*Plant Cell Physiol.* 45(5): 550–559 (2004)


# A Novel Rice PR10 Protein, RSOsPR10, Specifically Induced in Roots by Biotic and Abiotic Stresses, Possibly via the Jasmonic Acid Signaling Pathway

**Makoto Hashimoto [1], Larisa Kisseleva [2], Shinichiro Sawa [3], Toshiko Furukawa [4], Setsuko Komatsu [5] and Tomokazu Koshiba [1, 6]**

[1] *Department of Biological Sciences, Tokyo Metropolitan University, Minami-Osawa, Hachioji-shi, Tokyo, 192-0397 Japan*
[2] *Biotechnological Centre, Moscow State University, Moscow 119992, Russia*
[3] *Department of Biological Sciences, Graduate School of Science, The University of Tokyo, Hongo, Bunkyo-ku, Tokyo, 113-0033 Japan*
[4] *Tokyo Metropolitan College, Azuma-cho, Akishima-shi, Tokyo, 196-8540 Japan*
[5] *Department of Molecular Genetics, National Institute of Agrobiological Sciences, Kannondai, Tsukuba, Ibaraki, 305-8602 Japan*

**Plant roots have important roles not only in absorption of water and nutrients, but also in stress tolerance such as desiccation, salt, and low temperature. We have investigated stress-response proteins from rice roots using 2-dimensional polyacrylamide-gel electrophoresis and found a rice protein, RO-292, which was induced specifically in roots when 2-week-old rice seedlings were subjected to salt and drought stress. The full-length *RO-292* cDNA was cloned, and was determined to encode a protein of 160 amino acid residues (16.9 kDa, pI 4.74). The deduced amino acid sequence showed high similarity to known rice PR10 proteins, OsPR10a/PBZ1 and OsPR10b. *RO-292* mRNA accumulated rapidly upon drought, NaCl, jasmonic acid and probenazole, but not by exposure to low temperature or by abscisic acid and salicylic acid. The *RO-292* gene was also up-regulated by infection with rice blast fungus. Interestingly, induction was observed almost exclusively in roots, thus we named the gene *RSOsPR10* (*root specific rice PR10*). The present results indicate that RSOsPR10 is a novel rice PR10 protein, which is rapidly induced in roots by salt, drought stresses and blast fungus infection possibly through activation of the jasmonic acid signaling pathway, but not the abscisic acid and salicylic acid signaling pathway.**

**Keywords**: Fungal infection — Jasmonic acid — *Oryza sativa* — Pathogenesis-related (PR) protein — Root specific — Stress response.

Abbreviations: ABA, abscisic acid; CBB, Coomassie Brilliant Blue; 2D-PAGE, two-dimensional-polyacrylamide-gel electrophoresis; PR, pathogenesis-related, SDS, sodium dodecyl sulfate.

## Introduction

Plants are constantly exposed to a variety of biotic and abiotic stresses. To survive these challenges, plants have developed elaborate mechanisms to perceive external signals and to manifest adaptive responses with proper physiological changes. At the molecular level, the perception of environmental stimuli and the subsequent activation of defense responses require a complex interplay of signaling cascades. One area in which the details of the defense responses are becoming more evident is in the plant's production of pathogen-related (PR) proteins. PR proteins have been defined as plant proteins that are induced during pathogen infection or wounding. This term describes the collective induction of numerous plant genes that are induced in a variety of plant species in response to pathological attacks. The major families of PR proteins have been grouped at least into 14 different classes, primarily on the basis of their amino acid sequence identity (Van Loon and Van Strien 1999). Although the biological and/or biochemical function of many PR proteins remains unknown, several examples have been shown to possess anti-microbial activity. In vitro studies of classes PR3 and PR2, having chitinase and β-1,3-glucanase activity, respectively, have shown that these proteins can inhibit fungal growth by hydrolytic degradation of fungal cell walls (Sela-Buurlage et al. 1993, Woloshuk et al. 1991). In addition, transgenic studies with chitinases, β-1,3-glucanases, tobacco PR1a (Alexander et al. 1993) and ribosome-inactivating protein have shown that the transgenic plants have decreased disease severity after fungal infections. This demonstrates anti-microbial activity in at least some PR proteins and suggests that PR proteins are important for plant defenses against disease.

The PR10 class of proteins, first identified as a major pollen allergen (Bet v1) from white birch (Breiteneder et al. 1989), are induced by pathogen attack in a wide variety of plant species, including parsley (Somssich et al. 1986), potato (Matton and Brisson 1989), pea (Barratt and Clark 1991), soybean (Crowell et al. 1992), asparagus (Warner et al. 1993), sorghum (Lo et al. 1999) and rice (Midoh and Iwata 1996, McGee et al. 2001). PR10 proteins are small, primarily acidic intracellular proteins of about 16 kDa (parsley PR protein: Linthorst 1991). Many biotic stresses have been shown to activate PR10 protein expression transcriptionally, suggesting their importance during plant defense responses. The structure of birch pollen Bet v1, determined by X-ray and nuclear magnetic resonance (NMR) spectrometer, indicated the presence of a P-loop struc-

[6] Corresponding author: E-mail, koshiba-tomokazu@c.metro-u.ac.jp; Fax, +81-426-77-2565.

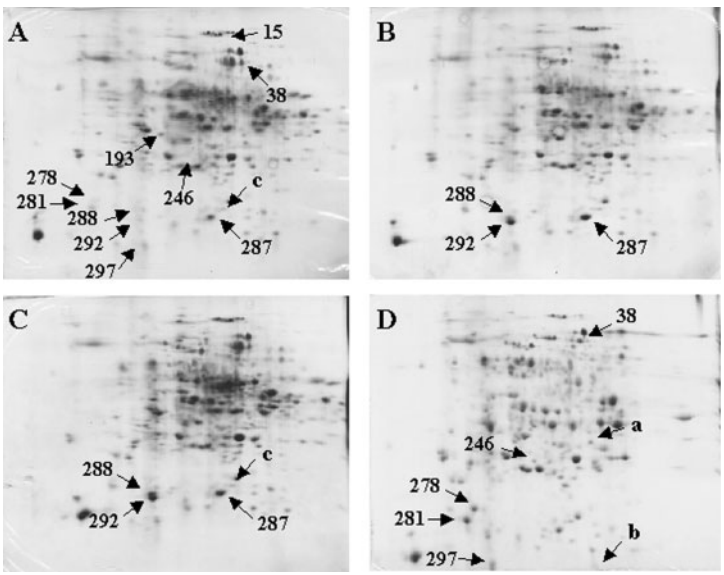


**Fig. 1** Changes in the protein profiles of rice roots after stress treatments. Proteins extracted from rice roots of 2-week-old seedlings were separated by 2D-PAGE and detected by CBB staining. (A) Control. (B) NaCl (100 mM) treatment for 24 h. (C) Air-drying for 15 h. (D) ABA (100 μM) treatment for 12 h. The arrows indicate the positions of protein spots whose expression levels changed after stress treatments.

ture with the amino acid sequence GXGGXG that resembles a motif found in many nucleotide-binding proteins (Gajhede et al. 1996). Some PR10 proteins with ribonuclease activity have been detected in birch pollens (Bufe et al. 1996, Swoboda et al. 1996) and lupine roots (Bantignies et al. 2000), and PR10 protein with cytokinin-binding ability has been detected in mung bean (Fujimoto et al. 1998) and moss (Gonneau et al. 2001). However, the exact biological functions of the PR10 proteins are still unclear.

