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Full Length Research Paper

Molecular characterization and expression of *DgZFP1*, a gene encoding a single zinc finger protein in chrysanthemum

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A single zinc finger protein gene was isolated from chrysanthemum by rapid amplification of cDNA ends (RACE) approach and was designated as *DgZFP1*. The *DgZFP1* encodes a protein of 168 amino acids residues with a calculated molecular mass of 18.1 kDa and theoretical isoelectric point is 4.71. DgZFP1 contains one single zinc finger motif and one ethylene-responsive element-binding factor (ERF)-associated amphiphilic repression (EAR) domain. The transcripts of *DgZFP1* was enriched in nodes and ray petal than in disc petal, disc stamen, disc pistil and ray pistil, but not detected in other tissues. Subcellular localization revealed that DgZFP1 was preferentially distributed to nucleus. We argued that *DgZFP1* is a new member of the single zinc finger protein genes and it may be the ortholog of *LIF*.

Key words: Chrysanthemum, *DgZFP1*, gene expression, single zinc finger protein.

INTRODUCTION

Arabidopsis SUPERMAN (SUP) and its homologues, belonging to a subset of the TFIIIA-type zinc finger proteins (ZFPs) genes, encode proteins that share a single Cys_2/His_2-type zinc-finger binding amino acid sequence QALGGH (Takatsuji, 1999) and a class II ethylene responsive element-binding factor (ERF)-associcated amphiphiphilic repression (EAR) motif (Ohta et al., 2001). Some SUP family members paly important roles in flower organogenesis (Sakai et al., 1995; Nakagawa et al., 2004; Payne et al., 2004; Takeda et al., 2004; Satoh-Nagasawa et al., 2006), plant devlepment and morphogeny (Chrispeels et al., 2000; Dinkins et al., 2002, 2003; Ohno et al., 2004; Nakagawa et al., 2005; Vollbercht et al., 2005; Satoh-Nagasawa et al., 2006), telomerase activation (Ren et al., 2004) and the regulation of stress-responses (Jiang et al., 2008). Several members of the SUP family genes have also been implicated in the metabolism and/or signaling of phytohormones (Jiang et al., 2008). The overexpression of a single zinc-finger protein of petunia, designated Lateral shoot-Inducing Factor (LIF), in transgenic petunia, *Arabidopsis* and tobacco plants resulted in dramatic increase in lateral shoots (Nakagawa et al., 2005). Control of branching is of immense economic importance in horticulture and agriculture with respect to ornamental value, crop yield and other considerations (Nakagawa et al., 2005).

Chrysanthemum is one of the most famous ornamental species in the world. Cultivating standard type chrysanthemum as cut flower is characterized by one flower per stem mainly done by removing the auxiliary flower buds. But cultivars as potted chrysanthemum, is a trait of many lateral shoots by removing the tips. However removing the buds and the tips require much effort and time (Han et al., 2007). The development of proper branching cultivars can reduce manual labour requirements and subsequently decrease the cost of flower production (Han et al., 2007).

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Abbreviations: RACE, Rapid amplification of cDNA ends; **ERF,** ethylene-responsive element-binding factor; **EAR,** ERF-associated amphiphilic repression; **LIF,** lateral shoot-inducing factor; **SUP,** *Arabidopsis SUPERMAN*; ***DgZFP1,*** new zinc-finger gene; **UTR,** untranslated region; **ORF,** open reading frame; **RT-PCR,** reverse transcriptase- polymerase chain reaction.

Thus, molecular breeding was introduced as an alternative approach to introduce specific characteristics. In this study, we isolated and characterized a new zinc-finger gene, designated as *DgZFP1*, which is structurally related to *LIF* so that it will be useful for chrysanthemum breeding.

MATERIALS AND METHODS

Plant materials

Chrysanthemum [*Dendronthema* × *grandiform* (Ramat.) Kitamura] cv. Jinba seedlings are grown in greenhouse. When the plants had 7 - 8 expanded leaves, the mature leaves (fully expanded), roots, internodes, nodes and stem tips were collected separately. When the plants flowered, flowers mixture (including flower buds and mature flower), inflorescence bract, ray petal, ray pistil, disc petal, disc stamen and disc pistil were collected separately. All samples of plants were frozen immediately into liquid nitrogen and stored at -80℃ for RNA extraction.

