

Cloning and analysis of *DIERF23* gene in flower induction

Xuelian SANG, Ci REN, Dengwei JUE*

Chongqing University of Arts and Sciences, Chongqing Key Laboratory of Economic Plant Biotechnology, Collaborative Innovation Center of Special Plant Industry in Chongqing, Chongqing Engineering Research Center for Special Plant Seedling, Institute of Special Plants, Yongchuan 402160, China; anyue1220@126.com; 962231430@qq.com; juedengwei@126.com (*corresponding author)

Abstract

Irregular flowering is a serious problem in longan production. Identifying the flower induction-related genes and analyzing their regulation mechanism is the key to solve this problem. The APETALA2/ethylene responsive factor (AP2/ERF) superfamily members are transcription factors (TFs) that regulate diverse developmental processes, including flowering time, and stress responses in plants. However, there is still no research about AP2/ERF involved in the regulation of longan flower induction. In the present study, a AP2/ERF TF member *DIERF23* was cloned from longan (*Dimocarpus longan*). It has a typical AP2 domain with the coding sequence (CDS) of *DIERF23* is 552 bp in length and encodes 184 amino acids. The molecular weight of *DIERF23* protein was 20.41 kDa and the theoretical isoelectric point (PI) was 7.69. The amino acid sequence of *DIERF23* protein had the highest similarity with CsERF23 (XP_006478313.1) of *Citrus sinensis* and CcERF23 (XP_006441807.2) of *Citrus clementina*. The results of qRT-PCR showed that the relative expression level of *DIERF23* gene in pericarp was higher, followed by stem, leaf, flower and flower bud. Meanwhile, *DIERF23* gene significant down-regulated in the early stage of flower induction in 'Sijimi' (SJ) longan and up-regulated in the late stage of flower induction in 'Shixia' (SX). The results of transient expression of *Arabidopsis* protoplasts showed that the fluorescence signal was mainly concentrated in the nucleus. Moreover, overexpression of *DIERF23* in *Arabidopsis* promoted early flowering. These results provide useful information for revealing the biological roles of *DIERF23* in longan and increase our understanding of the AP2/ERF superfamily members in fruit trees.

Keywords: *DIERF23*; flowering; longan; transgenic *Arabidopsis*

Introduction

The AP2/ERF superfamily is the largest and one of the most important transcription factors (TFs) family which plays important roles in plant growth, development, fruit ripening and biotic and abiotic stress responses in plants (Jiang *et al.*, 2022). AP2/ERF superfamily was early confirmed in *Arabidopsis thaliana* with 147 members (Feng *et al.*, 2005). Then, AP2/ERF family was gradually identified in diverse species, such as rice (Nakano *et al.*, 2006), banana (Lakhwani *et al.*, 2016), cotton (Liu *et al.*, 2017), pear (Li *et al.*, 2018), pineapple (Zhang *et al.*, 2021), and *Actinidia eriantha* (Jiang *et al.*, 2022). Based on the number of AP2 domain and the

Received: 25 Mar 2023. Received in revised form: 16 Apr 2023. Accepted: 08 May 2023. Published online: 20 Jun 2023.

From Volume 49, Issue 1, 2021, Notulae Botanicae Horti Agrobotanici Cluj-Napoca journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

specific B3 DNA-binding domain, AP2/ERF family can be divided into five subfamilies, APETELA2 (AP2), ABI3/VP1 (RAV) containing both of AP2 and B3 domain, DREB (dehydration-responsive element-binding protein), ERF (ethylene-responsive factor) subfamily and other proteins (Soloist). Additionally, based on the sequence similarity of their AP2 domain, DREB subfamily can be further grouped into A1 to A6, and ERF subfamily can be grouped into B1- B6 as well (Sakuma *et al.*, 2002).

Generally, AP2/ERF family proteins play important roles in the response to external biological and environmental stresses, including water deficit, drought (Dossa *et al.*, 2016), high salinity (Zhang *et al.*, 2009), low and high temperatures (Dubouzet *et al.*, 2003; Ito *et al.*, 2006), hormone response (Zarei *et al.*, 2011), and disease response (Zhang *et al.*, 2009). Additionally, previous studies also showed that AP2 family proteins have important functions in the regulation of developmental processes, such as seed development (Jofuku *et al.*, 2005), and embryo development (Boutilier *et al.*, 2002). There are plenty of evidences that AP2/ERF TFs cooperated other floral meristem genes to regulate floral organ development or flowering (Irish *et al.*, 1990; Bowman *et al.*, 1993; Zeng *et al.*, 2021). For instance, during the flower development of *Dendrobium officinale*, the expressions of two *DoAP2* genes (*DoAP2-8* and *DoAP2-10*) were down-regulated and another two *AP2* genes (*DoAP2-2* and *DoAP2-3*) were up-related (Zeng *et al.*, 2021). TOE1, one of the AP2-like TFs, could control flowering by regulating *FT* expression (Du *et al.*, 2020). Overexpression of *miR172*, whose target is *AP2*-like gene, resulted in the double flower phenotype in roses (François *et al.*, 2018).

Longan (*Dimocarpus longan*) is an important tropical/subtropical evergreen fruit tree which is widely cultivated in Southeast Asia, especially in China. Obtaining a stable annual fruit yield is the key factor that affecting the healthy development of longan industry (Jue *et al.*, 2019). However, the adverse environmental conditions, such as spring frost and high temperature and moisture could lead to irregular flowering of longan and cause an unstable production (You *et al.*, 2012). Therefore, the key to solve this problem is to identify the flower induction-related genes and analyze their regulation mechanism. Previous study has identified 125 longan AP2/ERF superfamily members in longan, and found some AP2/ERF members regulated early somatic embryogenesis and developmental processes in longan seed, root, and flower, and responded to exogenous hormones (Zhang *et al.*, 2020). However, the detailed functional analysis for AP2/ERF superfamily members involving in the regulation of longan flower induction is still limited. In this work, *DlERF23* was isolated and its potential function was investigated by overexpressing in transgenic *Arabidopsis* plants. Our findings indicated that *DlERF23* plays important roles in the flowering induction of longan.

