Auxin response factor gene *MdARF2* is involved in ABA and salt stress response in apple[1]

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Abstract Auxin response factors (ARFs) play key roles throughout the whole plant growth and development, and mediate auxin response gene transcription by directly binding with auxin response element (AuxREs). However, their functions in abiotic stresses are largely limited, especially in apples. Here, an auxin response factor gene *MdARF2* (HF41569) was cloned from apple cultivar '*Royal Gala*' (*Malus*× *domestica* Borkh.). Phylogenetic analysis showed that ARF2 proteins were highly conserved among different species and MdARF2 is the closest relative to PpARF2 of *Prunus persica*, but they differed at the DNA level. MdARF2 contained three typical conserved domains including B3 DNA-binding domain, Auxin_resp domain and AUX_IAA domain. The Subcellular localization demonstrated that MdARF2 was localized in the nucleus. The three-dimensional structure prediction of proteins showed that MdARF2 were highly similar with AtARF2, and contained helix, folds, and random coils. The promoter of *MdARF2* contained cis-acting elements in response various stresses, environmental and hormonal signals. Expression analysis showed that *MdARF2* was widely expressed in all tissues of apple, with the highest expression of *MdARF2* in root. Functional analysis with a series of *MdARF2* transgenic apple calli indicated that *MdARF2* can reduce the sensitivity to ABA signaling and enhance salt tolerance in apple. In summary, the research provides a new basis for studying the regulation of abiotic stress by ARFs.

Keywords: ABA signaling, Apple, *MdARF2*, salt stress

1. Introduction

Plants are subjected to many kinds of abiotic stresses in nature, which seriously affect their yield and quality. The study of plant stress resistance mechanism has important guiding significance for the improvement of crop resistance varieties. In order to adapt to various abiotic stresses, plants formed a complete resistance mechanism in the process of evolution by sensing external stimuli, adjusting gene expression and regulating metabolic pathways (Nakashima *et al.* 2014).

Salt stress affecting plant physiology mediated by NaCl is the most common abiotic stress (Munns and Tester 2008;

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Fritsche-Neto and Borém 2012). Soil salinization is one of the major factors restricting plant production, which not only leads to the disorder of nutrients by reducing the absorption of water, but also leads to the decline of growth and yield due to the toxicity of ions (Zhu 2002; Abogadallah 2010; Srivastava *et al.* 2017). Salt stress can cause a variety of plant disorders, which included growth inhibition, stunting, decreased photosynthetic rate, decreased respiratory rate, yellowing of leaves, and decreased relative leaf weight and root fresh weight (Clipson *et al.* 1985; Zhou *et al.* 2018).

The plant hormone abscisic acid (ABA) is an important regulator of plant growth, response to environmental stress and plant metabolism (Finkelstein *et al.* 2002). The mutations of plants lacking ABA reduce cell activity (Xiong and Zhu 2003). The activity of abscisic acid synthesis related factors was enhanced under drought stress (Iuchi *et al.* 2001). ABA enhances cold tolerance of grape buds (Rubio *et al.* 2019). NCED is considered as a key enzyme in abscisic acid biosynthesis and overexpression of *AtNCED* could reduce transpiration rate of leaves and improve drought tolerance (Seo *et al.* 2000). ABF is mainly involved in the regulation of ABA and stress; AtCDPK6 can phosphorylate AtABF3 and positively regulate drought resistance in plants (Zhang *et al.* 2020). *StABF1* positively regulates potato resistance to low temperature (Muniz Garcia *et al.* 2012).

The dynamic change and differential distribution of auxin in plants control the growth and development, so that plants can maintain steady state in the environment (Vanneste and Friml 2009). Auxin receptor proteins TIR1/AFB, Aux/IAA and auxin response protein ARF are involved in auxin signaling transduction in plants (Chapman and Estelle 2009). There are synergistic or antagonistic effects among ARF family members in Arabidopsis (Rademacher *et al.* 2011). The structure of ARF is highly conserved and can be used as a transcription factor to bind to auxin response element (AuxREs) binding elements on downstream target gene promoters (Guilfoyle and Hagen 2007).

The ARF family proteins are functionally diverse. ETTIN is involved in stamen development (Nemhauser *et al.* 2000). ARF5 is associated with embryonic development (Hardtke and Berleth 1998). ARF7, ARF19 and ARF2 are involved in regulating cell division (Schruff *et al.* 2006). Overexpression of *MiARF2* in Arabidopsis inhibits the elongation of hypocotyls and roots of seedlings (Wu *et al.* 2011). *AtARF2* regulated plant senescence independently of the ethylene and cytokinin pathways (Ellis *et al.* 2005; Okushima *et al.* 2005). *SlARF2* may regulate lateral root formation and floral organ senescence in tomatoes by regulating auxin and ethylene reaction factors (Ren *et al.* 2017). *ARF2* is regulated by a variety of hormones. *AtARF2* as a junction of ethylene and auxin signaling (Chandler 2016). BIN2 directly inactivates the inhibitor ARFs to increase the expression of auxin-induced genes (Vert *et al.* 2008). AtARF2 can bind *HAK5* promoter to inhibit its expression at the transcriptional level in response to potassium ion stress (Zhao *et al.* 2016). ARF2 may play a positive role in salt tolerance in *Jerusalem artichoke* (Wen *et al.* 2020). What's more, ARF2 acts as a molecular link to integrate ABA signalingand drought stress (Meng *et al.* 2015). Thus, *ARF2* is a very important gene in plants which could respond to hormones and plays a regulatory role in plant resistance to abiotic stress.

