Isolation and characterization of the PgDOF transcription factor in *Platycodon grandiflorum*

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**Abstract**

DNA binding with one finger (Dof) domain proteins are plant-specific transcription factors that are encoded by a multi-gene family in higher plants. A member of this gene family, *PgDOF*, was cloned from *Platycodon grandiflorum*. *PgDOF* was 489 bp in length and encoded 162 amino acids. The predicted protein contained 52 amino acids showing homology to the Dof domain and a putative nuclear localization signal near the carboxyl terminus. Alignment of sequences revealed that the DOF domain of *PgDOF* showed a 100% match to that of the *Arabidopsis COG1* gene, which has been shown to negatively regulate phytochrome signaling. *PgDOF* was expressed highly in leaves and stems but at low levels in flowers and roots. In addition, *PgDOF* was shown to localize to the nucleus in transient expression assays in onion epidermal cells with the results confirmed in transgenic *Arabidopsis*.

**Keywords**: DOF domain; gene expression; *Platycodon grandiflorum*; transcription factor.

**Abbreviations**: DOF–DNA binding with one finger; DEPC–diethylpyrocarbonate; GFP–green fluorescent protein.

**Introduction**

Transcription factors are an important group of regulatory genes involved in almost all plant biological processes, including various developmental responses to environmental changes. DNA binding with one zinc finger (Dof) proteins are a group of plant-specific transcription factors with a single Cys2/Cys2 zinc finger motif (Yanagisawa, 1995). The highly conserved Dof domain is located in the N-terminal DOF region and recognizes an AAAG DNA-binding motif as the essential sequence element (Yanagisawa, 2002). Although the Dof domain is highly conserved, the protein sequence outside it is divergent and may be involved in the functional diversity of the gene family. *Platycodon grandiflorum*, or balloon flower, is a traditional and popular medicinal plant in Korea. The roots of *P. grandiflorum* have been used widely in traditional Chinese, Japanese, and Korean medicines as an antiphlogistic, antitussive, and expectorant agent. Extracts from *P. grandiflorum* have been used to treat a variety of conditions; in Korea, 4-year-old roots have been used to treat bronchitis, asthma, pulmonary tuberculosis, diabetes, and inflammatory diseases (Lee, 1973; Takagi and Lee, 1972). Recently, immunopharmacological research has identified triterpenoid (Nikaido et al., 1999) and saponin (Ishii et al., 1984) as important active compounds in *P. grandiflorum* roots. In this paper, we describe the isolation and characterization of *PgDOF* from *P. grandiflorum*. In addition, we investigated the nuclear localization of *PgDOF* using onion epidermal cells and transgenic *Arabidopsis*.

**Materials and methods**

**Plant material**

Seeds of *P. grandiflorum* were surface-sterilized with 70% (v/v) ethanol for 30 s and 4.5% (v/v) sodium hypochlorite solution for 10 min, then rinsed 3 times in sterilized water. The seeds were placed on agar-solidified culture medium, which consisted of MS medium solidified with 0.8% (w/v) agar. The seeds were germinated in a growth chamber at 25 °C under standard cool white fluorescent tubes for a 16-h photoperiod. *P. grandiflorum* was grown in a greenhouse at the experimental farm of Chungnam National University (Daejeon, Korea). Plants were collected and stored at −80 °C. Before the experiments, each *P. grandiflorum* organ (e.g., flowers, leaves, stems, roots) was ground with a mortar and pestle under liquid nitrogen.

**Total RNA isolation and cDNA synthesis**

Total RNA was extracted from 100 mg of powdered seedlings using TRIzol (Invitrogen, Carlsbad, CA). The RNA pellet was dissolved in diethylpyrocarbonate (DEPC)-treated water, and the quality and concentration of the RNA were then determined by agarose gel electrophoresis and spectrophotometry, respectively. Subsequently, 1 μg of total RNA was reverse transcribed using the ReverTra Ace-α-kit (Toyobo, Osaka, Japan). The resulting cDNA was used as the template for quantitative real-time PCR.

