An S-Domain Receptor-Like Kinase, OsSIK2, Confers Abiotic Stress Tolerance and Delays Dark-Induced Leaf Senescence in Rice[1][W][OPEN]

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Receptor-like kinases play important roles in plant development and defense responses; however, their functions in other processes remain unclear. Here, we report that OsSIK2, an S-domain receptor-like kinase from rice (*Oryza sativa*), is involved in abiotic stress and the senescence process. OsSIK2 is a plasma membrane-localized protein with kinase activity in the presence of Mn^{2+}. *OsSIK2* is expressed mainly in rice leaf and sheath and can be induced by NaCl, drought, cold, dark, and abscisic acid treatment. Transgenic plants overexpressing *OsSIK2* and mutant *sik2* exhibit enhanced and reduced tolerance to salt and drought stress, respectively, compared with the controls. Interestingly, a truncated version of OsSIK2 without most of the extracellular region confers higher salt tolerance than the full-length OsSIK2, likely through the activation of different sets of downstream genes. Moreover, seedlings of *OsSIK2*-overexpressing transgenic plants exhibit early leaf development and a delayed dark-induced senescence phenotype, while mutant *sik2* shows the opposite phenotype. The downstream *PR*-related genes specifically up-regulated by full-length OsSIK2 or the *DREB*-like genes solely enhanced by truncated OsSIK2 are all induced by salt, drought, and dark treatments. These results indicate that *OsSIK2* may integrate stress signals into a developmental program for better adaptive growth under unfavorable conditions. Manipulation of *OsSIK2* should facilitate the improvement of production in rice and other crops.

Abiotic stress is one of the main factors that cause reductions in crop production; thus, it is important to study the molecular mechanisms of plant responses to the stress in order to improve stress tolerance in crops. When plants are exposed to abiotic stresses, signals are likely first sensed by receptors generally localized in the membrane, and then signals are transduced to the downstream factors and activate different stress responses. In this process, receptor-like kinase (RLK) may be the first sensor or transducer.

RLK comprises one of the largest families, with more than 610 and 1,131 members in Arabidopsis (*Arabidopsis thaliana*) and rice (*Oryza sativa*), respectively (Shiu et al., 2004). Besides big size, another feature of RLK is its diversity. RLK typically contains an N-terminal extracellular domain, a transmembrane domain, and a C-terminal intracellular kinase domain. Extracellular domains vary greatly to perceive a wide range of signals or stimuli. Intracellular kinase domains are relatively conserved with Ser/Thr kinase activity to transduce signals. Based on the identity of extracellular domains, RLKs are classified into 44 subfamilies. The biggest subfamily is leucine-rich repeats (LRRs), and the other subfamilies include S-domains, Domain of Unknown Function26, CELL WALL-ASSOCIATED KINASE-like, and others (Shiu and Bleecker, 2001).

The first RLK was found in maize (*Zea mays*; Walker and Zhang, 1990), and subsequently, a number of RLKs have been identified. RLK functions in many developmental processes, such as leaf development control by Crinkly4 (Becraft et al., 1996), meristem maintenance by CLAVATA1 (Clark et al., 1997), floral organ abscission by HAESA (Jinn et al., 2000), brassinosteroid (BR) perception by BRASSINOSTEROID-INSENSITIVE1 (BRI1) and BRI1-ASSOCIATED KINASE1 (BAK1; Li et al., 2002; Nam and Li, 2002), organ shape regulation by ERECTA1 (Shpak et al., 2003; van Zanten et al., 2009),

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reproduction process by FERONIA (Huck et al., 2003; Escobar-Restrepo et al., 2007), and polarization of cell division by PANGLOSS1 (PAN1) and PAN2 (Cartwright et al., 2009; Zhang et al., 2012). In addition, RLK also works in defense responses. Xanthomonas resistance21 (Xa21) confers resistance to *Xanthomonas oryzae* pv *oryzae* (Wang et al., 1996); Lr10 LOCUS RECEPTOR KINASE (LRK10) mediates resistance to wheat (*Triticum aestivum*) rust pathogens (Feuillet et al., 1997, 2003); FLAGELLIN SENSITIVE2 (FLS2) is involved in the perception of the bacterial elicitor flagellin (Gómez-Gómez and Boller, 2000; Göhre et al., 2008; Danna et al., 2011); AVRPPHB SUSCEPTIBLE1 (PBS1) improves tolerance to *Pseudomonas syringae* strains (Swiderski and Innes, 2001); Pi-d2 enhances rice resistance to blast (Chen et al., 2006). Moreover, RLK also participates in abiotic stress responses. *Salt-Induced Receptor-Like Kinase* (*Srlk*) contributes to salt tolerance in the legume *Medicago truncatula* (de Lorenzo et al., 2009). STRESS-INDUCED PROTEIN KINASE1 (OsSIK1) mediates tolerance to drought and salt stress in rice (Ouyang et al., 2010). RECEPTOR-LIKE PROTEIN KINASE1 is implicated in hydrogen peroxide (H_2O_2) and abiotic stress processes (Osakabe et al., 2010; Lee et al., 2011). ABSCISIC ACID (ABA)-AND OSMOTIC-STRESS-INDUCIBLE RECEPTOR-LIKE CYTOSOLIC KINASE1 interacts with CYSTEINE-RICH REPEAT RLK36 (CRK36) and negatively regulates the ABA signaling pathway (Tanaka et al., 2012).

In RLK superfamilies, S-domains belong to a large subfamily with 40 members in Arabidopsis and 147 members in rice (Morillo and Tax, 2006). The extracellular domain contains three modules, B_lectin, S-LOCUS GLYCOPROTEIN, and PAN_APPLE, which are presumed to mediate protein-protein and protein-carbohydrate interactions. Studies in this family are relatively few, and most reports focused on self-incompatibility, best characterized by *S-RECEPTOR KINASE* (*SRK*) in *Brassica* species (McCubbin and Kao, 2000; Takayama et al., 2000). Another member, *CALMODULIN-BINDING RECEPTOR-LIKE PROTEIN KINASE1*, was reported to be a negative regulator of pathogen resistance in Arabidopsis (Kim et al., 2009a, 2009b).

Rice is an important crop, and previously, we studied the roles of a LRR-type *RLK* gene, *OsSIK1*, in the stress tolerance of rice plants (Ouyang et al., 2010). Here, we report the characterization of *OsSIK2*, an S-domain *RLK* gene, in abiotic stress tolerance and the senescence process. The gene is induced by various stresses. OsSIK2 has kinase activity and is localized in the plasma membrane. *OsSIK2* overexpression promotes leaf emergence, improves plant tolerance to salt and drought stress, and delays leaf senescence. Interestingly, when the extracellular region was removed, the truncated version of OsSIK2 had stronger roles in conferring stress tolerance than the full-length version. The differential roles of the two versions may be exerted through the regulation of a common set and different sets of genes. Manipulation of OsSIK2 may improve the adaptive growth of rice plants.

RESULTS

Expression Patterns of the *OsSIK2* Gene, and Protein Kinase Activity and Subcellular Localization

Previously, we examined the expression patterns of 267 rice *RLK* genes under various abiotic stresses (Dong et al., 2004), and one *RLK* gene, *OsSIK1*, has been found to improve stress tolerance in transgenic rice (Ouyang et al., 2010). Now, we present the characterization of another *RLK* gene, *OsSIK2*, in plant growth and stress responses. *OsSIK2* was an intronless gene and encoded a protein of 833 amino acids. Using SMART programs, OsSIK2 was predicted to be an S-domain RLK (Fig. 1A) that was less studied in terms of stress response. The extracellular region of OsSIK2 contains a putative signal peptide at the N-terminal portion linked with three modules (B_lectin, S-LOCUS GLYCOPROTEIN, and PAN domains). A transmembrane domain was also identified, followed by an intracellular Ser/Thr kinase domain (Fig. 1A).

The expression of *OsSIK2* was examined in rice seedlings (TP309) in response to various abiotic stresses and treatments. Figure 1B shows that *OsSIK2* was apparently induced by treatments with NaCl (200 mM), polyethylene glycol (PEG; 20%), cold (4°C), and ABA (100 μM), with expression peaks at 1 to 3 h after initiation of the experiments. Thereafter, the expression was gradually reduced. *OsSIK2* was also expressed in various parts of rice plants, with relatively higher levels in leaf and sheath compared with the levels in stem, root, and panicles (Fig. 1C).

To investigate whether OsSIK2 is a functional protein kinase, the kinase domain (amino acids 513–803) of OsSIK2 with a maltose-binding protein (MBP) tag was expressed, purified, confirmed by western-blot analysis using anti-MBP monoclonal antibody (Fig. 1D, left and middle), and further subjected to autophosphorylation assay. As shown in Figure 1D (right), when the OsSIK2 kinase-MBP fusion protein was incubated with [C-^{32}P] ATP in the presence of Mn^{2+}, ^{32}P was strongly incorporated into the fusion protein. However, in the presence of Ca^{2+} or Mg^{2+}, ^{32}P incorporation was very limited or not detected. These results indicate that OsSIK2 is a Mn^{2+}-dependent protein kinase.

