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Characterization of the Rice Blast Fungal Elicitor-Responsive Gene *OsSBP* Encoding a Homolog to the Mammalian Selenium-binding Proteins

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Abstract

The expression of the *Oryza sativa* selenium-binding protein homolog (*OsSBP*), which was previously shown to be transcriptionally activated in fungus-infected or elicitor-treated rice leaves, was studied in the response of endogenous signaling molecules and generators of reactive oxygen species. *OsSBP* expression increased rapidly in response to jasmonic acid (JA) and salicylic acid (SA), both of which play important roles as endogenous signaling molecules that induce resistance to pathogens. *OsSBP* also responded to abscisic acid (ABA) and paraquat, both of which cause increased generation of reactive oxygen species. The deduced *OsSBP* protein has a bis (cystenyl) sequence motif CxxC, which acts as an active redox center controlling oxidation / reduction of protein *in vivo*. This is the first report about the molecular characterization of *OsSBP* in rice.

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Key words: redox, rice, selenium-binding protein.

Abbreviations

ABA, abscisic acid; JA, jasmonic acid; SA, salicylic acid; SBP, selenium-binding protein.

In a previous study, we have cloned a number of cDNAs from rice that correspond to transcripts, which accumulate after treatment with a cerebroside elicitor isolated from the rice blast fungus (*Magnaporthe grisea*) (Sawada and Iwata, 2002). One of these cDNAs, *Oryza sativa* selenium-binding protein homolog (*OsSBP*) cDNA, showed increased mRNA transcription in rice leaves inoculated with either the avirulent or virulent race of rice blast fungus, or the cerebroside elicitor. Several studies on SBP have been reported in mammals (Sani *et al.*, 1988; Bansal *et al.*, 1990; Bartolone *et al.*, 1992; Pumford *et al.*, 1992; Lanfear *et al.*, 1993; Jamba *et al.*, 1997; Gulesserian *et al.*, 2001). Various hypotheses about the function of SBP have been postulated. For example, SBP may play a protective role as a scavenger of toxic electrophiles or oxidant species. The function of SBP, however, has not been clarified yet. In plants, SBP homologs have been isolated from several higher plants, but few studies have reported a possible role. Machuka *et al.* (1999) reported that transcription of the SBP homolog was induced by treatment with ABA in the moss *Physcomitrella patens*. More recently, a homolog of SBP, *LjSBP*, was isolated from *Lotus japonicus* by differential screening of a cDNA library prepared from nodules (Flemetakis *et al.*, 2002).

As a first step toward understanding the role of *OsSBP* in rice, in this study, we show data here on its molecular characters, i.e. motif analysis based on its deduced amino acid sequences, number of its genome copies, its organ specific expression and its expression pattern in response to chemical mediators of self-defense and oxidative stress in plants.

Rice seeds (*Oriza sativa* L. cv. Kinmaze) were germinated and grown in a growth chamber at 30 ℃ under 16 h of light for 2 weeks before being used in experiments, except for old leaves (grown for 3 months) used in Northern blot analysis. For Northern analysis, total RNA was extracted from leaves, stems and roots using the RNeasy Plant Mini Kit

(QIAGEN), according to the supplier's protocol. Ten μg of total RNA was separated on a 1.0% agarose gel containing 6% formaldehyde, and transferred onto Hybond-N^+ nylon membranes (Amersham) by capillary transfer. Genomic DNA was isolated from leaves using the DNeasy Plant Mini Kit (QIAGEN), according to the supplier's protocol. Ten μg of DNA was digested with appropriate endonucleases, separated on 0.8% agarose gels and transferred to Hybond-N^+ nylon membranes by capillary transfer. Both DNA and RNA blots were hybridized with an [α-^{32}P] dCTP-labeled gene specific probe (full size of *OsSBP* cDNA). Hybridization was carried out at 65 ℃ for 18 h in Church

