OsMPH1 regulates plant height and improves grain yield in rice

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Abstract

Plant height is a major trait affecting yield potential in rice. Using a large-scale hybrid transcription factor approach, we identified the novel MYB-like transcription factor OsMPH1 (MYB-like gene of Plant Height 1), which is involved in the regulation of plant height in rice. Overexpression of OsMPH1 leads to increases of plant height and grain yield in rice, while knockdown of OsMPH1 leads to the opposite phenotypes. Microscopy of longitudinal stem sections indicated that a change in internode cell length resulted in the change in plant height. RNA sequencing (RNA-seq) analysis of transgenic rice lines showed that multiple genes related to cell elongation and cell wall synthesis, which are associated with plant height and yield phenotypes, exhibited an altered expression profile. These results imply that OsMPH1 might be involved in specific recognition and signal transduction processes related to plant height and yield formation, providing further insights into the mechanisms underlying the regulation of plant height and providing a candidate gene for the efficient improvement of rice yield.

Introduction

Plant height is an important agronomic trait of rice that directly affects the yield of this crop. The dwarf phenotype is beneficial for rice lodging, but if the plants are too short, it will lead to insufficient growth and ultimately affect the yield potential of rice. Therefore, in an absence of lodging, it is essential to increase plant height to increase yield. The second green revolution and the breeding of super rice are based on appropriate plant heights [1–3]. Therefore, it is of great significance to explore and understand plant height genes and apply them to rice breeding.

Most changes in plant height are related to the length of the internodes, which are altered by changes in the number or length of internode cells. Cell elongation involves turgor-driven expansion through wall component deposition or wall loosening. Cell expansion involves widespread changes in the cell wall architecture in terms of both mass and composition. To undergo expansion, the cell wall must first be softened and relax so that cell wall plasticity is
increased and the synthesis of new cell wall material and the amount of protoplasm also increase. Multiple internal and external factors, such as environmental conditions and plant hormones, are involved in the regulation of cell wall-loosening or the deposition of cell wall components. These processes are normally regulated by specific transcription factors, and a number of MYB family genes have been characterized as important regulators in cell wall biosynthesis.

MYB transcription factors are a group of ubiquitous transcription factors that are widely found in plant and animal species. The MYB family is one of the largest families of transcription factors in plants. According to reported statistics, there are 197 MYB genes in Arabidopsis and 155 in rice [4]. MYB proteins contain a characteristic conserved domain, the MYB DNA-binding domain. Based on the number of MYB domains, the MYB family can be divided into four classes: the 1R-, R2R3-, 3R- and 4R-MYB proteins [5]. MYB genes are involved in various processes, such as biological and abiotic stress, development, differentiation, metabolic reactions and defense[6]. At present, functional studies on MYB transcription factors are mainly focused on the regulation of plant responses to environmental stress, although MYBs also exhibit important functions in other processes, such as the cell cycle and cell wall biosynthesis. Arabidopsis MYB103, MYB85, MYB52, MYB54, MYB69, MYB42, MYB43 and MYB20 are regulators of the biosynthesis of lignin, xylan and cellulose, participating in secondary cell wall thickening [7–10]. MYB46 is a direct regulator of the genes involved in the biosynthesis of all three major components of the secondary wall as well as transcription factors in the biosynthesis pathways [8, 11–13]. OsMYB46 and ZmMYB46, the orthologues of Arabidopsis MYB46/MYB83, share similar functions and are able to activate the secondary wall biosynthetic program when overexpressed in Arabidopsis [14]. CEF1/OsMYB103L and MYB61 are also involved secondary wall biosynthesis mediated by the GA pathway, which can affect leaf shape, cellulose synthesis and mechanical strength in rice [15, 16].

In this study, we identified a novel rice height-regulating gene that encodes a MYB family transcription factor. OsMPH1 overexpression increased plant height by elongating internode cell length. OsMPH1 also affected the expression of multiple wall-associated kinase genes, which implies that OsMPH1 is involved in the regulation of cell development.