A rice PR10 protein was first characterized as a probenazole-inducible protein and initially named PBZ1 (Midoh and Iwata 1996). Later, three highly homologous *OsPR10* (*RPR10*) genes, *PR10a* (*PBZ1*), *PR10b*, *PR10c*, were identified, where *PR10a* encoded PBZ1 and *PR10c* appeared to be a non-functional pseudogene (McGee et al. 2001). The biotic and abiotic stress-inducible nature of the *PR10* genes has been investigated including pathogen infection (Midoh and Iwata 1996, McGee et al. 2001, Tanaka et al. 2003), salt tolerance (Moons et al. 1997), UV irradiation (Rakwal et al. 1999), and ozone stress (Agrawal et al. 2002b). Some plant hormones and defense-related signaling molecules have been reported to regulate *OsPR10* gene expression, such as jasmonic acid (Rakwal et al. 1999, Lee et al. 2001, McGee et al. 2001, Moons et al. 1997), salicylic acid (McGee et al. 2001), abscisic acid (ABA) (Lee et al. 2001, Moons et al. 1997), and kinetin (Rakwal et al. 2003). A different type of rice PR10 gene, JIOsPR10, was also identified, which was shown to be up-regulated by jasmonic acid and salicylic acid, and by pathogen infection (Jwa et al. 2001). However, there has been no direct evidence to show their physiological activity and function in plant-defense mechanisms.

During the course of proteome analysis in rice roots and isolation of stress response proteins, we found a protein, RSOsPR10, that was induced by salt and drought stresses. We cloned the full-length cDNA and revealed that the gene encoded a novel PR10 protein. In the present study, we determined how the gene expression of *RSOsPR10* was regulated by abiotic and biotic stresses and by defense-related signaling molecules, and discuss its role in defense responses in rice.

## Results

### *Analysis of stress-induced changes in rice root proteins using 2D-PAGE*

High-resolution two-dimensional polyacrylamide-gel electrophoresis (2D-PAGE) is very useful for separating complex protein mixtures. Proteins extracted from roots of 2-week-old rice seedlings were separated by 2D-PAGE and detected by Coomassie Brilliant Blue (CBB) staining. The positions of individual proteins on the gels were evaluated automatically with ImageMaster 2D Elite software. More than 300 proteins were detected and their relative molecular weights and pIs were determined. Reproducible protein profiles of the rice root proteins were obtained and numbered RO-#1–300 (see Rice Proteome database; http://gene64.dna.affrc.go.jp/RPD/). To inves-

**Table 1** Summary of protein spots where changes were detected from stress treatment in rice roots

| Treatment | Increase | Decrease |
|---|---|---|
| NaCl | RO-287, RO-288, RO-292 | RO-15 |
| Drought | RO-287, RO-288, RO-292 | RO-193 |
| ABA | RO-246, RO-278, RO-297 | RO-38 |
| | RO-a, RO-b | |
| | RO-281 | |

Two-week-old rice seedlings were treated by 100 mM NaCl for 24 h, air-drying for 15 h (drought) and 100 mM ABA for 12 h. The RO-numbers and RO-a, -b and -c were the same as indicated in Fig. 1.

**Table 2** Partial amino acid sequences (*N*-terminal and internal) of four protein spots separated by 2D-PAGE

| Spot no. | pI | kDa | Sequence | Homologous protein | Homology (%) | Treatment |
|---|---|---|---|---|---|---|
| 278 | 4.65 | 22.7 | LTASLPADG | putative actin-binding protein [*Oryza sativa*] | 100 | ABA |
| 287 | 5.49 | 19.9 | IESPVAAPRL | JIOsPR10 [*Oryza sativa*] | 100 | salt, drought |
| 292 | 4.78 | 19.0 | *N*-APVSISDERAVSVSAXXXK | ubiquinol cytochrome-*c* reductase [*Oryza sativa*] | 100 | salt, drought |
| | | | | pathogenesis-related protein PR10a [*Oryza sativa*] | 66 | |
| | | | GDGGAGTVTT | major root allergen cr16 [*Daucus carota*] | 100 | |
| 297 | 4.82 | 16.4 | *N*-TLVKIGP | salT gene product [*Oryza sativa*] | 100 | ABA |

The protein samples were obtained from rice seedlings treated by the indicated stresses or ABA treatment as described in the Materials and Methods. Identity of obtained amino-acid sequences to proteins found by searching the databases is given as % homology.

tigate the changes in protein profile caused by salt and drought stress, 2-week-old rice seedlings were treated with 100 mM NaCl for 24 h, air-drying for 15 h or 100 μM ABA for 12 h, and then analyzed by 2D-PAGE (Fig. 1). At least 13 protein spots that exhibited an increase or decrease in the intensity of CBB staining were detected after these treatments (Table 1). Significant increase in the intensity was observed in at least three spots (#287, 288 and 292) after salt and drought treatment. ABA treatment did not cause changes in these peptides, but induced changes in other spots (#38, 246, 278, 297, 281, a and b). In contrast, these ABA-responsible peptides were not affected by salt and drought stresses.

*Amino acid sequence analysis*

Among 13 spots that changed after stress treatments, partial amino acid sequences of four proteins could be determined. The separated proteins were electro-blotted onto a PVDF membrane and four proteins (#278, 287, 292 and 297) were analyzed using a gas-phase protein sequencer. The N-terminal amino acid sequences of two proteins (#292 and 297) were determined in this manner. The N-terminal region of the remaining proteins could not be sequenced, because of blocking at the N-terminus. The internal amino acid sequences of three peptides (#278, 287 and 292) could be determined by Cleveland peptide mapping using *Staphylococcus aureus* V8 protease. The obtained sequence results are given in Table 2. Database analysis revealed that these peptides have high homology to an ABA-inducible PR10, and salt-inducible proteins from rice. The amino acid sequences of RO-278 and RO-297 showed 100% homology to a putative actin-depolymerizing factor (Accession No. AAG13444; unpublished) and the *salT* gene product (Accession No. AAB23484; Claes et al. 1990),

```
   1  CCACGCGTCCGAGCAACTAGGTATCTAGCTAAGCAGTGGTGTGATCAGTAGGAAGTTGCA   60
  61  GGTGGGGGATATATCACCAATGGCTCCGGTCAGCATCTCCGACGAGCGCGCCGTCTCGGT  120
                         M  A  P  V  S  I  S  D  E  R  A  V  S  V

 121  GTCGGCGGAGCGGGTGTGGAAGGTCTTCTCCGACGCGCCCGCCATGCCCAAGGTTTGTGC  180
       S  A  E  R  V  W  K  V  F  S  D  A  P  A  M  P  K  V  C  A

 181  CGGCTTCATCGACGCCATTGAGGTCGAGGGGGATGGCGGGGCGGGCACTGTCACCACCAT  240
       G  F  I  D  A  I  E  V  E  G  D  G  G  A  G  T  V  T  T  M

 241  GAAGCTCAACCCTGCTGTGGATGATGGGGGGTCATTCAAAACACGTGTGGTGGCACGTGA  300
       K  L  N  P  A  V  D  D  G  G  S  F  K  T  R  V  V  A  R  D

 301  CAACGCAGCTCACATTATCAAGTCAGAGGTTCTGGATGTGCCGGCCGGAAGTAAAGTGGG  360
       N  A  A  H  I  I  K  S  E  V  L  D  V  P  A  G  S  K  V  G

 361  CAAGCTCAAGTCGCACGTGACAGAGACGAAGATCGAGGCCGCCGGTGCCGGCTCTTGCTT  420
       K  L  K  S  H  V  T  E  T  K  I  E  A  A  G  A  G  S  C  L

 421  GGCCAAGATAAACGTGGAGTATGAGCTCGAGGACGGCGGCTCACTGTCGCCGGAGAAGGA  480
       A  K  I  N  V  E  Y  E  L  E  D  G  G  S  L  S  P  E  K  E

 481  GAAGCTCATCCTCGACGGCTACTTCGGCATGCTCAAGATGATCGAGGACTACCTCGTCGC  540
       K  L  I  L  D  G  Y  F  G  M  L  K  M  I  E  D  Y  L  V  A

 541  TCACCCTACCGAGTATGCTTAAAAATTGTCATAAACCAAAATAATATACATCCATCTCCG  600
       H  P  T  E  Y  A  *

 601  TATTGCTGCTTCCTGATAATTAAATAATGTGAGCCACGACAAATCCAATCTTTTGTGGTG  660
 661  TTTGATTTTGTGAGAGTGATTTGTGTTTGAGGTTATGTAAGAAATAAATCATAATTGTGA  720
 721  TCGTGTTCTAAAAAAAAAAAAAAA  744
```

