DWT1/DWL2 act together with OsPIP5K1 to regulate plant uniform growth in rice

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Abstract
- Uniform growth of the main shoot and tillers significantly influences rice plant architecture and grain yield. The WUSCHEL-related homeobox transcription factor DWT1 is a key regulator of this important agronomic trait, disruption of which causes enhanced main shoot dominance and tiller dwarfism by an unknown mechanism.
- Here, we have used yeast-two-hybrid screening to identify OsPIP5K1, a member of the rice phosphatidylinositol-4-phosphate 5-kinase family, as a protein that interacts with DWT1. Cytological analyses confirmed that DWT1 induces accumulation of OsPIP5K1 and its product PI(4,5)P2, a phosphoinositide secondary messenger, in nuclear bodies.
- Mutation of OsPIP5K1 compounds the dwarf dwt1 phenotype but abolishes the main shoot dominance. Conversely, overexpression of OsPIP5K1 partially rescues dwt1 developmental defects. Furthermore, we showed that DWL2, the homologue of DWT1, is also able to interact with OsPIP5K1 and shares partial functional redundancy with DWT1 in controlling rice uniformity.
- Overall, our data suggest that nuclear-localised OsPIP5K1 acts with DWT1 and/or DWL2 to coordinate the uniform growth of rice shoots, likely to be through nuclear phosphoinositide signals, and provides insights into the regulation of rice uniformity via a largely unexplored plant nuclear signalling pathway.

Introduction
Plant architecture is one of the most important agronomic traits that determines the grain yield of rice (Wang & Li, 2005; Wang & Li, 2008). Most wild grasses have a dominant main shoot and weaker branches (which are named tillers in grass). Two main branching patterns have been selected during the domestication of cereal crops. Some crops, such as maize and sorghum, exhibit enhanced apical dominance and suppression of branches compared to their highly branched ancestors (Harlan, 1992). Conversely, other cultivated crops, including rice, wheat and barley, have been selected to develop multiple tiller shoots that bearing panicles of similar size as the main shoot at maturity (Harlan, 1992). The uniform growth of the main shoot and tillers, including the culm (stem) length and panicle size, is critical as it determines not only productive panicle number and grain yield, but also ensures synchronised maturation and a uniform panicle layer, which facilitate harvesting (Ma et al., 2009).

The mechanisms directing plant uniformity remain largely unknown, with only one gene involved in tiller growth identified to date. DWARF TILLER1 (DWT1)/WUSCHEL RELATED HOMEOBOX (WOX) 9A has been shown to function as a key regulator coordinating main shoot and tiller growth (Wang et al., 2014). DWT1 is preferentially expressed in panicle meristems, at higher levels in tillers than in the main shoot. Consistent with its expression pattern, DWT1 disruption leads to enhanced main shoot dominance; dwt1 mutant plants develop main shoots with normal height and larger panicles, but dwarf tillers that bear smaller panicles (Wang et al., 2014). Two paralogues of DWT1, DWL1 and DWL2 display very similar expression pattern, whose functions are currently unknown (Wang et al., 2014). WOX family proteins belong to the plant homeobox transcription factor superfamily, characterised by the presence of a DNA-binding homeodomain. The WOX proteins are divided into three clades (van der Graaff et al., 2009). The WUS clade, or modern clade, is specific to seed plants, and contains the founding member WUS and WOX1-7 in Arabidopsis. The intermediate clade exists in vascular plants and is further separated into WOX8/9 and WOX11/12 subgroups (Lian et al., 2014). WOX11 and WOX12 are mainly involved in root development (Liu et al., 2014; Kong et al., 2016), while homologues of WOX8/9, including DWT1, have diverse functions in different species (Wu et al., 2005; Palovaara et al., 2010; Zhou et al., 2018). In Arabidopsis, AtWOX8/STIMPY LIKE (STPL) and AtWOX9/STIMPY (STIP) are both required for embryogenesis and maintenance of vegetative SAM, but not for inflorescence development and architecture (Wu et al., 2005; Wu et al., 2007; Breuninger et al., 2008). By contrast, EVERGREEN (EVG) in petunia and COMPOUND
INFLORESCENCE (S) in tobacco are essential for inflorescence development and architecture (Lippman et al., 2008; Rebocho et al., 2008). The ancient clade is the most conserved, including WOX13 and WOX14, which have been reported to function in root and flower development in Arabidopsis (Deveaux et al., 2008).

