Running Title: Selective Binding of Rice WRKY13 to cis-Elements

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One Sentence Summary: WRKY13 regulate the antagonistic crosstalk between drought and disease resistance pathways by directly suppressing SNAC1 and WRKY45-1 and regulating its own expression via specific DNA elements on the promoters of these genes in vascular tissue where bacteria proliferate and in guard cells where transcriptional factor SNAC1 mediates drought resistance by promoting stomatal closure.
Rice WRKY13 Regulates Crosstalk between Abiotic and Biotic Stress Signaling Pathways by Selective Binding to Different cis-Elements

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Abstract
Plants use a complex signal transduction network to regulate their adaptation to the ever-changing environment. Rice WRKY13 plays a vital role in the crosstalk between abiotic and biotic stress signaling pathways by suppressing abiotic stress resistance and activating disease resistance. However, it is not clear how WRKY13 directly regulates this crosstalk. Here, we show that WRKY13 is a transcriptional repressor. During rice response to drought stress and bacterial infection, WRKY13 selectively bound to certain site- and sequence-specific cis-elements on the promoters of SNAC1, the overexpression of which increases drought resistance, and WRKY45-1, the knockout of which increases both bacterial disease and drought resistance. WRKY13 also bound to two cis-elements of its native promoter to autoregulate the balance of its gene expression in different physiological activities. WRKY13 was induced in leaf vascular tissue, where bacteria proliferate, during infection, and in guard cells, where transcriptional factor SNAC1 enhances drought resistance, during both bacterial infection and drought stress. These results suggest that WRKY13 regulates the antagonistic crosstalk between drought and disease resistance pathways by directly suppressing SNAC1 and WRKY45-1 and autoregulating its own expression via site- and sequence-specific cis-elements on the promoters of these genes in vascular tissue and guard cells.

Key words: bacterial pathogen; drought; expression regulation; Oryza sativa; transcription factor; Xanthomonas oryzae
Plants face various abiotic and biotic stresses. To survive, plants have evolved complex mechanisms for rapidly reallocating metabolic resources and changing the transcriptome among different physiological activities to adapt to the ever-changing environment (Matyssek et al., 2005). Although biotic interactions may diminish the effects of abiotic stress on the allocation of various resources (Matyssek et al., 2005), a prolonged metabolic and transcriptional adaptation to one type of stress is more likely to influence the adaptation of plants to another type of stress due to systemic resource reallocation (Liu et al., 2012). However, the cost/benefit relationship in balancing resource allocation between abiotic and biotic stresses remains unclear.

The defense signaling pathways for abiotic and biotic stresses share some common components, including the rapid stress response cis-acting regulatory elements (Walley and Dehesh, 2010), reactive oxygen species (Møller et al., 2007; Wong and Shimamoto, 2009), calcium ions (Galon et al., 2010), the phytohormones abscisic acid (Ton et al., 2009) and jasmonic acid (Fonseca et al., 2009), and mitogen-activated protein kinase cascades (Pitzschke et al., 2009). The common features have revealed some convergent nodes of abiotic and biotic stress response pathways, and plants can rapidly adapt to a changed environment by synergistically or antagonistically regulating the signaling crosstalk through these nodes (Fujita et al., 2006; Park et al., 2007; Qiu et al., 2008). Although research is clarifying the crosstalk nodes between abiotic and biotic stress signaling, only a few examples for rice have been published.

In rice, MAPK5/MPK5 kinase enhances resistance to drought, salt, and cold stresses and to Cochliobolus miyabeanus fungal infection, but decreases resistance to the fungal pathogen Magnaporthe oryzae and bacterial pathogen Xanthomonas oryzae pv. oryzae (Xoo) (Xiong and Yang, 2003; De Vleesschauwer et al., 2010; Xu et al., 2013). The WRKY89 transcription factor increases rice resistance to fungal pathogen and ultraviolet B irradiation (Wang et al., 2007). The WRKY45-2 transcription factor mediates broad-spectrum disease resistance, but negatively regulates adaptation to salt, cold, and drought stresses in rice (Tao et al., 2009, 2011). Interestingly, the allele of WRKY45-2, the WRKY45-1 that also encodes a transcription factor but with a difference of 10 amino acids from WRKY45-2, enhances rice resistance to M. oryzae but decreases resistance to bacterial pathogens Xoo and Xanthomonas oryzae pv. oryzicola and cold and drought stresses (Tao et al., 2009, 2011). Overexpression of rice WRKY30
transcription factor increases rice resistance to fungal pathogen and drought stress (Peng et al., 2012; Shen et al., 2012). However, our understanding of the molecular mechanisms underlying the crosstalk through these convergent nodes remains preliminary, and the direct downstream components of these nodes in both abiotic and biotic stress signaling are largely unknown.

The function of the rice WRKY13 gene in the crosstalk between disease resistance and abiotic stress resistance pathways has been more intensively studied. WRKY13 enhances rice resistance to Xoo and M. oryzae and decreases rice resistance to cold and salt stresses (Qiu et al., 2007, 2008). Overexpression of WRKY13 transcriptionally influenced more than 500 genes, including those known to function in disease resistance and abiotic stress responses (Qiu et al., 2008). The rice SNAC1 gene, encoding a NAC-type transcription factor, was induced predominantly by drought in guard cells that constitute the stomata; overexpression of SNAC1 significantly enhanced drought resistance in transgenic rice, which is associated with increased stomatal closure (Hu et al., 2006). Overexpressing WRKY13 suppressed SNAC1; genome-wide analysis of the expression profiles of WRKY13-transgenic lines suggested that the WRKY13-associated disease resistance pathway interacts antagonistically with the SNAC1-mediated abiotic stress responses (Qiu et al., 2008). Further research suggested that WRKY13-mediated signaling pathways are partitioned by different transcription factors. When WRKY13 was activated, the expression of at least 39 transcription factor genes was influenced and 30 of them were downregulated (Qiu et al., 2009). Analysis of the cis-regulatory elements of all the WRKY13-influenced genes suggested that WRKY13-influenced WRKY transcription factors may play important roles in the transcriptional regulation of WRKY13-upregulated genes and pathogen-associated defense-responsive genes. One of the WRKY13-regulated WRKY genes is WRKY45. WRKY13 appears to suppress both alleles of WRKY45, WRKY45-1 and WRKY45-2. In rice, WRKY13 physically binds to the promoter regions of these alleles, which harbor W or W-like box-type cis-regulatory elements for the binding of WRKY transcription factors (Qiu et al., 2009; Tao et al., 2009). In addition, WRKY13 can also bind to its own gene promoter via a W-like box and a non-W or non-W-like box cis-element in vitro (Qiu et al., 2007; Cai et al., 2008). However, it remains to be determined whether WRKY13 is a transcriptional repressor and whether it directly regulates SNAC1 and its
own gene expression in rice. It is also unclear which cis-elements on the promoter of WRKY45 are essential for WRKY13 binding during rice responses to abiotic and biotic stresses.

To address these questions, we analyzed the interactions of WRKY13 with candidate promoters in rice and the tissue- and cell-specific expression patterns of WRKY13 in abiotic and biotic stress responses. Our results suggest that WRKY13 is a negative regulator of transcription. It transcriptionally regulates rice responses to both drought stress and bacterial infection by specifically binding to certain cis-acting elements on the promoters of the drought resistance–related gene SNAC1, defense-responsive gene WRKY45-1, and its own gene.