Isolation of the *DgZFP1* gene

Nested PCR was performed to obtain a partial sequence of *DgZFP1* by using the first strand cDNA of chrysanthemum as a template. Two degenerate primers corresponding to the amino acid sequences QALGGH (5'-CA(A/G)GCI(T/C)TIGGIGGICA(C/T)-3') and C-terminal LDLELR (5'-A(G/A)IC(T/G)IA(G/A)(T/C)TCIA (G/A)(G/A)TC-3') were used for the first PCR and LDLELR primer and another primer corresponding to the LGGHMN sequence (5'-(C/T)TIGGIGGICA(C/T)ATGAA(C/T)) were used for the second PCR.

For 3' RACE, two gene-specific primers were designed (GSP1, 5'-ATGGATCCTGCAAGAGATCAA-3' and GSP2, 5'-GATGATAG TGTCAATATCG-3'). Primers for 5' RACE were: GSP3, 5'-TGCAAGAATCACTTGTAGACGAT-3' and GSP4 5'-CGATTCCG TGGTCCATGGCGTCAT-3'. The RACE reactions were performed according to the manufacturer's protocol (Invitrogen RACE cDNA amplification kit, USA). We obtained a single, full-length, cDNA sequence by combining the 5'-RACE fragment, cDNA fragment and 3'-RACE fragment. Finally, a pair of primers (F1, 5'-CCCTTCTCT AAGTCTCGCTCTCTCT-3' and F2 5'-AGACCATATATCTTAGG TCAAGAAG-3') was then designed from the putative 5' and 3' untranslated region (UTR) of the full-length cDNA sequence. The resultant DNA fragments and RACE products were gel purified and cloned into pMD18-T Vector (Takara) and sequenced (Invitrogen, Beijing).

RNA isolation and semi-quantitative RT-PCR assay

Total RNA from various chrysanthemum tissues was extracted using Trizol reagent (Mylab, Beijing) according to the manufacturer's instructions. The first strand cDNA was synthesized with 1 μg total RNA and 1 μl superscript II enzyme (Invitrogen, USA) according to the manufacturer's protocol. As a control, 18s rRNA gene (Genbank accession number: AF116239) was amplified from chrysanthemum various tissues. The primers used for detecting *DgZFP1* gene expression were: forward 5'-TAGTTATGATGGATCCTGC-3' and reverse 5'-AATACTCCGCCTCTTGCAGTAG-3'. The PCR was performed as follow: pre-denaturation at 94℃ for 5 min, followed by 38 cycles of 30 s at 94℃, 30 s at 59℃, 50 s at 72℃ for *DgZFP1*, 30 cycles for 18s rRNA and a final extension of 8 min at 72℃. The amplified products were resolved on a 1.2% agarose gel and then detected by agarose gel electrophoresis. All RT-PCR experiments were repeated at least three times.

Subcellular localization

The *DgZFP1* ORF were cloned into the SacI and EcoRI sites of the pSAT6-GFP-N1 vetor. This vetor contains a modified red-shifted (green fluorescent protein) GFP at EcoRI-NcoI sites. The DgZFP1-GFP construct was transformed into onion epidermal cells by particle bombardment as described earlier (Wang and Fang, 2002). The transient expression of the DgZFP1-GFP fusion protein was observed using under Confocal Microscopy.

RESULTS

Isolation of the *DgZFP1* Gene from chrysanthemum

According to the homologous regions of *SUP* and *LIF*, partial cDNA was isolated from nodes of chrysanthemum by using PCR method. The full-length cDNAs was obtained employing rapid amplification of 3' -cDNA end (3' -RACE) and 5' -cDNA end (5' -RACE) and were designated as *DgZFP1* (Genbank accession No. GQ988775). Sequence analysis showed that the *DgZFP1* cDNA was 687 bp in length, including a complete open reading frame of 504 bp flanking with a 5' -UTR of 52 bp and a 3'-UTR of 131 bp (Figure 1). The predicted protein of DgZFP1 comprises 168 amino acids with a calculated molecular mass of 18.1 kDa and its theoretical isoelectric point was 4.71.

