

核桃 Q 型 C2H2 锌指蛋白基因家族 全基因组鉴定及表达分析

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摘要:【目的】Q 型 C2H2 锌指蛋白在植物抵抗非生物胁迫中起重要作用。为深入了解核桃 Q 型 C2H2 锌指蛋白基因家族在核桃全基因组中的特征, 对核桃 Q 型 C2H2 锌指蛋白 (*JrZFP*) 基因家族进行全基因组鉴定与分析, 并研究其在核桃干旱和盐胁迫应答过程中的表达模式。【方法】利用生物信息学方法鉴定了核桃全基因组中的 Q 型 C2H2 锌指蛋白基因家族成员, 并对其理化性质、染色体定位、进化关系和基因结构进行分析; 根据 *JrZFP* 基因家族进化树随机挑选 15 个 *JrZFP* 基因, 并通过实时荧光定量 PCR (real-time quantitative PCR, qRT-PCR) 方法检测 15 个基因在干旱和盐胁迫下的响应特性。【结果】在核桃中共鉴定出 82 个 Q 型 C2H2 锌指蛋白基因, 其蛋白质序列长度介于 59~636 个氨基酸之间, 亚细胞定位主要位于细胞核中; 该基因家族成员不均匀分布在 15 条染色体上, 其中有 11 对 *JrZFPs* 基因为串联重复基因, 63 个 *JrZFP* 基因参与片段重复事件; 基因结构分析表明, 75 个 *JrZFP* 基因无内含子, 其余成员含有 1~10 个外显子; 上游启动子区分析发现, 该基因家族成员含有参与逆境胁迫和激素响应相关的顺式作用元件。表达分析结果显示, 在盐胁迫下 15 个 *JrZFP* 基因均显著上调, 以 *JrZFP55* 最为明显, 推测 *JrZFP55* 在核桃 NaCl 胁迫中起正向调节作用; 在干旱胁迫下 15 个 *JrZFP* 基因中上调 6 个, 其中 *JrZFP12* 上调最为显著, 下调 9 个, 以 *JrZFP4*、*JrZFP55* 和 *JrZFP57* 下调程度最大, 推测这 3 个基因在核桃干旱胁迫中起负调控作用。【结论】核桃全基因组中有 82 个 *JrZFP* 家族成员都具有 Q 型 C2H2 锌指蛋白典型结构域, 且通过 qRT-PCR 对随机挑选基因进行分析以发现可能参与干旱和盐胁迫的 *JrZFPs* 基因, 为深入研究核桃 *JrZFP* 参与干旱胁迫和盐胁迫提供理论依据。

关键词: 核桃; Q 型 C2H2 锌指蛋白基因家族; 基因表达

中图分类号: S664.1

文献标志码: A

文章编号: 1009-9980(2022)06-0920-15

Genome-wide identification and analysis of the Q-type C2H2 gene family in walnut (*Juglans regia* L.)

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Abstract: 【Objective】Q-type C2H2 zinc finger protein plays an important role in plant resistance to abiotic stress. As an important species of economic forest, walnuts are often exposed to adverse conditions. In order to further understand the characteristics of Q-type C2H2 zinc finger proteins in walnut genome, the whole genome of Q-type C2H2 zinc finger proteins (*JrZFPs*) in walnut was identified and analyzed, and their expression patterns in response to drought stress and salt stress were studied. We carried out this research to provide a reference for the study of walnut resistance to stress. 【Methods】4-

收稿日期: 2021-10-25 接受日期: 2022-02-28

基金项目: 中央财政林业科技推广示范项目(2020ZYFG22); 甘肃省林业和草原科技创新项目(KJCX202004); 甘肃省科技重大专项计划(18ZD2NA006-5)