RESULTS

Abiotic Stresses Influenced WRKY13 Expression

WRKY13 (also named EI12I1 for its cDNA) is transcriptionally induced in rice–pathogen interactions. The increased transcript level is closely associated with the presence of a major disease resistance gene Xa3/Xa26 against Xoo and positively correlated with the transcript level of Xa3/Xa26 (Wen et al., 2003; Cao et al., 2007; Cai et al., 2008). To determine whether WRKY13 was also transcriptionally responsive to abiotic stress, its expression in rice varieties Mudanjiang 8 and Minghui 63 was examined after drought and high salinity (150 mM NaCl) stresses. WRKY13 expression was significantly suppressed ($P < 0.05$) at 12 h to 5 d after drought stress in both rice varieties (Fig. 1, Supplemental Fig. S1). Salt stress also significantly suppressed ($P < 0.05$) WRKY13 at 3 to 36 h after treatment in the two rice varieties (Fig. 1, Supplemental Fig. S1). These results suggest that WRKY13 is involved in rice responses to abiotic stresses. The suppressed expression of WRKY13 in response to salt stress in Mudanjiang 8 is consistent with the phenotype of WRKY13-transgenic plants after stress. Overexpression of WRKY13 in the genetic background of Mudanjiang 8 resulted in growth retardation of these transgenic plants after salt stress (Qiu et al., 2008).

WRKY13-Transgenic Plants Showed Altered Response to Drought Stress

To test the putative role of WRKY13 in drought stress, we examined the
phenotype of WRKY13-transgenic plants after drought stress. The survival rates of the two WRKY13-overexpressing (WRKY13-oe) lines were 22% and 18%, which are significantly lower ($P < 0.05$) than that (34%) for the corresponding control (wild-type 1 with the genetic background of japonica rice variety Mudanjiang 8); the survive rates of the two WRKY13-RNAi lines were 19% and 20% compared to 14% for the corresponding control (wild-type 2 with the genetic background of indica rice variety Minghui 63; Fig. 2A). We then measured water loss rates of detached leaves from the transgenic plants. Consistent with the phenotypes of these transgenic plants, WRKY13-oe plants had a significantly higher ($P < 0.05$) water loss rate than the corresponding control at 1 to 1.5 h after detaching the leaves, whereas WRKY13-RNAi plants had a significantly ($P < 0.05$) lower water loss rate than the corresponding control at 0.5 to 2 h after detaching the leaves (Fig. 2B). These results suggest that WRKY13 negatively regulates rice response to drought stress.

WRKY13 Functioned as a Transcriptional Repressor

Rice SNAC1 transcription factor enhances drought resistance by promoting stomatal closure (Hu et al., 2006). WRKY13-oe plants showed markedly suppressed expression of SNAC1 without pathogen infection and in rice–Xoo interaction (Qiu et al., 2008). The present study showed that WRKY13-RNAi plants have significantly enhanced ($P < 0.05$) expression of SNAC1 compared to control plants without infection (Fig. 3A). The opposite expression patterns of SNAC1 in WRKY13-oe and WRKY13-RNAi plants as compared to its expression in control plants suggest that WRKY13 negatively and transcriptionally regulates SNAC1.

To further examine whether WRKY13 was a negative transcriptional regulator, we analyzed the interaction of WRKY13 protein and the SNAC1 promoter by transient expression of WRKY13 driven by the constitutive 35S promoter in rice calli generated from transgenic lines carrying a SNAC1 promoter-reporter ($\beta$-glucuronidase, GUS) gene ($P_{SNAC1};GUS$) construct via Agrobacterium-mediated transformation. The expression of GUS driven by the SNAC1 promoter in the calli was rapidly suppressed (2 h after transformation of WRKY13) compared to that in the calli immediately before the transformation (0 h, Fig. 3B). The GUS expression in the transformed calli reached the lowest level at 12 h after transformation of WRKY13. However, the expression of GUS
in the mock-transformed (control) calli was maintained at a relatively high and stable level throughout the time courses examined. The GUS level in the control calli was more than 6-fold higher than that in WRKY13-transformed calli at 12 h after transformation.

WRKY13 protein can also bind to its native promoter in vitro (Qiu et al., 2007; Cai et al., 2008). To ascertain whether WRKY13 could also suppress its own expression, we transiently expressed WRKY13 in rice calli carrying a \( P_{WRKY13}:GUS \) construct via Agrobacterium-mediated transformation. The expression of \( GUS \) driven by the \( WRKY13 \) promoter in the calli was markedly suppressed compared to that in untransformed calli (ck, Fig. 3B). Mock-transformation (control) did not markedly influence \( GUS \) expression driven by the \( WRKY13 \) promoter. The GUS level in the control calli was approximately 5-fold higher than that in \( WRKY13 \)-transformed calli at 12 h after transformation.

WRKY13 can also bind to \( WRKY45-1 \) promoter in rice (Tao et al., 2009). To examine whether WRKY13 could also suppress \( WRKY45-1 \) expression, we transiently expressed \( WRKY45-1 \) promoter-reporter (green fluorescence protein, GFP) gene \( (P_{WRKY45-1}:GFP) \) in rice calli overexpressing \( WRKY13 \) via Agrobacterium-mediated transformation. The transcript level of \( GFP \) driven by the \( WRKY45-1 \) promoter in the calli was increased at 12 h after transformation (Fig. 3C). However, the \( GFP \) transcript level in the wild-type calli (control) was significantly higher \( (P < 0.05) \) than that in \( WRKY13\)-oe calli. These results suggest that WRKY13 is a transcriptional repressor that not only suppresses \( SNAC1 \) and \( WRKY45-1 \) but perhaps its own gene as well.

**WRKY13 Selectively Bound to DNA Segments Harboring Known cis-Elements on \( SNAC1, WRKY13, \) and \( WRKY45-1 \) Promoters during Abiotic and Biotic Stresses in Vivo**

Sequence analysis revealed that the promoter region of \( SNAC1 \) harbored five \( cis \)-acting elements putative for WRKY protein binding, the W-like boxes. The five boxes include two “TTGACT” type W-like boxes (named WBOX1 and WBOX3 according to their locations in the promoter region) and three “TGACC” type W-like boxes (named WBOX2, WBOX4, and WBOX5; Fig. 4A, Supplemental Fig. S2). To evaluate whether WRKY13 directly bound to \( SNAC1 \) promoter, we performed
chromatin immunoprecipitation (ChIP) assays using anti-WRKY13 antibody. Four segments of the *SNAC1* promoter, which harbor W-like boxes, were analyzed by quantitative PCR before immunoprecipitation (input) or after immunoprecipitation (IP) with or without anti-WRKY13 antibody (Fig. 4A, Supplemental Fig. S2). The IP percentage (IP%), which was the percentage of PCR product from IP relative to that from input, was used to evaluate the binding intensity of WRKY13 to the target DNA segment. After immunoprecipitation with anti-WRKY13 antibody, we detected 4.6-, 3.4-, and 7.7-fold increased IP% of the DNA segment harboring WBOX1 from untreated, *Xoo*-inoculated, and drought-stressed samples, respectively, as compared to their corresponding controls (samples after precipitation without anti-WRKY13 antibody; Fig. 4A). Compared to untreated sample, there was less of an increase in IP% of the DNA segment harboring WBOX1 in the *Xoo*-inoculated sample and more of an increase in IP% of this segment in the drought-stressed sample. No significant or obvious increased IP% of the DNA segments harboring WBOX2, WBOX3, or WBOX4 and WBOX5 was detected; thus, WBOX2, WBOX3, WBOX4, and WBOX5 could serve as controls in this assay. WBOX1 and WBOX3 are the same type of cis-elements, but with different flanking sequences (Supplemental Fig. S2), indicating the binding specificity of WRKY13 to the DNA segment harboring WBOX1. These results suggest that WRKY13 binds to the promoter of *SNAC1* in vivo; it may preferentially bind to the WBOX1 cis-element of the *SNAC1* promoter both with and without abiotic and biotic stresses.

