins. Studies in cultured *Arabidopsis thaliana* and *Nicotiana tabacum* cells revealed that different MAP65 proteins localize to microtubule arrays in distinct, yet overlapping patterns, throughout the cell cycle (Van Damme et al., 2004; Smertenko et al., 2008). The spatiotemporal subcellular localization of each MAP65 protein is influenced by sequence variations in second microtubule binding domains and associated phosphorylation sites (Smertenko et al., 2008; Ho et al., 2011). Phosphorylation of MAP65 proteins reduces their microtubule binding and bundling activity (Smertenko et al., 2008). Aurora kinases, cyclin dependent kinases, and mitogen activated kinases are implicated in the phospho-regulation of MAP65 proteins (Sasabe et al., 2011; Boruc et al., 2017; Gao et al., 2022; Deng et al., 2024). Members of the MAP65 family are expressed in different tissues and cells (Parrotta et al., 2017; Liang et al., 2023, 2025). Recently, numerous motifs were identified in MAP65 promoter and coding sequences that may impact gene expression (Liang et al., 2023; Jia et al., 2024; Zhang et al., 2025). While the function of most MAP65 in plants is unknown, MAP65 genes are differentially regulated transcriptionally and post-translationally which suggests the family has functionally diversified to some extent.

Proteins in the MAP65-3 subgroup are specifically involved in the phragmoplast that mediates cytokinesis (Müller et al., 2004; Caillaud et al., 2008; Ho et al., 2011). Initial genetic studies in a model plant, *Arabidopsis thaliana*, revealed that *AtMAP65-3* localizes to the mitotic spindle and regions of antiparallel microtubule overlap in the phragmoplast (Müller et al., 2004; Caillaud et al., 2008; Smertenko et al., 2008; Ho et al., 2011). In *atmap65-3* mutants, phragmoplasts are disorganized and cytokinesis disrupted (Müller et al., 2004; Ho et al., 2011; Caillaud et al., 2008). Recent research in *Cucumis sativus*, *Oryza sativa*, and *Zea mays* indicate that MAP65-3 appears to play a conserved cellular role in cytokinesis (Lin et al., 2022; Chettoor et al., 2023; Liang et al., 2025), however *map65-3* mutations in each species manifest as different whole plant phenotypes.

MAP65-1 subgroup proteins function during interphase and cytokinesis. Genetic studies in *Arabidopsis thaliana* demonstrated that *AtMAP65-1* and *AtMAP65-2* redundantly promote axial elongation in seedling hypocotyls (Lucas et al., 2011) and root growth (Lucas and Shaw, 2012). *AtMAP65-1* proteins were also shown to overlap functionally with MAP65-3 in cytokinesis as

atmap65-1 and *atmap65-2* mutations exacerbate *map65-3* cytokinesis defects (Sasabe et al., 2011). Recently, studies showed that *AtMAP65-1* was targeted during infection with *Pseudomonas syringae*, and that overexpression of *AtMAP65-1* can quell *Pseudomonas syringae* infection (Guo et al., 2016). Similar results relating MAP65-1 and *Pseudomonas syringae* infection have been observed in *Glycine max* (Kim et al., 2024).

This manuscript focuses on recent genetic studies that demonstrate MAP65 function in the crop plants *Cucumis sativus*, *Glycine max*, *Solanum lycopersicum*, and *Zea mays*. These studies highlight the multiple roles microtubule arrays and MAP65 proteins play during plant growth, development, and environmental interactions. Collectively, recent publications indicate that MAP65-3 proteins play a conserved role in phragmoplasts (Ho et al., 2011; Lin et al., 2022; Chettoor et al., 2023; Liang et al., 2025), and that MAP65-1 proteins may modulate interphase microtubule function in response to multiple environmental cues (Lucas et al., 2011; Guo et al., 2016; Kim et al., 2024). Both MAP65-1 and MAP65-3 can be targets of signaling pathways and phosphorelays that impact microtubule organization and/or function (Gao et al., 2022; Lee and Liu, 2025). Comparisons of MAP65 function across different angiosperms show the limitations and benefits of translational research between research model systems and applied agricultural plants. In addition, discussion will include information from recent transcriptional analyses that may be productive to determine the role of MAP65 proteins in other plants and environmental conditions. First, to facilitate cross-species comparisons of gene function, we evaluate the four phylogenetic analyses of the MAP65 family to clarify the nomenclature of genes and subgroups.

