A single transcription factor promotes both yield and immunity in rice

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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 Ser163 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 quickly succumb to various diseases (3). Various proteins control the growth-immunity trade-off. For example, Arabidopsis BRASSINAZOLE-RESISTANT1 (BZR1) and HOMOLOG OF BRASSINOSTEROID ENHANCED EXPRESSION 2 (BEE2) interacting with IBH1 (IBH1) promote plant growth but suppress immunity (4, 5). Conversely, transcription factors TLI-BINDING FACTOR 1 (TBFI) 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-Diga 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–Gumel Resistant and Pyricularia–Gumel Susceptible (PigmR and PigmS) (10); and an artificial, pathogen-inducible cassette containing Nonexpressor of Pathogenesis-Related genes 1 (NPR1) or sncl (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 (17), 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 [ipa1-1D] and R441 [ipa1-1D] 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 (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 Ser163 (S163P) (fig. S5A). The Ab (aIPA1S163P) 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 aIPA1S163P (fig. S5B). In samples from M. oryzae–infected ipa1-1D plants, aIPA1S163P 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

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**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(ipa1-1D) and the R320 control were selected from BC1F6 progeny. Whole plants and panicles are displayed. Scale bars, 5 cm. (B) Yields of R320(ipa1-1D) 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 Chenghu3203 and R441(F3-1D) and R441 were selected. (D) Same as for (B), except that R441(F3-1D) 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.

**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 H2O 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(S163P)) after M. oryzae (left) or H2O (right) treatment. IPA1(S163P) protein amounts were quantitated by densitometry and normalized to the HSP level. The value at time zero was set as one.

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 Ser163→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;
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 DEPI 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 DEPI promoter (left) and enhances binding to the TGGGCC site in the WRKY45 promoter (right) in EMSAs. GST, glutathione S-transferase; B-DEPI1 and B-WRKY45, biotin-labeled DEPI 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 DEPI 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 DEPI 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. S8). 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

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SUPPLEMENTARY MATERIALS
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**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.

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