WRKY13 can bind to its native promoter at two sites, a novel cis-element PRE4 (TACTGCGCTTAGT) and a DNA probe harboring the complementary strand (TCTAGAACGTCAAATAAAAA; named WBOX-C in this paper) of a W-like box WBOX (TTTTATTTGACGTTCTAGA; W-like box is underlined) as analyzed using electrophoretic mobility shift assay (EMSA; Qiu et al., 2007; Cai et al., 2008). To determine whether WRKY13 protein physically bound to its native promoter (Supplemental Fig. S3) in vivo, we performed ChIP assays using the same samples used for studying the interaction of WRKY13 and the *SNAC1* promoter. After immunoprecipitation with anti-WRKY13 antibody, we detected 1.8- and 4.2-fold increased IP% of the DNA segments harboring PRE4 and WBOX-C from untreated samples, respectively, as compared to their corresponding controls (Fig. 4B).
Approximately 3-fold increased IP% of the segments harboring PRE4 and WBOX-C from the Xoo-inoculated sample was detected. However, 5.1-fold increased IP% of the segment harboring WBOX-C but not PRE4 from the drought-stressed sample was detected. No increased IP% of the segment harboring cis-element PRE2 (ACGCTGCCG), which is specific for the binding of other proteins (Cai et al., 2008), was detected, indicating the specificity of the binding in the ChIP assays. These results suggest that WRKY13 binds to its native promoter in vivo; it appears to bind selectively and preferentially to different segments under different physiological conditions.

Activation of WRKY13 suppressed the expression of the defense-responsive gene WRKY45-1, and this suppression was reduced after Xoo infection (Qiu et al., 2009). In addition, WRKY13 protein can bind to the WRKY45-1 promoter in vivo without stress (Tao et al., 2009). To ascertain whether WRKY13 also bound to site-specific W or W-like boxes on the WRKY45-1 promoter during rice responses to abiotic and biotic stresses, four segments of the WRKY45-1 promoter, which harbored W-like boxes WBOXa (TTGAC) and WBOXb (TGACC), W box WBOXd (TTGACC), and the complementary strand (ACTGA; named WBOXc-C in this paper) of a W-like box WBOXc (TGACT), were analyzed (Supplemental Fig. S4). Consistent with previous results (Tao et al., 2009), we detected 4.2- and 5.2-fold increased IP% of DNA segments harboring WBOXa and WBOXc-C, respectively, but not WBOXb and WBOXd from untreated control after immunoprecipitation with anti-WRKY13 antibody (Fig. 4C). Increased IP% of the DNA segments harboring WBOXa and WBOXc-C was also detected in Xoo-inoculated and drought-stressed samples. However, compared to the untreated control, there was less of an increase in IP% of the DNA segments harboring WBOXa and WBOXc-C in treated samples. These results suggest that WRKY13 preferentially binds to W-like boxes other than the W box on the WRKY45-1 promoter under both stressed and unstressed conditions.

WRKY13 Preferentially Bound to W-Like Boxes and PRE4 Element to Regulate Gene Expression during Abiotic and Biotic Stresses

To determine whether the positive segments for WRKY13 binding in the promoter regions of target genes identified by ChIP assays were due to known cis-elements, we performed EMSA. Total nuclear protein samples from untreated control,
Xoo-inoculated, and drought-stressed rice plants were used for the binding analysis. The proteins from both untreated and treated plants bound to the probe harboring WBOX1 but not to the probe harboring WBOX2 from the SNAC1 promoter (Fig. 5A). The protein binding to WBOX1 probe was reduced or abolished by adding anti-WRKY13 antibody. The binding of protein from drought-stressed and Xoo-inoculated plants was abolished by mutating the “TTGACT” sequence of WBOX1 element in the probe to “TTGACC” (M7 probe) that was a typical W box. However, protein from untreated plants showed a weak binding to M7 probe, and this binding was abolished by adding anti-WRKY13 antibody (Figs. 5A, C). Consistent with the results from ChIP assays (Fig. 4A), there was more protein from untreated and drought-stressed plants bound to the WBOX1 probe compared to the binding intensity of the protein from Xoo-inoculated plants (Fig. 5A). These results suggest that the binding protein is primarily WRKY13, and WRKY13 specifically and preferentially binds to the W-like box WBOX1 on the SNAC1 promoter under different physiological conditions.

The nuclear proteins also bound to DNA probes harboring cis-element WBOX-C and PRE4, respectively, but not PRE2 on the WRKY13 promoter (Fig. 5B). Anti-WRKY13 antibody reduced or abolished this binding. Mutation of WBOX-C (M8 probe) or PRE4 (Mc probe) abolished this binding, which is consistent with previous reports (Qiu et al., 2007; Cai et al., 2008). Consistent with the results from ChIP assays (Fig. 4B), there was more protein from untreated and drought-stressed plants binding to the WBOX-C probe compared to the binding intensity of protein from Xoo-inoculated plants (Fig. 5B). Only protein from Xoo-inoculated plants bound to PRE4, and anti-WRKY13 antibody reduced this binding. Mutation of PRE4 (Mc probe) abolished protein binding. These results suggest that WRKY13 protein also specifically and preferentially binds to WBOX-C and PRE4 elements on the WRKY13 promoter under different physiological conditions.

The nuclear proteins also bound to DNA probes harboring cis-element WBOX-a or WBOXc-C from the WRKY45-1 promoter, respectively (Fig. 5C). Anti-WRKY13 antibody reduced this binding. Mutation of WBOXa (M9 probe) or WBOXc-C (Mh probe) abolished this binding (Figs. 5A, C). These results suggest that WRKY13 protein also specifically and preferentially binds to WBOXa and WBOXc-C elements on the WRKY45 promoter under different physiological conditions.
To determine whether the differential binding of WRKY13 protein to different *cis*-elements affected transcriptional regulation of these promoters, we constructed five site-mutated promoters, in which the core sequences of *cis*-elements WBOX1 on the *SNAC1* promoter, PRE4 and WBOX-C on the *WRKY13* promoter, and WBOXa and WBOXc-C on the *WRKY45-1* promoter were mutated in the same way as DNA probes used for EMSA assays (Fig. 6, Supplemental Fig. S5). Four site-mutated promoters, in which the core sequences of WBOX2 and WBOX3 on the *SNAC1* promoter, PRE2 on the *WRKY13* promoter, and WBOXb on the *WRKY45-1* promoter were mutated, were used as negative controls (Fig. 6, Supplemental Fig. S5). Rice callus has been proved to be a reliable material for studying rice–*Xoo* interaction (Yuan et al., 2011), and it is also frequently used for studying abiotic stresses, such as salt and water stresses (Ahmad et al., 2007; Wani et al., 2010; Maragathamani and Khurana, 2012). These mutated promoters were fused with GFP and transiently expressed in the calli generated from the *WRKY13*-oe line and wild-type Mudanjiang 8.