Nomenclature of MAP65 family members and subgroups

Phylogenetic analyses of MAP65 sequences in multiple plant species revealed distinct subgroups of MAP65's in plant genomes (Smertenko et al., 2008; Jia et al., 2024; Liang et al., 2025; Zhang et al., 2025). All phylogenetic analyses included the nine *Arabidopsis thaliana* sequences, which enabled comparison of subgroup composition and labeling among four publications (Table 1). Three studies identified five subgroups (Smertenko et al., 2008; Jia et al., 2024; Zhang et al., 2025), however the subgroups do not contain the same members, and one publication designated three subfamilies (Liang et al., 2023) (Table 1). The categorization of sequences designated *AtMAP65-4*, 5, 8, and 9 shifted depending upon study (Table 1). *AtMAP65-3*, 4, and 9 were defined as a single subgroup in two analyses (Jia et al., 2024; Liang et al., 2025), or separated into two distinct subgroups (Smertenko et al., 2008; Zhang et al., 2025). MAP65-5 and MAP65-8 were either separated into individual subgroups (Smertenko et al., 2008; Jia et al., 2024), combined with MAP65-1 and 2 (Liang et al., 2025), or only MAP65-8 grouped with MAP65-1 and 2 (Zhang et al., 2025).

The observed discrepancies in subgrouping the MAP65 proteins may be due to the relatively small number of taxa included in each phylogenetic analysis. While all four analyses

TABLE 1 Comparison of MAP65 subgroups and MAP65 genes discussed in this mini-review.

A. Comparison of MAP65 subgroups			
5 subgroups			3 subgroups
Smertenko et al., 2008	Jia et al., 2024	Zhang et al., 2025	Liang et al., 2025
1. 65-1/2	I. 65-1/2	I. 65-1/2/8	a. 65-1/2/5/8
2. 65-5	III. 65-5	II. 65-5	
3. 65-6/7	IV. 65-6/7	V. 65-5/7	b. 65-6/7
4. 65-8	II. 65-8	III. 65-3/9	
5. 65-3	V. 65-3/4/9	IV. 65-4	c. 65-3-4-9
65-4/9 ungrouped			
B. MAP65 genes included in this mini review			
MAP65–1/2 subgroup			
<i>Arabidopsis thaliana</i>	MAP65-1, At5g55230. MAP65-2, At4g26760 *		
<i>Cucumis sativus</i> cv. Xintaimici	MAP65-1, CsaV3_3G004740		
<i>Glycine max</i> Williams 82	MAP1, Glyma.02g295100		
<i>Glycine max</i> Thorne	GmMAP65-1, Glyma.02g295100 *		
<i>Hordeum vulgare</i> Golden Promise	MAP65-1, HORVU.MOREX.r3.7HG0683940		
<i>Medicago truncatula</i> ecotype Jemalong A17	MAP65-1, Medtr5g093860		
<i>Solanum lycopersicum</i> cv. MicroTom and cv. Ailsa Craig	MAP65-1, Solyc07g064970 *		
MAP65–3 subgroup			
<i>Arabidopsis thaliana</i>	MAP65-3, At5g51600 *		
<i>Cucumis sativus</i> cv. Xintaimici	MAP65-2, CsaV3_3G016350 *		
	MAP65-3, CsaV3_5G005380 *		
<i>Medicago truncatula</i> ecotype Jemalong A17	MAP65-9, Medtr6g061690		
<i>Oryza sativa</i> Japonica cv. Nipponbare	MAP65-3.1, Os01g0685900 *		
	MAP65-3.2, Os05g0552900		
<i>Zea mays</i>	MAP65-3, Indeterminate gametophyte 2, Zm00001d011615 * (Homologue to ig2, Zm00001d043831)		
MAP65–5 subgroup			
<i>Arabidopsis thaliana</i>	MAP65-2, At 2g38720		
<i>Cucumis sativus</i>	MAP65-5, CsaV3_3G004740		
<i>Oryza sativa</i>	MAP65-5, Loc_Os05g33890		
MAP65–6/7 subgroup			
<i>Arabidopsis thaliana</i>	MAP65-6, At2g019010. MAP65-7, At1g014690		
<i>Hordeum vulgare</i> variety Golden Promise	MAP65-6, HORVU.MOREX.r3.4HG0337950		
<i>Phyllostachys edulis</i>	MAP65-18, PH02Gene23444.t3		

