

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

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Objectives

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

- Total RNA Isolation and cDNA Synthesis
- Isolation of cDNA encoding *PgDOF*
- Plasmid Construction for Transformation of *Arabidopsis*
- Quantitative real-time PCR
- Subcellular localization of *PgDOF*

Results

The 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 *PgDOF* DOF domain 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*.

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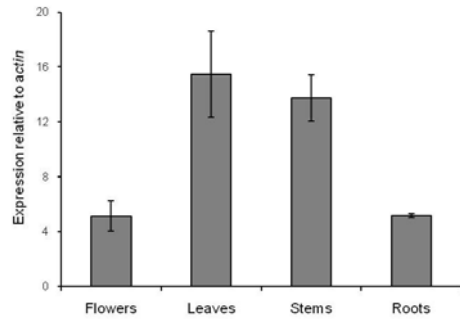


Fig. 1. *PgDOF* encodes a Dof transcription factor protein. (a) The deduced amino acid sequence of the *PgDOF* gene. The highly conserved Dof domain is underlined, and a predicted nuclear localization signal(PVKRRRS) is shown in bold. The predicted NLS was identified by the WoLFPSORT software program(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



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.

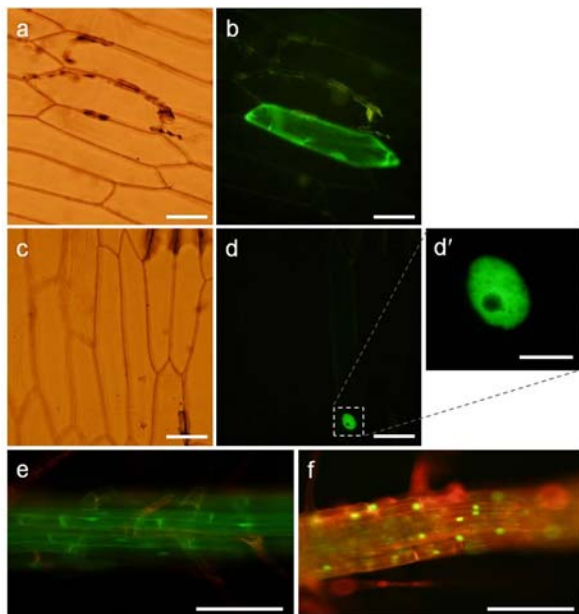


Fig. 3. Nuclear localization of the *GFP-PgDOF* protein. GFP localization in onion epidermal cells expressing *GFP* alone (a,b) and *GFP:PgDOF* (c,d). A close-up view of the nucleus (d') transformed with plasmids expressing *GFP:PgDOF*. GFP localization in *GFP* alone (e) and *GFP:PgDOF* (f) overexpressed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

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