Subcellular localization of the OsSIK2 was tested in Arabidopsis protoplasts. We created a construct harboring an *OsSIK2-GFP* fusion gene, in which *GFP* was fused to the 3′ end of the *OsSIK2* gene. Additionally, to investigate whether the extracellular parts affect protein localization, a truncated *OsSIK2-GFP* fusion gene was also made, in which the sequence (1–1,221 bp) encoding a signal peptide and most of the extracellular region of OsSIK2 was deleted. In this paper, we named the *OsSIK2* full-length gene and the truncated gene as *OsSIK2-f* and *OsSIK2-t*, respectively. Figure 1E shows that OsSIK2-f was exclusively localized in the plasma membrane, whereas OsSIK2-t was localized in both the membrane and cytoplasm, suggesting that the signal peptide and extracellular parts affect the normal localization of OsSIK2. GFP was used as a control and expressed in cytoplasm (Fig. 1E).

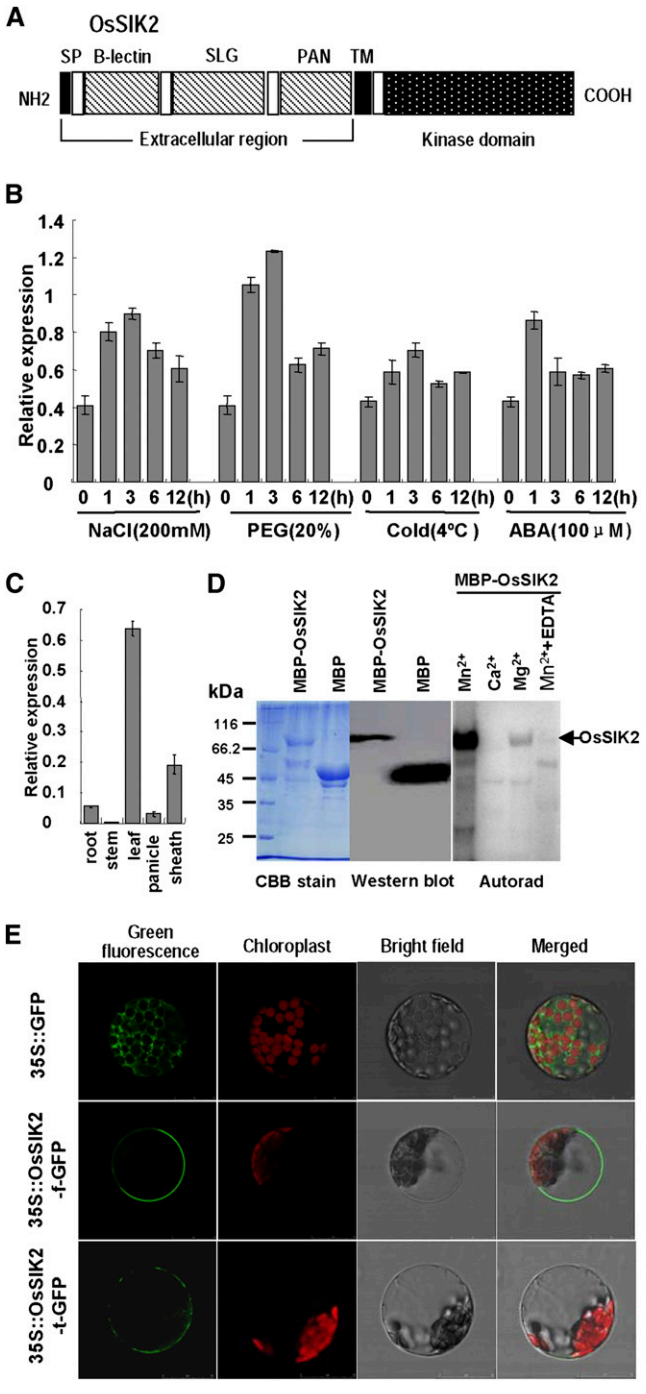

Figure 1. *OsSIK2* gene expression, protein kinase activity, and subcellular localization. A, Schematic representation of the OsSIK2 protein. SP, Signal peptide; TM, transmembrane domain. B, Expression of *OsSIK2* in response to NaCl, drought, cold, and ABA by quantitative reverse transcription (qRT)-PCR. Each column is the average of three repeats, and error bars indicate SD. C, Expression of *OsSIK2* in different organs by qRT-PCR. Each column is the average of three repeats, and error bars indicate SD. D, Phosphorylation assay of OsSIK2-KINASE DOMAIN (KD). The OsSIK2 kinase domain was expressed and purified from *E. coli* by affinity resin. The MBP fusion protein was stained with Coomassie blue (left) and confirmed by western blot using an anti-MBP monoclonal antibody (middle). OsSIK2-KD (2 μg) was incubated with [γ-^{32}P]ATP in the presence of 5 mM Mg^{2+}, 5 mM Ca^{2+}, or 5 mM Mn^{2+}; OsSIK2-KD was also incubated with Mn^{2+} and 10 mM EDTA and used as a control (right). Numbers on the left indicate protein sizes in kD. E, Subcellular localization of OsSIK2 protein. OsSIK2-f indicates the full-length OsSIK2 protein. OsSIK2-t indicates truncated OsSIK2 without most of the extracellular region. GFP was used as a control.

Transgenic Rice Plants Overexpressing *OsSIK2* Exhibit Dwarfism and Early Leaf Emergence at the Seedling Stage

To investigate the biological functions of *OsSIK2* in plants, expression vectors carrying the *OsSIK2* full-length gene (*OsSIK2-f*) and the truncated gene (*OsSIK2-t*; 1,222–2,504 bp; Fig. 2A) under the control of the cauliflower mosaic virus 35S promoter were constructed and transformed into rice (TP309). *sik2* (cv Nipponbare [Nip]), an *OsSIK2* knockout mutant (ND5850) with *Transposon of Oryza sativa17* (*Tos17*) insertion, was identified from the stock seeds in the Rice Genome Resource Center (http://tos.nias.affrc.go.jp/~miyao/pub/tos17/index.html.en). *Tos17* was inserted between 243 and 244 bp and disrupted *OsSIK2* expression in the *sik2* mutant (Fig. 2, A and B). *OsSIK2* expression levels of all plant materials were examined by quantitative PCR (Fig. 2C). We chose the overexpression lines OX-72 and OX-74 with *OsSIK2-t* and the overexpression lines OX-15 and OX-17 with *OsSIK2-f* for further analysis.

At the seedling stage, no significant difference in phenotype and shoot or root length was observed between the *sik2* mutant and control Nip (Fig. 2, D–G). In contrast, the *OsSIK2*-overexpressing plants with *OsSIK2-t* or *OsSIK2-f* exhibited a dwarf phenotype (Fig. 2, D and E). Shoot length and root length of 9-d-old seedlings were also measured and were consistently short when compared with the corresponding controls (Fig. 2, F and G). The ratio of root to shoot length was calculated, and the *OsSIK2*-overexpressing plants had a substantially higher ratio, whereas *sik2* had a lower ratio, compared with the corresponding controls (Fig. 2H).

The time course of third leaf emergence was analyzed, and early and delayed leaf emergence were observed in *OsSIK2* transgenic and *sik2* mutant plants, respectively, compared with the corresponding controls (Fig. 2, E and I). It is interesting that transgenic plants with *OsSIK2-t* (OX-72 and OX-74) generated the third leaf earlier than transgenic plants with *OsSIK2-f* (OX-15 and OX-17), suggesting that the extracellular region of OsSIK2 affects the promotion of leaf emergence.

Field-grown plants were carefully pulled out and regrown in pots for observation. Consistently, the OX-72 and OX-74 lines showed an apparent dwarf phenotype at the vegetative stage (50 d) and the maturation stage (120 d; Fig. 2, J and K). However, the lines OX-15 and OX-17 with *OsSIK2-f* were only slightly shorter than the control at the maturation stage (Fig. 2, J and K). The *sik2* mutant was similar to or slightly shorter than the Nip control (Fig. 2, J and K). Additionally, the OX-72 and OX-74 plants appeared to have a delayed-senescence phenotype at the maturation stage compared with the control (Fig. 2J, bottom).

