

Full Length Research Paper

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

Liu Qinglin, Xu Kedong, Zeng Li, Ma Nan and Zhao Liangjun*

Department of Ornamental Horticulture and Landscape Architecture, China Agricultural University, 2 Yuanmingyuan West Road, Haidian District, Beijing 100193, P.R. China.

Accepted 12 March, 2010

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 Cys₂/His₂-type zinc-finger binding amino acid sequence QALGGH (Takatsuji, 1999) and a class II ethylene responsive element-binding factor (ERF)-associated amphiphilic repression (EAR) motif (Ohta et al., 2001). Some SUP family members play 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 development 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).

*Corresponding author. E-mail: zhaolj5073@sina.com. Tel: +86-10-62733315. Fax: +86-10-62733603.

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°C 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'-GATGATAGTGCAATATCG-3'). Primers for 5' RACE were: GSP3, 5'-TGCAAGAATCACTTGTAGACGAT-3' and GSP4 5'-CGATTCCGTGGTCCATGGCGTCAT-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'-CCCTTCTCTAAGTCTCGCTCTCT-3' and F2 5'-AGACCATATATCTTAGGTCAGAAG-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'-AATACTCCGCTCTTGCAAGTAG-3'. The PCR was performed as follow: pre-denaturation at 94°C for 5 min, followed by 38 cycles of 30 s at 94°C, 30 s at 59°C, 50 s at 72°C for *DgZFP1*, 30 cycles for 18s rRNA and a final extension of 8 min at 72°C. 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 vector. This vector 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 Cys₂/His₂-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

```

1      ATTCAAACCCCTTCTCTAAGTCTCGCTCTCTCTCATACACACATACCTTTGGTATGGAAAT
1                                          M E I
61     AAATAACCCCTAAAAGTACAGGACTTTCGCAAGACATGGTATGTACCAACAATGACGATTT
4      N N P K S T G L S Q D M V C T N N D D F
121    TGGACAAGAAAGTAGGTCGTATACATGTACTTTCTGCAAACGGGGATTTTCAAATGCACA
24     G Q E S R S Y T C T F C K R G F S N A Q
181    AGCGCTGGGTGGCCACATGAATATCCACAGAAAAGATAGGGCCAGGTTACAAGAATCAAT
44     A L G G H M N I H R K D R A R L Q E S I
241    CCAAGAAACCCTAGTTATGATGGATCCTGCAAGAGATCAATCCTGCAGCGATGAAAAGGG
64     Q E T L V M M D P A R D Q S C S D E K G
301    CGACGCTGCGCCTATGACGCCATGGACCACGGAATCGAGTAGTCTCGGAAGCCACATACT
84     D A A P M T P W T T E S S S L G S H I L
361    GAAAGATGATAGTGCAATATCGAGAAAACCATCCCTAAAACCTCTCTTTGTGCATTGAGTC
104    K D D S V N I E K P S L K L S L C I E S
421    ATCGTCTACAAGTGATTCTTGCATCAGGTCAAACAAAATATCTTCATCATCATCATCGTC
124    S S T S D S C I R S N K I S S S S S S S
481    AACAGAAGTGACCTTGAGCTTCGGCTAGGGGTGGATTCTGATACTACTGCAAGAGGCGG
144    T E V D L E L R L G V D S D T T A R G G
541    AGTATTACGAGACTAGCTAGAGTTAATTAAGAGAACAACCTTAAACCGGTAAGATTAT
164    V L R D *
601    GTTAAACTAATAACATTTGGATCTTTTAAAATGCTCTCTTCTTGACCTAAGATATAT
661    GGTCTTCAAAAAAAAAAAAAAAAAAAAAA

