PLANT SCIENCE

A single transcription factor promotes both yield and immunity in rice

Jing Wang[1]*†, Lian Zhou[1]*, Hui Shi[1]*, Mawsheng Chern[2]*, Hong Yu[3]*, Hong Yi[1], Min He[1], Junjie Yin[1], Xiaobo Zhu[1], Yan Li[1], Weitao Li[1], Jiali Liu[1], Jichun Wang[1], Xiaoqiong Chen[1], Hai Qing[1], Yuping Wang[1], Guifu Liu[3], Wenming Wang[1], Ping Li[1], Xianjun Wu[1], Lihuang Zhu[3], Jian-Min Zhou[3,4], Pamela C. Ronald[2], Shigui Li[1], Jiayang Li[3,4]†, Xuewei Chen[1]†

Plant immunity often penalizes growth and yield. The transcription factor Ideal Plant Architecture 1 (IPA1) reduces unproductive tillers and increases grains per panicle, which results in improved rice yield. Here we report that higher IPA1 levels enhance immunity. Mechanistically, phosphorylation of IPA1 at amino acid Ser^{163} within its DNA binding domain occurs in response to infection by the fungus *Magnaporthe oryzae* and alters the DNA binding specificity of IPA1. Phosphorylated IPA1 binds to the promoter of the pathogen defense gene *WRKY45* and activates its expression, leading to enhanced disease resistance. IPA1 returns to a nonphosphorylated state within 48 hours after infection, resuming support of the growth needed for high yield. Thus, IPA1 promotes both yield and disease resistance by sustaining a balance between growth and immunity.

Plant growth is usually slowed by an active immune response, resulting in yield penalties for crops fighting pathogens (*1*, *2*). Plants without an active immune response may grow faster but will easily succumb to various diseases (*3*). Various proteins control the growth-immunity trade-off. For example, *Arabidopsis* BRASSINAZOLE-RESISTANT 1 (BZR1) and HOMOLOG OF BRASSINOSTEROID ENHANCED EXPRESSION 2 (BEE2) INTERACTING WITH IBH1 (HBI1) promote plant growth but suppress immunity (*4*, *5*). Conversely, transcription factors TL1-BINDING FACTOR 1 (TBF1) and WRKY45 enhance immunity but inhibit plant growth (*6*, *7*). Breeding practice has selected crop varieties with high yield and disease resistance. A better balance between growth and immunity is supported by various genes, including a natural allele of the *Broad-Spectrum Resistance-Digu 1* (*Bsr-d1*) transcription factor (*8*); a chemically induced allele of *Broad-Spectrum Resistance-Kitaake 1* (*Bsr-k1*), which encodes an RNA binding protein (*9*); a nucleotide-binding oligomerization domain–like receptor (NLR) pair, *Pyricularia-Gumei Resistant* and *Pyricularia-Gumei Susceptible* (*PigmR* and *PigmS*) (*10*); and an artificial, pathogen-inducible cassette containing *Nonexpressor of Pathogenesis-Related genes 1* (*NPR1*) or *snc1* (*suppressor of npr1-1, constitutive 1*) (*11*). However, there are no reports showing that a single protein can positively promote yield and disease resistance.