Materials and Methods

Plant material

The longan cultivars of ‘SJ’ and ‘SX’, which display opposite flowering phenotypes, were grown in an experimental orchard located in Mazhang district (110°16’ E, 21°10’ N), Zhanjiang, Guangdong Province, P. R. China. Three different developmental flower bud samples were obtained during different periods from November in 2016 to February in 2017: the dormant apical bud period (T1), the floral primordia differentiation period (red bud) (T2), and the floral organ formation period (T3). Three biological replicates from three different trees were used for each sample. Nine ‘SJ’ longan tissues, including flower, flower bud, leaf, pericarp, pulp, root, seed, stem, and young fruit (the fruit that 60 days after flowering) which were collected from November in 2016 to April in 2017 were used for tissue expression analysis. All samples were frozen immediately in liquid nitrogen and stored at -80 °C.

RNA extraction, qRT-PCR and DIERF23 gene cloning

Total RNA from different longan tissues was extracted by using the plant RNA extraction Kit (Huangyueyang, Beijing, China) according to the manufacturer's instructions, and the quality of the RNA were detected as described of our previous study (Jue *et al.*, 2019). The cDNA synthesis was performed with the PrimeScript First-Strand cDNA synthesis Kit (TaKaRa, Bio, China). The base sequence and amino acid sequence information of the *DIERF23* gene (Dlo_015669.1) were obtained from the longan genome database (NCBI Sequence Read Archive, SRA315202) (Lin *et al.*, 2017). Primer Premier 5.0 was used to design the primers ERF23-S and ERF23-A for *DIERF23* gene cloning based on the ORF sequence of the *DIERF23* gene. LightCycler® 480 Real-Time PCR System (Roche, Germany) and SYBR Green II PCR Master Mix (Takara, Dalian, China) were used for qRT-PCR experiment. The amplification program was performed as described in our previous study (Jue *et al.*, 2018). The longan *Actin1* gene (Dlo_028674) was used as an internal control for normalization. The gene-specific primers used in this study are listed in Table S1. The relative expression levels of the candidate genes were calculated by the $2^{-\Delta\Delta C_t}$ method. The analysis included cDNA from the three biological samples for each tissue, and all the reactions were run in triplicates.

Sequence alignment and bioinformatic analysis

The BioXM 2.6 software (<http://cbi.njau.edu.cn/BioXM/>) was used to calculate the MW, the number of amino acids, the ORF, ORF length, and isoelectric point (pI) of DIERF23 protein. The domain of DIERF23 protein was predicted by using Simple Modular Architecture Research Tool (SMART; <http://smart.emblheidelberg.de/>). Multiple sequence alignment of the DIERF23 protein was performed by using Clustal X version 1.83. Based on this alignment, a phylogenetic tree of DIERF23 was constructed by using the neighbor-joining (NJ) method with MEGA 11, with 1000 bootstrap replicates (Tamura *et al.*, 2021).

Subcellular localization analysis of DIERF23

The full coding sequence (CDS) of *DIERF23* gene without the termination codon was amplified using primers ProDIERF23-S and ProDIERF23-A (Table S1). The amplification conditions were: pre-denaturation at 94 °C for 5 min; denaturation at 94 °C for 30 seconds, annealing at 60 °C for 30 seconds, extension at 72 °C for 40 seconds, 35 cycles (denaturation and extension); extend at 72 °C for 10 min and store at 4 °C. Plasmids pBWA(V)HS-osgfp (negative) and pBWA(V)HS-DIERF23-osgfp were constructed and introduction into *Agrobacterium tumefaciens* strain GV3101, separately. Then, the pBWA(V)HS-osgfp vector and pBWA(V)HS-DIERF23-osgfp vector were transfected into *Arabidopsis thaliana* protoplasts by PEG mediated method (Yoo *et al.*, 2007). After dark incubation at 28 °C for 24-48 h, fluorescence signals in protoplasts were examined by using the Olympus FluoView FV1000 confocal microscope software (Olympus, Tokyo, Japan).

Transformation of DIERF23 into Arabidopsis and phenotype analysis

The full-length CDS of *DIERF23* was cloned and inserted into the BamHI and SacI sites in pBI121 under the control of the CaMV35S promoter to construct the overexpression vector. Then the pBI121-DIERF23 and pBI121 constructs were introduced into *Agrobacterium strain* GV3101 for *Arabidopsis* transformation using the floral dip method (Clough *et al.*, 2005). Ten transgenic plants overexpressing *DIERF23* were obtained. Seeds were collected and sown on Murashige and Skoog (MS) agar medium containing 25 $\mu\text{g}\cdot\text{mL}^{-1}$ hygromycin for selection. Two T3 generation homozygous lines (OE6 and 9) were used for further functional analysis. The *Arabidopsis* ecotype Columbia (Col-0) plants used for transformation were maintained in our laboratory. The flowering time and number of rosette leaves of the transgenic, WT, and empty vector-transformed *Arabidopsis* plants (which were used as controls) were recorded or measured under long-day (LD) conditions.

