

Identification and Expression Analysis of the *Magnolia officinalis* AP2/ERF Gene Family Based on Different Age Groups

Hui Tian

Mianyang Normal University

Qian Wang

Mianyang Normal University

MingXin Zhong

3387445190@qq.com

Mianyang Normal University

Yuanyuan Zhang

Mianyang Normal University

Bainian Zhang

Mianyang Normal University

Chengjia Tan

Mianyang Normal University

Zhuo Xu

Mianyang Normal University

Xin Hu

Mianyang Normal University

Daren Feng

Mianyang Institute for Food and Drug Control

Zhenpeng Xi

Beichuan Shennong Agriculture Technology Development Co., Ltd

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5

6 Corresponding authors:

7 1. Hui Tian

8 E-mail address: tianhui1009@126.com (Hui Tian)

9 Tel.: 0838-18981157657 (Hui Tian)

10 Mailing address: No. 166 Mianxing West Road, Fucheng District, Mianyang City, Sichuan Province,

11 China

12 2. Qian Wang

13 E-mail address: 1315522095@qq.com (Qian Wang)

14 Tel.: 0838-1820280893 (Qian Wang)

15 Mailing address: No. 166 Mianxing West Road, Fucheng District, Mianyang City, Sichuan Province,

16 China

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1 **Identification and Expression Analysis of the *Magnolia officinalis* AP2/ERF Gene Family Based**
2 **on Different Age Groups**

3 Mingxin Zhong^{1#}, Yuanyuan Zhang^{1#}, Qian Wang^{1*}, Hui Tian^{1*}, Bainian Zhang¹, Chengjia Tan¹, Zhuo
4 Xu¹, Xin Hu¹, Daren Feng², Zhenpeng Xi³

5
6
7 1 Key Laboratory of Quality Control of Traditional Chinese Medicine in Northwest Sichuan, Mianyang
8 Normal University, Mianyang 621000, China

9 2 Mianyang Institute for Food and Drug Control, Mianyang 621000, China

10 3 Beichuan Shennong Agriculture Technology Development Co., Ltd., Mianyang 621000, China

11

12

13 * Corresponding authors

14

15 E-mail address: 1315522095@qq.com (Qian Wang); tianhui1009@126.com (Hui Tian)

16 Links to the profile page of the institution where the corresponding author is located:

17 <https://sky.mtc.edu.cn/info/1017/2139.htm> (Hui Tian)

18

19 # MX Z and YY Z contributed equally to this work and should be considered co-first authors.

20 ORCID iD: 0009-0008-8124-5689 (Mingxin Zhong)

Abstract

Objective The purpose of this study was to identify the AP2/ERF gene family in *Magnolia officinalis* (M. officinalis) and to examine changes in AP2/ERF gene expression and functional annotation as M. officinalis grows. **Methods** The transcriptome data of M. officinalis were evaluated and compared to those of the model plant *Arabidopsis thaliana*. After screening, 75 distinct AP2/ERF gene family sequences (HoAP2/ERF1 ~ HoAP2/ERF75) were identified and analyzed via bioinformatics methods. The expression levels of XR and DR in the two groups were determined using Heatmap Tree, and genes with substantial expression were examined and functionally annotated. **Results** The 75 HoAP2/ERF proteins all had one or more conserved AP2 domains. Its protein stability is usually indicative of an unstable protein. HoAP2/ERF family proteins are hydrophilic. Subcellular research revealed that it was primarily located in the nucleus. The expression levels of certain AP2/ERF genes differed considerably across the XR and DR groups, with XR being overexpressed compared to those in the DR group. **Conclusion** M. officinalis' AP2/ERF gene family is divided into four subfamilies. Its role not only is related to M. officinalis growth and development, as well as secondary metabolism but also affects its stress tolerance. Furthermore, the low-aged group of M. officinalis XR exhibited greater stress tolerance than did the high-aged group of M. officinalis DR. Among the significantly expressed genes, HoAP2/ERF55 controls the activity of imidazole glycerol phosphate synthase, while HoAP2/ERF39 plays a role in plant–pathogen interactions by regulating the expression of PR genes.

Keywords: *Magnolia officinalis*; AP2/ERF transcription factor; Bioinformatics; Resistance against the environment

1 Introduction

Magnolia officinalis Rehd.et Wils. and *Magnolia officinalis* Rehd.et Wils.var.*biloba* Rehd.et Wils. are widely distributed in southern China. The dried bark, root bark and branch bark of *Magnolia officinalis* (M. officinalis) can be used as medicine after processing. The medicinal Magnolia bark in the 'Chinese Pharmacopoeia' is commonly used to treat dampness injury, phlegm cough and other symptoms^[1]. Lignans (including magnolol and honokiol), the main active components of M. officinalis, have many potential pharmacological effects, such as antitumor, antiviral, antibacterial, anti-inflammatory, antioxidative, liver function-protective, and cardiovascular disease effects(Liao et al. 2015; Pan et al. 2024; Shen et al. 2017; Xian et al. 2015; Zhang et al. 2020). Bioinformatics has previously investigated and discovered certain genes associated with the MVA pathway or the Dirigent gene family of M. officinalis, providing a theoretical basis for the molecular mechanism of M. officinalis biosynthesis(Zha et al. 2015; Zhang et al. 2024).

The *AP2/ERF* gene family has a conserved DNA binding domain of approximately 60 amino acids(Liang et al. 2020). *AP2/ERF* transcription factors are classified into five subfamilies: AP2, ERF, RAV, DREB, and Soloist(Sakuma et al. 2002). Five *AP2/ERF* subfamily transcription factors are widely involved in various physiological processes in plants, including plant growth, fruit ripening, flower development, and other plant development processes, as well as stress response activities such as injury, pest defense, high salt, and drought(Feng et al. 2020; Xie et al. 2022; Xie et al. 2019). Furthermore, the *AP2/ERF* gene family impacts secondary metabolite synthesis in addition to plant growth and development(Ma et al. 2017; Pan et al. 2019; Zhao et al. 2021).

Currently, the *AP2/ERF* gene family of many plants has been identified, and the number of *AP2/ERF* transcription family members varies substantially among species(Ji et al. 2016; Nakano et al.

2006; Song et al. 2013; Zhuang et al. 2011; Zhuang et al. 2010). *M. officinalis* requires more than ten years to mature for medical use due to its slow growth rate. Second, *M. officinalis* is becoming increasingly vulnerable, necessitating improved seedling management and protection of the mother tree. Understanding the *AP2/ERF* family genes involved in growth and development, as well as stress, at the gene level provides a foundation for choosing an appropriate growing environment and enhancing *M. officinalis* survival. As a result, the primary goal of this work was to identify and evaluate the *AP2/ERF* gene family of *M. officinalis*, as well as to compare the expression of *AP2/ERF* transcription factors in two groups of *M. officinalis* with varying growth tree ages in the same area.