Apple is one of the four largest fruits in the world, which is rich in vitamins and minerals. In previous studies, we have shown that *MdARF2* negatively regulates the accumulation of anthocyanin in apple (Wang *et al.* 2020). Here, further research on *MdARF2* found that it could reduce the sensitivity to ABA signaling and enhance salt tolerance in apple.

2. Materials and Methods

2.1. Experimental materials and growth conditions

The calli were taken from the young embryo of 'Orin' (*Malus*× *domestica* Borkh.). They were cultured on the Murashige Skoog (MS) medium plus 1.5 mg L^{-1} 2,4-dichlorophenoxyacetic acid (2,4-D), and 0.4 mg L^{-1} 6-benzylaminopurine (6-BA) of "Orin" calli for 15 days, and grown at 24 ℃ under the dark condition.

The different tissues of apple were obtained from the 8-year-old self-rooted '*Royal Gala*' apple tree planted in the experimental field of the Horticultural orchard of Shandong Agricultural University (Tai 'an, Shandong province, China) in April 2019. The roots, stems, new leaves, flowers and fruits (10 and 120 days after flowering) from trees were taken and frozen in liquid nitrogen. The seeds of Tea crabapple (*Malus*×*hupehensis* Rehd) were stratified to obtain seedlings, and seedlings with four true leaves (the height was about 10 cm and grew well) were used in this study. The seedlings were grown at 24 ℃ with 16 h light/8 h dark period.

2.2. Bioinformatics analysis

The protein and nucleic acid sequences of MdARF2 (HF41569) were downloaded from the Apple genome database (HFTH1 genome V1.0.a1; https://www.rosaceae.org/species/malus/all), while the protein sequences of other species were obtained from the NCBI database (https://www.ncbi.nlm.nih.gov/). ClustalW in MAGE7.0 was used to compare multiple sequences of proteins, Neighbor-joining Tree was used to construct phylogenetic trees (Bootstrp method was set as 1000), and then cluster analyzed. Conservative domain analysis was performed with SMART (http://smart.embl-heidelberg.de/), NCBI Conserved Domain Search (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi?) and WebLogo (http://weblogo.threeplusone.com/). PHYRE2 (http://www.sbg.bio.ic.ac.uk/phyre2/html/page.cgi?id=index), which uses advanced remote homology detection methods to build 3D models, was used to predict protein structure and PyMOL viewer visualized structures. The cis-acting element analysis of the promoter of *MdARF2* was performed online by using PlantCARE website (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/).

2.3. Construction of *MdARF2* gene expression vector and genetic transformation of apple calli

The construction of *MdARF2* gene expression vector and genetic transformation of apple calli has been described by Wang et al. (2020). The ORF sequence of *MdARF2* was cloned into pRI-101 to obtain the overexpression vector, and the non-conserved regions of *MdARF2*'s ORF were reversely cloned into pRI-101 to obtain the antisense vector. Transgenic apple calli was obtained by *Agrobacterium*-mediated transformation.

2.4. Quantitative real-time-PCR (qRT-PCR) analysis

The seedlings of Tea crabapple were treated with 100 mmol·L^{-1} NaCl, 100 μmol·L^{-1} ABA and water (control) for 0h, 1h, 3h, 6h, 12h and 24h, and repeated three times. The samples were quick frozen in liquid nitrogen stored at -80 ℃ for use. Plant RNA were extracted by RNA extraction Kit (TIANGEN, Beijing, China) and single-stranded cDNA was obtained by a reverse transcription kit (TaKaRa, Shiga, Japan). The quantitative primers of *MdARF2* refer to Wang et al. (2020). *MdActin*

(GenBank accession number CN938024) as a housekeeping reference gene. The real-time quantitative PCR (qRT-PCR) analysis were executed three biological and technical replications to test the expression level of *MdARF2*, which were performed with the methods as described by Hu et al. (2016). The quantitatively analysis of results by using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen 2001).

2.5. Determination of stress-related indexes of apple calli under NaCl and ABA treatment

The wild type and transgenic calli of apple were treated with MS medium, MS+100 mmol L^{-1} NaCl., MS+150 mmol L^{-1} NaCl, MS+50 μmol L^{-1} ABA, MS+100 μmol L^{-1} ABA, MS+150 μmol L^{-1} ABA, respectively. Each group was repeated for three times. Fresh weight and malondialdehyde (MDA) was measured after 15 days of treatment. The MDA content of calli were measured according to the content test boxes, respectively (Suzhou Ke Ming Biotechnology Co., Ltd., Suzhou, China).

