Postprint: Identification of the AP2/ERF Gene Family in Aquilegia vulgaris and Expression Analysis Under Salt Stress

Authors: Wang Yufeng, Meng Yuan, Yu Haihang, Cui Dingyuan, white cloud, white cloud

Date: 2022-12-29T00:00:00+00:00

Abstract

AP2/ERF transcription factors play crucial roles in plant growth, development, and responses to abiotic stresses. To investigate the response of AP2/ERF genes to salt stress in Aquilegia vulgaris, this study identified AP2/ERF family genes in A. vulgaris through bioinformatic approaches based on transcriptome data obtained from previous salt stress experiments. The biochemical characteristics, conserved motifs, and phylogenetic relationships were analyzed, and expression changes in roots and leaves at different time points under salt stress treatment were examined, with candidate gene expression validated using qRT-PCR technology. The results demonstrated: (1) A total of 86 AvAP2/ERF genes were identified, encoding proteins ranging from 154 to 722 aa, with relative molecular masses of 14,763.3 to 79,069.47 Da and isoelectric points between 4.49 and 9.68. The majority were acidic proteins, and all were hydrophilic. Subcellular localization prediction revealed that most AvAP2/ERF proteins were localized to the nucleus. (2) The secondary structures were dominated by random coils and α-helices, all possessing the AP2 conserved domain with two highly conserved motifs, Motif 1 and Motif 2. (3) Under salt stress, 71 AvAP2/ERF genes exhibited altered expression, including 18 differentially expressed genes in leaves and 19 in roots. Aquilegia vulgaris and Arabidopsis thaliana AP2/ERF genes were clustered into 5 subfamilies and 15 subgroups. Through expression analysis and homology relationships, three salt stress-responsive genes, AvAP2/ERF-56, AvAP2/ERF-61, and AvAP2/ERF-80, were identified, with qRT-PCR results consistent with transcriptome data. This study establishes a foundation for further investigation into the functions and stress response mechanisms of A. vulgaris AP2/ERF genes.

Full Text

Identification of the AP2/ERF Gene Family in Aquilegia vulgaris and Expression Analysis Under Salt Stress

Yufeng Wang, Yuan Meng, Haihang Yu, Dingyuan Cui, Yun Bai*

College of Forestry and Grassland Science, Jilin Agricultural University, Changchun 130118, China

Abstract

AP2/ERF transcription factors play crucial roles in plant growth, development, and responses to abiotic stress. To investigate the response of AP2/ERF genes to salt stress in Aquilegia vulgaris, we identified members of the AP2/ERF family from transcriptome data obtained under salt stress conditions. Bioinformatics approaches were employed to analyze their biochemical characteristics, conserved motifs, and phylogenetic relationships, while expression changes in roots and leaves at different time points under salt stress were examined. Candidate gene expression was validated using qRT-PCR. The results revealed: (1) Eighty-six AvAP2/ERF genes were identified, encoding proteins of 154–722 amino acids with relative molecular masses of 14,763.3–79,069.47 Da and isoelectric points ranging from 4.49 to 9.68. Most proteins were slightly acidic and all were hydrophilic. Subcellular localization prediction indicated that the majority of AvAP2/ERF proteins were nuclear-localized. (2) The secondary structure was dominated by random coils and α-helices, with all members containing the conserved AP2 domain and two highly conserved motifs, Motif 1 and Motif 2. (3) Under salt stress, 71 AvAP2/ERF genes showed altered expression, with 18 differentially expressed genes in leaves and 19 in roots. Phylogenetic analysis clustered Aquilegia vulgaris and Arabidopsis thaliana AP2/ERF genes into five subfamilies and 15 subgroups. Based on expression analysis and homology, three salt-responsive genes—AvAP2/ERF-56, AvAP2/ERF-61, and AvAP2/ERF-80—were identified, with qRT-PCR results confirming the transcriptome data. This study provides a foundation for further investigation of AvAP2/ERF gene functions and stress response mechanisms.

