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Molecular cloning of a BcPGIP cDNA from *Brassica campestris* and its expression to several stresses

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Abstract

Polygalacturonase-inhibiting proteins (PGIPs) play a prominent role as a first line host defense by preventing the polygalacturonases (PGs) activities of the pathogens. Even though PGIPs are regarded as a defensive protein family in plants the expressions of PGIPs are also versatile. Little information is available about the expression of PGIPs to the abiotic stresses. To investigate in response to abiotic stresses we have cloned a cDNA encoding polygalacturonase inhibitory protein (PGIP) by screening a *Brassica campestris* var. *pekinensis* (Chinese cabbage) cDNA library. The cDNA of BcPGIP revealed a 999-base pair (bp) open reading frame (ORF) that encoded a protein of 332 amino acids having 10 leucine-rich repeats (LRRs). A 9-bp 5′-untranslated region (UTR) and a 138-bp 3′-UTR were also contained in the ORF of the BcPGIP cDNA obtained. An estimated molecular mass of the cloned BcPGIP was 37.48 kDa and it had an isoelectric point (p*I*) of 9.26. The putative amino acid sequence of the BcPGIP showed 50–75% similarity to the extracellular PGIPs of other plants. Accumulation of the BcPGIP mRNA was observed in the cotyledons and to a lesser extent, it was also found in the primary roots and in 4-week-old stems while other tissues examined did not show any expression at the mRNA level. Mature leaves, however, had accumulated the transcripts of the BcPGIP gene by the treatment of jasmonic acid. Transcripts of the BcPGIP gene were also observed in the leaves under cold, salt or waterlogged conditions. As the BcPGIP gene was expressed not by salicylic acid but by jasmonic acid and it was also expressed by mechanical wounding as well as by other environmental stresses, it could be speculated that the BcPGIP gene was regulated via jasmonic acid dependent signaling pathway. The results suggest that BcPGIP may involve in plant response to different abiotic stresses.

Keywords: BcPGIP; *Brassica campestris* var. *pekinensis*; Molecular cloning; Stress; Tissue specificity; Jasmonic acid

1. Introduction

Plant resistance to diseases is dependent on cell wall fortification and extracellular activities. Polygalacturonase-inhibiting proteins (PGIPs) are a class of extracellular proteins in plants, which belong to a large family having the leucine-rich repeats (LRRs) and they act as a prominent defense reactive molecule [1]. As the PGIP gene expression shows the highest level at the epidermis by pathogen invasions, the PGIP is regarded as a first-line defense gene product in plants [2]. In the plant kingdom, the PGIPs are widespread and the activities of them have been confirmed in many plant species of diverse families [3]. The PGIP genes have been cloned from almost 25 dicotyledonous plants [4] and recently, from *Brassica napus*, rice and strawberries [5–7]. The major function of the PGIPs containing the LRRs is to inhibit fungal PG activity by the protein–protein interaction. It has also been reported that the expressions of some PGIP genes are regulated spatially and temporarily during developmental processes such as floral maturation [8,9] and in determining the floral organ number in rice [6] or they are also regulated in response to several abiotic stress stimuli [4,5,10,11]. Similar to other pathogen related (PR) genes, PGIP genes are also organized

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into multigene families and clustered in a specific chromosomal region [12–15]. All of the PGIPs examined and characterized show the similarity not only in LRR region but also at the outside region of the LRR domains between members within a family [4,16,17]. They also showed almost identical biochemical characteristics, but they have distinct specificity in some of the amino acids in their sequences and in their promoters of the gene [15,18]. The site specificities among the PGIP genes are different and the expression of them is regulated by different signal molecules through the separate signal transudation pathways [5,19]. It has been reported that different abiotic stresses may elicit different PGIPs to interact with PGs possibly by other signaling pathways [20]. PGIPs may be important for the general resistance to biotic and some abiotic stresses [5]. There are many reports to elucidate the function of the PGIP genes, but most of them are focused on the interaction between PGIPs and the fungal PGs and some of them are on the PGIP gene expression in the developmental processes. Very few reports, however, showed the relation between PGIP and abiotic stresses. It has been shown that *Brassica napus* and *Arabidopsis* PGIPs are regulated by some abiotic stresses [5,19]. However, other plant PGIPs or PGIP-like proteins have also been induced to cold temperature [10]. *Brassica campestris* var. *pekinensis* (Chinese cabbage) is the most important commercial vegetable in Korea and most of them are grown in cold temperature. It is necessary to find out a Chinese cabbage showing specifically higher PGIP activities and tolerant to cold or to abiotic stresses or is necessary to make a cold and other abiotic stresses resistance variety to support higher productivity even under cold or the other abiotic stress conditions. We have cloned some oxidative stress related genes from the Chinese cabbage and have examined their responses to oxidative and other abiotic stresses for the same purpose [21,22].