2.6. Analysis of subcellular localization

The CDS sequence of *MdARF2* was cloned into pAL3-rc$_{@}$1 for fusing with green fluorescent protein (GFP). The vector *35S:MdARF2-GFP* was transformed into the *Agrobacterium* LBA4404 strain. *Agrobacterium*-mediated transient expression of *Nicotiana benthamiana* according to the method of Sun et al (Sun *et al.* 2018). The GFP signal was observed by LSM 880 META confocal microscope (Zeiss LSM 510 META, Jena, Germany). DAPI, a nuclear localization fluorescent dye, was used in this study as control.

2.7. Analysis of published data

According to the chromosome location information of genes, the methylation site information of *MdARF2* and *PpARF2* was extracted from the methylation sequencing results of apples (SRX1656922, ftp://ftp.ebi.ac.uk/pub/databases/microarray/data/experiment/GEOD/E-GEOD-79526/E-GEOD-79526.processed.16.zip) and peaches (SRX1656929, ftp://ftp.ebi.ac.uk/pub/databases/microarray/data/experiment/GEOD/E-GEOD-79526/E-GEOD-79526.processed.9.zip). The QTL data and the expression level of *MdARF2* were obtained from the supplemental table S8 of Liu et al. (2020). R-pheatmap package was used to draw the expression level heatmap.

2.8 Data presentation and statistical analysis

The data obtained in this study were analyzed by DPS software (Enfield, UK, P-values < 0.05 was considered as significant difference).

3. Results

3.1. Phylogenetic analysis of MdARF2

Evolutionary analysis of ARF2 protein in different species was performed according to the plant ancestor node provided by JGI in order to explore the relationship among ARF2 proteins in different species. The sequences of ARF2 in different species from the NCBI database and MdARF2 were extracted for phylogenetic analysis. The results showed that MdARF2

and PpARF2 had the closest homologous relationship (Fig. 1-A). Functional domain analysis showed that it was conserved between MdARF2 and other ARF2 proteins in other species; they contain the conserved B3 DNA-binding domain and Auxin_resp domain. Interestingly, AUX_IAA domain disappeared after the Tracheophyte ancestor node and reappeared after the Eudicot ancestor node. ARF2 protein in the Grass ancestor node does not contain the AUX_IAA domain. According to the evolutionary relationship, apple appeared after the ancestor node. It had B3 DNA-binding domain, Auxin_resp domain and AUX_IAA domain (Fig. 1-B-C).

3.2. Comparison of DNA methylation level between *MdARF2* and *PpARF2*

To explore the similarities between *MdARF2* and *PpARF2* at the DNA level, based on the chromosomal locations (*MdARF2* was locates in chr14 from 23242584 to 23246708, and *PpARF2* was locates in chr5 from 13246315 to 13251610) of two genes, methylation site information of two genes was extracted from published data (Niederhuth *et al.* 2016). The distribution of C methylation across the genome consists of three motif forms: CG, CHG and CHH, where H stands for either A or T or C bases. The results showed that there were 374 (78%CHH, 7%CHG and 15%CG) methylation sites in *MdARF2*, and the number of *PpARF2* was 1836 (75%CHH, 11% CHG and 14%CG), which was much higher than that of apple (Fig. 2-A-B). CHH sites had the highest proportion in both genes. The methylation coverage of *MdARF2* in CHG was 71%, in CHH was 21%, and in CG was 48%, all of which were higher than *PpARF2* (Fig. 2-C-D). *MdARF2* has higher coverage of methylation than *PpARF2*, which indicated that they are very different at the DNA level, despite they are closely related.

3.3. Subcellular localization and structure prediction of MdARF2

To further explore the function of MdARF2, the subcellular localization of it was analyzed. The results of subcellular localization showed that MdARF2 was localized in the nucleus, which was the same to AtARF2 (Fig. 3-A). AtARF2 has been well studied (Ellis *et al.* 2005; Chandler 2016). Therefore, we constructed the three-dimensional structure of AtARF2 and MdARF2, and compared them. The structure prediction of proteins showed that MdARF2 and AtARF2 contained helix, folds, and random coils (Fig. 3-B-C). Two proteins were highly identical except that the two fragments were different after merge (Fig. 3-D). The prediction of the three-dimensional structure of MdARF2 and AtARF2 proteins revealed that the two proteins were highly similar. This suggested that the two proteins might have similar functions.

3.4. *MdARF2* promoter cis-acting element analysis

Plants need to increase their resistance to abiotic stress to improve their adaptability to the environment. To explore the relationship between *MdARF2* and abiotic stress, PlantCARE website was used to analyze the cis-acting components of *MdARF2* promoter. The promoter sequence of *MdARF2* contains resistance-related response elements, such as ABA-responsive element ABRE; environmental response element, such as cis-acting regulatory element involved in light responsiveness G-box, anoxic specific inducible element. The promoter sequence also had some phytohormone-related response elements, such as characteristic cis-acting element of auxin response factor TGA-element, MeJA responsive element CGTCA-motif (Table 1). These components are all involved in the stress response of plants, which suggested that

MdARF2 may be involved in abiotic stress in apple plants.