As shown in Figure 2A, the deduced amino acid sequence of DgZFP1 included one conserved Cys_2/His_2-type zinc finger motifs in the N-terminal region and an EAR-box in C-terminus. The zinc finger motif contains the plant-specific QALGGH sequences which serves as a DNA-binding motif in petunia (Takatsuji and Matsumoto, 1996; Yoshioka et al., 2001). The EAR-box in the C-terminus functions as a transcription repression domain (Sugano et al., 2003; Sakamoto et al., 2004). Upon comparisons of the amino acid sequences between the DgZFP1 and the single zinc finger proteins from other species, it was found that DgZFP1 was specifically with higher identity to LIF in the zinc finger motif.

To investigate the evolutionary relationship among plant single zinc finger proteins, a phylogenetic tree was constructed using neighbor-joining method with the full-length amino acid residues. The results show that DgZFP1 and LIF were grouped into a cluster whereas SUPMAN, PhSUP1, REB and AtZFP10 formed another big cluster (Figure 2B).

Expression analysis of *DgZFP1*

The expression profiles of *DgZFP1* gene in various chrysanthemum tissues were investigated using semi-quantitative RT-PCR assay. Various chrysanthemum tissues were collected, respectively, as described in Materials and Methods. *DgZFP1* mRNA was detected at