Rice feeds half of the world's population; improved yields would help sustain the food supply needed for the growing world population. Grain yield depends on the number of productive tillers per plant, the number of grains per panicle, and grain weight (*12*). The *Ideal Plant Architecture 1* (*IPA1*) gene encodes a SQUAMOSA promoter binding protein–like (SPL) transcription factor, also known as OsSPL14, which activates yield-related genes, including *Dense and Erect Panicle 1* (*DEP1*), leading to plants with fewer unproductive tillers and more grains per panicle, supporting higher yield (*13*–*15*). The *ipa1-1D* allele carries a mutation at the miR156 and miR529 target sites, releasing suppression by miR156 and miR529 and leading to higher *IPA1* RNA and protein levels (*13*, *16*). Though *ipa1-1D* plants have been demonstrated to have 10% higher yields in extensive field trials (*13*), it was not known if the improved yield would persist when plants faced pathogen challenges. We directly tested the yield of *ipa1-1D* plants under challenge with *Magnaporthe oryzae*, which causes the devastating rice blast disease. We conducted field tests in three consecutive years by using isogenic rice lines developed in two rice varieties (R320 and R441) and found that *ipa1-1D* plants R320$^{ipa1\text{-}1D}$ and R441$^{ipa1\text{-}1D}$ had yields 10.1 to 13.3% higher under normal field conditions without blast disease and 30.7 to 48.2% higher under high blast disease pressure than controls R320 and R441, respectively (Fig. 1, A to D). The 10 to 13% yield increase under normal conditions is consistent with previous reports (*13*). *ipa1-1D*–mediated yield increase is greater under blast disease pressure, indicating that *IPA1* may also improve resistance to *M. oryzae*. To test this hypothesis directly, we generated *IPA1* overexpression [IPA1–green fluorescent protein (IPA1-GFP)] plants and plants with *IPA1* expression reduced by RNA interference (RNAi) (*13*). *IPA1* overexpression lines showed enhanced resistance and *IPA1* RNAi lines showed higher susceptibility to multiple isolates of *M. oryzae* in both detached leaves and spray-inoculated plants (Fig. 1, E to G, and figs. S1 to S3).

Because *IPA1* RNA and protein levels do not change upon *M. oryzae* infection (Fig. 2A), we investigated whether IPA1 protein becomes phosphorylated in *ipa1-1D* plants upon *M. oryzae* infection. The phosphorylated IPA1 protein was separated from nonphosphorylated IPA1 in a gel containing Phos-tag and detected with an IPA1 polyclonal antibody (Ab). Phosphorylated IPA1 protein starts to accumulate at 3 hours post-infection (hpi), peaks at 6 to 12 hpi, and then subsides to near normal levels within 48 hpi (Fig. 2B and fig. S4). A conserved serine residue exists among different SPL proteins and has been suggested as a phosphorylation site necessary for the transcriptional activity of SPL proteins (*17*). We therefore generated a polyclonal Ab against a 14–amino acid peptide containing phosphorylated Ser^{163} (S163-P) (fig. S5A). The Ab (αIPA1S163-P) recognizes IPA1 containing S163-P [IPA1(S163-P)] with high specificity. Changing S163 to alanine (S163A), which removes the ability of IPA1 to be phosphorylated, abolished IPA1 recognition by αIPA1S163-P (fig. S5B). In samples from *M. oryzae*–infected *ipa1-1D* plants, αIPA1S163-P detected an IPA1 phosphorylation pattern similar to that detected by using Phos-tag, peaking at 12 hpi with ~3-fold enrichment of the IPA1 (S163-P) protein (Fig. 2C). Wild-type plants displayed a similar but weaker phosphorylation response (~2-fold enrichment) upon *M. oryzae* infection (fig. S6). These results indicate that IPA1 S163 becomes phosphorylated upon *M. oryzae* infection in a manner similar to the overall phosphorylation pattern of IPA1.

We next used chromatin immunoprecipitation sequencing (ChIP-seq) to identify genes up-regulated by IPA1 in IPA1-GFP plants (*15*). We found that defense-related genes, including transcription factor *WRKY45*, were up-regulated (fig. S7A). *WRKY45* is required for benzothiadiazole-inducible and NLR protein–mediated immunity to *M. oryzae*, and its elevated expression enhances resistance in rice (*18*, *19*). Thus, elevated *WRKY45* expression may mediate the enhanced pathogen resistance in *IPA1* overexpression plants. Two SPL binding sites containing GTAC sequences were identified in the *WRKY45* promoter (fig. S7B). As expected, probes carrying each of these two sites bound to IPA1 in vitro and in vivo (fig. S7, C and D). Overexpression of *IPA1* in IPA1-GFP plants increased *WRKY45* expression (fig. S7E), indicating that IPA1 activates the *WRKY45* promoter. Moreover, *WRKY45* induction by *M. oryzae* infection was enhanced in *ipa1-1D* plants but reduced