2 Materials and Methods

2.1 Plant materials

The material used in the experiment was *Magnolia officinalis* Rehd. et Wils, which is derived from the trunk epidermis (longitude and latitude: 104° 30' 39.00", latitude 31° 58' 46.38") of *Magnolia officinalis* Rehd. et Wils. at the planting base of Wahugou village, Beichuan Qiang Autonomous County, Mianyang city, Sichuan Province. The participants were divided into two groups according to growth age. Three *Magnolia officinalis* (*M. officinalis*) trees from each group were collected for biological replicates.

2.2 Identification of *AP2/ERF* family members in *M. officinalis*

After being frozen in liquid nitrogen, the trunk epidermis of *M. officinalis* was transferred to Major Biotechnology Co., Ltd., for second-generation transcriptome sequencing. After eliminating the joints and low-quality reads, clean data were produced. Using the scaffold-level *M. officinalis* genome sequence published by Chengdu University of Traditional Chinese Medicine, the complete coding gene sequence of *M. officinalis* was retrieved by sequence splicing, and each sequence was annotated in the

1 database. The amino acid sequences of 135 Arabidopsis *AP2/ERF* proteins were retrieved from TAIR
2 (<https://www.arabidopsis.org/index.jsp>).

3 The amino acid sequences of the *M. officinalis* full-length transcriptome and 135 members of the
4 Arabidopsis *AP2/ERF* gene family were compared and analyzed using local BLAST software. High-
5 reliability candidate sequences were screened using the following threshold conditions: E-value $< 1 \times$
6 10^{-10} , score ≥ 100 , and one terminator. Second, the information gathered from the preceding step is
7 summarized, duplicate data are removed, and redundant sequences are eliminated. The potential
8 *AP2/ERF* sequence was subsequently analyzed using SMART online software to determine whether it
9 has a complete conserved AP2 domain, indicating that it is an *AP2/ERF* family protein. Finally, the
10 amino acid sequence of the *M. officinalis* *AP2/ERF* protein was obtained.

11 The 135 Arabidopsis *AP2/ERF* protein sequences that have been clearly published, as well as the
12 *abovementioned* Magnolia *AP2/ERF* protein sequences, were loaded into MEGA11.0 software to
13 construct a phylogenetic tree. After importing all of the sequences, Align by ClustalW was used for
14 multiple sequence alignment, and the neighbor-joining method was used to construct the phylogenetic
15 tree of the Magnolia *AP2/ERF* and Arabidopsis *AP2/ERF* protein families (with the exception of
16 changing the test of phylogeny to the bootstrap method, increasing the number of bootstrap replicates
17 to 1000, and leaving the remaining parameters as defaults).

18 **2.3 Physicochemical property analysis and subcellular localization prediction**

19 The *AP2/ERF* protein sequence of *M. officinalis* was analyzed by ProtParam
20 (<https://www.ExPASy.org/resources/protparam>) in the online analysis software ExPASy to obtain
21 information such as the length of the encoded amino acid, total number of positively and negatively
22 charged residues, relative molecular mass, theoretical isoelectric point, instability coefficient, fat

coefficient, and total average hydrophilicity. The subcellular localization of the *M. officinalis AP2/ERF* protein sequence was then determined using Plant-mPLOC and Cell-PLOC 2.0 (<http://www.csbio.sjtu.edu.cn/bioinf/Cell-PLOC-2/>).

2.4 Structure prediction analysis of *M. officinalis AP2/ERF* proteins

Protein secondary structures include α -helices, β -sheets, β -turns, and random coils. The secondary structure prediction module was entered into Prabi online analysis software, and SOPMA (https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=npsa_sopma.html) was used to predict the secondary structure of the *M. officinalis AP2/ERF* protein.

The protein domain represents the position and relationship of amino acid residues to specific functions in the peptide chain. The internet software MEME (<http://meme-suite.org/tools/meme>) was used to predict the *AP2/ERF* protein motif. The number of motif predictions was fixed at ten, with a minimum length of six amino acids and a maximum length of fifty. MEGA11.0 was subsequently used for multiple sequence alignment of the *M. officinalis AP2/ERF* amino acid sequence.

The *AP2/ERF* protein sequence of *M. officinalis* was examined using the online software SWISS-MODEL (<https://www.swissmodel.ExPASy.org/>). The model was developed based on the template results with the highest similarity, and the tertiary structure of the *AP2/ERF* protein of *M. officinalis* was determined.

2.5 Expression analysis and functional annotation

The *AP2/ERF* transcription factors in the two groups of *M. officinalis* tissues were classified and functionally analyzed for similar homologous genes in the phylogenetic tree of the *M. officinalis AP2/ERF* family, and the transcription analysis expression data were used to construct a heatmap tree, from which the changes in *AP2/ERF* gene expression at different tree ages were obtained. Second, the

1 AP2/ERF transcription factors that were overexpressed in both groups were identified in the GO and
2 KEGG databases.

3 **3 Results**

4 **3.1 Identification of the *AP2/ERF* gene family**

5 The full-length amino acid sequence of *Magnolia officinalis* (M. officinalis) was compared to the
6 *AP2/ERF* family protein sequence of *Arabidopsis thaliana* via local BLAST. After the comparison,
7 11553 amino acid sequences were retrieved. After information summary, we excluded repetitive items,
8 sequences with E-values greater than 1×10^{-10} , and those with a score less than 100. A total of 89
9 amino acid sequences were found. Amino acid sequences that had two or more terminators and the
10 same amino acid sequence were deleted. The amino acid sequences with the AP2 domain were tested
11 using the SMART network online software. After screening and reducing redundancy, 75 *AP2/ERF*
12 sequences with AP2 domains were identified and called HoAP2/ERF1 ~ HoAP2/ERF75, respectively.

13 The 75 *AP2/ERF* protein sequences of M. officinalis were aligned with the *AP2/ERF* protein
14 sequences of the model plant *Arabidopsis thaliana* using MEGA11.0 software, and a neighbor-joining
15 phylogenetic tree was generated. The results showed that the phylogenetic tree was separated into four
16 subfamilies: AP2, Soloist, ERF, and DREB (Fig. 1).

17 **3.2 Physicochemical property analysis and subcellular localization of proteins**

18 The results of ExPASy's physicochemical study of the *AP2/ERF* protein sequence of M.
19 officinalis revealed that most *AP2/ERF* proteins had an average amino acid chain length of 306 aa, with
20 a range of 72 to 664 aa. HoAP2/ERF51 had the shortest amino acid length, 72 aa, while HoAP2/ERF57
21 had the largest amino acid length, 664 aa, indicating a significant difference in length. The relative
22 molecular weight is approximately 33779.84 Da. The *AP2/ERF* protein of M. officinalis has a high

degree of variability in the acid–base range, as indicated by the theoretical isoelectric point of 4.83 ~ 9.65. Similarly, the theoretical isoelectric point of less than 7.00 indicates that the majority of the DREB subfamily proteins are acidic and that the number of acidic *AP2/ERF* proteins is greater than that of alkaline *AP2/ERF* proteins. According to a study on protein stability, only nine *AP2/ERF* proteins were stable; practically all proteins had instability coefficients greater than 40, indicating that they were unstable. The range of the protein fat coefficient was 46.31 to 76.84. All of the hydrophilic *AP2/ERF* family proteins in *M. officinalis* had an average total negative hydrophilicity.