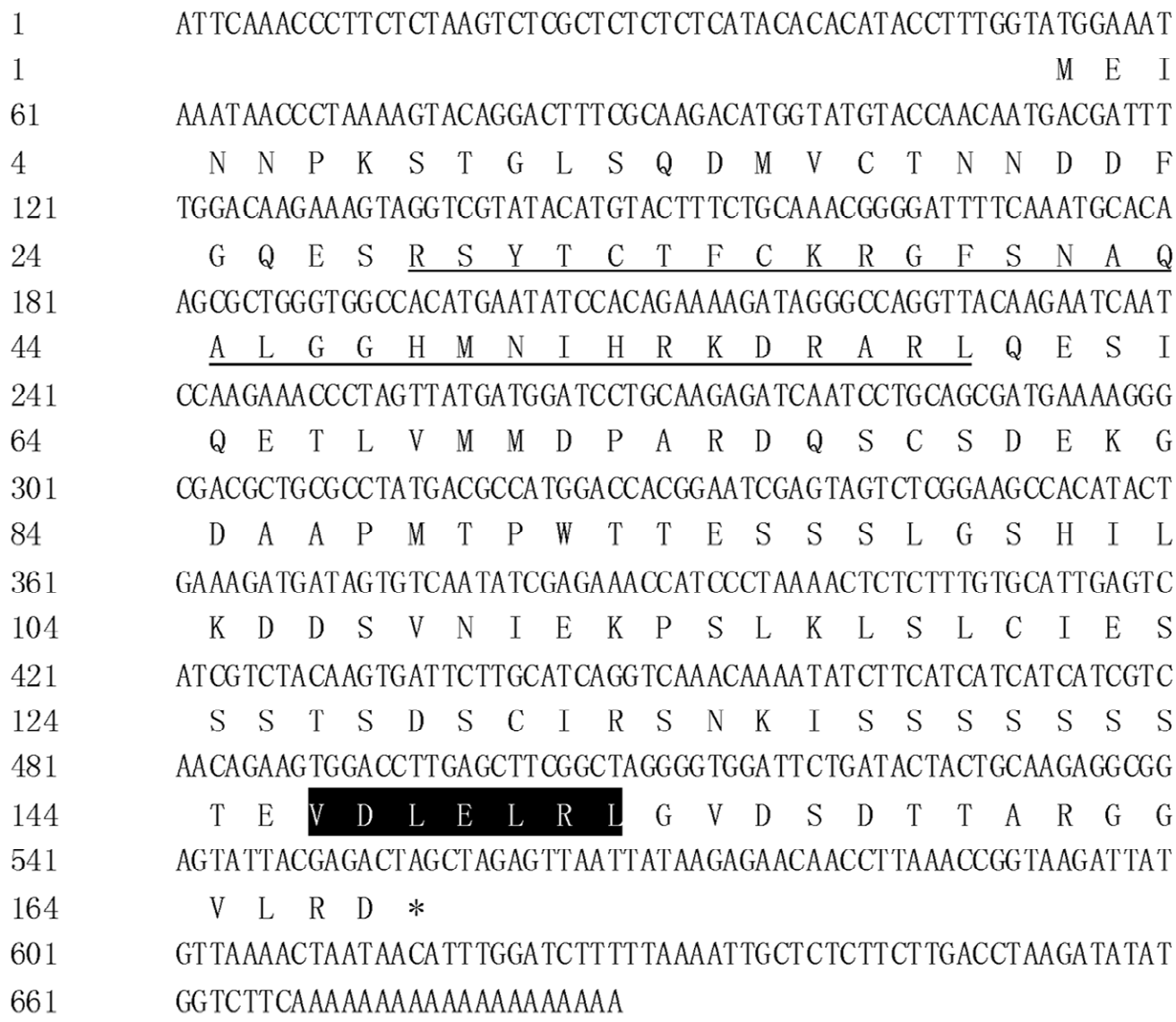

Figure 1. Nucleotide and deduced amino acid sequences of *DgZFP1* (GenBank accession no. GQ988775). The single zinc finger motif is underlined. The EAR-motif is highlighted with white letters in the black background.

vegetative nodes and flowers, but not in other tissues (Figure 3A). To further analyze *DgZFP1* expression in flowers, we prepared total RNA from inflorescence bract, ray petal, disc petal, disc stamen, disc pistil and ray pistil. The results suggested that *DgZFP1* mRNA were detected mainly in ray petal but weak in disc petal, disc stamen, disc pistil, ray pistil and not in inflorescence bract (Figure 3B).

Localization of *DgZFP1* in the nucleus

The presence of a single zinc-finger motif, which commonly serves as a DNA-binding domain, suggests that *DgZFP1* is a transcription factor. To examine subcellular localization of DgZFP1 protein, the DgZFP1-GFP fusion protein was introduced into onion epidermal cells by particle bombardment. As shown in Figure 4, the DgZFP1-GFP fusion protein was targeted into the nucleus, whereas the control GFP alone was distributed throughout the cytoplasm. These results showed that the DgZFP1 protein is a nuclear localization protein.

DISCUSSION

In this study, we isolated a single zinc finger protein gene *DgZFP1* from chrysanthemum. Sequence analysis showed that it contains one zinc finger motifs with a conserved QALGGGH sequence and a EAR-motif. The subcellular localization of the DgZFP1-GFP fusion protein in the nuclei implied the role of *DgZFP1* as a transcription

