Background: Rice (Oryza sativa) feeds half of the world’s population. Rice grain yield and quality which are constrained by diseases and mineral nutrition have important human healthy impacts. Plant “fruit-weight 2.2-like” (FWL) genes play key roles in modulating plant fruit weight, organ size and iron distribution. Previous work has uncovered that the grains of OsFWL5-oeverexpressing rice accumulated more beneficial element zinc (Zn) and less toxic element cadmium (Cd) content. However, whether FWL genes play roles in rice resistance remains unknown.

Findings: Here, we validated that one of rice FWL genes OsFWL5 plays a positive role in defense to Xanthomonas oryzae pv. oryzae (Xoo). Overexpresion of OsFWL5 promotes H2O2 accumulation and cell death. The OsFWL5-overexpresing plants show activated flg22-induced reactive oxygen species (ROS) generation, and increased resistance to Xoo, indicating that OsFWL5 functions to increase pathogen-associated molecular pattern (PAMP)-triggered immunity in rice. The activated defense response is associated with increased the expression of genes involved in jasmonic acid (JA)-related signaling. Furthermore, Cd can induce rice resistance to Xoo, and OsFWL5 is required for Cd-induced rice defense response.

Conclusion: Putting our finds and previous work together, OsFWL5 could be a candidate gene for breeders to genetically improve rice resistance and grain quality.
with Xoo strain PXO341. MKbZH1 carried a transgenic major disease resistance gene Xa3/Xa26 in the genetic background of japonica/geng variety Zhonghua 11 (ZH11) conferring race-specific resistance to Xoo including to strain PXO341, wild type ZH11 is susceptible to Xoo strain PXO341 (Cao et al., 2007; Gao et al., 2010; Li et al., 2012). OsFWL5 showed differential expression patterns in rice resistant and susceptible interactions (Additional file 1: Figure S1). The transcript level of OsFWL5 was lower in MKbFZH1 relative to wild type before Xoo inoculation, while higher transcript level of OsFWL5 was observed in resistant plants than in susceptible plants at 4, 8, 24, 48 and 72 h after Xoo infection. The differential expression patterns of OsFWL5 in susceptible and resistant response in the same genetic background indicated that OsFWL5 might be involved in the rice-Xoo interaction.

We then generated OsFWL5-overexpressing plants (OsFWL5-oe) by transforming ZH11 with OsFWL5 cDNA under the control of maize ubiquitin (Ubi) promoter. The OsFWL5-oe plants displayed a spontaneous lesion mimic (LMM) phenotype from seedling stage, and developed more serious LMM at adult stage (Fig. 1a). Many LMM show an accumulation of reactive oxygen species ROS (including H2O2) in and around lesions (Lorrain et al., 2003). To test whether the lesions of OsFWL5-oe plants accumulate H2O2, we stained the leaves of OsFWL5-oe plants with diaminobenzidine (DAB) revealing a strong accumulation of H2O2 in OsFWL5-oe plants relative to WT (Fig. 1b). The appearance of LMM in OsFWL5-oe plants promotes us to check the expression of cell death related gene. Rice NAC4 (a plant-specific transcription factor) positively regulates programmed cell death (PCD) and activation of NAC4 expression promotes PCD (Kaneda et al., 2009). The expression of NAC4 were up-regulated in OsFWL5-oe plants (Fig. 1c). These results indicate that overexpression of OsFWL5 promotes H2O2 accumulation and cell death.

Upon pathogen infection, the recognition of PAMPs by the pattern recognition receptors (PRRs) triggers PAMP-triggered immunity (PTI) and includes the accumulation of ROS (Jones and Dangl, 2006). Rice cells can recognize bacterial pathogen PAMP elicitor flg22 through the PRR FLS2 (Takai et al., 2008). Mutations resulting in constitutive expression of defense mechanisms cause spontaneous lesions. To examine whether overexpression of OsFWL5 affects ROS production after PAMP elicitor flg22 treatment, we collected leaves from the OsFWL5-oe and WT plants and measured the ROS level after flg22 treatment using a ROS inhibition assay (Schwacke and Hager, 1992). Tissues of 4-week-old rice leaves exhibited a ROS burst when they were exposed to flg22 (Fig. 1d). In OsFWL5-oe plants, the flg22-induced ROS generation was earlier and higher than that in WT. These data suggested that overexpressing OsFWL5 enhances rice PAMP-triggered immune response.