```

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

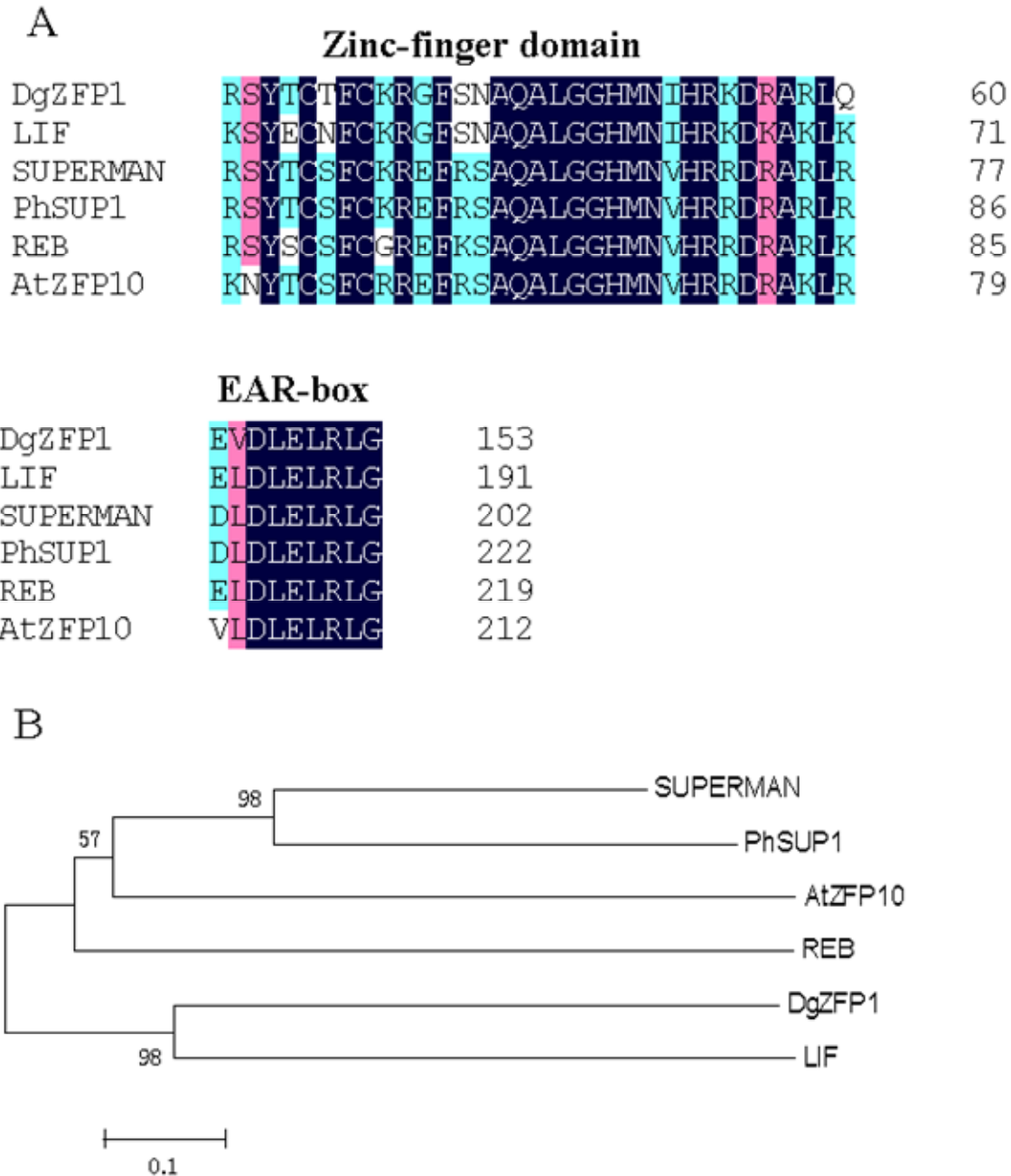


Figure 2. Comparison and phylogenetic relationship of DgZFP1 with reported single zinc finger proteins from other species. (A) Partial alignment of DgZFP1 with other plant single zinc finger proteins. Positions containing identical residues are shaded in navy blue, while conservative residues virility. (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

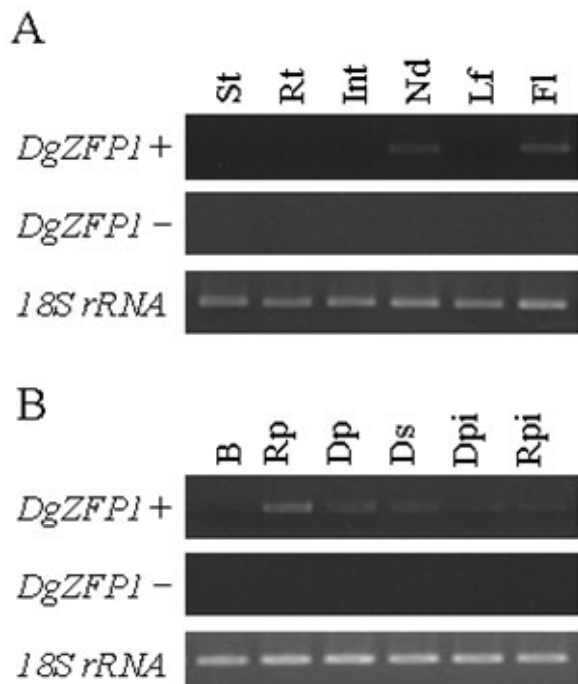


Figure 3. Expression patterns of *DgZFP1* in different organs. (A) Expression patterns of *DgZFP1* in stem tip (St), root (Rt), internode (Int), node (Nd), leaf (Lf) and flowers (Fl). Ethidium bromide staining of PCR products using *DgZFP1*-specific primers with (top) and without (middle) prior reverse 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).