Results

Isolation and sequence analysis of DIERF23 in longan

By using 'SJ' leaves cDNA as the template and ERF23-S/ERF23-A as the specific primers, a PCR fragment with a size about 500bp was amplified (Figure 1). After sequencing, we found the nucleotide similarity between *DIERF23* isolated from 'SJ' and *DIERF23* sequence (dlo_015501.2) of 'SX' longan is 99.27%. There are three different bases: the base at position 171 changes from C to G; the base at position 303 changes from C to A; the base at position 361 changes from A to C; the base at position 378 changes from C to T (Figure 2A). Meanwhile, there is one different amino acid: the amino acid at position 121 changes from K (lysine) to Q (glutamine) (Figure 2B).

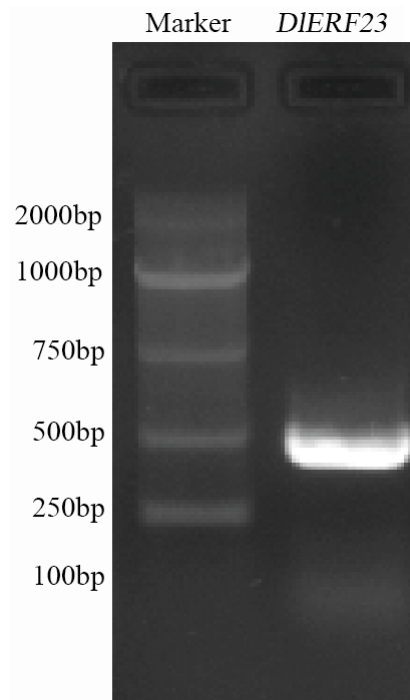


Figure 1. PCR amplification of *DIERF23* gene in longan

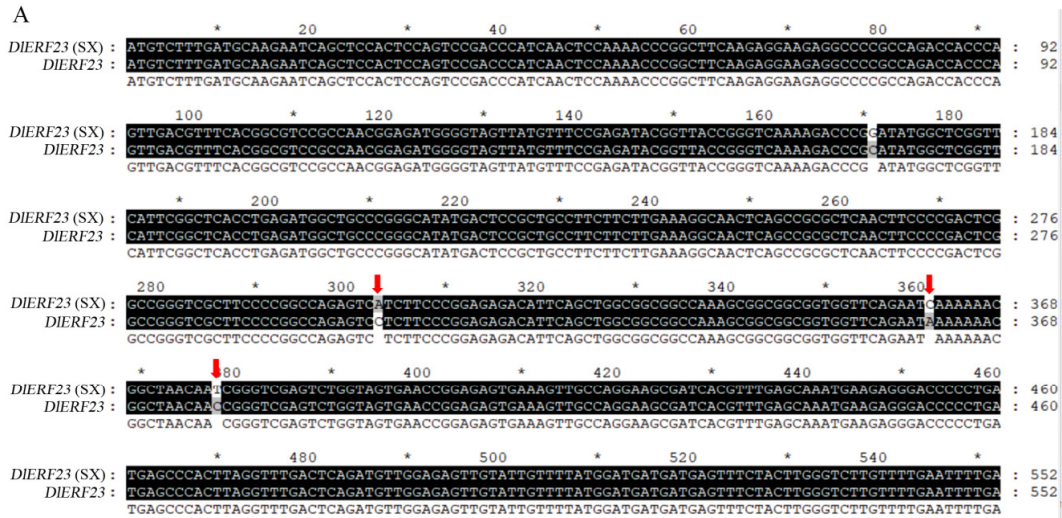


Figure 2. Differences between base sequence (A) and amino acid sequence (B) of *DIERF23* gene from ‘SJ’ and ‘SX’ longan (*DIERF23* (SX))

The red arrows represent differential bases or amino acids.

The cDNA length *DIERF23* was 552 bp, encoding a 184 amino acid protein, with a pI value of 7.69, and a MW value of 20.41 kDa. There are no signal peptides and transmembrane structures in *DIERF23* protein. Amino acid sequence analysis showed that *DIERF23* contains an AP2/ERF domain and is a typical member of the AP2/ERF family (Figure 3).

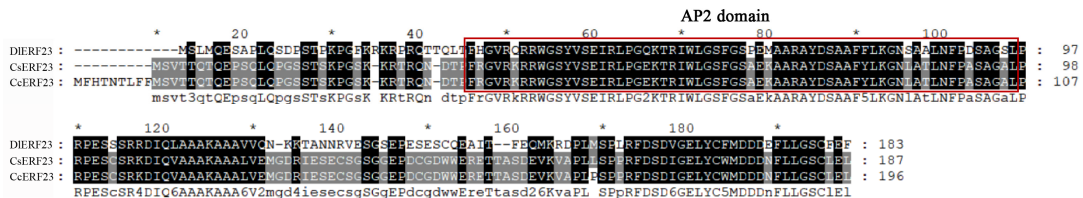


Figure 3. Sequence alignment of AP2/ERF proteins among different species. The red box indicated the amino acid sequence of AP2/ERF

Phylogenetic analysis of *DIERF23*

To study the phylogenetic relationships between *DIERF23* and other plant AP2/ERF proteins, a phylogenetic tree was created by using MEGA 11.0. As shown in Figure 4, *DIERF23* is clustered with WRKY of other woody plants, and is closest to *CsERF23* (XP_006478313.1) of *Citrus sinensis* and *CcERF23* (XP_006441807.2) of *Citrus dementina*. Whereas there is a distant genetic relationship between *DIERF23* and the AP2/ERF members of model plants *Arabidopsis* or dicotyledon maize (Figure 4).