We further inoculated OsFWL5-oe plants with Xoo strain PXO341 at the booting (panicle development) stage. The OsFWL5-oe plants showed increased resistance to Xoo strain PXO341 compared to WT plants (Fig. 1e; 1f), with the lesion length ~ 0.5 cm for OsFWL5-oe transgenic positive plants versus ~ 11.0 cm for negative transgenic plants and WT. The increased resistance of OsFWL5-oe plants co-segregated with increased OsFWL5 transcripts. The correlations between length and OsFWL5 transcripts were ~ 0.926 (significant at α = 0.01; n = 15) and ~ 0.8993 (significant at α = 0.01; n = 15) for OsFWL5-oe93 and OsFWL5-oe95 families, respectively. Bacterial growth analysis showed that the growth rate of PXO341 on transgenic plants was significantly lower than the growth rate on WT plants at 4–12 days after infection. These results suggest that the increased resistance of the transgenic plants may be attributable to the increased expression level of OsFWL5.

To further investigate the role of OsFWL5 in rice-Xoo interaction, we generated OsFWL5-knockout mutants osfwl5 using CRISPR/Cas9 editing in ZH11. We selected two 20-nt sequences as target sites for Cas9 cleavage with one in the 5’ UTR and another one in the first exon of OsFWL5 gene (Additional file 1: Figure S2). We found two mutant lines osfwl5–1 and osfwl5–2. osfwl5–1 carries a 242-base fragment deletion in 5’ UTR and one-base insertion in site 2; osfwl5–2 carries a 678-base fragment deletion from site 2 to 5’ UTR of OsFWL5 gene (Additional file 1: Figure S2). We inoculated osfwl5 lines with Xoo strain PXO341 at booting stage. osfwl5 lines developed similar lesion length as WT (Additional file 1: Figure S3a), indicating that OsFWL5 is not necessary for Xoo resistance in rice. Together with the results from the above analysis, these data suggested that OsFWL5 contributes to rice resistance by activating rice basal defense.

The enhanced resistance of OsFWL5-oe plants promoted us to check the expression of defense-related genes to dissect possible defense pathways mediated by OsFWL5. AOS2 (allene oxide synthase 2; AJ062258) is involved in JA biosynthesis, JAZ8 (jasmonate ZIM-domain protein; XP_015612402) associates with the JA-dependent signaling pathway (Mei et al., 2006; Ke et al., 2014), WRKY13 antagonistically regulates salicylic acid (SA)- and JA-dependent signal pathway acting as a positive regulator in SA-dependent and a negative regulator in JA-dependent signal pathway, ICS1 (isorhizomate synthase 1, AK120689) is involved in SA biosynthesis (Qiu et al., 2007), PR1a (for acidic pathogenesis-related protein 1; AJ278436) is a SA and JA responsive gene (Ke et al., 2014). The expression levels of AOS2, JAZ8 and PR1a were significantly higher in OsFWL5-oe plants than those in WT (Fig. 2a). By contrast, the expression levels of WRKY13 and ICS1 were significantly lower in OsFWL5-oe plants than those in WT (Fig. 2a). We also checked the expression of these genes in osfwl5 mutants.
plants, and results showed that osfwl5 mutants plants accumulate similar AOS2, JAZ8, PR1a and ICS1 transcripts, and slightly more WRKY13 transcripts relative to wild type (Additional file 1: Figure S3b). These data indicated that overexpression of OsFWL5 promotes defense response associated with activated JA-dependent pathway but repressed SA-dependent pathway.

As OsFWL5 is involved in grain Cd distribution (Song et al., 2015), we treated wild type ZH11 with Cd to analyze OsFWL5 expression. Result showed that Cd treated plants
accumulated more OsFWL5 transcripts than mock treated plants did (Fig. 2b), indicating OsFWL5 expression is induced by Cd. Overexpressing OsFWL5 activates JA-dependent related signaling, promoting us to test JA-signaling related genes expression after Cd treatment. We analyzed AOS2 and PR1a expression and this analysis showed that Cd could induce AOS2 and PR1a expression (Fig. 2c). Cd treatment promotes ROS accumulation in pea plant (Romero-Puertas et al., 2002). These data suggest that Cd might induce plant defense response. To test this inference, we treated OsFWL5-oe, osfwl5 mutants and WT with Cd and inoculated with Xoo. Results showed that Cd induced wild type ZH11 resistance to Xoo (Fig. 2d). Cd did not further increase OsFWL5-oe resistance to Xoo, although OsFWL5-oe plants accumulated more AOS2 and PR1a transcripts relative to wild type after Cd induction (Fig. 2d; 2e). One of the possible reasons is that OsFWL5-oe plants show high resistance to Xoo with the lesion length less than 0.5 cm. Cd induced resistance, AOS2 and PR1a expression was impaired in osfwl5 mutants (Fig. 2d; 2f). These results suggested that OsFWL5 is required for Cd-induced defense response.