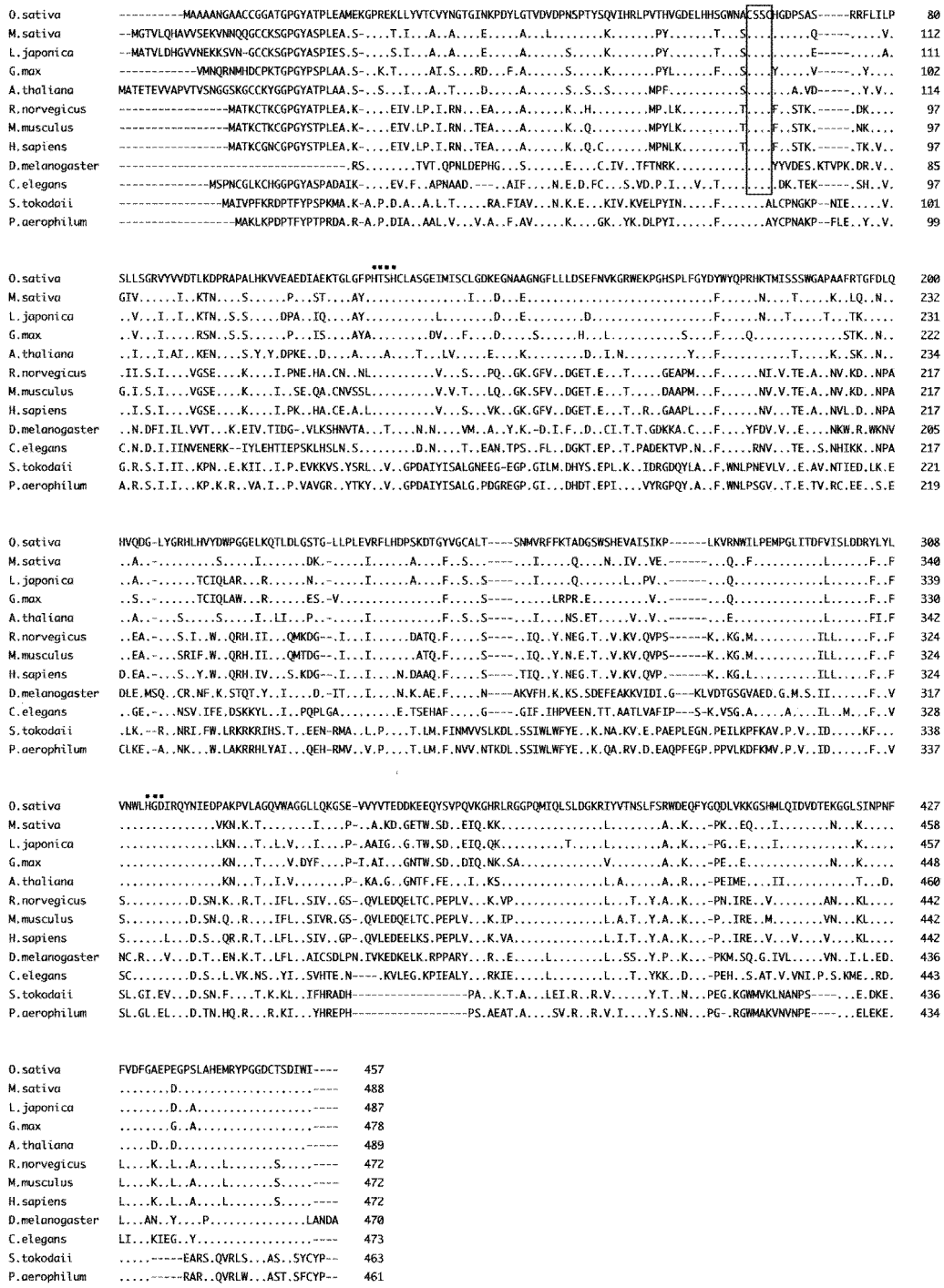

Fig. 1 Amino acid sequence alignment deduced from the nucleotide sequences of *OsSBP* along with those of homologous proteins.

O. sativa, *Oryza sativa*; M. sativa, *Medicago sativa*; L. japonica, *Lotus japonicus*; G. max, *Glycine max*; A. thaliana, *Arabidopsis thaliana*; R. norvegicus, *Ruttus norvegicus*; M. musculus, *Mus musculus*; H. sapiens, *Homo sapiens*; D. melanogaster, *Drosophila melanogaster*; C. elegans, *Caenorhabditis elegans*; S. tokodaii, *Sulfolobus tokodaii*; P. aerophilum, *Pyrobaculum aerophilum*. The putative bis (cystenyl) sequence motif (CxxC) is boxed and the putative metal-binding sites (HxxH or HxD) are indicated as dots.

hybridization buffer (0.25 M NaCl, 1% BSA, 1 mM EDTA, 0.25 M Na_2HPO_4, and 7% SDS) (Church and Gilbert, 1984). The membranes were washed once with 2x SSC containing 0.1% SDS at 65 ℃ for 15 min and then once with 1x SSC containing 0.1% SDS at 65 ℃ for 15 min.

