Phosphorylation-guarded light-harvesting complex II contributes to broad-spectrum blast resistance in rice

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Environmental conditions are key factors in the progression of plant disease epidemics. Light affects the outbreak of plant diseases, but the underlying molecular mechanisms are not well understood. Here, we report that the light-harvesting complex II protein, LHCB5, from rice is subject to light-induced phosphorylation during infection by the rice blast fungus *Magnaporthe oryzae*. We demonstrate that single-nucleotide polymorphisms (SNPs) in the *LHCB5* promoter control the expression of *LHCB5*, which in turn correlates with the phosphorylation of LHCB5. LHCB5 phosphorylation enhances broad-spectrum resistance of rice to *M. oryzae* through the accumulation of reactive oxidative species (ROS) in the chloroplast. We also show that LHCB5 phosphorylation-induced resistance is inheritable. Our results uncover an immunity mechanism mediated by phosphorylation of light-harvesting complex II.

light-harvesting complex II | phosphorylation | *Magnaporthe oryzae* | broad-spectrum resistance

Environmental conditions strongly influence pathogen–host plant interactions, and changes in a range of environmental factors can directly determine the outcomes of such interactions. In the 1960s, George McNew proposed a "disease triangle" concept, comprising the host, pathogen, and environment as the 3 fundamental components that affect crop diseases. Many studies have subsequently demonstrated that environmental conditions, such as light, temperature, and humidity, can affect disease development and plant innate immunity. For example, the wheat (*Triticum aestivum*) *Yr36* gene confers broad-spectrum resistance to stripe rust at high temperatures, but not at low temperatures (1), while moderate temperatures have been shown to enhance pathogen-associated molecular patterns (PAMPs)-triggered immunity (PTI) in *Arabidopsis thaliana* (2). Other studies have shown that high humidity promotes bacterial infection by also modulating plant immunity (3). Rice (*Oryza sativa*) blast caused by *Magnaporthe oryzae* is often triggered by the overcast weather and lack of sunlight conditions. A study documenting rice blast of over 55 y in Jiangsu, China, showed that fewer hours of sunlight directly correlated with the seriousness of the blast disease (4, 5). However, the underlying molecular mechanisms linking light to infection are not well understood.

Notably, chloroplasts are not only the organelles where light sensing and photosynthesis take place, but also the site of biosynthesis of defense-related molecules, such as salicylic acid (SA) and jasmonic acid (JA), as well as secondary messengers, including calcium and reactive oxygen species (ROS) (6–9). Photosynthetic electron transduction in the chloroplast can result in the reduction of O_2 and the formation of ROS, such as singlet oxygen ($^1O_2$), which is generated from the chlorophyll and photosystems II (PSII) antenna complex (10). PSII, which is central to the conversion of light into chemical energy, is surrounded by the light-harvesting complex II (LHCII) and the monomeric light-harvesting proteins LHCB4 (CP29), LHCB5 (CP26), and LHCB6 (CP24) (11, 12). It has been established that LHCB4, LHCB5, and LHCB6 are all involved in energy dissipation in *A. thaliana* (13, 14) and that LHCB5 functions as a trimeric protein complex rescuing a defect in LHCII function due to the absence of LHCB1 and LHCB2 (15). However, in contrast to its known function in energy transduction, the role of LHCII in plant immunity has not been well elucidated.

Results

***LHCB5* Is Involved in Light Regulation of Resistance to *M. oryzae* in Different Rice Varieties.** To investigate whether rice resistance to *M. oryzae* is light-dependent, 25 rice varieties, collected worldwide, were screened for blast resistance under 2 different light intensities. These varieties showed various degrees of susceptibilities to the wild-type *M. oryzae* Guy11 under the low light intensity of 50 μmol photons $m^{-2}s^{-1}$ (Fig. 1), based on both the

Significance

Environmental conditions strongly influence pathogen–host plant interactions. Rice blast erupts in overcast and rainy conditions, due not only to favorable environmental conditions but also to insufficient light, which reduces host resistance. Due to the importance of breeding of blast-resistant rice varieties, elucidation of light-regulated rice immunity is an important research goal. We revealed that light induces protein phosphorylation of a harvesting complex II protein, LHCB5, upon infection by the rice blast fungus *Magnaporthe oryzae*. Resistance governed by LHCB5 phosphorylation cosegregates with the progenies harboring the desirable haplotype promoter. This establishes the genetic basis of LHCB5-regulated resistance mediated by phosphorylation. Our study highlights a mechanism for light-dependent rice blast resistance that promises future breeding of blast-resistant rice varieties.