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week-old Liaohe No. 4 tissue cultured seedlings were used as materials. These plantlets were treated with NaCl ($100 \text{ mmol} \cdot \text{L}^{-1}$) and PEG (15.0%, ρ) for 0, 6, 12 and 24 h, then frozen in liquid nitrogen immediately and stored at $-80 \text{ }^{\circ}\text{C}$ for RNA extraction and gene expression analysis. Each treatment had 3 replicates, with nine plants per replicate. Additionally, through BLAST *Arabidopsis thaliana* Q-type C2H2 protein sequences, 82 *JrZFPs* were screened from the walnut genome database. With the help of ExpASy, MEGA X, TBtools, MEME and Plant CARE online website, bioinformatics analysis of the gene families was performed, including the phylogenetic analysis, gene structure and protein sequence analysis, chromosome location, series replication analysis, and inter species collinear analysis. Finally, the expression patterns of 15 Q-type C2H2 genes were analyzed under drought and salt stress through qRT-PCR. **【Results】**A total of eighty-two members of *JrZFP* were identified from the walnut genome database (named as *JrZFP1*-*JrZFP82*). They all contained invariant “QALGGH” motif in the zinc finger domains ($\text{CX}_2\text{-}_4\text{CX}_3\text{FX}_3\text{QALGGHX}_3\text{-}_5\text{H}$). In addition, the number of amino acids, relative molecular weight, isoelectric point and subcellular localization of *JrZFP* family genes were analyzed. The number of amino acids of the 82 *JrZFP* gene family members ranged from 59 (*JrZFP26*) aa to 636 (*JrZFP63*) aa, with a molecular weight ranging from 6.72 (*JrZFP26*) to 70.33 (*JrZFP63*), and an isoelectric point from 5.33 (*JrZFP80*) to 10.23 (*JrZFP47*). Prediction of subcellular location showed that *JrZFPs* were mainly located in the nucleus, with a small amount distributed in the cytoplasm, peroxisomes and chloroplasts. Chromosome location analysis showed that these *JrZFPs* were unevenly distributed in all the walnut except for chromosome 16. In the present study, 11 pairs of *JrZFP* genes (22/82, 26.83%) were identified as tandemly duplicated genes, of which chromosomes 1, 2, 3, 4, 6, 10 and 14 had one pair of tandem duplication genes. Chromosome 9 and 13 had two pairs of genes. Besides the tandem duplication, 63 (63/82, 76.83%) segmental duplication genes were also identified using MCS-canX methods. In order to study the evolutionary relationship between walnut and *Arabidopsis* Q-type C2H2 genes, a phylogenetic tree analysis was performed on the amino acid sequences of 140 Q-type C2H2 (including 58 *Arabidopsis* genes and 82 walnut genes). According to sequence similarity and topological structure, the phylogenetic tree could be divided into 7 ZFP subfamilies, namely C1, C2- I, C2- II, C2-III, C2-IV, C3- I and C3- II. In order to understand the gene structure of *JrZFPs*, their introns, exons and conservative motifs were analyzed. The results showed that 75 *JrZFP* genes (91.46%) had no introns, and a total of 10 Motifs were identified in the conservative motif distribution. Motif 1 and Motif 2 both contained Q-type C2H2 core sequences, and Motif 5 was an EAR-motif. The *JrZFPs* in the C1 and C2 subfamily contained two zinc finger domains, while the members of the C3 subfamily contained only one zinc finger domain. Motif 1 and Motif 2 were Q-type zinc finger domains, which are widely distributed in 82 *JrZFPs*. All 76 *JrZFPs* contained EAR-motif, and the remaining members did not have EAR-motif. Ninety-eight percent of the members of this family had light-responsive elements, 93% of members contained abscisic acid-responsive elements and 50% of members had low-temperature response elements. Additionally, 44% of members had drought-responsive elements, indicating that the *JrZFPs* family plays an important role in response to drought environment. At last, the 15 *JrZFP* genes showed different response patterns under salt stress, all of which were significantly up-regulated. Especially, the relative expression of *JrZFP55* was the highest compared with S0, indicating that *JrZFP55* played a positive regulatory role in walnut NaCl stress. On the other hand, the 15 *JrZFP* genes showed different response patterns under drought stress, which were either significantly up-regulated or down-regulated. Among them, *JrZFP12* was up-regulated the most, which was 13.97 times that of S0. At the same time, *JrZFP4*, *JrZFP55* and *JrZFP57* were down-regulated most significantly under

drought stress, suggesting that these three genes play a negative regulatory role in walnut drought stress. 【Conclusion】 There are 82 *JrZFP* family members in the whole genome of walnut, all of which contain the typical Q-type C2H2 domain. *JrZFP* genes may be involved in drought and salt stress based on real-time fluorescence quantitative analysis.