Chemical treatments were carried out as described previously (Mittler and Zilinskas, 1992; Lee *et al.*, 2001). Two weeks-old rice seedlings were sprayed with either 0.1 mM JA (Wako), 1.5 mM SA (Nakarai), 0.1 mM ABA (Wako) and 1 μM paraquat (Sigma) in 0.1% aqueous Tween 20. All experiments were conducted at least twice. Total RNA for Northern analysis was extracted from leaves and stems by the method mentioned above.

As shown in **Fig. 1**, the amino acid sequences (457 amino acids) deduced from the *OsSBP* cDNAs contain a bis (cystenyl) sequence motif (CxxC). This sequence motif is conserved with all compared SBP from three mammals and SBP homolog from four plants and two other organisms except for those isolated from *S. tokodaii* and *P. aerophilum*. The CxxC sequence motif has been reported to be an integral part of several other proteins such as thioredoxin, protein disulfide isomerase, endoplasmic reticulum protein (Erp72), selenoprotein W, and formate dehydrogenase. This sequence motif acts as an active redox center for the majority of the proteins mentioned above, which may control the oxidation / reduction of proteins *in vivo* (Meyer *et al.*, 1999). The amino acid sequence alignment also shows that the putative metal-binding motifs (HxxH or HxD) are also conserved among plant and mammalian SBP proteins.

To elucidate the number of *OsSBP* genes in rice, genomic DNA was prepared from rice leaves, digested with *Xba*I, *Hin*dIII, *Bam*HI, and *Spe*I, and then subjected to Southern analysis (**Fig. 2**). Only one band is visible in each lane. These data suggest that *OsSBP* is present as a single-copy in the rice genome. This finding is in agreement with a report on the *Lotus japonicus* gene, *LjSBP* (Flemetakis *et al.*, 2002).

The expression pattern of the *OsSBP* transcript in various tissues from whole plants was examined by Northern analysis (**Fig. 3**). *OsSBP* transcript was detected at high levels in roots relatively, at trace levels in stems, and a very low levels in young and old leaves. These results indicate that *OsSBP* mRNA expression is similar to that of the *LjSBP* transcript in *Lotus japonicus*.

We previously reported that *OsSBP* was transcriptionally activated in fungus-infected or elicitor-treated. To investigate whether *OsSBP* expression was activated by endogenous signaling molecules in the induced resistance against pathogen infection, we examined the expression of the *OsSBP* transcript in seedlings following treatment with JA and SA. RNA blot analysis showed that *OsSBP* was rapidly activated (within at least 3 h) by JA, peaked at 6h and 24 h, and decreased at 48 h after treatment (**Fig. 4A**). *OsSBP* expression was also induced within 3 h by SA, peaked at 6h and 48 h, and decreased at 72 h after treatment (**Fig. 4B**). Recently, a number of reports have demonstrated the importance of JA in mediating defense responses against plant patho-

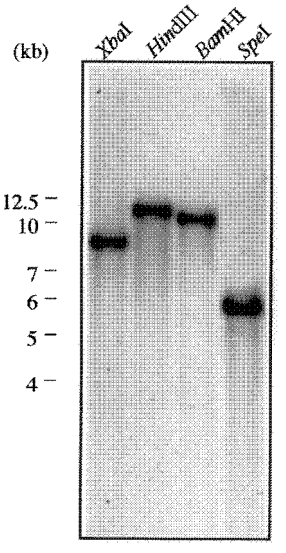

Fig. 2 Genomic DNA gel blot analysis of *OsSBP*. Genomic DNA was isolated from *Oryza sativa*. Ten μg of DNA was digested with the restriction enzymes *Xba*I, *Hin*dIII, *Bam*HI, and *Spe*I, fractionated by electrophoresis, transferred onto a nylon membrane and probed with ^{32}P-labeled *OsSBP* cDNA.

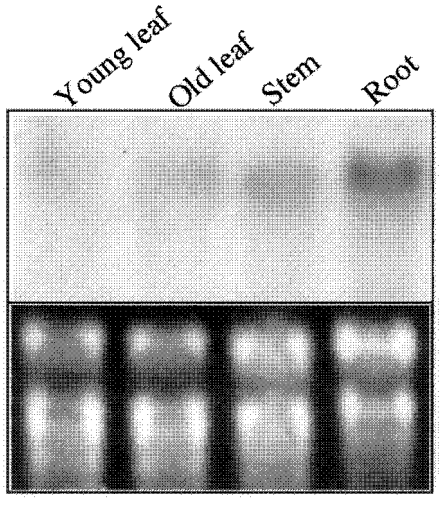

Fig. 3 Organ specific expression of *OsSBP*. Total RNAs was isolated from the indicated organs of rice plants. RNA gel blot analysis (10 μg lane^{-1}) was carried out using radiolabeled *OsSBP* cDNA as a probe.