### Methods

#### Plant materials and growth conditions

The Kita-ake cultivar (*Oryza sativa japonica* cv. Kita-ake) was used as the wild-type. Rice plants were grown at the Experimental Station of the Chinese Academy of Agricultural Sciences in Beijing (39˚54’ N, 116˚23’ E) under natural conditions from May to October of 2014 to 2016 year. Field experiments were performed with three replicates, and each replicate included 10 individuals for each material. Relevant agronomical traits were recorded at heading and mature stages and analyzed with least significance difference (LSD) software.

#### Generation of transgenic rice plants

The construction of *OsMPH1V* and *OsMPH1E* has been described in a previous report [17]. The *OsMPH1* overexpression vector was recombined with the destination vectors pBCV, pBCE [17] and pCAMBIA1301-Bar-FLAG using the Gateway cloning system (Invitrogen). The primers used for this purpose are listed in S1 Table. The constructs were subsequently introduced into *Agrobacterium tumefaciens* strain EHA105 and then transformed into Kita-ake wild-type plants [18].

Competing interests: The authors have declared that no competing interests exist.
**OsMPH1 bioinformatics analysis**

The OsMPH1 gene locus identifier is LOC_Os06g45890. Protein alignment was carried out with ClustalX 2.0. A phylogenetic tree was constructed with MEGA5.1 using the neighbor-joining (NJ) method. The bootstrap values for nodes in the phylogenetic tree came from 1000 replications. The handling gap option was pairwise deletion, and the numbers at the branching points indicate the bootstrap values. The accession numbers and protein sequences are shown in S3 Table.

**Histological analysis**

The first internodes in the heading date stage were collected from WT, OsMPH1V and OsMPH1E plants, then fixed in FAA solution (60% (v/v) ethanol, 5% (v/v) glacial acetic acid and 5% (v/v) formaldehyde) and subjected to vacuum pumping for 40 minutes. Next, the internodes were dehydrated in a series of ethanol solutions (70% (v/v) ethanol, 80% (v/v) ethanol, 85% (v/v) ethanol, 90% (v/v) ethanol, 95% (v/v) ethanol, and anhydrous ethanol) and destained in a series of xylene solutions (3:1 ethanol: xylene, 1:1 ethanol: xylene, 1:3 ethanol: xylene, and pure xylene). The internodes were soaked in each ethanol and xylene solution for two hours and then embedded in paraffin. Tissue sections were cut with a Leica rotary microscope, fixed on a glass slide, and stained with 0.05% Toluidine Blue O (Sigma).

**Subcellular localization**

Full-length OsMPH1 was inserted into the PA7-YFP vector, which had been digested with BamHI and SmaI using the In-fusion system (Clontech). OsMPH1-YFP was then transiently expressed in Arabidopsis mesophyll cells [19]. The AtAHL-RFP fusion protein was used as a nuclear marker. The resulting fluorescent signal was observed under a confocal microscope (Zeiss LSM700) after 16 h of transformation at room temperature in the dark.

**Transactivation activity assays in yeast**

Full-length OsMPH1 was inserted into the pGBK7 vector and transformed into the yeast strain AH109. The empty pGBK7 vector and the BD-4VP16 and BD-4EAR vectors were used as controls. Transformed yeast was grown in SD/-W (-Trp) and SD/-W-H-Ade (-Trp/-His/-Ade) plates for 48 hours before taking photographs. β-galactosidase activity was measured according to the Yeast Protocols Handbook (Clontech) using chlorophenol red-β-D-galactopyranoside as a substrate (CPRG, Roche Biochemical).

**RNA isolation and qRT-PCR analysis**

The WT plants and the OsMPH1V and OsMPH1E transgenic lines were cultivated under continuous light at 28˚C for 4 weeks in plant growth chambers. RNA extraction and quantitative reverse transcription PCR (qRT-PCR) analysis were performed as described previously [20].

**Immunoblots**

Immunoblot analysis was performed using one-week-old seedlings as described previously [20].