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.

ACKNOWLEDGEMENTS

This research was supported by the funds of the 863 Program (2006AA100109), the Program of Ministry of Agriculture (No.2008-G3), and China Agricultural University scientific fund to graduate research and

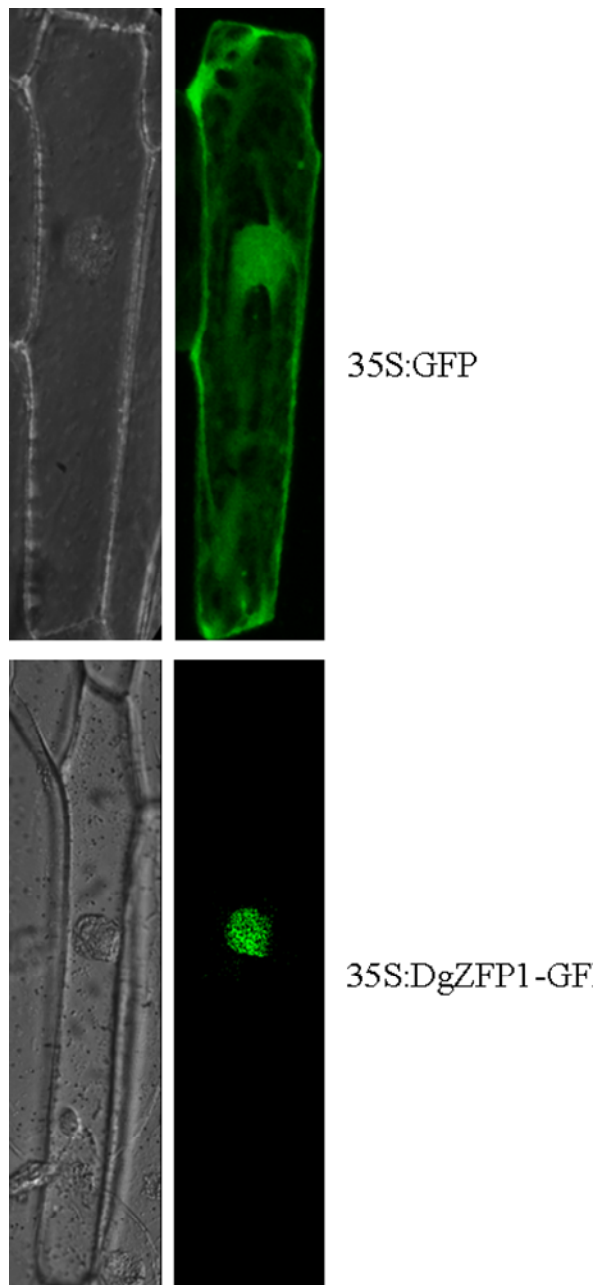


Figure 4. *DgZFP1* localizes to the nuclear. Onion epidermal cells were transformed with 35S-GFP and 35S-*DgZFP1*-GFP. Transformed cells were observed in the bright light (left) or in the dark for green fluorescence microscopy (right) after incubation for 20 h.

innovation projects (15059201).

REFERENCES

- Chrispeels HE, Oettinger H, Janvier N, Tague BW (2000). *AtZFP1*, encoding *Arabidopsis thaliana* C₂H₂ zinc-finger protein 1, is expressed downstream of photomorphogenic activation. *Plant Mol. Biol.* 42: 279-290.

