RESEARCH PAPER

MYB57 transcriptionally regulates MAPK11 to interact with PAL2;3 and modulate rice allelopathy

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Abstract

Rice allelopathy is a natural method of weed control that is regarded as an eco-friendly practice in agroecology. The allelopathic potential of rice is regulated by various genes, including those that encode transcription factors. Our study characterized a MYB transcription factor, OsMYB57, to explore its role in the regulation of rice allelopathy. Increasing the expression of OsMYB57 in rice using the transcription activator VP64 resulted in increased inhibitory ratios against barnyardgrass. The gene expression levels of OsPAL, OsC4H, OsOMT, and OsCAD from the phenylpropanoid pathway were also up-regulated, and the content of l-phenylalanine increased. Chromatin immunoprecipitation incorporated with HiSeq demonstrated that OsMYB57 transcriptionally regulated a mitogen-activated protein kinase (OsMAPK11); in addition, OsMAPK11 interacted with OsPAL2;3. The expression of OsPAL2;3 was higher in the allelopathic rice PI312777 than in the non-allelopathic rice Lemont, and OsPAL2;3 was negatively regulated by Whirly transcription factors. Moreover, microbes with weed-suppression potential, including Penicillium spp. and Bacillus spp., were assembled in the rhizosphere of the rice accession Kitaake with increased expression of OsMYB57, and were responsible for phenolic acid induction. Our findings suggest that OsMYB57 positively regulates rice allelopathy, providing an option for the improvement of rice allelopathic traits through genetic modification.

Introduction

Allelopathy is a biological phenomenon, typical of certain plants, algae, and microorganisms (Reigosa et al., 2006), that involves inhibitory or stimulatory effects of one organism on another. This phenomenon can influence organism growth, survival, and reproduction through the secretion of allelochemicals or their release into the environment (Rice, 1984). Allelochemicals belong to the secondary plant metabolites, and the role of several groups of secondary metabolites, such as phenolic acids and terpenoids, in allelopathy has been documented (Maqbool et al., 2013; Soltys et al., 2013; Einhellig, 2018). They are predominantly derived from the phenylpropanoid metabolic and isoprenoid biosynthetic pathways and their associated branch paths (Verpoorte, 2000).

In rice, allelopathic effects were first recorded by Dilday et al. (1989) during a field experiment that presented different rice accessions with ~50–90% inhibitory ratios against duckswal [Heteranthera limosa (Sw.) Willd.]. Since that study, several studies have screened potential allelopathic rice accessions and have separated and identified rice allelochemicals
(Dilday et al., 1994; Olofsdotter et al., 1995; Chung et al., 2001, 2006). To date, various studies have shown that phenolic acids such as ferulic acid (FA), cinnamic acid (CA), p-hydroxybenzoic acid (HA), and monilactone B, which is a diterpenoid, have allelopathic activity against barnyardgrass (Kato-Noguchi, 2004, 2011; Seal et al., 2004; Bi et al., 2007; Kato-Noguchi and Ito, 2005; Khanh et al., 2007; He et al., 2012a,b). The knockout or silencing of relevant genes participating in the diterpene or phenolic acid pathways, such as copalyl diphasphate synthase 4 (OsCPS4), kaurene synthase-like 4 (OsKSL4), and phenylalanine ammonia lyase (OsPAL), results in weakened allelopathic activity of donor rice on barnyardgrass. This weakened activity is attributed to reduced contents of monilactones or phenolic acid in root exudates (Xu et al., 2012; Fang et al., 2013). Studies have documented that rhizospheric microorganisms have indispensable roles in the process of allelopathy (Inderjit, 2005; Fang et al., 2015; Li et al., 2015; Niro et al., 2016; Wu et al., 2019). Specifically, *Sphingobium* spp. from the rhizosphere of peanut (*Arachis hypogaea*) functioned in the biodegradation of pterostilbene produced by the plants, suggesting that bacterial interference may play a role in plant allelopathy (Yu et al., 2019). Exogenous vanillin influenced the bacterial and fungal communities of the rhizosphere of cucumber (*Cucumis sativus* L.) seedlings (Jia et al., 2018; Zhang et al., 2018). Vanillic acid also changed the total fungal community in the cucumber seedling rhizosphere, and the *Trichoderma* and *Fusarium* spp. communities showed different responses to vanillic acid (Chen et al., 2018). In rice, phenolic acids secreted from the root tissue were found to interact with specific microorganisms in the rice rhizosphere (e.g. *Myxovorax xanthus*) to increase the inhibition of weeds. This inhibition indicates the vital role of the interaction of *M. xanthus* and phenolic acids in rice allelopathy in the field (Fang et al., 2015). These compounds are known to be derived from the phenylpropanoid metabolic pathway.

Previous studies have shown that MYB transcription factors function in the regulation of genes involved in the plant phenylpropanoid metabolic pathway (Yang et al., 2012; Liu et al., 2015a). MYB transcription factors in plants can be classified into four types depending on the number of adjacent repeats in the MYB domain, namely 1R-MYB, 2R3-MYB, 3R-MYB, and 4R-MYB, which contain one, two, three, and four MYB repeats, respectively (Dubos et al., 2010). Among these four types, 2R3-MYB factors are the main components of the MYB family and play a role in the regulation of genes involved in secondary metabolism (Liu et al., 2015a). For example, constitutively expressed *ZmMYB-IF35* promotes the accumulation of ferulic and chlorogenic acids in maize (*Dias and Grotewold, 2003*). Similarly, the genes *StAN1*, *StMTF1*, and *PAP1* in potato and *AtMYB12* in Arabidopsis, which were constitutively expressed in tobacco, potato, tomato, and snow lotus (*Saussurea involucrata*), respectively, resulted in an increase in the chlorogenic acid content (Luo et al., 2008; Rommens et al., 2008; Payyavula et al., 2013; Qiu et al., 2013). In grape, the overexpression of *VvMYB5a*, which encodes a protein belonging to a small subfamily of 2R3-MYB transcription factors, induced strong accumulation of various phenolic compounds. This finding suggests that *VvMYB5a* is involved in the control of different branches of the phenylpropanoid pathway in grapevine (Deluc et al., 2006). In *Arabidopsis thaliana*, subgroup 7 of the 2R3-MYB gene family, which includes MYB11, MYB12, and MYB111, plays a role in the regulation of the gene expression of *chalcone synthase*, *chalcone isomerase*, *flavanone 3-hydroxylase*, and *flavonol synthase*, all of which are involved in flavonoid biosynthesis, thereby controlling flavonol accumulation in the plant (Stracke et al., 2007).