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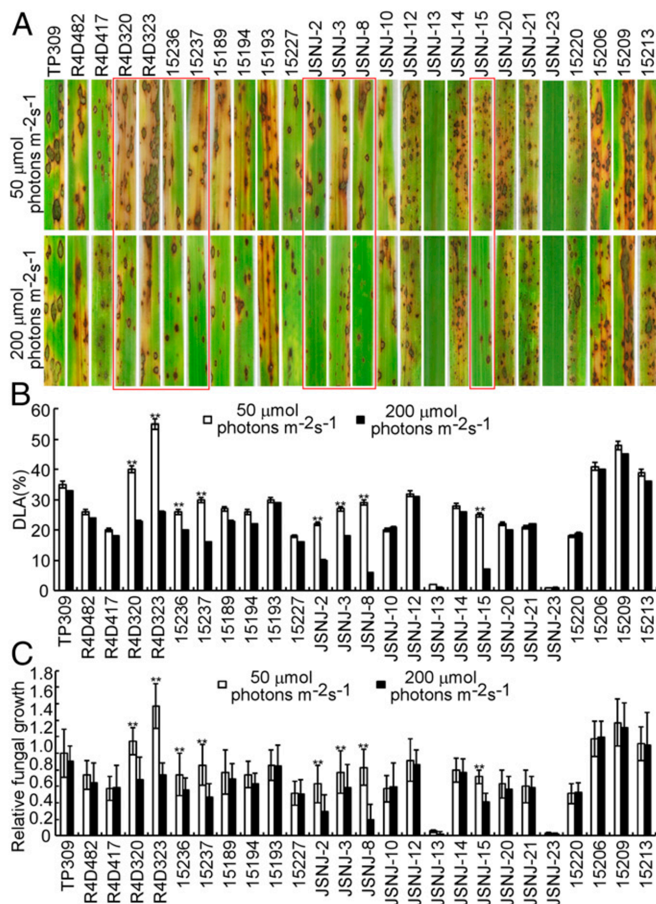

Fig. 1. Identification of blast-resistant rice varieties in a light-dependent manner. (*A*) The wild-type TP309 and 25 rice varieties collected worldwide were used for blast resistance screen. The rice varieties inoculated with Guy11 were cultivated in a light incubator with different light intensity (50 and 200 μmol photons $m^{-2}s^{-1}$). Red boxes indicate rice varieties with resistant variations under different light intensity. (*B*) Disease lesion area (DLA) was assessed by Image J. Lesions were photographed and measured or scored at 7 d postinoculation (dpi). The experiments were repeated twice with similar results. (*C*) Fungal growth and severity of blast were evaluated by quantifying *M. oryzae* genomic 28S rDNA relative to rice genomic Rubq1 DNA. The mean values of 3 determinations with SDs are shown. The asterisks indicate a significant difference according to Student's *t* test ($P < 0.01$).

diseased leaf area (DLA) and the pathogen biomass assay. However, 8 varieties showed significantly increased resistance when the assessment was conducted under the higher light intensity of 200 μmol photons $m^{-2}s^{-1}$ (Fig. 1). In contrast, the remaining 17 varieties did not show any significant differences in light-associated blast resistance (Fig. 1).

This light intensity-associated resistance prompted us to investigate the possible roles of the LHCII proteins, which are required for absorbing and transferring the light energy (16, 17). We first tested the transcript levels of 6 *LHCB* genes in the nonresponsive TP309 variety rice and found that all of them were significantly up-regulated under the lighted conditions, compared to the dark, suggesting that all *LHCB* genes are light-inducible (*SI Appendix*, Fig. S1). We also observed that the LHCB5 transcript level was uniformly up-regulated in all 8 light-dependent resistant varieties, but not in TP309 (*SI Appendix*, Fig. S2), as was the accumulation of LHCB5 protein (*SI Appendix*, Fig. S3). *LHCB5* transcript levels gradually increased at different time points until 96 h postinoculation (hpi) (*SI Appendix*, Fig. S4*A*), and this accumulation also corresponded with the levels of the LHCB5 protein. These results indicated that *LHCB5* is light- and blast pathogen-responsive and may play a role in light-responsive blast resistance.

LHCB5-Mediated Resistance Is Highly Associated with Promoter Sequence Divergence Leading to Different Expression Levels among Diverse Rice Germplasm. The various levels of the *LHCB5* transcript and protein in different rice varieties prompted us to investigate the variance of its promoter and the coding sequences from 3,000 sequenced rice genomes (18). No nonsynonymous sequence mutations were found in *LHCB5* coding sequences, suggesting high conservation (Dataset S1). In contrast, 11 single-nucleotide polymorphisms (SNPs) were identified that were separated into 18 groups (SNP ≥ 1) using *O. sativa* ssp *japonica* cv. Nipponbare (NPB) as the control (SNP = 0) (Dataset S2). The SNPs were mainly discovered in the *indica* rice varieties, and most of these varieties showed variations in 7 of the SNPs identified (*SI Appendix*, Fig. S5 *A* and *B*). The difference in SNP variation suggested a causal relationship with a lower expression in *indica* than in *japonica* (*SI Appendix*, Fig. S6*A*). To test this hypothesis, 238 rice varieties were selected for analyzing the relationship between SNPs and transcription levels (Dataset S3). The expression of *LHCB5* in SNP variation rice lines (SNP ≥ 1) was significantly lower than that in nonvariation varieties (SNP = 0) (*SI Appendix*, Fig. S5*C*), and this was, in particular, true among 7 of the 11 SNP positions (1–4, 9–11) (*SI Appendix*, Fig. S5*D*). To validate the correlation between SNPs and the LHCB5 expression levels, we expressed GFP (green fluorescent protein) reporter gene driven by different promoters (p35S:GFP, $pLHCB5^{SNP\ =\ 0}$:GFP and $pLHCB5^{SNP\ =\ 7}$:GFP) in rice protoplast. The result showed that constructs p35S:GFP and $pLHCB5^{SNP\ =\ 0}$:GFP had a much higher level of green fluorescent; the Western blot also showed a higher GFP protein level under the control of 35S and SNP = 0 promoter than SNP = 7 promoter, indicating that the promoter SNPs are indeed associated with higher LHCB5 expression (*SI Appendix*, Fig. S7). When 238 rice varieties were inoculated with 2 rice blast isolates, Mo15-125 and Mo15-9, a correlation between higher *LHCB5* expression and smaller disease lesion area was found (*SI Appendix*, Fig. S8). These data indicated that SNP variations in *indica* rice varieties result in reduced *LHCB5* expression and lower resistance to blast.