Key words: Walnut; Q-type C2H2 gene family; Gene expression

锌指蛋白(zinc-finger proteins, ZFPs)是一类具有手指状结构域的转录因子^[1]。锌指是一个具有特殊二级结构的小肽域,该结构由锌离子与半胱氨酸(Cys)残基和组氨酸(His)残基结合而稳定存在,是该家族的主要特征^[2]。根据Cys残基和His残基的数量和位置,锌指蛋白可分为C2H2、C2HC、C2HC5、C3HC4、CCCH、C4、C4HC3、C6和C8^[3]。其中,C2H2-ZFPs成员在真核生物中数量最多^[4-5],其锌指结构域中存在2个Cys和2个His,并围绕中心锌离子组成稳定ZFP结构域,主要参与基因的启动子调节^[2]。研究发现,C2H2-ZFPs家族参与调控拟南芥(*Arabidopsis thaliana*)在干旱、寒冷、盐和光胁迫响应中的耐受性^[6],通过过表达*OsZFP245*增强水稻(*Oryza sativa*)的耐旱性^[7];水稻*OsZFP179*作为新的盐敏感基因,其过表达可以提高水稻耐盐性^[8];苹果(*Malus pumila* Mill.)的C2H2型锌指转录因子*MdZAT10*能显著加速叶片衰老,促进衰老相关基因的表达^[9];木薯(*Manihot esculenta* Crantz.)C2H2型锌指蛋白转录因子在植物生长发育和响应非生物胁迫功能方面具有重要作用^[10]。

大多数植物C2H2-ZFPs在锌指结构域(CX₂-₄CX₃FX₃QALGGHX₃-₅H)内含有高度保守的QALGGH基序^[6,11-13],形成了植物特有的Q型C2H2锌指亚家族。Q型C2H2锌指蛋白在矮牵牛(*Petunia hybrida*)中首次被发现,并证明其高度保守基序QALGGH对DNA结合活性至关重要^[14-15]。随后Q型C2H2锌指蛋白基因在拟南芥^[16]、水稻^[12]、杨树(*Populus trichocarpa*)^[17]、小麦(*Triticum aestivum*)^[18]、甘蓝(*Brassica oleracea*)、油菜(*Brassica napus*)、白菜(*Brassica rapa*)^[19]和马铃薯(*Solanum tuberosum*)^[20]中均被证明参与多种生物过程,包括植物生长和器官发育以及对逆境的反应与防御^[21-24]。马铃薯Q型C2H2锌指蛋白基因参与对非生物胁迫的响应,增强其对盐和干旱胁迫的耐受性^[20]。此外,在杨树和小麦中发现Q型C2H2锌指亚类响应非生物胁迫^[17-18]。但在核桃中未发现对该基因家族的相

关研究。

核桃(*Juglans regia* L.)是重要的干果经济林树种,而中国核桃主产区多分布于干旱和半干旱地区,春季及初夏降雨量少且分布不均^[25],加之部分地区土壤含盐量较高,使苗木生长受到抑制^[26],严重影响了核桃的产量与品质^[27]。因此,鉴定核桃全基因组中Q型C2H2锌指蛋白基因家族,对其蛋白理化性质、染色体定位、系统进化关系和基因结构进行分析,探究其在干旱和盐胁迫下的表达情况,为研究Q型C2H2锌指蛋白基因家族在核桃中生物学功能、利用基因工程手段提高核桃的抗逆性、扩大种植范围、提高品质与产量奠定基础。

1 材料和方法

1.1 试材处理

以甘肃农业大学园艺学院果树科学系组培室4周龄辽核4号组培苗为材料。选取生长健壮、长势一致的核桃试管苗分别进行干旱[15%(w)聚乙二醇(polyethylene glycol, PEG)]和盐(100 mmol·L⁻¹ NaCl)处理0、6、12、24 h,以正常生长的试管苗作对照,3次生物学重复^[20,28-30]。收集供试试管苗叶片用液氮冷冻,然后置于-80℃保存,用于后续RNA提取。