In rice, genome-wide analysis identified at least 185 MYB genes, including genes encoding 70 1R-MYBs, 109 2R3-MYBs, 5 3R-MYBs, and 1 4R-MYB (Chen et al., 2006; Katiyar et al., 2012). The overexpression of the 2R3-type *OsMYB60* resulted in twisted leaf blades in transgenic rice (Liu et al., 2018). Another 2R3-type transcription factor, *OsMYB1*, was identified in response to phosphate starvation (Gu et al., 2017). Yang et al. (2012) identified a 2R3-type MYB gene, *OsMYB2*, that is involved in the tolerance of salt, cold, and dehydration in rice. To the best of our knowledge, however, aside from these studies, there has been no investigation of the involvement of 2R3-type MYBs in the regulation of rice allelopathy, despite the potential role of 2R3 MYBs in the regulation of secondary pathways. Our previous studies screened a set of VP64 transcription factor transgenic rice lines of the accession Kitaake, constructed by Zhao et al. (2015), to investigate their weed-suppression capacity. An increased weed-suppression ability was found in a VP64-activated 2R3 MYB transcription factor *OsMYB57* transgenic line. Wang et al. (2016) used these transgenic lines to investigate the transcription factor that possibly increased rice grain yield, and a 35% increase in grain yield was found in the *OsMYB1R1-VP64* transformed line. The *UL48* gene-encoded viral protein 16 (VP16) is a transcription activation domain from the human herpes simplex virus, which is one of the best-known transcription regulatory domains (Sadowski et al., 1988). The C terminal of the VP16 protein maintains an 11-amino acid peptide with transcription activation function, and four repeats of this peptide (VP64) have even stronger activation properties (Yaghmai et al., 2002). Both VP16 and VP64 represent a new strategy for genetic engineering. The activity of transcription factors is significantly increased after they have been fused with VP64, enabling them to play a stronger role in regulating crop growth and development. According to the results of the current study, increasing *OsMYB57* expression in rice resulted in larger inhibitory ratios against barnyardgrass, but the regulatory network of *OsMYB57* remains unknown. In Arabidopsis, the expression of *MYB57* was induced by jasmonate and was able to promote stamen filament growth (Cheng et al., 2009). However, the coding sequences of *OsMYB57* and *AtMYB57* share little homology (35.66%), suggesting that OsMYB57 in rice may have a distinct role.

The aim of this study was to clarify the exact role of OsMYB57 in the regulation of rice allelopathy. To reveal the genes that were transcriptionally regulated by OsMYB57, chromatin immunoprecipitation sequencing (ChIP-seq) was conducted to identify the motif of the relevant genes regulated by OsMYB57. Correlations with the rice phenylpropanoid pathway were explored, and the role of OsMYB57 in regulating allelopathy was revealed.
Materials and methods

Plant materials
This study used the rice (Oryza sativa L. subsp. japonica) accession Kitaake and its transgenic lines with increased OsMYB57 expression via a transcriptional activator containing four copies of VP16 (i.e., VP64) (Sadowski et al., 1988; Yaghmai et al., 2002). The allelopathic rice (O. sativa L. subsp. japonica) accession PI312777 and the non-allelopathic rice (O. sativa L. subsp. japonica) accession Lemont were also used (Bollich et al., 1985; Fang et al., 2015).

Rice growth conditions
The seeds of each rice line were treated with 25% NaClO for 30 min for surface sterilization and then soaked in sterilized double-distilled (dd) H₂O overnight. The imbibed seeds were transferred to an artificial climate incubator at 30 °C to germinate, and germinated seeds were sown on to separate seedling plates and grown until they were at the three-leaf stage. Uniform seedlings with similar growth rates from each line were selected and transplanted to a Styrofoam plate containing 20 equally distributed holes by using a spoon to affix the seedlings and insert them into each hole. The Styrofoam plate was floated in a pot (45 cm × 35 cm × 15 cm) filled with 10 litres of rice culture solution (Fang et al., 2011, 2016), according to a modified technique described by Yoshida (1976). The solution was replaced with fresh solution every week, and its pH was maintained at 5.5–6.0 throughout the experiment.

Evaluation of the rice allelopathic inhibition of barnyardgrass
To evaluate the allelopathic potential of OsMYB57VP64 and Kitaake, the seeds of these two rice lines were again surface sterilized, germinated and sown in agar medium and in the soil. The OsMYB57VP64 transgenic rice lines were constructed by Zhao et al. (2015). For the agar culture condition experiments, 10 germinated rice seed were sown on plates (9 cm diameter) containing 200 ml 0.6% solid agar. After 1 week, 10 germinated barnyardgrass seeds were also sown on the plates. For the soil culture condition experiments, five germinated rice seed were sown in a 0.5 litre beaker (8.5 cm diameter) containing 100 ml of soil. The soil was sandy loam with pH 6.5 and 55.8% moisture content, 53.75 mg kg⁻¹ alkali-hydrolysable nitrogen, 11.10 mg kg⁻¹ Olsen phosphorus, 72.67 mg kg⁻¹ available potassium, and 20.82 g kg⁻¹ organic matter. After 1 week, 5 germinated barnyardgrass seeds were also sown in the beaker, and both the plates and beakers were placed in an artificial climate incubator under 12 h/12 h (day/night) photoperiod, 10 000 lux, and 75% relative humidity. A total of 20 mono-cultured barnyardgrass seeds grown on a plate or 10 mono-cultured barnyardgrass seeds in a beaker were used as controls. Both the treatment and control were performed in triplicate. The barnyardgrass seeds were allowed to grow for 1 week, after which the root length, plant height, and fresh weight of barnyardgrass were measured to determine the allelopathic potential of rice on barnyardgrass (Fang et al., 2013, 2015).

Detection of OsMYB57 expression in VP64-derived transformed rice and wild-type rice
The top second leaf of the VP64-derived OsMYB57-transformed Kitaake rice line (OsMYB57VP64) and its wild type (WT) were sampled to extract the total proteins for western blotting. Specific antibodies to OsMYB57 and goat anti-rabbit IgG conjugated to horseradish peroxidase were successively used in western blots to detect the levels of OsMYB57 expression in OsMYB57VP64 and WT Kitaake.