Subcellular localization studies revealed that the majority of *M. officinalis AP2/ERF* gene family proteins were found in the nucleus (56), with a smaller number of proteins found in the cytoplasm (6) and some in both (13–14). The nucleus is where the *AP2/ERF* protein is primarily localized within the cell (Table 1).

3.3 Structure prediction analysis of *M. officinalis AP2/ERF* proteins

The secondary structure prediction of the *AP2/ERF* gene family proteins of *M. officinalis* indicated a significant proportion of α -helices and random coils. The AP2 domain is highly conserved, as evidenced by the large proportion of α -helices in Ho*AP2/ERF*30, and the remaining concentration ranges from 20.00 to 40.00%. The proportion of irregular curls ranges from 32.35 to 71.07%, making it the highest. The proportions of Ho*AP2/ERF*30 and Ho*AP2/ERF*51 are quite low. This suggests that the secondary structure of Ho*AP2/ERF*30 is more conserved and consistent. The proportion of β -sheets is the lowest. The proportion of extended chains is approximately 2.73 ~ 17.41% (Fig. 2).

The conserved motifs of 75 *AP2/ERF* amino acid sequences of *M. officinalis* were predicted and analyzed by the online analysis software MEME. The amino acid sequences of *AP2/ERF*s often include Motif1, Motif2, Motif3, and Motif4, which are processed by protein α -helices, β -sheets, extensions,

1 and random coils to produce conserved AP2 domains (Fig. 3a; Fig. 3b). The AP2 domain is created in
2 the following order: Motif 2, Motif 4, Motif 1, and Motif 3, followed by Motif 8 and Motif 5. The
3 conserved motifs show that most *AP2/ERF* proteins, in addition to the AP2 domain, are highly
4 conserved due to their unstable structure, which causes the deletion of some genes.

5 The plant *AP2/ERF* domain is a three-dimensional structure composed of three antiparallel β -
6 sheets, an α -helix parallel to the β -sheet, and a fraction of random coils (Donde et al. 2019). The
7 majority of *AP2/ERF* gene family proteins in the SWISS-MODEL online model have a Seq-identity
8 greater than 70%. There are seven *AP2/ERF* gene family proteins that resemble the ERF structural
9 model of Gcc-box binding. There are 32 comparable ethylene-responsive transcription factor or ERF
10 crystal structure models, as well as 31 similar *AP2/ERF*-related protein models, AP2-like ethylene-
11 responsive transcription factor models, and CBF-like transcription factor models (Fig. 4).

12 **3.5 Expression analysis and functional annotation**

13 The 75 *AP2/ERF* family genes in the development tree were expressed in the order of DREB to
14 ERF in the lower age group (XR1, XR2, and XR3) and the higher age group (DR1, DR2, and DR3) of
15 *M. officinalis* to form a heatmap tree (Fig. 5). HoAP2/ERF31 and HoAP2/ERF32, which share
16 significant homology, were overexpressed in all samples from the DREB subfamily. In addition, the
17 expression of other *AP2/ERF* genes in the low-age group of XR was greater than that in the high-age
18 group of DR. On the large branch of the AP2 subfamily, HoAP2/ERF62 and HoAP2/ERF70, which are
19 highly homologous, and HoAP2/ERF66 ~ HoAP2/ERF58, which are located in the last small branch of
20 the AP2 subfamily, also showed that the expression level of XR in the low-age group was greater than
21 that of DR in the high-age group. Furthermore, HoAP2/ERF71 and HoAP2/ERF69 of the Soloist
22 subfamily exhibited strong expression in the XR low age group and the DR high age group.

1 Intriguingly, there are more active *AP2/ERF* genes in the ERF subfamily, such as *HoAP2/ERF39*,
2 *HoAP2/ERF36*, *HoAP2/ERF37*, *HoAP2/ERF55*, *HoAP2/ERF45*, *HoAP2/ERF56*, *HoAP2/ERF22*,
3 *HoAP2/ERF20*, *HoAP2/ERF1*, and *HoAP2/ERF43*, which are highly expressed in both the DR and XR
4 (Fig. 6).

5 We performed GO and KEGG functional annotations on these 15 highly expressed *AP2/ERF*
6 genes and obtained functional classifications for 11 of them in the GO database, which included three
7 types of biological processes, cellular components, and molecular functions (Fig. 7). Among these
8 genes, *HoAP2/ERF22* and *HoAP2/ERF1* have biological regulatory functions. *HoAP2/ERF22*,
9 *HoAP2/ERF60*, *HoAP2/ERF32*, *HoAP2/ERF71*, and *HoAP2/ERF1* can respond to stimuli. Notably,
10 *HoAP2/ERF55* can activate imidazole glycerol phosphate synthase in His biosynthesis. In vivo,
11 *HoAP2/ERF39* serves as a transcriptional activator of *Pti6*, activating the expression of numerous PR
12 genes with GCC boxes and playing an important and unique role in plant defense.

13 **4 Discussions**

14 The *AP2/ERF* gene family plays a crucial role in plant development and metabolism. It has a large
15 number of branches and a diverse set of functions. In recent years, the *AP2/ERF* gene family has been
16 found in a variety of medicinal plants, including *Salvia miltiorrhiza*, *Artemisia annua*, and
17 *Catharanthus roseus*. As a high-quality source of Chinese medicinal materials, 75 *AP2/ERF* genes of
18 *Magnolia officinalis* (*M. officinalis*) were extracted and identified using second-generation
19 transcriptome sequencing data, followed by a series of bioinformatics analyses to reveal the *AP2/ERF*
20 gene family sequence of *M. officinalis*.