**RNA-seq and data analysis**

The WT plants and the OsMPH1V-3and OsMPH1E-22 were grown under continuous light at 28˚C till four leaves stage and total RNA was extracted using the Trizol reagent (Invitrogen).
The sequencing library was constructed following the manufacturer’s instructions (Illumina Inc.). Paired-end sequencing libraries with an insert size of approximately 200 bp were sequenced on an Illumina HiSeq 2000 sequencer at ANOROAD Company in Beijing. RNA-seq clean reads of three biological replicates were mapped to the *O. sativa ssp. japonica* reference genome after removing adaptor and low quality nucleotides by TopHat. The expression value was calculated in FPKM (fragments per kilobase of exon model per million mapped fragments) and the differentially expressed genes were further analyzed by Cuffdiff (*q* < 0.05). Differentially expressed genes were defined as those with fold changes ≥ 2, or ≤ 2/3. RNA-seq data have been deposited in the ArrayExpress database at EMBL-EBI (https://www.ebi.ac.uk/arrayexpress/experiments/E-MTAB-5759) under accession number E-MTAB-5759.

**Results**

*OsMPH1 (MYB-like gene of plant height 1)* is involved in the regulation of plant height

Previously, we described the approach of using hybrid transcription factors (HTFs) to investigate the roles of different TFs in plant growth and development [17]. By surveying the phenotypes of over 50,000 transgenic lines, covering 1,500 rice transcription factors fused with the transcription activation module 4VP16 or the repression module 4EAR, we identified a pair of HTFs that exhibited opposite plant height phenotypes, referred to as *OsMPH1-4VP16* (*OsMPH1V*) and *OsMPH1-4EAR* (*OsMPH1E*). In total, we obtained 34 *OsMPH1V* transgenic events (designated as *OsMPH1Vs*) and 25 *OsMPH1E* transgenic events (designated as *OsMPH1Es*), among which 19 *OsMPH1Vs* and 15 *OsMPH1Es* showed a dwarf or taller phenotype, respectively. We verified the transgenic plants via quantitative reverse transcription PCR (qRT-PCR) or immunoblot analyses and selected two *OsMPH1Vs* and two *OsMPH1Es* for further analyses (S1A and S1B Fig).

We first measured plant height. In comparison with the wild-type, the plant height of *OsMPH1Vs-3* and *OsMPH1Vs-6* was decreased by 22.3% and 22.5%, while that of *OsMPH1E-22* and *OsMPH1E-50* was increased by 41.3% and 44.8%, respectively (Fig 1A and 1B). To confirm the culms phenotype, we measured internode length in the late stage of rice growth. The statistical results showed that the internode length of all *OsMPH1E-22* and *OsMPH1E-50* plants was increased dramatically in comparison with the wild-type; in contrast, the internode length of all *OsMPH1Vs-3* and *OsMPH1Vs-6* plants was markedly decreased (Fig 1C and 1D).

To avoid the artifactual phenotype caused by an additional 4VP16 activation domain or 4EAR suppression domain, we constructed an *OsMPH1* overexpression vector (*OsMPH1-OX*) and obtained 47 independent *OsMPH1-OX* events. Plant height was increased by most of the transgenic events (Fig 2A and 2B). We validated the transgenic plants through qRT-PCR and immunoblot probing with an anti-Flag antibody and selected four representative events for further analysis (S1C and S1D Fig). The statistical results showed that there was correlation between *OsMPH1-OX* plant height and the protein expression level. The greater the amount of the *OsMPH1* protein, the greater the height of *OsMPH1-OX* plants, suggesting that OsMPH1 is involved in the regulation of plant height. Next, we observed the internode length of *OsMPH1-OX* plants. The *OsMPH1-OX* plants displayed a similar phenotype to the *OsMPH1E* plants, with all plants exhibiting a dramatic increase in internode length (Fig 2C and 2D).