Keywords: Aquilegia vulgaris, AP2/ERF, bioinformatics, salt stress transcriptome, expression analysis

---

Introduction

AP2/ERF transcription factors constitute a ubiquitous superfamily in plants that participates in growth, development, and environmental stress responses. All family members contain one or two AP2 conserved domains comprising 60–70 amino acids. Based on the number and structure of AP2 domains, the AP2/ERF superfamily can be divided into five subfamilies: AP2, ERF, DREB, RAV, and

Soloist (Gou et al., 2020). In recent years, numerous AP2/ERF transcription factors have been identified and functionally characterized across various plant species, including 147 members in Arabidopsis thaliana (Nakano et al., 2006), 163 in rice (Akhter et al., 2011), 186 in mung bean (Chen et al., 2022), 271 in durum wheat (Faraji et al., 2020), and 116 in pomegranate (Ran et al., 2022).

AP2/ERF transcription factors play vital roles in plant development and in responses to both biotic and abiotic stresses (Feng et al., 2020). The AP2 subfamily primarily regulates plant growth and development; the AP2 transcription factor was first isolated from Arabidopsis by Jofuku et al. (1994) and is associated with flowering physiology. The DREB and ERF subfamilies mainly function in abiotic stress responses (Hong et al., 2020), while the RAV subfamily participates in responses to various biotic and abiotic stresses (Liu et al., 2021). Research on the Soloist subfamily remains relatively limited, though it is known to be involved in the positive regulation of salicylic acid accumulation and basal defense (Wang et al., 2018).

Salt stress is one of the most critical factors affecting plant growth and development, and AP2/ERF transcription factors play pivotal roles in plant salt tolerance. In Arabidopsis, AtERF1 expression is controlled by the interaction of jasmonic acid (JA), ethylene (ET), and abscisic acid (ABA) signaling pathways, serving as a central hub that regulates both salt and drought tolerance (Cheng et al., 2013). Wheat TaERF-6-3A negatively regulates salt tolerance by influencing proline synthesis and suppressing expression of antioxidant-related genes RD29A and P5CS1 (Yu et al., 2022). In upland cotton, GhERF13.12 is affected by ABA signaling and participates in proline biosynthesis; its overexpression in Arabidopsis enhances reactive oxygen species (ROS) scavenging gene expression and improves salt tolerance (Lu et al., 2021). In Salix matsudana, SmAP2-17 can bind to the promoters of SOS3 and ABI5 to activate their expression, playing a key role in salt stress regulation (Chen et al., 2022).

Aquilegia species are important perennial ornamental flowers valued for their unique floral morphology and vibrant colors (M. Carmen et al., 2010; Jesus et al., 2015). However, increasing soil salinization has limited their application, as salt stress significantly reduces their ornamental value. Our preliminary experiments revealed that Aquilegia vulgaris possesses strong cold and salt tolerance, capable of overwintering in northeastern China with minimal management. Based on transcriptome sequencing data from A. vulgaris under salt stress at different time points, this study identified AP2/ERF family genes, conducted bioinformatics analyses, and performed experimental validation to address three questions: (1) What are the physicochemical properties of proteins encoded by the A. vulgaris AP2/ERF family? (2) What are the characteristics of their secondary structures and conserved domains? (3) How do AP2/ERF genes express in roots and leaves under different durations of salt stress, and how can phylogenetic analysis classify them and predict their functions? This work provides a reference for future studies on the biological functions of A. vulgaris AP2/ERF genes in salt resistance.

Materials and Methods

1.1 Transcriptome Sequencing Materials Salt stress transcriptome data for A. vulgaris were obtained from our previous experiments. Seeds preserved by the Ornamental Plant Resources Team at Jilin Agricultural University were sown in June 2020. When seedlings developed six true leaves, healthy plants with similar height and crown width were selected for treatment with 200 mmol·L^{-1} NaCl solution. Root and leaf samples were collected at 0 h (CK), 12 h, 24 h, and 48 h, with three biological replicates. Salt concentration and treatment duration were determined through preliminary experiments. Samples were rapidly frozen in liquid nitrogen and stored at -80°C until transcriptome sequencing and data analysis were performed by Beijing Novogene Bioinformatics Technology Co., Ltd.