21 The *AP2/ERF* gene family of *M. officinalis* identified in this study can be divided into four
22 branches: DREB (18), AP2 (18), Soloist (5) and ERF (34). These 75 *HoAP2/ERF1*–*HoAP2/ERF75*

1 genes were classified based on the distinct conserved AP2 domains found in the *AP2/ERF* gene
2 subfamilies. HoAP2/ERF57 ~ HoAP2/ERF59, HoAP2/ERF62 ~ HoAP2/ERF67, and HoAP2/ERF70,
3 which are typical AP2 subfamilies of the *AP2/ERF* gene family, contain two conserved AP2 domains.
4 There are still 9 HoAP2/ERF transcription factors in the phylogenetic tree that are homologous to the
5 AP2 subfamily and in the same branch; both the ERF and DREB subfamilies contain only one AP2
6 domain, but the 14th and 19th amino acids on the AP2 domain are different. The 14th and 19th amino
7 acids in the AP2 domain of the ERF subfamily are alanine and aspartic acid, respectively, while the
8 14th and 19th amino acids in the DREB subfamily are valine and glutamic acid(Zhang et al. 2012;
9 Shao et al. 2020). The difference between these two amino acid residues precisely dictates the binding
10 of these two subfamilies to distinct cis-acting elements. The RAV subfamily includes AP2 and a B3
11 domain(Kagaya et al. 1999; Luo et al. 2013), and the N-terminus contains the AP2 domain, which has
12 the ability to attach to GCC-box components. The C-terminus contains the B3 domain, which has the
13 ability to bind only to the CACCTG sequence. However, no B3 domain was detected in the *AP2/ERF*
14 gene family, indicating that no *AP2/ERF* gene was a member of the RAV subfamily. Although soloist
15 *AP2/ERF* transcription factors also have an AP2 domain, their gene structure and amino acid patterns
16 are different from those of other *AP2/ERF* transcription factor subfamilies(Dossa et al. 2016). Its
17 nucleic acid sequence is highly conserved in most plants. This time, the five *AP2/ERF* transcription
18 factors that make up the Soloist class are separated from the preceding AP2 subfamilies by the AP2
19 conserved domain.

20 The family proteins are all hydrophilic, with a PI range of 4.83 ~ 9.65. The AP2 domain forms in
21 motif order (Motif2, Motif4, Motif1, Motif3, Motif8, and Motif5) and is strongly retained by
22 polymerization, such as the α -helix, which is more easily dissolved and unstable. The *AP2/ERF* gene

1 family proteins of *M. officinalis* are primarily found in the nucleus, with only a few found in the
2 cytoplasm, indicating that the family plays a variety of roles.

3 The majority of the *AP2/ERF* genes are classified as dehydration-responsive element-binding
4 proteins (DREB), which are primarily involved in the response to ABA, drought, low temperature, and
5 other environmental stresses. Overexpression of the DREB subfamily increases the tolerance of
6 *Magnolia* to osmotic, high salt, and cold stressors. The primary purpose of the AP2 subfamily is to
7 govern the growth of flowers, seeds, and ovules, as well as the buildup of seed oil and protein(Jofuku et
8 al. 2005). *M. officinalis* has a high seed oil content and is closely related to the AP2 subfamily. Its
9 postflowering fruit are also closely tied to altitude. At lower altitudes, saplings grow quickly, and adult
10 trees grow slowly, whereas at higher elevations, the opposite occurs. The *M. officinalis* used in this
11 study was found in Wahugou village, approximately 1500 meters above sea level. The expression of the
12 AP2 subfamily is modest, particularly in adult trees. *HoAP2/ERF71* and *HoAP2/ERF69* of the Soloist
13 subfamily are highly expressed in *M. officinalis*, indicating that they play a significant role in this
14 process. The ERF subfamily is not only involved in the response to biological stressors such as
15 ethylene and infections(Ohme-Takagi and Shinshi 1995); however, some academics have screened the
16 ERF subfamily gene *LTF1*, which has an AP2 conserved domain, and established that *LTF1* has an
17 upregulatory effect on genes involved in the lignan synthesis pathway, increasing the accumulation of
18 lignans(Gui et al. 2020). The ERF subfamily of *M. officinalis* has many branches and is strongly
19 expressed. It is thought that it may interact with small molecular compounds or bacteria in its own
20 environment, causing overexpression through mutual collaboration, thus boosting *M. officinalis*
21 tolerance.

22 Through the expression of *M. officinalis* at different ages, we found that the *M. officinalis* XR low

1 age group had stronger environmental tolerance than did the DR high age group, implying that *M.*
2 *officinalis* had a strong tolerance level during the rapid growth of the juvenile stage to resist possible
3 adverse factors from the external environment(Hu et al. 2023). Notably, *HoAP2/ERF32*,
4 *HoAP2/ERF31*, *HoAP2/ERF60*, *HoAP2/ERF71*, *HoAP2/ERF69*, *HoAP2/ERF39*, *HoAP2/ERF36*,
5 *HoAP2/ERF37*, *HoAP2/ERF55*, *HoAP2/ERF45*, *HoAP2/ERF56*, *HoAP2/ERF22*, *HoAP2/ERF20*,
6 *HoAP2/ERF1* and *HoAP2/ERF43* belong to the whole *AP2/ERF* gene family. These genes are highly
7 expressed and are involved in biological processes, cellular components, and molecular functions. In
8 addition, *HoAP2/ERF55* can catalyze the action of imidazole glycerol phosphate synthase during
9 histidine biosynthesis. The transcription activator Pti6 (*HoAP2/ERF39*) can regulate PR gene
10 expression in response to plant pathogen invasion.

11 **5 Conclusion**

12 In this study, 75 *AP2/ERF* gene sequences of *Magnolia officinalis* (*M. officinalis*) were obtained
13 and identified by transcriptome sequencing and named *HoAP2/ERF1* ~ *HoAP2/ERF75*. According to
14 the conserved domain and phylogenetic tree, *M. officinalis* is divided into four subfamilies, with the
15 majority of the genes belonging to the DREB and ERF subfamilies, allowing *M. officinalis* to grow
16 under adverse environmental conditions such as drought, low temperature, and pathogen invasion. The
17 relative expression level of the *AP2/ERF* gene demonstrated that *M. officinalis* had greater stress
18 tolerance in the lower age group than in the other age groups, allowing *M. officinalis* to continue
19 growing well. Second, we discovered that *HoAP2/ERF55*, one of the 15 strong genes in *M. officinalis*'s
20 *AP2/ERF* gene family, can catalyze the activity of imidazole glycerol phosphate synthase and that
21 *HoAP2/ERF39* (transcriptional activator Pti6) participates in plant–pathogen interactions by regulating
22 the expression of PR genes.

1 **Declarations**

2 **Ethical Approval**

3 This study compliance with ethical standards and is not applicable to human and / or animal studies.

4 **Competing interests**

5 The authors declare that there are no conflicts of interest. The authors declare that the research was
6 conducted in the absence of any commercial or financial relationships that could be construed as
7 potential conflicts of interest.

8 **Authors' contributions**

9 All the authors listed have contributed to this work and approved it for publication. HT and YYZ
10 conceived and designed the experiments. QW, YYZ, and XMZ performed the experiments. MXZ and
11 BNZ analyzed the data. YYZ, CJT, ZX, DRF, ZPX, HT, QW, and XH provided relevant professional
12 knowledge. MXZ and QW wrote the paper.