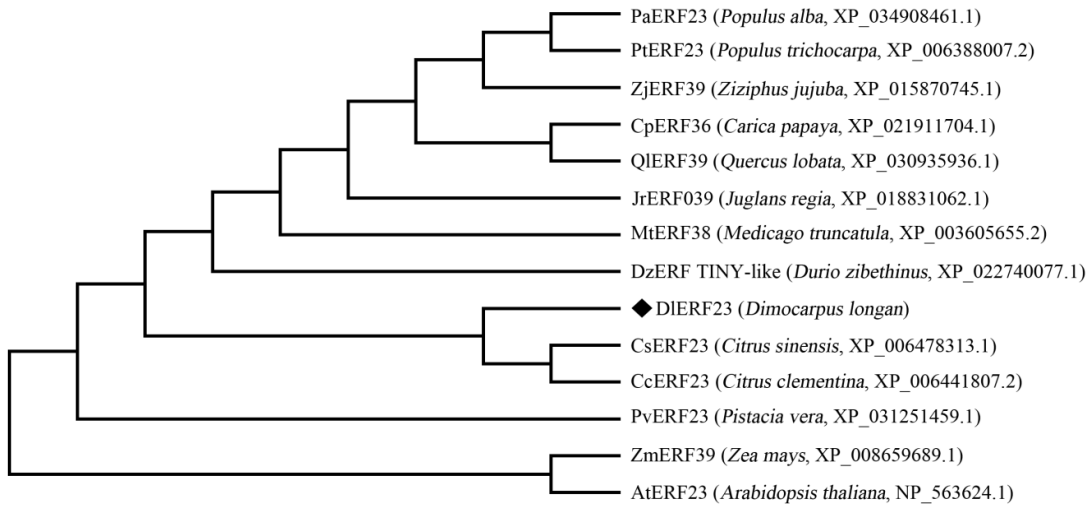


Figure 4. phylogenetic tree analysis between longan DIERF23 and similar sequences in GenBank

Tissue-specific expression of DIERF23

By using nine 'SJ' longan tissues, including flower, flower bud, leaf, pericarp, pulp, root, seed, stem, and young fruit, the tissue-specific expression of *DIERF23* was analyzed. The result of qRT-PCR shown that the expression of *DIERF23* can detected in the nine tissues, with the highest expression displayed in the pericarp which is 20 times that of the young fruit, followed by the stem, leave, flower and flower bud, and the lowest expression in the young fruit (Figure 5). This result indicates that the expression of *DIERF23* gene is tissue-specific and may specifically regulate the development of longan pericarp, young fruit, flower and other organs.

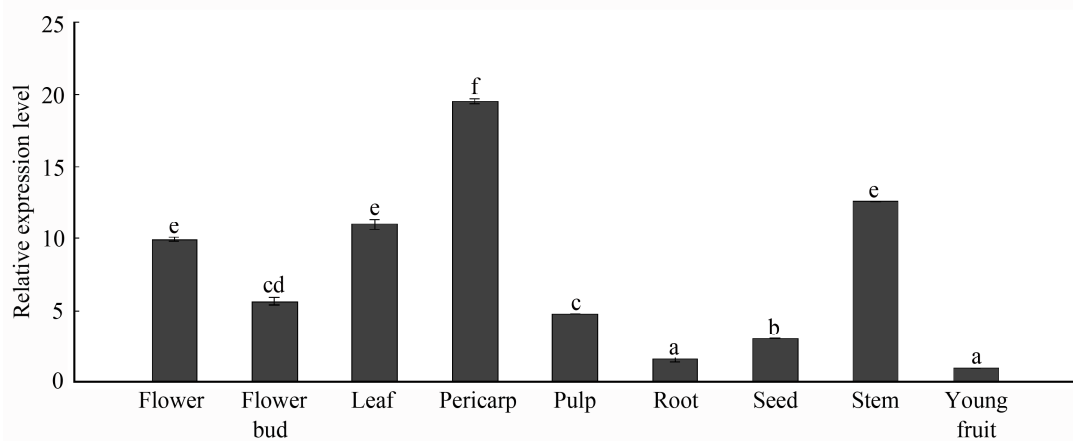


Figure 5. Relative expression levels of *DIERF23* in different longan tissues

Significant differences among samples were assessed at $p < 0.05$ level by Student's t-test; different letters indicate significant difference between samples

Expression during different flowering development of DIERF23

The expression of *DIERF23* in three flower induction stages of 'SX' and 'SJ' longan was detected. The result showed that *DIERF23* shown the opposite expression pattern with a significant down-regulated in the early stage of flower induction in 'SJ' and an up-regulated in the late stage of flower induction in 'SX' (Figure 6).