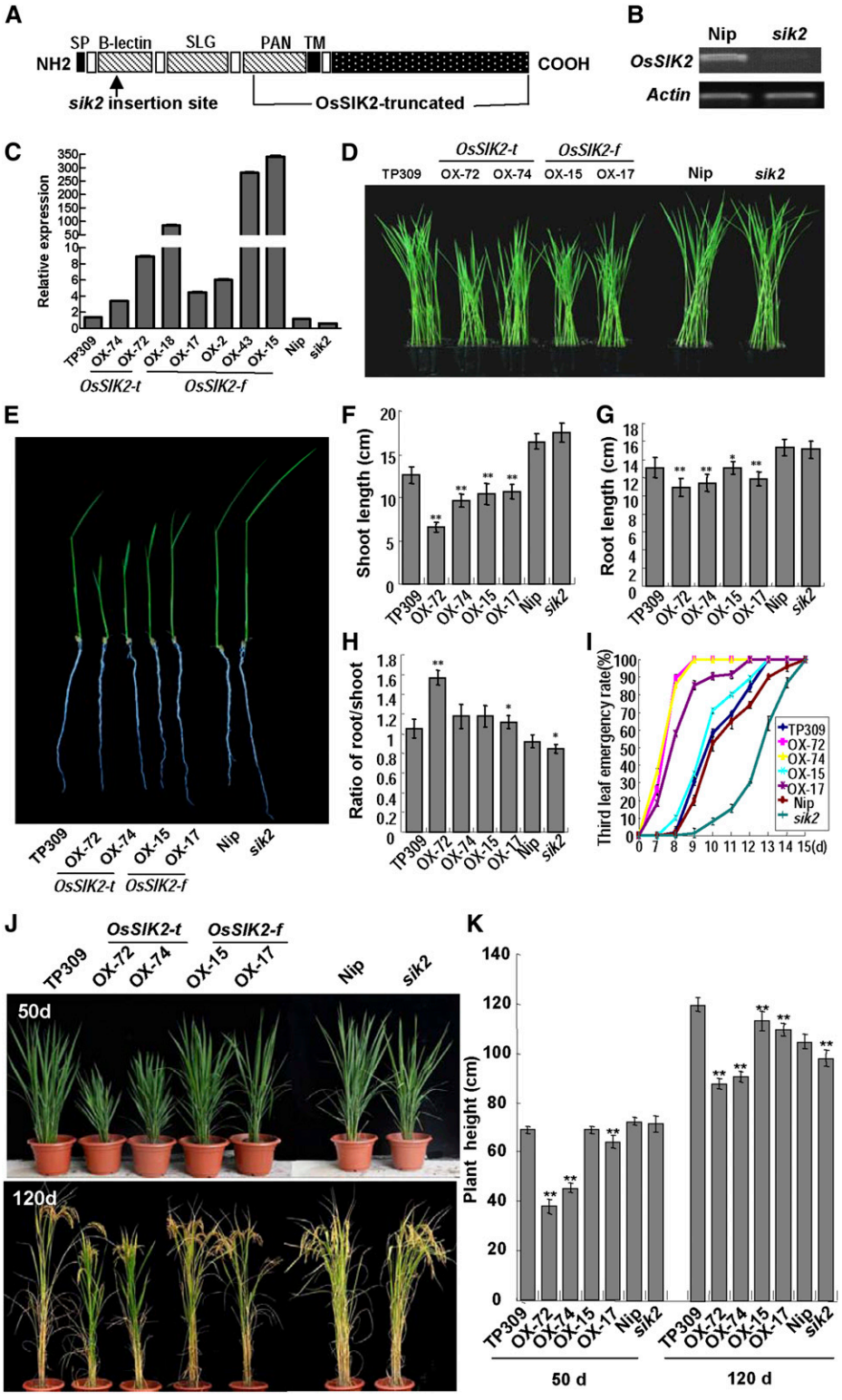

Figure 2. Generation of *OsSIK2*-overexpressing plants, mutant *sik2* identification, and phenotype analysis. A, Schematic of mutant *sik2* insertion and the OsSIK2-truncated protein (OsSIK2-t). SP, Signal peptide; TM, transmembrane domain. The arrow indicates the *Tos17* insertion site. The full length of OsSIK2 is represented by OsSIK2-f below. B, The *sik2* mutant has no expression of *OsSIK2* as revealed by reverse transcription-PCR. The *ACTIN* gene was amplified as a control. C, *OsSIK2* expression in *OsSIK2*-overexpressing lines and the mutant *sik2* by qRT-PCR. *OsSIK2-t* indicates a transgenic line with a truncated *OsSIK2* gene. *OsSIK2-f* indicates a transgenic line with a full-length *OsSIK2* gene. OX indicates *OsSIK2*-overexpressing transgenic lines. For each column, error bars indicate SD ($n = 3$). D, Comparison of the phenotypes of young seedlings. Fourteen-day-old plants were used. E, Comparison of various plant lines. Nine-day-old plants grown in soil were used for photography. F, Shoot length of 9-d-old plants. G, Root length of 9-d-old plants. H, Ratio of root length to shoot length in 9-d-old seedlings. I, Third leaf emergence rates in various plants. In total, 30 seedlings for each line were surveyed at the indicated time points. J, Phenotypes of field-grown plants. Field-grown 50-d-old (top) and 120-d-old (bottom) plants were regrown in pots for photography. K, Measurement of plant height. For F, G, H, and K, each column represents an average of at least 15 plants. Error bars indicate SD. Asterisks indicate significant differences from the corresponding controls (Nip or TP309) at $^{*}P < 0.05$ and $^{**}P < 0.01$.

Overexpression of *OsSIK2* Improves Salt and Drought Tolerance in Transgenic Rice Seedlings

Considering that *OsSIK2* was induced by multiple abiotic stresses, we examined whether *OsSIK2* contributes to salt tolerance. For 35S-*OsSIK2* transgenic plants, 2-week-old seedlings were treated with 0.6% NaCl for 14 d and then subjected to water recovery for 7 d. For the mutant *sik2*, 2-week-old seedlings were treated with 0.5% NaCl for 18 d and then subjected to water

recovery for 10 d. All plants grew well under normal conditions (Fig. 3A, top). After salt treatment and recovery, more 35S-*OsSIK2* transgenic seedlings survived than TP309 seedlings, whereas the *sik2* mutant was more sensitive to salt stress than the control (Fig. 3A, middle and bottom). It should be noted that transgenic seedlings with *OsSIK2-t* had higher survival than the transgenic seedlings with *OsSIK2-f* (Fig. 3A).

Two-week-old seedlings were further treated with 200 mM NaCl for 7 d, and flag leaves were used to measure chlorophyll content. Under both normal and salt stress conditions, all the *OsSIK2*-overexpressing seedlings had higher levels of chlorophyll content than the TP309 control (Fig. 3B). In addition, the lines with *OsSIK2-t* (OX-72 and OX-74) had more chlorophyll than the lines with *OsSIK2-f* (OX-15 and OX-17; Fig. 3B). Under salt stress, the *sik2* mutant contained less chlorophyll than the Nip control, although it had relatively more chlorophyll than Nip under normal conditions (Fig. 3B). All these results indicate that *OsSIK2* overexpression improves the performance of the transgenic rice plants under salt stress.

The performance of the *OsSIK2* transgenic plants under drought stress was also evaluated. For 35S-*OsSIK2* transgenic plants, 3-week-old seedlings were withheld from water for 5 d and then subjected to water recovery for 7 d. For the mutant, 2-week-old seedlings were withheld from water for 10 d and then subjected to water recovery for 10 d. Under normal conditions, all plants grew well (Fig. 4, top). After drought stress or recovery, the *OsSIK2* transgenic plants had better performance and higher survival rate compared with the TP309 control, whereas the *sik2* mutant had much lower survival than control Nip (Fig. 4, middle and bottom). Moreover, the survival rate of the lines with *OsSIK2-t* was higher than that of the lines with *OsSIK2-f*, suggesting that OsSIK2-t may have a greater ability to confer tolerance than OsSIK2-f.

Performance of the *OsSIK2* Transgenic Plants in Response to Salt Stress under Field Conditions

To further investigate the roles of *OsSIK2* in salt tolerance, 1-month-old *OsSIK2* transgenic rice seedlings grown in the field of the experiment station were transferred to a controlled field system with a transparent shelter (Fig. 5) and maintained for 1 week. Then, the transgenic plants were treated with 0.5% NaCl for 4 weeks. After the treatment, the salt water was drained from the bottom of the system, the soil was washed extensively with water, and finally, the plants were grown in water. Performance and survival rate were evaluated at different stages. Before treatment, all plants grew well (Fig. 5A). After NaCl treatment for 3 weeks, more leaves in the lower part of the control TP309 plants were dead compared with those in *OsSIK2* transgenic plants (Fig. 5B). After NaCl treatment for 4 weeks, most control