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17 **Availability of data and materials**

18 All data supporting the findings of this study are available within the paper and its Supplementary
19 Information. *Magnolia officinalis* AP2/ERF sequences are provided in Manuscript Data 1.

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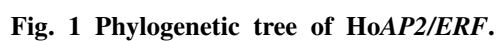
32 Table

33 **Table 1 Physicochemical properties and subcellular localization of AP2/ERF proteins.**

Gene ID	Gene length	MW	Theoretic al pI	Wobble factor	Aliphatic index	GRAVY	Subcellular localization
HoAP2/ERF1	384	42385.20	5.20	46.89	57.19	−0.670	Nucleus.
HoAP2/ERF2	222	24496.57	6.09	57.84	68.20	−0.444	Cytoplasm. Nucleus.
HoAP2/ERF3	311	33840.17	5.66	64.87	71.86	−0.421	Nucleus.
HoAP2/ERF4	183	20286.04	9.10	46.38	72.73	−0.612	Nucleus.
HoAP2/ERF5	222	24534.50	5.17	61.33	66.85	−0.359	Cytoplasm. Nucleus.
HoAP2/ERF6	327	36506.60	5.50	51.95	63.00	−0.674	Nucleus.
HoAP2/ERF7	222	25163.13	5.09	62.31	67.66	−0.668	Nucleus.

HoAP2/ERF8	237	25867.98	4.86	63.07	64.30	-0.514	Nucleus.
HoAP2/ERF9	314	34313.25	6.10	55.91	66.88	-0.499	Cytoplasm. Nucleus.
HoAP2/ERF10	236	26032.03	4.90	62.59	63.35	-0.580	Nucleus.
HoAP2/ERF11	245	26780.50	5.51	55.49	59.10	-0.674	Nucleus.
HoAP2/ERF12	151	16981.16	9.54	49.39	56.42	-0.740	Nucleus.
HoAP2/ERF13	318	35459.04	5.16	56.83	60.75	-0.721	Nucleus.
HoAP2/ERF14	363	40907.93	6.21	62.75	66.45	-0.553	Nucleus.
HoAP2/ERF15	362	40005.50	5.01	54.24	59.64	-0.769	Nucleus.
HoAP2/ERF16	462	51355.31	6.02	61.80	56.30	-0.692	Nucleus.
HoAP2/ERF17	406	44869.19	6.32	57.03	69.01	-0.573	Nucleus.
HoAP2/ERF18	197	2038.21	8.77	57.95	62.79	-0.587	Nucleus.
HoAP2/ERF19	312	34852.70	4.90	52.74	58.14	-0.679	Nucleus.
HoAP2/ERF20	251	28206.70	7.61	36.43	67.29	-0.750	Nucleus.
HoAP2/ERF21	169	18886.12	6.10	65.45	60.71	-0.638	Nucleus.
HoAP2/ERF22	319	35607.06	5.94	62.82	67.62	-0.613	Nucleus.
HoAP2/ERF23	201	21928.32	4.83	56.10	63.13	-0.477	Cytoplasm.
HoAP2/ERF24	195	21776.28	5.47	65.81	53.18	-0.739	Nucleus.
HoAP2/ERF25	406	45358.31	5.96	52.73	55.52	-0.878	Nucleus.
HoAP2/ERF26	250	27627.27	5.75	56.01	53.96	-0.900	Nucleus.
HoAP2/ERF27	402	44697.65	6.04	52.68	56.07	-0.851	Nucleus.
HoAP2/ERF28	409	45621.71	5.95	52.52	57.48	-0.844	Nucleus.
HoAP2/ERF29	326	35858.18	5.57	47.39	59.54	-0.531	Nucleus.
HoAP2/ERF30	136	15747.08	7.73	60.43	76.84	-0.471	Nucleus.
HoAP2/ERF31	152	16939.83	9.48	43.31	76.84	-0.471	Nucleus.
HoAP2/ERF32	169	18920.85	6.63	38.22	52.76	-0.889	Cytoplasm. Nucleus.
HoAP2/ERF33	290	32545.39	6.12	49.01	49.88	-1.059	Cytoplasm. Nucleus.
HoAP2/ERF34	240	26016.79	6.84	69.35	60.21	-0.770	Nucleus.
HoAP2/ERF35	164	17848.31	7.05	44.10	56.12	-0.614	Nucleus.
HoAP2/ERF36	204	22606.13	9.33	69.42	74.02	-0.415	Nucleus.
HoAP2/ERF37	203	22073.56	9.33	51.83	55.54	-0.921	Nucleus.
HoAP2/ERF38	191	21519.14	8.73	35.32	46.31	-0.806	Nucleus.
HoAP2/ERF39	266	30098.89	9.11	55.55	62.26	-0.664	Nucleus.
HoAP2/ERF40	245	26818.29	8.23	61.35	51.84	-0.473	Cytoplasm. Nucleus.
HoAP2/ERF41	183	19930.30	5.36	49.65	69.89	-0.453	Nucleus.
HoAP2/ERF42	179	19880.84	6.11	55.38	61.73	-0.783	Nucleus.
HoAP2/ERF43	222	24837.91	9.19	57.76	51.04	-0.878	Nucleus.
HoAP2/ERF44	288	32613.25	5.40	54.99	72.22	-0.681	Nucleus.
HoAP2/ERF45	204	22368.19	8.59	65.91	68.04	-0.597	Nucleus.
HoAP2/ERF46	464	53231.62	8.92	47.46	75.11	-0.753	Cytoplasm.
HoAP2/ERF47	473	54332.00	9.22	46.07	74.90	-0.758	Cytoplasm.
HoAP2/ERF48	154	17023.29	9.21	76.57	62.73	-0.624	Nucleus.
HoAP2/ERF49	246	27507.72	5.75	47.26	60.65	-0.818	Nucleus.
HoAP2/ERF50	504	57808.66	7.16	50.19	75.54	-0.771	Cytoplasm.
HoAP2/ERF51	72	8364.29	6.31	34.31	67.78	-0.818	Nucleus.