*WRKY13* expression is induced by *Xoo* invasion in rice plants (Cai et al., 2008). Its expression was also slightly induced in rice calli after *Xoo* treatment (Fig. 6). Unexpectedly, *WRKY13* expression in rice calli was obviously induced after drought treatment (Fig. 6, Supplemental Fig. S5), but its expression in rice plants was suppressed after drought stress (Fig. 1), suggesting that the drought treatment on rice calli may not fully represent drought stress on rice plants under the present condition. However, consistent with the results that WRKY13 is a transcriptional repressor that binds to the promoters of *SNAC1* and *WRKY45-1* (Figs. 3–5), the transcript levels of GFP driven by the native promoters of *SNAC1* (*P*-*SNAC1*) and *WRKY45-1* (*P*-*WRKY45-1*) were negatively correlated with the transcript level of *WRKY13* (Fig. 6A, B; Supplemental Fig. S5A, B). Mutation of the WBOX1 element on the *SNAC1* promoter to a typical W box (TTGACC; *P*-*SNAC1*△152C) resulted in significantly increased (\(P < 0.01\)) GFP transcripts compared with that driven by *P*-*SNAC1* (Fig. 6A, Supplemental Fig. S5A). However, mutation of WBOX2 (*P*-*SNAC1*△298G) or WBOX3 (*P*-*SNAC1*△645G) on the *SNAC1* promoter did not change the transcript level of *GFP*. The *GFP* transcripts was also significantly increased (\(P < 0.05\)) after mutation of WBOXa (*P*-*WRKY45-1*△30G) and WBOXc-C (*P*-*WRKY45-1*△408C), but not WBOXb (*P*-*WRKY45-1*△93G), on the *WRKY45-1* promoter (Fig. 6B, Supplemental Fig. S5B). These results provide further evidence that WRKY13 may
suppress \textit{SNAC1} through the WBOX1 element and suppress \textit{WRKY45-1} through the WBOXa and WBOXc-C elements.

Mutation of WBOX-C (\textit{P}_{\textit{WRKY13A}_{\text{541T}}}), but not PRE2 (\textit{P}_{\textit{WRKY13A}_{\text{371G}}}), on the \textit{WRKY13} promoter also resulted in a significantly increased (\textit{P} < 0.01) \textit{GFP} transcript level in untreated and \textit{Xoo}- or drought-treated calli compared to the \textit{GFP} transcript level regulated by the native promoter (\textit{P}_{\textit{WRKY13}}; Fig. 6C, Supplemental Fig. S5C). Consistent with the results of ChIP and EMSA assays that \textit{WRKY13} preferentially bound to the PRE4 element on the \textit{WRKY13} promoter only after \textit{Xoo} inoculation (Figs. 4, 5), mutation of PRE4 influenced the \textit{GFP} transcript level only in \textit{Xoo}-treated but not untreated or drought-treated calli (Fig. 6C, Supplemental Fig. S5C). However, mutation of PRE4 (\textit{P}_{\textit{WRKY13A}_{\text{158T}}}) resulted in a slightly decreased \textit{GFP} transcript level in \textit{Xoo}-treated calli. This result is consistent with a previous report that PRE4 positively regulated marker gene expression after \textit{Xoo} challenge (Cai et al., 2008). In addition to \textit{WRKY13}, several other nuclear proteins can also bind to PRE4, but with different binding core sequences (Cai et al., 2008). These results suggest that \textit{WRKY13} may suppress its own gene expression via the WBOX-C element and it may cooperate with other proteins to maintain or increase its gene expression via PRE4 in rice–\textit{Xoo} interactions.

\textbf{\textit{WRKY13} Was Transcriptionally Induced in Vascular Tissue and Guard Cells during \textit{Xoo} Infection and Drought Stress}

To ascertain in which tissue \textit{WRKY13} regulated the crosstalk between abiotic and biotic stress signaling pathways, we expressed \textit{GFP} driven by the promoter of \textit{WRKY13} (\textit{P}_{\textit{WRKY13}}) in rice variety Mudanjiang 8. Without biotic and abiotic stress (control), a strong \textit{GFP} signal was observed in various tissues (i.e., callus, node, stem, collar, ligule, root, pistil, lemma, and palea) in the transgenic plants carrying \textit{P}_{\textit{WRKY13}}:\textit{GFP}, but no obvious \textit{GFP} signal was observed in the leaf tissue (Fig. 7A). Two hours after inoculation of \textit{Xoo}, a detectable \textit{GFP} signal was observed in the vascular tissue of leaf immediately next to the infection site, whereas no obvious \textit{GFP} signal difference was observed in other tissues compared to the tissues from uninfected plants. However, 2 h after drought stress, the \textit{GFP} expression pattern in various tissues showed no obvious difference from that in control plants (Fig. 7A). \textit{Xoo} invades rice plants...
through hydathodes or wounds and spreads via the vascular system. Thus, the tissue-
specific induction of \textit{WRKY13} by \textit{Xoo} infection is associated with its function in
bacterial resistance.

Enhanced \textit{SNAC1} expression specifically in guard cells is associated with
increased stomatal closure in \textit{SNAC1}-mediated drought resistance (Hu et al., 2006). To
ascertain whether \textit{WRKY13} had a similar cell-specific expression pattern as \textit{SNAC1} in
rice response to drought stress, we analyzed GFP expression in guard cells of transgenic
plants carrying \textit{P}_{WRKY13}:GFP. No GFP expression was detected in untreated (control)
plants, whereas a strong GFP signal was observed in guard cells 2 h after drought stress
(Fig. 7B). Expression of \textit{P}_{WRKY13}:GFP in guard cells was also observed at 30 min after
\textit{Xoo} infection. These results suggest that expression of \textit{WRKY13} in guard cells is
specifically induced by both biotic and abiotic stresses.

**DISCUSSION**

\textit{WRKY13} plays multiple roles in rice biological activities. It is involved in the
regulation of antagonistic crosstalk between the pathways of abiotic stress response and
biotic resistance, and it reduces rice plant height and delays flowering time (Qiu et al.,
2007, 2008). Although \textit{WRKY13} possesses the structural characteristics of \textit{WRKY}-type
transcription factors and has DNA-binding ability, its transcriptional regulation function
has not been examined previously (Qiu et al., 2007, 2009; Cai et al., 2008; Tao et al.,
2009). The present results suggest that \textit{WRKY13} is a transcriptional repressor. This
conclusion is also supported by a previous report that \textit{WRKY13} preferentially bound to
the promoters of genes whose expression was downregulated in \textit{WRKY13}-oe lines
analyzed by yeast one-hybrid assays (Qiu et al., 2009). The transcriptional repressor
function of \textit{WRKY13} appears to be associated with its direct binding to site- and
sequence-specific \textit{W-like box-type and PRE4 cis-elements} on the promoters of genes
functioning in abiotic and biotic stress responses and on its native promoter (Fig. 8).