A: Three phylogenetic analyses of protein sequences identified five groups within the MAP65 family (Smertenko et al., 2008; Jia et al., 2024; Zhang et al., 2025), while another study recognized three groups (Liang et al., 2025). Subgroups are numbered or lettered according to the original publication defining the category. The nine *Arabidopsis thaliana* MAP65 genes are shown for comparison of subgroups. MAP65-1, At5g55230; 65-2, At4g26760; 65-3, At5g51600; 65-4, At3g60840; 65-5, At2g38720; 65-6, At2g01910; 65-7, At1g14690; 65-8, At1g14690; 65-9, At5g62250. B. List of MAP65 genes discussed in this paper organized into the subgroups proposed by Smertenko et al., 2008. Genes marked with asterisks (*) have associated mutant phenotypes summarized in this mini-review.

contained *Arabidopsis thaliana* and *Oryza sativa* sequences (Smertenko et al., 2008; Liang et al., 2023; Jia et al., 2024; Zhang et al., 2025), not all major groups of land plants was represented in the analyses. To date, the most thorough analysis which included sequences from seven complete genomes resolved five groups (*Dendrocalamus latiflorus*, *Guadua angustifolia*, *Phyllostachys edulis*, *Populus trichocapa*, *Raddia guianensis*) (Jia et al., 2024). Smertenko et al. (2008) included sequences from eight plant species, but many of those genomes were incomplete when the research was completed.

Further complicating cross-species comparisons, the numbering of MAP65 genes within a species appears inconsistent. For example, the *Cucumis sativus* gene named MAP65-2 is reported to be most like *AtMAP65-3*, not *AtMAP65-2* (Table 1). In *Medicago truncatula*, MAP65-9 is most closely related to *AtMAP65-3* (Gao et al., 2022). All MAP65 in *Glycine max* have been described as MAP65-1 (Zhang et al., 2025), or into subgroups (Kim et al., 2024).

Despite differences in the naming conventions, at least three phylogenetically distinct groups of MAP65 consistently appear (Table 1). A group containing *AtMAP65-1* and 2, a second group with *AtMAP65-6* and 7, and a third group with *AtMAP65-3*. A more complete phylogenetic analysis with multiple species across the plant kingdom will likely be needed to resolve the organization of the MAP65 family tree. This information will be valuable to functional genetic analyses by elucidating if and when gene duplications occurred. A clear understanding of the evolutionary history of the gene family could also help assess whether protein functions translate from one species to another.

The following sections are organized by MAP65 subgroup to emphasize similarities and differences between plant species and protein subgroups. To avoid the same subgroup number referring to different sequences (for example, according to Table 1, Group 3 could contain *AtMAP65-6* or *AtMAP65-5*), we use the five MAP65 subgroups originally proposed in Smertenko et al. (2008). Also, we refer to the subgroups based upon the founding *Arabidopsis thaliana* members (MAP65-1 or MAP65-3 subgroup).

MAP65-3 function in cytokinesis and development

Genetic studies in *Arabidopsis thaliana*, *Oryza sativa*, and *Zea mays* demonstrated that MAP65-3 proteins are needed for cytokinesis to occur properly throughout the plant life cycle (Figure 1) (Müller et al., 2004; Müller et al., 2004; Caillaud et al., 2008; Ho et al., 2011; Lin et al., 2022; Chettoor et al., 2023). In plants, cytokinesis requires a phragmoplast which is a dynamic microtubule array that guides vesicles to coalesce into a cell plate between daughter cells (Samuels et al., 1995; Smertenko, 2018). Phragmoplasts are composed of two equal sets of microtubules separated by the growing cell plate, and a few phragmoplast microtubules cross through perforations in cell plate to interdigitate with one other and connect the phragmoplast halves (Smertenko, 2018). *AtMAP65-3* was shown to crosslink the interdigitating, antiparallel microtubules at the phragmoplast