HoAP2/ERF52	408	43986.09	5.85	69.26	52.03	−0.635	Nucleus.
HoAP2/ERF53	227	24936.59	5.47	52.42	62.86	−0.649	Nucleus.
HoAP2/ERF54	171	19077.65	9.57	43.77	62.28	−0.715	Nucleus.
HoAP2/ERF55	161	17814.09	9.65	42.90	61.93	−0.698	Nucleus.
HoAP2/ERF56	284	32017.84	5.26	57.12	72.50	−0.774	Cytoplasm. Nucleus.
HoAP2/ERF57	664	73275.52	6.42	48.90	60.75	−0.675	Cytoplasm. Nucleus.
HoAP2/ERF58	455	49691.62	6.28	45.18	57.56	−0.585	Nucleus.
HoAP2/ERF59	471	51503.79	7.21	38.71	61.83	−0.568	Nucleus.
HoAP2/ERF60	408	45584.30	9.29	54.15	64.90	−0.701	Cytoplasm.
HoAP2/ERF61	396	44098.62	9.31	51.05	66.62	−0.680	Cytoplasm.
HoAP2/ERF62	494	54160.76	5.95	58.94	54.74	−0.624	Nucleus.
HoAP2/ERF63	368	39982.20	7.40	38.48	68.32	−0.487	Nucleus.
HoAP2/ERF64	321	35297.01	9.02	33.51	62.99	−0.559	Nucleus.
HoAP2/ERF65	333	36849.06	6.92	45.91	67.75	−0.605	Nucleus.
HoAP2/ERF66	325	35874.10	6.37	47.89	70.03	−0.511	Nucleus.
HoAP2/ERF67	276	30720.31	9.30	35.64	71.12	−0.796	Nucleus.
HoAP2/ERF68	255	28197.45	8.24	41.96	60.51	−0.493	Nucleus.
HoAP2/ERF69	379	41436.04	5.82	69.05	67.78	−0.484	Nucleus.
HoAP2/ERF70	355	39989.44	7.37	53.33	60.82	−0.788	Nucleus.
HoAP2/ERF71	368	40421.20	6.00	62.44	69.24	−0.429	Nucleus.
HoAP2/ERF72	373	41801.38	4.89	50.83	55.71	−0.790	Nucleus.
HoAP2/ERF73	230	25379.74	7.77	58.78	68.83	−0.450	Cytoplasm. Nucleus.
HoAP2/ERF74	222	24588.58	5.90	62.50	63.78	−0.500	Cytoplasm. Nucleus.
HoAP2/ERF75	222	24587.66	5.61	56.96	68.65	−0.428	Cytoplasm. Nucleus.

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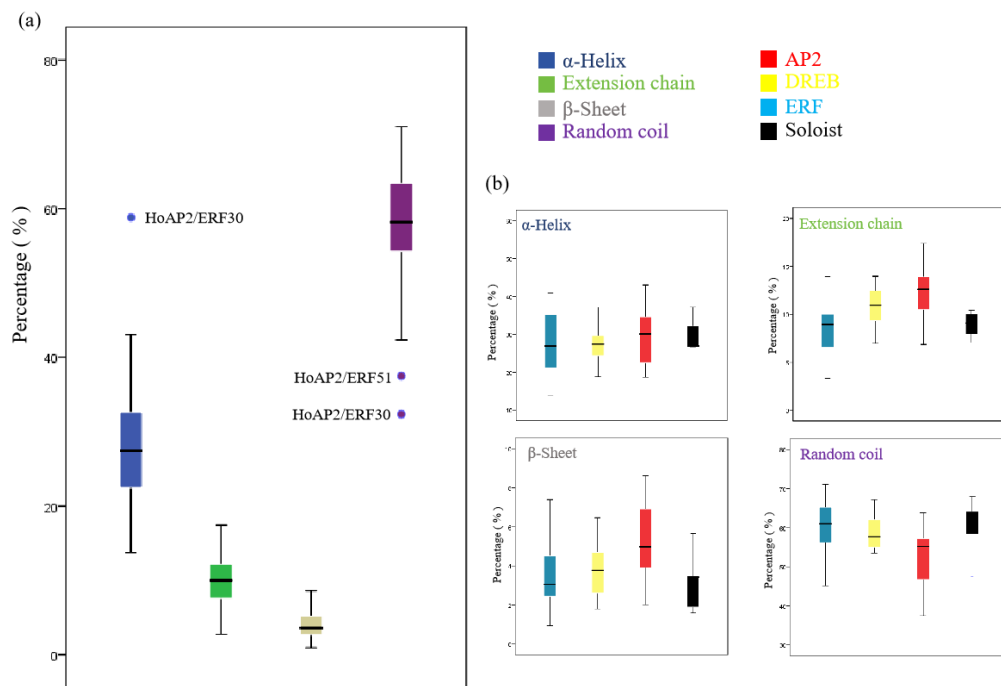
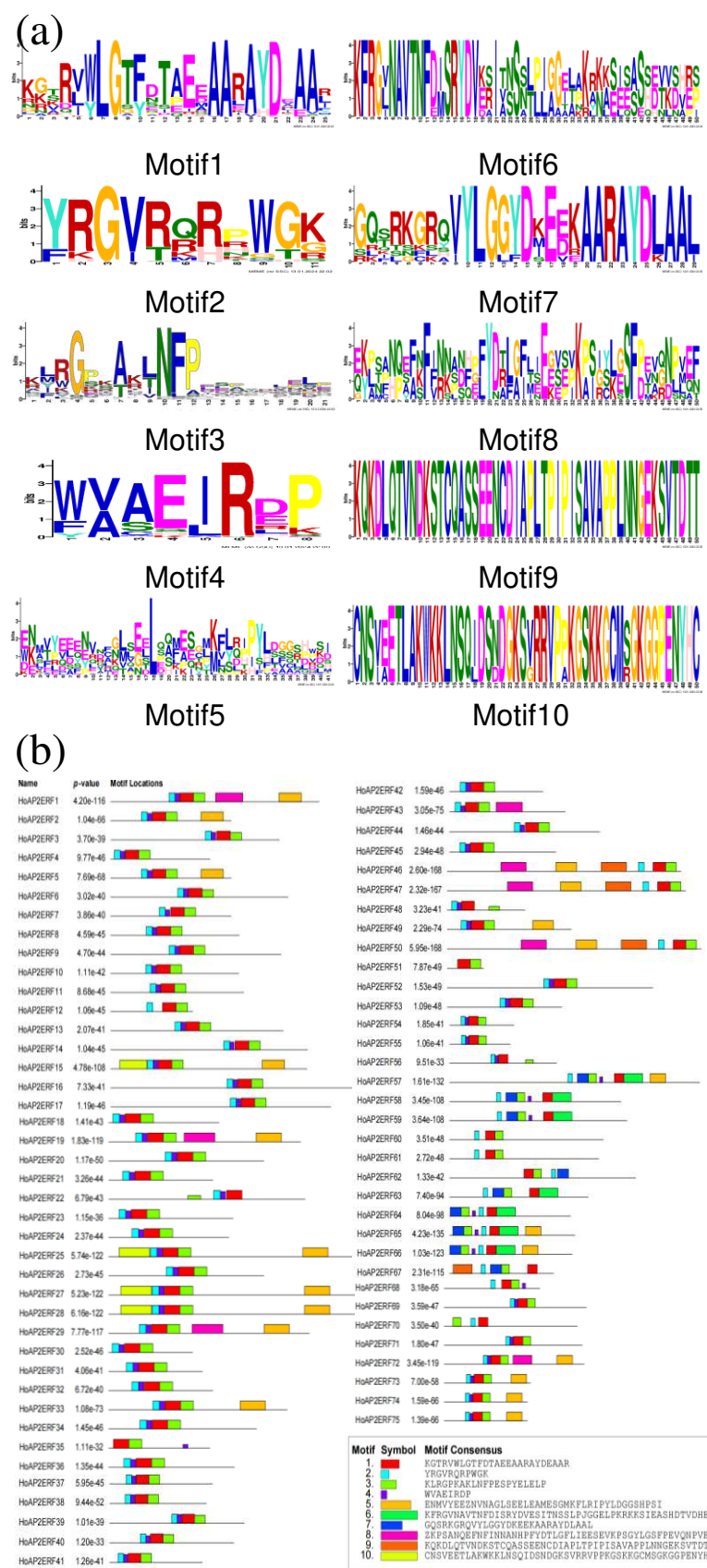


Fig. 2 AP2/ERF protein secondary structure (a). Comparison of the AP2/ERF subfamily on four secondary structures (b).