We also obtained *OsMPH1-RNAi* transgenic rice and verified that mRNA levels were significantly reduced in these plants (S2A and S2B Fig). In comparison with wild-type plants, the *OsMPH1-RNAi* plants exhibited decreased plant height phenotypes (S2C Fig).
OsMPH1 affects internode cell length

The variation in plant height could be caused by a change of internode cell length. To verify
this hypothesis, longitudinal anatomical sections of the internodes were analyzed. Longitudi-
nal sections from rice plants were photographed at high magnification under a microscope,
and software was used to calculate cell length. The results showed that the longitudinal length
of OsMPH1V and OsMPH1E internode cells was dramatically altered compared with the WT
controls. The longitudinal cell phenotype and length statistics are shown in Fig 3. The length
of OsMPH1V parenchyma cells was significantly decreased, while the length of OsMPH1E

![Fig 1. Phenotypic analysis of OsMPH1V- and OsMPH1E-overexpressing plants. A Gross morphology of WT, OsMPH1V and
OsMPH1E. Bars = 20 cm. B Comparison of plant height between WT, OsMPH1V and OsMPH1E transgenic rice. Data are shown as the
means ± s.d. (Student’s t-tests, **P < 0.01, n = 60). C Internode morphology of WT, OsMPH1V and OsMPH1E. Bars = 5 cm. D Schematic
representation and comparison of the various elongation patterns of internodes in WT, OsMPH1V and OsMPH1E transgenic rice. Data are
shown as mean as the means ± s.d. (Student’s t-tests, **P < 0.01, n = 10).](https://doi.org/10.1371/journal.pone.0180825.g001)
parenchyma cells was significantly increased, whereas there was no significant change in the number of cells. These results indicated that OsMPH1 could regulate plant height by altering the length of internode cells.

**OsMPH1 improves quantitative yield components in rice**

In addition to the greater plant height, we also observed that both OsMPH1Es and OsMPH1-OXs showed large-panicle and late-flowering phenotypes under natural conditions (Fig 4).

To analyze the large-panicle phenotype, we compare the branching pattern of OsMPH1Vs and OsMPH1Es with the wild-type in more detail. The statistical results indicated that primary and secondary branches were significantly more abundant in OsMPH1Es than in the wild-

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**Fig 2. Phenotypic analysis of OsMPH1-overexpressing plants.** A Gross morphology of WT and OsMPH1-OX. Bars = 20 cm. B Comparison of plant height between WT and OsMPH1 transgenic rice. Data are shown as the means ± s.d. (Student’s t-tests, **P < 0.01, n = 60). C Internode morphology of WT and OsMPH1-OX. Bars = 5 cm. D Schematic representation and comparison of the various elongation patterns of internodes in WT and OsMPH1-OX transgenic rice. Data are shown as the means ± s.d. (Student’s t-tests, **P < 0.01, n = 10).

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type, while OsMPH1Vs exhibited no difference from the wild-type (Fig 5A and 5C). We next counted the grains produced on primary and secondary branches (Fig 5B and 5D). The results showed that the OsMPH1Vs and OsMPH1Es presented no difference from the wild-type. We also examined other components of the rice yield trait, such as tiller number and 1000-grain weight. Statistical analysis revealed that the OsMPH1Es exhibited fewer tillers than the OsMPH1Vs and the wild-type, while the 1000-grain weight showed no difference between OsMPH1V, OsMPH1E and wild-type plants ((Fig 5E and 5F). We further measured the actual grain yield at the plot level (10 plants per plot); the results showed that the grain yields of OsMPH1Es were increased by 45% to 50% in comparison with wild-type plants (Fig 5G). The increased number of primary and secondary branches and extended growth period appeared to explain why OsMPH1Es exhibit a higher grain yield.

We also checked the grain weight per plot, KGW, tiller number and grains per panicle of OsMPH1-OXs and OsMPH1-RNAi, which share the similar results to OsMPH1Es and OsMPH1Vs (S3 Fig).