midline (Müller et al., 2004), and these *AtMAP65-3* microtubule bundles were necessary to maintain phragmoplast integrity throughout the cytokinesis process (Ho et al., 2011). Cytokinesis was often incomplete in *atmap65-3* mutants, and daughter cells were separated by unfinished cell wall stubs instead of complete cell walls (Müller et al., 2004; Caillaud et al., 2008; Ho et al., 2011). Loss of function mutations in *map65-3* in *Arabidopsis thaliana*, *Oryza sativa*, and *Zea mays* all showed disorganized phragmoplast arrays and growth defects related to incomplete cytokinesis (Ho et al., 2011; Lin et al., 2022; Chettoor et al., 2023).

While *Arabidopsis thaliana*, *Oryza sativa*, and *Zea mays map65-3* mutants show similar phenotypes at the cellular level, each plant species displayed different growth phenotypes (Figure 1). In *Oryza sativa*, *OsMAP65-3* appeared essential for vegetative growth as *map65-3.1* heterozygotes died as seedlings and never sexually reproduced (Lin et al., 2022). *Arabidopsis thaliana map65-3* plants progressed through the life cycle, produced viable seeds, but root growth was stunted (Mueller et al., 2002). *Zea mays map65-3* mutants were unable to form viable seeds because female gametophytes did not cellularize properly during development (Chettoor et al., 2023). These phenotypic disparities may be due to allelic differences, tissue specific gene expression, growth conditions, and/or other genes compensating for the loss of MAP65-3. As an example of possible genetic redundancy, only phragmoplasts in root cells were disrupted in *atmap65-3* mutants, which suggests another MAP65 gene functions in the shoot. However, while *Oryza sativa* encodes a second MAP65-3 gene (MAP65-3.2), it was minimally expressed, and knock-down alleles were indistinguishable from wild-type (Lin et al., 2022). *Zea mays* also encodes a second MAP65-3 gene that did not rescue the female gametophyte phenotype, but it may function in other parts of the plant body (Chettoor et al., 2023).

MAP65-3 function in pathogen infection

The role of MAP65-3 in phragmoplasts also impacted the susceptibility of *Arabidopsis thaliana* and *Cucumis sativa* to infection by the parasite *Meloidogyne incognita*, root knot nematode (Figure 1) (Caillaud et al., 2008; Liang et al., 2025). Upon infection with *Meloidogyne incognita*, plant roots form giant cells that support the parasite life cycle (Jones and Payne, 1978). Giant cells are multinucleate syncytia that undergo multiple rounds of mitosis without cytoplasmic division (Jones and Payne, 1978). While giant cells do not complete cytokinesis, they form short-lived phragmoplasts that build 'mini-cell plates' (Caillaud et al., 2008). *Arabidopsis thaliana* and *Cucumis sativus map65-3* mutants formed fewer giant cells and root galls when infected by *Meloidogyne incognita*, suggesting that MAP65-3 action was needed for phragmoplast integrity during infection and/or mini-cell plates formation (Caillaud et al., 2008; Liang et al., 2025). Furthermore, the expression of defense-related genes increased upon *Meloidogyne. incognita* infection in *Cucumis sativus map65-3* mutants (Liang et al., 2025). Interestingly, in *Cucumis sativus map65-3* mutants root and shoot growth appeared wildtype under