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2 Fig. 3 Logo of the HoAP2/ERF sequence (a). Conserved motif of the HoAP2/ERF sequence (b).

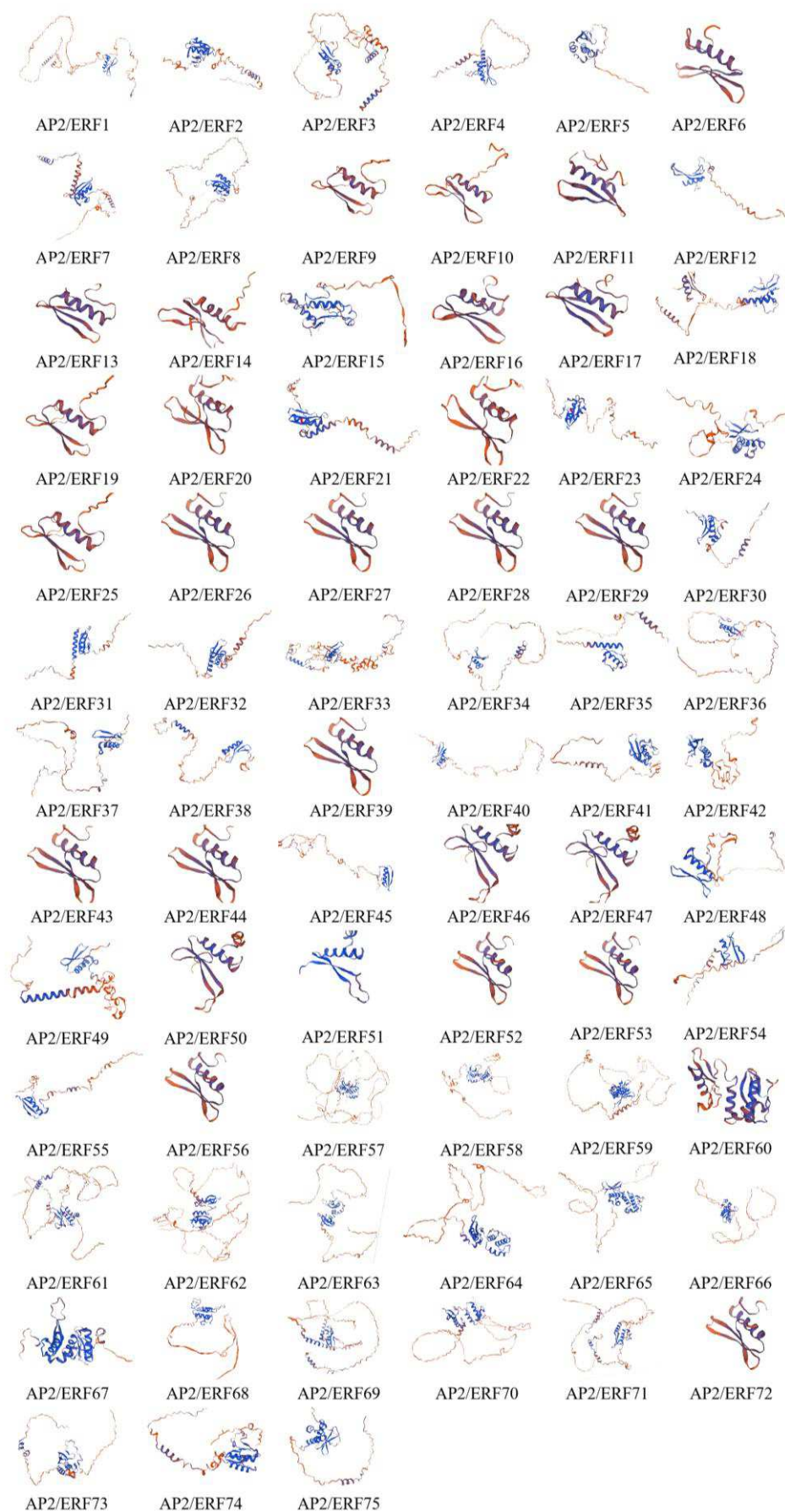


Fig. 4 Tertiary structure of the HoAP2/ERF protein.