The amino acid sequence of OsFWL5 from ZH11 is identical to that from another geng/japonica variety Nipponbare (Additional file 1: Figure S4). The sequence diversity of OsFWL5 from gene/japonica-type accessions and jing/indica-type accessions is correlated with Zn content in both rice and yeast cells, while yeast cells accumulate similar Cd concentrations expressing both types of OsFWL5 (Song et al., 2015). In this study, OsFWL5 mediated rice defense may

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Fig. 2 OsFWL5 affects a set of Pathogenesis-related genes expression and rice response to Cd. ** and * indicate significant differences between Cd treatment and mock treatment at $P < 0.01$ and $P < 0.05$, respectively. The “a” and “b” above bars indicate significant differences compared to wild type (WT) at $P < 0.01$ and $P < 0.05$, respectively. Data are means ± SD ($n$ = 3 for gene expression, and 5 to 15 for lesion length). Primers and methods are listed in additional files 2 and 3. a OsFWL5-oe plants accumulate more JA signaling involved genes AOS2, JAZ8 and PR1a transcripts, and less SA signaling involved genes ICS1 and WRKY13 transcripts. b OsFWL5 expression was induced by Cd treatment. c OsFWL5-oe plants accumulate more AOS2 and PR1a transcripts (e), while osfwl5 plants accumulate less AOS2 and PR1a transcripts (f) after Cd treatment.

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be associated with Cd, suggesting that OsFWL5 from jing indica-type accessions might also play a role in rice resistance. Further studies are needed to provide insight on this perspective.

In conclusion, in this study we have confirmed the novel function of rice OsFWL5. Activation of OsFWL5 expression in rice triggers H₂O₂ accumulation and cell death. We further demonstrated that OsFWL5 positively regulates PTI response and disease resistance. In addition, OsFWL5 is required for Cd-induced defense response. The grains of OsFWL5-oeverexpressing rice accumulated more beneficial element Zn and less toxic element Cd content (Song et al., 2015). So breeders can use OsFWL5 for rice genetic improvement through screening alleles with optimal expression level.

Additional files

**Additional file 1:** Figure S1. Expression patterns of OsFWL5 in rice susceptible and resistant reactions. Figure S2. osfwl5 genotype characterization. Figure S3. Performance of osfwl5 plants. Figure S4. Comparison of OsFWL5 amino acid sequences. (PPTX 103 kb)

**Additional file 2:** Table S1. PCR primers used for construction of vectors, detection of positive transgenic plants, mutant analysis, and sequencing. Table S2. Primers used for quantitative PCR in gene expression analysis. (DOC 43 kb)

**Additional file 3:** Materials and Methods. (DOC 37 kb)

**Abbreviations**

AOS2: allene oxide synthase 2; FWL: fruit-weight 2.2-like; ICS1: isochorismate synthase 1; JA: jasmonic acid; IAZ: jasmonate ZIM-domain protein; PR1a: acidic pathogenesis-related protein 1; PTI: pathogen-associated molecular pattern (PAMP)-triggered immunity; ROS: reactive oxygen species; SA: salicylic acid; Xoo: Xanthomonas oryzae pv. oryzae

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**Authors’ contributions**

BL and SYS designed and performed most of the experiments, analyzed the data, drafted the manuscript; XMG analyzed OsFWL5 sequence diversity; MXW and YD helped to generate transgenic rice plants and pathogen inoculation; QZ, JX, and XL provided biochemical and molecular analysis data, drafted the manuscript; XMG analyzed OsFWL5 sequence diversity; BL and SYS designed and performed most of the experiments, analyzed the data, revised the manuscript. All authors read and approved the final manuscript.

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**Availability of data and materials**

All data generated or analyzed during this study are included in this published article [and its supplementary information files].

**Ethics approval and consent to participate**

Not applicable.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

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