Functional diversification and specificity of WOX proteins are partly determined by sequence variations, outside the characteristic homeodomain, that confer the ability to interact with other proteins. The three clades possess distinct conserved motifs at their C-termini (Deveaux et al., 2008). Most members of the modern clade encode a WUS domain, which can interact with TOLESS (TPL) type co-repressors to inhibit gene expression (Causier et al., 2012; Dolzblasz et al., 2016). In Arabidopsis, WOX5 interacts with TPL and a histone deacetylase to inhibit CDF4 transcription, therefore suppressing differentiation of root columella stem cells (Pi et al., 2015) while, in Medicago truncatula, WUS/STF recruits TPL to repress AS2 during leaf blade development (F. Zhang et al., 2014). The repressive EAR domain present in some WUS clade members can also mediate interaction with TPL (Szemenyei et al., 2008), and other conserved C-terminal sequences mediate interaction with transcription cofactors HAIRY MERISTEMS (HAMS) to regulate the maintenance of diverse stem cell niches (Zhou et al., 2015). The absence of the WUS box and EAR domain in the intermediate and ancient clade members suggests that these proteins may recruit other partners to regulate gene expression (Lin et al., 2013). In rice, WOX11 has been shown to interact with the histone acetyltransferase module ADA1-GCN5 to activate multiple target genes required for crown root meristem proliferation (Zhou et al., 2017), or with H3K27me3 demethylase to target gene expression in the shoot apex (Cheng et al., 2018). However, potential partners of other WOX proteins in the intermediate and ancient clade remain undetermined.

Nuclear proteins interacting with phosphoinositides (PIs) or their kinases have been implicated in the regulation of gene transcription, mRNA maturation, and chromatin remodeling (Shah et al., 2013). PIs are membrane phospholipids derived by multiple phosphorylation steps from glycerophospholipid phosphatidylinositol (PtdIns) and also can be dephosphorylated by phosphatases (Gehrt et al., 2017b). A doubly phosphorylated derivative, phosphatidylinositol 4,5-bisphosphate (PI(4,5)P2), occupies a central position in phosphoinositide signalling, acting as a critical secondary messenger or a precursor for further messengers. PI(4,5)P2 is generated by two phosphatidylinositol phosphate (PIP) kinases: PIP5Ks (PIP 5-kinases) use PI4P (phosphatidylinositol 4-phosphate) as a substrate to produce PI (4,5)P2, while PIP4Ks use PI5P (Doughman et al., 2003; van den Bout & Diceva, 2009). Because the intracellular levels of PI4P are much higher than those of PI5P (Meijer et al., 2001; Meijer & Munnik, 2003), it is widely believed that PIP5Ks contribute to the majority of cellular PI(4,5)P2 generation.

In plants, PIP5K isoforms are separated into two subfamilies based on the presence or absence of N-terminal MORN (membrane occupation and recognition nexus) domains upstream of the C-terminal kinase domain (Mueller-Roeber and Pical, 2002). In Arabidopsis, 9 of 11 PIP5K isoforms (subfamily B) possess repeated MORN domains and a highly variable linker domain, while PIP5K10 and PIP5K11 (subfamily A) have no MORN domains (Heilmann & Heilmann, 2015). In animals they were first identified in junctophilins (Takeshima et al., 2000), and later were found in other functionally different proteins. In plants, MORN domains only have been identified in PIP5Ks, having roles in the protein subcellular localisation and phospholipid binding (Ma et al., 2006). Arabidopsis PIP5Ks have been shown to regulate a multitude of cellular activities, such as membrane trafficking, clathrin-mediated endocytosis, exocytosis and actin dynamics (Ischebeck et al., 2008; Kusano et al., 2008; Sousa et al., 2008; Zhao et al., 2010; Mei et al., 2012; Tejos et al., 2014; Ugalde et al., 2016). In rice, only OsPIPK1 has been characterised and reported to negatively regulate rice flowering (Ma et al., 2004), but no information is available on the biological functions of other rice PIP5Ks.

PI(4,5)P2 is unevenly distributed in the cell, and has been observed predominantly at the plasma membrane as well as in the cytosol and nucleus of plant cells (Simón et al., 2014; Tejos et al., 2014; van Leeuwen et al., 2007). Similarly, PIP5Ks reside in the plasma membrane, in intracellular vesicles and in the nucleus (Heilmann, 2016). A recent study reports that the N-terminus of Arabidopsis PIP5K2 contains nuclear localisation sequences that drive active import of the protein into the nucleus upon interaction with selected alpha-importin isoforms (Geth et al., 2017a). Although both PI(4,5)P2 and PIP5Ks are present within the nucleus, the biological relevance of this subcellular localisation in plants is largely unknown; currently very limited information suggests the effects of altered PI(4,5)P2 contents on plant nuclear function (Dieck et al., 2012), most previous reports focus on PI signalling occurring at the plasma membrane and cytoplasmic membranes (reviewed by Geth et al., 2017b). However, in yeast and animals, emerging evidence indicates that the nuclear PI(4,5)P2 and other PIs modulate cellular events independent of their cytosolic counterparts, often via interaction with other nuclear proteins (Shah et al., 2013). One well known example is mammalian Star-PAP, a noncanonical poly(A) polymerase whose activity is highly stimulated by PI(4,5)P2 produced after Star-PAP interaction with a PIP5K in nuclear speckles (Mellman et al., 2008). Another example is ABSENT, SMALL, OR HOMEOPTIC DISCS 2 (ASH2), a trithorax group (trxG) protein in Drosophila melanogaster, disruption of ASH2 interaction with a nuclear PIP5K results in a dramatically increased histone H1 hyperphosphorylation, suggesting a role of PI(4,5)P2 in maintaining transcriptionally active chromatin (Cheng & Shearn, 2004). Whether the PI signalling pathway has similar, important regulatory roles in plant nuclei remains to be clarified.