**Fig. 2** Nucleotide and deduced amino acid sequence of *RSOsPR10* cDNA. The stop codon is indicated with an asterisk. The two peptide sequences of RO-292 determined by a gas-phase protein sequencer are underlined. A putative polyadenylation signal is boxed. The nucleotide sequence data has been submitted to the DDBJ, EMBL and NCBI nucleotide sequence databases with the accession number AB127580.

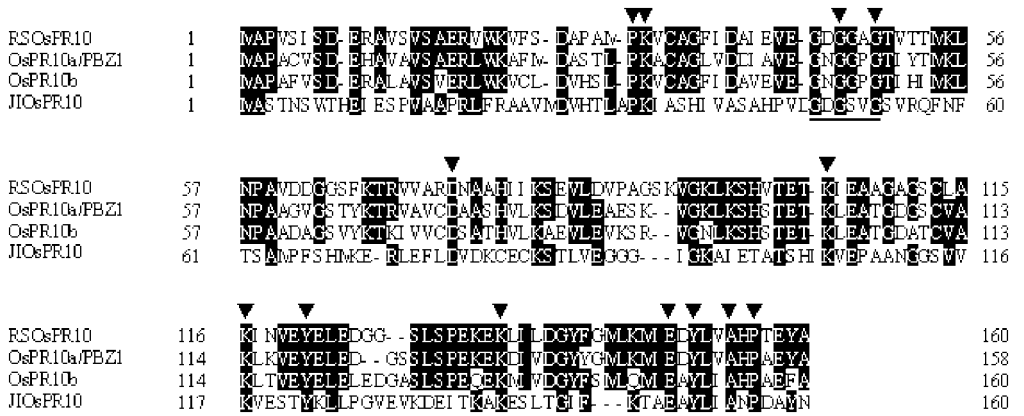


**Fig. 3** Alignment of the amino acid sequences of RSOsPR10 and rice PR10 proteins. The predicted amino acid sequence of the RSOsPR10 protein is compared with that of OsPR10a/PBZ1, OsPR10b and JIOsPR10 proteins. Arrowheads indicate the strictly conserved residues, and the conserved motif GXGGXG is underlined.

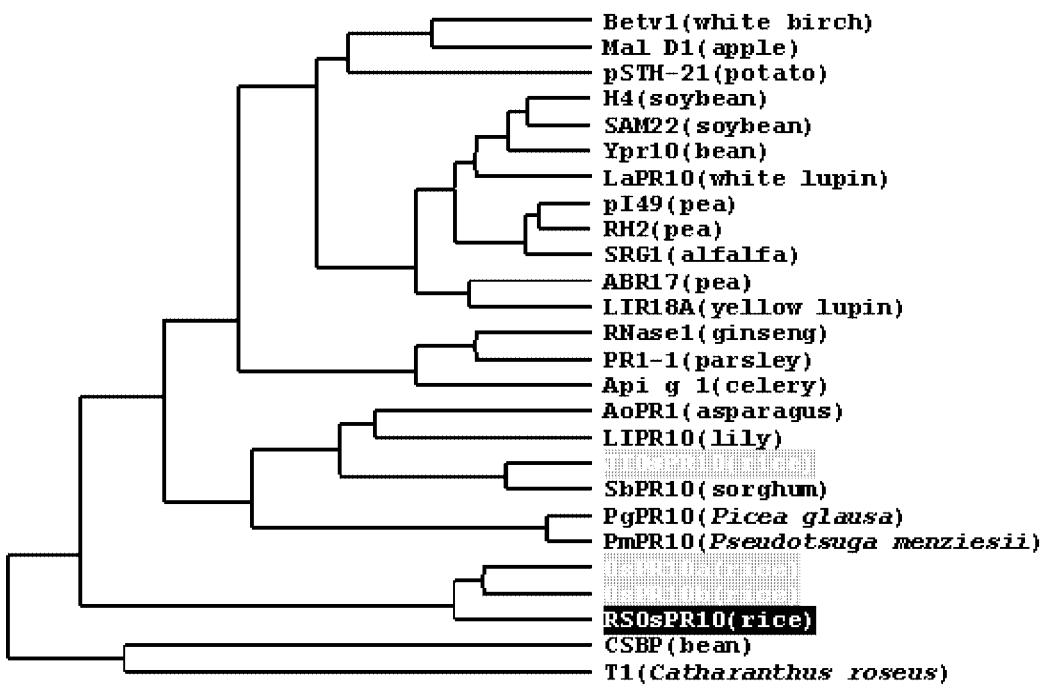


**Fig. 4** Phylogenetic tree of PR10 proteins from various plants. The phylogenetic tree was constructed by UPGMA (unweighted pair group method arithmetic means) using the Genetyx program (Japan). RSOsPR10 [in the present study] and other proteins are presented with the previously published designations [Bet v1 (Breiteneder et al. 1989, S05376), Mal D1 (Hoffmann-Sommergruber et al. 1997, gi1313967), pSTH-21 (Matton and Brisson 1989, AAA03020), H4, SAM22 (Crowell et al. 1992, CAA42647 and P26987, respectably), Ypr10 (Walter et al. 1990, X96999), LaPR10 (Bantignies et al. 2000, AJ000108), pI49 (Fristensky et al. 1998, P14710), RH2 (Mylona et al. 1994, AAB32504), SRG1 (Truesdell and Dickman 1997, AAB58315), ABR17 (Iturriaga et al. 1994, Q06931), LIR18A (Sikorski et al. 1996, X79974), RNase1 (Moiseyev et al. 1994, P80889), PR1-1 (Somssich et al. 1986, P10417), Api g1 (Breiteneder et al. 1995, P49372), AoPR1 (Warner et al. 1992, Q05736), LIPR10 (Huang et al. 1997, AAD17336), JIOsPR10 (Jwa et al. 2001, AAL74406), SbPR10 (Lo et al. 1999, U60764), PgPR10 (unpublished, AAF12810), PmPR10 (Ekramoddoullah et al. 2000, AAF60972), OsPR10a (PBZ1, Midoh and Iwata 1996, D38170), OsPR10b (McGee et al. 2001, AAF85973), CSBP (Fujimoto et al. 1998, AB012218), T1 (Carpin et al. 1998, Y10612)].

respectively. The internal peptide sequence of RO-287 had 100% similarity with JIOsPR10 (Accession No. AAL74406; Jwa et al. 2001). Although N-terminal sequence of RO-292 showed 100% homology to a ubiquinol cytochrome-*c* reductase of rice, the sequence exhibited 66% similarity to OsPR10a/PBZ1 (Accession No. BAA07369; Midoh and Iwata 1996) and its internal sequence was highly homologous to carrot PR protein (Accession No. AAL76932), strongly suggesting that RO-292 is one of the PR10 family protein.