**WRKY13 Specifically Binds to Multiple Types of \textit{cis}-Elements in Rice**

\textit{WRKY}-type transcription factors are well known to bind to \textit{W} and \textit{W-like boxes}
(Eulgem et al., 2000; Eulgem and Somssich, 2007; Yuan and Wang, 2012). However,
several studies have revealed that some WRKY proteins can bind to non-W or non-W-like box *cis*-elements (Sun et al., 2003; Cai et al., 2008; van Verk et al., 2008). For example, a sucrose-regulated barley WRKY transcription factor, SUSIBA2, can bind to both W box and a sugar-responsive element (Sun et al., 2003). Rice WRKY13 was shown to bind to *cis*-element PRE4 that is not the sequence homolog of W and W-like boxes in yeast cells and in vitro (Cai et al., 2008). In addition, WRKY13 appears to bind to the complementary strand (WBOX-C: GTCAA) of a W-like box (WBOX: TTGAC) on its native promoter in vitro (Qiu et al., 2007, 2008). WRKY proteins in plants can bind to W box core sequence TGAC or its complementary sequence GTCA (Rushton et al., 1996). The present results further suggest that WRKY13 can bind to PRE4 and WBOX-C in vivo. This protein can also bind to the complementary strand (WBOXc-C: ACTGA) of a W-like box (WBOXc: TGACT) on the WRKY45-1 promoter. In addition, as compared to the typical W box (TTGACC), WRKY13 preferentially binds to the W-like boxes WBOX1 (TTGACT) and WBOXa (TTGAC), respectively, on the promoters of SNAC1 and WRKY45-1 in vivo. These conclusions are supported by in vivo ChIP assays and in vitro sequence-specific EMSA assays, which showed a consistent WRKY13 binding intensity and specificity to the DNA segments and probes harboring WBOX1, PRE4, or WBOX-C when comparing untreated and stress-treated samples (Figs. 4, 5). Furthermore, site mutation of WBOX1, PRE4, WBOX-C, WBOXa, or WBOXc-C influenced the expression of marker gene driven by the mutated promoters (Fig. 6, Supplemental Fig. S5).

Although WBOX1 and WBOX3 are the same type of W-like boxes on the SNAC1 promoter (Supplemental Fig. S2), WRKY13 only bound to WBOX1. This selective binding of WRKY13 on the SNAC1 promoter may be due to the fact that WBOX1 and WBOX3 have different flanking sequences. Ciolkowski et al. (2008) reported that the W-box consensus alone is insufficient for the binding of WRKY proteins, and additional neighboring nucleotides or space between adjacent W-box elements also contribute to high-affinity binding. This explanation is also supported by the evidence that mutation of WBOX1 to TTGACC (probe M7), the typical W box, abolished WRKY13 binding to this probe under Xoo-inoculated or drought-stress conditions (Fig. 5A), whereas WRKY13 can bind to another probe (m1) harboring TTGACC (Qiu et al., 2007). The TTGACC elements in probes M7 and m1 have
different flanking sequences. These results suggest that WRKY13 can specifically bind to at least five types of cis-elements in vivo (Fig. 8). Furthermore, WRKY13 binding has DNA site specificity, which may be at least partly due to flanking sequence sensitivity.

**WRKY13 Differentially Regulates Abiotic and Biotic Stress Responses by Selectively Binding to Different cis-Elements of Target Genes**

Although it is a transcriptional repressor, WRKY13 enhances biotic resistance and decreases abiotic stress resistance (Fig. 2; Qiu et al., 2007, 2008). The opposite functions of WRKY13 appear to be at least partly performed by directly suppressing the expression of two transcription factor genes: *WRKY45-I*, which decreases both bacterial disease and abiotic stress resistance (Tao et al., 2009, 2011), and *SNAC1*, which increases drought resistance (Hu et al., 2006). It does this by binding to site- and sequence-specific cis-elements on their promoters in vascular tissue or guard cells (Fig. 8). This inference is supported by the following evidence.

First, WRKY13 binds to two segments harboring WBOXa and WBOXc-C but not other W-like boxes and W box on the *WRKY45-I* promoter in response to both bacterial infection and drought stress in vivo (Fig. 4C). *WRKY45-I* transcription factor also negatively regulates *SNAC1* expression (Tao et al., 2011). WRKY13 appeared to constitutively bind to WBOXa and WBOXc-C, but it had a stronger effect on WBOXa than WBOXc-C and it had a stronger effect during drought stress than *Xoo* stress and no stress (Figs. 6, 8). These findings suggest that WRKY13 may help to maintain the balance of *WRKY45-I* expression under different physiological conditions through the two cis-elements.

Second, WRKY13 bound to WBOX1 on the *SNAC1* promoter with different strengths of effect under different physiological conditions (Figs. 4A, 5A, 6, 8). WRKY13 also bound to WBOX1 without abiotic and biotic stresses, suggesting that WRKY13 may help to maintain the balance of *SNAC1* expression. However, this balance control may not occur in guard cells, because no *P<sub>WRKY13</sub>:GFP* expression was detected without stress (Fig. 7B). Increased expression of *SNAC1* in guard cells is associated with SNAC1-mediated drought resistance in rice (Hu et al., 2006). *Xoo* infection of rice and rice resistance to *Xoo* are not associated with stomata. Thus, *Xoo*-
induced rapid expression of $P_{\text{WRKY13}}$ in guard cells suggests that WRKY13 may suppress $SNAC1$ function in guard cells to temporarily ensure metabolic resources for a host defense response to pathogen infection. However, the total binding of WRKY13 to the $SNAC1$ promoter in leaf tissue was reduced in rice–$Xoo$ interactions compared to that under the untreated condition (Figs. 4A, 5A). Further research is required to determine whether the reduced total binding of WRKY13 to the $SNAC1$ promoter is associated with reallocating its function to guard cells. Drought stress also induced the expression of $P_{\text{WRKY13}}$ in guard cells. A large amount of WRKY13 protein bound to WBOX1 during drought stress (Figs. 4A, 5A). These results suggest that WRKY13 is the suppressor of $SNAC1$ not only in rice–bacteria interactions but also in drought stress.

WRKY13 had different binding intensities to the same $cis$-element in different physiological activities (Figs. 4, 5), which may be due to different states of this protein (e.g., phosphorylated, dephosphorylated, or phosphorylation of different amino acid residues) or the coexistence of other proteins or cofactors under different physiological conditions (Yuan and Wang, 2012). This inference is supported by the evidence that mutation of WBOX1 to a typical W box (M7 probe) abolished WRKY13 binding in $Xoo$ and drought stresses, but WRKY13 bound to M7 probe in untreated samples (Fig. 5A, C).

**WRKY13 Transcriptionally Autoregulates Its Own Expression**

WRKY proteins transcriptionally regulate not only other genes but also their own genes, as seen in parsley WRKY1 (Turck et al., 2004). The in vivo binding of WRKY13 to its native promoter (Fig. 4B), repression of its native promoter by transcriptional activation of WRKY13 (Fig. 1B), and altered expression of WRKY13 by mutation of target $cis$-elements on its promoter (Fig. 6C, Supplemental Fig. S5C) suggest that WRKY13 also has this autoregulation function. There are two noteworthy aspects of this autoregulation. First, the strong binding to and strong effect on WBOX-C element of WRKY13 without stress (Figs. 4B, 5B, 6, 8) suggest that this protein may help to maintain its own gene expression at a low level in some tissues, such as leaves. After biotic stress, the level of WRKY13 autoregulation of its own gene through WBOX-C was reduced. However, the large amount of WRKY13 binding to WBOX-C after drought stress suggests that this protein may also autoregulate its own gene...
expression at a low level during abiotic stress through this *cis*-element. Second, WRKY13 may cooperate with other protein(s) to promote its own gene expression during rice–*Xoo* interactions, mainly via the PRE4 element, because more WRKY13 proteins bound to the PRE4 element after *Xoo* infection and mutation of PRE4 slightly suppressed marker gene expression (Figs. 4B, 5B, 6C, Supplemental Fig. S5C; Cai et al., 2008).

