Plant Omics Journal POJ

POJ 4(3):149-153 (2011) ISSN:1836-3644

Research Note

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

Nam Il Park, Pham Anh Tuan, Hui Xu, and Sang Un Park*

Department of Crop Science, College of Agriculture and Life Sciences, Chungnam National University, 79 Daehangno, Yuseong-gu, Daejeon, 305-764, Korea

∗Corresponding author: supark@cnu.ac.kr

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

	Name	Sequence (5′ to 3′)	Use
PgDof	PgDof F	CACCATGGCCGACGTCCACAATGG	Full-length PCR
	PgDof R	TTAACAACTTTGACCACTGG	
PgDof	PgDof_RT F	GCTATTCGGAAAGACAATTACGG	Real-time PCR
	PgDof_RT R	TTGCAGAAGTGTCTTGGTTGATT	
PgActin	PgActin_RT F	CCATACAGTCCCCATTTATGAAG	
	PgActin_RT R	GCTAACTTCTCCATGTCTCTCA	

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 $mg \cdot L^{-1}$ 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

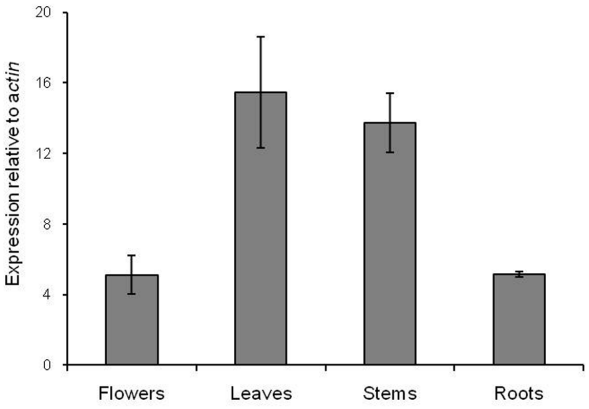

Fig 2. Expression levels of *PgDOF* mRNA transcripts relative to that of actin in different organs of *P. grandiflorum.* The values and error bars indicate the mean and standard error, respectively, from 3 independent measurements.

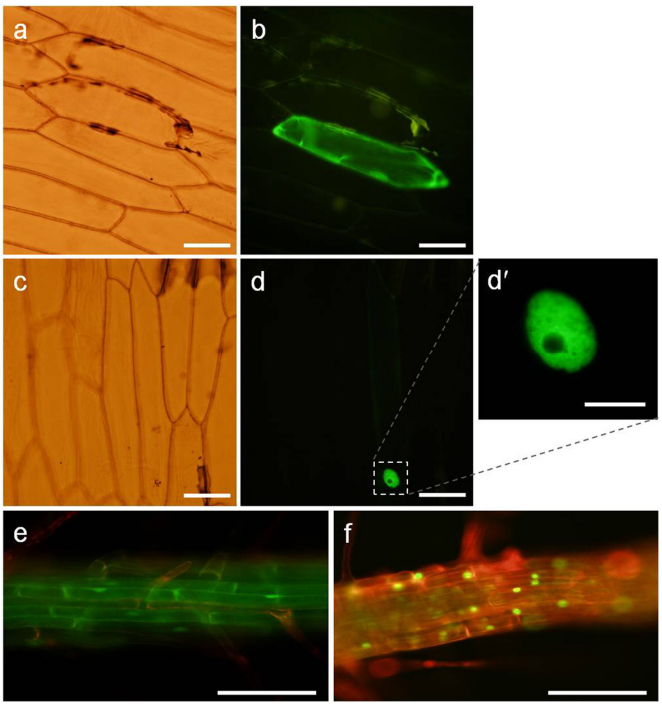

Fig 3. Nuclear localization of the *GFP-PgDOF* protein. GFP localization in onion epidermal cells expressing *GFP* alone (a, b) and *GFP:PgDOF* (c, d). A close-up view of the nucleus (d′) transformed with plasmids expressing *GFP:PgDOF*. GFP localization in GFP alone (e) and *GFP:PgDOF* (f) overexpressed in *Arabidopsis* roots. Propidium iodide-stained *Arabidopsis* roots cell wall. Bars: 100 µm (a–f), 20 µm (d′).

was obtained by biolistic bombardment into onion (*Allium cepa*) epidermal cells as described previously (Park and Muench, 2007). Briefly, 5 µg of DNA was mixed vigorously with tungsten particles in a solution containing 1 M $CaCl_2$ and 16 mM spermidine. The DNA-coated particles were washed with 70% and 95% ethanol, loaded onto macrocarriers, and bombarded into onion epidermal cells using a particle gun (PDS-1000; BioRad). GFP-PgDOF vector was transformed into Col-0 *Arabidopsis* by floral dipping using the *Agrobacterium tumefaciens* strain GV3101 (Clough and Bent, 1998). Peeled epidermal cell layers or GFP-PgDOF transgenic seedlings were mounted on microscope slides in MS medium, covered with a coverglass, and observed using a UPlanFL N 40× objective lens attached to a fluorescence microscope (BX51; Olympus, Tokyo, Japan). Images were captured using a DP71 microscope digital camera (Olympus).