To further investigate the genetic association between the SNP pattern and expression of *LHCB5*, we used an F2 population of 44 progenies derived from a cross between YG456 (Yangeng456, SNP = 0) and LTH (Lijiangxintuanheigu, SNP ≥ 1, containing all 7 candidate SNPs mentioned above). As indicated in Fig. 2 *A* and *B*, the parental line YG456 and 32 progenies containing the YG456-type promoter showed significantly higher expression levels than LTH and the 12 progenies containing the LTH-type promoter. This revealed a cosegregation between the expression level and the promoter type in the F2 population.

YG456 was also found to be resistant to the virulent rice blast isolate Guy11, which had a lesion length 40% smaller than the lesion of the susceptible rice variety LTH (Fig. 2 *C* and *F*). Moreover, all of the progenies with a higher *LHCB5* expression level showed resistance to Guy11, whereas those with a lower *LHCB5* expression level were susceptible (Fig. 2 *E* and *F*). Taken together, these results revealed that the elevated level of *LHCB5* governed by the promoter (SNP = 0) contributes to enhanced resistance to blast, and the trait is genetically transferable.

Expression of _LHCB5_ in Transgenic Rice Lines Correlates with Resistance to Infection. To further investigate the relationship between *LHCB5* expression and resistance, we characterized transgenic rice lines with expression differences. We first generated transgenic *LHCB5* RNAi-silenced (*lhcb5*-RNAi) and overexpression (*LHCB5*-OX) rice lines in the TP309 background. The *lhcb5*-RNAi lines #1 and #2 showed significantly reduced *LHCB5* transcript levels in the leaves, whereas *LHCB5*-OX lines #1 and #2 showed significantly increased levels, compared to TP309 (*SI Appendix*, Fig. S9*A*). Western blot analysis showed that the amount of

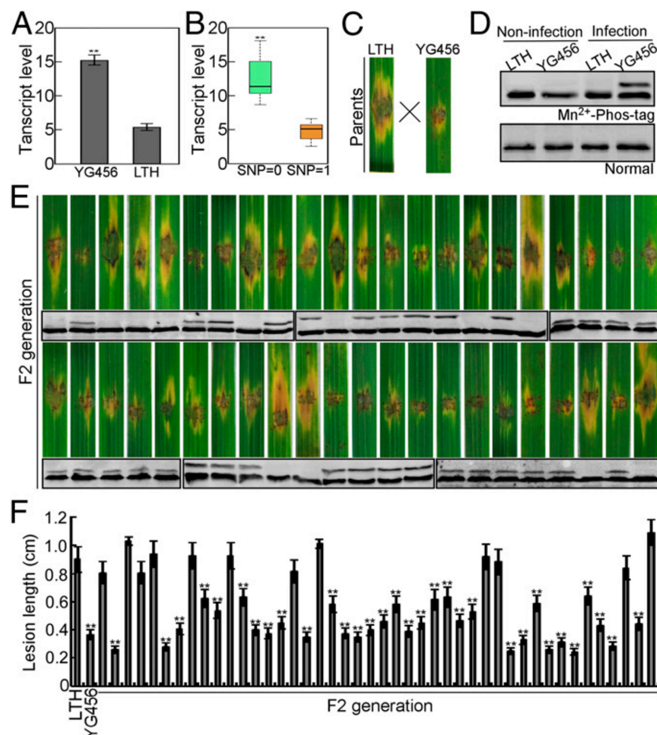

Fig. 2. Genetic association between the SNP pattern and expression of *LHCB*5 and the inheritance of LHCB5 phosphorylation. (*A*) The transcript analysis of *LHCB5* in YG456 and LTH. qRT-PCR on *LHCB5* in YG456 in comparison with LTH. (*B*) The transcript analysis of *LHCB5* in an F2 population derived from a cross between YG456 and LTH. The expression level of 44 progeny was analyzed by qRT-PCR. (*C*) Blast resistance of LTH and YG456 plants. The leaves of 4-wk-old plants were inoculated using method of punch. Photos were taken at 6 dpi. (*D*) Detection of LHCB5 phosphorylation in LTH and YG456. LTH and YG456 plants were inoculated with (infection) or without (noninfection) Guy11 after 48 hpi. The protein extracts were subjected to Phos-tag SDS/PAGE and normal SDS/PAGE followed by immunoblotting with the anti-LHCB5 polyclonal antibody. (*E*) Rice blast resistance is correlated with phosphorylation assess in the F2 generation of LTH and YG456. Blast resistance of 44 F2 generations using punch inoculation. Phosphorylation assay was performed as above. (*F*) Lesion length was measured 6 dpi. Values are the means of 3 replications, and error bars represent the SD (n = 3). The asterisks indicate a significant difference according to Student's t test ($P < 0.01$).

