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Characterization of a wheat pathogenesis-related protein, TaBWPR-1.2, in seminal roots in response to waterlogging stress

Md. Emdadul Haque [1], Fumitaka Abe *, Masahiko Mori [2], Atsushi Oyanagi, Setsuko Komatsu, Kentaro Kawaguchi

NARO Institute of Crop Science (NICS), National Agriculture and Food Research Organization (NARO), 2-1-18 Kannondai, Tsukuba, Ibaraki 305-8518, Japan

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SUMMARY

We examined the role of pathogenesis-related protein TaBWPR-1.2 in the context of molecular and physiological responses of wheat (*Triticum aestivum*) seminal roots under waterlogging stress. Two cDNAs corresponding to the *TaBWPR-1.2* gene, *TaBWPR-1.2#2* and *TaBWPR-1.2#13* were cloned from seminal roots. These cDNAs were predicted to encode proteins of 173 and 172 amino acids, respectively. In a time-course experiment, *TaBWPR-1.2* gene expression was highest in whole seminal roots after 1 day of waterlogging treatment and higher than the control for at least 10 days; significantly increased protein abundance was observed after 7 days of waterlogging. Drought, another abiotic stress, did not influence *TaBWPR-1.2* gene expression in wheat seminal roots at 5-d-old seedlings. Tissue-specific studies revealed that the highest *TaBWPR-1.2* gene expression and protein levels were in the aerenchymatous root zone. *TaBWPR-1.2* expression in seminal roots was also increased by the signalling molecules 1-aminocyclopropane-1-carboxylic acid (ACC; an ethylene precursor), H_2O_2, jasmonic acid (JA), and nitric oxide (NO); however, treatment with abscisic acid (ABA), salicylic acid (SA), and ethanol did not alter its expression. Interestingly, aerenchyma formation in the seminal root cortex was induced only by ACC and H_2O_2. Taken together, these results indicate that *TaBWPR-1.2* is a waterlogging-responsive gene that might be associated with root cortex tissue alteration in wheat plants through ACC and/or H_2O_2 regulatory mechanisms.

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Introduction

Terrestrial plants have evolved several responses to waterlogging (WL) that involve multifaceted alterations in their cellular systems (Crawford and Braendle, 1996; Fukao and Bailey-Serres, 2004; Benz et al., 2007; Sairam et al., 2008; Rajhi et al., 2011; Yamauchi et al., 2011). Wheat (*Triticum aestivum*), an important socioeconomic crop worldwide, suffers severely from WL stress (Haque et al., 2012). In a previous study on morphological adaptation to WL in seminal roots of wheat (Haque et al., 2010), we found that levels of a pathogenesis-related (PR) protein, TaBWPR-1.2, significantly increased during lysigenous aerenchyma formation (Haque et al., 2011). From these results, it seemed that there was a relationship between TaBWPR-1.2 and WL response and/or aerenchyma tissue formation in wheat seminal roots. However, the significance of the TaBWPR-1.2 protein increase and the possible molecular mechanisms, such as increased *TaBWPR-1.2* gene expression following the perception of WL signals by plant cells and the subsequent activation of signal transduction pathways were unknown.

PR proteins in plants are associated with and/or induced by pathogen infection or wounding. PR proteins from both dicot and monocot species have been grouped into at least 17 families, primarily on the basis of their amino acid sequences (van Loon et al., 2006). The induction of PR proteins in many plant species belonging to various families is suggestive of a general role for these proteins in adaptation to biotic/abiotic stress conditions (van Loon, 1997, 1999; van Loon and van Strien, 1999). More recently, PR proteins have been found to be involved in response to adverse environments such as drought, salinity, and cold (Hashimoto et al., 2004; Xie et al., 2010; Takeuchi et al., 2011). Among the PR proteins, the family 1 PR (PR-1) was the first discovered; it is also the most

Abbreviations: ABA, abscisic acid; ACC, 1-aminocyclopropane-1-carboxylic acid; JA, jasmonic acid; PR, pathogenesis-related; qRT-PCR, quantitative reverse-transcription real-time PCR; SA, salicylic acid; SAR, systemic acquired resistance; SDS-PAGE, SDS-polyacrylamide gel electrophoresis; TaBWPR-1.2, *Triticum aestivum* cv. Bobwhite PR-1.2; WL, waterlogging.