**Isolation of the cDNA encoding PgDOF**

Using a *P. grandiflorum* EST library of (http://pestas.kribb.re.kr/), primers were designed to amplify the full-length *PgDOF* cDNA (EPP157KIAA11C000018) (Table 1). The PCR products were cloned into a T-Blunt vector (SolGent, Daejeon, Korea) and then sequenced by the National Instrumentation Center for Environmental Management (NICEM, Seoul National University, Korea).
Table 1. Primers used in this study

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence (5′ to 3′)</th>
<th>Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>PgDof F</td>
<td>CACCATGGCCGACGTCCACAATGG</td>
<td>Full-length PCR</td>
</tr>
<tr>
<td>PgDof R</td>
<td>TTAACAACCTTTGACCACCTGG</td>
<td></td>
</tr>
<tr>
<td>PgDof F</td>
<td>GCTATTCGGAAAGACAATTACGG</td>
<td>Real-time PCR</td>
</tr>
<tr>
<td>PgDof R</td>
<td>TTGCAGAAGTGTCTTGTTGATT</td>
<td></td>
</tr>
<tr>
<td>PgActin F</td>
<td>CCATACAGTCCCCATTATGAAG</td>
<td></td>
</tr>
<tr>
<td>PgActin R</td>
<td>GCTAACCCTCCATGTCTCTCA</td>
<td></td>
</tr>
</tbody>
</table>

Fig 1. *PgDOF* encodes a Dof transcription factor protein. (a) The deduced amino acid sequence of the *PgDOF* gene. The highly conserved Dof domain is underlined, and a predicted nuclear localization signal (PVKRRRS) is shown in bold. The predicted NLS was identified by the WoLF PSORT software program (http://wolfpsort.org/). (b) Multiple amino acid sequence alignments of the DOF domains of *PgDOF* and other DOF proteins in other plants.

Quantitative real-time PCR

The gene expression level of *PgDOF* was analyzed using a MiniOpticon real-time PCR detection system (BioRad, Hercules, CA). Primers were designed from the full-length cDNA sequences using the Primer3 program (http://frodo.wi.mit.edu/primer3/Table 1). Real-time PCR was performed in a 20-µL reaction volume with 0.4 µM of each primer and 1× SYBR Green Real-time PCR Master Mix (Toyobo). The PCR protocol was as follows: 1 cycle of 5 min at 95 °C, 40 cycles with a denaturing time of 15 s at 95 °C, an annealing time of 15 s at 56 °C, and an elongation time of 20 sec at 72 °C. Quantitative real-time PCR experiments were performed in triplicate. In this study, the actin gene (GenBank accession number JF781303) was cloned from *P. grandiflorum* based on its high homology with other actins and was used as an internal reference.

Sequence Analysis

Homologous sequences of *PgDOF* were identified using BLAST (http://www.ncbi.nlm.nih.gov/BLAST). The sequence alignments were constructed using BioEdit Sequence Alignment Editor version 5.0.9 (Department of Microbiology, North Carolina State University, USA). Phylogenetic trees were constructed with the TreeTop-Phylogenetic Tree Prediction program (http://www.genebee.msu.su/services/phtree_reduced.html).

Subcellular localization of *PgDOF*

The construct, *PgDOF* fused with green fluorescent protein (GFP), was generated for transient expression in onion epidermal cells. The PCR primers used for this experiment is shown in Table 1. For recombination cloning into Gateway vectors (Invitrogen), the PCR products with a CACC sequence at the 5′ end were inserted into a pENTR/D-TOPO vector, as recommended by the manufacturer (Invitrogen), to create entry vectors. Then these vectors were transformed into the *E. coli* strain TOP10 (Invitrogen) and clones were selected with 50 mg·L⁻¹ kanamycin. The resultant vector was then recombined into a pK7WGF2 destination vector (obtained from the Functional Genomics unit of the Department of Plant System Biology; VIB-Ghent University) using Gateway® LR Clonase™ II plus enzyme mix (Invitrogen). Transient expression of GFP fusion proteins...
Results and discussion