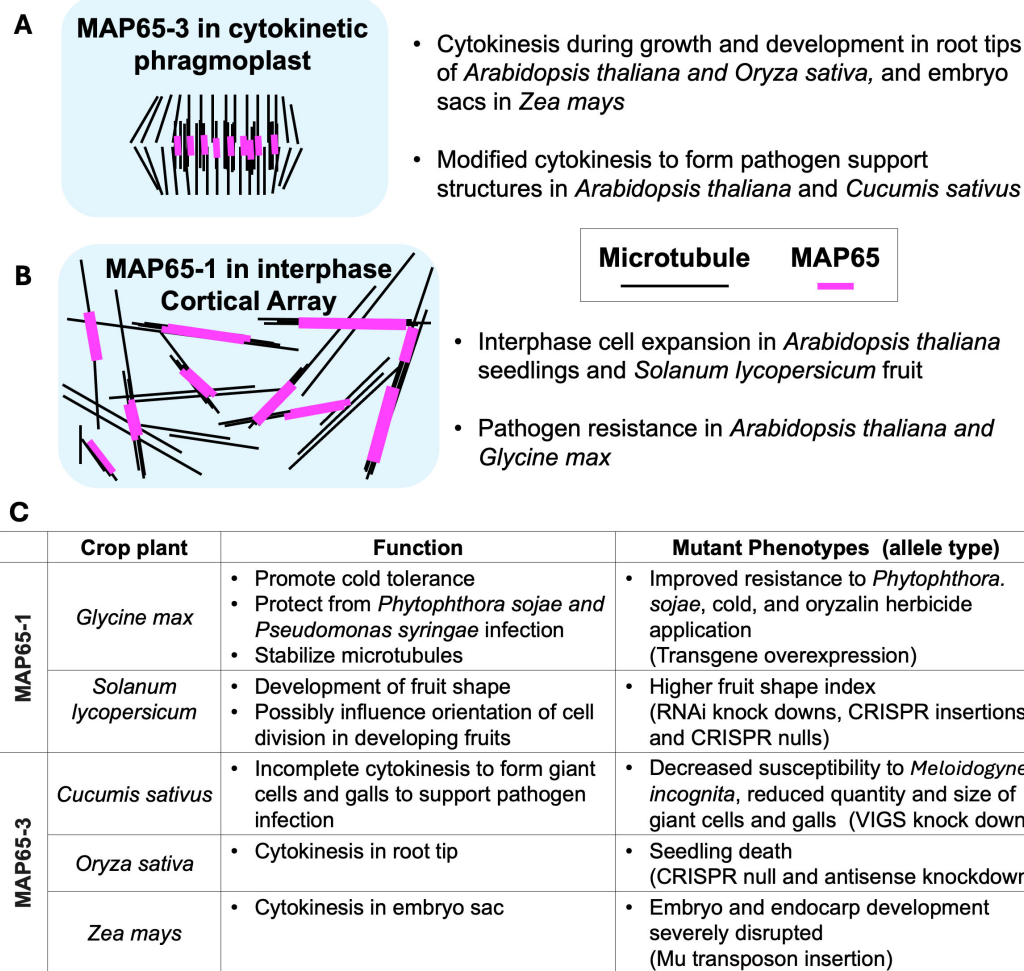


FIGURE 1 Summary of MAP65–1 and MAP65–3 functions and mutant phenotypes. **(A)** MAP65–3 (magenta bars) bundles microtubules (black lines) in phragmoplast midzones and is needed for proper cytokinesis in *Arabidopsis thaliana*, *Oryza sativa*, and *Zea mays*. MAP65–3 function also needed during a modified cytokinesis involved in giant cell formation when *Arabidopsis thaliana* and *Cucumis sativus* are infected with root knot nematodes. **(B)** MAP65–1 labels bundles in interphase microtubule arrays. MAP65–1 promotes elongation of *Arabidopsis thaliana* hypocotyls and seedling roots, and MAP65–1 may be involved in cell expansion or division plane orientation in *Solanum lycopersicum* fruits. Interphase MAP65–1 function appears to promote resistance to certain pathogens in *Arabidopsis thaliana* and *Glycine max*. **(C)** Summary of MAP65–1 and MAP65–3 mutant phenotypes and inferred functions.

normal growth conditions, however this may be due to genetic redundancy (Liang et al., 2025). Overall, with fewer giant cells and increased expression of defense-related genes, *Cucumis sativus map65-3* mutant plants were more resistant to *Meloidogyne incognita* (Liang et al., 2025). Similarly, *atmap65-3* mutants are more resistant to infections with filamentous pathogens *Hyaloperonospora arabidopsidis* and *Erysiphe cruciferarum* and display increased salicylic acid-dependent defense transcripts upon infection (Quentin et al., 2016).