3.5. Expression analysis of *MdARF2*

Expression pattern of *MdARF2* is an indication of its function, and the expression level of *MdARF2* in different apple tissues was detected. The results showed that *MdARF2* was widely expressed in all tissues of the apple, with the highest expression in the root, the high expression in the fruit, the stem and the flower, and the lowest expression in the leaf (Fig. 4-A).

To investigate the response of *MdARF2* to ABA and salt stress, the qRT-PCR assay was used to detect the gene expression level under ABA treatment and NaCl treatment. The results showed that relative expression level of MdARF2 changed significantly under ABA and salt treatment (Fig. 4-B-C). These results suggested that MdARF2 was induced by ABA and NaCl treatments.

3.6. Overexpression of *MdARF2* increases resistance to ABA and NaCl

To further explore the response mechanism of *MdARF2* to abiotic stress, the overexpression and antisense vector of *MdARF2* were expressed in apple calli by using *Agrobacterium*-mediated genetic system to obtain transgenic calli *MdARF2-OE* and *MdARF2-anti* (Wang *et al.* 2020). The expression level of *MdARF2* in transgenic calli was detected (Fig. S1). To investigate the effects of *MdARF2* on ABA stress and salt stress, the growth of wild-type and transgenic calli under different concentrations of ABA and NaCl was observed. The results showed that overexpressing *MdARF2* calli were more resistant to ABA and NaCl than the wild-type, while the antisense *MdARF2* calli were less resistant (Fig. 5-A-B). The fresh weight of *MdARF2-OE* calli were higher than wild-type, and *MdARF2-anti* were lower (Fig. 5-C-D). Analysis of MDA level in calli showed that MDA content in *MdARF2-OE* was the highest, followed by wild-type, and *MdARF2-anti* was the lowest under treatments (Fig. 5-E-F). This indicated that overexpression of *MdARF2* can enhance the resistance of apple calli to ABA and NaCl stress and *MdARF2* can reduce the sensitivity to ABA signaling.

3.7 *MdARF2* is a candidate QTL gene for screening salt-tolerant cultivars in apple

In order to explore the application potential of *MdARF2* in practical production, we analyzed published QTL and transcriptome data of hybrid populations in apples (Liu *et al.* 2020). *MdARF2* is located on chromosome 14, and study have shown that there is a salt-tolerant QTL region S-H14.2 on chromosome 14. Based on published transcriptome data, we analyzed of the genes with relative expression levels (read counts) higher than 10 in S-H14.2. The result showed that *MdARF2* belonged to this QTL region and had high expression levels (Fig. 6). In addition, *MdARF2* is the only one of QTL candidate genes in ARF family that can be used to screen salt tolerant rootstocks, which indicated that *MdARF2* may plays an important role in salt stress.

4. Discussion

Auxin plays an important role in plants growth and development. Auxin response factors ARFs are crucial regulators in auxin signaling pathway. Therefore, it is important to study auxin-related factors in plants (Guilfoyle and Hagen 2007). In

this study, we cloned and conducted bioinformatics analysis on *MdARF2*. The homologous relationship between MdARF2 and PpARF2 was the closest by comparing the evolutionary relationship between MdARF2 and ARF2 in other species (Fig. 1A). ARF2 proteins in all species contained the B3 DNA-binding domain and the Aux_resp domain. ARF2 proteins under Grass ancestor node had no AUX_IAA domain (Fig. 1-B-C) methylation sites in *PpARF2* was more than that in *MdARF2*, but the methylation coverage in *MdARF2* was higher than that in *PpARF2* (Fig. 2). Subcellular localization analysis showed that MdARF*2* was localized in the nucleus (Fig. 3-A). We predicted the three-dimensional structure of ARF2 proteins in Arabidopsis and Apple (Fig. 3-B-C), and the results showed that the two proteins were highly similar (Fig. 3-D), suggesting that they might have the similar functions. Tissue expression analysis showed that *MdARF2* was expressed in all apple tissues with the highest expression in the root (Fig. 4-A). This result was similar with the constitutive expression of *SlARF2* in tomato (Ren *et al.* 2017). ARF2 was induced by hormones and environmental factors (Vert *et al*. 2008; Chandler 2016; Ren *et al.* 2017). The promoter of *MdARF2* contains cis-acting elements in response to various hormones and environmental factors (Table 1).

Auxin is closely related to salt stress and abscisic acid. Auxin can regulate seed germination of Arabidopsis under high salinity conditions (Jung and Park 2011). ABA receptor PYL8 enhanced auxin signaling by enhancing the activity of MYB44, MYB73 and MYB77 with its byproducts to promoting lateral root growth (Zhao *et al*. 2014). *AtARF2* was involved in the ABA signaling pathway, and *arf2* mutants showed an ABA-sensitive phenotype of inhibited primary root growth, decreased cotyledon greening rate and altered auxin distribution in ABA medium, while *AtARF2* overexpressed materials display an ABA-insensitive phenotype (Wang *et al.* 2011). We demonstrated that *MdARF2-anti* calli exhibited an ABA-sensitive phenotype of growth retardation on ABA medium, which consistent with Arabidopsis (Fig. 5-A). *MdARF2* is an auxin response factor and can reduce the ABA signaling, suggesting that *MdARF2* might participate in the cross-network signaling pathway between ABA and auxin in apple. These findings provided a reference for the follow-up study on the function and mechanism of *MdARF2*.