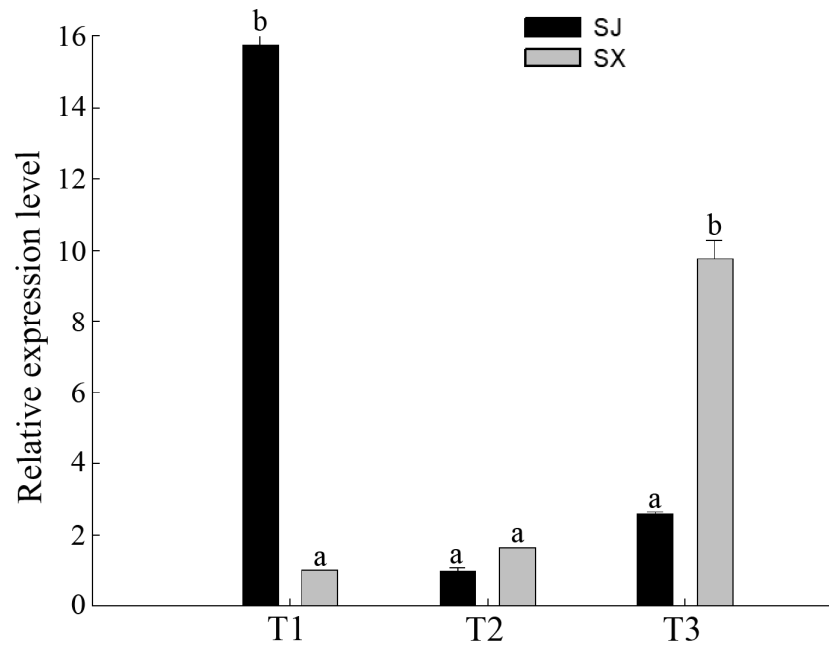


Figure 6. Relative expression levels of *DIERF23* in 'SX' and 'SJ' during different flower development stages. Significant differences among samples were assessed at $p < 0.05$ level by Student's t-test; different letters indicate significant difference between samples.

Subcellular localization of DIERF23

In order to determine the subcellular localization of DIERF23 protein, recombinant plasmid pBWA (V) HS-DIERF23-osGFP was generated and introduced into *Arabidopsis* protoplasts. As shown in Figure 7, under excitation at a wavelength of 480 nm, the fluorescence signal from the DIERF23-GFP fusion protein was mainly detected in the nucleus under confocal laser scanning microscopy, whereas the GFP control was detected in both the nucleus and cytoplasm, without clear localization. These results demonstrated that DIERF23 is a nuclear protein.

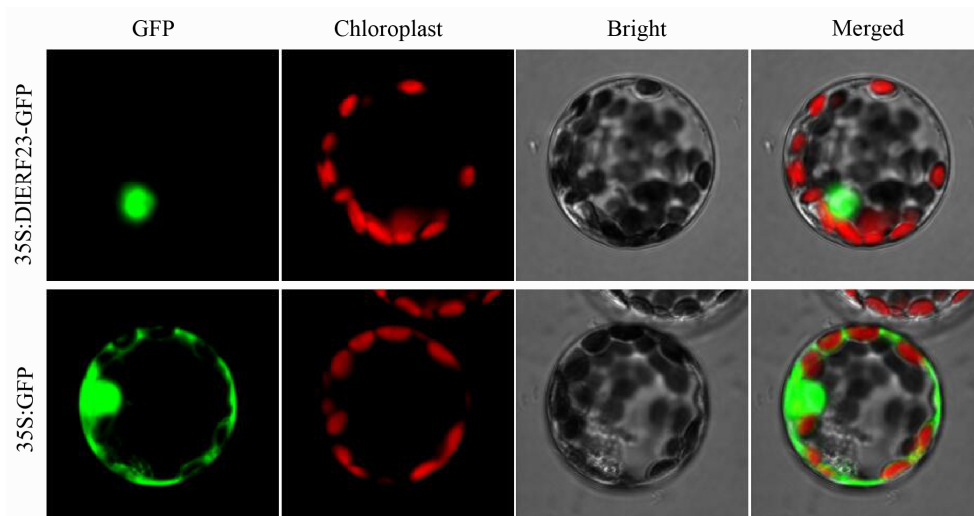


Figure 7. Subcellular localization of the DIERF23-GFP fusion protein in *Arabidopsis* protoplasts. GFP: Green fluorescent protein; Chloroplast: Chloroplast autofluorescence; Bright: Bright field; Merged: Fusion of two kinds of fluorescence; Scale bar= 10 μ m

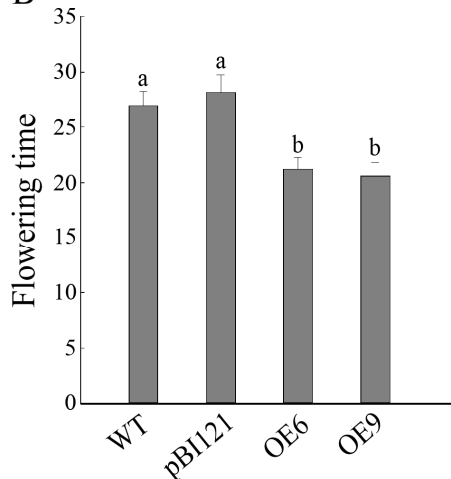
Overexpression of DIERF23 promoting plant flowering

To further investigate the function of *DIERF23*, the overexpression vector of *DIERF23* was constructed and introduced it into *Arabidopsis* (Col-0). Meanwhile, WT plants and WT plants transformed with the pBI121 empty vector were used as negative controls and positive controls, respectively. Two homozygous T3 transgenic lines were randomly selected from the seven independent transgenic lines for phenotype analyzing. Compared with the control plants, the transgenic plants flowered earlier (Figure 8A and 8B). The flowering time ranged from 20.6 to 21.2 d in the transgenic plants, and from 26.9 to 28.1 d in the control plants (Figure 8A and 8B). The average number of rosette leaves in the WT plants is 8.5 which is significantly lower than transgenic (between 11 and 12) (Figure 8C).

A



B



C

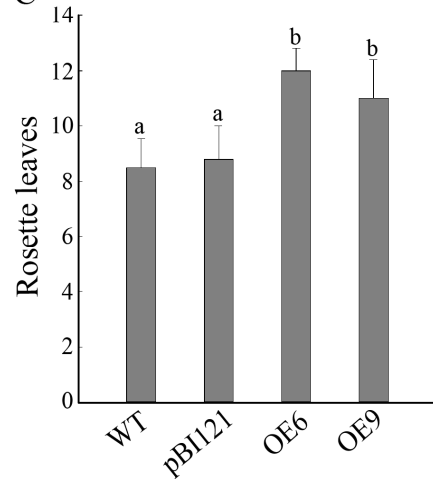


Figure 8 Flowering phenotype of *DIERF23* transgenic *Arabidopsis*. (A) Phenotypes of WT *Arabidopsis*, *DIERF23*-overexpressing *Arabidopsis* and transgenic pBI121 empty vector *Arabidopsis*; (B) Flowering time analysis; (C) Analysis of rosette leaf number

Discussion

AP2/ERF superfamily is the largest TFs family which plays important roles in plant growth, development, biotic and abiotic stress responses, and regulating floral organ development or flowering in plants (Jiang *et al.*, 2022). However, the research of AP2/ERF superfamily members involving in the regulation of longan flower induction is still limited.