Role of MAP65–1 and MAP65–3 during nodulation

While proteins in the MAP65–3 subgroup are often associated with mitotic spindles and phragmoplasts, research in *Medicago*

truncatula demonstrated that both MAP65–1 and MAP65–3 were expressed during symbiotic nodule formation which does not involve cell division (Gao et al., 2022). *Medicago truncatula* forms symbiotic nodules with nitrogen-fixing *Rhizobium rhizogene*, and pre-infection threads develop early in the nodulation process to allow the bacteria passage into the root (Tsyganova et al., 2021). Initiation of pre-infection threads involves cytoplasmic and microtubule reorganization (Fournier et al., 2008). A ‘mitotic module’ including *MtAurora kinase1*, *MtMAP65-1*, and *MtMAP65-3* was upregulated during pre-infection thread formation (Gao et al., 2022). *MtAurora kinase1*, *MtMAP65-1*, and *MtMAP65-3* localized microtubule bindles and endoplasmic reticulum surrounding the developing pre-infection thread (Gao et al., 2022). MAP65 proteins can be phosphorylated at multiple sites by kinases including mitotic aurora kinases and cyclin-dependent kinases, and phosphorylation negatively impacts the

ability of MAP65 proteins to crosslink microtubules (Smertenko et al., 2008; Boruc et al., 2017). The function of MAP65-1 and MAP65-3 and the relevance of MAP65 phosphorylation in *Medicago truncatula* nodulation has not yet been determined, but authors suggest these MAP65 proteins likely crosslink antiparallel microtubules, direct secretion, and/or influence the location of kinesin motor proteins along crosslinked microtubules (Gao et al., 2022).

MAP65-1 function in growth

Proteins in the MAP65-1 subgroup are active in both interphase and cytokinesis. In *Arabidopsis thaliana*, *atmap65-1* mutants exaggerate *atmap65-3* cytokinetic root phenotypes (Sasabe et al., 2011), but cytokinesis defects were not obvious in when a wildtype copy of MAP65-3 is present (Lucas and Shaw, 2008). In *Solanum lycopersicum*, *smap65-1* mutant tomato plants produced viable but misshapen fruits (Figure 1) (Bao et al., 2024; Zhang et al., 2025). Tissues within the misshapen tomatoes were slightly disorganized and individual cells displayed a wildtype morphology (Bao et al., 2024; Zhang et al., 2025). Authors suggested that *SIMAP65-1* may function in the division site formation, cell plate growth, and/or new cell wall maturation (Bao et al., 2024; Zhang et al., 2025). Further genetic and cellular analyses are needed to determine the function of MAP65-1 in tomato fruit development.

MAP65-1 overexpression in pathogen resistance

MAP65-1 was involved in the response to bacterial pathogen *Pseudomonas syringae* in both *Arabidopsis thaliana* and *Glycine max* (Figure 1) (Guo et al., 2016; Kim et al., 2024). Overexpression of *Arabidopsis thaliana* or *Glycine max* MAP65-1 coding sequences in soybean increased resistance to *Pseudomonas syringae* (Kim et al., 2024). Furthermore, *GmMAP65-1* overexpression in soybean also improved resistance to fungal pathogen *Phytophthora sojae*, cold temperature, and the herbicide oryzalin (Kim et al., 2024). Previous research demonstrated that *AtMAP65-1* was a downstream target of the *Pseudomonas syringae* effector protein HopE1 in *Arabidopsis thaliana* (Guo et al., 2016). Upon binding the bacterial HopE1, *AtMAP65-1* disassociated from interphase cortical microtubules, yet interphase microtubule arrays appeared unchanged, and secretion of defense-related cell wall polysaccharides stopped (Guo et al., 2016). These data indicated that MAP65-1 association with cortical microtubule may influence secretion.