Here, we show that OsPIPK5 interacts with DWT1 in the nucleus to co-ordinately regulate the uniform growth of rice plants. OsPIPK5 interacts both at the plasma membrane and in the nucleus. DWT1 is not required for the nuclear localisation of OsPIPK5, but its presence induces the accumulation of OsPIPK5 in nuclear bodies. Mutations of OsPIPK5 enhance the dwarfism phenotype of dwt1, while overexpression of OsPIPK5 partially rescues dwt1 developmental defects.
Furthermore, we show that DWL2, the homologue of DWT1, is also able to interact with OsPIP5K1 and has partially redundant function with DWT1 in coordinating main shoot and tiller growth. Our results reveal the potential involvement of the PI signalling pathway to regulate plant architecture and uniform shoot growth.

Materials and Methods

Plant materials and growth conditions

Rice (Oryza sativa cultivar 9522, also known as WUYUNJING 7) plants used in this study were grown in the paddy field of Shanghai Jiao Tong University under the natural long day condition from May to September. The dwt1 mutant was described by Wang et al. (2014). ospip5k1 single mutants, dwt1ospip5k1 and dwt1dwt2 double mutants were obtained using CRISPR/Cas9 technology (H. Zhang et al., 2014), using sgRNA-Cas9 plant expression vectors kindly provided by Professor Jiankang Zhu. To construct the 35S::OsPIP5K1 plant overexpression vector, the full-length cDNA of OsPIP5K1 was amplified by RT-PCR and inserted into the overexpression vector PHB (Gao et al., 2010). Plant expression vectors were transformed into Agrobacterium tumefaciens (EHA105) cells, which was used to infect rice calli. Transgenic plants were confirmed by PCR detection. Primers for constructing sgRNA vectors and the OsPIP5K1 overexpression vector are listed in Supporting Information Table S1. Mature rice plants grown 90 d after transplanting were used for phenotyping.

Tobacco (Nicotiana benthamiana) plants were grown in the glasshouse at 22°C, with a 16 h : 8 h, light : dark cycle.

Analysis of protein–protein interactions

The rice cDNA library for Y2H experiments was constructed by OE BioTech (Shanghai, China) by cloning cDNA synthesised from the mRNAs of young panicle meristems (<5 mm) into the prey vector pGADT7 (TaKaRa, Shiga, Japan). Full-length and truncated cDNAs of DWT1 and OsPIP5K1 were amplified by PCR and cloned into pGADT7 and pGBK7 vectors (TaKaRa), respectively. Y2H assays were performed according to protocols for the Matchmaker Two-Hybrid System (TaKaRa), using yeast strain AH109. Selection was performed in SD/-Leu/-Trp/-His/-Ade selection medium. To generate constructs for the bimolecular fluorescence complementation (BiFC) assay, DWT1 cDNA was cloned into the pSAT1-cEYFP-C1 vector, and OsPIP5K1 cDNA was cloned into the pSAT1-cEYFP-N1 vector. The BiFC assay was performed as previously described (He et al., 2016). The primers used for constructing Y2H and BiFC vectors are listed in Table S1.

Co-immunoprecipitation (Co-IP) analysis was performed with protein extracts from 3-wk-old tobacco leaf as described by Hu et al. (2019). To create the HA-tagged DWT1 and YFP-tagged OsPIP5K1 for transient expression, the full-length coding regions of these two genes were amplified by PCR using the primers listed in Table S1 and cloned to PGREEN-HA (kindly provided by H. Yu, National University of Singapore) and PHB-YFP vector (Xu et al., 2019), respectively. The fusion proteins DWT1-HA and OsPIP5K1-YFP were transiently expressed in tobacco leaves. Leaves were collected 48 h after co-infiltration. Proteins were extracted with ice-cold buffer (50 mM Tris-HCl, pH 7.5, 100 mM NaCl, 1 mM EDTA, 10 mM NaF, 5 mM Na3VO4, 0.25% NP-40, 1 mM PMSF, 1× protease inhibitor cocktail (Roche)), and centrifuged at 15 000 g for 10 min at 4°C. The supernatant was incubated with 25 µl green fluorescent protein (GFP)-Trap MA beads (chromotek) for 2 h at 4°C, then the beads were washed three times with wash buffer (50 mM Tris-HCl, pH 7.5, 100 mM NaCl, 1 mM EDTA, 1 mM PMSF, 1× protease inhibitor cocktail (Roche)). Proteins were eluted by boiling the beads in 5× SDS loading buffer, then separated on an SDS-PAGE gel and subjected to immunoblotting using an anti-HA antibody (Abmart, Shanghai, China) and an anti-GFP antibody (Sigma).