1.2 Identification and Bioinformatics Analysis of A. vulgaris AP2/ERF Genes Arabidopsis AP2/ERF family gene sequences were downloaded from TAIR (https://www.arabidopsis.org/) as reference sequences. Local BLAST alignment was performed against the transcriptome sequences with an E-value cutoff of 1e-5. Retrieved nucleotide sequences were translated into protein sequences using TBtools (Chen et al., 2020). Domain prediction was conducted using NCBI (https://www.ncbi.nlm.nih.gov/) and SMART (http://smart.embl-heidelberg.de/), retaining only protein sequences containing complete AP2/ERF domains. A phylogenetic tree was constructed using MEGA 7.0 software with the neighbor-joining method and visualized using iTOL (https://itol.embl.de/). Physicochemical properties including molecular weight, amino acid number, isoelectric point, and average hydrophilicity were analyzed using the online tool pI/Mw (http://web.expasy.org/protparam/). Subcellular localization was predicted using MBC (http://cello.life.nctu.edu.tw/). Protein secondary structure was predicted using Prabi (https://prabi.ibcp.fr/htm/site/web), conserved motifs were analyzed using MEME (http://meme-suite.org/tools/meme), and visualization was performed with TBtools.

1.3 Expression Pattern Analysis of A. vulgaris AP2/ERF Genes Based on the salt stress transcriptome data, AP2/ERF family gene expression levels at 0 h (CK), 12 h, 24 h, and 48 h were normalized using FPKM (fragments per kilobase million). DEGseq software was used to compare treatment and control groups, with $|\log_2(\text{Fold Change})| > 1$ and $P(\text{padj}) < 0.05$ as criteria for differential expression (Niu et al., 2022). Heatmaps were generated using TBtools with Row Scale normalization and Cluster Rows clustering (default parameters for other settings), followed by expression pattern analysis.

1.4 Quantitative Real-Time PCR (qRT-PCR) To validate transcriptome sequencing results and target gene expression patterns, specific primers were designed (Table 1) with IPP2 as the reference gene (Sharma & Kramer, 2013). qRT-PCR was performed to detect expression of selected A. vulgaris

AP2/ERF genes in roots and leaves under salt stress at different time points. SYBR Green I was used to detect PCR products. The reaction mixture contained: 10 L 2×SYBR Mix, 1 L each of forward and reverse primers, 2 L template (cDNA), and 6 L ddH_2O. The PCR program consisted of initial denaturation (95°C, 1 min), followed by 40 cycles of amplification (95°C, 20 s; 60°C, 20 s; 72°C, 30 s), and a melting curve analysis (60°C to 95°C). Each sample had three biological replicates and two technical replicates. Relative expression levels were calculated using the 2^(-ΔΔCt) method, with $\log_2$(Fold Change) used for standardized comparison of relative expression (up- or down-regulation).

Table 1 Primers used for qRT-PCR analysis of Aquilegia vulgaris

Primer name	Sequence (5' -3')
AvAP2/ERF-56F	GTATGGTGCCTCCCCTCGTT
AvAP2/ERF-56R	GCCCCTTGGTCTTGAACCTG
AvAP2/ERF-61F	GCGAAGTAGACGCAATGGACC
AvAP2/ERF-61R	AGCTGGCACTTTACGACGCT
AvAP2/ERF-80F	ATACGAAAGGCGGCAAGTGA
AvAP2/ERF-80R	CAACCCTGGCATTCCAAACTC
IPP2F	CAGGTGAAGACGGACTGAAGTTATC
IPP2R	CCAAGACTGGAAAAAAGACCACAC

Results

2.1 Transcriptome Sequencing Overview A total of 12 transcriptome libraries were obtained. After filtering, each library yielded at least 41,521,502 clean reads and 6.23 Gb clean bases. Q30 values exceeded 94.81% for all 12 libraries, with GC content ranging from 41.51% to 42.72%. Assembly generated 28,088 sequences. The percentage of reads mapped to the reference genome exceeded 70% for all samples, indicating close similarity to the reference species. Biological replicates showed R^2 values greater than 0.85, demonstrating good correlation within groups. To further explore gene changes in various pathways under salt stress, 13 significantly enriched KEGG pathways and 2,197 differentially expressed genes were identified using P(padj) < 0.05 as the screening criterion.

2.2 Identification and Phylogenetic Analysis of the A. vulgaris AP2/ERF Gene Family This study identified 86 AP2/ERF family members from the A. vulgaris transcriptome, designated AvAP2/ERF-1 through AvAP2/ERF-86. A phylogenetic tree constructed with Arabidopsis AP2/ERF genes revealed that AvAP2/ERF members were distributed across five subfamilies: ERF, DREB, AP2, RAV, and Soloist (Figure 1 [Figure 1: see original paper]). The ERF subfamily was further divided into six subgroups (B1–B6), and DREB into six subgroups (A1–A6). Among the 86 AvAP2/ERF genes, 15

(17.44%) belonged to the AP2 subfamily, 37 (43.02%) to ERF, 29 (33.78%) to DREB, 4 (4.65%) to RAV, and 1 (1.16%) to Soloist.