Several studies have shown that *ARF2* plays an important role in plant stress (Zhao *et al.* 2016). The expression level of *MdARF2* under salt stress indicted that it respond to salt stress (Fig. 4-C), which was similar to the results of *Jerusalem artichoke* (Wen *et al.* 2020). Phenotypic experimental results showed that *MdARF2* overexpression could reduce the inhibition of salt stress on apple calli growth (Fig. 5-B and D). MDA could reflect the antioxidant capacity of plant tissues and measure the resistance of plants to external adversity. When a variety of enzymes and membrane systems in plant tissues are destroyed, MDA content increases (Guclu *et al.* 2014). MDA content of *MdARF2-OE* calli were lower than that of wild type under salt stress, which further proved that *MdARF2* enhanced the tolerance of apple calli to salt stress (Fig. 5-F). Moreover, *MdARF2* can also be used as a QTL candidate gene for screening salt tolerant rootstocks (Fig. 6). Salt tolerance of *MdARF*2 was verified, which indicated that *MdARF2* can be used as a QTL gene, laying a foundation for further exploring the regulatory relationship between auxin signaling and salt stress, and providing a theoretical basis for salt tolerant rootstock breeding.

5. Conclusion

In this study, we found that ARF2 of different species is conserved and MdARF2 is the closest relative to PpARF2. But there is a great difference between *MdARF2* and *PpARF2* in DNA level, the methylation coverage of *MdARF2* is higher than that of *PpARF2*. MdARF2 is localized in the nucleus, and the three-dimensional protein structure of MdARF2 highly coincides with that of AtARF2. *MdARF2* could influence ABA signaling and salt stress. Silencing *MdARF2* can enhance the sensitivity of calli to ABA signaling, and overexpression of *MdARF2* can enhance the resistance of apple calli to salt stress. We also found that *MdARF2* is a candidate gene for screening salt resistant varieties. This study provides a new basis for studying the regulation of abiotic stress by ARFs.

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Declaration of competing interest

The authors declare that they have no conflict of interest.

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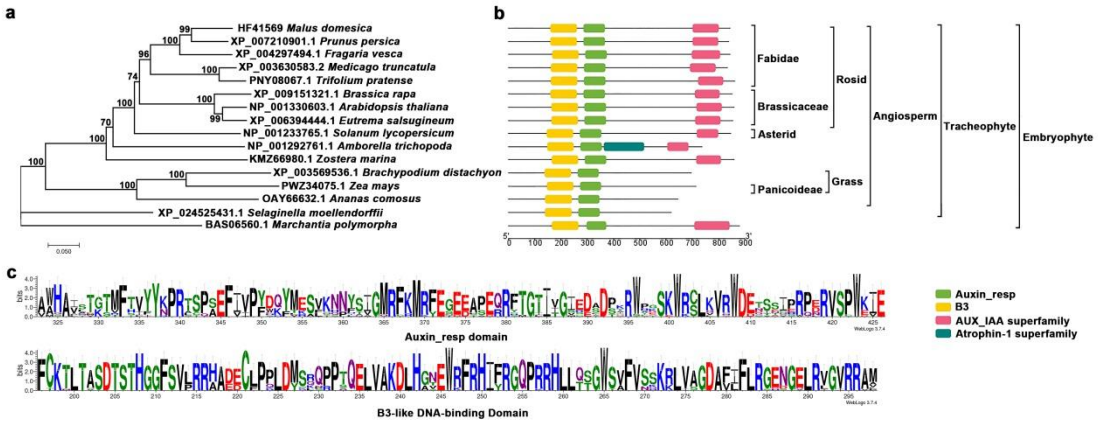

Fig. 1 The analysis of ARF2s in apple and other species. A, phylogenetic analysis of ARF2s. Tree was constructed via neighbor-joining method with 1000 bootstrap replications. B, analysis of conserved domain in ARF2s proteins. C, enrichment analysis of conserved domains of ARF2s proteins.