Transient expression in tobacco leaves

DWT1 cDNA was cloned in frame downstream of the CFP reporter gene in the CFP-PHB vector (Xu et al., 2019) to generate the transient expression vector. The sequences encoding full-length or truncated OsPIP5K1 were cloned in frame upstream of the YFP reporter gene in the YFP-PHB vector (Xu et al., 2019). PI biosensor markers were obtained from Yvon Jaillais. Plasmids were transfected into Agrobacterium strain GV3101, then the cultured bacteria (OD600 = 0.6) were infiltrated into young leaves of 1-month-old tobacco plants. Fluorescence was observed 36–48 h after infiltration. Fluorescence signals in tobacco epidermal cells were visualised and recorded using a Leica TCS SP5 confocal microscope according to the manufacturer’s instructions. CFP, YFP and RFP were excited at 453 nm, 514 nm and 543 nm, respectively, and emissions were observed at 465–505 nm, 525–600 nm, 609–630 nm, respectively.

Phylogenetic analysis

The full-length amino acid sequences of all annotated PIP5K proteins were downloaded from National Center for Biotechnology Information (NCBI, https://www.ncbi.nlm.nih.gov/) and aligned using CLUSTALX (Larkin et al., 2007) with the default settings, that were used to construct a neighbour-joining tree using MEGA 5 (Tamura et al., 2011), with parameters as following: Poisson correction, pairwise deletion and 1000 bootstrap replicates.

Quantitative RT-PCR analysis

Total RNA from rice tissues was isolated with Trizol reagent (Invitrogen), according to the manufacturer’s manual. Reverse transcription reaction was carried out using a PrimeScript RT reagent kit with gDNA Eraser (TaKaRa), according to the manufacturer’s instructions. qPCR was performed using SYBR Green SuperReal PreMix Plus (Tiangen, Beijing, China) on a CFX96 Real-Time PCR machine (Bio-Rad). Each gene was assayed on three biological cDNA replicates, each with three technical replicates. The expression levels were quantified using the 2–ΔΔCt method.
repeats. Rice actin gene was used as an internal control. The primers are listed in Table S1.

Thin section microscopy

Rice inflorescences at branch meristem and spikelet meristem stages were dissected and embedded in 4% agarose blocks, and materials were longitudinally cut into 60 μm slices in thickness with Leica Vibratome VT1000S. Samples for imaging were made following Yang et al. (Yang et al., 2017). GFP was excited at 488 nm, and emission was observed at 505–525 nm.

Results

DWT1 interacts with OsPIP5K1 in the nucleus

To identify proteins that may interact with DWT1 to control rice plant architecture, a yeast two-hybrid (Y2H) assay was used to screen a rice panicle meristem cDNA library. We identified one candidate protein that belongs to the rice PIP5K family. This protein, OsPIP5K1, possesses a conserved kinase domain (lipid kinase domain) at the C-terminus and seven MORN motifs at N-terminus (Fig. 1a). Searching the rice genome for homologues, we found 10 PIP5K family proteins, seven of which possess N-terminal MORN motifs (Table S2). These proteins share overall domain organisation with Arabidopsis PIP5K proteins where OsPIP5K1 shows highest similarity to AtPIP5K1 and AtPIP5K2 (Fig. S1).

Y2H results indicated that the C-terminal region of DWT1 downstream of the homeobox domain, between aa 256 and 533, mediated its interaction with OsPIP5K1 (Fig. 1b,c). This result revealed the importance of C-terminal region for DWT1 function, which is consistent with the previous report that dwt1 mutation that causes a frame shift and premature stop at aa 254 produced a nonfunctional protein (Wang et al., 2014). Other truncated regions containing only the N-terminus of the protein (DWT1-N1, DWT1-N2; Fig 1b), were not able to interact with OsPIP5K1. Deletion analysis revealed that the N-terminal MORN motifs of OsPIP5K1 were required and sufficient for interaction with DWT1 (Fig. 1c). The interaction between DWT1 and OsPIP5K1 was validated by BiFC assay in rice protoplasts (Fig. 1d). The result of Co-IP analysis showed that HA-tagged DWT1 co-immunoprecipitated with YFP-tagged OsPIP5K1 (Fig. 1e), further confirmed the interaction between DWT1 and OsPIP5K1.

![Fig. 1 DWT1 interacts with OsPIP5K1.](image)
To investigate the association of DWT1 and OsPIP5K1 in planta, we examined their subcellular localisation by transient expression in tobacco (Nicotiana benthamiana) leaves. Consistent with our previous reports (Wang et al., 2014), DWT1 localised in the nucleus, specifically in the nuclear bodies (Figs 1f, S2). OsPIP5K1 mainly localised to the plasma membrane and in the nucleus, where it was distributed evenly (Fig. 1f). However, when co-expressed in tobacco cells, colocalisation of CFP-DWT1 and OsPIP5K1-YFP was observed in the nucleus, enriched in the nuclear bodies (Fig. 1f5–f10). CFP-DWT1 could not induce the enrichment of native YFP signal in nuclear bodies (Fig. S2), suggesting that DWT1 interacts with OsPIP5K1 in vivo, changing the localisation of PIP5K1 within the nucleus to concentrate within nuclear bodies.