Figure 1 Phylogenetic tree of AP2/ERF genes in Aquilegia vulgaris and Arabidopsis thaliana

2.3 Physicochemical Properties and Subcellular Localization of A. vulgaris AP2/ERF Proteins The 86 AP2/ERF genes encoded proteins ranging from 154 to 722 amino acids, with molecular weights of 14,763.3–79,069.47 Da and isoelectric points from 4.49 to 9.68, suggesting functional diversity. Acidic proteins (54) outnumbered basic proteins (32). All proteins were hydrophilic, with average hydrophilicity values below zero. Subcellular localization predicted five proteins in chloroplasts, one in mitochondria, one in the cytoplasm, and the remaining 79 in the nucleus (Table 2). Genes localized outside the nucleus showed genetic independence and substantial divergence from other family members (Wu et al., 2022). Notably, the Soloist subfamily protein AvAP2/ERF-17 was localized to the cytoplasm.

Table 2 Physicochemical properties and subcellular localization of AP2/ERF proteins in Aquilegia vulgaris

[Note: The table contains data for AvAP2/ERF-1 through AvAP2/ERF-86, showing amino acid number, molecular weight, isoelectric point, average hydrophilicity, and subcellular localization for each gene. The majority are nuclear-localized, with a few in chloroplasts, mitochondria, or cytoplasm.]

2.4 Secondary Structure Analysis of A. vulgaris AP2/ERF Proteins In A. vulgaris AP2/ERF proteins, α-helices and random coils constituted the main secondary structural elements. AP2/ERF transcription factors possess a conserved AP2 domain containing a RAYD element at the C-terminus that forms an amphipathic α-helix, which helps maintain protein stability (Gou et al., 2020) (Table 3).

Table 3 Secondary structure analysis of AP2/ERF proteins in Aquilegia vulgaris

[Note: The table presents secondary structure composition (α-helix, extended strand, random coil) for each AvAP2/ERF gene, showing consistent patterns across the family.]

2.5 Conserved Motif Analysis of A. vulgaris AP2/ERF Proteins MEME analysis revealed that Motif 1 and Motif 2 were the most highly conserved across A. vulgaris AP2/ERF proteins, present in 84 of 86 members except AvAP2/ERF-13 and AvAP2/ERF-23. Other motif types showed subfamily-specific conservation patterns (Figure 2 [Figure 2: see original paper]). Within the same subfamily, one or more additional relatively conserved domains were present: the AP2 subfamily contained Motif 4 and Motif 13,

while the RAV subfamily featured Motif 9 and Motif 15, which were absent in other subfamilies (Figure 3 [Figure 3: see original paper]).

Figure 2 Conserved motifs of AP2/ERF proteins in Aquilegia vulgaris

Figure 3 Distribution of motifs in AP2/ERF proteins of Aquilegia vulgaris

2.6 Expression Pattern Analysis of A. vulgaris AP2/ERF Genes
Heatmaps and cluster analysis of AP2/ERF gene expression in roots and leaves under salt stress at different time points showed that genes with FPKM > 0.05 were considered expressed, with higher values indicating stronger expression. In roots, 69 genes were expressed, while 50 were expressed in leaves, with expression levels showing time-dependent trends (Figure 4 [Figure 4: see original paper]). Differential expression was defined as $|\log_2(\text{Fold Change})| > 1$ and P(padj) < 0.05. Nineteen differentially expressed genes were identified in roots and 18 in leaves, with eight AP2/ERF genes showing differential expression in both tissues. Genes with $\log_2(\text{Fold Change}) > 1$ were upregulated, while those with $\log_2(\text{Fold Change}) < -1$ were downregulated.

In roots, 15 genes were upregulated: AvAP2/ERF-2, -3, -22, -40, -53, and -64 at 12 h; AvAP2/ERF-29, -50, -56, -57, -61, and -80 at 24 h; and AvAP2/ERF-46, -54, -56, and -81 at 48 h. Downregulated genes included AvAP2/ERF-35, -51, -58, and -82 at various time points. In leaves, 13 genes were upregulated: AvAP2/ERF-21, -42, -44, -46, -47, -72, and -86 at 12 h; AvAP2/ERF-80 at 24 h; and AvAP2/ERF-24, -56, -61, -64, and -71 at 48 h. Downregulated genes were AvAP2/ERF-23, -30, -37, -58, and -73 (Figure 5 [Figure 5: see original paper]).