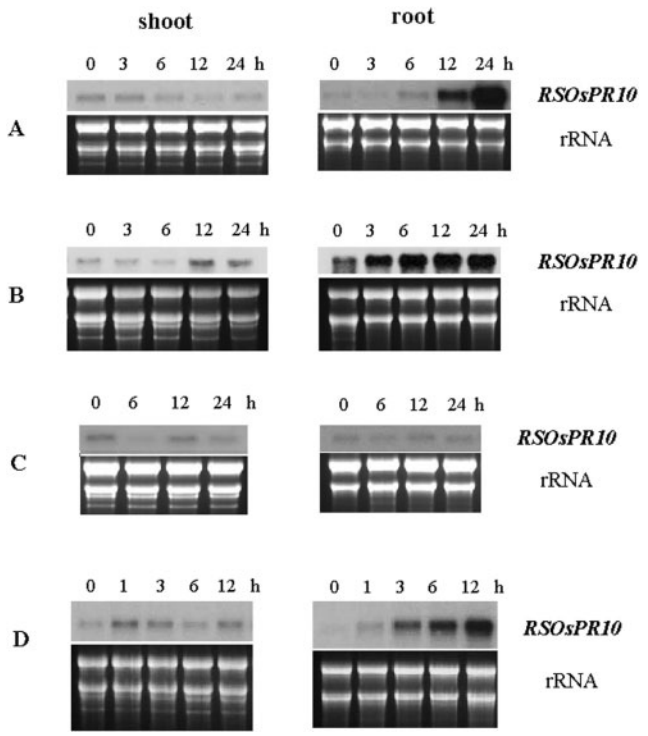


**Fig. 5** Expression of *RSOsPR10* mRNA after various stress treatments. Total RNA was isolated from shoots (left) and roots (right) of 2-week-old rice seedlings after treatment for the indicated times; (A) 100 mM NaCl, (B) air-drying, (C) cold (4°C), and (D) infection by blast fungus. Ten μg of total RNA were applied to each lane in a formaldehyde–agarose gel. After electrophoresis, RNA was transferred onto a nylon membrane and arrowed to hybridize with the *RSOsPR10* specific cDNA probe.

*cDNA cloning of RO-292 mRNA*

An EST (S6339, Accession No. C25417) corresponding to the N-terminal of the RO-292 protein (APVSISDERAVSVSAXXXK) was obtained from the NIAS DNA Bank. We examined the sequence of the *EST* in the pBluescript II SK-vector and found that it contained a full-length cDNA of 744 bp. The sequence is given in Fig. 2. The cDNA encoded a putative protein of 160 amino acids with a predicted molecular weight of 16,900. A putative polyadenylation signal (AATAAA) was found in the 3′ non-coding region. The predicted amino acid sequence from the cDNA was identical to the corresponding N-terminal and internal peptide sequences obtained by amino acid sequencing described above (Fig. 2, underlined). Because the expression of the gene encoding the RO-292 protein was induced mainly in roots by abiotic and biotic stresses as described below, we named the gene *RSOsPR10* (*root specific rice PR10*) and its protein RSOsPR10.

A sequence alignment, using PSI-BLASTP/BLASTP interrogation, of the predicted amino acid residues of RSOsPR10 with different members of the OsPR10 protein family is shown in Fig. 3. RSOsPR10 showed 68%, 62%, and 27% similarity with OsPR10a/PBZ1 (Accession No. D38170) and OsPR10b (Accession No. AAF85973), and JIOsPR10 (Accession No. AF395880), respectively. The alignment revealed that there are 13 highly conserved amino acid residues (marked by arrowheads) among the PR proteins from various plant species (Midoh and Iwata 1996, Jwa et al. 2001, Walter et al. 1996). RSOsPR10 and OsPR10 proteins contained a GXGGXG motif that is found in many nucleotide-binding proteins (Gajhede et al. 1996). JIOsPR10 was different from these proteins because it exhibits lower sequence homology and had a more neutral pI than those of other PR10 proteins. The sequence data indicates that RSOsPR10 belongs to a group of OsPR10 proteins, together with OsPR10a/PBZ1 and OsPR10b, and that JIOsPR10 might belong to a different class of PR10 proteins in rice. A phylogenic tree of PR10 proteins was constructed based on the homologous full-length sequences (Fig. 4) and revealed that monocot PR10 proteins were separated to two groups: OsPR10a/PBZ1, OsPR10b and RSOsPR10 belong to one group, and JIOsPR10 belongs to another group along with sorghum, lily and asparagus PR10s.

*Effects of salt, drought, cold stress and blast fungal infection on RSOsPR10 gene expression*

To examine the abiotic and biotic stresses on *RSOsPR10* gene expression, Northern blot analysis was performed on NaCl, drought and cold temperature (4°C) treated and blast fungus inoculated rice seedlings. The results are shown in Fig. 5. NaCl and drought treatment caused rapid and strong induction of *RSOsPR10* transcription (Fig. 5A, B). Drought treatment caused an especially rapid increase within 3 h. In contrast, cold stress did not induce any change in *RSOsPR10* gene expression (Fig. 5C).

When the rice seedlings were inoculated with blast fungus, after about 2 d the necrotic lesion formation was not observed, but the rice seedlings began to wither (data not shown). The treatment caused increased *RSOsPR10* expression, again only in the roots (Fig. 5D). Because no incompatible race of *Pyricularia grisea* for cv. Nipponbare was found, we examined the expression of *RSOsPR10* using a compatible race here. An investigation of the effect of compatible and incompatible races on *RSOsPR10* expression using cv. Jikkoku (*Pi-a*), which was used for *PBZ1* analysis (Midoh and Iwata 1996), should reveal details of the relationship between fungus infection and *RSOsPR10* expression.

*Effect of probenazole on RSOsPR10 and OsPR10a/PBZ1 gene expression*

Probenazole, a well-known agricultural chemical, is effective against rice blast fungal disease and can induce *OsPR10a/PBZ1* gene expression (Midoh and Iwata 1996). When the rice seedlings were treated with a solution containing 100 mg liter$^{-1}$ probenazole, *RSOsPR10* mRNA was induced within 3 h of treatment (Fig. 6A). This expression was mainly detected only in the roots. In contrast, probenazole treatment only increased

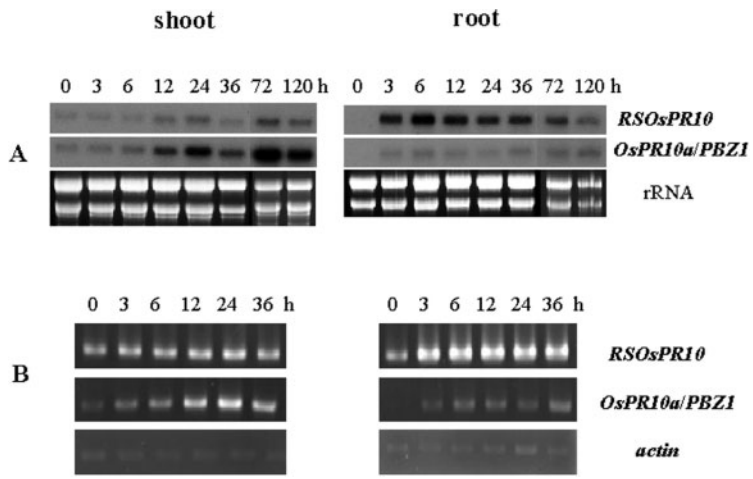


**Fig. 6** Effect of probenazole on *RSOsPR10* or *OsPR10a/PBZ1* mRNA expression. Total RNA was isolated from shoots and roots of 2-week-old rice seedlings after treatment for the indicated times by submerged application of 100 mg liter$^{-1}$ probenazole. (A) Ten μg of total RNA were analyzed for Northern blotting with *RSOsPR10* cDNA specific or *OsPR10a/PBZ1* cDNA specific 3′-untranslated regions as probes. (B) RT-PCR analysis of *RSOsPR10* and *OsPR10a/PBZ1* gene expression in shoots and roots of probenazole treated rice seedlings.