[1]State Key Laboratory of Hybrid Rice, Key Laboratory of Major Crop Diseases and Collaborative Innovation Center for Hybrid Rice in Yangtze River Basin, Rice Research Institute, Sichuan Agricultural University at Wenjiang, Chengdu, Sichuan 611130, China. [2]Department of Plant Pathology and the Genome Center, University of California, Davis, CA 95616, USA. [3]State Key Laboratory of Plant Genomics and National Center for Plant Gene Research (Beijing), Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101, China. [4]University of the Chinese Academy of Sciences, Beijing 100049, China.
*These authors contributed equally to this work.
†Corresponding author. Email: xwchen88@163.com (X.C.); jyli@genetics.ac.cn (J.L.); jingwang406@sicau.edu.cn (J.W.)

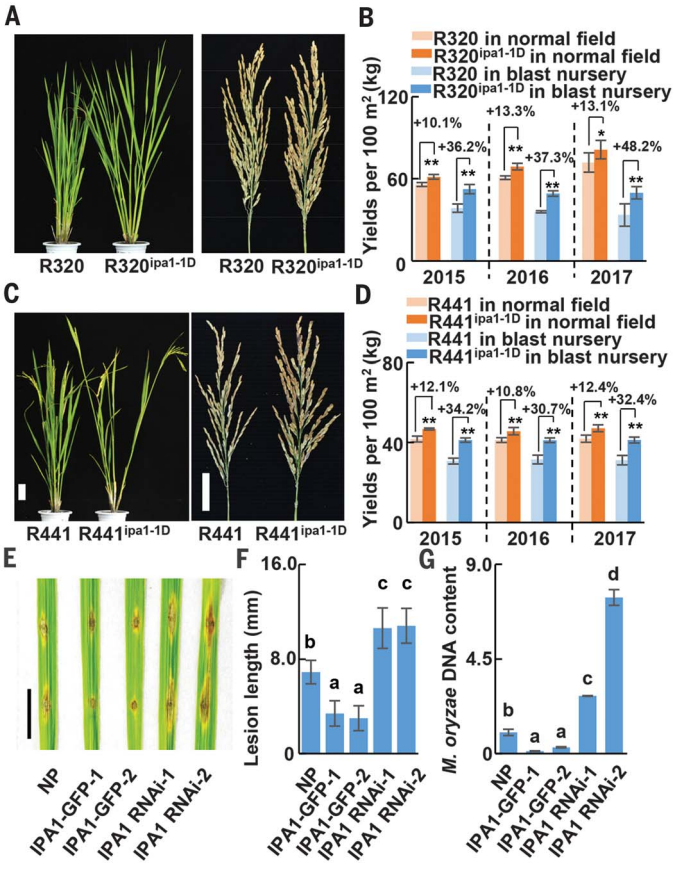

Fig. 1. Elevated IPA1 levels enhance resistance to *M. oryzae*. (**A**) The *ipa1-1D* allele alters plant architecture. *ipa1-1D* was introduced into Shuhui527. The *ipa1-1D* line R320$^{ipa1\text{-}1D}$ and the R320 control were selected from BC_2F_8 progeny. Whole plants and panicles are displayed. Scale bars, 5 cm. (**B**) Yields of R320$^{ipa1\text{-}1D}$ and R320 were tested in the presence of high blast disease pressure (blast nursery) or the absence of blast disease (normal field). Field tests were conducted in 2015, 2016, and 2017. Each data set contained three plots. *P < 0.05; **P < 0.01. (**C**) Same as for (A), except that *ipa1-1D* was introduced into Chenghui3203 and R441$^{ipa1\text{-}1D}$ and R441 were selected. (**D**) Same as for (B), except that R441$^{ipa1\text{-}1D}$ was tested against R441. In (B) and (D), the percent difference was calculated by comparing with the corresponding control. (**E** to **G**) *IPA1* overexpression (IPA1-GFP) enhances resistance and RNAi reduces resistance to *M. oryzae*. Wild-type Nipponbare (NP), IPA1-GFP, and RNAi plants were inoculated with *M. oryzae* isolate Zhong10-8-14. (E) Photographs of lesions. Scale bar, 1 cm. (F) Lesion lengths (n = 10 lesions). (G) *M. oryzae* population (n = 3 repeats). Values are means ± SD. Different letters indicate significant differences determined by the Tukey-Kramer test.