MORN motifs of OsPIP5K1 are responsible for its nuclear localisation

To determine which domain targets OsPIP5K1 to the nucleus where it interacts with DWT1, we examined the localisation of truncated OsPIP5K1 variants fused to enhanced GFP in tobacco cells (Fig. 2). The N-terminus of OsPIP5K1 containing seven MORN motifs (OsPIP5K1-N, Fig 1a) was mainly localised to the nucleus, though weak fluorescence signals could also be detected at the plasma membrane (Fig. 2a,e). When co-transformed with CFP-DWT1, OsPIP5K1-N co-localised with DWT1 in the nuclear bodies (Fig. 2b–d,f–h), similar to the behaviour of the full-length protein. Conversely, the C-terminus of OsPIP5K1 containing the catalytic kinase domain (OsPIP5K1-C, Fig 1a) exclusively localised to the plasma membrane (Fig. 2i,m), and co-expression with DWT1 did not change its subcellular localisation (Fig. 2j–l,n–p). These results suggest that the N-terminal MORN motifs of OsPIP5K1 are not only required for the interaction with DWT1, but also essential for its nuclear localisation.

Nuclear PI(4,5)P2 associates with DWT1

It has been suggested that PI(4,5)P2 co-localises with its synthetic enzymes and is channeled to downstream targets via protein–
protein interactions (Heilmann and Heilmann, 2013). To test whether DWT1 co-localises with PI(4,5)P2, we monitored PI(4,5)P2 distribution in tobacco cells using biosensor markers P15Y and P15R under the control of Arabidopsis UBIQUITIN10 promoter. P15Y/R were generated by fusing YFP or RFP, respectively, to the C-terminal domain of the Tubby protein, which specifically binds PI(4,5)P2 (Simon et al., 2014). PI(4,5)P2 was predominantly localised in the plasma membrane and the nucleus, and also distributed in some dots and net-shaped intracellular structures (Fig. 3a), suggesting that PI(4,5)P2 accumulates in a similar pattern to OsPIP5K1. Accordingly, we were able to detect the co-localisation of OsPIP5K1 and PI(4,5)P2 (Fig. S3). When P15Y was co-transformed with CFP-DWT1, an obvious colocalisation of PI(4,5)P2 and DWT1 could be observed in the nucleus, especially in nuclear bodies (Fig. 3b–d, f–h) suggesting that the PI(4,5)P2 is potentially involved in DWT1 functioning in these areas. In addition, similar to PI(4,5)P2, PI4P is also co-localised with DWT1 in nuclear bodies (Fig. S5a–h). As a control, CFP is not able to induce PI(4,5)P2 and PI4P accumulating in nuclear bodies (Figs S4, S5i–n).

Disruption of OsPIP5K1 enhances dwt1 phenotypes

To elucidate the role of OsPIP5K1 in rice plant growth, we first investigated the spatial-temporal patterns of gene expression and protein accumulation. qRT-PCR analysis showed that OsPIP5K1 was highly expressed in young panicles of both the main shoot and tillers (Fig. S6a). Lower expression of OsPIP5K1 was also detected in leaves, roots and culms (Fig. S6a). Protein localisation was observed by fusing the genomic fragment of OsPIP5K1 containing promoter and coding region with the enhanced GFP reporter gene (pOsPIP5K1::OsPIP5K1gDNA-eGFP) and transforming the construct into rice plants. In transgenic plants, fluorescence signals were observed in branch meristems, spikelet meristems, leaf primordia and stem vasculature (Fig. S6b–d). OsPIP5K1-eGFP localised predominantly in the nucleus and the plasma membrane, largely overlapping the region where DWT1 localised (Wang et al., 2014), and providing further support for in vivo interactions between the two proteins.

The genetic relationship between DWT1 and OsPIP5K1 was analysed using CRISPR-Cas9 technology to knockout OsPIP5K1 in wild-type and dwt1 plants. Two mutant alleles were obtained in the wild-type background: ospip5k1-1 contained a single base pair insertion, while ospip5k1-2 contained a four base pair deletion, both in the first exon of OsPIP5K1, which caused a frame shift and premature transnational termination (Fig. S7). At maturity, both mutant lines exhibited mild dwarfism compared to wild-type (Fig. 4a,b). Measurement of internode length indicated that the first, second and third internodes beneath the panicle became much shorter in ospip5k1 mutants, suggesting that OsPIP5K1 activity is required for culm elongation (Fig. 4c,d).