Based on known salt-tolerant AtAP2/ERF genes in Arabidopsis (Table 4) and phylogenetic relationships, AvAP2/ERF-56, -61, and -80 were identified as salt resistance candidates. All three showed significant upregulation in both roots and leaves after salt stress treatment. qRT-PCR validation confirmed that AvAP2/ERF-56 expression gradually increased with salt stress duration in both tissues, showing significant differences at 48 h compared to the control. AvAP2/ERF-61 expression increased progressively in leaves, with significant upregulation at 48 h, while in roots it was significantly upregulated at 12 and 24 h before declining at 48 h. AvAP2/ERF-80 showed increased expression in leaves at 24 and 48 h, and significant upregulation in roots at all time points (12, 24, and 48 h), consistent with transcriptome data (Figure 6 [Figure 6: see original paper]).

Figure 4 Heatmap of AP2/ERF gene expression in Aquilegia vulgaris under salt stress

L and R represent leaf and root, respectively; 0, 12, 24, 48 indicate treatment time (h). The same below.

Figure 5 Heatmap of differentially expressed AP2/ERF genes in Aquilegia vulgaris under salt stress

Asterisks indicate significant differences compared to the control at the specified time point.

Table 4 Functional studies of AP2/ERF family homologous genes in Arabidopsis thaliana

Arabidopsis gene	Aquilegia vulgaris homolog	Function	Reference
AT4G37750 (Soloist)	AvAP2/ERF-37	Controls root cell number and size; negatively regulates salt tolerance	Meng et al., 2015
AT4G13040 (Soloist)	AvAP2/ERF-17	Functions downstream of PAD4 in salicylic acid defense signaling; positively regulates salicylic acid biosynthesis	Mrunmay et al., 2014
AT3G23240 (ERF-B3)	AvAP2/ERF-40, -42, -44, -47	Salt and drought tolerance; hub for JA, ET, and ABA signaling	Cheng et al., 2013
AT3G23230 (ERF-B4)	AvAP2/ERF-50, -51	Promotes ascorbic acid synthesis; salt tolerance	Zhang et al., 2012

Arabidopsis gene	Aquilegia vulgaris homolog	Function	Reference
AT5G13330 (RAP2.6L)	AvAP2/ERF-56, -57	Delays waterlogging-induced senescence via ABI1-mediated ABA signaling; overexpression enhances salt and drought tolerance	Liu et al., 2012; Sowmya et al., 2011
AT4G25480 (DREB1)	AvAP2/ERF-79, -80, -81	Master switch for cold adaptation; participates in ROS scavenging; enhances cold and salt tolerance	Kidokoro et al., 2021
AT2G38340 (DREB19)	AvAP2/ERF-61	Overexpression enhances salt and drought tolerance	Sowmya et al., 2011
AT2G36450 (HRD)	AvAP2/ERF-73, -82, -86	HRD mutants show enhanced salt and drought tolerance; improves photosynthetic efficiency	Karaba et al., 2007

Arabidopsis gene	Aquilegia vulgaris homolog	Function	Reference
AT1G78080 (RAP2.4)	AvAP2/ERF-64, -66, -68	Acts at the intersection of light and ethylene signaling; downregulated by light, upregulated by salt and drought stress	Lin et al., 2008

Figure 6 Relative expression of selected AP2/ERF genes in Aquilegia vulgaris under salt stress

RNA-seq represents transcriptome sequencing results; qRT-PCR represents quantitative real-time PCR results.

Discussion and Conclusion

This study identified 86 AvAP2/ERF genes from salt stress transcriptome data of A. vulgaris and analyzed their physicochemical characteristics, phylogenetic relationships, and expression patterns under salt stress. The 86 AvAP2/ERF genes were classified into 15 AP2 genes, 29 DREB genes, 37 ERF genes, 4 RAV genes, and 1 Soloist gene. In most studied plants, the ERF subfamily contains the most members, followed by DREB, AP2, RAV, and Soloist (Nakano et al., 2006). A. vulgaris shows a similar pattern to Arabidopsis, rice, and other species (Akhter et al., 2011; Chen et al., 2022), suggesting a common evolutionary origin for plant AP2/ERF genes.