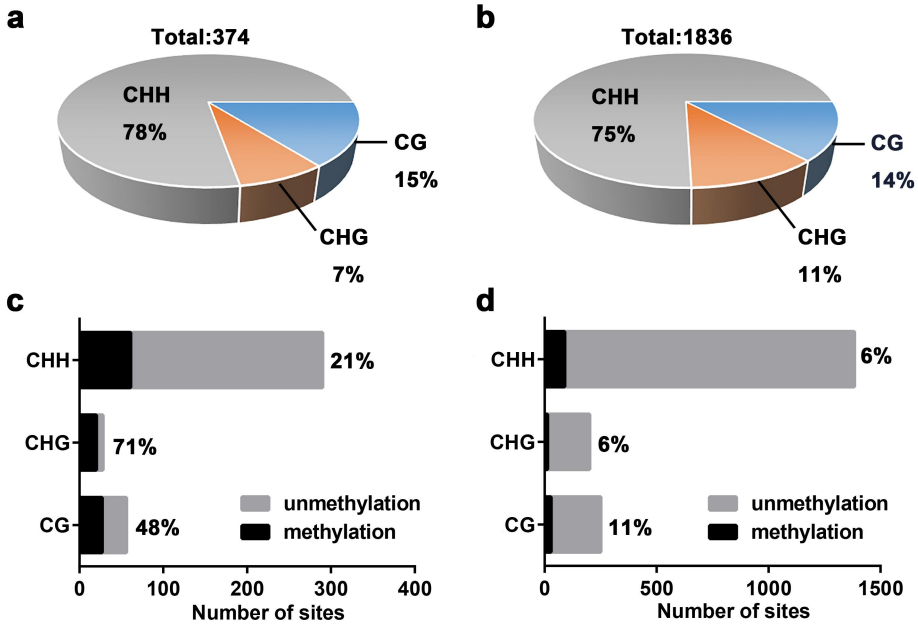

Fig. 2 Methylation analysis of *MdARF2* and *PpARF2*. A, the proportion of three types of methylation sites (CHH, CHG, and CG) in *MdARF2*. B, the proportion of three types of methylation sites in *PpARF2*. C, the number of three types of methylation sites in *MdARF2* and their respective methylation coverage. D, the number of three types of methylation sites in *PpARF2* and their respective methylation coverage.

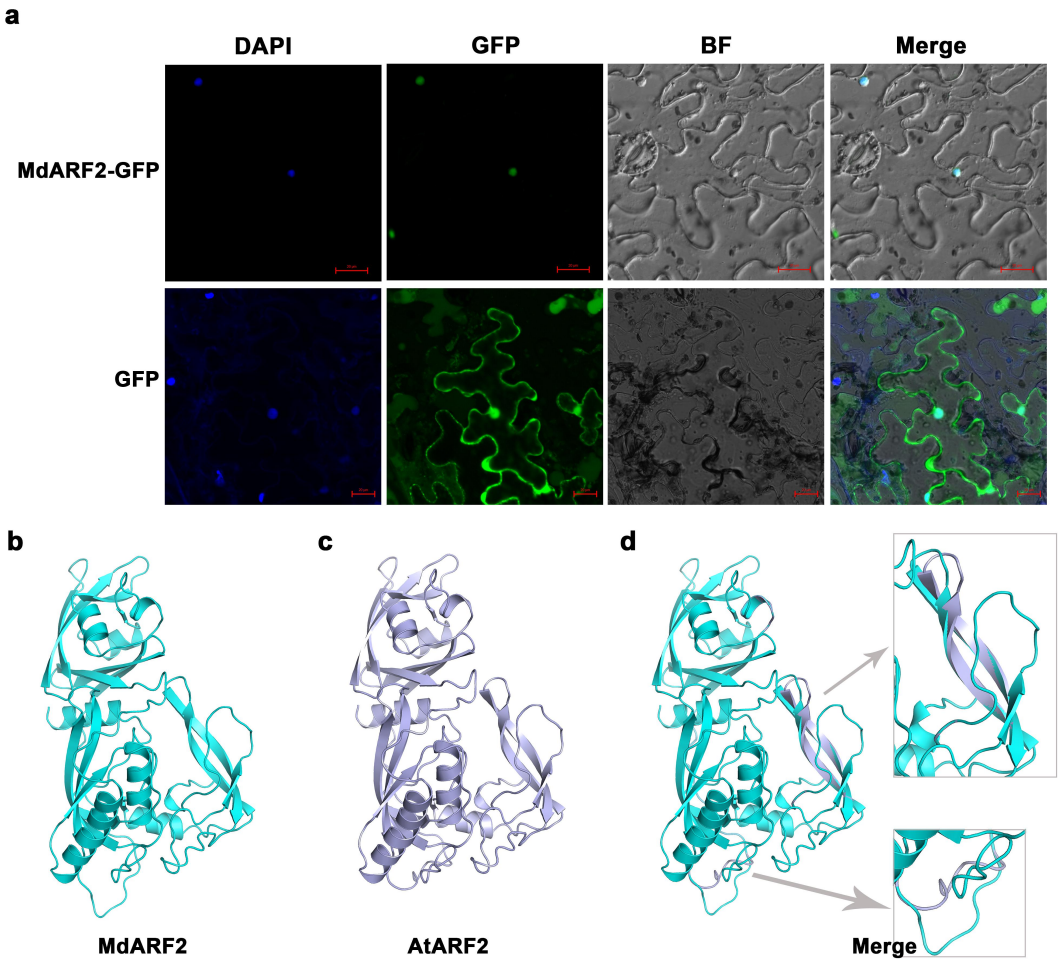

Fig. 3 Subcellular localization and structure prediction of MdARF2. A, subcellular localization of MdARF2. B, three-dimensional structure prediction of MdARF2 protein. C, three-dimensional structure prediction of AtARF2 protein. D, merge of three-dimensional structures of AtARF2 and MdARF2 proteins.