*OsPR10a/PBZ1* gene expression in shoots. This was confirmed by RT-PCR analysis (Fig. 6B).

*Effects of defense-related signaling molecules on RSOsPR10 gene expression*

In the process of plant stress responses, involvement of several signaling molecules, such as ABA, jasmonic acid and salicylic acid, has been reported and their signaling cascade has been investigated (Zeevaart and Creelman 1988, Reymond and Farmer 1998, Turner et al. 2002). To determine whether these molecules have an effect on *RSOsPR10* expression, *RSOsPR10* mRNA levels were determined in rice seedlings treated with ABA (100 μM), jasmonic acid (100 μM) and salicylic acid (100 μM) (Fig. 7). ABA and salicylic acid exhibited no effect on *RSOsPR10* expression (Fig. 7A, B). An even higher concentration of salicylic acid (1 mM) did not cause any change for the *RSOsPR10* gene expression (data not shown). The results showed clearly that only jasmonic acid had a significant effect on root specific induction of *RSOsPR10* mRNA (Fig. 7C).

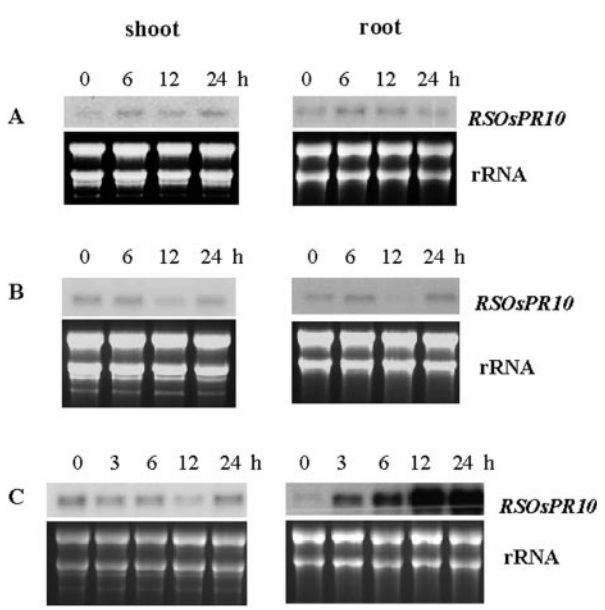


**Fig. 7** Effects of signaling molecules on *RSOsPR10* mRNA expression. Total RNA was isolated from shoots and roots of 2-week-old rice seedlings after treatment for the indicated times by submerged application of 100 μM ABA (A), 100 μM salicylic acid (B) or 100 μM jasmonic acid (C). Ten μg of total RNA were separated and subjected to Northern hybridization using the *RSOsPR10* cDNA specific probe.

## Discussion

In the present study, we found several stress-response proteins in roots of rice seedlings by 2D-PAGE by exposing 2-week-old rice seedlings to salt and water stress and treatment with ABA. The amounts of three common peptides were increased after salt or drought treatment, but they did not change by ABA. In contrast, several ABA-responsive peptides were not affected by salt and drought stress, indicating the presence of ABA independent signaling pathways for salt- and water-stress responses. Among 13 spots identified by 2D-PAGE that were altered by salt, drought and ABA treatments, the partial amino acid sequences of four peptides could be determined. Using homology searches, these peptides were identified as known stress-response proteins. Among them, RO-292 was found to be a new member of the OsPR10 family protein. Gene expression of *RSOsPR10*, encoding the RO-292 protein, was up-regulated by salt, drought, fungal infection and probenazole. Cold stress did not affect gene expression. Induction of *RSOsPR10* was detected almost exclusively in the roots. Thus, RSOsPR10 appears to be a novel OsPR10 protein specifically expressed in rice roots that is involved in plant-stress response to salt, drought and fungal infection, where jasmonic acid, but not ABA, functions as a stress-signaling molecule.

Previous studies on rice PR10 proteins, mainly OsPR10a/PBZ1, have focused on their role in disease defenses and endogenous secondary signals, such as jasmonic acid, salicylic acid and probenazole, were investigated as a effective mole-

cules in fungal infection and *OsPR10a/PBZ1* gene induction (Lee et al. 2001, Midoh and Iwata 1996, McGee et al. 2001, Moons et al. 1997, Rakwal et al. 2001). Ozone and UV stresses, known to induce jasmonic-acid-mediated plant defense cascades, also increased rice PR10 proteins (Agrawal et al. 2002b, Rakwal et al. 1999). Although salicylic acid has an important role on SAR (systemic acquired resistance) and has been known to induce the PR3 and PR5 classes of PR proteins, salicylic acid does not always induce rice *PR10* genes (Midoh and Iwata 1996, Moons et al. 1997). It is thus proposed that the infection by a pathogen may transduce via two independent pathways, jasmonic acid and salicylic acid, and that jasmonic acid is a signaling molecule for rice *PR10* gene induction.

A few studies have dealt with the effect of abiotic stresses such as drought, salt and cold on rice *PR10* gene expression. Moons et al. (1997) showed that NaCl could induce *OsPR10a/PBZ1* gene expression. They showed that high concentrations (more than 150 mM) of NaCl caused rapid and transient increase of endogenous methyl-jasmonic acid. Jasmonic acid and ABA could antagonistically regulate the expression of salt stress-inducible proteins. It was also demonstrated that rice *OsPR10a/PBZ1* was up-regulated by salt stress via jasmonic acid, not via ABA cascade in roots, but enhancement of *OsPR10a/PBZ1* transcription by ABA was demonstrated in the sheath. A similar report showed the positive effect of ABA on *OsPR10a/PBZ1* induction in rice seedlings (Lee et al. 2001). Thus, currently the involvement of ABA in *OsPR10a/PBZ1* expression is not clearly evident. In the present study, RO-292 (RSOsPR10) was isolated also as drought stress-inducible protein, and rapid induction of the *RSOsPR10* gene was detected after drought treatment. However, RSOsPR10 protein and mRNA was moderated by jasmonic acid, but not by ABA, indicating that induction of RSOsPR10 after drought stress is regulated via the jasmonic acid pathway. This could be explained by a report with micro-array analysis showing that a large member of wounding induced genes mediated by jasmonic acid overlapped with water stress-inducible genes (Reymond et al. 2000).

Many works on OsPR10a/PBZ1 have investigated its expression in rice leaves, discussing its role in pathogen defense mainly in the leaves (Xiong and Yang 2003, Lee et al. 2001, McGee et al. 2001, Midoh and Iwata 1996, Rakwal et al. 1999, Agrawal et al. 2002a). There has been only limited information regarding *OsPR10a/PBZ1* gene expression in roots (Moons et al. 1997). The present study showed that RSOsPR10 was expressed in a root-specific manner after salt stresses, drought stresses and fungal infection, as well as with probenazole treatment. As mentioned above, there are some differences between OsPR10a/PBZ1 and RSOsPR10, but the amino acid sequence homology and similar induction mechanisms suggest that they have similar activity in stress response. In the present work, we have clearly shown that OsPR10a/PBZ1 and RSOsPR10 are separately regulated in the shoots and roots (see Fig. 5). This fact strongly indicates that these proteins function against various environmental stresses in an organ-specific manner. Study of the detailed expression pattern of RSOsPR10 and its regulation in various organs or tissues in mature rice plants will give us some important answers for this question.