the LHCB5 protein was 3- to 4-fold higher in the *LHCB5*-OX lines than in TP309, while the *lhcb5*-RNAi lines had 20 to 25% of the levels of TP309 (*SI Appendix*, Fig. S9*B*).

In infection, the *LHCB5*-OX rice lines showed strong resistance to blast, with punctate and significantly reduced lesion areas, whereas the *lhcb5*-RNAi lines were more susceptible (Fig. 3 *A* and *B*). In addition, lesions on *LHCB5*-OX lines failed to produce any conidia (Fig. 3*C*). A rice sheath infection assay for invasive hyphal growth (19) revealed that >90% of penetration was at the level I in the *LHCB5*-OX rice lines, in contrast to 11% and 10% in TP309 and *lhcb5*-RNAi lines at 24 hpi, respectively. The infectious hyphae on the *LHCB5*-OX rice lines failed to expand, even at 48 hpi, and showed level II penetration (Fig. 3 *D* and *E*). Furthermore, we used the CRISPR/Cas9 technology to knock out the endogenous *LHCB5* gene (*LHCB5*-KO) in TP309, and the infection phenotype of *LHCB5*-KO lines was as susceptible as *lhcb5*-RNAi lines (*SI Appendix*, Fig. S10). Together, these data indicated that LHCB5 overexpression enhances basal resistance.

Induced Immune Response in *LHCB5*-OX Lines Is Broad Spectrum to *M. oryzae*. To explore the mechanism by which LHCB5 mediates resistance, we inoculated TP309, *LHCB5*-OX, and *lhcb5*-RNAi leaves with Guy11 and the incompatible strain 51# and stained the leaf cells with 3, 3′-diamino-benzidine (DAB) and Trypan Blue (TB). We observed that *LHCB5*-OX accumulated higher levels of H_2O_2, accompanied by cell death (*SI Appendix*, Fig. S11*A*). We also used a luminol-based chemiluminescence assay to monitor the generation of ROS induced by purified mycelia (PM) as the elicitor (20) and obtained a similar result (*SI Appendix*, Fig. S11*B*). Finally, we performed a rice sheath infection assay to examine ROS accumulation and cell death and found that the DAB and TB stain signals were 4 to 5 times stronger in *LHCB5*-OX sheaths than in those of TP309 and *lhcb5*-RNAi. Pretreating *LHCB5*-OX rice sheaths with the catalase of *Aspergillus niger* (CAG) (21) and reduced glutathione (GSH) (22) partially suppressed the production of ROS and rescued the invasive growth (*SI Appendix*, Fig. S11 *C*–*E*). Moreover, qRT-PCR revealed that the expression levels of PR genes, including *PR1*, *PBZ1*, *AOS2*, and *LOX1*, as well as 2 NADPH oxidases (*RBOHA* and *RBOHB*), were significantly up-regulated in the *LHCB5*-OX lines (*SI Appendix*, Fig. S11 *F* and *G*).

We next examined TP309, *lhcb5*-RNAi, and *LHCB5*-OX resistant to 21 *M. oryzae* isolates containing various *AVR* genes (*SI Appendix*, Fig. S12) (23) and found that the *LHCB5*-OX lines showed greater resistance to all 21 isolates (*SI Appendix*, Fig. S13). To investigate whether these lines have also been more resistant to other pathogens, we inoculated them with the pathogenic fungus *Bipolaris oryzae* and the pathogenic bacterium *Xanthomonas oryzae* pv *oryzae* PXO99. We observed that the *LHCB5*-OX lines were not resistant to either of these pathogens (*SI Appendix*, Fig. S14*A*), suggesting that the basal resistance in