Previous studies showed that all of the AP2/ERF superfamily members have the conservative AP2 domain (Zhang *et al.*, 2021). Consistent with these studies, we found *DIERF23*, belonged to DREB subfamily, contain one AP2 domain, was more closely related to ERF members from woody plants in evolutionary relationships, especially for citrus species. AP2/ERF superfamily members appear to be differentially expressed in different tissues and organs. For instance, among the ninety-seven AP2/ERF members of Pineapple (*Ananas comosus* L. Merr), eight-five *AcAP2/ERF* genes were expressed in at least one tissues, and *AcAP2/ERF57/48/71/06/95* (DREB subfamily) almost had no expression in all the four tested tissues, including the leaf, fruit, flower and root (Zhang *et al.*, 2021). Similarly, 173 of 214 *ZmAP2/ERF* genes from maize (*Zea mays* L.) were examined in at least one tissues, whereas 41 genes were not detected in any tissues (Zhang *et al.*, 2022). In the present study, the expression level of *DIERF23* varied in different tissues with the highest expression in the pericarp, followed by the stem, leave, flower and flower bud, and the lowest expression in the young fruit. As the expression level of genes can reflect their role in the development of plant organs and tissues (Rishmawi *et al.*, 2014), our result suggesting that *DIERF23* may specifically participate in the development of fruit and flower organs.

AP2/ERF superfamily members involved in flower organ development and flower induction in plants. For instance, approximately 95.00% of PpcAP2/ERF genes from Chinese cherry (*Prunus pseudocerasus*) were expressed in the three dormancy stages (Zhu *et al.*, 2021). In this study, we found that *DIERF23* shown the opposite expression pattern with a significant down-regulated in the early stage of flower induction in 'SJ', a perpetual flowering (PF) genotype, which flowers and bears fruits throughout the year and does not require special external environmental conditions, and an up-regulated in the late stage of flower induction in 'SX', seasonal flowering (SF), which flowers and bears fruits once a year and requires favorable conditions such as a period of low temperature (vernalization) (Jue *et al.*, 2021). This result indicates that *DIERF23* gene may be involved in the induction of longan flower. To better understand the function of *DIERF23*, the *DIERF23* overexpression *Arabidopsis* lines were conducted. Many studies shown that overexpression different plants *AP2/ERF* genes resulted delay or promote flowering. For example, overexpressed rice *AP2/ERF-N22* gene in *Arabidopsis* would delay flowering by 1 week as compared to WT (Mawlong *et al.*, 2015). Rice *LATE FLOWERING SEMI-DWARF (LFS)* encodes an AP2/ERF TFs that promotes flowering under non-inductive LD conditions (Shim *et al.*, 2022). Consistent with these studies, overexpressed *DIERF23* in *Arabidopsis*, the transgenic plants flowered earlier ranged from 6 to 7 d than the control plants. This result indicates that *DIERF23* gene may be positive regulating plant flowering. As one kind of TFs, AP2/ERF members mainly through cooperated with other floral meristem genes to regulate plant flowering. For example, AtERF98 could regulate flowering in *Arabidopsis* by activating the function through the EDLL activation motif (Tiwari *et al.*, 2012). TOE1, one of the AP2-like TFs, could control flowering by regulating FT expression (Du *et al.*, 2020). However, since the mechanism of AP2/ERF participating in the regulation of plant flowering is relatively complex, further research need to perform to clarify its regulatory mechanisms.

Conclusions

In the present study, we identified a *DIERF23* gene from longan, and analyzed its gene and protein features by bioinformatics approaches. *DIERF23* is a typical DREB subfamily of AP2/ERF TFs, and possesses one typical AP2 domain. Expression pattern analysis showed that *DIERF23* is preferentially expressed in fruit and flower organs, and displayed opposite expression pattern in 'SJ' and 'SX' during three flower induction stages. Meanwhile, overexpressing *DIERF23* in *Arabidopsis*, the transgenic lines shown an early flowering phenotype. This study provides useful information for identifying *DIERF23* function during flower induction.

Authors' Contributions

Conceptualization: DJ; Data curation: CR; Formal analysis: DJ and XS; Funding acquisition: DJ; Investigation, Methodology, Resource, Software, and Visualization: DJ and XS; Writing-original draft: DJ; Writing-review and editing: DJ. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

Acknowledgements

This research was funded by the Natural Science Foundation of China (32001346), the Science and Technology Research Program of Chongqing Education Commission (KJZD-K202201301), the Scientific Research Projects of Chongqing University of Arts and Sciences (R2020FYL01).