in *IPA1* RNAi plants compared with wild-type plants (Fig. 3A and fig. S8). These results demonstrate the involvement of *WRKY45* in IPA1-mediated immunity to *M. oryzae*. In contrast, expression of *DEP1*, a yield-related gene promoted by IPA1, was suppressed by *M. oryzae* infection (Fig. 3B), suggesting that *M. oryzae*–triggered phosphorylation may change IPA1 DNA binding activity.

To test this hypothesis, we first created two IPA1 mutants, IPA1(S163A) and IPA1(S163D). S163A abolishes phosphorylation capacity, and Ser^{163}→Asp (S163D) mimics S163-P without altering IPA1 nuclear localization (fig. S9). IPA1 (S163D) had reduced binding to the GTAC sites on both *DEP1* and *WRKY45* promoters in electrophoretic mobility shift assays (EMSAs); IPA1 (S163A) had little effect (Fig. 3C and fig. S10A). In addition to GTAC, we previously identified another motif, TGGGCC/T, enriched in the IPA1 ChIP-seq assay (*15*). IPA1(S163D) increased binding to the cis motif TGGGCC, present in the *WRKY45* promoter but not in the *DEP1* promoter (Fig. 3C and fig. S10B). IPA1(S163A) remained similar to the wild type (Fig. 3C). These results were confirmed by ChIP–quantitative polymerase chain reaction (PCR) assays, where IPA1(S163D) pulled down more *WRKY45* TGGGCC sequence (Fig. 3D). Thus, IPA1(S163D) preferentially binds to the TGGGCC site in the *WRKY45* promoter but does not bind the *DEP1* promoter.

To confirm the effects of differential DNA binding ability in planta upon S163 phosphorylation, we overexpressed IPA1(S163D) (labeled S163D-OE) and IPA1(S163A) (labeled S163A-OE) in rice and assessed their effects on immunity. IPA1 S163D-OE plants had smaller lesions and *M. oryzae* populations, whereas S163A-OE had no significant effects (Fig. 4, A to D, and fig. S11). Consistent with this result, *WRKY45* RNA levels were elevated six- to sevenfold in S163D-OE plants; in