Discussion

The plant microtubule cytoskeleton plays instrumental roles in cell growth, development, and environmental responses (Takemoto and Hardham, 2004; Lucas and Shaw, 2008; Meents et al., 2018; Gu

and Rasmussen, 2022). By studying MAP65 proteins in multiple plant species, our understanding of microtubule function has increased. MAP65-3 function in phragmoplast organization has now been demonstrated in three plant species, and MAP65-3 also impacts plant defense responses to some pathogens (Figure 1). MAP65-1 operates in a variety of capacities during interphase, from cell morphogenesis to plant immunity (Figure 1). Studies of MAP65 proteins in agricultural plants have highlighted the utility and limitations of applying knowledge from model systems to other organisms. Despite numerous relevant differences between research models and applied species, some functional conservation of MAP65-related processes exist. For instance, the regulation of MAP65-3 microtubule bundling by phosphorylation with aurora kinases may be conserved in *Arabidopsis thaliana* and *Medicago truncatula* (Gao et al., 2022; Deng et al., 2024). Future research on MAP65 proteins, including non-model organisms in multiple environmental conditions and development stages, will lead to a deeper comprehension of microtubule-related processes and potential avenues for crop development.

Conserved role of MAP65-3 in phragmoplasts

Molecular genetic research demonstrated similar cellular MAP65-3 functions in *Arabidopsis thaliana*, *Cucumis sativus*, *Oryza sativa*, and *Zea mays* (Müller et al., 2004; Ho et al., 2011; Lin et al., 2022; Chettoor et al., 2023; Liang et al., 2025). *map65-3* mutant analysis showed disconnected phragmoplast halves and incomplete cytokinesis, which revealed the importance of MAP65-3 to maintain phragmoplast integrity in *Arabidopsis thaliana*, *Oryza sativa*, and *Zea mays* (Müller et al., 2004; Ho et al., 2011; Lin et al., 2022; Chettoor et al., 2023). Also, MAP65-3 proteins displayed similar subcellular localization patterns across plant species. In both *Arabidopsis thaliana* and *Oryza sativa*, MAP65-3 proteins localized to the phragmoplast midzone (Ho et al., 2011; Lin et al., 2022), but the localization of *Cucumis sativus* and *Zea mays* MAP65-3 proteins have not yet been reported.

However, the phenotypes of *map65-3* mutant plants significantly differed among the four species despite similar cellular phenotypes. MAP65-3 was required for rice and corn development and survival. Rice plants missing one functional copy of *OsMAP65-3.1* died as seedlings (Lin et al., 2022), and *Zea mays map65-3* mutant female gametophytes were not fertile (Chettoor et al., 2023). However, MAP65-3 was dispensable in *Cucumis sativus* vegetative growth, and *csmap65-3* mutants were less susceptible to nematode infection (Liang et al., 2025). Phenotypic differences in *map65-3* mutant plants across species could be due to functionally redundant copies of MAP65 genes, as two MAP65-3 genes exist in *Cucumis sativus* (Liang et al., 2025). Overlapping functions of other MAP65 proteins may also impact the phenotypic severity of any *map65* mutation. For example, *map65-1* mutations exaggerate *map65-3* mutant phenotypes in *Arabidopsis thaliana* indicating the MAP65-1 and MAP65-3 both function in phragmoplasts (Sasabe et al., 2011; Ho et al., 2011).

Conservation of MAP65–3 protein function was demonstrated between *Oryza sativa* and *Arabidopsis thaliana* as both *OsMAP65–3.1* and *OsMAP65–3.2* coding sequences complemented *atmap65–3* when driven from the *AtMAP65–3* promoter (Lin et al., 2022). However, the utility of *OsMAP65–3.2* was unclear, as it did not display obvious mutant characteristics and it did not complement *osmap65–3.1* or *atmap65–3* mutants when expressed from its own promoter (Lin et al., 2022). Given the necessity of MAP65–3.1 in *Oryza sativa*, it is surprisingly that a functional *OsMAP65–3.2* promoter was not under positive selection.