Identification of the secondary metabolites from root tissues of OsMYB57VP64 and Kitaake
Rice allelochemicals are mainly derived from plant secondary metabolites. To investigate the compounds from the OsMYB57VP64 and Kitaake rice lines, the two rice lines were co-cultured with barnyardgrass for 7 days. The root tissues of the two rice lines were then harvested and ground individually, and the secondary metabolites were extracted using methanol. The crude extract was then extracted using chloroform, vacuum dried, and derivatized for identification by GC-QqQ mass spectrometry [Shimadzu (China) Co., Ltd].

Determination of population numbers of microorganisms from rhizospheric soil of rice
To investigate the differences in the populations of rhizospheric microorganisms from the OsMYB57VP64 and Kitaake rice lines, the DNA from the rhizospheric soil of the two rice lines was extracted using a BioFast Soil DNA Extraction Kit (Hangzhou Bioer Technology Co., Ltd). Quantitative PCR (qPCR) was conducted to determine the population numbers of bacteria, fungi, and myxobacteria in the rhizosphere of the two rice lines, based on Fang et al. (2013, 2015).

Isolation of rhizospheric microbes and evaluation of their allelopathic potential against barnyardgrass
Rhizospheric soil samples from OsMYB57VP64 and Kitaake (5 g each) were resuspended in 100 ml of sterilized ddH₂O and shaken at 1000 rpm at 37 °C for 1.5 h, after which the mixture was incubated at room temperature for 10 min. Next, the supernatant was serially diluted from 10-fold to 10⁷-fold and spread on to beef extract peptone agar (BPA) medium and potato dextrose agar (PDA) medium using the plate smearing method. BPA was used for bacterial cultures and PDA was used for fungal cultures. The BPA and PDA medium plates were placed in an incubator at 37 °C and 28 °C, respectively. Colony growth on the media was recorded every 24 h for 1 week. The colonies were then isolated and purified to obtain single strains. The purified bacteria and fungi were further cultured in beef extract peptone liquid medium and potato dextrose liquid medium, and the fermentation broths were collected and used to evaluate their allelopathic potential against barnyardgrass. Barnyardgrass co-cultured in the medium was used as a control.

Identification of allelopathic bacterial and fungal species
Bacteria and fungi with allelopathic potential were cultured for genomic DNA extraction. An EasyPure Bacteria Genomic DNA Kit (Transgen Biotech) was used for bacterial genomic DNA extraction, and the cetyltrimethylammonium bromide method was used for fungal genomic DNA extraction. Genomic DNA was used for PCR amplification; the primers 27F (5′-AGAGTTTGTATCTCGACGCTAG-3′) and 1492R (5′-GTTTACCTTGTTACGACTT-3′) were used for amplification of bacterial DNA, and ITS1 (5′-TCCGATAGGTCAACGC-3′) and ITS4 (5′-CCGGAAGACGCTTATGACC-3′) were used to amplify fungal DNA. The PCR products were purified and then sequenced at BioSune Biotechnology Co., Ltd (Shanghai). The sequence was aligned in GenBank using BLASTn to identify similarities and homologous strains.

Induction of microbial growth with phenolic acids
Three phenolic acids, CA (0.0066 μmol l⁻¹), FA (0.0031 μmol l⁻¹), and HA (0.168 μmol l⁻¹), were added to the culture medium to detect the induction effect of phenolic acids on the growth of Bacillus and Pseudomonas spp. The culture was sampled at 6, 12, 18, 24, and 30 h for Bacillus spp. and 12, 24, 36, 48, 60, 72, and 84 h for Pseudomonas spp. to determine the OD₆₀₀ value.

Determination of genes regulated by OsMYB57 using ChiP-seq and ChiP-qPCR
To investigate the genes potentially regulated by OsMYB57, chromatin immunoprecipitation (ChiP) was used to obtain the chromatin DNA fragment that bound to OsMYB57. The chromatin DNA was isolated from
the leaves of Kitaake rice using 1% formaldehyde to crosslink and then isolate the nuclei and chromatin. The chromatin was sheared into 200–500 bp fragments, subjected to immunoprecipitation using 20 μg of antibody to OsMYB57 or anti- IgG, and collected with DNA beads Protein A (Invitrogen). The IgG antibody bound to these DNA fragments was used as a negative control. The precipitated chromatin was decrosslinked to release the ChiP-DNA, which was purified and quantified (Qubit Fluorometer) for ChiP sequencing (ChiP-seq) library construction and ChiP-qPCR detection. For the ChiP-seq, two libraries (ChiP-DNA and input DNA) were prepared using a library preparation kit (New England Biolabs) according to the manufacturer's instructions. The libraries were sequenced using an Illumina Genome Analyzer (HiSeq 2000). The library construction and sequencing were performed by Wuhan IGENEBOOK Biotechnology Co., Ltd (http://www.igenbook.com/).

For the ChiP-qPCR, the chromatin DNA fragment bound to OsMYB57 was collected as a template. The sequences of peak 26 and peak 39 from the ChiP-seq and the promoter regions of OsPAL2;3 (LOC_Os02g41670) and OsKSL4 (LOC_Os10g00600) were selected to design the primers for ChiP-qPCR (see Supplementary Table S1). The DNA fragment bound to the IgG antibody was again used as a negative control for normalization.

Bimolecular fluorescence complementation and co-immunoprecipitation assays
To investigate the possible interactions among the proteins regulated by OsMYB57, the OsMAPK11 and OsPAL2;3 genes were cloned from PI312777. They were then used to construct recombinant vectors with the N-terminal or C-terminal domain of enhanced yellow fluorescent protein (eYFP). These vectors were then transformed into Agrobacterium (EHA105) for inoculation into the cytoplasm of tobacco (N. benthamiana) for transient expression assays. Laser scanning confocal microscopy (Leica TCS SP8X DLS, Leica Microsystems Inc.) was used to investigate positive interactions among the target proteins.

Additionally, genes fused with the FLAG tag or eYFP were constructed and transformed into Agrobacterium EHA105 for inoculation. The total fraction of soluble proteins of inoculated tobacco leaves was extracted and incubated with FLAG magnetic beads (Sigma) or GFP-Trap agarose (Chromotek) to collect putative interacting proteins. Anti-eYFP and anti-MAPK11 were used in western blotting to detect positive interactions among the proteins.