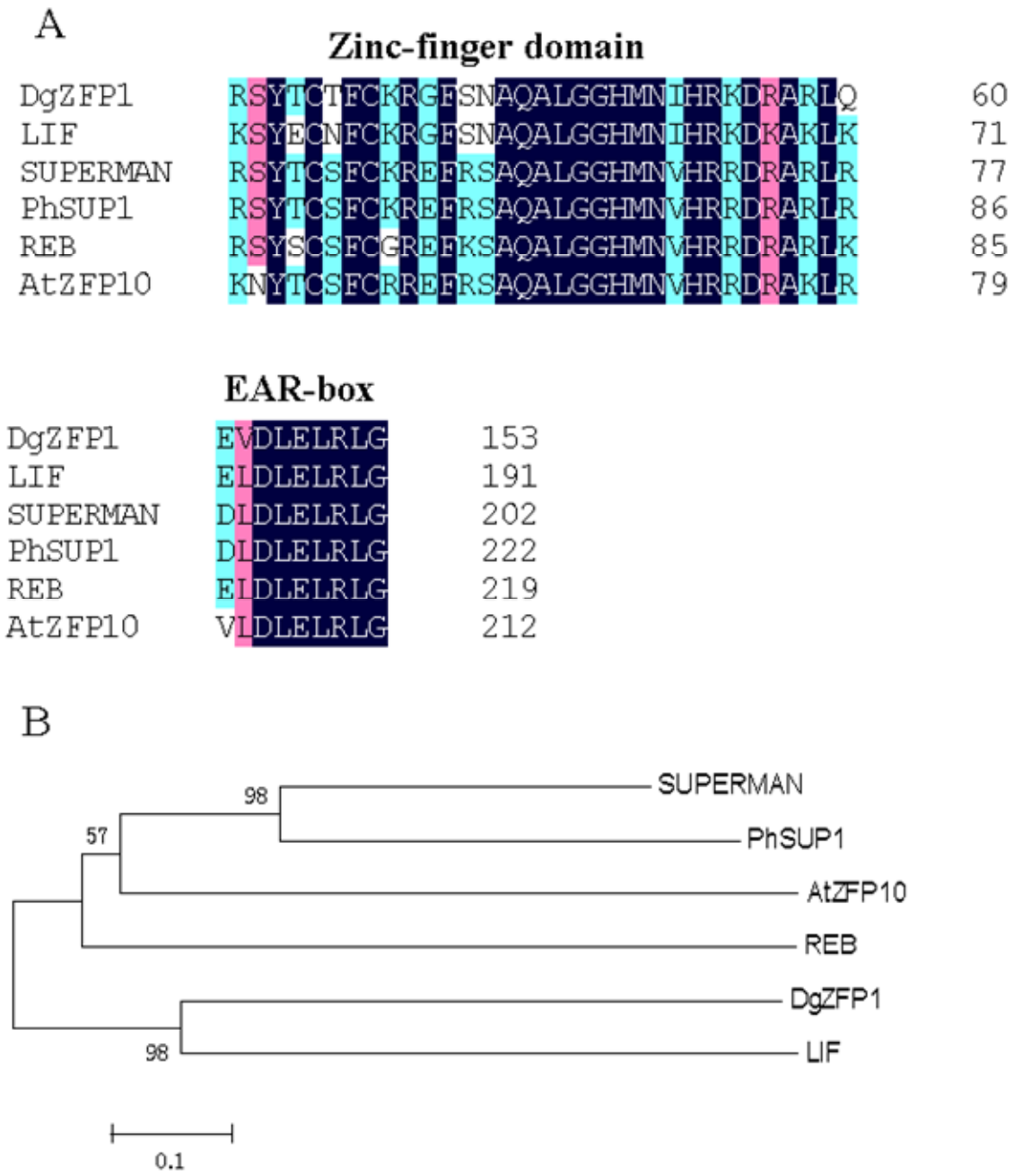

Figure 2. Comparison and phylogenetic relationship of DgZFP1 with reported single zinc finger proteins from other species. (A) Partial alignment of DgZFP1 with other plant single zinc finger proteins. Positions containing identical residues are shaded in navy blue, while conservative residues virility. (B) Phylogenetic tree analysis of DgZFP1 and other plant single zinc finger proteins. The tree was constructed by neighbor-joining method with MEGA program (ver 4.0). Branch numbers represent as percentage of bootstrap values in 1000 sampling replicates and scale indicates branch lengths. The accession numbers as follows: SUPERMAN (S60325), AtZFP10 (AAC23644), REB (BAC98433), LIF (BAB58897), PhSUP (BAD11142) and chrysanthemum DgZFP1 (GQ988775).

factor. These results demonstrate that *DgZFP1* is a novel single zinc finger transcription factor. The DgZFP1 was structurally similar with LIF in the zinc finger motif which was first isolated from petunia (Nakagawa et al., 2005). The sequence alignment and phylogenetic tree analysis both showed that DgZFP1 was more similar with LIF than any other reported plant single zinc finger protein.

DgZFP1 expression in chrysanthemum can be detected in vegetative nodes and flowers. However, *DgZFP1* mRNA was detected mainly in ray petal but weak in disc