Significant variation in molecular weight, isoelectric point, and hydrophilicity among A. vulgaris AP2/ERF transcription factors is consistent with previous reports (Ran et al., 2022), indicating structural complexity and functional diversity within the family. The majority of A. vulgaris AP2/ERF proteins were nuclear-localized, suggesting they function in the nucleus after cytoplasmic synthesis, while those localized to chloroplasts and mitochondria may have distinct functional roles at different signal transduction stages.

The highly conserved AP2 domain is a key structural feature of AP2/ERF transcription factors, typically comprising 60–70 amino acid residues that form a characteristic three-dimensional structure of three β-sheets and one α-helix

(Hong et al., 2020). In A. vulgaris, Motif 1 and Motif 2 were the most conserved elements, forming important parts of the AP2 domain, which aligns with previous findings. Conserved domains and motifs are typically associated with transcription factor function (Sakuma et al., 2002). Genes within the same subfamily of A. vulgaris AP2/ERF transcription factors shared identical or similar conserved motifs, suggesting comparable biological functions and regulatory pathways. For example, the AP2 subfamily contained Motif 4 and Motif 13, which constitute another 72-amino-acid AP2 domain, while the RAV subfamily possessed Motif 9 and Motif 15, important components of the B3 domain—features consistent with the characteristics of these subfamilies (Gou et al., 2020). Subfamily-specific conserved motifs also play important roles in transcriptional regulation.

Homology-based functional prediction combined with expression trend analysis is a common approach for inferring gene function (Ma et al., 2022). Our phylogenetic analysis of 86 A. vulgaris and 147 Arabidopsis AP2/ERF genes divided them into five subfamilies with similar functions. Homology analysis suggested that AvAP2/ERF-37 in the AP2 subfamily may negatively regulate salt tolerance by controlling root cell number and size (Meng et al., 2015). AvAP2/ERF-17 in the Soloist subfamily likely functions downstream of PAD4 in salicylic acid defense signaling, positively regulating salicylic acid biosynthesis to enhance stress resistance (Mrunmay et al., 2014). ERF subfamily members AvAP2/ERF-40, -42, -44, and -47 may serve as central hubs in abiotic stress hormone signal transduction (Cheng et al., 2013); AvAP2/ERF-50 and -51 promote ascorbic acid synthesis to improve salt tolerance (Zhang et al., 2012); AvAP2/ERF-56 and -57 enhance salt and drought tolerance through ABI1-mediated ABA signaling and delayed waterlogging-induced senescence (Liu et al., 2012). DREB subfamily members AvAP2/ERF-79, -80, and -81 may participate in ROS scavenging to improve cold and salt tolerance (Kidokoro et al., 2021); AvAP2/ERF-61 enhances salt tolerance through upregulation (Sowmya et al., 2011); AvAP2/ERF-64, -66, and -68 may function at the intersection of light and ethylene signaling pathways (Lin et al., 2008); and AvAP2/ERF-73, -82, and -86 may improve salt tolerance through downregulation (Karaba et al., 2007).

Gene expression can vary significantly between different tissues (Zhou & Rajesh, 2021; Wang et al., 2022). Our transcriptome analysis revealed 15 upregulated and four downregulated genes in roots, compared to 13 upregulated and five downregulated genes in leaves. Only five genes were upregulated in both tissues, and just one was downregulated in both, indicating distinct temporal and molecular mechanisms of salt stress response in different tissues and functional diversity among genes during salt resistance.

AvAP2/ERF-56, AvAP2/ERF-61, and AvAP2/ERF-80 were significantly upregulated in both roots and leaves of A. vulgaris, indicating their active involvement in salt resistance. Their distinct expression patterns over time suggest different functional roles during salt stress. Homology comparison with known

salt-tolerant Arabidopsis AP2/ERF genes strongly suggests that these three genes are induced by salt stress to enhance expression and confer resistance. qRT-PCR validation confirmed that their expression patterns under salt stress were consistent with transcriptome data.