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 carboxyl 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 phytochrome A and phytochrome 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, *OBP1* has been shown to function in the regulation of the cell cycle (Skirycz 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 peptidase 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, *cog1-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 negative regulator in phytochrome 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

This work was supported by the National Research Foundation of Korea Grant, which was funded by the Korean Government (Ministry of Education, Science and Technology (NRF-2010-355-F00013).

References

Clough SJ, Bent AF (1998) Floral dip: a simplified method for*Agrobacterium*-mediated transformation of *Arabidopsis thaliana*. Plant J 16:735-743

Imaizumi T, Schultz TF, Harmon FG, Ho LA, Kay SA (2005) FKF1 F-box protein mediates cyclic degradation of a repressor of CONSTANS in Arabidopsis. Science 309:293-297

Ishii H, Tori K, Tozyo T, Yoshimura Y (1984) Saponins from roots of *Platycodon grandiflorum*. Part 2. Isolation and structure of new triterpene glycosides. Journal of the Chemical Society, Perkin Transaction 1:661-668

Lee EB (1973) Pharmacological studies on *Platycodon grandiflorum* A. DC. IV. A comparison of experimental pharmacological effects of crude platycodin with clinical indications of Platycodi radix. Yakugaku Zasshi 93:1188-1194

Lijavetzky D, Carbonero P, Vicente-Carbajosa J (2003) Genome-wide comparative phylogenetic analysis of the rice and Arabidopsis Dof gene families. BMC Evol Biol 3:1-11

Mena M, Vicente-Carbajosa J, Schmidt RJ, Carbonero P (1998) An endosperm-specific DOF protein from barley, highly conserved in wheat, binds to and activates transcription from the prolamin-box of a native B-hordein promoter in barley endosperm. Plant J 16:53-62

Nikaido T, Koike K, Mitsunaga K, Saeki T (1999) Two new triterpenoid saponins from *Platycodon grandiflorum*. Chem Pharm Bull 47:903-904

Papi M, Sabatini S, Altamura MM, Hennig L, Schafer E, Costantino P, Vittorioso P (2002) Inactivation of the phloem-specific Dof zinc finger gene DAG1 affects response to light and integrity of the testa of Arabidopsis seeds. Plant Physiol 128:411-417

Park D, Lim P, Kim J, Cho D, Hong S, Nam H (2003) The Arabidopsis COG1 gene encodes a Dof domain transcription factor and negatively regulates phytochrome signaling. Plant J 34:161-171

Park NI, Muench D (2007) Biochemical and cellular characterization of the plant ortholog of PYM, a protein that interacts with the exon junction complex core proteins Mago and Y14. Planta 225:625-639

Riechmann JL, Heard J, Martin G, Reuber L, Jiang C, Keddie J, Adam L, Pineda O, Ratcliffe OJ, Samaha RR, Creelman R, Pilgrim M, Broun P, Zhang JZ, Ghandehari D, Sherman BK, Yu G (2002) Arabidopsis transcription factors: genome-wide comparative analysis among eukaryotes. Science 290:2105-2110

Skirycz A, Radziejwoski A, Busch W, Hannah MA, Czeszejko J, Kwaśniewski M, Zanor M-I, Lohmann JU, De Veylder L, Witt I, Mueller-Roeber B (2008) The DOF transcription factor OBP1 is involved in cell cycle regulation in *Arabidopsis thaliana*. Plant J 56:779-792

Takagi K, Lee EB (1972) Pharmacological studies on *Platycodon grandiflorum* A. DC. Activities of crude platycodin on respiratory and circulatory systems and its other pharmacological activities. Yakugaku Zasshi 92:969-973

Vicente-Carbajosa J, Moose SP, Parsons RL, Schmidt RJ (1997) A maize zinc-finger protein binds the prolamin box in zein gene promoters and interacts with the basic leucine zipper transcriptional activator Opaque2. Proc Natl Acad Sci USA 94:7685-7690

Wang HW, Zhang B, Hao YJ, Huang J, Tian AG, Liao Y, Zhang JS, Chen SY (2007) The soybean Dof-type transcription factor genes, GmDof4 and GmDof11, enhance lipid content in the seeds of transgenic Arabidopsis plants. Plant J 52:716-729

Washio K (2001) Identification of Dof proteins with implication in the gibberellin-regulated expression of a peptidase gene following the germination of rice grain. Biochim Biophys Acta-Gene Struct Expr 1520:54-62

Yanagisawa S (1995) A novel DNA-binding domain that may form a single zinc finger motif. Nucleic Acids Res 23:3403-3410

Yanagisawa S (2002) The Dof family of plant transcription factors. Trends Plant Sci 7:555-560