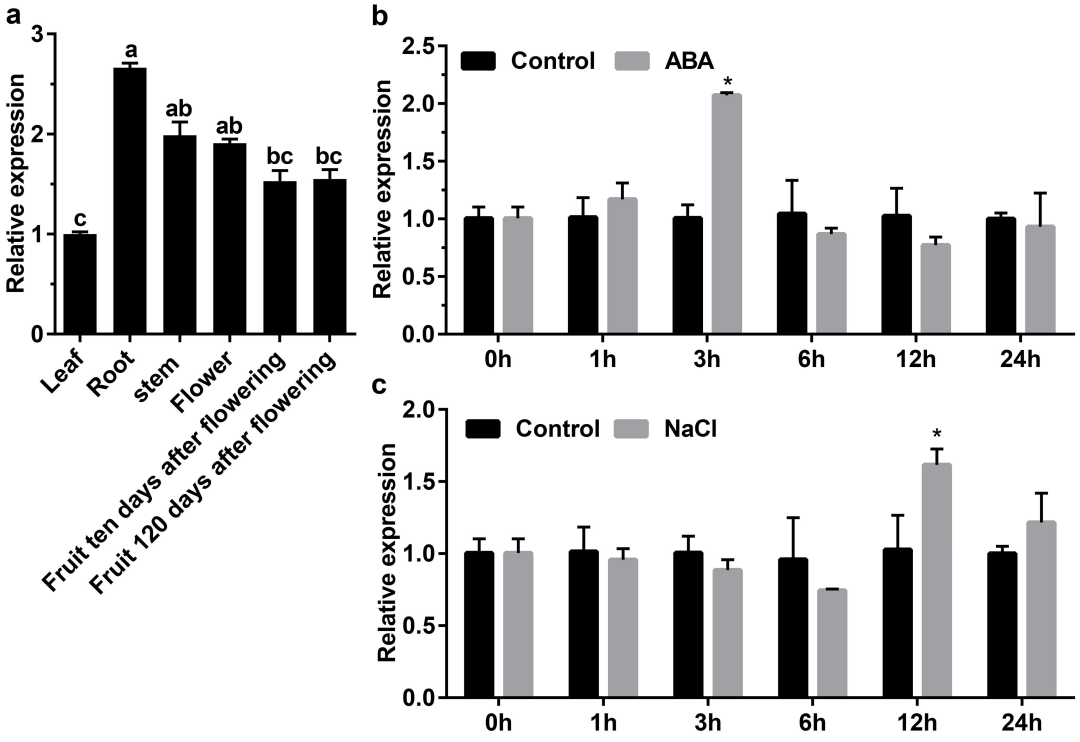

Fig. 4 Expression analysis of *MdARF2*. A, expression analysis of the *MdARF2* gene in different apple tissues with qRT-PCR. B, expression analysis of *MdARF2* in seedlings of Tea crabapple (*Malus*×*hupehensis* Rehd) under NaCl treatment. C, expression analysis of *MdARF2* in seedlings of Tea crabapple (*Malus*×*hupehensis* Rehd) under ABA treatment. The significance diff erence was analysed by DPS.