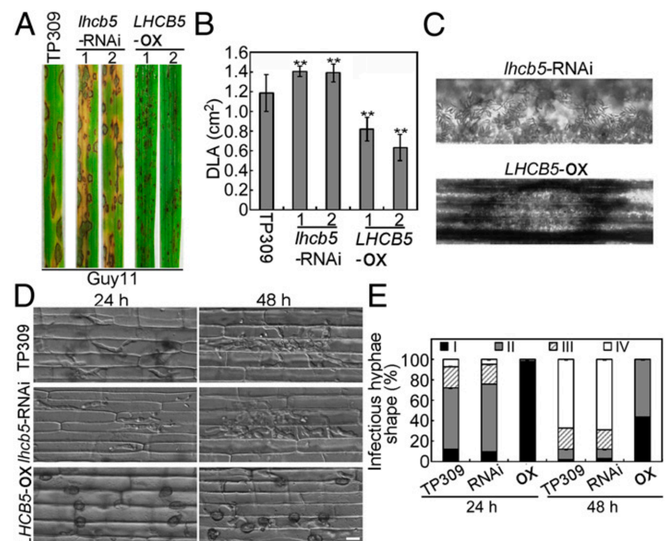

Fig. 3. The function of LHCB5 in blast resistance. (*A*) Blast resistance of *lhcb5*-RNAi and *LHCB5*-OX plants using spraying inoculation. Numbers "1" and "2" mean 2 independent transformants. (*B*) The DLA of the leaves infected by Guy11 was assessed by Image J. Lesions were photographed and measured or scored at 7 dpi, and experiments were repeated twice with similar results. Values are the means of 3 replications, and error bars represent the SD (n = 3). The asterisks indicate a significant difference according to Student's t test ($P < 0.01$). (*C*) Assays for fungal growth on surface-sterilized rice leaves inoculated by spray with Guy11. (*D*) Typical infection sites of TP309, *lhcb5*-RNAi, and *LHCB5*-OX leaf sheath inoculated with Guy11 strain, showing greater fungal proliferation and tissue invasion in TP309 and *lhcb5*-RNAi by the wild-type strain and restricted in *LHCB5*-OX leaf sheath. Infectious growth was observed at 30 hpi. (Scale bar, 10 μm). (*E*) Statistics of invasive hyphal growth at 100 appressorium penetration sites by rating the hyphal growth from level I to IV (I, no penetration; II, with primary invasive hypha; III, secondary invasive hypha does not extend to the neighboring plant cells; IV, invasive hypha extended into neighboring plant cells).

the *LHCB5*-OX lines, associated with ROS production, was *M. oryzae*-specific.

LHCB5 Phosphorylation Regulates Basal Immunity Independent of *R* Genes *Pia* and *Pizt*. Previous studies have suggested that LHCII is phosphorylated to balance light excitation energy between photosystem I and photosystem II (24). In the alga *Chlamydomonas reinhardtii*, a marked increase in phosphorylation of the chlorophyll protein CP26 (LHCB5 homolog) was observed in state II (25). We analyzed the phosphorylation of LHCB5 in TP309 and *LHCB5*-OX lines that were uninfected, or that had been infected by Guy11, using Mn^{2+}-Phos-tag gel electrophoresis (26). We found that LHCB5 phosphorylation was induced by Guy11 in the *LHCB5*-OX lines, but not in TP309 (Fig. 4*A*), and that this phosphorylation was *M. oryzae*-specific (*SI Appendix*, Fig. S14*B*). To further test whether the LHCB5 phosphorylation was light-dependent, we examined LHCB5 phosphorylation at various light intensities and found that it was induced at 100 and 200 μmol photons $m^{-2}s^{-1}$, but not below 50 μmol photons $m^{-2}s^{-1}$ (Fig. 4*B*).

We next carried out a blast-resistance assay with 14 randomly selected lines out of 3,000 rice accessions (18) and 45 lines of the Chinese origin to determine whether phosphorylation was associated with resistance. We observed that only LHCB5-phosphorylated lines exhibited resistance (Fig. 4*C*), and a total of 12 lines showed no observable lesions, indicative of the typical *R* resistance gene-mediated immune response (corresponding to the AVR protein in Guy11) (Fig. 4*C*). Further, we observed that this correlates with LHCB5 not being phosphorylated during interactions between *Avr-Pia*/*Pia* and *AvrPiz-t*/*Piz-t* (Fig. 4*D*). These findings suggested that LHCB5 phosphorylation regulates basal immunity and that the resistance is independent of the *R* genes *Pia* and *Pizt*.

Finally, we examined the expression of *LHCB5* in the above-mentioned 47 rice lines (excluding the 12 lines showing no lesions) and found higher *LHCB5* expression levels correlated with smaller lesion sizes (0.622 Pearson Correlation Coefficient) (Fig. 4*E*), which is consistent with the results shown in *SI Appendix*, Fig. S7. Since *LHCB5* expression was significantly reduced in *indica* compared to *japonica* (*SI Appendix*, Fig. S6*A*), we next determined whether *LHCB5* phosphorylation similarly differed,