Determination of OsMYB57, OsMAPK11, and OsPAL2;3 expression
Seeds of the allelopathic rice PI312777 and the non-allelopathic rice Lemont were sterilized and germinated following the protocol described above for Kitaake, and the seedlings were grown to the three-leaf stage. Seeds of barnyardgrass were treated by using the same method and also grown to the three-leaf stage. Next, 20 uniform PI312777 or Lemont rice seedlings were co-cultured with 20 uniform barnyardgrass seedlings in a 20 litre container. Forty mono-cultured PI312777 or Lemont rice seedlings were prepared as controls under the same conditions as the treatment plants. Both the treatments and the controls were grown in a greenhouse and studied in triplicate. At 1, 3, 5, and 7 days, the top second leaf of the co-cultured or mono-cultured rice was sampled to extract the total fraction of soluble proteins, and western blotting was conducted to detect the protein expression of OsMYB57, OsMAPK11, and OsPAL2;3 using specific antibodies. The protein expression of β-actin was used as a reference.

Cloning of the OsPAL2;3 promoter and detection of its activity
The promoter of OsPAL2;3, a 3000 bp region upstream of the gene coding DNA sequence (CDS), was amplified from the genomic DNA of PI312777 and Lemont. The promoter was used to replace the Δactin2 promoter in pCambia 3301, followed by a 2×FLAG tag and GFP gene, to construct recombinant vectors for tobacco leaf and rice protoplast transformations. For tobacco, the transformed leaves were sampled to extract the proteins, and a western blot was performed using anti-FLAG antibody to determine the protein expression level of FLAG fused with GFP. For the rice protoplasts, the intensity of GFP fluorescence was detected using confocal laser scanning microscopy (Nikon C2-ER, Nikon Instruments Co., Ltd).

DNA pulldown to identify transcription factors binding at the promoter of OsPAL2;3
The total fraction of leaf soluble proteins of PI312777 and Lemont was extracted. The promoter region (3000 bp upstream of the CDS) of OsPAL2;3 was cloned from the DNA of PI312777 and Lemont, respectively, labelled with biotin at the 5′ end, and incubated with the proteins. The interacting proteins were collected using a Dynabeads kilobaseBINDER Kit (Thermo Fisher Scientific) and then separated on 10% SDS-PAGE and identified using Orbitrap Fusion (Thermo Fisher Scientific).

qPCR detection of the expression levels of transcription factors
The Whirly transcription factors, including LOC_Os06g05350 (OsWHY1) and LOC_Os02g06370 (OsWHY2), and the transcription regulator histone H4 (LOC_Os07g36300), which binds to the promoter region of OsPAL2;3, were selected to detect the level of transcripts from PI312777 and Lemont when they were co-cultured with barnyardgrass. The specific primers for OsWHY1, OsWHY2, and histone H4 are shown in Supplementary Table S1, and β-actin was used as a reference gene for normalization. The relative transcription levels of the genes of the two rice lines cultured together with barnyardgrass for 1, 3, 5, and 7 days were analysed.

Statistical analysis
All of the experiments were conducted at least three times. The results in the figures are presented as means ±SD. Statistical analyses were based on the Student's t-test.

Results
OsMYB57 expression level in OsMYB57VP64 transgenic lines and weed-suppression capacity
A western blot analysis showed that VP64 led to increased OsMYB57 expression in the transformed rice lines (OsMYB57VP64) compared with WT Kitaake (Fig. 1A). The capacity for suppression on the root length of barnyardgrass was enhanced at a ratio of approximately 20–39% for rice with increased OsMYB57 expression levels compared with a ratio of –0.4–2.3% for WT Kitaake in agar culture medium (Fig. 1B). Moreover, when grown in soil, the OsMYB57VP64 transgenic lines had a 24.7–41.9% inhibitory ratio against the fresh weight of barnyardgrass, while the WT showed a 17–19% inhibitory ratio (Fig. 1C). The significant enhancement of weed-suppression capacity in the transgenic lines suggests a positive role for OsMYB57 in the regulation of rice allelopathy.

Changes in the expression of genes participating in the monomilactone or phenolic acid pathways
The expression of genes involved in allelochemical synthesis was compared between OsMYB57VP64 and Kitaake. Four genes from the phenolic acid pathway, encoding
phenylalanine ammonia lyase (OsPAL), cinnamate 4-hydroxylase (OsC4H), O-methyltransferase (OsOMT), and cinnamoyl-CoA (OsCCA), were up-regulated by a factor of 5.76, 5.96, 17.47, and 10.24, respectively, in OsMYB57VP64 compared with Kitaake. The expression of CoA-ligases (OsCoA) and cinnamoyl alcohol dehydrogenases

| Function          | OsMYB57VP64 | Kitaake |
|-------------------|-------------|---------|
| MW                | 32 kDa      | 42 kDa  |
| OsMYB57 expression level and the allelopathic inhibition of barnyardgrass | | |
| (A) (A) The protein expression of OsMYB57 was determined from three independent OsMYB57VP64 transgenic rice lines and two groups of WT Kitaake, with the protein expression of β-actin as a reference. The allelopathic potential of OsMYB57VP64 and Kitaake on barnyardgrass was evaluated in 0.6% agar solid medium (B) and in soil (C). The ratio of rice to barnyardgrass in the co-cultured system was 1:1 (TR), and mono-cultured barnyardgrass was used as a control (CT). Inhibitory ratios (IRs) were calculated as follows: IR=(CT–TR)/CT×100%. IR >0 represents growth inhibition, and IR <0 represents growth promotion. (D) Genes involved in allelochemical synthesis and the associated differences in expression between OsMYB57VP64 and Kitaake (D). The fold change was calculated according to the formula 2−△△ct, and the gene expression of β-actin was used as a reference for normalization. For each column, significant differences in the gene expression level between OsMYB57VP64 and Kitaake are indicated with asterisks: *P<0.05, **P<0.01.

![Fig. 1. OsMYB57 expression level and the allelopathic inhibition of barnyardgrass by the OsMYB57VP64 transgenic rice lines and wild-type (WT) Kitaake rice lines. (A) The protein expression of OsMYB57 was determined from three independent OsMYB57VP64 transgenic rice lines and two groups of WT Kitaake, with the protein expression of β-actin as a reference. The allelopathic potential of OsMYB57VP64 and Kitaake on barnyardgrass was evaluated in 0.6% agar solid medium (B) and in soil (C). The ratio of rice to barnyardgrass in the co-cultured system was 1:1 (TR), and mono-cultured barnyardgrass was used as a control (CT). Inhibitory ratios (IRs) were calculated as follows: IR=(CT–TR)/CT×100%. IR >0 represents growth inhibition, and IR <0 represents growth promotion. (D) Genes involved in allelochemical synthesis and the associated differences in expression between OsMYB57VP64 and Kitaake. To compare the differences in gene expression between the rice lines, the expression of OsPAL, OsC4H, OsCoA, OsOMT, OsCCA, and OsCAD, which are involved in the phenolic acid pathway, and OsCPS4, OsMAS, and OsKSL4, which are involved in the momilactone pathway, was detected in OsMYB57VP64 and Kitaake (D). The fold change was calculated according to the formula 2−△△ct, and the gene expression of β-actin was used as a reference for normalization. For each column, significant differences in the gene expression level between OsMYB57VP64 and Kitaake are indicated with asterisks: *P<0.05, **P<0.01. |
was down-regulated in OsMYB57VP64 compared with Kitaake. The expression of the genes encoding momilactone-A synthase (OsMAS) and syn-pimara-7, 15-diene synthase (OsKSL4) from the momilactone pathway were down-regulated by a factor of 0.67 and 0.42, respectively, in OsMYB57VP64 compared with Kitaake. The expression of copalyl diphosphate synthase (OsCPS4) showed no detectable difference between the two rice lines (Fig. 1D). These results suggest that OsMYB57 positively regulated the expression of genes participating in the phenolic acid pathway.