1.2 核桃Q型C2H2锌指蛋白基因家族成员的鉴定

从拟南芥数据库(<https://www.arabidopsis.org/>)中查找得到174个已知C2H2锌指蛋白基因保守蛋白序列,从核桃全基因组数据库(<http://xhhuanglab.cn/data/juglans.html>)^[31]查询得到核桃全基因组中的保守蛋白序列。利用HMMER 3.1软件(<http://hmm-mer.org/download.html>)将得到的拟南芥174个C2H2锌指蛋白结构域序列作为基础构建隐马尔可夫模型(hidden markov model, HMM)图谱,然后利用BLASTP算法根据拟南芥C2H2锌指蛋白氨基酸序列搜索核桃C2H2锌指蛋白成员,*E*值 $\leq 1 \times 10^{-5}$ 。删除所有冗余序列后,将得到的候选成员提交至SMART(<http://smart.embl-heidelberg.de/>)和NCBI保守域数据库(CDD),使用手动筛选的方式进一步确

定Q型C2H2-ZFPs成员。对最终得到的核桃Q型C2H2-ZFPs家族成员的氨基酸数目、理论等电点、分子量大小等理化性质数据在Expasy(<https://web.expasy.org/protparam/>)平台进行分析,在WoLF PSORT(<https://wolfpsort.hgc.jp/>)平台进行亚细胞结构定位分析。

1.3 染色体定位与基因复制

根据核桃全基因组注释信息文件利用Mapchart绘制核桃Q型C2H2锌指蛋白基因染色体定位图。核桃Q型C2H2锌指蛋白基因的串联重复事件是根据3个标准:(1)2个基因相似度大于75%;(2)比对长度大于75%(相对于较长的基因);(3)基因在同一条染色体且物理距离小于100 kb^[32]。利用MCS-canX分析了核桃种内Q型C2H2锌指蛋白基因片段重复事件,并用Circos软件绘图。

1.4 系统进化关系、基因结构及顺式元件调控分析

利用MEGA-X软件分析基因家族系统进化关系,以邻接法(neighbor-joining method)构建进化

树。利用MEME 5.3.2在线网站(<http://memesuite.org/>)进行基因家族成员保守基序结构分析,使用TBtools软件^[33]对家族成员编码序列(coding sequence, CDS)与全基因序列进行内含子和外显子可视化,再将进化树、保守结构基序及内含子和外显子进行合并。

1.5 试材RNA提取与实时荧光定量PCR分析

通过核桃Q型C2H2锌指蛋白基因家族进化树随机挑选15个*JrZFP*基因,并将其核酸序列提交至上海生工生物工程股份有限公司官网进行实时荧光定量PCR(real-time quantitative PCR, qRT-PCR)引物设计(表1),使用TaKaRa公司的MiniBEST Plant RNA Extraction Kit试剂盒进行材料RNA的提取,用PrimeScript™ RT reagent Kit with gDNA Eraser试剂盒将RNA反转录为cDNA,最后用TB Green® Premix Ex Taq™ II试剂盒进行qRT-PCR验证各基因在不同胁迫下的表达。将所得数据利用SPSS 25和