- Dinkins R, Pflipsen C, Thompson A, Collins GB (2002). Ectopic expression of an *Arabidopsis* single zinc finger gene in tobacco results in dwarf plants. *Plant Cell Physiol.* 43: 743-750.
- Dinkins R, Pflipsen C, Collins GB (2003). Expression and deletion analysis of an *Arabidopsis* SUPERMAN-like zinc finger gene. *Plant Sci.* 165: 33-41.
- Han BH, Suh EJ, Lee SY, Shin HK, Lim YP (2007). Selection of non-branching lines induced by introducing *Ls*-like cDNA into *Chrysanthemum* (*Dendronthema grandifolium* (Ramat.) Kitamura) Shuho-no-chikara. *Sci. Hortic.* 115: 70-75.
- Jiang CJ, Aono M, Tamaoki M, Maeda S, Sugano S, Mori M, Takatsuji H (2008). SAZ, a new SUPERMAN-like protein, negatively regulates a subset of ABA-responsive genes in *Arabidopsis*. *Mol. Genet. Genomics*, 279: 183-192.
- Nakagawa H, Ferrario S, Angenent GC, Kobayashi A, Takatsuji H (2004). The petunia ortholog of *Arabidopsis* SUPERMAN plays a distinct role in floral organ morphogenesis. *Plant Cell*, 16: 920-932.
- Nakagawa H, Jiang CJ, Sakakibara H, Kojima M, Honda I, Ajisaka H, Nishijima T, Koshioka M, Homma T, Mander LN, Takatsuji H (2005). Overexpression of petunia zinc-finger gene alters cytokinin metabolism and plant forms. *Plant J.* 41: 512-523.
- Ohno CK, Reddy GV, Heisler MG, Meyerowitz EM (2004). The *Arabidopsis* JAGGED gene encodes a zinc-finger protein that promotes leaf tissue development. *Development*, 131: 1111-1122.
- Ohta M, Matsui K, Hiratsu K, Shinshi H, Ohme-Takagi M (2001). Repression domains of class II ERF transcriptional repressors share an essential motif for active repression. *Plant Cell*, 13: 1959-1968.
- Payne T, Johnson SD, Koltunow AM (2004). KNUCKLES (*KNU*) encodes a C₂H₂ zinc-finger protein that regulates development of basal pattern elements of the *Arabidopsis* gynoecium. *Development*, 131: 3737-3749.
- Ren S, Johnston JS, Shippen DE, McKnight TD (2004). *TELOMERASE ACTIVATOR1* induces telomerase activity and potentiates responses to auxin in *Arabidopsis*. *Plant Cell*, 16: 2910-2922.
- Sakai H, Medrano LJ, Meyerowitz EM (1995). Role of SUPERMAN in maintaining *Arabidopsis* floral whorl boundaries. *Nature*, 378: 199-203.
- Sakamoto H, Maruyama K, Sakuma Y, Meshi T, Iwabuchi M, Shinozaki K, Yamaguchi-Shinozaki K (2004). *Arabidopsis* Cys₂/His₂-type zinc-finger proteins function as transcription repressors under drought, cold and high-salinity stress conditions. *Plant Physiol.* 136: 2734-2746.
- Satoh-Nagasawa N, Nagasawa N, Malcomber S, Sakai H, Jackson D (2006). A trehalose metabolic enzyme controls inflorescence architecture in maize. *Nature*, 441: 227-230.
- Sugano S, Kaminaka H, Rybka Z, Catala R, Salinas J, Matsui K, Ohme-Takagi M, Takatsuji H (2003). Stress-responsive zinc finger gene *ZPT2-3* plays a role in drought tolerance in petunia. *Plant J.* 36: 830-841.
- Takatsuji H (1999). Zinc-finger proteins: the classical zinc finger emerges in contemporary plant science. *Plant Mol. Biol.* 277: 1073-1078.
- Takatsuji H, Matsumoto T (1996). Target-sequence recognition by separate Cys₂/His₂-type zinc-finger proteins in plants. *J. Biol. Chem.* 271: 23368-23373.
- Takeda S, Matsumoto N, Okada K (2004). *RABBIT EARS*, encoding a SUPERMAN-like zinc-finger protein regulates petal development in *Arabidopsis thaliana*. *Development*, 131: 425-434.
- Vollbercht E, Springer PS, Goh L, Buckler EST, Martienssen R (2005). Architecture of floral branch systems in maize and related grasses. *Nature*, 436: 119-1126.
- Wang GL, Fang HY (2002). *Gene engineering in plant*; 2nd edition. Press of Science, Beijing, pp. 734-736.
- Yoshioka K, Fukushima S, Yamazaki T, Yoshida M, Takatsuji H (2001). The plant zinc-finger protein *ZPT2-2* has a unique mode of DNA interaction. *J. Biol. Chem.* 276: 35802-35807.