Changes in the content of secondary metabolites

The determination of the secondary metabolites from the two rice lines identified l-phenylalanine and p-toluic acid in the OsMYB57VP64 transgenic line, but these metabolites were beyond the lower limit of detection in Kitaake (Supplementary Table S2). l-phenylalanine is the vital precursor substance of phenolic acid and OsPAL catalyses the first step in the production of phenolic acid by using l-phenylalanine as substrate.

Microbial population numbers from the rhizospheric soil of OsMYB57VP64 transgenic lines and WT rice

Allelopathic potential is well known to be correlated with rhizospheric microbes (Inderjit, 2005); thus, we determined the total amount of bacterial and fungal species in the rhizospheric soil of OsMYB57VP64 transgenic lines and WT Kitaake. The rhizospheric soil of OsMYB57VP64 transgenic lines had the largest bacterial population compared with the rhizospheric soil of Kitaake and control soil samples without rice plants. The numbers of myxobacteria have been reported to correlate with rice allelopathy (Fang et al., 2015), and these were also higher in the OsMYB57VP64 rhizospheric soil than in the rhizospheric soil of Kitaake and soil without rice plants. In addition, the population of fungi from OsMYB57VP64 rhizospheric soil was higher than that of the other two samples, but no significant difference was found between OsMYB57VP64 and Kitaake (Fig. 2A).

Isolation of microorganisms from soil and associated weed-suppression capacities

Rhizospheric soil from the OsMYB57VP64 transgenic lines and Kitaake was sampled to isolate the culturable micro-organisms.
A total of 31 strains were obtained from Kitaake, and 35 strains were isolated from the OsMYB57VP64 transgenic lines. The allelopathic potential of these micro-organisms to barnyardgrass was evaluated, and 32 out of 35 strains isolated from the rhizospheric soil of OsMYB57VP64 transgenic lines showed weed-suppression capacity (Supplementary Fig. S1A). The inhibition ratios to barnyardgrass were greater than 30% in six strains; the highest inhibition ratio was 44.1% (Fig. 2B). In contrast to the transgenic lines, 16 out of 31 WT strains showed suppression capacity on barnyardgrass (Supplementary In Fig. S1B); however, only one strain had an inhibitory ratio of more than 30% (Fig. 2B). These results indicate that OsMYB57VP64 in rice stimulated the accumulation of potentially allelopathic microorganisms in the rhizospheric soil. To further investigate these microorganisms, the genomic DNA of each strain with high allelopathic potential was extracted, and the 16S rRNA or ITS region was amplified for sequencing. Two Pseudomonas spp., two Penicillium spp., one Bacillus spp., and one Streptomyces spp. were identified from the rhizospheric soil of the OsMYB57VP64 transgenic lines. Only one species, a Streptomyces spp., was isolated from the WT (Supplementary Table S3; Supplementary Dataset S1). The accumulation of microbes with high allelopathic potential in the rhizospheric soil of OsMYB57VP64 rice further indicated the involvement of OsMYB57VP64 in the regulation of allelopathic microbial diversity.

**Effect of phenolic acids on microbial growth**

The Streptomyces viridobrunneus strain SCPE-09 and the Bacillus spp. were selected to study the effect of phenolic acids on growth and proliferation. The phenolic acids CA, FA, and HA were added to the culture medium and the effects on the OD600 value of the culture were measured. The results showed that, individually, CA, FA, and HA promoted the growth and proliferation of S. viridobrunneus strain SCPE-09. After being cultured in the presence of phenolic acid for 24–60 h, the OD600 of the phenolic acid-supplemented culture medium was significantly higher than that of the medium without phenolic acids, indicating the growth-inducing effect of phenolic allelochemicals on S. viridobrunneus strain SCPE-09 (Fig. 2C). A similar growth-inducing effect was evident for Bacillus spp. during the period of 30–36 h (Fig. 2D).

**Genes transcriptionally regulated by OsMYB57**

Transcription factors function by binding to the promoter region of genes to regulate gene expression. In this study, the chromatin DNA that bound OsMYB57 was obtained using ChIP. From sequencing and alignment, a total of 75 peaks were obtained (Supplementary Dataset S2). Fifteen motifs were predicted from these peaks (Supplementary Table S4), which were significantly enriched by OsMYB57 in comparison with the anti-IgG control. On the basis of the annotation, it was shown that OsMYB57 functions in the regulation of the promoter region of genes including retrotransposon protein (OsRTP, LOC_Os02g23820), mitogen-activated protein kinase 11 (OsMAPK11, LOC_Os06g26340), and OsWRKY66 (LOC_Os02g47060) (Table 1). For LOC_Os02g23820, peak 25 was annotated in the promoter region of the gene, while there were three peaks (peaks 39, 40, and 41) annotated in the promoter region of LOC_Os06g26340. These results indicate that OsMYB57 plays a role in the regulation of OsMAPK11, OsRTP, and OsWRKY66 expression. Further prediction of the motifs from peaks 26, 39, 40, and 41 showed that 10 motifs were included in the peak (Fig. 3A), and these predicted motifs indicated the binding sites of OsMYB57 on the transcription regulation region of the promoter.