Diverse functions of MAP65–1

While genetic studies have implicated MAP65–1 proteins in many processes (Lucas et al., 2011; Lucas and Shaw, 2008; Guo et al., 2016; Bao et al., 2024; Kim et al., 2024; Zhang et al., 2025), the mechanistic role of MAP65–1 remains vague in many cases. MAP65 proteins have often been considered organizers of the microtubule cytoskeleton by crosslinking and bundling microtubules within larger arrays (Ehrhardt and Shaw, 2006). However, interphase microtubule arrays appeared unaltered in *Arabidopsis thaliana map65–1/map65–2* double mutants that displayed reduced etiolated hypocotyl elongation (Lucas et al., 2011). Also, microtubule arrays appeared unaltered upon MAP65–1 disassociation from cortical microtubules in *Arabidopsis thaliana* challenged with *Pseudomonas syringae*, but cell wall secretion was inhibited (Guo et al., 2016). These data support a hypothesis that MAP65–1 proteins may be involved in secretion and/or less obvious array organizational states.

Further cellular and molecular genetic studies of MAP65–1 proteins will help elucidate their mechanism of action. The genetic interaction between *AtMAP65–1* and *AtMAP65–3* in root phragmoplasts could be interpreted as functional redundancy (Sasabe et al., 2011) or additive (Ho et al., 2011), in that MAP65–1 could be involved with secretion of cell plate materials rather than the MAP65–3 interdigitation of phragmoplast microtubules. Furthermore, in *Medicago truncatula* root cells forming pre-infection threads, it is unclear whether *MtMAP65–1* and *MtMAP65–3* both crosslink microtubule bundles, or if MAP65–1 functions separately (Gao et al., 2022). Currently, it is unknown how MAP65–1 proteins influence tomato development (Bao et al., 2024; Zhang et al., 2025). Transcriptomics and other studies suggest that MAP65–1 proteins affect the stability of microtubule polymers and perhaps buffer cells from osmotic, ionic, and temperature stresses (Zhang et al., 2012; Guo et al., 2016; Liang et al., 2023; Kim et al., 2024; Wu et al., 2024). Future research to investigate MAP65–1 in stressful environments could yield more tolerant crop varieties.

Future research directions

Currently, the MAP65–1 and MAP65–3 subgroups are the most studied. Transcriptomics and expression data that include other members of the MAP65 family provide direction for future investigations. The expression and localization changes in

MAP65–1, MAP65–5, MAP65–6 under salt and heavy metal stress indicate these proteins are involved in abiotic responses (Zhang et al., 2012; Zhou et al., 2017; Wu et al., 2024). The expression of MAP65–6/7 in moso bamboo fibers (Jia et al., 2024) and in *Arabidopsis thaliana* hypocotyl cells (Lucas and Shaw, 2023) suggest these proteins may be involved in secondary wall deposition, and further analyses are needed to deduce the function of these proteins.

A more thorough and complete phylogenetic analysis of the MAP65 family in photosynthetic organisms would help guide genetic research in multiple plants. Currently, discrepancies in sub-group categories do not illuminate the evolutionary relationships of the MAP65 family members. Some subgroups may reflect functional diversifications while other subgroups may be redundant or becoming pseudogenes (Lin et al., 2022). The MAP65 family expanded in land plants, while only one MAP65 gene is present in green algal genomes (Gardiner, 2013). As phragmoplasts and other plant specific microtubule arrays arose within the green alga (Buschmann and Zachgo, 2016), it would be useful to understand if MAP65 subgroups arose in accordance with the functioning of plant-specific microtubule arrays.

Potential agricultural applications

Significant limitations exist when translating genetic information from one plant species to another (Bevan et al., 2025; Roeder et al., 2025; Uauy et al., 2025). Comparative studies of MAP65 in multiple species indicate that some functions are conserved (Lin et al., 2022). Efforts to breed crops more tolerant to environmental stresses could be assisted by selecting for plants with increased MAP65–1 and decreased MAP65–3 subgroup expression. Increased expression of MAP65–1 proteins correlates with improved responses to cold, salt, and *Pseudomonas syringae* in *Arabidopsis thaliana*, *Cucumis sativus* and *Glycine max* plants (Liang et al., 2023; Kim et al., 2024). Decreased expression of MAP65–3 genes reduced root knot nematode infection in *Arabidopsis thaliana* and *Cucumis sativus* (Caillaud et al., 2008; Liang et al., 2025). However, caution must be exercised when applying broad generalizations of protein function across plant species (Bevan et al., 2025; Roeder et al., 2025; Uauy et al., 2025).

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