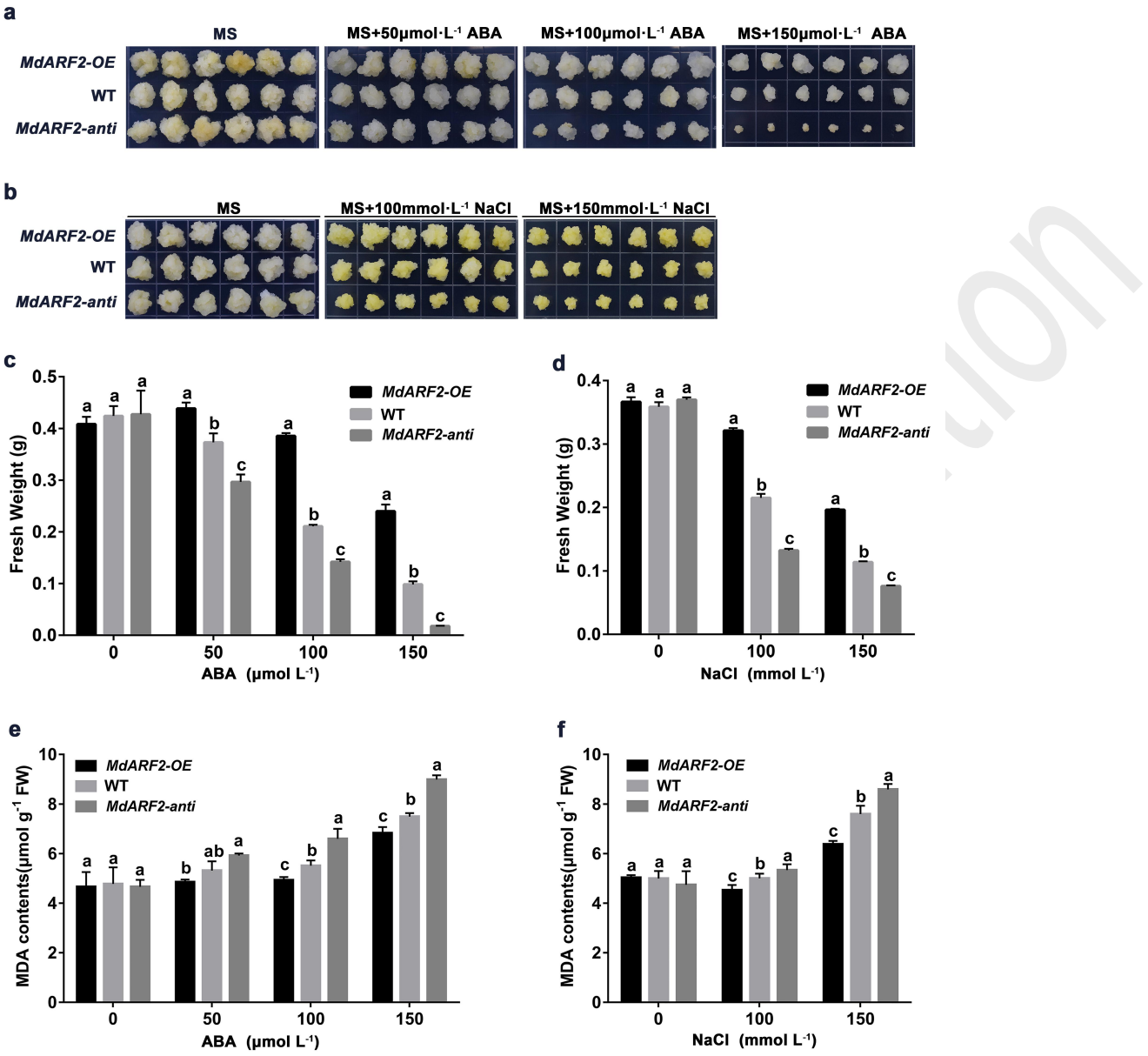

Fig. 5 Overexpression of *MdARF2* increased resistance to ABA and NaCl. A, the phenotypes of apple calli under ABA treatment. B, the phenotypes of apple calli under NaCl treatment. C, fresh weight of apple calli after ABA treatment. D, fresh weight of apple calli after NaCl treatment. E, MDA contents of apple calli under ABA treatment. F, MDA contents of apple calli under NaCl treatment. WT, wild-type; *MdARF2-OE*, overexpressed type; *MdARF2-anti*, antisense type. The significance diff erence was analyzed by DPS.

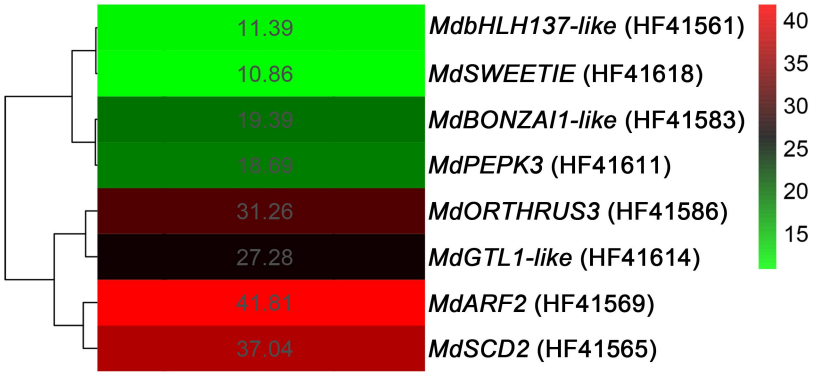

Fig. 6 Candidate gene expression level in QTL region S-H14.2. The heatmap of candidate genes with high expression level (expression level higher than 10).

Table 1 *MdARF2* promoter *cis*-acting element analysis

cis-Element name	*cis*-Element sequence	Function	Start site (bp)	Termination site (bp)
ABRE	ACGTG	*cis*-Acting element involved in the abscisic acid responsiveness	+1370	+1374
ARE	AAACCA	*cis*-acting regulatory element essential for the anaerobic induction	+227	+232
BOX-4	ATTAAT	part of a conserved DNA module involved in light responsiveness	+273	+278
CGTCA-motif	CGTCA	*cis*-acting regulatory element involved in the MeJA responsiveness	-1186	-1190
GC-motif	CCCCCG	enhancer-like element involved in anoxic specific inducibility	+789	+794
O2-site	GTTGACGTGA	*cis*-acting regulatory element involved in zein metabolism regulation	-348	-356
TGA-element	AACGAC	auxin-responsive element	+744	+749
G-box	TACGTG	*cis*-acting regulatory element involved in light responsiveness	-816	-821