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

- Boutilier K, Offringa R, Sharma V K, Kieft H, Ouellet T, Zhang L, ... Campagne M (2002). Ectopic expression of BABY BOOM triggers a conversion from vegetative to embryonic growth. *The Plant Cell* 14(8):1737-1749. <https://doi.org/10.1105/tpc.001941>
- Bowman JL, Alvarez J, Weigel D, Meyerowitz E, Smyth D (1993). Control of flower development in *Arabidopsis thaliana* by *APETALA1* and interacting genes. *Development* 119(3):721-743. <https://doi.org/10.1242/dev.119.3.721>
- Clough S (2005). Floral dip: agrobacterium-mediated germ line transformation. In: *Transgenic Plants: Methods and Protocols*. Springer, pp 91-101. <https://doi.org/10.1385/1-59259-827-7:091>
- Du SS, Li L, Li L, Wei X, Xu F, Xu P, ... Yang H (2020). Photoexcited cryptochrome2 interacts directly with TOE1 and TOE2 in flowering regulation. *Plant Physiology* 184(1):487-505. <https://doi.org/10.1104/pp.20.00486>
- Dubouzet J G, Sakuma Y, Ito Y, Kasuga M, Dubouzet EG, Miura S, Seki M, Shinozaki K, Yamaguchi-Shinozaki K (2003). *OsDREB* genes in rice, *Oryza sativa* L., encode transcription activators that function in drought-, high- salt- and

- cold- responsive gene expression. *The Plant Journal* 33(4):751-763. <https://doi.org/10.1046/j.1365-313X.2003.01661.x>
- Dossa K, Wei X, Li D, Fonceka D, Zhang Y, Wang L, ... Zhang X (2016). Insight into the AP2/ERF transcription factor superfamily in sesame and expression profiling of DREB subfamily under drought stress. *BMC Plant Biology* 16:1-16. <https://doi.org/10.1186/s12870-016-0859-4>
- Feng J, Liu D, Pan Y, Gong W, Ma L, Luo J, Deng X, Zhu Y (2005). An annotation update via cDNA sequence analysis and comprehensive profiling of developmental, hormonal or environmental responsiveness of the *Arabidopsis* AP2/EREBP transcription factor gene family. *Plant Molecular Biology* 59:853-868. <https://doi.org/10.1007/s11103-005-1511-0>
- François L, Verdenaud M, Fu X, Ruleman D, Dubois A, Vandenbussche M, ... Bendahmane M (2018). A miR172 target-deficient AP2-like gene correlates with the double flower phenotype in roses. *Scientific Reports* 8(1):12912. <https://doi.org/10.1038/s41598-018-30918-4>
- Jofuku KD, Omidyar PK, Gee Z, Okamuro J (2005). Control of seed mass and seed yield by the floral homeotic gene *APETALA2*. *Proceedings of the National Academy of Sciences* 102(8):3117-3122. <https://doi.org/10.1073/pnas.0409893102>
- Ito Y, Katsura K, Maruyama K, Taji T, Kobayashi M, Seki M, Shinozaki K, Yamaguchi-Shinozaki K (2006). Functional analysis of rice DREB1/CBF-type transcription factors involved in cold-responsive gene expression in transgenic rice. *Plant and Cell Physiology* 47(1):141-153. <https://doi.org/10.1093/pcp/pci230>
- Jiang Q, Wang Z, Hu G, Yao X (2022). Genome-wide identification and characterization of *AP2/ERF* gene superfamily during flower development in *Actinidia eriantha*. *BMC Genomics* 23(1):1-16. <https://doi.org/10.1186/s12864-022-08871-4>
- Jue D, Sang X, Liu L, Shu B, Wang Y, Liu C, Wang Y, Xie J, Shi S (2019). Comprehensive analysis of the longan transcriptome reveals distinct regulatory programs during the floral transition. *BMC Genomics* 20(1):1-18. <https://doi.org/10.1186/s12864-019-5461-3>
- Jue D, Sang X, Liu L, Shu B, Wang Y, Liu C (2018). Identification of *WRKY* gene family from *Dimocarpus longan* and its expression analysis during flower induction and abiotic stress responses. *International Journal of Molecular Sciences* 19(8):2169. <https://doi.org/10.3390/ijms19082169>
- Lakhwani D, Pandey A, Dhar Y, Bag S, Trivedi P, Asif M (2016). Genome-wide analysis of the AP2/ERF family in *Musa* species reveals divergence and neofunctionalisation during evolution. *Scientific Reports* 6(1):1-17. <https://doi.org/10.1038/srep18878>
- Li X, Tao S, Wei S, Ming M, Huang X, Zhang S, Wu J (2018). The mining and evolutionary investigation of *AP2/ERF* genes in pear (*Pyrus*). *BMC Plant Biology* 18(1):1-14. <https://doi.org/10.1186/s12870-018-1265-x>
- Lin Y, Min J, Lai R, Wu Z, Chen Y, Yu L, ... Lai Z (2017). Genome-wide sequencing of longan (*Dimocarpus longan* Lour.) provides insights into molecular basis of its polyphenol-rich characteristics. *Gigascience* 6(5):gix023. <https://doi.org/10.1093/gigascience/gix023>
- Liu C, Zhang T (2017). Expansion and stress responses of the AP2/EREBP superfamily in cotton. *BMC Genomics* 18(1):1-16. <https://doi.org/10.1186/s12864-017-3517-9>
- Mawlong I, Ali K, Srinivasan R, Rai R, Tyagi A (2015). Functional validation of a drought-responsive AP2/ERF family transcription factor-encoding gene from rice in *Arabidopsis*. *Molecular Breeding* 35:1-14. <https://doi.org/10.1007/s11032-015-0290-9>
- Nakano T, Suzuki K, Fujimura T, Shinshi H (2006). Genome-wide analysis of the ERF gene family in *Arabidopsis* and rice. *Plant Physiology* 140(2):411-432. <https://doi.org/10.1104/pp.105.073783>
- Rishmawi L, Pesch M, Juengst C, Schauss AC, Schrader A, Hülskamp M (2014). Non-cell-autonomous regulation of root hair patterning genes by *WRKY75* in *Arabidopsis*. *Plant Physiology* 165(1):186-195. <https://doi.org/10.1104/pp.113.233775>
- Sakuma Y, Liu Q, Dubouzet J, Abe H, Shinozaki K, Yamaguchi-Shinozaki K (2002). DNA-binding specificity of the ERF/AP2 domain of *Arabidopsis* DREBs, transcription factors involved in dehydration-and cold-inducible gene expression. *Biochemical and Biophysical Research Communications* 290(3):998-1009. <https://doi.org/10.1006/bbrc.2001.6299>