CONCLUSIONS

WRKY13 is a transcriptional repressor that functions on the node of the disease and abiotic stress resistance pathways. It directly suppresses the expression of two important genes, *SANC1* and *WRKY45-1*, which are involved in abiotic stress resistance and rice–bacterium interaction, by binding to site- and sequence-specific W-like-type *cis*-elements on the promoters of these genes. WRKY13 also autoregulates its own gene expression, which may be associated with balancing its function as rice plants face varying environments. This study provides further insight into the complicated regulation of the crosstalk between signaling pathways leading to abiotic and biotic stress responses in rice. Furthermore, the present results also suggest that rice callus is a suitable tissue to study environmental defense responses.

MATERIALS AND METHODS

Rice Materials

The *WRKY13* gene (GenBank accession number EF143611) from indica (*Oryza sativa* L. ssp. *indica*) rice variety Minghui 63 was previously used for generating the *WRKY13*-overexpressing (oe) and *WRKY13*-suppressing (RNAi) plants (Qiu et al., 2007, 2009). The *WRKY13*-oe plants were generated using the maize ubiquitin gene promoter to drive *WRKY13* in the genetic background of japonica (*Oryza sativa* L. ssp. *japonica*) rice variety Mudanjiang 8 (Qiu et al., 2007). The *WRKY13*-RNAi plants were created using the RNA interference strategy in the genetic background of Minghui 63 (Qiu et al., 2009). Homozygous *WRKY13*-oe lines (D11UM1-1 and D11UM7-2) and *WRKY13*-RNAi lines (WRKY13S4-10 and WRKY13S12-4) were used for studying the role of
WRKY13 in drought stress. The wild-type plants were negative siblings from the WRKY13-oe segregating population (D11UM1-4-8) and WRKY13-RNAi segregating population (WRKY13S4-7-1) and were used as genotype controls.

**Abiotic Stress Treatment**

The stress treatments were performed as reported previously (Tao et al., 2011). In brief, rice plants grown in sandy soil were kept in a greenhouse with light intensity maintained at 12000–14000 lux and with a 14 h light/10 h dark cycle at 25°C until the three- to four-leaf stage. The humidity in the greenhouse was maintained at 50–60%. For studying WRKY13 expression after abiotic stress, water was withheld from Mudanjiang 8 and Minghui 63 for 4 h to 6 d for drought stress or plants were irrigated with a solution containing 150 mM NaCl for 3 to 12 h for salt stress.

For studying the role of WRKY13 in drought stress, water was withheld from WRKY13-transgenic and control plants growing in the same pot for 6 d (until almost all the leaves in the pot became completely rolled). Plants were then recovered by providing water for 5 to 9 d. The plants with more than 20% green leaves were considered to have survived and others were considered not to have survived. The survival rates were determined by calculating the percent surviving plants ([number of surviving plants/number of total plants] × 100). The experiments were biologically repeated twice. Similar results were obtained from both repetitions, but the results from only one are presented.

**Water Loss Rate**

Plants were grown in sandy soil in a greenhouse until the three- to four-leaf stage. Only fully expanded leaves were cut for measuring the loss of water. At the indicated time points, water loss rates of detached leaves from the plants were measured by monitoring the fresh weight loss: ([initial fresh leaf weight – leaf weight after water loss]/initial fresh leaf weight] × 100) (Xiang et al., 2008).

**Plasmid Construction and Rice Transformation**

Two GUS reporter constructs, $P_{WRKY13}$:GUS and $P_{SNAC1}$:GUS, were generated by ligating the promoter regions of WRKY13 (a 1700-bp fragment located at -1497 to +203;
the nucleotide immediately upstream of the translation start codon is numbered as “–1”) and \textit{SNAC1} (a 1635-bp fragment located at –1371 to +264) amplified using promoter-specific primers (Supplemental Table S1) from Minghui 63 (the \textit{WRKY13} promoters in Minghui 63 and Mudanjiang 8 have identical W-like box and PRE4 elements and sequences flanking these \textit{cis}-elements) and japonica rice variety IRAT109 (the \textit{SNAC1} genes in Mudanjiang 8 and IRAT109 have identical sequence in coding regions and promoter regions), respectively, to pCAMBIA1381 vector to regulate reporter gene \textit{GUS}. One \textit{GFP} construct, \textit{P\textit{WRKY13}:GFP}, was generated by ligating the promoter region of \textit{WRKY13} to pCAMBIA1381 vector to regulate reporter gene \textit{GFP}. The promoter constructs were transferred into Mudanjiang 8 by \textit{Agrobacterium}-mediated transformation (Lin and Zhang, 2005).

**Pathogen Inoculation**

Rice plants were inoculated with \textit{Xoo} strain PXO61 at the seedling or booting (panicle development) stage by the leaf clipping method (Chen et al., 2002). Mock-inoculated plants were treated under the same conditions except that the \textit{Xoo} suspension was replaced with water. Leaf fragments (about 5 to 8 cm long) adjacent to the inoculation site of the plants at the booting stage were used for gene expression analysis. Rice shoots from seedlings at the three-leaf stage were used for ChIP assay after inoculation with \textit{Xoo}.

**Gene Expression Analysis**

Total RNA was isolated from rice leaves using TRIzol reagent (Life Technologies Corporation, Carlsbad, CA, USA). Real-time quantitative RT-PCR (qRT-PCR) was performed on a 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) using SYBR Premix Ex Taq (TaKaRa, Dalian, China), according to procedure reported previously (Qiu et al., 2007). Primers for quantitative PCR are listed in Supplemental Table S2. The quantitative PCR data were calculated as reported by Livak and Schmittgen (2001). In brief, the mean (from three technical replicates) transcript level of the actin gene was first used to standardize the three technical replicates of RNA sample for each qRT-PCR. Then, the transcript fold differences of three technical replicates for treatment relative to the mean transcript (from three
technical replicates) for control were determined. The relative transcript level for
treatment is presented. Standard deviation represents the variation of three technical
replicates.

**Agrobacterium-Mediated Transient Expression**

Rice calli generated from the seeds of a transgenic line carrying \( P_{SNAC1}:GUS \) and
a transgenic line carrying \( P_{WRKY13}:GUS \) were used to study the regulation of WRKY13
protein on the expression of \( SNAC1 \) and \( WRKY13 \). The overexpression vector of
\( WRKY13 \) was constructed by inserting a 3-kb DNA fragment, harboring the \( WRKY13 \)
coding region that had been digested with \( BamHI \) from \( WRKY13 \)-overexpressing vector
D11U (Qiu et al., 2007), into the pCAMBIA1300S vector, which was modified by
insertion of a double 35S promoter of cauliflower mosaic virus and a napoline synthase
polyadenylation signal terminator into the multiple cloning sites of vector
pCAMBIA1300S (Xiong and Yang, 2003). WRKY13 was transiently expressed in the
calli via *Agrobacterium*-mediated transformation (Yuan et al., 2011). As a control, the
same calli were transformed with *Agrobacterium* that carried empty pCAMBIA1300S
vector (mock-transformation).