Isolation and sequence analysis of PgDof from *P. grandiflorum*

RT-PCR generated a 961-bp fragment of *PgDOF* from the cDNA of a *P. grandiflorum* seedling EST as part of a *P. grandiflorum* seedling library (http://pestas.kribb.re.kr/). This fragment has a 489-bp open reading frame and encodes 162 amino acids (GenBank accession number, JF746194) (Fig. 1). Although Blast analysis showed that full-length of PgDOF shares only 60% identity and 60% similarity with other plant...
DOFs, the Dof domain of PgDOF showed a 96% identity and 97% similarity with other plant gene sequences. In particular, the DOF domain of PgDof showed a 100% match with the Dof domain in the Arabidopsis COG1 gene, which negatively regulates phytochrome signaling (Park et al., 2003).

**Expression levels of the Dof gene in different organs of P. grandiflorum**

PgDOF was expressed in all tested organs (Fig. 2). The highest expression level occurred in the leaves and stems, where its relative expression (RQ value) to the actin gene was 15.45 and 13.74, respectively. The lowest expression level occurred in flowers and roots (RQ = 5.11 and 5.13, respectively).

**PgDOF is localized in the nucleus**

The amino acid sequence of PgDOF contains a putative nuclear localization signal (PVKRRRS) near the carboxy terminus (Fig. 1a). We used the green fluorescent protein fusion construct GFP-PgDOF to determine the subcellular localization of PgDOF in onion epidermal cells. As shown in Fig. 3, GFP-PgDOF was most strongly detected in the nucleus. In particular, GFP-PgDOF was localized in the nucleoplasm and not in the nucleolus. The GFP-only construct did not exhibit any distinctive localization pattern.

To date, a number of Dof genes have been isolated from various plants, including 37 genes in Arabidopsis thaliana (Riechmann et al., 2002), 30 genes in Oryza sativa (Lijavetzky et al., 2003), and 28 genes in soybean (Wang et al., 2007). The presence of many Dof genes in 1 plant suggests that Dof genes play critical roles as transcriptional regulators in a number of biological processes. In Arabidopsis, DAG1 and DAG2 genes are involved in seed germination (Papi et al., 2002). Meanwhile, COG1 has been shown to be a negative regulator in both the phytocrome A and phytocrome B signaling pathways (Park, et al., 2003), whereas the CDF1 gene is involved in negatively regulating the expression of key genes in the photoperiodic pathway of flowering time control (Imaizumi et al., 2005). In addition, OBPI has been shown to function in the regulation of the cell cycle (Skiarycz et al., 2008), whereas Dof 5.1 regulates adaxial-abaxial polarity (Kim et al., 2010). In rice, OsDof3 plays a regulatory role in the expression of a peptide gene under the control of gibberellins in germinated cereal grains (Washio, 2001). Furthermore, Dof genes have been found to regulate endosperm-specific seed storage proteins in maize (Vicente-Carbajosa et al., 1997), barley, and wheat (Mena et al., 1998). The conserved DNA-binding domain of the Dof domain proteins shows no homology to animal transcription factors; therefore, this class of proteins represents plant-specific transcription factors (Yanagisawa 1995, 1996, Yanagisawa and Sheen 1998). Thirty-seven putative genes encoding Dof domain proteins have been identified in Arabidopsis (Yanagisawa, 2002). Among them, a dominant Dof mutant, c og1-D, was obtained from an activation-tagging pool derived from a long-hypocotyl phenotype in the light (Park, et al., 2003). Phenotypic analyses suggested that COG1 is a negative regulator in phytocrome signaling pathways. In this study, we found that PgDOF and AtCOG1 showed 95% identity in their amino acid sequences. Although we did not show direct evidence that PgDOF has the same function as AtCOG1, we anticipate this will be the function of PgDOF based on the result of alignment between these 2 genes. Moreover, PgDOF encodes a Dof transcription factor and is localized to the nucleus.

**Acknowledgements**

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**References**


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