Three mutant ospip5k1 alleles were created in the dwt1 background (ospip5k1-1, ospip5k1-3 and ospip5k1-4), one of which was the same as in the wild-type background. All three mutations resulted in premature termination early in the OsPIP5K1 protein (Fig. S7). Noticeably, all three double mutant lines displayed enhanced dwarfism (Fig. 5). While dwt1 plants exhibit a main shoot of normal height and dwarf tillers, the main shoot of double mutants became much shorter and could not be distinguished from tillers, indicating that the apical dominance of dwt1 main shoots was abolished by an additional mutation in OsPIP5K1 (Fig. 5a,b). In dwt1, about 10% of tillers displayed a normal height, while no more than 5% of tillers in double mutants had no unaffected internodes (Figs 5c,d, S8). Furthermore, up to 30% of tiller culms exhibited defective elongation at all internodes, a phenotype that was not observed in the dwt1 single mutant (Figs 5d, S8). Compared with wild-type, dwt1 produced a larger panicle on the main shoot and smaller ones on tillers (Wang et al., 2014). However, the size of both the main shoot and tiller panicles was substantially decreased in double mutants (Fig. S9a,b), suggesting that disruption of OsPIP5K1 not only enhanced defects in culm elongation but also affected panicle development.

Fig. 3 DWT1 induces the accumulation of PI(4,5)P2 in nuclear bodies. (a, e) PI(4,5)P2 is broadly distributed in the plasma membrane and nucleus of tobacco leaf cells; (e) is one representative magnified nucleus in (a). (b, d, f–h) PI(4,5)P2 was enriched in nuclear bodies and co-localised with DWT1, with one representative nucleus shown (f–h). Bars: (a–e) 20 μm; (f–h) 5 μm.
Overexpression of OsPIP5K1 partially rescues dwt1 developmental defects

The pleiotropic effects and genetic functions of OsPIP5K1 were further examined by overexpression the gene in wild-type and dwt1 plants under the control of double cauliflower mosaic virus 35S promoter (Gao et al., 2010). Wild-type plants overexpressing OsPIP5K1 did not show obvious developmental changes (Fig. S10a,b). By contrast, a comparably higher level of OsPIP5K1 expression partially restored the developmental defects in dwt1 plants (Figs 6a,b, S10c). In transgenic plants, the proportion of normal tillers increased from c. 10% in dwt1 to c. 20%; that of tillers having only the second internode un-elongated increased from c. 35% in dwt1 to c. 60%; while that of tillers with two or more un-elongated internodes decreased from c. 55% in dwt1 to c. 20% (Fig. 6c,d).

Our previous study indicated that although c. 40% of dwt1 main shoots had one or two un-elongated internodes, a compensatory elongation of other internodes retained the normal height of the main shoot, which usually occurred in the first internode (Wang et al., 2014). By contrast, compensatory elongation was not observed in dwt1 tillers resulting in the dwarf tiller phenotype. Overexpression of OsPIP5K1 had distinct effects on compensatory growth in the main shoot and tillers. The average length of the first internode on dwt1/OsPIP5K1-OE main shoots was comparable with that of wild-type and shorter than that of dwt1 main shoots, which may be attributed to fewer non-elongated internodes (Fig. 6e). However, the first internode of dwt1/OsPIP5K1-OE tillers was significantly longer than that of dwt1 tillers (Fig. 6e), indicating that compensatory elongation occurred in tillers of overexpression lines.

We further analysed the impacts of OsPIP5K1 overexpression on the panicle size in transgenic plants. The main shoot (MS) panicle and tiller panicles were of comparable size, and morphologically fairly similar to those of wild-type plants (Fig. S9a,c). Panicle agricultural traits such as panicle length, number of primary and secondary branches, number of spikelets per panicle and 1000-grain-weight were also similar to wild-type values, and consistently lower than dwt1 MS panicles and higher than dwt1 tiller panicles (Fig. S11). Taken together, these data indicate that OsPIP5K1 promotes several aspects of tiller growth and abolishes MS dominance in the dwt1 mutant.

DWT1 and DWL2 have redundant functions

Given that overexpression of OsPIP5K1 partially rescued the phenotypes of dwt1 mutant plants, we speculated that other WOX proteins might also be activated by elevated levels of OsPIP5K1 and could partially replace the role of DWT1. DWL2 belongs to the same WOX8/9 subclade as DWT1 (Lian et al., 2014), and the two genes show overlapping expression patterns (Wang et al., 2014). Yeast-two-hybrid assay showed that DWL2 was able to interact with OsPIP5K1, specifically via the N-terminal region (Fig. 7a). Similar to DWT1, DWL2 co-localised with OsPIP5K1 in nuclear bodies (Figs 7b, S12).
It has been shown that mutations in DWL2 did not cause obvious developmental defects (Ye et al., 2018). To explore the genetic relationship between DWT1 and DWL2, we created two dwl2 mutant alleles, dwt1dwl2-1 and dwt1dwl2-2 by CRISPR/Cas9 technology, in a dwt1 background (Fig. S13). Sequence analysis revealed that dwt1dwl2-1 carried an 18-bp in-frame deletion in the first exon of DWL2, while dwt1dwl2-2 contained a 1-bp insertion 145 bp into the coding sequence, which caused a frame shift and premature translational stop site.