Rice calli generated from the seeds of a transgenic line (D11UM1-1)
overexpressing \( WRKY13 \) and wild-type Mudanjiang 8 were used to study the regulation
of WRKY13 protein on the expression of \( WRKY45-1 \). The \( P_{WRKY45-1}:GFP \) plasmid was
constructed by inserting the promoter fragment of \( WRKY45-1 \) (the \(-100 \) to \(-1097 \) region
in Supplemental Fig. S3) into the pCAMBIA1381 vector. The \( P_{WRKY45-1}:GFP \) was
transiently expressed in the calli via *Agrobacterium*-mediated transformation (Yuan et
al., 2011).

**Analysis of GUS Activity**

Leaf fragments (about 1 cm long) adjacent to the inoculation sites or rice calli
were used for analysis of GUS expression. Quantitative analyses of GUS activity were
conducted as described previously (Cai et al., 2007). Total protein concentration in the
supernatant was quantified with the Bradford assay (Bradford, 1976). GUS protein in
the supernatant was determined fluorometrically with an INFINITE 200 (Tecan Austria
GmbH, Ltd., 5082 Grodig, Austria).
Sequence Analysis

A 1.3- to 1.6-kb sequence upstream of the coding region of each target gene was analyzed to identify the *cis*-acting elements, W or W-like box, using the program PLACE Signal Scan (a database of plant *cis*-acting regulatory DNA elements: http://www.dna.affrc.go.jp/PLACE/signalscan.html). The W or W-like boxes were classified according to previously published criteria (Eulgem et al., 2000; Qiu et al., 2009; Yuan and Wang, 2012).

ChIP Assay

The ChIP assay was performed as described previously (Tao et al., 2009). Rice shoots from Mudanjiang 8 at the three-leaf stage were used for sample preparation. Sonicated chromatin fragments were immunoprecipitated with WRKY13-specific antibody, which was custom synthesized by NewEast Biosciences (Malvern, PA, USA), against peptide LEVPEPEPEQESEP (201 to 214 of WRKY13; Tao et al., 2009). The immunoprecipitated (IP) chromatin was analyzed by quantitative PCR using promoter-specific primers (Supplemental Table S2). The nonimmunoprecipitated and sonicated chromatin was used as the total input DNA control. The percent of immunoprecipitation (IP%) was used to compare different samples by calculating the ratio of target PCR product from IP relative to that from input (Haring et al., 2007).

Electrophoretic Mobility Shift Assay

The nuclear extract was isolated from leaves of rice plants at the six-leaf stage as reported previously (Cai et al., 2007). The protein content in the nuclear extract was quantified using the Bradford assay (Bradford, 1976). EMSA was performed as described previously (Cai et al., 2007). DNA probes used for analyzing the binding of DNA-WRKY13 protein are listed in Supplemental Table S3. To examine the binding specificity of WRKY13 to target DNA, the nuclear extract was pre-incubated with 100 ng anti-WRKY13 antibody at 25°C for 30 min before mixed with labeled DNA probe.

Site-Directed Mutation

The overlap extension (or nested) PCR technique was used for site-directed
mutation of putative WRKY13 binding sites in the promoters of SNAC1, WRKY13, and WRKY45-1 as described previously (Yuan et al., 2011). The mutagenic primer pairs (Supplemental Table S4), in which the target sites were mutated, were used to amplify the promoter fragments containing target mutation. T040PF/R, WRKY9F/9R, and WRKY45PF1/R1 primer pairs (Supplemental Table S1) were then used for the amplification of full-length mutated promoters of SNAC1, WRKY13, and WRKY45-1, respectively. Each site-mutated promoter was ligated to pDX2181 vector to regulate reporter gene GFP. The mutated promoter:GFP constructs were transiently expressed in rice calli generated from Mudanjiang 8 and transgenic line D11UM1-1, which overexpressed WRKY13, by Agrobacterium-mediated transformation (Qiu et al., 2007; Yuan et al., 2011). For bacterial treatment, the transformed calli were then treated with Xoo strain PXO61 at 10⁹ colony forming units/ml or mock-treated with autoclaved water for 2 h (Yuan et al., 2011). For drought stress, the transformed calli were dried in a laminar flow cabinet for 2 h. The expression of GFP was analyzed by qRT-PCR using gene-specific primers (Supplemental Table S2).

Statistical Analysis

The significance of differences between control and treated samples or between control and transgenic plants was analyzed by the pairwise t-test in the Microsoft Office Excel program (Microsoft, Redmond, WA, USA).

Supplemental Data

Supplemental Figure S1. Drought and salt stresses influenced WRKY13 expression in rice varieties Mudanjiang 8 and Minghui 63 analyzed by quantitative reverse transcription-PCR. Bars represent mean (three technical replicates) ± standard deviation. The “b” indicates that a significant difference was detected between the treated plants and untreated control at P < 0.05.

Supplemental Figure S2. The promoter region of rice SNAC1 in rice variety Mudanjiang 8. The nucleotide immediately upstream of the translation start codon is numbered as “−1”. Arrows indicate the positions of PCR primers used for chromatin immunoprecipitation (ChIP) assays. The putative W-like boxes for WRKY protein
binding in the DNA segments for ChIP assays are underlined and named WBOX1 to WBOX5.

**Supplemental Figure S3.** The promoter region of rice WRKY13 in rice variety Mudanjiang 8. The nucleotide immediately upstream of the translation start codon is numbered as “–1”. Arrows indicate the positions of PCR primers used for chromatin immunoprecipitation (ChIP) assay. The putative cis-elements PRE4 and WBOX-C for WRKY13 protein binding and negative control cis-element PRE2 in the DNA segments for ChIP assays are underlined.

**Supplemental Figure S4.** The promoter region of rice WRKY45-1 in rice variety Mudanjiang 8. The nucleotide immediately upstream of the translation start codon is numbered as “–1”. Arrows indicate the positions of PCR primers used for chromatin immunoprecipitation (ChIP) assay. The putative cis-elements for WRKY protein binding in the DNA segments for ChIP assays are underlined and named WBOXa, WBOXb, WBOXc-C, and WBOXd.

**Supplemental Figure S5.** Expression of GFP regulated by native and mutated SNAC1 (A), WRKY45-1 (B), and WRKY13 (C) promoters in rice calli was analyzed by quantitative reverse transcription-PCR. Calli were generated from rice variety Mudanjiang 8. The calli were untreated (control) or treated with Xoo (strain PXO61) or drought for 2 h. Asterisks indicate that a significant difference was detected between native and mutated promoters within the same treatment at $P < 0.01$ (***) and $P < 0.05$ (*).

**Supplemental Table S1.** PCR primers used for construction of transformation vectors and sequence analysis

**Supplemental Table S2.** Primers used for real-time PCR analyses

**Supplemental Table S3.** DNA probes used for electrophoretic mobility shift assays
Supplemental Table S4. Primers used for point mutation of W-like boxes
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Figure legends

Figure 1. Drought and salt stresses influenced *WRKY13* expression in rice varieties Mudanjiang 8 and Minghui 63 analyzed by quantitative reverse transcription-PCR. Bars represent mean (three technical replicates) ± standard deviation. The “a” and “b” indicate that a significant difference was detected between the treated plants and untreated control at *P* < 0.01 and *P* < 0.05, respectively.