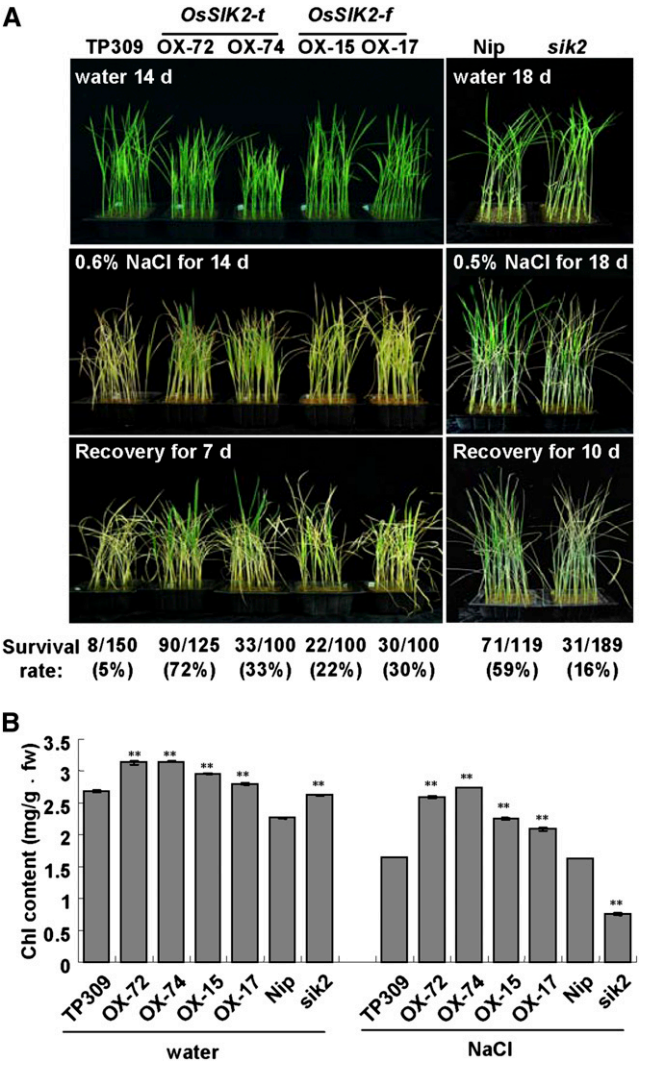

Figure 3. Performance of 35S-*OsSIK2* transgenic plants and the *sik2* mutant under salt stress. A, Comparison of seedling growth under salt stress. For 35S-*OsSIK2* transgenic plants, 14-d-old seedlings in pots were soaked in 0.6% NaCl for 14 d and then recovered for 7 d. For *sik2*, 14-d-old seedlings were soaked in 0.5% NaCl for 18 d and then allowed to recover for 10 d. Numbers below the images indicate survival rates. B, Chlorophyll content in 14-d-old plants treated with 200 mM NaCl for 24 h. Each column represents an average of three replicates. Error bars indicate SD. Asterisks indicate significant differences from the corresponding controls (Nip or TP309) at $*P < 0.05$ and $**P < 0.01$. fw, Fresh weight.

seedlings died, whereas more *OsSIK2* transgenic plants remained green and alive compared with the control plants (Fig. 5C). After recovery for 3 weeks, more *OsSIK2* transgenic plants survived when compared with control TP309 plants (Fig. 5D). It should be noted that transgenic plants with *OsSIK2-t* expression had much better performance than plants with *OsSIK2-f* expression at the stages of salt treatment and recovery (Fig. 5, B–D). A comparison of survival rates during salt treatments and after recovery for different times also

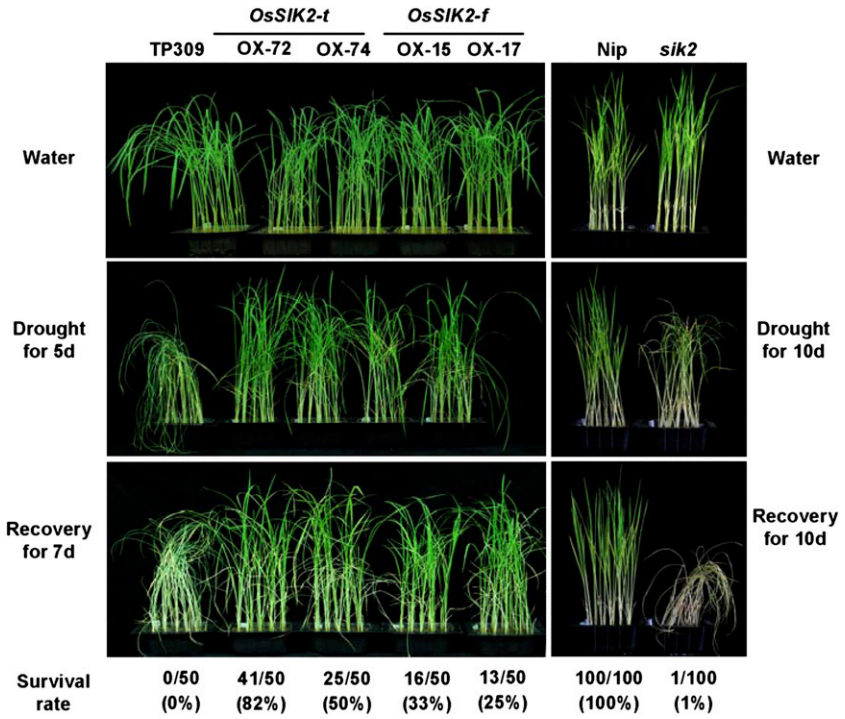

Figure 4. Performance of *OsSIK2*-overexpressing transgenic plants and the *sik2* mutant under drought stress. For 35S-*OsSIK2* transgenic plants, 21-d-old seedlings were withheld from water for 5 d and then exposed to water for 7 d. For *sik2*, 14-d-old seedlings were withheld from water for 10 d and then exposed to water for 10 d. Numbers below the images indicate survival rates.

revealed that *OsSIK2* transgenic plants performed better than the control plants (Table I). All these results indicate that *OsSIK2* confers tolerance to salt stress under field conditions and that the truncated version *OsSIK2-t* plays a stronger role than the full-length version *OsSIK2-f* in stress tolerance.

OsSIK2 Modulates Antioxidant Capacity

Under abiotic stresses, more reactive oxygen species (ROS) such as H_2O_2 are usually produced, causing damage in plants (Zhu, 2001). We examined whether *OsSIK2* increases rice salt tolerance through the

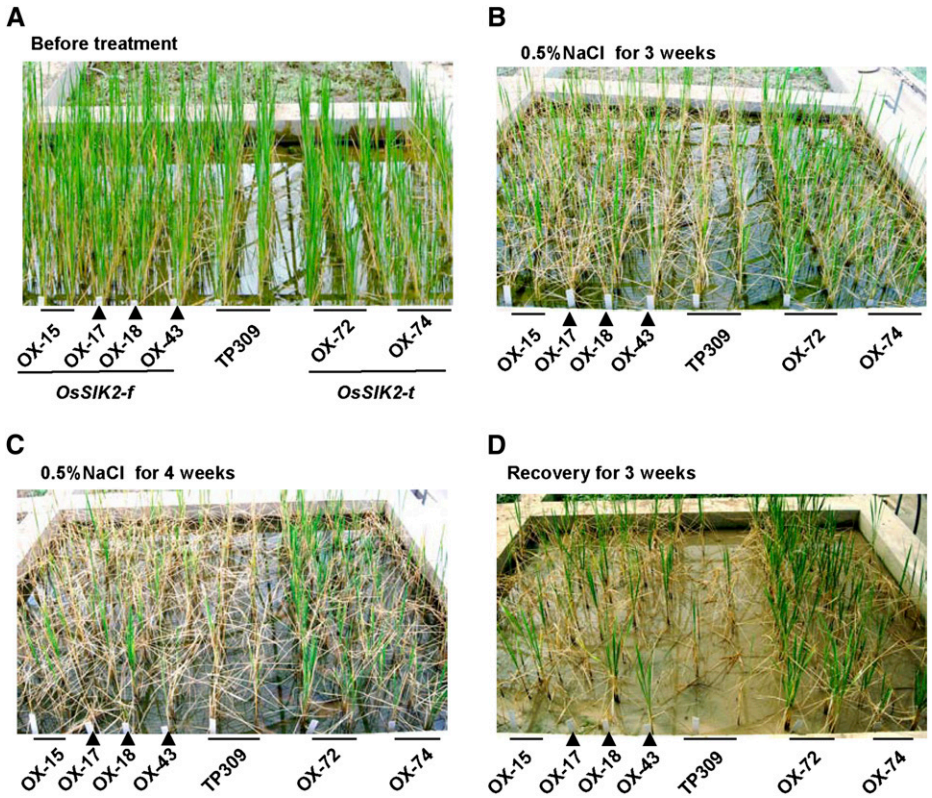

Figure 5. Salt tolerance of *OsSIK2*-overexpressing transgenic plants under field conditions. A, Growth of various rice seedlings. One-month-old field-grown seedlings were transferred to the controlled system and grown for 1 week before salt treatment. B, Plant growth for 3 weeks under salt stress. The above seedlings were exposed to 0.5% NaCl for 3 weeks and then photographed. C, Plant growth for 4 weeks under salt stress. The seedlings in A were exposed to 0.5% NaCl for 4 weeks and then photographed for comparison. D, Comparison of plant growth after recovery from salt stress. The seedlings exposed to 0.5% NaCl for 4 weeks were recovered in water for 3 weeks.

detoxification of ROS. Rice seedlings were treated with 200 mM NaCl for 4 d, and then flag leaves were subjected to 3,3′-diaminobenzidine (DAB) staining for the detection of H_2O_2. As shown in Figure 6A, under normal conditions in water, all leaves were green. After salt treatment, a large brown area in the upper portion of leaves from control TP309 was observed, whereas leaves from *OsSIK2* transgenic plants showed almost no brown regions (Fig. 6A). In agreement, a larger brown area of H_2O_2 was detected in mutant *sik2* leaves than those in control Nip (Fig. 6A). These results indicate that OsSIK2 is efficient in eliminating H_2O_2 produced under salt stress.