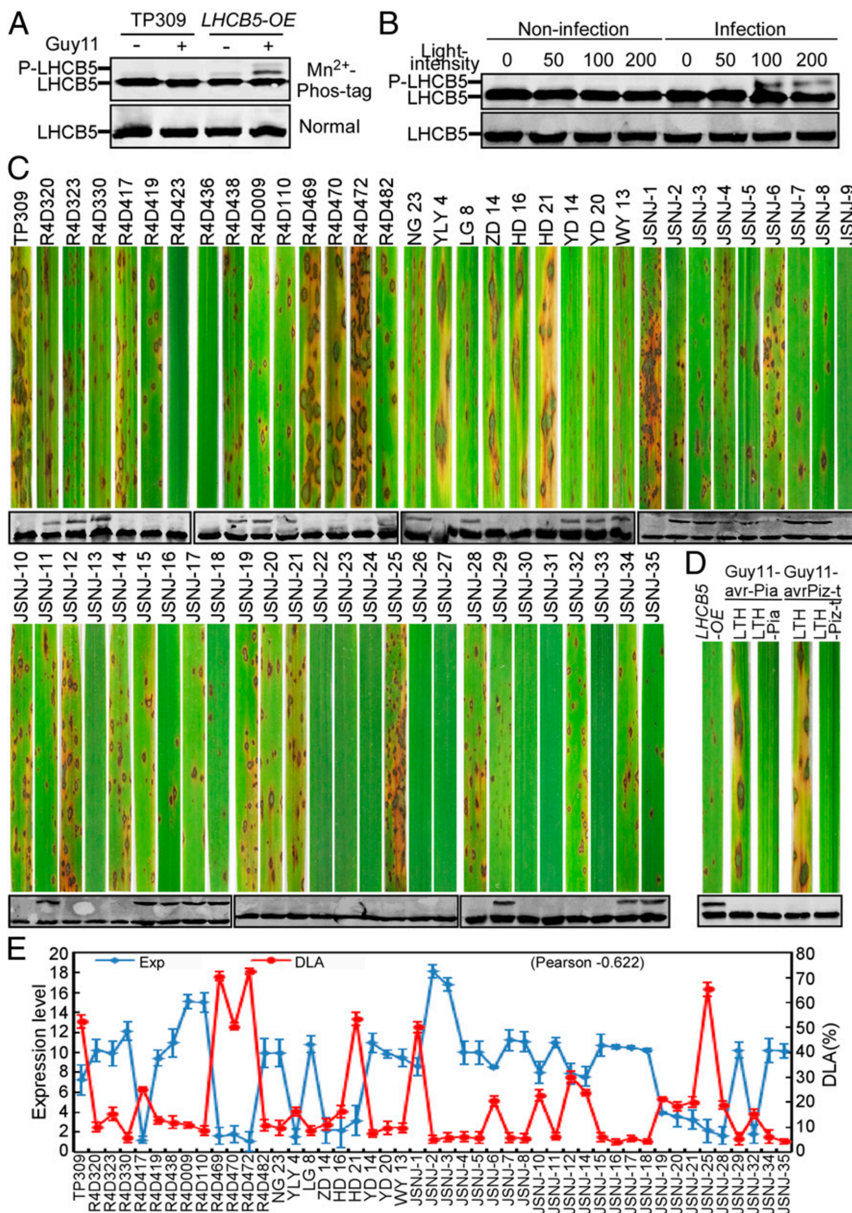

Fig. 4. Phosphorylation of LHCB5 regulates blast resistance. (*A*) LHCB5 was phosphorylated in *LHCB5*-OX plants inoculated with Guy11. Proteins from TP309 and the *LHCB5*-OX cell extracts were subjected to Phos-tag SDS/PAGE and normal SDS/PAGE followed by immunoblotting with LHCB5 polyclonal antibody. (*B*) LHCB5 phosphorylation was light-dependent. The *LHCB5*-OX plants inoculated with or without Guy11 were cultivated in a light incubator with different light intensity (0, 50, 100, and 200 μmol photons $m^{-2}s^{-1}$). (*C*) The phosphorylation is correlated with blast resistance. Blast resistance of 59 rice varieties using spraying inoculation. The phosphorylation of LHCB5 was detected at 48 hpi. Lesions were photographed and measured or scored at 7 dpi. (*D*) Phosphorylation assay of LHCB5 during interaction between *Avr-Pia*/*Pia* and *AvrPiz-t*/*Piz-t*. LTH-*Pia* and LTH-*Pizt* plants were inoculated with Guy11/*Avr-Pia* and Guy11/*AvrPiz-t*, respectively. (*E*) The expression level is associated with blast resistance. The DLA was measured by Image J, and the expression (Exp) level was detected by qRT-PCR. The Pearson correlation coefficient was −0.622. Values are the means of 3 replications, and error bars represent the SD (*n* = 3).

which we tested using 9 high and low *LHCB5* expression varieties from each subgroup. After *M. oryzae* infection, LHCB5 phosphorylation was found to mainly occur in the highly expressing *japonica* varieties (*SI Appendix*, Fig. S6*B*).

Phosphorylation of LHCB5 Correlates with Enhanced Resistance of Progeny Containing the YG456-Type Promoter. To confirm the relationship between the phosphorylation of LHCB5 and resistance to *M. oryzae*, we investigated LHCB5 phosphorylation in the same F2 population as used for the genetic association experiments. First, we analyzed the phosphorylation of LHCB5 in the LTH and YG456 lines, with or without infection, and found that LHCB5 was phosphorylated in infected YG456 (Fig. 2*D*). Of all 42 progenies, LHCB5 phosphorylation was only detected in resistant progenies (Fig. 2*E*), indicating that these traits cosegregated. Taken together with the observed close association between the YG456-type promoter and resistance to rice blast, we speculate that the resistance governed by the phosphorylation of LHCB5 is genetically controlled by the *LHCB5* promoter in YG456.