Figure 2. Modulating *WRKY13* expression influenced rice response to dehydration. *WRKY13*-oe, *WRKY13*-overexpressing lines D11UM1-1 and D11UM7-2; *WRKY13*-RNAi, *WRKY13*-suppressing lines WRKY13S4-10 and WRKY13S12-4. The “a” and “b” indicate that a significant difference was detected between *WRKY13*-transgenic plants and corresponding control plants at *P* < 0.01 and *P* < 0.05, respectively. A, *WRKY13*-oe plants and *WRKY13*-RNAi plants showed opposite responses to drought stress. Water was withheld from rice seedlings at the three- to four-leaf stage for 6 d. After 5 d of recovery, the survival rates were recorded. Bars represent mean (45 to 50 plants) ± standard deviation. B, Water loss of detached leaves in transgenic rice plants after drought stress was analyzed. Each data point represents mean (three replicates with each replicate consisting of approximately 30 detached leaves) ± standard deviation.

Figure 3. *WRKY13* regulated *SNAC1*, *WRKY13*, and *WRKY45-1* expression. Each data represent mean (three technical replicates) ± standard deviation for gene expression analyzed by quantitative reverse transcription-PCR (A and C). 0 h, immediately after transformation (B and C). A, Suppressing *WRKY13* (*WRKY13*-RNAi) increased *SNAC1* transcript level at the booting stage analyzed by quantitative reverse transcription-PCR. The “a” and “b” indicate that a significant difference was detected between *WRKY13*-RNAi plants and wild-type control at *P* < 0.01 and *P* < 0.05, respectively. B, Transient overexpression of *WRKY13* in rice calli suppressed *GUS* expression driven by the *SNAC1* (*P_{SNAC1}* or *WRKY13* (*P_{WRKY13}*)) promoter. Mock, mock-transformation of rice calli carrying *PSNAC1:GUS* or *P_{WRKY13}:GUS*. GUS activity was determined by measuring the amount of 4-methylumbelliferone (Mu) produced under the catalysis of GUS in 1 mg total protein per minute. Each data point represents mean (three biological replicates with each replicate containing approximately 20 g calli) ± standard deviation. C,
Overexpressing **WRKY13** suppressed the transcript level of **GFP** driven by the WRKY45-1 promoter (\( P_{WRKY13} \)) analyzed by quantitative reverse transcription-PCR. WT, wild-type Mudanjiang 8; WRKY13-oe, WRKY13-overexpressing line D11UM1-1. Asterisks indicate that a significant difference was detected between **GFP** expression in WRKY13-oe background and WT background at \( P < 0.01 (**\) and \( P < 0.05 (*)\). Each data point was obtained by analyzing approximately 5 g calli.

**Figure 4.** WRKY13 protein bound to the promoter regions of **SNAC1** (A), **WRKY13** (B), and WRKY45-1 (C) analyzed by ChIP assays. Samples were from rice variety Mudanjiang 8 that was untreated (control), 2 d after inoculation with *Xoo* (strain PXO61), or 2 d after drought stress (withholding water). The quantitative PCR was conducted before immunoprecipitation (input), after immunoprecipitation (IP) using anti-WRKY13 antibody (white bar), or mock immunoprecipitation (without using anti-WRKY13 antibody, black bar). The presented percentage of PCR product from IP is relative to that from input. Bars represent mean (three replicates) ± standard deviation. Asterisks indicate that a significant difference was detected between the PCR products from IP with and without using anti-WRKY13 antibody at \( P < 0.01 (**\) and \( P < 0.05 (*)\). The numbers in the white bars are fold differences compared to samples without using anti-WRKY13 antibody for IP. 1 to 5, cis-elements WBOX1 to WBOX5 on the SNAC1 promoter. The amplification of DNA fragment harboring a non-WRKY13 binding element PRE2 served as sample quantity control. a to d, cis-elements WBOXa, WBOXb, WBOXc-C, and WBOXd on the WRKY45-1 promoter.

**Figure 5.** WRKY13 selectively bound to the cis-elements on **SNAC1** (A), **WRKY13** (B), and WRKY45-1 (C) promoters under different physiological conditions analyzed by electrophoretic mobility shift assay. The sequences of DNA probes harboring different cis-element (underlined) and their corresponding point-mutated probes are presented. WBOX1 and WBOX2 are cis-elements on the SNAC1 promoter; WBOX-C, PRE4, and PRE2 are cis-elements on the WRKY13 promoter; WBOXa and WBOXc-C are cis-elements on the WRKY45-1 promoter. Samples were from rice variety Mudanjiang 8 that was untreated (control), 2 d after inoculation with *Xoo* (strain PXO61), and 2 d after drought stress (withholding water). The cis-elements WBOX2 and PRE2 are negative
controls. +, plus 100 ng anti-WRKY13 antibody; −, without using anti-WRKY13 antibody.

**Figure 6.** Expression of GFP regulated by native and mutated SNAC1 (A), WRKY45-1 (B), and WRKY13 (C) promoters in rice calli was analyzed by quantitative reverse transcription-PCR. Calli were generated from WRKY13-overexpressing line D11UM1-1. The calli were untreated (control) or treated with *Xoo* (strain PXO61) or drought for 2 h. Asterisks indicate that a significant difference was detected between native and mutated promoters within the same treatment at $P < 0.01$ (***) and $P < 0.05$ (*).

**Figure 7.** WRKY13 showed specific tissue and cell expression patterns. *Xoo*, 2 h (for tissue expression pattern) or 30 min (for cell expression pattern) after inoculation of *Xoo* strain PXO61; drought, 2 h after drought stress (withholding water); control, before stress treatment. A, Green fluorescent protein (GFP) was expressed in different rice tissues in T$_1$ transgenic plants carrying $P_{WRKY13}$:GFP. The green fluorescence is GFP and red fluorescence is autofluorescence of tissues. Part of leaf and stem tissues, entire node, collar, ligule, and a root branch were collected at the booting stage; entire pistil, lemma, and palea were collected at flowering and grain-filling stages. B, Expression of GFP in guard cells of stomata after *Xoo* infection or drought stress in T$_1$ transgenic plants carrying $P_{WRKY13}$:GFP at the four-leaf stage. Scale bars: 15 μm.

**Figure 8.** Model of how WRKY13 decreases resistance to drought stress and enhances resistance to bacterial pathogen *Xoo* by regulating the crosstalk between abiotic and biotic signaling pathways by binding to site- and sequence-specific cis-elements on the promoters of SNAC1, WRKY45-1, and its own gene. Arrow, promoting gene expression through specific cis-element; inhibiting marker (⊥), inhibiting gene expression through specific cis-element. The thin and thick lines indicate weak and strong effects of WRKY13 protein on different cis-element based on the results presented in Figure 6 and Supplemental Figure S5. The treatments (no stress, *Xoo*, drought) shown in different colors indicate that WRKY13 protein has variable effects on a cis-element under different physiological conditions based on the results presented in Figures 4 and 6 and Supplemental Figure S5. Other PRE4 binding protein, based on Cai et al. (2008).
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