- Shim Y, Lim C, Seong G, Choi Y, Kang K, Paek N (2022). The AP2/ERF transcription factor LATE FLOWERING SEMI-DWARF suppresses long-day-dependent repression of flowering. *Plant, Cell & Environment* 45(8):2446-2459. <https://doi.org/10.1111/pce.14365>
- Tamura K, Stecher G, Kumar S (2021). MEGA11: molecular evolutionary genetics analysis version 11. *Molecular Biology and Evolution* 38(7):3022-3027. <https://doi.org/10.1093/molbev/msab120>
- Tiwari S, Belachew A, Ma S, Young M, Ade J, Shen Y, ... Repetti P (2012). The EDLL motif: a potent plant transcriptional activation domain from AP2/ERF transcription factors. *The Plant Journal* 70(5):855-865. <https://doi.org/10.1111/j.1365-313X.2012.04935.x>
- Yoo S-D, Cho Y-H, Sheen J (2007). Arabidopsis mesophyll protoplasts: a versatile cell system for transient gene expression analysis. *Nature Protocols* 2(7):1565-1572. <https://doi.org/10.1038/nprot.2007.199>
- You X, Wang L, Liang W, Gai Y, Wang X, Chen W (2012). Floral reversion mechanism in longan (*Dimocarpus longan* Lour.) revealed by proteomic and anatomic analyses. *Journal of Proteomics* 75(4):1099-1118. <https://doi.org/10.1016/j.jprot.2011.10.023>
- Zarei A, Körbes A P, Younessi P, Montiel G, Champion A, Memelink J (2011). Two GCC boxes and AP2/ERF-domain transcription factor ORA59 in jasmonate/ethylene-mediated activation of the *PDF1.2* promoter in Arabidopsis. *Plant Molecular Biology* 75:321-331. <https://doi.org/10.1007/s11103-010-9728-y>
- Zeng D, Teixeira da Silva JA, Zhang M, Yu Z, Si C, Zhao C, ... Duan J (2021). Genome-wide identification and analysis of the APETALA2 (AP2) transcription factor in *Dendrobium officinale*. *International Journal of Molecular Sciences* 22(10):5221. <https://doi.org/10.3390/ijms22105221>
- Zhang G, Chen M, Li L, Xu Z, Chen X, Guo J, Ma Y (2009). Overexpression of the soybean *GmERF3* gene, an AP2/ERF type transcription factor for increased tolerances to salt, drought, and diseases in transgenic tobacco. *Journal of Experimental Botany* 60(13):3781-3796. <https://doi.org/10.1093/jxb/erp214>
- Zhang H, Pan X, Liu S, Lin M, Li Y, Zhang X (2021). Genome-wide analysis of AP2/ERF transcription factors in pineapple reveals functional divergence during flowering induction mediated by ethylene and floral organ development. *Genomics* 113(2):474-489. <https://doi.org/10.1016/j.ygeno.2020.10.040>
- Zhang J, Liao J, Ling Q, Xi Y, Qian Y (2022). Genome-wide identification and expression profiling analysis of maize AP2/ERF superfamily genes reveal essential roles in abiotic stress tolerance. *BMC Genomics* 23(1):1-22. <https://doi.org/10.1186/s12864-022-08345-7>
- Zhang S, Zhu C, Lyu Y, Chen Y, Zhang Z, Lai Z, Lin Y (2020). Genome-wide identification, molecular evolution, and expression analysis provide new insights into the APETALA2/ethylene responsive factor (AP2/ERF) superfamily in *Dimocarpus longan* Lour. *BMC Genomics* 21(1):1-20. <https://doi.org/10.1186/s12864-020-6469-4>
- Zhu Y, Liu X, Gao Y, Li K, Guo W (2021). Transcriptome-based identification of AP2/ERF family genes and their cold-regulated expression during the dormancy phase transition of Chinese cherry flower buds. *Scientia Horticulturae* 275:109666. <https://doi.org/10.1016/j.scienta.2020.109666>



The journal offers free, immediate, and unrestricted access to peer-reviewed research and scholarly work. Users are allowed to read, download, copy, distribute, print, search, or link to the full texts of the articles, or use them for any other lawful purpose, without asking prior permission from the publisher or the author.



License - Articles published in *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* are Open-Access, distributed under the terms and conditions of the Creative Commons Attribution (CC BY 4.0) License.
© Articles by the authors; Licensee UASVM and SHST, Cluj-Napoca, Romania. The journal allows the author(s) to hold the copyright/to retain publishing rights without restriction.

Notes:

- **Material disclaimer:** The authors are fully responsible for their work and they hold sole responsibility for the articles published in the journal.
- **Maps and affiliations:** The publisher stay neutral with regard to jurisdictional claims in published maps and institutional affiliations.
- **Responsibilities:** The editors, editorial board and publisher do not assume any responsibility for the article's contents and for the authors' views expressed in their contributions. The statements and opinions published represent the views of the authors

or persons to whom they are credited. Publication of research information does not constitute a recommendation or endorsement of products involved.