Phosphorylation of LHCB5 24th Threonine (T24) Activates an ROS Burst in Chloroplasts. To study the phosphorylation mechanism, we first compared the LHCB5 sequence to those of other plant species and predicted 3 possible phosphorylation sites at the less conserved N-terminal domain, using DISPHOS 1.3 and NetPhos 3.1 server (*SI Appendix*, Fig. S15*A*). We showed that the constitutively activated LHCB5 T24 ($LHCB5^{T24D}$) allele induced ROS production and cell death in *Nicotiana benthamiana* leaves, in contrast to the nonphosphorylated LHCB5 ($LHCB5^{T24A}$) (*SI Appendix*, Fig. S15 *B* and *C*), or LHCB5 with constitutive phosphorylation at 2 other sites (*SI Appendix*, Fig. S16). The phosphorylation of LHCB5 was observed in the cytoplasm, but not in the chloroplast (*SI Appendix*, Fig. S17). We further expressed the rice LHCB5 and $LHCB5^{T24A}$ protein with a FLAG peptide tag at the C terminus (OsLHCB5:Flag and $OsLHCB5^{T24A}$:Flag) in *lhcb5*-RNAi protoplasts induced by PM and detected mobility shifts representing phosphorylated LHCB5 in OsLHCB5, but not $OsLHCB5^{T24A}$ (*SI Appendix*, Fig. S15*D*). Using a luminol-based chemiluminescence assay, we observed higher ROS levels in the presence of constitutively activated LHCB5 ($LHCB5^{T24D}$) than with inactivated LHCB5 ($LHCB5^{T24A}$) in protoplasts (*SI Appendix*, Fig. S15*E*). These results further suggested that LHCB5 24T phosphorylation is important for LHCB5 function.

To confirm the elevated production of ROS in chloroplasts, we expressed the empty plasmid pBIN:GFP (as a marker for subcellular localization), as well as $OsLHCB5^{T24D}$:GFP, and $OsLHCB5^{T24A}$:GFP in rice protoplasts, and found that both $OsLHCB5^{T24D}$:GFP and $OsLHCB5^{T24A}$:GFP were localized to the chloroplast. Nitroblue tetrazolium (NBT) staining revealed an accumulation of superoxide ($O_2 \bullet^-$) in $OsLHCB5^{T24D}$:GFP expressing protoplasts, but not in pBIN:GFP or $OsLHCB5^{T24A}$:GFP expressing protoplasts (*SI Appendix*, Fig. S15*F*), consistent with LHCB5 phosphorylation that results in $O_2 \bullet^-$ accumulation in the chloroplast and activation of the immune response.

Phosphorylation Facilitates LHCB5 Accumulation and Trimerization in Chloroplasts. We noticed that the phosphorylation site was located within the region of the LHCB5 polypeptide responsible for chloroplast transit, which is important for the import of precursor proteins (27, 28). To investigate whether phosphorylation influences the accumulation of LHCB5 in the chloroplast, we separated chloroplast and cytoplasmic protein fractions extracted from TP309, *lhcb5*-RNAi, and *LHCB5*-OX lines, with or without Guy11 inoculation. Western blot analysis indicated the increased accumulation of LHCB5 in the chloroplasts of *LHCB5*-OX lines inoculated with Guy11 (*SI Appendix*, Fig. S18). Furthermore, we expressed pBIN-LHCB5-Flag, pBIN- $LHCB5^{T24A}$-Flag, and pBIN- $LHCB5^{T24D}$-Flag in the *LHCB5*-KO rice protoplast treated with or without PM. As shown in *SI Appendix*, Fig. S19 *A* and *B*, inactivated LHCB5 ($LHCB5^{T24A}$) cannot stimulate host immunity. Moreover, we found that pBIN-LHCB5-Flag and pBIN- $LHCB5^{T24D}$-Flag showed higher protein accumulation in chloroplast than pBIN- $LHCB5^{T24A}$-Flag when treated with PM (*SI Appendix*, Fig. S19*C*). Together, the results indicated that phosphorylation of 24th threonine facilitates LHCB5 accumulation in chloroplast to stimulate the immune response.

Since a previous study found that *A. thaliana* LHCB5 is organized into trimeric complexes in the absence of LHCB1 and LHCB2 (15), we next tested whether increased LHCB5 accumulation also led to the accumulation of trimers. Using native-PAGE analysis, we found that LHCB5 is mostly present in the form of monomers and dimers in TP309 and that trimeric forms were only founded in infected *LHCB5*-OX lines (*SI Appendix*, Fig. S20*A*). When a His-tagged form of LHCB5, LHCB5-His, was expressed in *Escherichia coli* BL21 and then purified (*SI Appendix*, Fig. S20*B*), fractionated, and analyzed by native-PAGE analysis, we also observed LHCB5-His in a trimeric complex (*SI Appendix*, Fig. S20*C*). Together, these data indicated that phosphorylation facilitates the accumulation of LHCB5 into chloroplasts and that LHCB5 exists in multimeric conformations, including a trimer.