Recent works indicated that some other phytohormones, ethylene (Poupard et al. 2003) and cytokinin (Fujimoto et al. 1998, Gonneau et al. 2001), as well as wounding treatment (Warner et al. 1992) were effective on plant PR gene expression. As for the signaling pathway, there were only a few reports, showing the involvement of mitogen-activated protein kinase (Xiong and Yang 2003) and protein phosphatase in PR10 expression (Rakwal et al. 2001). Thus, there are still many unknown parts for the induction mechanisms and the signaling pathways of PR10 expression, especially in relation to the cross talk between various biotic and abiotic stresses, and the signaling cascade via phytohormones. Finally, it is of utmost importance to clarify the in vivo biological function(s) of the RSOsPR10 protein in rice. The genetic manipulation of these genes will be useful for this purpose. We are now producing transgenic rice expressing sense and anti-sense cDNAs of *RSOsPR10* to reveal its exact function(s) and the signaling pathways that mediate the defense and stress responses in rice.

## Materials and Methods

*Plant materials, growth condition and stress treatment*

Rice (*Oryza Sativa* cv. Nipponbare) seeds were sown in water and grown at 27°C using a 12 h light and 12 h dark regime. Two-week-old seedlings were treated with the following chemicals by a submerged method; 100 mM NaCl, 100 μM ABA, 100 μM jasmonic acid, 100 μM salicylic acid, and 100 mg liter$^{-1}$ probenazole. Fresh water was used for control seedlings. For desiccation experiments, the seedlings were air-dried on paper towels. Blast infections were carried out with 2-week-old rice seedlings by submerged conidial suspensions ($1\times10^5$ conidial ml$^{-1}$ water) of *Pyricularia grisea* (MAFF 101001: race 001, compatible to Nipponbare). After treatment for appropriate time intervals, the roots and the shoots (leaf and sheath) were separately sampled and kept at –80°C until use.

*Protein extraction and 2D-PAGE*

A portion (0.5 g) of the fresh roots was homogenized with 2 ml phosphate buffer pH 7.6 containing 65 mM $K_2HPO_4$, 2.6 mM $KH_2PO_4$, 400 mM NaCl and 3 mM $NaN_3$ after freezing with liquid nitrogen. The homogenate was centrifuged at 15,000×*g* for 10 min, and 50% trichloroacetic acid was added to the supernatant to a final concentration of 10%. The solution was kept on ice for 20 min and centrifuged at 15,000×*g* for 10 min. The resultant precipitate was suspended in 0.2 ml of lysis buffer (O'Farrell 1975) and sonicated for 2 min. The solution was adjusted to pH 7.0 with 1 M NaOH.

A portion (50 μl) of solution was subjected to 2D-PAGE. Isoelectric focusing was carried out in a glass tube 13-cm long and 3.5-mm in diameter. Electrophoresis was carried out at 200 V for 30 min, followed by 400 V for 16 h and 600 V for 1 h. Sodium dodecyl sulfate (SDS)-PAGE in the second dimension was performed using 17% separation and 5% stacking gels at a constant current of 35 mA. The isoelectric point and relative molecular weight of each protein were determined using 2D-PAGE Marker (Bio-Rad, Richmond, CA, U.S.A.). The positions of individual proteins on the gels were evalu-

ated automatically with Image-Master 2D Elite software (Pharmacia Biotech, Uppsala, Sweden).

*N-Terminal and internal sequence analysis*

Following separation by 2D-PAGE, the proteins were electroblotted onto a PVDF membrane (Fluorotrans; Pall Bio Support Division, Port Washington, NY, U.S.A.) and detected by CBB staining. The spots were excised from the PVDF membrane and applied to the upper glass block in the reaction chamber of a gas-phase protein sequencer (Precise 494; Applied Biosystems, Foster City, CA, U.S.A.). Edman degradation was performed according to the standard program supplied by Applied Biosystems.

The Cleveland method was used to obtain internal sequences. After proteins were separated by 2D-PAGE and stained with CBB, the desired protein spots were cut out and the gel pieces were crushed in a tube with 20 µl of SDS sample buffer. The gel suspension was then applied onto a well of a SDS-slab gel, and mixed with 20 µl of a protease solution containing 10 µl of *Staphylococcus aureus* V8 protease (Pierce, Rockford, IL, U.S.A.) with 0.1 µg $\mu l^{-1}$ in deionized water and 10 µl of SDS sample buffer. Electrophoresis was performed until the sample and protease were stacked in the upper gel and interrupted for 30 min to digest the protein, after which electrophoresis was continued. After the electrophoresis, some of the digested peptides were sequenced using a gas-phase protein sequencer as described above.

*Homology search of amino acid sequences*

All the sequence data were analyzed using the Genetyx program (SDC Software Development, Tokyo, Japan). Searches for homology of nucleotide and amino-acid sequences were carried out with BLAST/PSI-BLAST against the sequences in the DDBJ, EMBL and NCBI DNA databases. The amino-acid sequences of proteins, that were obtained by analyses of *N*-terminal and internal sequences, were compared with those of in a DDBJ database using FASTA, BLAST and PSI-BLAST sequence alignment programs.

*cDNA cloning and sequence analysis of RO-292 mRNA*

The EST (S6339, Accession No. C25417) containing the N-terminal sequence of RO-292 protein of was obtained from the NIAS DNA Bank. DNA sequencing was carried out using a Dye Primer Cycle Sequencing kit (Applied Biosystems) and a DNA sequencer (model 310, Applied Biosystems) according to the manufacturer's instructions.

*RNA preparation and Northern hybridization*

Frozen plant tissues were ground under liquid nitrogen into a powder using a mortar and pestle. The powder (0.5 g) was added to a microcentrifuge tube containing 1 ml ISOGEN (Nippongene, Tokyo, Japan) on ice. The solution was mixed by a vortex for 5 min, after that, 0.2 ml chloroform was added and the solution was mixed vigorously again for 3 min. The samples were centrifuged at 15,000×*g* for 20 min at 4°C. The upper aqueous layer was transferred to a clean tube. Three phenol and chloroform extractions were performed on the solution. After the third extraction, the same amount of 4 M LiCl was added to the aqueous layer and the solution was left at 4°C overnight. The sample was centrifuged at 15,000×*g* for 20 min at 4°C. The pellet was washed with 1 ml of 70% ethanol and dried by inverting the tube for 5 min. The pellet was dissolved in 20 µl of DEPC (diethyl pyrocarbonate)-treated water. This RNA solution was stored at –20°C until analysis.

Ten µg aliquots of total RNA were separated by electrophoresis through a formaldehyde-agarose gel (1.5% agarose) and blotted onto a hybridization transfer membrane (GeneScreen, PerkinElmer Life Sciences, Inc.). Ethidium bromide was included in the running buffer at 50 µg $liter^{-1}$, which allowed photography under UV light after electrophoresis to confirm equal sample loading. RNA gel blots were hybridized with [$\alpha$-$^{32}$P]dCTP-labeled gene-specific probes in church buffer. Gene-specific probes were made from the 3′-untranslated region of *RSOsPR10* and *OsPR10a/PBZ1* by PCR cloning. The forward primer, common to both genes, 5′-GGCATGCTCAAGATGATCGA-3′ was designed using the 506–525 bp region of *RSOsPR10* cDNA in Fig. 2, and the M13 reverse primer (on the pBluescript II SK-; cloning vector), were used to amplify DNA fragments covering the 3′-untranslated regions of *RSOsPR10* and *OsPR10a/PBZ1*.