表1 qRT-PCR引物序列
Table 1 qRT-PCR primer sequence

登录号 Accession No.	基因名称 Gene name	引物序列(5'-3') Primer sequence(5'-3')	
JreChr01G12107	<i>JrZFP4</i>	F:AGAGACAGAGCCAGGTTGAGACAG	R:AATGACGAGGAGGATGAGGGTGAG
JreChr02G11510	<i>JrZFP12</i>	F:AGCAGTCTTGTGTGTCGTCGGATG	R:CGGAGCACTCGTGAACCTTAGAC
JreChr03G13269	<i>JrZFP21</i>	F:GGTGAACAGGAGAGGAGTGGAG	R:AAACGAATGAGCCCTCTGTGACC
JreChr04G11953	<i>JrZFP27</i>	F:TGGCAAAGCGGGCTATGAGAATG	R:GGACCTCACTGATGGCACAACCC
JreChr05G10388	<i>JrZFP29</i>	F:ACAGGGTTTGAATGGCAAGGATG	R:TGGTCTGACGGTGGCTCTGAC
JreChr05G12759	<i>JrZFP34</i>	F:GAGAGGAGGCGGCTGAGGAG	R:GCGAAGCACCCCTTTCACATTCTTG
JreChr06G10479	<i>JrZFP35</i>	F:GGTATGCTGTACCTGCTGCTCAC	R:TCTGCTGGGAAGAGGGTAAAGGAG
JreChr07G11727	<i>JrZFP44</i>	F:CACCATCTGCCACAAGTCCTTCC	R:GTGGTGACGACGCTGCTTCC
JreChr09G11432	<i>JrZFP55</i>	F:TTCTCAAATGCTCAGGCTCTTGGG	R:ATGTCCATAGGCGGCGAGTTTTC
JreChr10G10800	<i>JrZFP57</i>	F:TCGGCATAGAATTTGGCGGTGAC	R:GTTGTGCTCCTTGGCGTCTCC
JreChr10G11227	<i>JrZFP59</i>	F:GCGGCATAGAGCGAACGAAGG	R:AAGGGCGTCAAGTTCAAGTCCAAG
JreChr11G12142	<i>JrZFP63</i>	F:GAAGCTGGAAGCCGACGTGAAG	R:CGGTGGAGCAGTTTCTAGCATAGC
JreChr12G10255	<i>JrZFP65</i>	F:TGGGTCGTGGTTCTGAGGCTAG	R:CACGGCGGTTGCTCTCTTCTTC
JreChr13G10892	<i>JrZFP71</i>	F:GTGGTCACAAGGCAAGAGGGAAG	R:GCCCCGACAAAGACATCACAGAC
JreChr14G10249	<i>JrZFP77</i>	F:TGAGGAAATCGGTGCTGCATCTTC	R:CTGCTGCTTGCTCGGTTCAAG

Origin 2021进行处理并绘图。

2 结果与分析

2.1 核桃Q型C2H2锌指蛋白基因家族鉴定及理化性质分析

对核桃全基因组进行HMM和BLASTP算法搜索,最后手工筛选具有核心序列CX₂-₄CX₃FX₃QAL-GGHX₃-₅H的Q型C2H2-ZFPs成员,共获得82个成

员。将该基因家族成员根据其在染色体上的位置分别编号*JrZFP1*(*JreChr01G10056*)~*JrZFP82*(*JreChr15G10369*),便于后续分析。从表2中可看出,82个Q型C2H2成员的氨基酸数目介于59(*JrZFP26*)~636(*JrZFP63*)个之间,蛋白质分子质量介于6.72(*JrZFP26*)~70.33(*JrZFP63*)ku之间,理论等电点介于5.33(*JrZFP80*)~10.23(*JrZFP47*)之间。亚细胞结构定位预测显示*JrZFPs*基因主要位于细胞核中,少