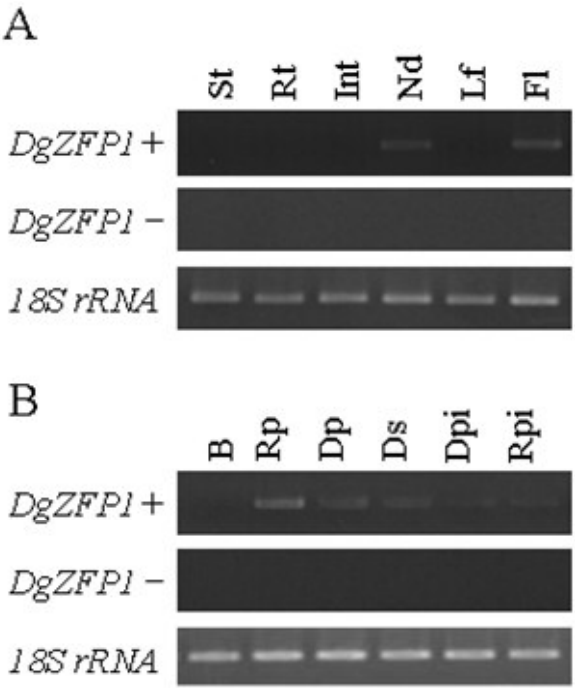

Figure 3. Expression patterns of *DgZFP1* in different organs. (A) Expression patterns of *DgZFP1* in stem tip (St), root (Rt), internode (Int), node (Nd), leaf (Lf) and flowers (Fl). Ethidium bromide staining of PCR products using *DgZFP1*-specific primers with (top) and without (middle) prior reverse transcription and the RT-PCR products with 18s rRNA-specific primers (bottom). (B) Expression patterns of *DgZFP1* in inflorescence bract (B), ray petal (Rp), disc petal (Dp), disc stamen (Ds), disc pistil (Dpi), ray pistil (Rpi). Ethidium bromide staining of PCR products using *DgZFP1*-specific primers with (top) and without (middle) prior reverse transcription and the RT-PCR products with 18s rRNA-specific primers (bottom).

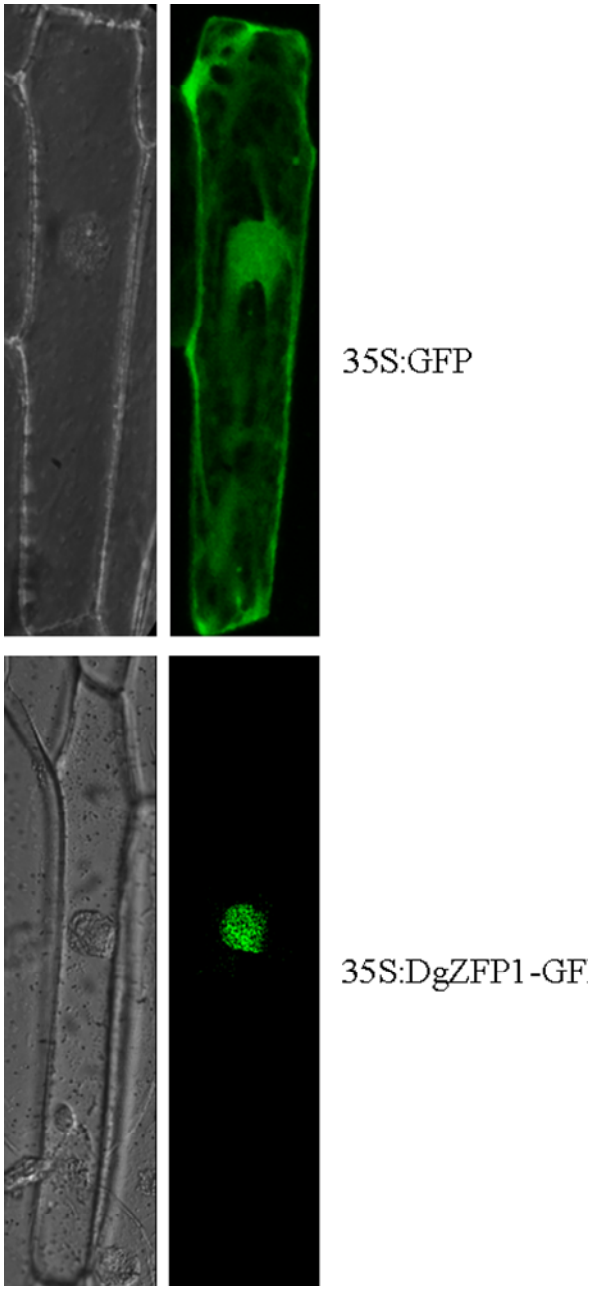

Figure 4. DgZFP1 localizes to the nuclear. Onion epidermal cells were transformed with 35S-GFP and 35S-DgZFP1-GFP. Transformed cells were observed in the bright light (left) or in the dark for green fluorescence microscopy (right) after incubation for 20 h.

petal, disc stamen, disc pistil, ray pistil and not in inflorescence bract. This expression pattern indicates that *DgZFP1* may function in normal program of vegetative nodes and flowers. In *LIF*, transcription can also be detected in vegetative nodes and flowers (Nakagawa et al., 2005). Comparison of this expression pattern indicated that *DgZFP1* was more similar to *LIF*.

Therefore, DgZFP1 is probably the ortholog of LIF protein and relative of other plant single zinc finger proteins, such as SUPERMAN. Transformation of the *DgZFP1* into plants and further analysis should reveal its possible functions in plant vegetative nodes and flowers.

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