**Enrichment of the promoter fragment by OsMYB57**

ChIP-qPCR was conducted to determine the fold enrichment on the gene promoter regions by OsMYB57 and to validate and provide further insight into the efficiency of OsMYB57 in combination with the promoter of OsRTP (LOC_Os02g23820) OsMAPK11 (LOC_Os06g26340). The promoters OsPAL2;3 (LOC_Os02g41670) and OsKSL4 (LOC_Os04g10060), which are involved in phenolic acid and momilactone B synthesis, were also evaluated using ChIP-qPCR. The enrichment of the promoter region on OsRTP (peak 25), OsMAPK11 (peak 39), OsPAL2;3, and OsKSL4 increased by 10.66–, 3.60–, 1.86–, and 2.00–fold, respectively (Fig. 3B). These findings validate the transcriptional regulatory capacity of OsMYB57 on OsRTP and OsMAPK11. As the enrichment on OsPAL2;3 and OsKSL4 was no more than 2-fold, OsMYB57 showed no direct regulation of these promoter regions.

**Interactions between OsMAPK11 and OsPAL2;3**

To investigate whether OsMAPK11 interacted with OsPAL2;3 to regulate phenolic acid synthesis, bimolecular fluorescence complementation (BiFC) was conducted. Based on the fluorescence signal observed by laser confocal microscopy in tobacco leaf cells when OsMAPK11 was fused with either the N-terminal domain or the C-terminal domain of YFP, it was determined that OsMAPK11 interacted with OsPAL2;3 in the cytoplasm. In contrast, no fluorescence signal was observed from the control groups (Fig. 4A). Co-immunoprecipitation (Co-IP) was simultaneously conducted to validate the interaction of OsMAPK11 with OsPAL2;3. According to a western blot analysis, the proteins collected by FLAG beads and then immunostained with anti-eYFP showed positive bands in the film, indicating positive interactions between OsMAPK11 and OsPAL2;3. Similarly, the proteins collected by GFP-Trap beads were detected using anti-OsMAPK11 antibody; the results of Co-IP and western blotting therefore further validated the positive interaction between OsMAPK11 and OsPAL2;3 (Fig. 4B). Taken together, the results of BiFC and Co-IP indicated that OsMAPK11 interacted with OsPAL2;3 in vivo.

**Expression of OsMYB57, OsMAPK11, and OsPAL2;3 in PI312777 and Lemont rice**

To investigate how OsMYB57, OsMAPK11, and OsPAL2;3 were correlated with allelopathic regulation, the expression
of these three proteins in the allelopathic rice PI312777 and the non-allelopathic rice Lemont co-cultured at a ratio of 1:1 with barnyardgrass and in mono-culture was evaluated. OsMYB57 was expressed in PI312777 co-cultured with barnyardgrass but was hardly detected from mono-cultured PI312777, or from either co-cultured or mono-cultured Lemont. OsMAPK11 and OsPAL2;3 were also expressed to a much higher level in PI312777 co-cultured with barnyardgrass compared with mono-cultured PI312777, indicating that the expression of these proteins was induced
Fig. 4. BiFC and Co-IP validation of the interaction between OsMAPK11 and OsPAL2;3. (A) OsMAPK11 and OsPAL2;3 were fused with the N-terminal domain and the C-terminal domain of YFP. The two genes with different YFP domains were then co-expressed in the leaves of Nicotiana benthamiana for 48 h, and YFP fluorescence was detected using laser confocal microscopy under an excitation wavelength of 488 nm. Bar=50 μm. (B) Validation by Co-IP of the interaction between OsMAPK11 and OsPAL2;3. Recombinant vectors of OsMAPK11 fused with FLAG and OsPAL2;3 fused with eYFP were constructed and transformed into tobacco leaves to co-express the two proteins. The total fraction of soluble proteins from the infected leaves was harvested and extracted. Proteins that interacted with OsPAL2;3 or OsMAPK11 were co-precipitated using GFP-Trap magnetic agarose and anti-FLAG M2 magnetic beads and separated by SDS-PAGE. Antibodies to eYFP and OsMAPK11 were used to detect the interaction between OsPAL2;3 and OsMAPK11. (C) Expression of OsMYB57, OsMAPK11, and OsPAL2;3 in the allelopathic rice PI312777 and the non-allelopathic rice Lemont. PI312777 and Lemont were co-cultured with barnyardgrass (BYG) at a ratio of 1:1 for 1, 3, 5, and 7 days; mono-cultured PI312777 and Lemont were used as controls. The expression of OsMYB57, OsMAPK11, and OsPAL2;3 protein was detected in each rice line; the expression of β-actin was used as a reference.
by the presence of barnyardgrass. By contrast, little expression was observed in either co-cultured or mono-cultured Lemont (Fig. 4C).

Transcriptional activity of OsPAL2;3 in PI312777 and Lemont

As the first key enzyme in the phenylpropanoid pathway, OsPAL2;3 plays a vital role in the regulation of phenolic acid synthesis. We therefore compared the transcriptional activity of OsPAL2;3 in the allelopathic rice PI312777 and the non-allelopathic rice Lemont. The detection of FLAG- and GFP-fusion protein expression via western blotting and the observation of GFP fluorescence, which was driven by the OsPAL2;3 gene promoter from PI312777 and Lemont, via laser scanning confocal microscopy were used to detect the transcriptional activity of OsPAL2;3. There were higher levels of FLAG- and GFP-fused protein expression in leaves transformed with recombinant vector containing the OsPAL2;3 gene promoter from PI312777 compared with those containing the OsPAL2;3 gene promoter from Lemont (Fig. 5A). There was also a higher intensity of GFP fluorescence in rice protoplasts transformed with the recombinant vector containing the OsPAL2;3 gene promoter from PI312777 (Fig. 5B), which provides further evidence of the higher transcriptional activity of OsPAL2;3 from PI312777 compared with Lemont.

Transcription factors binding to the promoter of OsPAL2;3 and their gene expression levels

As different levels of transcriptional activity of OsPAL2;3 were detected between PI312777 and Lemont, the binding of transcription factors to the promoter region of OsPAL2;3 was investigated using the DNA pulldown method. Among the proteins identified, several with DNA-binding capacity were obtained, including histones H1 and H4. Transcriptional regulators were also identified from the promoters of both PI312777 and Lemont. In contrast, two Whirly transcription factor domain-containing proteins (OsWHY; LOC_Os02g06370 and LOC_Os06g05350) were identified from the OsPAL2;3 promoter of Lemont but not from PI312777 (Fig. 6A, B; Table 2).