Compared with dwt1, dwt1dwl2-1 displayed enhanced dwarfism and decreased MS dominance, reminiscent of the plant architecture of dwt1ospip5k1 (Fig. 7c,d). dwt1dwl2-2 homozygotes were embryonic lethal. dwt1dwl2-2+/− heterozygotes were viable and showed a similar plant stature to dwt1dwl2-1 (Fig. 7c,d). We suggest that dwt1dwl2-1 and dwt1dwl2-2 represented weak and strong alleles, respectively. The largely decreased plant height was the result of arrested growth of most internodes; in most culms, only the first internode elongated (Fig. 7e). Furthermore, we found that panicles from both the MS and tillers of dwt1dwl2 double mutants were much smaller than those of wild-type and dwt1 plants (Fig. 7f). These results suggested that DWT1 and DWL2 have similar and partially redundant functions in regulating culm elongation and panicle development.

Discussion

OsPIP5K1 and DWT1 work together to control growth uniformity

The MS and tiller shoots determine plant architecture in rice; as both shoot types develop panicles, changes in their development can have a major influence on final yield. To date, DWT1 is the only reported regulator of rice plant uniformity that affects tiller growth (Wang et al., 2014). In this study, we demonstrated that OsPIP5K1, one of phosphatidylinositol-4-phosphate 5-kinase family proteins, acts together with DWT1 to regulate the uniform growth of rice MS and tillers. OsPIP5K1 is a B type PIP5K that possesses MORN motifs not found in yeast and animal PIPKs (Audhya and Emr, 2003; van den Bout and Divecha, 2009). By Y2H and BiFC assays, we demonstrated that the C-terminus of DWT1 interacts with N-terminal MORN motifs of OsPIP5K1 (Fig. 1). MORN motifs
of PIP5Ks have been reported to be involved in regulating protein subcellular localisation and enzyme activity in Arabidopsis and rice (Ma et al., 2006; Im et al., 2007). However, a study on MORN motifs of PpPIPK1 from the moss Physcomitrella patens suggests that they exert no effect on enzymatic activity (Mikami et al., 2010) implying unconserved roles of MORN motifs between paralogues in the same species or homologues in different species. Therefore, the functions of MORN motifs in plant PIP5Ks are not yet fully understood. Our data indicated that MORN motifs of rice OsPIP5K1 are required for its nuclear localisation and the interaction with DWT1 (Figs 1, 2).

The biological relevance of the DWT1/OsPIP5K1 interaction was revealed by the impacts of OsPIP5K1 deficiency and overexpression on dwt1 plant architecture. Although the ospip5k1 single mutations caused only a mild effect on plant height, which possibly results from the redundant function of other PIP5Ks potentially involved in the same developmental process, the combination with the dwt1 mutation led to synergistic effects on both plant height and panicle size. dwt1 ospip5k1 double mutants exhibited more severe dwarfism and smaller panicle size than the dwt1 single mutant (Figs 5, S9). Notably, the MS dominance of dwt1 was abolished in the double mutant. Conversely, overexpression of PIP5K1 in dwt1 plants promoted culm elongation and panicle growth on tiller shoots, therefore largely restoring the uniformity of plant architecture (Fig. 6). Overexpression of PIP5K1 did not affect the growth of wild-type plants, suggesting that its effects become manifest only in the absence of other key genes, DWT1 in this instance. Overall, these results suggested that DWT1 and OsPIP5K1 act in the same pathway to regulate rice plant architecture.

DWL2 and DWT1 play complementary roles in mediating shoot development

In rice, DWT1/WOX9A, DWL1/WOX9B and DWL2/WOX9C comprise a single subclade of WOX genes (Lian et al., 2014; Wang et al., 2014). DWL1 and DWL2 display overlapping expression patterns with DWT1, preferentially expressed in the
in florescence meristems and embryo, with DWL2 generally expressed more highly than DWT1 (Wang et al., 2014). Disruption of DWL2 function does not affect rice plant growth (Ye et al., 2018). In this study, we showed that dwt1dwl2-1 and dwt1dwl2-1/+ double mutants exhibit stronger phenotypes than the dwt1 single mutant, with abolition of MS dominance as for dwt1ospip5k1 double mutants (Fig. 7). dwl2-1, carrying an 18-bp in-frame deletion, reduces DWL2 function; full knockout of protein function, as encoded by dwl2-2, was lethal, causing a failure of seed germination. Moreover, DWL2 interacts with OsPIP5K1 as DWT1 (Fig. 7a,b). Thus, DWL2 and DWT1 likely have functionally complementary roles in regulating several aspects of rice plant growth, including shoot development, as has been observed for WUS clade members of the WOX superfamily (Sarkar et al., 2007). A higher level of OsPIP5K1 in overexpression lines could intensify the activity of DWL2 to partially compensate for the absence of DWT1 function. Conversely, the expression of DWL1 is extremely low (Wang et al., 2014) and its mutation has no effects on dwt1 phenotypes (data not shown), suggesting that the DWL1 may lose its function in controlling rice architecture during evolution.