Rice OsMPH1 encodes a MYB family transcription factor

OsMPH1 refers to LOC_Os06g45890, which encodes a 256-amino acid protein with a predicted molecular mass of 28.5 kD. Phylogenetic tree analysis showed that OsMPH1 clustered with homologues in rice (LOC_Os02g07170, LOC_Os03g03760, LOC_Os04g47890, LOC_Os10g39550 and LOC_Os11g01480) and Arabidopsis (AT1G14600, AT2G02060 and AT2G40260) (Fig 6A). Protein structural analysis revealed that OsMPH1 contains a typical R1 Myb_DNA-binding motif from amino acid residues 19 to 70 at the N-terminus. Amino acid alignment showed that OsMPH1 shares over 60% identity with Arabidopsis and rice homologues in the Myb_DNA-binding motif, while sharing less than 25% identity in other regions (Fig 6B).

The MYB family transcription factors have been reported to localize to the nucleus. To examine whether OsMPH1 localizes to the nucleus, OsMPH1 was fused with YFP at C-terminus and transiently expressed in the Arabidopsis mesophyll protoplasts. The results showed
Fig 4. Analysis of plant height and heading dates in OsMPH1V, OsMPH1E and OsMPH1-OX plants. A,B Panicle morphology of WT, OsMPH1V, OsMPH1E and OsMPH1-OX transgenic rice. C,D Comparison of panicle length between WT, OsMPH1V, OsMPH1E and OsMPH1-OX transgenic rice. Data are shown as the means ± s.d. (Student’s t-tests, \(*P < 0.01, n = 60\)). E,F Morphology of WT, OsMPH1V, OsMPH1E and OsMPH1-OX transgenic rice on the heading date. G,H Comparison of heading dates between WT, OsMPH1V, OsMPH1E and OsMPH1-OX transgenic rice. Data are shown as the means ± s.d. (Student’s t-tests, \(*P < 0.01, n = 60\)).

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that OsMPH1-YFP was exclusively located in the nucleus, co-localizing with the nuclear marker protein AHL-RFP. In contrast, the control YFP protein was only detectable in the intracellular region (Fig 6C). Together, these data suggest that OsMPH1 is a nuclear-localized MYB family transcription factor.
Rice OsMPH1 expression pattern

To investigate the tissue-specific expression pattern of OsMPH1, the GUS reporter system was used to monitor its expression throughout the life cycle (from seeding to mature caryopses). As shown in Fig 7A, histochemical staining indicated that OsMPH1 was expressed in almost all the examined tissues, including the plumule of germinating seeds, coleoptile, leaves, stem nodes, internodes, sheaths, pulvinus, spikes and roots. Among these tissues, OsMPH1 exhibited extremely high expression in the pulvinus and stem nodes.

We also monitored the OsMPH1 expression pattern using qRT-PCR assays. Similar to the GUS staining results, OsMPH1 was detectable in all tissues. Notably, our results showed an extremely high mRNA expression level of OsMPH1 in the leaves and sheaths and especially in the pulvinus, whereas OsMPH1 was weakly expressed in the internodes and spikes (Fig 7B).

Rice OsMPH1 exhibits no self-activation activity

To determine the transcriptional activity of OsMPH1, constructs of OsMPH1, MPH1V and OsMPH1E with the pGBKT7 vector were generated and used to transform the AH109 yeast
cell line. The empty BD vector, 4EAR vector and 4VP16 vector were used as controls. As shown in Fig 8, all the yeast transformants grew well on (-Trp) SD medium. When they were transferred to (-Trp/-His/-Ade) SD medium, only OsMPH1Vs and 4VP16s grew well. We also quantified the β-galactosidase activity of all the transformants using CPRG as a substrate. In comparison with the BD empty vector control, OsMPH1 and its truncated version possessed very low transcriptional activity, while when OsMPH1 was fused with the 4VP16 activation domain, OsMPH1 transcriptional activity increased dramatically. In contrast, when OsMPH1 was fused with the 4EAR suppression domain, OsMPH1 transcriptional activity decreased slightly. These results indicated that OsMPH1V functions as a transcriptional activator, while OsMPH1E functions as a suppressor. OsMPH1 possessed very weak transcriptional activity, and the recruitment of a partner may be necessary for its functional implementation.