Fig. 5. Differences in the transcriptional activity of the OsPAL2;3 gene promoter from PI312777 and Lemont. (A) To compare the transcriptional activity of the promoter of OsPAL2;3 from PI312777 and Lemont, the promoter region of OsPAL2;3 was cloned from PI312777 and Lemont and fused with a FLAG tag and GFP to construct recombinant vectors, which were transformed into Agrobacterium EHA105 and then injected into tobacco leaves. (B) The two recombinant vectors were also transformed into rice protoplasts and the GFP fluorescence intensity was detected by laser scanning confocal microscopy.
Functions of MYB57 in modulated rice allelopathy

Gene expression levels of OsWHY and histone H4 in PI312777 and Lemont

To confirm the role of OsWHY (LOC_Os02g06370 and LOC_Os06g05350) and histone H4 in the regulation of promoter activity, the gene expression levels of these transcriptional regulators were detected in PI312777 and Lemont co-cultured with barnyardgrass for 1, 3, 5, and 7 days and compared with their respective control groups. Barnyardgrass induced substantially increased expression of OsWHY1 (LOC_Os06g05350), OsWHY2 (LOC_Os02g06370), and histone H4 (LOC_Os07g36500) in Lemont, while these genes showed little up- or down-regulation in PI312777 (Fig. 6C, D). As the OsPAL2;3 promoter was more active in PI312777 than in Lemont, the three DNA-binding proteins were considered to negatively regulate the transcription of OsPAL2;3.

Discussion

The application of crop allelopathic traits to control weeds in farmland has recently received more attention and is recognized as a viable option for sustainable and eco-friendly agriculture (Jabran et al., 2015; Aslam et al., 2017; Mwendwa et al., 2018; Chung et al., 2018; Farooq et al., 2019; Macías et al., 2019). Allelopathy relies on the synthesis and secretion of allelochemical compounds by donor plants. Most of these compounds are categorized as secondary metabolites (Lotina-Hennsen et al., 2006). The synthesis of the metabolites is controlled by the expression of structural genes, such as OsPAL, OsKSL4, and OsCPS4 in rice. These metabolites are involved in pathways that ultimately determine the weed-suppression capacity of rice (Xu et al., 2012; Fang et al., 2013). Some special transcription factors, that is, MYB proteins, are also able to modulate the intensity of the phenylpropanoid pathway (Borevitz et al., 2000; Liu et al., 2015a; Deluc et al., 2006); however, few studies have investigated the correlations among transcription factors and allelopathy traits.

The findings of the present study indicate that the transcription factor OsMYB57 functions in the regulation of rice allelopathy, because the allelopathic inhibitory ratios against barnyardgrass increased with increasing OsMYB57 expression in rice (Fig. 1A–C). Rice MYB contains at least 185 multi-gene members (Chen et al., 2006; Katiyar et al., 2012, and OsMYB57 belongs to the R2R3 subfamily. The R2R3-MYB subfamily has been shown to participate in the regulation of phenylpropanoid metabolism (Liu et al., 2015a). Our study showed that the expression of genes involved in phenylpropanoid metabolism, including OsPAL,
OsC4H, OsOMT, and OsCCA, was up-regulated in rice with enhanced OsMYB57, in contrast to the gene expression levels of OsKSL4, OsMAS, and OsCPS4, which are involved in the momilactone pathway (Fig. 1D). OsMYB57 likely played a positive role in the regulation of genes involved in phenylpropanoid metabolism. Accordingly, L-phenylalanine, which is the precursor substance for phenylpropanoid metabolism, accumulated in the OsMYB57VP64 transgenic line which is the precursor substance for phenylpropanoid metabolism.

In the response of rice to weeds, OsMAPK11 was predicted to be involved in the regulation of rice allelopathy against barnyardgrass through its interaction with OsPAL2;3, as evidenced by Co-IP (Fig. 4A, B). The expression levels of OsMAPK11 and OsPAL2;3 protein were higher in the allelopathic rice PI312777 than in the non-allelopathic Lemont rice (Fig. 4C), while the allelopathic inhibitory ratios of PI312777 against barnyardgrass were higher than those of Lemont (Song et al., 2008, 2012, 2018; He et al., 2012a, b; Fang et al., 2015, Zhang et al., 2018, 2019). Because PAL is a multi-gene family in plants, OsPAL2;3 is one of four gene members located on chromosome 2 (OsPAL2;1, OsPAL2;2, OsPAL2;3, and OsPAL2;4), and stronger transcriptional activity of OsPAL2;3 was observed in PI312777 than in Lemont (Fig. 5A, B). Our previous studies showed that silencing the expression of OsPAL results in weakened allelopathic activity from the donor rice to the barnyardgrass (Fang et al., 2013). The high activity of OsPAL2;3 in the allelopathic rice was considered to contribute mainly to the increased synthesis of phenolic acid in rice plants. The transcriptional activity of genes relied on their transcription factors, while the interacting proteins acted to activate the enzyme capacity. In this study, the differences in transcriptional activity of OsPAL2;3 from PI312777 or Lemont depended on the different types of proteins binding to the promoter region of OsPAL2;3. Among these proteins, OsWhirly transcription factors (LOC_Os06g05350 and LOC_Os02g06370) were identified only from the promoter of Lemont (Table 2). These proteins were considered to negatively regulate the transcription of OsPAL2;3 because the expression levels of the genes encoding these two proteins were greatly induced in Lemont, while little change was observed in PI312777 (Fig. 6C, D). The Whirly transcription factors are well known to modulate the rate of gene transcription by binding to the DNA of promoter or enhancer regions of specific genes, and ChIP is a powerful technology to obtain the DNA region regulated by transcription factors (Carey et al., 2009; Nützmann and Osborn, 2015; Jarillo et al., 2018). In this study, a combination of ChIP-seq and ChIP-qPCR revealed that OsMYB57 transcriptionally regulates the expression of the genes OsMAPK11, retrotransposon protein, and OsWRKY66 (Table 1). The MAPK cascade is a highly conserved family of serine/threonine protein kinases involved in a variety of fundamental cellular processes, such as proliferation, differentiation, motility, stress response, apoptosis, and survival, and it is also a signal factor that amplifies the signal and mediates a broad range of biological processes (Nakagami et al., 2005; Pedley and Martin, 2005). MAPK plays a vital role in transducing extracellular stimuli into intracellular responses in eukaryotic organisms, and 17 gene members have been identified from the rice genome (Rohila and Yang, 2007). Among these multi-gene members, OsMAPK11, which was up-regulated in rice with enhanced OsMYB57, is a highly conserved family of serine/threonine protein kinases involved in the regulation of genes involved in phenylpropanoid metabolism. Accordingly, L-phenylalanine, which is the precursor substance for phenylpropanoid metabolism, accumulated in the OsMYB57VP64 transgenic line which is the precursor substance for phenylpropanoid metabolism.