In summary, this study identified 86 AP2/ERF genes in A. vulgaris, conducted detailed characterization and evolutionary classification, and analyzed their expression patterns under salt stress. Three candidate genes—AvAP2/ERF-56, AvAP2/ERF-61, and AvAP2/ERF-80—were identified as potentially important for salt resistance, though their specific functions require further experimental validation.

References

AKHTER MS, MOHAMMED N, KOUJ S, et al., 2011. Gene structures, classification and expression models of the AP2/EREBP transcription factor family in rice. *Plant Cell Physiol*, 52(2): 344-360.

CHENG MC, LIAO PM, KUO WW, et al., 2013. The Arabidopsis ETHYLENE RESPONSE FACTOR1 regulates abiotic stress-responsive gene expression by binding to different cis-acting elements in response to different stress signals. *Plant Physiol*, 162(3): 1566-1582.

CHEN CJ, CHEN H, ZHANG Y, et al., 2020. TBtools: an integrative toolkit developed for interactive analyses of big biological data. *Mol Plant*, 13(8): 1194-1202.

CHEN HL, HU LL, WANG LX, et al., 2022. Genome-wide identification and expression profiles of AP2/ERF transcription factor family in mung bean (*Vigna radiata* L.). *J Appl Genetics*, 63(2): 223-236.

CHEN Y, H DAI YH, LI YX, et al., 2022. Overexpression of the *Salix matsudana* SmAP2-17 gene improves Arabidopsis salinity tolerance by enhancing the expression of SOS3 and ABI5. *BMC Plant Biol*, 22(1): 102.

CHEN Y, SUN MZ, JIA BW, et al., 2022. Research progress regarding the function and mechanism of rice AP2/ERF transcription factor in stress response. *Acta Agron Sin*, 48(4): 781-790.

FARAJI S, FILIZ E, KAZEMITABAR SK, et al., 2020. The AP2/ERF gene family in *Triticum durum*: genome-wide identification and expression analysis under drought and salinity stresses. *Genes*, 11(12): 1464.

FENG K, HOU XL, XING GM, et al., 2020. Advances in AP2/ERF superfamily transcription factors in plant. *Crc Crit Rev Biotechnol*, 40(6): 750-776.

GOU YL, ZHANG L, GUO H, et al., 2020. Research progress on the AP2/ERF transcription factor in plants. *Pratac Sci*, 37(6): 1150-1159.

HONG L, YANG L, YANG HJ, et al., 2020. Research advances in AP2/ERF transcription factors in regulating plant responses to abiotic stress. *Chin Bull*

Bot, 55(4): 481-496.

JOFUKU KD, BOER BG, MONTAGU MV, et al., 1994. Control of Arabidopsis flower and seed development by the homeotic gene APETALA2. *Plant Cell*, 6(9): 1211-1225.

JESUS MB, PEDRO JR, JULIO MA, 2015. Local adaptation to distinct elevational cores contributes to current elevational divergence of two *Aquilegia vulgaris* subspecies. *J Plant Ecol*, 8(3): 273-283.

KARABA A, DIXIT S, GRECO R, et al., 2007. Improvement of water use efficiency in rice by expression of HARDY, an Arabidopsis drought and salt tolerance gene. *Pans*, 104(39): 15270-15275.

KIDOKORO S, HAYASHI K, HARAGUCHI H, et al., 2021. Posttranslational regulation of multiple clock-related transcription factors triggers cold-inducible gene expression in Arabidopsis. *Pans*, 118(10): e2021048118.

LIN RC, PARK HJ, WANG HY, 2008. Role of Arabidopsis RAP2.4 in regulating light- and ethylene-mediated developmental processes and drought stress tolerance. *Mol Plant*, 1(1): 42-57.

LIU JL, DENG ZW, LIANG CL, et al., 2021. Genome-wide analysis of RAV transcription factors and functional characterization of anthocyanin-biosynthesis-related RAV genes in pear. *Int J Mol Sci*, 22(11): 5567.

LIU PQ, SUN F, GAO R, et al., 2012. RAP2.6L overexpression delays waterlogging induced premature senescence by increasing stomatal closure more than antioxidant enzyme activity. *Plant Mol Biol*, 79(6): 609-622.

LU LL, QANMBER G, LI J, et al., 2021. Identification and characterization of the ERF subfamily B3 group revealed GhERF13.12 improves salt tolerance in upland cotton. *Front Plant Sci*, https://doi.org/10.3389/fpls.2021.705883.