The potential role of OsPIP5K1 and PI(4,5)P2 in nuclear signalling

Numerous studies have indicated that PIP5Ks are targeted to various subcellular compartments, including the plasma membrane, cytoplasmic vesicles and nucleus, to generate and maintain distinct PI(4,5)P2 pools in the cell (reviewed by Gerth et al., 2017b). Our observation of PI(4,5)P2 distribution in tobacco cells using biosensor markers P15Y similarly suggests the presence of PI(4,5)P2 pools in distinct cellular subdomains, including plasma membrane, nucleus and potential cytoskeleton and intracellular vesicles (Fig. 3a). In animals, many recent reports have demonstrated the importance of PI(4,5)P2 in regulating nuclear function (reviewed by Irvine, 2002). However, the functions of PIP5Ks and PI(4,5)P2 in plant nuclei are as yet largely unknown. Here, for the first time, we describe the detailed nuclear localisation pattern of a rice PIP5K protein, OsPIP5K1. Although its nuclear localisation does not depend on DWT1, the presence of DWT1 induces the colocalisation and accumulation of OsPIP5K1 in nuclear bodies in tobacco cells. These observations suggest that DWT1 may recruit OsPIP5K1 to produce PI(4,5)P2 pools in specific subnuclear regions, which may serve as important modulators of gene or protein expression. Consistent with this hypothesis, we observed the enrichment of signals from the PI(4,5)P2 biosensor reporter in nuclear bodies when co-expressed with DWT1 (Fig. 3).

The mechanisms by which OsPIP5K1 and PI(4,5)P2 affect the function of DWT1 and DWL2 are not clear. Proteomic studies have identified PI(4,5)P2-interacting nuclear proteins with functions related to transcription, chromatin remodeling and mRNA maturation (Bidlingmaier and Liu, 2007; Lewis et al., 2011). One possibility is that an increased in PI(4,5)P2 may modulate activity of DWT1, DWL2, or other transcription factors; in mouse, the transcription factor c-fos activates nuclear PI(4,5)P2.
synthesis by modulating the activity of PIP5K, which in turn regulates transcription (Ferrero et al., 2014). Another possibility is that nuclear PI(4,5)P₂, induced by the interaction between DWT1 and OsPIP5K1, may be involved in chromatin modification; in human cells, the transcriptional co-repressor BASP1 recruits PI(4,5)P₂ to the promoter region of target genes, where it is required for the interaction of BASP1 with a histone deacetylase to elicit transcriptional repression (Toska et al., 2012). Recent reports have revealed that, in rice, OsWOX11 regulates gene expression by recruiting histone acetyltransferase module ADA2-GCN5 or histone H3K27me3 demethylase JMJD705 (Zhou et al., 2017; Cheng et al., 2018). It is possible that DWT1 and/or DWL2 control the production and distribution of PI(4,5)P₂ in nuclei, which may facilitate their interactions with histone modification factors to regulate the expression of target genes. Further study of the biological relevance of DWT1 and OsPIP5K1 interaction in the nucleus will help unravel the largely unexplored area of PI signalling in plants.

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Author contributions

WL designed the research project and supervised the experiments. FF, SY and JT performed the experiments. WL, FF and MJB wrote the paper.

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**Supporting Information**

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

**Fig. S1** Phylogenetic analysis of phosphatidylinositol-4-phosphate 5-kinase proteins containing MORN motifs in rice and Arabidopsis.

**Fig. S2** DWT1 cannot induce the accumulation of YFP into nuclear bodies.

**Fig. S3** OsPIP5K1 co-localises with PI(4,5)P₂ both in the plasma membrane and the nucleus.

**Fig. S4** CFP cannot induce the accumulation of PI(4,5)P₂ in nuclear bodies.

**Fig. S5** DWT1 induces the accumulation of PI4P in nuclear bodies.

**Fig. S6** Expression pattern of OsPIP5K1 and distribution of OsPIP5K1 in inflorescence meristems.

**Fig. S7** Mutations of OsPIP5K1 CRISPR lines.

**Fig. S8** The frequency of normal and non-elongated internodes in main shoots (MS) and tillers shoots (TS) of dwt1, dwt1ospip5k₁-3, and dwt1ospip5k₁-4 plants (n = 20).

**Fig. S9** Morphology of mature panicles from the main shoot (MS) and tillers (TS) of wild-type (WT) and dwt1 plants, dwt1ospip5k₁ double mutants and OsPIP5K1-OE lines in a dwt1 background.

**Fig. S10** Relative expression of OsPIP5K1 in overexpression lines.

**Fig. S11** Characterisation of panicle agricultural traits.

**Fig. S12** DWL2 cannot induce the accumulation of YFP into nuclear bodies.

**Fig. S13** Mutations of DWL2 CRISPR lines.

**Table S1** List of primers used in this study.

**Table S2** List of rice PIP5K genes.

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