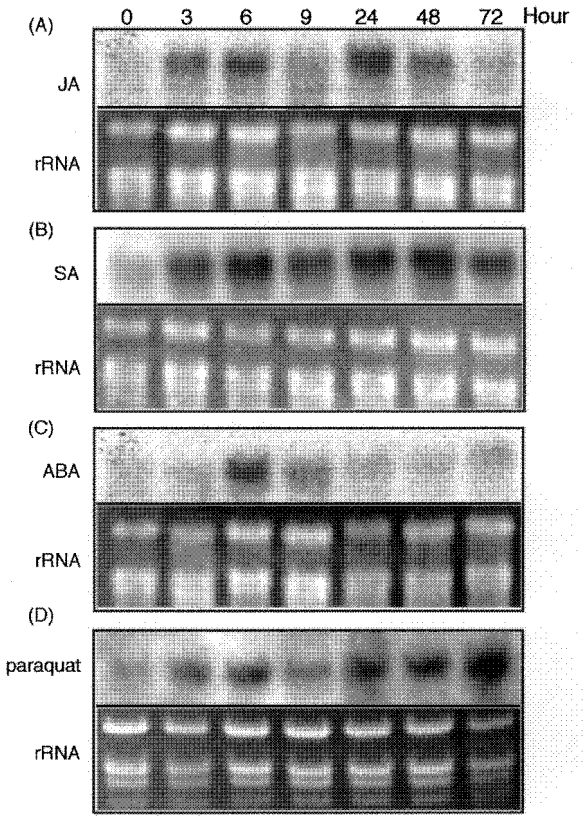

Fig. 4 Specific induction of *OsSBP* by (A) JA, (B) SA, (C) ABA and (D) paraquat in rice seedlings. Total RNA was isolated from rice seedlings treated with JA (0.1 mM), SA (1.5 mM), ABA (0.1 mM) and paraquat (1 μM).

gens, particularly fungi (Dong, 1998; Reymond and Farmer, 1998). In rice, JA appeared to be a major endogenous molecule mediating phytoalexin production and systemic acquired resistance (Tamogami *et al.*, 1997; Shweizer *et al.*, 1998). On the other hand, the endogenous SA levels in rice are very high than most other plant species (Raskin *et al.*, 1990). SA levels do not change significantly after infection by either compatible or incompatible pathogen (Silverman *et al.*, 1995), suggesting that it may not be a signal in pathogen defense. However, recently it is reported that external application of SA seems to induce oxidative stress in rice through H_2O_2; a signal molecule implicated in biotic and abiotic stress responses (Ganesan and Thomas, 2001).

The phytohormone ABA has been shown to increase generation of H_2O_2 besides prominent roles in various physiological processes including induction of seed dormancy and adaptive responses to environmental stresses (Nambara *et al.*, 1998). To evaluate whether *OsSBP* was transcriptionally activated by ABA, similar to the SBP homolog in the moss *Physcomitrella patens* (Machuka *et al.*, 1999), ABA was applied to 2 weeks-old rice seedlings. *OsSBP* expression was induced within 6 h by ABA, peaked at 6 h, and decreased at 9 h after treatment (**Fig. 4C**). To further evaluate the effects of oxidant generators on *OsSBP* expression, the herbicide paraquat, which generates superoxide radical ($O_2^{-\cdot}$), was also applied to rice seedlings. **Fig. 4D** shows that *OsSBP* was induced within 3 h by paraquat, peaked at 6 h, decreased at 9 h, and from 24 h after treatment, it newly began to increase until 72 h. The reason for the two peaks of *OsSBP* mRNA levels caused by JA, SA and paraquat remains to be studied. However, it may be possible to explain that the first peak of *OsSBP* mRNA was caused by exogenous treatment of these chemicals directly, and the second peak of it was caused by secondary increased endogenous levels of signaling molecules in rice induced by oxidative stress through external application of these chemicals.

Based on this study, we have not yet clarified the biological function of *OsSBP*, however, we suggest that *OsSBP* may be involved in response to environmental stresses from the results of several Northern analyses. To test this hypothesis, we recently generated transgenic rice lines overexpressing *OsSBP*. Further studies involving overexpression and antisense approaches will help further elucidation of the role of *OsSBP* in rice.

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