Peroxidase (POD) can directly eliminate H_2O_2, and we examined if the POD activity was altered in *OsSIK2* transgenic plants. Two-week-old rice seedlings were treated with 200 mM NaCl for 24 h, and then POD activity was determined in flag leaves. Under normal conditions in water, POD activities were elevated in *OsSIK2* transgenic plants but reduced in the mutant *sik2* compared with the corresponding controls (Fig. 6B). Under salt stress, a similar trend was also observed, except that no significant difference was found between mutant *sik2* and control Nip (Fig. 6B). The lack of difference in POD activity between *sik2* and Nip is probably due to the fact that a different rice variety was used. Other possibilities cannot be excluded. We further tested which POD genes were regulated by *OsSIK2* and found that the expression of the peroxidase gene *POX-1* (Os04g0688200) and *POX-2* (Os07g0676900) was up-regulated in *OsSIK2* transgenic plants but down-regulated in the *sik2* mutant compared with the corresponding controls under normal conditions (Fig. 6C), suggesting that *OsSIK2* increases rice salt tolerance at least partially by the activation of *POX-1* and *POX-2* expression.

OsSIK2 Overexpression Delays Dark-Induced Leaf Senescence

Under field conditions, the transgenic plants with *OsSIK2-t* expression seemed to have delayed senescence compared with the control (Fig. 2J, bottom). We then examined whether *OsSIK2* was involved in the process of senescence. Three-week-old seedlings grown in pots were placed in the dark for 6 d, and the phenotypic change was observed. Before treatment, all plants were green and healthy (Fig. 7A). After dark treatment, the *OsSIK2* transgenic plants were greener, whereas the *sik2* mutant was more yellow, than the corresponding controls (Fig. 7A, bottom left). Examination of the second leaves from the bottom of the seedlings and measurement of chlorophyll contents further supported that OsSIK2 delayed dark-induced leaf senescence, whereas disruption of *OsSIK2* caused early leaf senescence (Fig. 7, A, right, and B).

To further investigate the relationship between *OsSIK2* and senescence, we checked the expression pattern of *OsSIK2* under dark treatment. *OsSIK2* was apparently induced and reached a peak at 6 to 12 h after the initiation of dark treatment (Fig. 7C). After this period, the *OsSIK2* level decreased and then remained almost unchanged. Natural leaf senescence proceeds from tip to base, and the control TP309 leaves exhibiting approximately 20% senescence were separated into base, middle, and tip parts (Fig. 7D, top). *OsSIK2* was highly expressed in the leaf tip but expressed at low levels in the middle and base parts of the leaf (Fig. 7D, bottom). All these results indicate that *OsSIK2* was involved in the dark-induced leaf senescence.

OsSIK2 Alters the Expression of Downstream Genes

To further elucidate the molecular mechanisms underlying OsSIK2 functions, microarray analysis was performed (GSE51151), and many differentially expressed genes were identified (Supplemental Table S1–S6). These genes, together with other stress-responsive genes, were further analyzed. A set of *PATHOGENESIS-RELATED* (*PR*) genes including *PR1a* (Os07g0129300), *PR5* (Os12g0628600), *PROBENAZOLE-INDUCED PROTEIN1* (*PBZ1*; Os12g0555200), and *JA-INDUCED PROTEIN* (Os12g0247700) were apparently up-regulated in transgenic plants with *OsSIK2-f* but reduced in the mutant *sik2* compared with the corresponding controls (Fig. 8A). However, the transgenic plants with *OsSIK2-t* showed no significant change in the expression of these genes (Fig. 8A).

OsSIK2-t plants showed stronger salt tolerance than *OsSIK2-f* plants; however, the above *PR* genes were relatively unchanged in *OsSIK2-t* plants (Figs. 3, 5, and 8A),

Table I. *Survival rates of field-grown 35S-OsSIK2 transgenic plants under salt stress*

OsSIK2-f indicates transgenic rice lines overexpressing the full-length *OsSIK2* gene. *OsSIK2-t* indicates transgenic lines overexpressing the truncated version of OsSIK2 without the extracellular region. OX indicates different transgenic lines. Numbers indicate surviving plants versus total plants tested. The percentages indicate survival rates after treatments.

Treatment	TP309	*OsSIK2-t*		*OsSIK2-f*			
		OX-72	OX-74	OX-43	OX-18	OX-17	OX-15
0.5% NaCl for 3 weeks	19/25 (76%)	32/32 (100%)	30/30 (100%)	13/16 (81%)	11/13 (85%)	12/16 (75%)	24/27 (88%)
0.5% NaCl for 4 weeks	16/25 (64%)	32/32 (100%)	30/30 (100%)	9/16 (56%)	10/13 (77%)	12/16 (75%)	17/27 (63%)
Recovery for 1 week	9/25 (36%)	32/32 (100%)	30/30 (100%)	9/16 (56%)	10/13 (77%)	11/16 (69%)	17/27 (63%)
Recovery for 3 weeks	5/25 (20%)	26/32 (81%)	23/30 (76%)	7/16 (44%)	6/13 (46%)	7/16 (44%)	13/27 (48%)
Recovery for 10 weeks	1/25 (4%)	24/32 (75%)	22/30 (73%)	7/16 (44%)	3/13 (23%)	4/16 (25%)	6/27 (22%)

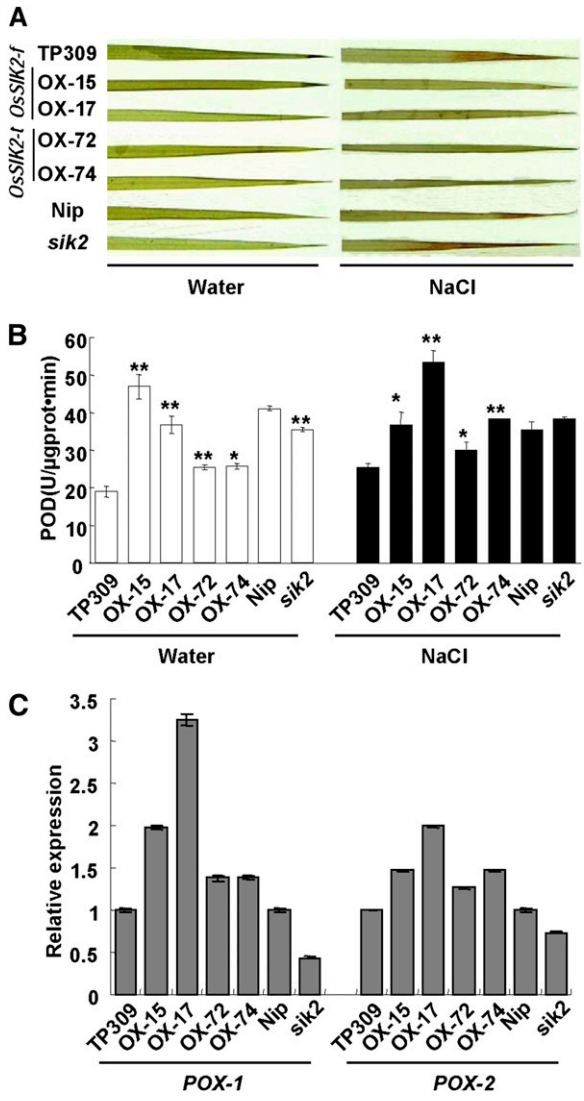

Figure 6. Antioxidative capacity was enhanced in transgenic plants overexpressing *OsSIK2*. Two-week-old plants grown in pots were soaked in water or in 200 mM NaCl for 4 d, and then flag leaves were detached for DAB staining or measurement of POD activity. Brown regions or spots on leaves indicate the presence of H_2O_2. A, DAB staining of flag leaves. B, POD activity in flag leaves of various rice seedlings. Each column represents an average of three replicates. Error bars indicate SD. Asterisks indicate significant differences from the corresponding controls (Nip or TP309) at $*P < 0.05$ and $**P < 0.01$. C, Expression of the POD genes *POX-1* and *POX-2*. Leaves from 2-week-old plants were pooled for RNA extraction, and expression was determined by qRT-PCR. Error bars indicate SD ($n = 3$).

suggesting that other genes may be involved in OsSIK2-t functions. More genes were then analyzed, and we found that the *DEHYDRATION-RESPONSIVE ELEMENT-BINDING PROTEIN* (*DREB*) genes *OsDREB1B* (Os09g0522000) and *OsDREB1E* (Os04g0572400) and the senescence-associated dehydrogenase gene (Os12g0183100) and dioxygenase gene (Os02g0168100; Lee et al., 2001) were exclusively up-regulated in *OsSIK2-t* plants but reduced in the *sik2* mutant compared with the corresponding controls (Fig. 8B). The four genes were also somehow reduced in *OsSIK2-f* transgenic plants compared with the corresponding controls (Fig. 8B).

The dehydrogenase gene (Os12g0183100) and dioxygenase gene (Os02g0168100) can be induced by natural senescence (Lee et al., 2001). Leaf senescence can be strongly induced by darkness (Weaver and Amasino, 2001). We further examined whether these genes, *POX-1* and *POX-2* (Fig. 6C) and the eight above genes (Fig. 8, A and B), were related to abiotic stress and senescence. We found that all 10 genes were significantly but differentially induced by salt stress, osmotic stress (PEG treatment), and dark treatment (Fig. 8, C–E), suggesting that these 10 genes may function in both abiotic stress response and the senescence process.