Trimeric LHCB5 Conformation Affects PsbS Binding and the Electron Transport Rate in Chloroplasts. It was reported that rice plants deficient in the PSII protein PsbS had higher levels of chloroplastic superoxide and hydrogen peroxide and were more resistant to *M. oryzae* infection (29, 30). In *A. thaliana*, PsbS binds to LHCB5 to control nonphotochemical quenching (NPQ) (31). We therefore tested the interaction between LHCB5 and PsbS in the *LHCB5*-OX lines and found that this is indeed also the case in rice. However, the Co-IP analysis showed that the LHCB5-PsbS interaction does not occur upon infection by Guy11 (*SI Appendix*, Fig. S21). We hypothesized that multimeric forms of LHCB5 interact less with PsbS than does the LHCB5 monomer. To test this, we performed an in vitro pull-down assay with varying amounts of LHCB5. Consistent with our hypothesis, PsbS binding decreased as LHCB5 concentration increased (*SI Appendix*, Fig. S22).

To validate $O_2 \bullet^-$ accumulation in the chloroplast, we measured photosynthetic parameters in the TP309, G-TP309 (Guy11-infected), *lhcb5*-RNAi, G-*lhcb5*-RNAi (Guy11-infected), *LHCB5*-OX, and G-*LHCB5*-OX (Guy11-infected) lines using a Chlorophyll Fluorescence Imager (Ecotek, Beijing). The electron transport rate (ETR) was significantly reduced in G-*LHCB5*-OX lines (*SI Appendix*, Fig. S23*A*). Moreover, a transmission microscopic analysis of chloroplast morphology revealed that the structure was less compact in G-TP309 and G-*lhcb5*-RNAi lines when compared to noninfected lines, while no difference was detected in the G-*LHCB5*-OX lines (*SI Appendix*, Fig. S23*B*). These data suggested that LHCB5 may form trimeric complexes during infection to maintain normal morphology and may affect PsbS binding, thereby influencing $O_2 \bullet^-$ accumulation.

Discussion

Rice blast threatens rice production worldwide, and serious outbreaks can destroy whole crop harvests. Rice blast erupts in overcast and rainy conditions, due not only to favorable environmental conditions but also to insufficient light, which reduces host resistance (32). The mechanistic basis of the relationship between resistance and light levels has not been elucidated until now. Our study establishes the relationships among light conditions, light-harvesting complex protein LHCB5, and disease resistance.

It is known that transcription is often associated with SNP variations in the promoter and that nucleotide polymorphism in either the promoter or gene can affect resistance (33, 34). For example, it was reported that an A to G transition in the promoter region of the *BSR-D1* gene results in lower expression, resulting in elevated resistance (35). A survey of SNPs among *LHCB5* promoter sequences of 3,000 sequenced rice genomes suggested that SNP variation is associated with the differential

expression of *LHCB5* and, accordingly, resistance (Fig. 4*E* and *SI Appendix*, Fig. S8). It has been demonstrated that *japonica* rice varieties from the Chinese Yuanyang terraces have higher basal immunity, but a lower content of major resistance (*Pi*) genes than do *indica* varieties (36). Our results suggested that *LHCB5* expression was higher in *japonica* (*SI Appendix*, Fig. S6*A*). Moreover, a higher *LHCB5* expression leads to its phosphorylation upon challenge with the blast fungus, particularly in *japonica* (*SI Appendix*, Fig. S6*B*). We propose that SNP variation among *japonica* and *indica* varieties causes differences in *LHCB5* expression patterns, resulting in the differential phosphorylation of LHCB5. This is consistent with the elevated basal immunity observed in *japonica* rice. Whereas the evidence for a direct connection remains to be established, our studies suggest a strong correlation between promoter SNP and LHCB5 phosphorylation.

Biotic stress typically stimulates the production of ROS, such as $O_2^{\bullet -}$, in the chloroplast, (37) and ROS generation is associated with resistance to rice blast (35). Here, we showed that the ROS burst in the chloroplast is correlated with the phosphorylation of LHCB5, and that mediated broad-spectrum blast resistance (*SI Appendix*, Fig. S15). A widely used strategy to protect rice against pathogens is through the breeding of resistant varieties (38, 39), despite that the rapid variation of rice blast can lessen the effectiveness of this approach (40). To evaluate LHCB5 phosphorylation-induced resistance, we assessed the inheritance of this trait and found that it cosegregated with resistance (Fig. 2). Our studies indicated no differences between *LHCB5* transgenic and wild-type rice lines in the plant height, grain weight, and seed-setting rate (*SI Appendix*, Fig. S24), which promises for future breeding of high yield and blast-resistant rice varieties.

We propose a model where during the interaction between rice and *M. oryzae*, the host monitors the progression of infection through the phosphorylation of LHCB5 in a light-dependent manner. *M. oryzae* challenge results in phosphorylated LHCB5 accumulation in chloroplasts, which helps maintain chloroplast function by reducing the binding of LHCB5 to PsbS, which in turn leads to reduced ETR for ROS production (*SI Appendix*, Fig. S25).

Materials and Methods

The plant strains and blast isolates are listed in *SI Appendix*, *Materials and Methods*. Resistance test and infection assessment were performed as described (19). The ROS and cell death observation was performed as described (19). The phosphorylation assay was performed as described (41). Purified recombinant proteins from *Escherichia coli* were used for in vitro pull-down assays (41). All other materials and details of experimental methods can be found in *SI Appendix*, *Materials and Methods*.

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