In this paper, we report the cloning of the BcPGIP cDNA by screening a *Brassica campestris* cDNA library and the result of its expression pattern at the mRNA level to different environmental stresses as well as to different signaling molecules to deduce the relationship between the BcPGIP gene expression under adverse plant conditions and the signal transduction pathways.

2. Materials and methods

2.1. Plant material and sample collection

To examine the mRNA expression of the BcPGIP gene in the different tissues by treatment of signaling molecules or abiotic stresses, *Brassica campestris* var. *pekinensis* (Chinese cabbage) was grown in a 22 °C day/18 °C night cycle within a controlled growth chamber under a 16 h photoperiod and under 350 $\mu E\ m^{-2}\ s^{-1}$ conditions. The tissue samples of cotyledons, hypocotyls, primary roots, leaves, stems and roots of 4- and 8-week-old plants were collected for use. After harvesting, the samples were frozen in liquid nitrogen and kept at −80 °C for RNA extraction.

2.2. Wounding and different stress treatments

The leaves of the plants were mutilated with the use of sterile forceps. Low temperature, salt, draught and water-logging treatments were carried out as environmental stresses. In the low temperature experiment, 4-week-old plants were kept at 5 °C for 3 days under the 16 h daylight conditions. Plants in pots were watered and leaves were sprayed with a NaCl (Shinyo Pure Chemicals Co. Ltd., Japan) solution (300 mM) for 3 days as salt treatment. Watering of the plants was withheld for 4 days and then the plants began to wilt. Samples were harvested from the wilted plants for the examination of draught. In order to study the effect of waterlogging, plants were waterlogged for 3 days. In all experiments of wounding and stresses, the samples were collected from 4-week-old plants at 6 h, 24 h and 72 h after the start of treatments. Same aged plants treated with jasmonic acid were used as positive controls and those without any treatment were used as negative controls. Leaves were selected as sample tissues, except for the tissue specific experiments. Harvested leaves were frozen immediately in liquid nitrogen and kept at −80 °C for mRNA extraction.

2.3. Treatments of different defense response activators

Leaves of 4-week-old intact plants were selected in order to examine the effect of exposure to different defense response activators or to signaling molecules. Defense response activators such as 100 μM jasmonic acid (Sigma, St. Louis), 50 mM salicylic acid (Sigma, St. Louis), 0.1% ethanol (Merck, Germany), 3 mM methyl viologen (Sigma, St. Louis) and 10 mM H_2O_2 (Aldrich Chemical Company Inc., USA) were sprayed at the aerial parts of the plants. After treatment with ethanol, the plants were covered with transparent plastic bags in order to prevent the evaporation of ethanol. The same aged plants treated with distilled water were used as a control. Leaves treated with defense response activators or signaling molecules were harvested at the 2 h, 4 h and 6 h after the treatments with those molecules.

2.4. Cloning of the PGIP gene

The BcPGIP cDNA was cloned by the screening of a *Brassica campestris* cDNA library. The cDNA library of *Brassica campestris* was kindly provided by Dr. Won-Il Jung of the Korea Advanced Institute of Science and Technology. The mRNA was extracted from 3-week-old *Brassica* plant leaves and double-strand cDNAs were synthesized by reverse transcription. The λZAPII vector (Stratagene) was used to entrap the synthesized cDNA in order to construct the *Brassica* cDNA library. The titer of the library constructed was 2.6×10^6 pfu/ml indicating that cDNAs