DISCUSSION

Although many *RLK* genes have been identified, only a small number of LRR-type RLKs have been studied, mainly in Arabidopsis. Studies in other subfamilies are relatively few. At present, we identified a novel S-domain RLK gene, *OsSIK2* from rice, and discovered that OsSIK2 improves stress tolerance and delays dark-induced leaf senescence in transgenic rice plants.

OsSIK2 expression is induced by multiple abiotic stresses. *OsSIK2* overexpression improves the performance of pot-grown plants under salt and drought stress conditions and promotes salt tolerance for field-grown plants (Figs. 3–5). This function is likely achieved at least partially through the activation of *POX-1* and *POX-2* expression, the promotion of POD activity, and the detoxification of ROS (Fig. 6). Our previous study has revealed that OsSIK1, an LRR-type RLK, also plays roles in stress tolerance through the activation of antioxidative ability (Ouyang et al., 2010). Therefore, the two proteins may have redundant functions during stress responses. However, the two proteins belong to different types, with OsSIK1 being a LRR-type RLK whereas OsSIK2 is an S-domain RLK, and they share only 35% identity at the full-length level and 36% identity in the kinase domain. Furthermore, the stress induction of *OsSIK2* expression seems to be earlier than that of *OsSIK1* in TP309 (Fig. 1B; Ouyang et al., 2010), implying that the two genes may function in a sequential way. Although the two genes play similar roles in stress tolerance, they differentially affect plant development. OsSIK1 suppresses stomatal formation (Ouyang et al., 2010), whereas OsSIK2 appears to promote leaf emergence but inhibit shoot length (Fig. 2). It is unclear through which biochemical pathways or what signaling components the two RLK proteins would activate the antioxidation system to confer stress tolerance, although it can be predicted that the Ser/Thr kinase activity of the two proteins should be involved. The mechanisms by which these two proteins affect developmental processes also remain to be studied.

The OsSIK2 kinase domain has kinase activity. However, it is not clear whether the full-length protein has any activity. It is possible that the full-length

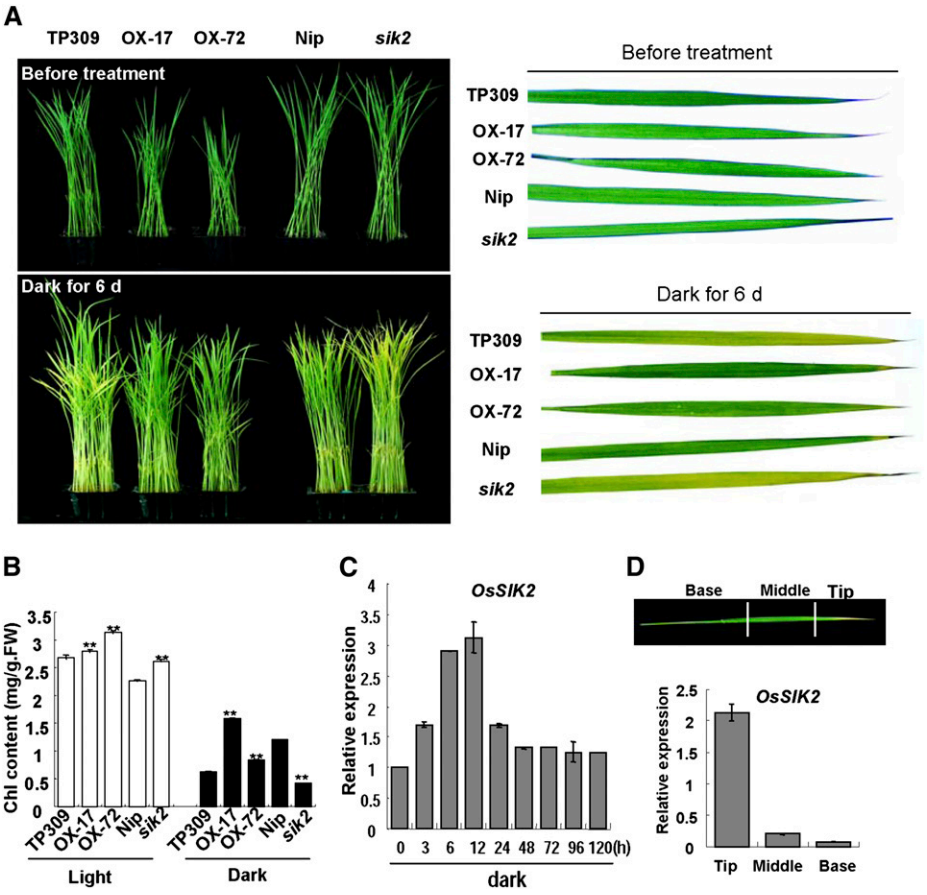

Figure 7. Delayed leaf senescence in *OsSIK2* transgenic plants in the dark. A, Comparison of plants after dark treatment. Top left, 14-d-old plants before treatment; top right, second leaves from top left plants; bottom left, plants in the dark for 6 d; bottom right, second leaves from bottom left plants. B, Chlorophyll (Chl) contents in the second leaves of rice seedlings. Each column represents an average of three replicates. Error bars indicate SD. Asterisks indicate significant differences from the corresponding controls (Nip or TP309) at $*P < 0.05$ and $**P < 0.01$. FW, Fresh weight. C, Expression of *OsSIK2* in response to dark treatment. Two-week-old TP309 plants were treated, and leaves were pooled for RNA extraction. Expression was determined by qRT-PCR. D, Expression pattern of *OsSIK2* in a senescing leaf. One-month-old leaves showing approximately 20% senescence were dissected into three parts as indicated, and *OsSIK2* expression was measured. For C and D, error bars indicate SD ($n = 3$).

protein is inactive and does not have kinase activity. Upon sensing signals through the extracellular domain, the kinase domain may be activated and then perform phosphorylation for downstream signaling. Other mechanisms may also be involved. OsSIK2 localizes on the plasma membrane. When the extracellular region was removed, the truncated protein was discretely localized on the plasma membrane and/or in the cytoplasm. Considering that the extracellular region of a RLK usually plays sensing, ligand-binding, or regulatory roles, deletion of this region should affect OsSIK2 function. It is interesting that OsSIK2-t without the extracellular region has much stronger roles than the full-length OsSIK2-f, namely in the control of severe dwarfism and in conferring stress tolerance in transgenic plants. The differential roles of OsSIK2-f and OsSIK2-t may depend on the regulation of different downstream genes. In addition to the common set of genes, *POX-1* and *POX-2* for detoxification, OsSIK2-f and OsSIK2-t actually regulate different sets of genes. OsSIK2-f specifically enhances the expression of four PR-related genes, whereas OsSIK2-t solely promotes the expression of two DREB genes and senescence-associated genes. The differential roles of the OsSIK2-f and OsSIK2-t in gene regulation may be due to the difference in kinase activity. While the OsSIK2-f kinase may be activated upon sensing environmental signals through its N-terminal extracellular domain, the OsSIK2-t kinase may be constitutively active, leading to the activation of different sets of genes. Other possibilities may also exist.

PRs are well known to function in the defense response. Recent studies have shown that many *PRs* are also involved in the abiotic stress process. For example, *RSOsPR10* can be induced by salt and drought stresses (Hashimoto et al., 2004). The expression of *PBZ1* is highly elevated in cold (Tanaka et al., 2006). *Di19* (a drought-induced gene) is involved in the drought response through regulating pathogen-related genes *PR1*, *PR2*, and *PR5*. Similar to *Di19*, the transgenic lines overexpressing these three genes also show drought tolerance (Liu et al., 2013). *PR3*, *PR4*, and *PR5* are also involved in the salt regulation of seed germination (Seo et al., 2008). The *PR* genes, including *PR1a-l*, *PR5-l*, *PBZ1-l*, and *JA-INDUCED PROTEIN*, are exclusively up-regulated in the present *OsSIK2-f* plants and should contribute to salt tolerance in these plants.

