Brief Communication

Maize ZmRPH1 encodes a microtubule-associated protein that controls plant and ear height

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In grass crops, short stature is an ideal agronomic trait since it allows for better resistance to lodging and higher planting density. Dwarf and semi-dwarf mutants have been widely used in plant (Oryza sativa) and wheat (Triticum aestivum) breeding, and the related ‘Green Revolution’ genes, such as sd1 in rice and Rht in wheat, are usually involved in the gibberellin (GA) biosynthesis or signalling (Peng et al., 1999; Sasaki et al., 2002). However, many GA biosynthesis/signalling deficient maize (Zea mays) mutants have pleiotropic phenotypes that are detrimental to yields (Chen et al., 2014; Fujikawa et al., 1988; Lawit et al., 2010; Winkler and Helentjaris, 1995), therefore, are not applicable to maize breeding. Considering that maize plant height largely results from stem elongation driven by cell division and cell expansion within the internodes, identifying new regulators that directly regulate cell elongation may be a solution.

The microtubule plays an essential role in guiding plant cell polar growth, which is dependent on its organization and dynamics and regulated by a suite of diverse microtubule-associated proteins (MAPs) (Chen et al., 2016). However, MAPs in maize are largely unknown. Recently, several proteins of Arabidopsis QWRF family were identified as MAPs (Lee et al., 2017; Pignocchi et al., 2009). By searching the Gramene database, we revealed a gene with ID Zm00001d028073 that encoded a QWRF homolog protein in maize. We cloned the CDS of this gene into an overexpression vector, pBCXUN, driven by the maize UBI promoter, and introduced it into the maize inbred line B73-329 using Agrobacterium-mediated transformation. The resulting overexpressed lines (OE2 and OE4) had significantly shorter and wider mesocotyls (Figure 1a–c) with parenchyma cells showing reduced length but increased width compared to B73-329 (Figure 1d–f). We renamed this gene ZmRPH1 (Reducing Plant Height 1). Furthermore, ZmRPH1 overexpression also led to shorter roots with reduced cell length in maize seedlings (Figure 1g,h). These data suggest a general role for ZmRPH1 in the control of polar cell growth in maize.

To test whether ZmRPH1 is a novel MAP just like its Arabidopsis homologs, we performed an in vitro microtubule cosedimentation assay. We adopted the TNT quick coupled transcription/translation system (Promega, Madison, WI, USA) to express the ZmRPH1 protein. Biotinylated-lysine-labelled ZmRPH1 protein cosedimented with prepolymerized microtubules in the pellet after a high-speed centrifugation, indicating its direct association with microtubules in vitro (Figure 1i). We then fused ZmRPH1 to green fluorescent protein (GFP) and transiently expressed it into maize protoplasts. We observed filament-like structures that colocalized with mCherry-tubulin-labelled microtubules (Figure 1j). Thus, we conclude that ZmRPH1 is an MAP in maize.

Next, we investigated the function of ZmRPH1 in microtubules. It is well established that in fast elongating cells cortical microtubules organize into a transverse parallel array to guide microfibrillar deposition in the cell wall. When cell growth slows or ceases, cortical microtubules reorganize into randomly or longitudinally oriented arrays (Hamada, 2014). We surveyed root epidermal cells in the elongation zone, within which the microtubules were easily visualized by immunofluorescence microscopy using an anti-a-tubulin antibody. In 2-day-old seedlings, we observed transversely oriented microtubules in 93% of the B73-329 root epidermal cells, while <10% of OE2 or OE4 cells had transverse microtubules. By contrast, in most OE2 or OE4 cells microtubules were obliquely or randomly oriented (Figure 1k,l). We further overexpressed ZmRPH1 in Arabidopsis, and the resulting overexpressing lines (OX1 and OX2) had shorter etiolated hypocotyls compared with the control. By crossing OX1 and OX2 with a transgenic line expressing GFP-MBD, we observed GFP-MBD-labelled microtubules in live cells. We found that ZmRPH1 overexpression significantly reduced the frequency of cells with transverse microtubules in OX1 and OX2 hypocotyls (approximately 30%) compared to the control (>60%; Figure 1m, n). Overall, the above results indicated that ZmRPH1 modulates cell elongation by regulating the cortical microtubule orientation.

We then measured plant height and the length of the sixth internode in maize plants grown in a greenhouse. The ZmRPH1 overexpressing lines (OE2 and OE4) all resulted in shorter plant heights and reduced internode lengths (Figure 1o–q). Reduced internode epidermal and parenchyma cell lengths were also revealed in OE2 and OE4 compared with the control, B73-329 (Figure 1r,s), indicating that ZmRPH1 overexpression reduced internode cell elongation and affected maize plant height. However, it remains an interesting question whether ZmRPH1, as a MAP, can also regulate mitotic microtubule organization and affect cell division. Next, we evaluated various growth-related traits of OE2 and OE4 plants in field trials at multiple sites across different years. In addition to the ZmRPH1 OE inbred lines, we also used the T13 inbred line as the female tester to make hybrids with OE2 and OE4, and the resulting hybrid lines were named #OE5. We found that both plant and ear heights were significantly reduced in
all tested ZmRPH1 overexpressing inbred and hybrid lines compared with corresponding controls (Figure 11,u). Additionally, the number of internodes did not differ between transgenic lines and controls. However, average internode lengths were significantly shorter in ZmRPH1 overexpressing plants (Figure 11u). Moreover, the lodging rate of lines #OE2 and #OE4 was significantly lower compared with the control (Figure 11v). Thus, ZmRPH1 overexpression reduced plant and ear height and enhanced the lodging resistance of plants, which could be a primary precondition for efforts to achieve higher yielding maize.

Plant height is highly related to maize yield. Therefore, we explored whether ZmRPH1 overexpression would affect maize yield and found that ZmRPH1 overexpression had no obvious impact on flowering time and fertility (Figure 1w). We then measured the ear weight per plant and grain yield per hybrid line plot in different years and found no significant difference between ZmRPH1 overexpression lines and the control in most cases (Figure 1x).

In summary, we identified ZmRPH1 as a novel MAP that regulates cell elongation in maize. We demonstrated that ZmRPH1 overexpression lowered plant and ear heights by reducing the length of all internodes and increased lodging resistance, without significantly reducing maize yield. This study also proved that modulation of microtubules to control cell elongation may be a potential strategy in breeding cultivars for compact planting, and genes for MAPs in maize including...
ZmRPH1 could serve as new plant height genes with practical potential in creating maize of ideal plant type.

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Author contributions

W.L., J-S. L., X-Q. W., and Y.F. contributed to project design. W.L., F-H. G., Z-Q. Q., L.Z., S-S. Z., and L-M. C. performed the experiments and data analysis. W.L. and Y.F. wrote the manuscript. Y.F., J-S.L. and X-Q.W. revised the article.

Conflict of interest

No conflict of interests to declare.

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