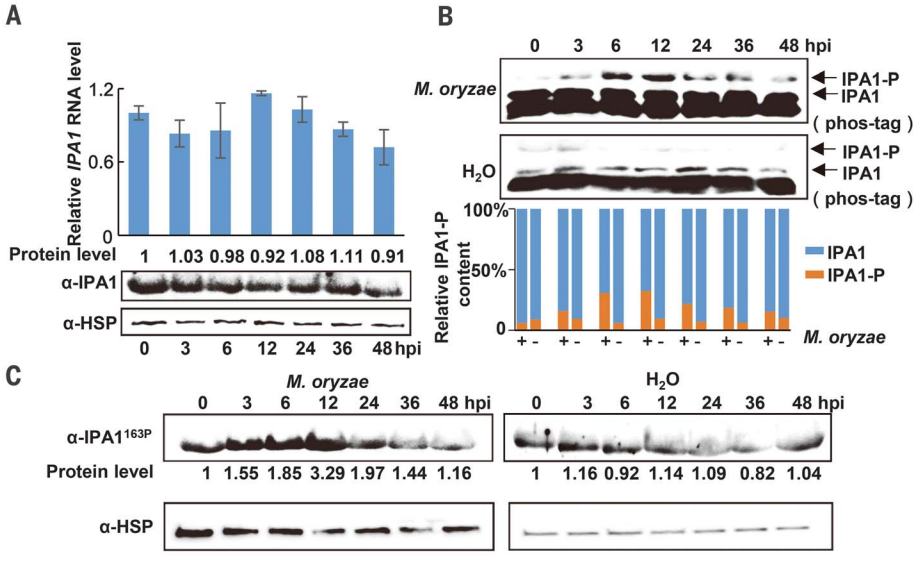

Fig. 2. *M. oryzae* infection induces phosphorylation of IPA1 at S163. (**A**) IPA1 RNA and protein levels are not significantly affected by *M. oryzae* infection. The IPA1 RNA (top) and protein (bottom) levels were assessed at different hpi with *M. oryzae*. The IPA1 protein level was quantitated and normalized to the heat shock protein (HSP) level; the value at time zero was set as one. Error bars indicate SD. (**B**) Phosphorylation of IPA1 is induced upon *M. oryzae* infection. Leaves were collected at different hpi with *M. oryzae* (top) or after treatment with H_2O as a control (middle). Phosphorylated and nonphosphorylated IPA1 proteins were separated on a Phos-tag gel, detected by IPA1 Ab, and quantitated by densitometry, and percentages were calculated (bottom). (**C**) *M. oryzae* infection enhances IPA1 phosphorylation at S163. Immunoblots were probed with an Ab specifically recognizing IPA1 phosphorylated at S163 (IPA1^{163P}) after *M. oryzae* (left) or H_2O (right) treatment. IPA1^{163P} protein amounts were quantitated by densitometry and normalized to the HSP level. The value at time zero was set as one.

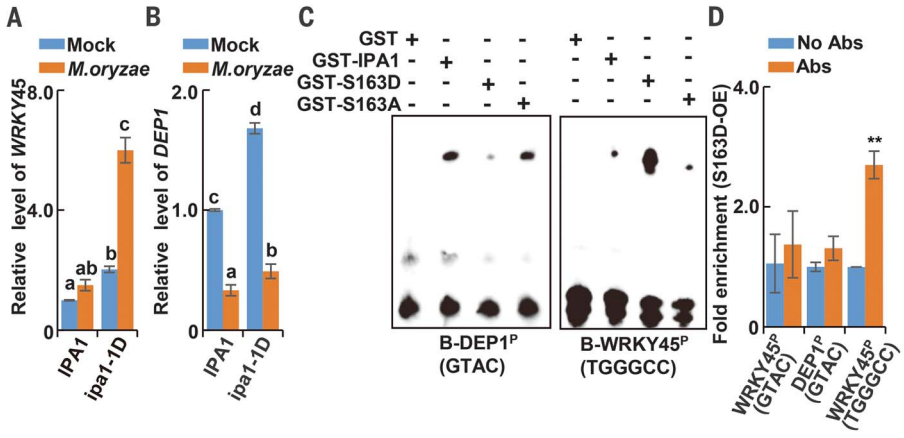

Fig. 3. IPA1(S163D) preferentially binds to the TGGGCC site in the *WRKY45* promoter. (**A**) *M. oryzae* infection induces higher *WRKY45* expression in *ipa1-1D* plants than in wild-type plants. (**B**) *M. oryzae* infection represses *DEP1* expression. In (A) and (B), RNA levels were determined by real-time PCR. (**C**) IPA1(S163D), a mimic of IPA1 phosphorylation at S163, changes DNA binding specificity. IPA1(S163D) reduces binding to the GTAC site in the *DEP1* promoter (left) and enhances binding to the TGGGCC site in the *WRKY45* promoter (right) in EMSAs. GST, glutathione *S*-transferase; B-DEP1^{P} and B-WRKY45^{P}, biotin-labeled *DEP1* and *WRKY45* promoters. (**D**) IPA1(S163D) preferentially binds the *WRKY45* TGGGCC site in a ChIP assay. Values are means ± SD (n = 3 repeats) in (A), (B), and (D). Letters indicate significant differences determined by the Tukey-Kramer test. $^{**}P < 0.01$.