The expression of *DREB* genes (*OsDREB1B* and *OsDREB1E*) is exclusively elevated in *OsSIK2-t* transgenic plants (Fig. 8B). It has been well documented that *DREBs* strongly enhance abiotic stress tolerance and cause dwarfism (Liu et al., 1998; Hsieh et al., 2002; Gilmour et al., 2004; Kasuga et al., 2004; Navarro et al., 2011). At present, field-grown *OsSIK2-t* plants exhibit a severe dwarf phenotype but strong salt tolerance

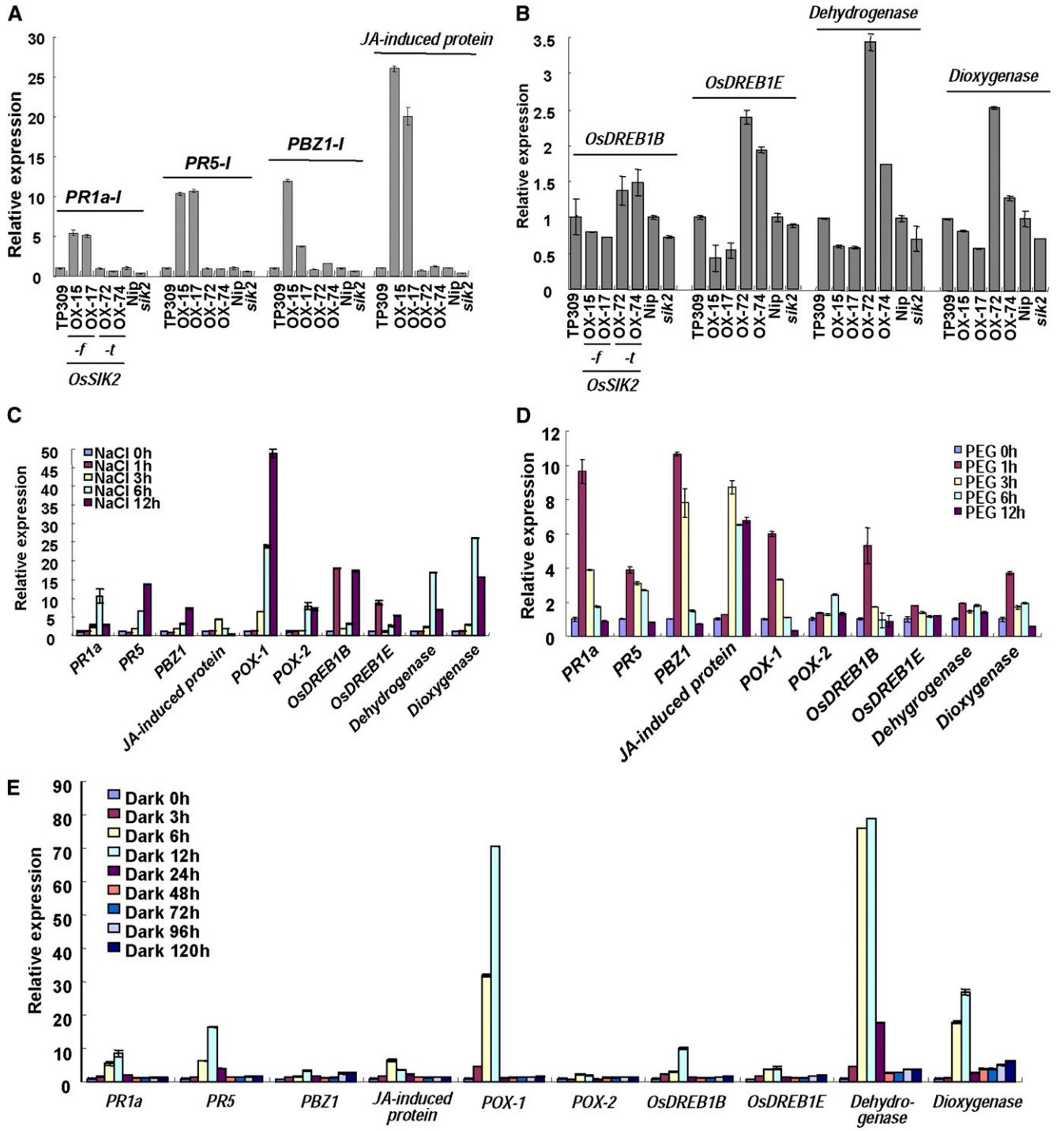

Figure 8. OsSIK2 alters the expression of downstream genes. Two-week-old plants were treated, and gene expression was determined by qRT-PCR. Each column represents an average of three replicates. Error bars indicate SD. A, Genes specifically up-regulated in *OsSIK2-f* transgenic plants (OX-15 and OX-17). B, Genes specifically up-regulated in *OsSIK2-t* transgenic plants (OX-72 and OX-74). C, Induction of gene expression under NaCl stress in TP309 seedlings. D, Induction of gene expression with PEG (20%) treatment in TP309 seedlings. E, Dark-induced gene expression in TP309 seedlings.

compared with *OsSIK2-f* plants (Figs. 2J and 5). Therefore, we propose that OsSIK2-t activates the expression of *OsDREB1B* and *OsDREB1E* and contributes to salt tolerance and dwarfism in transgenic plants. Additionally, OsSIK2-t transgenic plants show apparently delayed senescence under field conditions (Fig. 2J, bottom). This phenomenon may be also caused by promotion of the *DREB* gene or other senescence-related genes (Fig. 8B).

Overexpression of *C-REPEAT BINDING/DREB TRANSCRIPTION FACTOR* has been found to delay leaf senescence and extend plant longevity in Arabidopsis (Sharabi-Schwager et al., 2010).

Considering that all 10 downstream genes can be induced by salt stress and dark treatment, OsSIK2-f and OsSIK2-t may somehow choose to activate alternative pathways for the induction of genes to reach their differential functions in the stress response and other processes. We propose that the full-length OsSIK2-f may sense stress signals through extracellular domains and then initiate the phosphorylation of substrates through the cytoplasmic kinase domain for the activation of downstream events. When the extracellular domains are removed, the kinase activity may not be under strict control, so that OsSIK2-t constitutively phosphorylates substrates, leading to severe dwarfism and strong salt tolerance. Alternatively, the ectopic localization of OsSIK2-t may activate the DREB pathway for stress tolerance. Other mechanisms may also be involved.

Leaf senescence is a tightly regulated developmental process and also hastened by many external factors, such as salt, drought, darkness, and detachment (Buchanan-Wollaston et al., 2005; Munns, 2005; Guo and Gan, 2012). The signaling pathways may be overlapped between the abiotic stress response and dark-induced senescence. By analysis of the transcription data of 1,880 transcription factors, a large number of senescence-regulated members were found to respond to abiotic stress, especially salt stress (Balazadeh et al., 2008). Previously, we identified the *AtNAC2* (for *NO APICAL MERISTEM, ARABIDOPSIS TRANSCRIPTION ACTIVATION FACTOR*, and *CUP-SHAPED COTYLEDON2*) gene, which can integrate ethylene signaling and salt stress signals to regulate lateral root development (He et al., 2005). Later, AtNAC2/ORESARA1 (ORE1) was found to play with ETHYLENE-INSENSITIVE2 (EIN2) and microRNA164 (miR164) to regulate leaf senescence (Kim et al., 2009). Balazadeh et al. (2010) further identified the network controlled by ANAC092/AtNAC2/ORE1 during salt-promoted senescence. Ethylene signaling is beneficial for salt tolerance, and ethylene insensitivity results in a delayed senescence process (Cao et al., 2007; Wuriyanghan et al., 2009; Ma et al., 2013). *VND-INTERACTING2*, a dark-induced and senescence-induced *NAC* transcription factor gene, serves as a molecular link that integrates abiotic stress and the senescence process through regulating *COLD-REGULATED15A/B* (*COR15A/B*). Similar to *VND-INTERACTING2*, the two down-regulated genes, which are well-known marker genes in abiotic stress, also delayed senescence (Yang et al., 2011). The transgenic plants with *SENESCENCE-ASSOCIATED GENE29* (*SAG29*) overexpression exhibited accelerated senescence and were hypersensitive to salt stress (Seo et al., 2011). RECEPTOR-LIKE PROTEIN KINASE1, a positive regulator of abiotic stress, accelerates senescence at the mature stage (Lee et al., 2011). ORYZA SATIVA TANDEM ZINC FINGER-DOMAIN PROTEIN1, a zinc finger transcription factor, confers salt tolerance and shows delayed senescence (Jan et al., 2013).

Our results here show that *OsSIK2* is induced by abiotic stresses and dark treatment, and *OsSIK2* transgenic plants exhibited enhanced salt and drought tolerance and delayed senescence in dark. All 10 downstream genes (Fig. 8) are also induced by salt stress, drought, and dark treatment, indicating that OsSIK2 is a component that integrates stress signaling with the senescence process. It should be mentioned that the *OsSIK2* gene is induced by dark treatment and highly expressed in the senescent region of a leaf; however, its overexpression leads to delayed senescence, suggesting that the OsSIK2 negatively regulates the senescence process.

A RLK typically contains three parts, the extracellular domain, transmembrane domain, and intracellular kinase domain. Studies have revealed that chimeric receptors fused with different extracellular and intracellular regions were functional. For example, the extracellular region of BRI (a BR receptor) was fused to the intracellular kinase domain of XA21, a rice pathogen resistant receptor, and the chimeric receptor exhibited pathogen resistance after adding BR (He et al., 2000). Similar results were found in the chimeric receptors CEBiP-XA21 (Kishimoto et al., 2010), CEBiP-Pi_d2 (Kouzai et al., 2013), and CELL WALL-ASOCIATED KINASE1-EFR (Brutus et al., 2010). Our study provides insight into the manipulation of OsSIK2 by the combination of different extracellular and intracellular domains, and this approach may finally improve adaptive growth in rice plants.

MATERIALS AND METHODS

Materials and Treatments

Seeds of rice (*Oryza sativa* ssp. *japonica* 'TP309' and 'Nipponbare') were sown in pots (8 × 8 × 10 cm) containing 180 g of vermiculite soaked with water. All plants were grown under white fluorescent light (600 photons m^{-2} s^{-1}, 12 h of light/12 h of dark) at 28°C and 75% relative humidity.