* Corresponding author. Tel.: +81 29 838 8694; fax: +81 29 838 8694.
E-mail address: fabe@affrc.go.jp (F. Abe).
[1] Present address: Laboratory of Plant Genetics and Breeding, Faculty of Agriculture, Saga University, Honjyo-machi 1, Saga 840-8502, Japan. Tel.: +81 952 28 8725; fax: +81 952 28 8725.
[2] Present address: Department of Agro-Environmental Science, Division of Plant Production Science, Obihiro University of Agriculture and Veterinary, Inada-cho, Nishi 2-11, Obihiro, Hokkaido 080-8555, Japan.

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dominant group and is commonly used as a marker for systemic acquired resistance (SAR) (van Loon and van Kammen, 1970; van Loon and van Strien, 1999). PR-1 proteins are conserved among diverse plant species (van Loon and van Strien, 1999). All PR-1-like proteins contain a signal peptide (SP) at the N-terminus and a conserved three-dimensional structure called a PR-1 fold (or SCP_PR-1_like domain) that consists of four α-helices and one four-strand β-sheet (Fernandez et al., 1997). The PR-1 fold is believed to contribute to the stability of PR-1 proteins in a harsh extracellular environment where acidic pH and proteolytic attacks are expected (van Loon and van Strien, 1999; Buchel and Linthorst, 1999). The functional significance of PR-1 genes in adaptation to biotic stresses is reasonably well understood (Alexander et al., 1993; Niderman et al., 1995; Rauscher et al., 1999; Kiba et al., 2007; Rivière et al., 2008). PR-1 protein production and/or gene expression can be increased by abiotic stress (light, aluminium etc.), and signalling molecules such as H_2O_2, NO, jasmonic acid (JA), ethylene, and salicylic acid (SA) (Memelink et al., 1990; Bi et al., 1995; Durner et al., 1998; Agrawal et al., 2000a,b; Milla et al., 2002; Seregelyes et al., 2004; Mitsuhara et al., 2008).

Molina et al. (1999) first isolated genes encoding two putative PR-1 proteins, PR-1.1 and PR-1.2, from wheat cultivar Kanzler by using barley PR-1b cDNA (from the *HvPR-1b* gene) as a probe (Bryngelsson et al., 1994). The major difference between these two TaPR-1s is the presence of a C-terminal extension in TaPR-1.2 (9 amino acids in length in TaPR-1.2). By using the deduced amino acid sequence of the published wheat *PR-1.1* gene, Lu et al. (2011) showed that the hexaploid wheat genome contains 23 PR-1-like genes (Lu et al., 2011) located on seven different chromosomes, with the majority mapping to chromosomes of homoeologous groups 5 and 7. Among them, the deduced TaPR-1.20 protein sequence was highly identical to that encoded by the *TaPR-1.2* gene. Molina et al. (1999) first attempted to characterize the *TaPR-1.2* gene in Kanzler in terms of response to biotic and abiotic stresses. The expression of *TaPR-1.2* was found in whole seedlings and only in roots of adult plants and increased more slowly upon infection by the fungal pathogen *Erysiphe graminis* compared to *TaPR-1.1*. However, *TaPR-1.2* expression was unchanged by chemical activators of SAR (such as SA), indicating its potential involvement in biological functions other than defence against pathogens. The objective of the present study was to investigate the expression pattern of *TaBWPR-1.2* in response to WL at the mRNA and protein levels, and to examine the role of regulatory signalling molecules and their association with root responses. On the basis of our results, we hypothesize that *TaBWPR-1.2* is a WL-responsive gene increased in wheat seminal roots, where it may be involved in root morphological adaptation to WL through 1-aminocyclopropane-1-carboxylic acid (ACC) and/or H_2O_2 signalling pathways.