mRNA expression pattern analysis of transgenic plants

Our data indicated that a change in internode cell length led to the observed plant height phenotype, which suggests that OsMPH1 is involved cell length regulation. To further elucidate...
the regulation of OsMPH1, we compared mRNA expression in WT and OsMPH1V and OsMPH1E transgenic plants using RNA-seq assays (S4 Fig). We identified 73 genes that exhibited opposite expression pattern in OsMPH1Vs versus OsMPH1Es, which could be divided into two types (Fig 9; S2 Table). The Type-one genes included 48 genes whose expression levels were increased in OsMPH1Vs compared with the wild-type but were decreased sharply in OsMPH1Es. The Type-two genes included 25 genes whose expression levels were decreased in OsMPH1Vs, but dramatically increased in OsMPH1Es. Among the Type-one genes, there were 9 receptor-like protein kinases (RLKs), which could be classified into 4 types, including 4 wall-associated (WAK) RLKs (LOC_Os02g42150, LOC_Os04g30010, LOC_Os09g29560 and LOC_Os04g5103), 3 leucine-rich repeat (LRR) RLKs, 1 stress-antifung RLK and 1 cysteine-rich RLK. All of these RLKs were predicted to possess a transmembrane region and to localize to the plasma membrane (S2 Table). WAKs are involved in determining the cell wall composition as well as phosphorylation or transcription processes and are required for cell expansion during plant development [21]. We also identified a cinnamyl alcohol dehydrogenase (CAD) (LOC_Os06g22919) and a xyloglucan endotransglycosylase/hydrolase (LOC_Os04g1592), which are key enzymes involved in lignin biosynthesis and the process of plant cell wall remodeling. These genes might provide clues to explain the phenotypic variation of plant height.

In addition, we identified several genes involved in the indole biosynthetic pathway, including two genes (LOC_Os04g08828 and LOC_Os06g02019) encoding cytochrome P450 monooxygenases, which could convert Trp to indole-3-acetaldoxime (IAOx), a precursor of IAA and indole glucosinolates; one gene (LOC_Os04g09604) encoding an indole glucosinolate O-methyltransferase; and one gene (LOC_Os05g37470) encoding an auxin transporter, all of which are involved in cell expansion.
Plant height is closely related to biomass production, which makes it an important morphological trait that affects yield performance. Rice is an economically important crop, in which the ratio of economic output to biological yield is 1:1. Therefore plant height and yield are closely related, and within a certain range, when plant height is increased, the yield is also increased. A moderate plant height is an important basis for rice breeding. In the present study, we identified a new rice MYB gene, OsMPH1, which might function in plant height regulation. OsMPH1 is highly expressed in the rice pulvinus and nodes, and the encoded protein is exclusively localized in the nucleus. OsMPH1 overexpression leads to an increase of plant height, caused by longitudinal elongation of internode cells. Our result also indicated that OsMPH1Es plants shared the similar phenotype with OsMPH1 overexpression plants, while OsMPH1V and OsMPH1-RNAi plants exhibited the opposite phenotype. Combining with the transcription activation analysis result that OsMPH1V functions as a transcriptional activator and OsMPH1E functions as a suppressor, OsMPH1 might work as a transcriptional suppressor in vivo. Considering that OsMPH1 bears almost no transcriptional activity, recruitment of a partner may be necessary for OsMPH1 to implement the activator function of the plant height.