*RT-PCR*

To detect the two different genes, *RSOsPR10* and *OsPR10a/PBZ1*, two gene specific primer sets were designed for *RSOsPR10*; 5′-GTATCTAGCTAAGCAGTGGT-3′ (5′-UTR, 21–40 bp region of *RSOsPR10* cDNA)/5′-GCAATACGGAGATGGATGTA-3′ (3′-UTR, 587–606 bp region of *RSOsPR10* cDNA), and for *OsPR10a/PBZ1*; 5′-CATCAGTGGTCAGTAGAGTG-3′ (5′-UTR, 21–40 bp region of D38170 sequence)/5′-CTCTAGGTGGGATATACTGG-3′ (3′-UTR, 584–613 bp region of D38170), to amplify DNA fragments specific for each DNA. Equal amounts of total RNA (1 µg) were subjected to RT-PCR analysis using Superscript II reverse transcriptase (Invitrogen Corp., Carlsbad, CA, U.S.A.). PCR amplification was conducted at 94°C for 30 s (denature), 60°C for 30 s (annealing), and 68°C for 90 s (synthesis) for 23 cycles. After PCR, 5 µl of PCR products were separated by 1% agarose gel electrophoresis. The *actin* gene (rice actin, Accession No. X16280) was used as an internal control for RT-PCR and the primers for *actin* were, 5′-GCGATAATGGAACTGGTATGG-3′ (forward primer) and 5′-CGAGACGAAGGATAGCATGG-3′ (reverse primer).

## Acknowledgments

We are grateful to NIAS DNA bank for providing rice EST clones. This work was supported by a grant from the rice genome Project PR, MAFF, Japan.

## References

Agrawal, G.K., Rakwal, R., Tamogami, S., Yonekura, M., Kubo, A. and Saji, H. (2002a) Chitosan activates defense/stress response(s) in the leaves of *Oryza sativa* seedlings. *Plant Physiol. Biochem.* 40: 1061–1069.

Agrawal, G.K., Rakwal, R., Yonekura, M., Kubo, A. and Saji, H. (2002b) Proteome analysis of differentially displayed proteins as a tool for investigating ozone stress in rice (*Oryza sativa* L.) seedlings. *Proteomics* 2: 947–959.

Alexander, D., Goodman, R.M., Gut-Rella, M., Glascock, C., Weymann, K., Friedrich, L., Maddox, D., Ahl-Goy, P., Luntz, T., Ward, E. and Ryals, J. (1993) Increased tolerance to two oomycete pathogens in transgenic tobacco expressing pathogenesis-related protein 1a. *Proc. Natl Acad. Sci. USA* 90: 7327–7331.

Bantignies, B., Séguin, J., Muzac, I., Dédaldéchamp, F., Gulick, P. and Ibrahim, R. (2000) Direct evidence for ribonucleolytic activity of a PR-10-like protein from white lupin roots. *Plant Mol. Biol.* 42: 871–881.

Barratt, D.H.P. and Clark, J.A. (1991) Proteins arising during the late stages of embryogenesis in *Pisum sativum* L. *Planta* 184: 14–23.

Breiteneder, H., Hoffmann-Sommergruber, K., O'Riordain, G., Susani, M., Ahorn, H., Ebner, C., Kraft, D. and Scheiner, O. (1995) Molecular characterization of Api g 1, the major allergen of celery (*Apium graveolens*), and its immunological and structural relationships to a group of 17-kDa tree pollen allergens. *Eur. J. Biochem.* 233: 484–489.

Breiteneder, H., Pettenburger, K., Bito, A., Valenta, R., Kraft, D., Rumpold, H., Scheiner, O. and Breitenbach, M. (1989) The gene coding for the major birch pollen allergen *Bet*v1, is highly homologous to a pea disease resistance response gene. *EMBO J.* 8: 1935–1938.

Bufe, A., Spangfort, M.D., Kahlert, H., Schlaak, M. and Becker, W.-M. (1996) The major birch pollen allergen, Bet v 1, shows ribonuclease activity. *Planta* 199: 413–415.

Carpin, S., Laffer, S., Schoentgen, F., Valenta, R., Chénieux, J.-C., Rideau, M. and Hamdi, S. (1998) Molecular characterization of a cytokinin-inducible periwinkle protein showing sequence homology with pathogenesis-related proteins and the Bet v 1 allergen family. *Plant Mol. Biol.* 36: 791–798.

Claes, B., Dekeyser, R., Villarroel, R., Van den Bulcke, M., Bauw, G., Van Montagu, M. and Caplan, A. (1990) Characterization of a rice gene showing organ-specific expression in response to salt stress and drought. *Plant Cell* 2: 19–27.

Crowell, D.N., John, M.E., Russell, D. and Amasino, R.M. (1992) Characterization of a stress-induced, developmentally regulated gene family from soybean. *Plant Mol. Biol.* 18: 459–466.

Ekramoddoullah, A.K.M., Yu, X., Sturrock, R., Zamani, A. and Taylor, D. (2000) Detection and seasonal expression pattern of a pathogenesis-related protein (PR-10) in Douglas-fir (*Pseudotsuga menziesii*) tissues. *Physiol. Plant.* 110: 240–247.

Fristensky, B., Horovitz, D. and Hadwiger, L.A. (1998) cDNA sequences for pea disease resistance response genes. *Plant Mol. Biol.* 11: 713–715.

Fujimoto, Y., Nagata, R., Fukasawa, H., Yano, K., Azuma, M., Iida, A., Sugimoto, S., Shudo, K. and Hashimoto, Y. (1998) Purification and cDNA cloning of cytokinin-specific binding protein from mung bean (*Vigna radiata*). *Eur. J. Biochem.* 258: 794–802.

Gajhede, M., Osmark, P., Poulsen, F.M., Ipsen, H., Larsen, J.N., van Neerven, R.J.J., Schou, C., Løwenstein, H. and Spangfort, M.D. (1996) X-ray and NMR structure of Bet v 1, the origin of birch pollen allergy. *Nat. Struct. Biol.* 3: 1040–1045.

Gonneau, M., Pagant, S., Brun, F. and Laloue, M. (2001) Photoaffinity labeling with the cytokinin agonist azido-CPPU of a 34 kDa peptide of the intracellular pathogenesis-related protein family in the moss *Physcomitrella patens*. *Plant Mol. Biol.* 46: 539–548.

Hoffmann-Sommergruber, K., Vanek-Krebitz, M., Radauer, C., Wen, J., Ferreira, F., Scheiner, O. and Breiteneder, H. (1997) Genomic characterization of members of the Bet v 1 family: genes coding for allergens and pathogenesis-related proteins share intron positions. *Gene* 197: 91–100.

Huang, J.-C., Chang, F.-C. and Wang, C.-S. (1997) Characterization of a lily tapetal transcript that shares sequence similarity with a class of intracellular pathogenesis-related (IPR) proteins. *Plant Mol. Biol.* 34: 681–686.

Iturriaga, E.A., Leech, M.J., Barratt, D.H.P. and Wang, T.L. (1994) Two ABA-responsive proteins from pea (*Pisum sativum* L.) are closely related to intracellular pathogenesis-related proteins. *Plant Mol. Biol.* 24: 235–240.

Jwa, N.-S., Agrawal, G.K., Rakwal, R., Park, C.-H. and Agrawal, V.P. (2001) Molecular cloning and characterization of a novel Jasmonate inducible pathogenesis-related class 10 protein gene, *JIOsPR10*, from rice (*Oryza sativa* L.) seedling leaves. *Biochem. Biophys. Res. Commun.* 286: 973–983.

Lee, M.-W., Qi, M. and Yang, Y. (2001) A novel jasmonic acid-inducible rice *myb* gene associates with fungal infection and host cell death. *Mol. Plant-Microbe Interact.* 14: 527–535.

Linthorst, H.J.M. (1991) Pathogenesis-related proteins of plants. *Crit. Rev. Plant Sci.* 10: 123–150.

Lo, S.-C.C., Hipskind, J.D. and Nicholson, R.L. (1999) cDNA cloning of a sorghum pathogenesis-related protein (PR-10) and differential expression of defense-related genes following inoculation with *Cochliobolus heterostrophus* or *Colletotrichum sublineolum*. *Mol. Plant-Microbe Interact.* 12: 479–489.