MA YC, HAO YM, HUANG ML, et al., 2022. Identification and expression analysis of CesA gene family in *Brassica rapa*. *Guihaia*: 1-14. http://kns.cnki.net/kcms/detail/45.1134.q.20220104.2313.011.html.

M.CARMEN M, JORDI LP, MARIA B, et al., 2010. Low genetic variability in the rare, recently differentiated *Aquilegia paui* (Ranunculaceae). *Biochem Syst Ecol*, 38(3): 390-397.

MENG LS, WANG YB, YAO SQ, et al., 2015. Arabidopsis AINTEGUMENTA mediates salt tolerance by trans-repressing SCABP8. *J Cell Sci*, 128(15): 2919-2927.

MORAN NC, COLE T, LOYAL G, et al., 2011. Integrative annotation of human large intergenic noncoding RNAs reveals global properties and specific subclasses. *Gene Dev*, 25(18): 1915-1927.

MRUNMAY KG, SWADHIN S, JANESH KG, et al., 2014. The Arabidopsis thaliana At4g13040 gene, a unique member of the AP2/EREBP family, is a pos-

itive regulator for salicylic acid accumulation and basal defense against bacterial pathogens. *Aust J Plant Physiol*, 171(10): 981-994.

NAKANO T, SUZUKI K, FUJIMURA T, et al., 2006. Genome-wide analysis of the ERF gene family in Arabidopsis and rice. *Plant Physiol*, 140(2): 411-432.

NIU SY, LIANG F, ZHANG ZH, et al., 2022. Identification and analysis of TCP transcription factors in *Solanum tuberosum* response to low nitrogen fertilizer stress. *Guihaia*: 1-14. http://kns.cnki.net/kcms/detail/45.1134.Q.20220711.1205.002.html.

RAN W, SONG JH, LV ZY, et al., 2022. Genome-wide identification and comprehensive analysis of the AP2/ERF gene family in pomegranate fruit development and postharvest preservation. *Genes*, 13(5): 895.

SAKUMA Y, LIU Q, G.DUBOUZET J, et al., 2002. DNA-binding specificity of the ERF/AP2 domain of Arabidopsis DREBs, transcription factors involved in dehydration- and cold-inducible gene expression. *Biochem Biophys Res Comm*, 290(3): 998-1009.

SHARMA B, KRAMER E, 2013. Sub- and neo-functionalization of APETALA3 paralogs have contributed to the evolution of novel floral organ identity in *Aquilegia* (columbine, Ranunculaceae). *New Phytol*, 197(3): 951-959.

SOWMYA K, SHIV V, MUHAMMAD HR, et al., 2011. Functional characterization of four APETALA2-family genes (RAP2.6, RAP2.6L, DREB19 and DREB26) in Arabidopsis. *Plant Mol Biol*, 75(1-2): 107-127.

WANG HB, GONG M, GUO JY, et al., 2018. Molecular cloning and prokaryotic expression of orphan gene Soloist of AP2/ERF gene family in *Jatropha curcas*. *Sci Silv Sin*, 54(9): 60-69.

WANG LJ, WANG Y, LU B, et al., 2022. Identification and expression analysis of AP2/ERF transcription factor family genes in *Olea europaea* under water stress. *Guihaia*: 1-15. http://kns.cnki.net/kcms/detail/45.1134.Q.20210818.1342.004.html.

WU CX, LIU XW, LI ZJ, et al., 2022. Analysis of chloroplast genome of rice Dalixiang. *Guihaia*: 1-14. http://kns.cnki.net/kcms/detail/45.1134.q.20220424.1933.004.html.

YU Y, YU M, ZHANG SX, et al., 2022. Transcriptomic identification of wheat AP2/ERF transcription factors and functional characterization of TaERF-6-3A in response to drought and salinity stresses. *Int J Mol Sci*, 23(6): 3272.

ZHANG ZJ, WANG J, ZHANG RX, et al., 2012. The ethylene response factor AtERF98 enhances tolerance to salt through the transcriptional activation of ascorbic acid synthesis in Arabidopsis. *Plant J*, 71(2): 273-287.

ZHOU LX, RAJESH Y, 2021. Genome-wide identification and characterization of AP2/ERF transcription factor family genes in oil palm under abiotic stress conditions. *Int J Mol Sci*, 22(6): 2821.

Note: Figure translations are in progress. See original paper for figures.