For salt and drought stress and ABA treatment in Figures 1 and 8, 2-week-old TP309 seedlings grown under normal conditions were soaked with their roots in NaCl (200 mM), PEG (20%), ABA (100 μM), or water and incubated at constant light for the indicated times. For cold stress and dark treatment, 2-week-old TP309 seedlings were transferred to cold chambers at 4°C or transferred to complete darkness and incubated for the indicated times. The aerial parts of seedlings were harvested for total RNA isolation.

Reverse Transcription-PCR and Quantitative PCR

Total RNA was extracted using TRNzol reagent (Tiangen) and reverse transcribed, and the complementary DNA (cDNA) was used for reverse transcription-PCR or quantitative PCR. Quantitative PCR was performed on the Roche LightCycler 480 II, using the TransStart Green qPCR SuperMix kit (TransGen Biotech). PCR buffer (20 μL) contains 10 μL of TransStart Green qPCR SuperMix, 1 μL of first-strand cDNAs, and 0.25 μM of each primer. The PCR mixtures were preheated at 94°C for 3 min, followed by 42 cycles of amplification (94°C for 20 s, 58°C for 20 s, and 72°C for 30 s), and stopped by an extension (72°C for 10 min). The results were analyzed using LightCycle 480 SW1.5 (Roche). Each data set had three replicates, and the experiments were repeated twice. Primers are listed in Supplemental Table S7.

Generation of Transgenic Plants and Mutant Identification

The full-length *OsSIK2* (*OsSIK2-f*) and the truncated *OsSIK2* (*OsSIK2-t*; 1,222–2,504 bp) cDNA sequences were amplified from TP309 cDNA and subcloned into the binary vector pBIN438 under the control of the cauliflower mosaic virus 35S promoter as described previously (Wuriyanghan et al., 2009).

Each of the two constructs was introduced into *Agrobacterium tumefaciens* strain AGL1 and then transformed into TP309. The mutant *sik2* (ND5850) was identified from *Tos17* insertional mutant rice deposited into the Rice Genome Resource Center (http://www.rgrc.dna.affrc.go.jp). The primers used for subcloning were as follows: OsSIK-f-F, 5′-GAAGATCTCCTATCTTCT-CCAGCCATGC-3′; OsSIK-f-R, 5′-CCGGAATTCTATTGAAGCCACGTCA-GAGTG-3′; OsSIK-t-F, 5′-GTTAGATCTATGGAAGATGCTTCGACCCA-3′; and OsSIK-t-R, 5′-GTGGGTACCTATTGAAGCCACATCAGAGT-3′. The underlined sequences indicate digestion sites of corresponding restriction enzymes.

Phosphorylation Assay

The cDNA sequence encoding the OsSIK2 protein kinase domain (1,539–2,502 bp) was amplified and subcloned into the vector pMAL-c2X (New England Biolabs). The MBP-coding sequence was fused in frame to the 5′ end of the *OsSIK2* gene sequence. The fusion protein was induced and expressed in *Escherichia coli* and then purified according to the manufacturer's procedure using the pMAL protein fusion and purification system (E8000S). The expressed MBP fusion protein was confirmed by western blotting using a mouse anti-MBP monoclonal antibody (Abmart). The MBP protein itself was also expressed and used as a control. The MBP fusion protein OsSIK2-KD was assayed for kinase activity in vitro (Xie et al., 2003; Wuriyanghan et al., 2009; Ouyang et al., 2010). The reaction mix of the phosphorylation assay (25 μL) contained 50 mM Tris-HCl (pH 7.6), 50 mM KCl, 2 mM dithiothreitol, 10% (v/v) glycerol, 2 μg of OsSIK2-KD, and 5 mM $MnCl_2$ (or 5 mM $CaCl_2$ or 5 mM $MgCl_2$). The reactions were initiated after the addition of 12.5 μCi of [γ-^{32}P]ATP (30 Ci mmol^{-1}), maintained at 22°C for 45 min, and stopped after the addition of EDTA to a final concentration of 10 mM for 10 min. After electrophoresis by 10% SDS-PAGE, the protein on the gel was transferred onto a polyvinylidene fluoride membrane and subjected to phosphor imaging. The protein samples were also resolved by SDS-PAGE to examine the loading after Coomassie blue staining.

Subcellular Localization Assays

For detection of the subcellular localization of OsSIK2, the full-length *OsSIK2* (*OsSIK2-f*) and the truncated *OsSIK2* (*OsSIK2-t*) cDNA sequences were amplified and subcloned into the pBI221-GFP vector, in which GFP-coding sequence was fused in frame to the 3′ end of the *OsSIK2* gene sequence. Primers are listed in Supplemental Table S1. The constructs were transformed into Arabidopsis (*Arabidopsis thaliana*) protoplasts by the PEG-mediated transformation method (Yoo et al., 2007). Subcellular distribution of the OsSIK2 protein was visualized by fluorescence microscopy (Leica TCS SP5).

Stress Treatments

For salt stress, 2-week-old seedlings grown in pots under normal conditions were transferred, with pots, onto trays containing 0.6% (for *OsSIK2*-OX lines) or 0.5% (for *sik2*) NaCl solution and maintained there for 10 or 18 d, respectively. Then, NaCl solution was removed from the tray, and the pot-grown plants were subjected to water treatment for 7 d (for *OsSIK2*-OX lines) or 10 d (for *sik2*) to recover.

For drought stress, 3-week-old *OsSIK2*-OX lines or 2-week-old *sik2* grown in pots under normal conditions were withheld from water for 5 or 10 d, respectively, until the leaves of TP309 (a wild-type control) or *sik2* (mutant) were rolled. Then, plants were exposed to water for 7 or 10 d, respectively. The phenotypes of the plants were examined and photographed at different times. After recovery, plants that had green and healthy young leaves were regarded as having survived, and the survival rate was calculated.

For dark treatment, 3-week-old plants grown under normal conditions were transferred to complete darkness and incubated for appropriate times up to 6 d. Representative plants were photographed.

Measurement of Chlorophyll Contents, and DAB Staining for H_2O_2

About 100 mg of flag leaves excised to 2 to 3 cm in length was immersed in the extract solution (95% ethanol + 5% water) at room temperature (25°C) until the leaves were bleached. The total chlorophyll content was assayed by measuring the absorbance of the extracts at 647 and 665 nm (Inskeep and Bloom, 1985).

Plant flag leaves were excised and immersed in a 1% solution of DAB in 50 mM Tris-HCl buffer (pH 3.8) at room temperature (25°C) for 20 h in the dark. Leaves were bleached by immersing in ethanol to visualize the brown spots, which were characteristic of the reaction of DAB, for the presence of H_2O_2.

Measurement of POD Activity

Two-week-old rice seedlings were treated with or without 200 mM NaCl for 24 h, and then the shoots (500 mg) were harvested and ground (1:5, m/v) in 50 mM sodium phosphate buffer (pH 7.8) containing 1% polyvinylpyrrolidone and β-mercaptoethanol. After centrifugation (13,000*g*, 15 min, 4°C), the supernatant was used for the determination of POD activities. The reaction mixture (3 mL) contained 50 mM sodium acetate buffer (pH 5.6), 5.4 mM guaiacol, 15 mM H_2O_2, and 30 μL of enzyme extract. POD activity was assayed by measuring A_{470} resulting from the oxidation of guaiacol to tetraguaiacol (Maehly and Chance, 1954). One unit of POD activity was defined as 0.01 absorbance increase per minute.

Sequence data from this article can be found in GenBank/EMBL databases under the following accession numbers: *OsSIK2* (Os07g0186200), *ACTIN* (Os10g0510000), *POX-1* (Os04g0688200), *POX-2* (Os07g0676900), *PR1a-l* (Os07g0129300), *PR5-l* (Os12g0628600), *JA-INDUCED PROTEIN* (Os12g0247700), *PBZ-l* (Os12g0555200), *OsDREB1B* (Os09g0522000), *OsDREB1E* (Os04g0572400), dehydrogenase gene (Os12g0183100), and dioxygenase gene (Os02g0168100).

Supplemental Data

The following materials are available in the online version of this article.

Supplemental Table S1. Genes up-regulated in OsSIK2-f transgenic plants (OX-15) compared with TP309.

Supplemental Table S2. Genes down-regulated in OsSIK2-f transgenic plants (OX-15) compared with TP309.

Supplemental Table S3. Genes up-regulated in OsSIK2-t transgenic plants (OX-72) compared with TP309.

Supplemental Table S4. Genes down-regulated in OsSIK2-t transgenic plants (OX-72) compared with TP309.

Supplemental Table S5. Genes up-regulated in both OsSIK2-f (OX-15) and OsSIK2-t (OX-72) transgenic plants compared with TP309.

Supplemental Table S6. Genes down-regulated in both OsSIK2-f (OX-15) and OsSIK2-t (OX-72) transgenic plants compared with TP309.

Supplemental Table S7. Primer sequences used in this study.

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