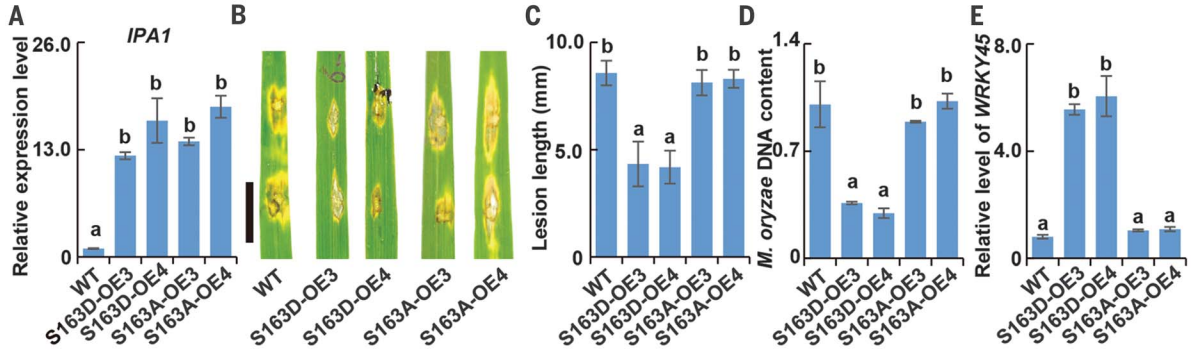

Fig. 4. IPA1(S163D) but not IPA1(S163A) overexpression induces *WRKY45* expression and enhances resistance to *M. oryzae*. IPA1(S163D) (labeled S163D-OE3 and S163D-OE4), IPA1(S163A) (labeled S163A-OE3 and S163A-OE4), and wild-type (WT) plants were inoculated with *M. oryzae*. (**A**) *IPA1* RNA levels. (**B**) Lesion pictures. Scale bar, 1 cm. (**C**) Lesion lengths. (**D**) *M. oryzae* population postinfection. (**E**) *WRKY45* RNA levels. Values are means ± SD. n = 3 repeats in (A), (D), and (E); n = 10 lesions in (C). Different letters indicate significant differences determined by the Tukey-Kramer test.

contrast, S163A-OE activated *DEP1* expression (fig. S12) but not *WRKY45* expression (Fig. 4E). These results demonstrate that IPA1(S163D) but not IPA1(S163A) induces *WRKY45* expression and activates immunity, suggesting that the phosphorylation of IPA1 S163 is critical for the ability to induce *WRKY45* expression and enhance immunity. Moreover, *WRKY45* up-regulation and IPA1 S163 phosphorylation induced by *M. oryzae* infection in *ipa1-1D* plants followed the same pattern (Fig. 2 and fig. S13), further supporting the importance of S163 phosphorylation.

In summary, we discovered that a single protein, IPA1, promotes both yield and disease resistance, and we uncovered its mechanism for controlling two different biological processes. Here, we propose a model for IPA1 function in *ipa1-1D* plants (fig. S14). In the absence of a pathogen, IPA1 is nonphosphorylated at S163 and binds to and activates the *DEP1* promoter, promoting plant growth and yield. Upon pathogen attack, IPA1 becomes phosphorylated at S163. Phosphorylated IPA1 changes DNA binding specificity, switching to bind to the TGGGCC site in the *WRKY45* promoter, and activates *WRKY45* expression, leading to enhanced immunity to *M. oryzae*. Because constitutive phosphorylation of IPA1 would reduce yield (fig. S12), IPA1 returns to the nonphosphorylated state that activates the genes needed for growth and high yield within 48 hpi. In this way, inducible phosphorylation of IPA1 promotes plant growth in the absence of a pathogen and promotes immunity upon pathogen attack. Wild-type plants follow the same phosphorylation pattern for IPA1 as *ipa1-1D* plants, albeit at a lower magnitude (fig. S6). Because *ipa1-1D* plants carry higher levels of nonphosphorylated IPA1 for yield and higher levels of phosphorylated IPA1 protein upon pathogen attack, *ipa1-1D* plants have both improved grain yield and improved immunity. Furthermore, changing DNA binding specificity via phosphorylation of an amino acid to nimbly control different outcomes may prove to be a widespread phenomenon.