Plant cell elongation growth is regulated by a variety of internal and external factors, and the regulation of endogenous hormones in plants plays an important role in this process. For example, auxin (IAA), gibberellin (GA), brassinolide (BR) and ethylene (ETH) can regulate cell elongation, and the interaction between the sources of hormones directly or indirectly...
regulates cell elongation. It has been reported that AtMYB52, AtMYB54, and AtMYB69 regulate the biosynthesis of lignin, xylan, and cellulose, participating in secondary cell wall thickening (Stracke et al. 2001; Zhong et al. 2008). OsMPH1 may exhibit functions similar to these transcription factors, particularly in cell wall development. In plants, hormones including small organic molecules as well as larger peptides and small proteins act as ligands and interact with receptor proteins to trigger rapid biochemical changes and induce intracellular transcriptional and long-term physiological responses. Receptor kinases have been demonstrated to be important for cell elongation. For example, auxins can stimulate cell elongation by activating ROPs [Rho-like guanosine triphosphatases (GTPase)], and the TMK receptors-like kinases activate ROP activators, such as ROP-GEF, via phosphorylation to activate ROPs [22, 23]. The cell wall-associated receptor kinase WAK4 has been reported to be involved in cell elongation and plant development. WAK4 antisense expression results in cell elongation and developmental arrest [21]. Our further investigation showed that WAK4 homologous genes in rice, including LOC_Os02g42150, LOC_Os04g30010, LOC_Os09g29560 and LOC_Os04g51030, were up-regulated in OsMPH1V transgenic plants but down-regulated in OsMPH1E transgenic plants. A previous study identified 27 AtWAKs in Arabidopsis and 130 OsWAKs in rice, suggesting functional diversification in Arabidopsis and rice [24, 25]. Therefore, these four genes might be involved in the regulation of cell elongation, differing from the functions observed in Arabidopsis. Additional efforts focusing on the functional activity of these genes will be helpful to understand the regulation of cell elongation in rice. Furthermore, we identified a series of indole biosynthetic pathway genes whose expression patterns were changed in the transgenic plants, which implied that auxin-regulated cell elongation is an important component of the variation in plant height.

OsMPH1 comprises an R1-type MYB domain that is presumably required for DNA binding. The MYB proteins are divided into 4 sub-families according to the structure of the DNA binding domain that contains one to three repeats. Each repeat composed of approximately 53 amino acid residues that form a helix-turn-helix. OsMPH1 MYB domain contains 52 amino acid residues and shares highly similarities with reported R1-type MYB proteins. OsMPH1 localizes to the nucleus and possesses weak transcriptional activity which implies that OsMPH1 is typical R1-type MYB protein. Previous research on R1-type MYB genes OsMYBS1, OsMYBS2, and OsMYBS3 in rice has demonstrated that these genes display the DNA-binding capacity to bind the TATCCA motif [26]. Thus, we further examined whether a TATCCA motif was present in the promoter regions (1,000 bp upstream) of OsMPH1-regulated genes. We found that LOC_Os04g08828, LOC_Os04g09604, LOC_Os04g15920, LOC_Os04g51030 and LOC_Os06g02019 possessed at least one TATCCA motif in their promoter regions, which suggests that these genes might be direct targets of OsMPH1.

Supporting information

S1 Fig. Identification of transgenic plants. A Immunoblot analysis of WT and OsMPH1V plants. B OsMPH1V and OsMPH1E expression level analysis by qRT-PCR. C Immunoblot analysis of WT and OsMPH1-OX plants. D OsMPH1-OX expression level analysis by qRT-PCR. (TIF)

S2 Fig. Phenotypic analysis of OsMPH1 RNAi plants. A Gross morphology of WT and OsMPH1-OX. Bars = 20 cm. B OsMPH1-OX expression level analysis by qRT-PCR. C Comparison of plant height between WT, OsMPH1V and OsMPH1E transgenic rice. Data are shown as the means ± s.d. (Student’s t tests. **P < 0.01, n = 60). (TIF)
S3 Fig. Analysis of yield traits in OsMPH1-OX and OsMPH1-RNAi plants. A-D Comparison of the number of grains weight per plot, KGW, tillers per plant, grain per panicle between WT, OsMPH1-OX and OsMPH1-RNAi transgenic rice. Data are shown as the means ± s.d. (Student’s t tests, *P < 0.05, **P < 0.01, n = 60).

(TIF)

S4 Fig. Heat map of RNA-seq data.

(TIF)

S1 Table. Primers used in this study.

(XLSX)

S2 Table. Differentially expressed genes between OsMPH1V and OsMPH1E.

(XLSX)

S3 Table. The protein sequences used to build the phylogenetic tree.

(XLSX)

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