31. A cDNA Encoding a Polygalacturonase-inhibiting Protein (PGIP) Exhibits Tissue Specific and Abiotic Stress Induced Expression in Chinese Cabbage.

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배추에서의 PGIP cDNA에 의한 조직 특이성과 Abiotic 스트레스의 유도 발현

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<Introduction>

Plants have exhibited a various extracellular proteins to protect themselves from the fungal invasion. Among them, polygalacturonase-inhibiting proteins (PGIPs) have been considered as a first line defense gene product in plants by showing the highest level of expression at epidermis. PGIPs are a class of extracellular proteins in plants, which belong to the large family of the leucine-rich repeat (LRR) and they play as a prominent defense reactive molecule. Even though the major function of PGIPs is to inhibit the fungal PGs activity by the protein-protein interaction but regulation spatially and temporarily during developmental processes such as floral maturation and floral organ number or are regulation in response to several abiotic stress stimuli has also been reported. In this report, we have cloned a potential PGIP cDNA by screening a *Brassica campestris* cDNA library and examined its expression at the mRNA level to different tissues and to different abiotic stresses.

<Materials and Methods>

Brassica campestris var. *Pekinensis*(Chinese cabbage) was grown under a controlled growth chamber. Tissue samples of cotyledons, hypocotyls and primary roots from one week grown seedlings after seeding and those of leaves, stems and roots from four-week and eight-week old plants were collected for use. Mechanical wounding to the leaves of 4-week old plants was performed with the sterile forceps. Low temperature, salt, draught and waterlogging treatments were carried out as environmental stresses and treated with H_2O_2 and paraquat (PQ) was considered as other abiotic stresses. In all the stress experiments samples were collected at 2h, 4h, 6h, 24h or 72h duration after starting the treatments. The same age plants treated with Jasmonic acid were used as positive control and plants without any treatment were used as a negative control in all the experiments. Leaves were selected as sample tissue except tissue specificity experiment and harvested leaves were frozen immediately in liquid nitrogen and kept in -80℃ temperature for mRNA extraction.

The PGIP gene was cloned by screening of a *Brassica campestris* (Chinese cabbage) cDNA library. By using a forward (5'-GAC TTT ATA AGT CAG AAG AAT CTC-3') and a reverse primer sequences (5'-AAG AGG AGC ACC ACA CAA ACA CTT GTT GTG-3') a 558 bp DNA fragment was amplified from the cabbage cDNA through PCR and labeled with $^{\alpha\text{-}32}$P. The labeled PCR product was used as a probe to screen the *Brassica campestris* cDNA library. Seven positive clones were screened from the approximately 4,000,000 plaques and about a 1-kilobase full-length sequence insert was identified after two rounds of screening.

The sequence of the cloned cDNA was determined with the help of a sequence analyzing company named

Takara Bio Inc. The NCBI site was chosen for the database search and sequence similarity of the deduced amino acid sequence. A multiple sequence alignment was done with the ClustalW program. Signal peptide prediction and motif searches were performed using the SignalP V3.0 and the PROSITE programs, respectively.

Expression of PGIP to different stress treatments and at different tissues was analyzed by Northern blot analysis. The total RNA was purified from the frozen sample using the RNeasy Plant Mini Kit (Qiagen) and according to the manufacturer's instructions. The same PCR product (558 bp) was used as a probe. The Northern blot and hybridization was performed as a standard protocol.

<Results>

The full-length cDNA insert of the BcPGIP was longer than1 kb and it revealed a 999-base pair (bp) open reading frame (ORF) which encoded a protein with 332 amino acids. The obtained PGIP cDNA contained a 9-bp 5'-untransleted region (UTR) and a 138-bp 3'-UTR along with the ORF. N-terminal domain and the C-terminal domain flanked by the LRRs region. At the beginning of the N-terminal domain of the BcPGIP, a 22-amino acid sequence was identified to be a signal peptide (Fig. 1). Ten tandem flawed LRRs consisting of 22-25 amino acid residues in which leucine residues spaced irregularly were also detected in the open reading frame. An estimated molecular mass of the cloned BcPGIP was 37.48 and it had an isoelectric point (p*I*) of 9.26. The putative amino acid sequence of the BcPGIP showed 50 to 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 four-week old stems while other tissues examined did not show any expression at the mRNA level (Fig. 2). Transcripts of the BcPGIP gene were also observed under cold, salt or waterlogged conditions. However, no transcripts were found in dehydration, H_2O_2, or in paraquat (Fig. 3). This is the first report to show that the PGIP is expressed under waterlogged and salt conditions. The results suggest that PGIP not merely involved to inhibit the fungal PG but also might have a prominent role to different abiotic stresses.

Fig. 1. Multiple alignments of the deduced amino acid sequences of BcPGIP and other plant PGIPs from Arabidopsis thaliana AtPGIP1(Accession No. AAM65836), AtPGIP2(Accession No. AAF69828), Brassica napus BnPGIP1(Accession No. AAM 94868), BnPGIP2(Accession No. AAM94870), BnPGIP4 (Accession No. AAM95648). Features: alignment gap (-), identical amino acids (*), amino acids similar in their chemical properties (.), the signal peptide(box), N-glycosylation sites (underline), phosphorylation sites(shaded background), N-terminal domain and C-terminal-domain(upper line), LRR region(bold).

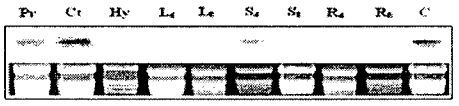

Fig. 2. Tissue-specific expression of the BcPGIP gene. Primary roots(Pr), cotyledons(Ct), hypocotyls (Hy), 4-week old leaves(L4), 8-week old leaves (L8), 4-week old stems(S4), 8-week old stems (S8), 4-week old roots (R4), 8-week old roots (R8) and positive control leaves treated with jasmonic acid (C) were collected from the plants and analyzed by Northern blot hybridization. Quantity of the total RNA(15g) loaded in each lane is shown in the bottom panel.

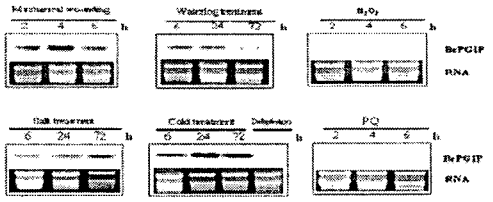

Fig. 3. The effects of mechanical wounding, waterlogging, H_2O_2, salt, low temperature, dehydration and PQ treatments on the BcPGIP gene induction. The induction of the BcPGIP was identified at the mRNA levels.