Matton, D.P. and Brisson, N. (1989) Cloning, expression, and sequence conservation of pathogenesis-related gene transcripts of potato. *Mol. Plant-Microbe Interact.* 2: 325–331.

McGee, J.D., Hamer, J.E. and Hodges, T.K. (2001) Characterization of a *PR-10* pathogenesis-related gene family induced in rice during infection with *Magnaporthe grisea*. *Mol. Plant-Microbe Interact.* 14: 877–886.

Midoh, N. and Iwata, M. (1996) Cloning and characterization of a probenazole-inducible gene for an intracellular pathogenesis-related protein in rice. *Plant Cell Physiol.* 37: 9–18.

Moiseyev, G.P., Beintema, J.J., Fedoreyeva, L.I. and Yakovlev, G.I. (1994) High sequence similarity between a ribonuclease from *ginseng* calluses and fungus-elicited proteins from parsley indicates that intracellular pathogenesis-related proteins are ribonucleases. *Planta* 193: 470–472.

Moons, A., Prinsen, E., Bauw, G. and Van Montagu, M. (1997) Antagonistic effects of abscisic acid and jasmonates on salt stress-inducible transcripts in rice roots. *Plant Cell* 9: 2243–2259.

Mylona, P., Moerman, M., Yang, W.-C., Gloudemans, T., Van De Kerckhove, J., van Kammen, A., Bisseling, T. and Franssen, H.J. (1994) The root epidermis-specific pea gene RH2 is homologous to a pathogenesis-related gene. *Plant Mol. Biol.* 26: 39–50.

O'Farrell, P.H. (1975) High resolution two-dimensional electrophoresis of proteins. *J. Biol. Chem.* 250: 4007–4021.

Poupard, P., Parisi, L., Campion, C., Ziadi, S. and Simoneau, P. (2003) A wound- and ethephon-inducible *PR-10* gene subclass from apple is differentially expressed during infection with a compatible and an incompatible race of *Venturia inaequalis*. *Physiol. Mol. Plant Pathol.* 62: 3–12.

Rakwal, R., Agrawal, G.K., Tamogami, S., Yonekura, M., Agrawal, V.P. and Iwahashi, H. (2003) Novel insight into kinetin-inducible stress responses in rice seedlings. *Plant Physiol. Biochem.* 41: 453–457.

Rakwal, R., Agrawal, G.K. and Yonekura, M. (1999) Separation of proteins from stressed rice (*Oryza sativa* L.) leaf tissues by two-dimensional polyacrylamide gel electrophoresis: induction of pathogenesis-related and cellular protectant proteins by jasmonic acid, UV irradiation and copper chloride. *Electrophoresis* 20: 3472–3478.

Rakwal, R., Agrawal, G.K. and Yonekura, M. (2001) Light-dependent induction of *OsPR10* in rice (*Oryza sativa* L.) seedlings by the global stress signaling molecule jasmonic acid and protein phosphatase 2A inhibitors. *Plant Sci.* 161: 469–479.

Reymond, P. and Farmer, E.E. (1998) Jasmonate and salicylate as global signals for defense gene expression. *Curr. Opin. Plant Biol.* 1: 404–411.

Reymond, P., Weber, H., Damond, M. and Farmer, E.E. (2000) Differential gene expression in response to mechanical wounding and insect feeding in Arabidopsis. *Plant Cell* 12: 707–719.

Sela-Buurlage, M.B., Ponstein, A.S., Bres-Vloemans, S.A., Melchers, L.S., Van den Elzen, P.J.M. and Cornelissen, B.J.C. (1993) Only specific tobacco (*Nicotiana tabacum*) chitinases and *b*-1, 3-glucanases exhibit antifungal activity. *Plant Physiol.* 101: 857–863.

Sikorski, M.M., Szlagowska, A. and Legocki, A.B. (1996) cDNA sequences encoding for two homologs of *Lupinus luteus* (L.) IPR-like proteins (Accession Nos. X79974 and X79975 for LlR18A and LlR18B mRNAs, respectively). *Plant Physiol.* 110: 335.

Somssich, I.E., Schmelzer, E., Bollmann, J. and Hahlbrock, K. (1986) Rapid activation by fungal elicitor of genes encoding "pathogenesis-related" proteins in cultured parsley cells. *Proc. Natl Acad. Sci. USA* 83: 2427–2430.

Swoboda, I., Hoffmann-Sommergruber, K., O'Ríordáin, G., Scheiner, O., Heberle-Bors, E. and Vicente, O. (1996) Bet v 1 proteins, the major birch pollen allergens and members of a family of conserved pathogenesis-related proteins, show ribonuclease activity in vitro. *Physiol. Plant.* 96: 433–438.

Tanaka, N., Che, F.-S., Watanabe, N., Fujiwara, S., Takayama, S. and Isogai, A. (2003) Flagellin from an incompatible strain of *Acidovorax avenae* mediates $H_2O_2$ generation accompanying hypersensitive cell death and expression of *PAL*, *Cht-1*, and *PBZ*1, but not of *LOX* in rice. *Mol. Plant-Microbe Interact.* 16: 422–428.

Truesdell, G.M. and Dickman, M.B. (1997) Isolation of pathogen/stress-inducible cDNAs from alfalfa by mRNA differential display. *Plant Mol. Biol.* 33: 737–743.

Turner, J.G., Ellis, C. and Devoto, A. (2002) The jasmonate signal pathway. *Plant Cell* 14 Suppl: S153–164.

Van Loon, L.C. and Van Strien, E.A. (1999) The families of pathogenesis-related proteins, their activities, and comparative analysis of PR-1 type proteins. *Physiol. Mol. Plant Pathol.* 55: 85–97.

Walter, M.H., Liu, J.-W., Grand, C., Lamb, C.J. and Hess, D. (1990) Bean pathogenesis-related (PR) proteins deduced from elicitor-induced transcripts are members of a ubiquitous new class of conserved PR proteins including pollen allergens. *Mol. Gen. Genet.* 222: 353–360.

Walter, M.H., Liu, J.-W., Wünn, J. and Hess, D. (1996) Bean ribonuclease-like pathogenesis-related protein genes (*Ypr10*) display complex patterns of developmental, dark-induced and exogenous-stimulus-dependent expression. *Eur. J. Biochem.* 239: 281–293.

Warner, S.A.J., Scott, R. and Draper, J. (1992) Characterisation of a wound-induced transcript from the monocot asparagus that shares similarity with a class of intracellular pathogenesis-related (PR) proteins. *Plant Mol. Biol.* 19: 555–561.

Warner, S.A.J., Scott, R. and Draper, J. (1993) Isolation of an asparagus intracellular *PR* gene (*AoPR1*) wound-responsive promoter by the inverse polymerase chain reaction and its characterization in transgenic tobacco. *Plant J.* 3: 191–201.

Woloshuk, C.P., Meulenhoff, J.S., Sela-Buurlage, M., van den Elyzen, P.J.M. and Cornelissen, B.J.C. (1991) Pathogen-induced proteins with inhibitory activity toward *Phytophthora infestans*. *Plant Cell* 3: 619–628.

Xiong, L. and Yang, Y. (2003) Disease resistance and abiotic stress tolerance in rice are inversely modulated by an abscisic acid-inducible mitogen-activated protein kinase. *Plant Cell* 15: 745–759.

Zeevaart, J.A.D. and Creelman, R.A. (1988) Metabolism and physiology of abscisic acid. *Annu. Rev. Plant Physiol.* 39: 439–473.

(Received December 4, 2003; Accepted February 22, 2004)