Materials and methods

Plant growth and stress treatments

Triticum aestivum L. cv. Bobwhite SH 98 26 was used for all experiments. Growth conditions, WL treatments (7 d), and sampling were as described by Haque et al. (2011). In the present study, we used only two conditions: (i) the control (C), with well-drained soil watered daily and then allowed to drain; and (ii) the T+3 treatment, in which the water level was maintained at 3 cm above the soil surface. For the time-course experiments using quantitative reverse-transcription real-time PCR (qRT-PCR), whole roots including primary, first pair, and second pair were collected from individual wheat plants. To perform organ-specific experiments, we collected three sections of root: a 3-cm section measured from the base of the root ("base 0–3 cm"), a 2-cm section measured from the root tip ("tip 0–2 cm"), and a 13-cm middle section measured beginning 2 cm from the root tip ("tip 2–15 cm"). If the root was less than 18 cm long, the "tip 2–15 section" included the remainder of the root after the "base 0–3 cm" and "tip 0–2 cm" pieces had been removed.

To test the effects of drought and signalling molecules, aerenchyma formation and gene expression were evaluated in 5-d-old wheat seedlings. Seeds were germinated on moist filter paper in Petri dishes in the dark at 23 °C for 3 d followed by 2 d in constant light. Three healthy seedlings of equal size were transferred onto two layers of filter paper in deep glass Petri dishes (height 6 cm × diameter 9 cm; As One Stock, Tokyo, Japan) containing 100 μM ACC (Sigma), 0.05% ethanol (Wako), 100 μM SA (Wako), 100 μM JA (Sigma), 100 μM abscisic acid (ABA) (Sigma), 1.0 mM H_2O_2 (Wako), or 100 μM of the NO donor sodium nitroprusside (Wako). Fresh water was used for control seedlings. For drought treatments, the seedlings were air-dried on paper towels for 6, 12, or 24 h. Experiments were maintained in a controlled phytotron chamber at 25–27 °C using a 16-h photoperiod with 55–75% relative humidity. For the root anatomy studies, seminal roots were treated with ACC, JA, H_2O_2, or NO as described above. For the gene expression studies, samples were frozen into liquid nitrogen and stored at −80 °C until use. For each treatment, three independent biological replicates were prepared.

Protein extraction and gel blot analysis

Proteins from the seminal roots of Bobwhite seedlings were extracted according to Haque et al. (2011). Gel blot analysis was performed according to Komatsu et al. (2011) with minor modifications. Briefly, protein samples were separated by 15% SDS-PAGE or 2-DE and then transferred onto a polyvinylidene difluoride membrane by using a semidry transfer blotter. The blotted membrane was blocked overnight at 4 °C in buffer containing 20 mM Tris–HCl (pH 7.5), 500 mM NaCl, and 5% non-fat milk (Skim milk; Difco). The membrane was subsequently incubated with an anti-rice PR-1 antibody (Rakwal and Komatsu, 2000) at 1:3000 dilution for 1 h at room temperature. Anti-rabbit IgG conjugated with horseradish peroxidase (Bio-Rad) served as the secondary antibody. After incubation for 1 h with the secondary antibody, the signals were detected by using an ECL Plus detection kit (GE Healthcare) following the manufacturer's protocol, and were visualized on X-ray film (Hyperfilm, GE Healthcare).

cDNA cloning and sequence analysis

An oligonucleotide primer set (*TaPR-1.2*-forward: 5′-ccatggcatc ttccaagagta-3′ and *TaPR-1.2*-reverse: 5′-gccggaatgtgtgcttattt-3′) was used to amplify the coding region of *PR-1.2* cDNA. Total RNA was extracted from the same roots used for proteomics. Primer set for *PR-1.2* corresponded to untranslated regions of expressed sequence tag (EST) clones CV777242. The amplified fragment was ligated into the pCR2.1-TOPO vector (Invitrogen) and its sequence was then determined by using a DNA sequencer (Applied Biosystems). Sequence data for this article have been deposited at GenBank under the accession numbers AB711115 (TaBWPR-1.2#2), and AB711116 (TaBWPR-1.2#13).

RNA extraction and qRT-PCR

Frozen root tissues (100–400 mg) were ground into fine powder in liquid nitrogen with a mortar and pestle. Total RNA was extracted by using an RNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA). All samples were treated with RNase-free DNase I (Qiagen). qRT-PCR was performed according to Nakamura et al. (2011). Briefly, 1 μg of total RNA isolated from each sample was reverse-transcribed to

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