REFERENCES AND NOTES

1. J. K. Brown, *Trends Genet.* **19**, 667–671 (2003).
2. R. Nelson, T. Wiesner-Hanks, R. Wisser, P. Balint-Kurti, *Nat. Rev. Genet.* **19**, 21–33 (2018).
3. H. Shi *et al.*, *Plant Cell* **25**, 1143–1157 (2013).
4. M. Fan *et al.*, *Plant Cell* **26**, 828–841 (2014).
5. R. Lozano-Durán *et al.*, *eLife* **2**, e00983 (2013).
6. K. M. Pajerowska-Mukhtar *et al.*, *Curr. Biol.* **22**, 103–112 (2012).
7. S. Goto *et al.*, *Plant Biotechnol. J.* **13**, 753–765 (2015).
8. W. Li *et al.*, *Cell* **170**, 114–126.e15 (2017).
9. X. Zhou *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **115**, 3174–3179 (2018).
10. Y. Deng *et al.*, *Science* **355**, 962–965 (2017).
11. G. Xu *et al.*, *Nature* **545**, 491–494 (2017).
12. Y. Wang, J. Li, *Plant Mol. Biol.* **59**, 75–84 (2005).
13. Y. Jiao *et al.*, *Nat. Genet.* **42**, 541–544 (2010).
14. K. Miura *et al.*, *Nat. Genet.* **42**, 545–549 (2010).
15. Z. Lu *et al.*, *Plant Cell* **25**, 3743–3759 (2013).
16. L. Wang *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **112**, 15504–15509 (2015).
17. R. P. Birkenbihl, G. Jach, H. Saedler, P. Huijser, *J. Mol. Biol.* **352**, 585–596 (2005).
18. M. Shimono *et al.*, *Plant Cell* **19**, 2064–2076 (2007).
19. H. Inoue *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **110**, 9577–9582 (2013).

ACKNOWLEDGMENTS

We thank Y. Qian (Biogle) for rice transformation. **Funding:** This work was supported by NSFC (31171622, 31571994, 31788103, 31401351, 31601290, and 31701779), NKRDPC (2016YFD0100600), PNCETC (NECT-13-0920), the NSF (1237975), the NIH (GM59962), and NIFA (2017-67013-26590). **Author contributions:** Jin.W., J.Li, and Xu.C. conceived and designed the experiments. Jin.W., L.Zho., H.S., H.Yu, H.Yi, M.H., X.Z., Y.L., W.L., J.Liu, and Xi.C. performed experiments with phenotypic and biochemical assays. J.Y., Jic.W., Y.W., G.L., and H.Q. contributed to rice materials. Jin.W., M.C., H.Yu, W.W., P.L., X.W., and S.L. collected data. Jin.W., M.C., L.Zhu, J.-M.Z., P.C.R., J.Li, and Xu.C. analyzed the data and wrote the manuscript. **Competing interests:** None declared. **Data and materials availability:** All data needed to evaluate the conclusions in the paper are present in the paper or the supplementary materials.

SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/361/6406/1026/suppl/DC1
Materials and Methods
Figs. S1 to S14
Table S1
References (*20*–*27*)

12 June 2018; accepted 16 July 2018
10.1126/science.aat7675

A single transcription factor promotes both yield and immunity in rice

Jing Wang, Lian Zhou, Hui Shi, Mawsheng Chern, Hong Yu, Hong Yi, Min He, Junjie Yin, Xiaobo Zhu, Yan Li, Weitao Li, Jiali Liu, Jichun Wang, Xiaoqiong Chen, Hai Qing, Yuping Wang, Guifu Liu, Wenming Wang, Ping Li, Xianjun Wu, Lihuang Zhu, Jian-Min Zhou, Pamela C. Ronald, Shigui Li, Jiayang Li and Xuewei Chen

Science **361** (6406), 1026-1028.
DOI: 10.1126/science.aat7675

Flexible growth and immune responses in rice

Plants that are fighting microbial pathogens often divert resources that could be used for growth into the immune response. For crops, this translates into lower yield when plant immunity is activated. Wang *et al.* show that, in rice, reversible phosphorylation of a key transcription factor allows the plant to defend against fungal attack when needed but then, within days, reallocate resources back to growth (see the Perspective by Greene and Dong). Thus, both pathogen defense and crop yield can be sustained.

Science, this issue p. 1026; see also p. 976