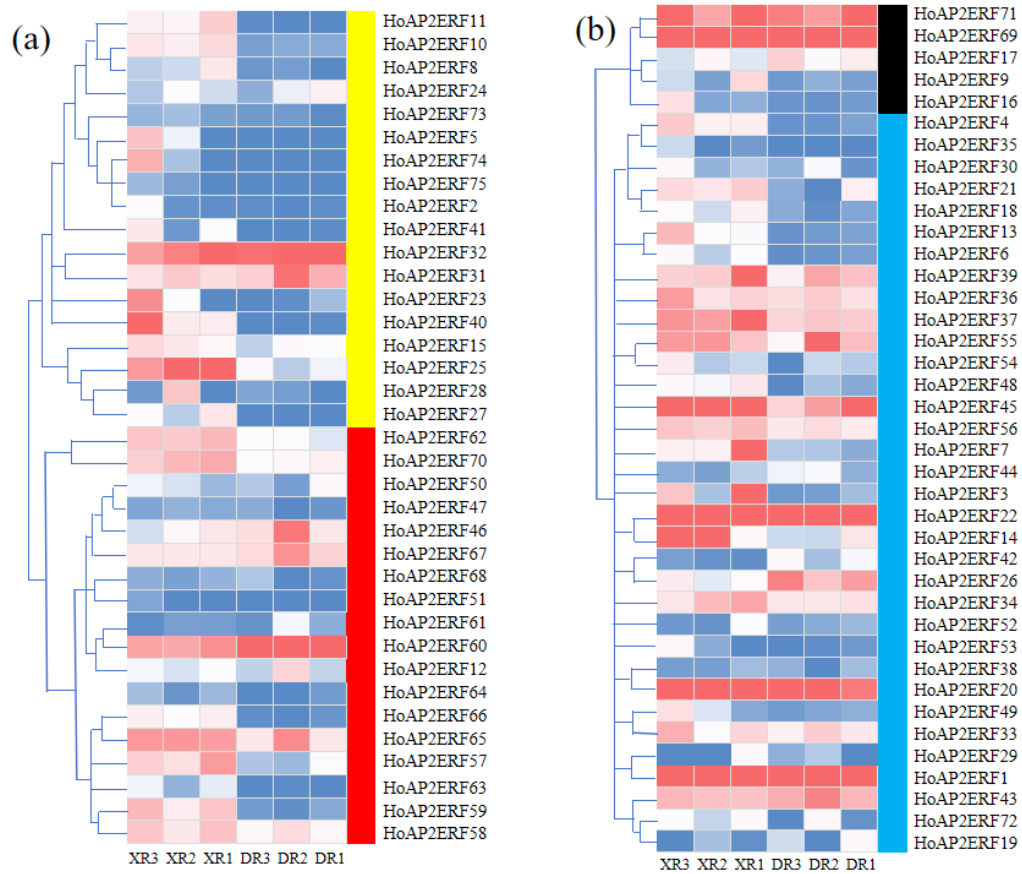
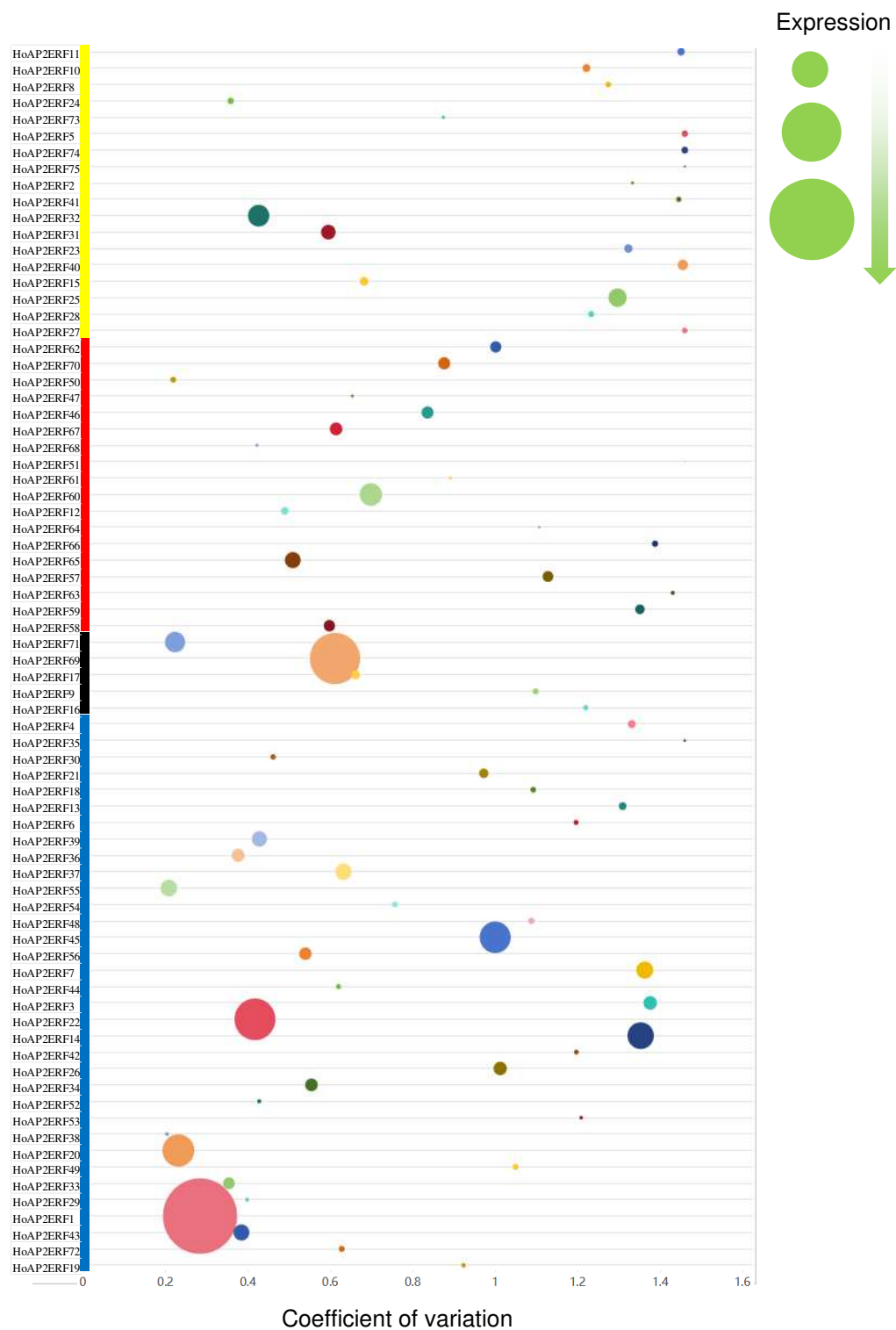


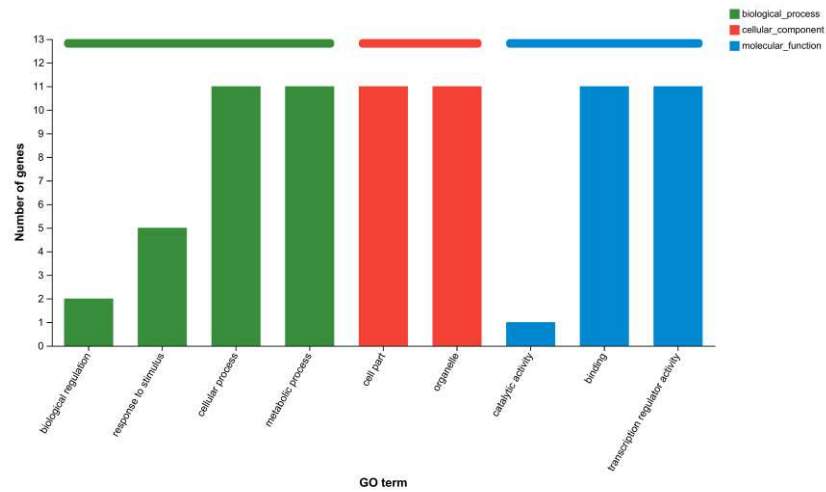
Fig. 5 *M. officinalis* AP2/ERF transcriptome expression heatmap tree. In the DREB and AP2 subfamilies (a). The Soloist and ERF subfamilies (b).



1

2 **Fig. 6** The bubble size represents the average expression level of *AP2/ERF* transcription factors

3 in a single sample, and the abscissa represents the difference in expression level among samples.



1

2

Fig. 7 Functional classification of 11 *AP2/ERF* transcription factors.

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