### Table 2. Proteins binding on the OsPAL2;3 gene promoter from PI312777 and Lemont

| Accession | Description | Sum PEP score | Coverage | Peptides | PSMs | Unique peptides |
|-----------|-------------|---------------|----------|----------|------|----------------|
| LOC_Os06g05350.1 | Whirly transcription factor domain-containing protein, expressed | 44.334 | 50.36765 | 14 | 28 | 14 |
| LOC_Os02g06370.1 | Whirly transcription factor domain-containing protein, expressed | 28.059 | 36.40351 | 6 | 14 | 6 |
| LOC_Os04g42320.1 | AT hook motif family protein, expressed | 14.505 | 9.456265 | 9 | 13 | 9 |
| LOC_Os05g51850.1 | AT hook-containing DNA-binding protein, putative, expressed | 8.184 | 15.24164 | 6 | 7 | 6 |
| LOC_Os07g08710.1 | AT hook-containing DNA-binding protein, putative, expressed | 8.048 | 17.26619 | 6 | 9 | 6 |
| LOC_Os04g58730.1 | AT hook motif domain-containing protein, expressed | 11.839 | 25.05967 | 5 | 9 | 5 |
| LOC_Os08g040150.1 | AT hook motif domain-containing protein, expressed | 14.021 | 22.0339 | 4 | 6 | 3 |
| LOC_Os02g31160.1 | Transcription factor, putative, expressed | 10.902 | 13.63636 | 4 | 4 | 4 |
| LOC_Os09g27850.1 | Transcription regulator, putative, expressed | 5.362 | 6.136364 | 4 | 4 | 4 |
| LOC_Os06g04020.1 | Histone H1, putative, expressed | 3.708 | 11.66667 | 3 | 3 | 3 |
| LOC_Os07g36500.1 | Histone H4, putative, expressed | 4.883 | 21.35922 | 2 | 2 | 2 |
| LOC_Os06g04020.1 | Histone H1, putative, expressed | 4.945 | 20.41667 | 4 | 4 | 4 |
| LOC_Os07g36500.1 | Histone H4, putative, expressed | 3.038 | 19.41748 | 2 | 2 | 2 |
| LOC_Os07g08710.1 | AT hook-containing DNA-binding protein, putative, expressed | 17.578 | 19.42446 | 4 | 18 | 4 |
| LOC_Os04g58730.1 | AT hook motif domain-containing protein, expressed | 11.217 | 10.02387 | 3 | 5 | 3 |
| LOC_Os04g42320.1 | AT hook motif family protein, expressed | 4.684 | 3.309693 | 3 | 3 | 3 |
| LOC_Os09g27850.1 | Transcription regulator, putative, expressed | 3.897 | 4.545455 | 3 | 3 | 3 |
| LOC_Os08g040150.1 | AT hook motif domain-containing protein, expressed | 5.682 | 13.27684 | 2 | 2 | 2 |
Factors from Arabidopsis and potato play roles in the regulation of defence gene expression (Desveaux et al., 2005), and Whirly was considered a negative regulator for the control of the rice-induced hypersensitive response (Yao et al., 2008). The hypersensitive response was detectably induced in the OsWHY-RNAi transformed rice cells after inoculation with a non-host pathogen bacterium (Yao et al., 2008). Additionally, the histone H4 that was identified among the promoter–binding proteins from both PI312777 and Lemont also showed higher expression in Lemont than in PI312777 (Fig. 6C, D; Table 2). The deacetylation of histones is often correlated with transcriptional repression (Liu et al., 2014; Zhao et al., 2015). High expression of histone H4 has a negative role in the regulation of OsPAL2;3 gene expression, which represses phenolic acid synthesis in rice.

Phenolic acids that are secreted from the roots of allelopathic rice interfere with the growth of weeds (i.e. barnyardgrass) and interact with the soil microorganisms in the rhizosphere (Fang et al., 2013). Our previous studies indicated that phenolic acids, including FA, CA, and HA, induce the growth and proliferation of bacteria in the culture medium of rice/weed systems, which promote weed-suppression capacity on barnyardgrass. Some species, for example, the myxobacterial species M. xanthus, can be induced by FA, and in a previous study the cooperation of FA and M. xanthus led to the highest allelopathic inhibition of barnyardgrass compared with treatment with either FA or M. xanthus (Fang et al., 2015). The current study demonstrated that increasing the gene expression of OsMYB57 resulted in a higher population of myxobacteria and, indeed, an increase in the entire bacterial population in the rhizospheric soil (Fig. 2A). By contrast, the population of fungi was only slightly increased in OsMYB57 VP64 rhizospheric soil (Fig. 2A). The Bacillus spp., Pseudomonas spp., and S. viridobrunneus isolated from rhizospheric soil showed allelopathic potential against the growth of barnyardgrass (Fig. 2B). These micro-organisms, which can be induced by FA, CA, and HA (Fig. 2C, D), are considered to have positive roles in the process of inhibition of weed growth by rice. Increasing OsMYB57 expression in rice also changed the rhizospheric community of micro-organisms, and these micro-organisms can act as a link between phenolic allelochemicals and their bio-activity in the soil.

In conclusion, OsMYB57 acts as a positive transcription factor in the regulation of OsPAL2;3 gene expression, which is dependent on transcriptional regulation of the expression of OsMAPK11. OsMAPK11 protein then interacts with OsPAL2;3 to regulate the activity of OsPAL2;3. The allelopathic rice PI312777 had a higher transcriptional ability of OsPAL2;3 than the non-allelopathic rice Lemont. OsWHY and histone H4 appear to be negative factors that regulate OsPAL2;3 transcripts (Fig. 7). These findings may provide researchers with more options for the improvement of rice allelopathic traits by genetic modification.
Supplementary data

Supplementary data are available at JXB online.

Table S1. Primers used in this study.

Table S2. The relative contents of secondary metabolites detected from the root tissue of OsMYB57(V64) and Kitaake.

Table S3. High allelopathic microbes isolated from the rhizospheric soil of OsMYB57(V64) and Kitaake.

Table S4. Motifs from the sequence of peaks.

Fig. S1. The allelopathic inhibitory ratios to barnyardgrass from specific microbial strains isolated from the rhizospheric soil of Kitaake and OsMYB57(V64).

Dataset S1. Sequence of 16srDNA or ITS from the specific microbial strains with allelopathic inhibitory ratios to barnyardgrass.

Dataset S2. Sequence of peaks from ChIP-seq.

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