表 2 理化性质分析
Table 2 Physical and chemical properties analysis

登录号 Accession No.	基因名称 Gene name	氨基酸数 Number of amino acids	分子质量 Molecular weight/ku	理论等电点 pI	亚细胞定位 Subcellular localization
JreChr01G10056	<i>JrZFP1</i>	208	23.58	6.13	nucl:14
JreChr01G10385	<i>JrZFP2</i>	166	18.05	8.43	nucl:13,pero:1
JreChr01G12106	<i>JrZFP3</i>	185	21.14	6.75	nucl:14
JreChr01G12107	<i>JrZFP4</i>	233	26.13	8.92	nucl:12,cyto:1,pero:1
JreChr01G12880	<i>JrZFP5</i>	264	28.90	9.36	nucl:14
JreChr01G13047	<i>JrZFP6</i>	343	37.64	6.32	nucl:14
JreChr01G13243	<i>JrZFP7</i>	181	19.62	9.12	nucl:14
JreChr02G10459	<i>JrZFP8</i>	185	20.25	6.92	nucl:13,pero:1
JreChr02G10911	<i>JrZFP9</i>	166	18.37	6.59	nucl:14
JreChr02G10912	<i>JrZFP10</i>	231	25.44	9.51	nucl:13,pero:1
JreChr02G11409	<i>JrZFP11</i>	290	32.08	6.15	nucl:14
JreChr02G11510	<i>JrZFP12</i>	347	38.06	6.64	nucl:14
JreChr02G11636	<i>JrZFP13</i>	177	19.59	9.17	nucl:14
JreChr02G11690	<i>JrZFP14</i>	292	32.54	6.79	nucl:14
JreChr03G10653	<i>JrZFP15</i>	317	35.06	8.92	nucl:14
JreChr03G12539	<i>JrZFP16</i>	151	16.83	9.06	nucl:13,pero:1
JreChr03G12575	<i>JrZFP17</i>	204	22.89	8.71	nucl:12,cyto:1,pero:1
JreChr03G12576	<i>JrZFP18</i>	182	21.10	5.74	nucl:14
JreChr03G12945	<i>JrZFP19</i>	223	23.69	7.82	nucl:14
JreChr03G13093	<i>JrZFP20</i>	256	27.83	6.13	nucl:14
JreChr03G13269	<i>JrZFP21</i>	573	64.23	7.04	nucl:14
JreChr04G10054	<i>JrZFP22</i>	309	34.65	8.92	nucl:14
JreChr04G10151	<i>JrZFP23</i>	191	21.14	8.85	nucl:13,pero:1
JreChr04G11643	<i>JrZFP24</i>	477	52.22	9.63	nucl:14
JreChr04G11737	<i>JrZFP25</i>	201	22.61	9.47	nucl:13,pero:1
JreChr04G11738	<i>JrZFP26</i>	59	6.72	5.49	nucl:14
JreChr04G11953	<i>JrZFP27</i>	256	27.98	5.56	nucl:13,pero:1
JreChr05G10137	<i>JrZFP28</i>	547	60.85	9.04	nucl:14
JreChr05G10388	<i>JrZFP29</i>	191	21.40	9.30	nucl:14
JreChr05G10431	<i>JrZFP30</i>	260	28.99	8.71	nucl:13.5,cyto_nucl:7.5
JreChr05G10552	<i>JrZFP31</i>	253	27.74	8.19	nucl:14
JreChr05G11243	<i>JrZFP32</i>	245	26.54	5.80	nucl:14
JreChr05G11939	<i>JrZFP33</i>	237	24.63	8.37	nucl:14
JreChr05G12759	<i>JrZFP34</i>	312	34.06	7.22	nucl:14
JreChr06G10479	<i>JrZFP35</i>	273	30.30	9.02	nucl:14
JreChr06G10678	<i>JrZFP36</i>	502	55.66	8.85	nucl:14
JreChr06G11002	<i>JrZFP37</i>	277	30.16	8.03	nucl:14
JreChr06G11553	<i>JrZFP38</i>	251	27.24	7.62	nucl:14
JreChr06G11609	<i>JrZFP39</i>	261	28.25	7.62	nucl:14
JreChr06G11628	<i>JrZFP40</i>	192	21.51	6.52	nucl:14
JreChr06G11629	<i>JrZFP41</i>	556	61.58	8.84	nucl:12,extr:2
JreChr06G11732	<i>JrZFP42</i>	249	26.28	8.69	nucl:14
JreChr07G11002	<i>JrZFP43</i>	372	40.92	9.88	nucl:14
JreChr07G11727	<i>JrZFP44</i>	284	30.39	9.24	nucl:12.5,cyto_nucl:7,extr:1
JreChr07G11892	<i>JrZFP45</i>	172	18.70	7.85	nucl:13.5,cyto_nucl:7.5
JreChr07G12339	<i>JrZFP46</i>	276	30.27	6.89	nucl:13,pero:1
JreChr08G10074	<i>JrZFP47</i>	189	21.02	10.32	nucl:14

续表 Continued Table

登录号 Accession No.	基因名称 Gene name	氨基酸数 Number of amino acids	分子质量 Molecular weight/ku	理论等电点 pI	亚细胞定位 Subcellular localization
JreChr08G11864	<i>JrZFP48</i>	252	26.73	8.51	nucl:14
JreChr08G12211	<i>JrZFP49</i>	286	31.87	6.04	nucl:13,pero:1
JreChr09G10134	<i>JrZFP50</i>	312	33.69	6.45	nucl:14
JreChr09G10780	<i>JrZFP51</i>	547	61.23	9.14	nucl:14
JreChr09G10970	<i>JrZFP52</i>	246	27.24	6.56	nucl:14
JreChr09G10994	<i>JrZFP53</i>	228	24.95	9.79	nucl:13,chlo:1
JreChr09G11302	<i>JrZFP54</i>	260	29.30	8.95	nucl:14
JreChr09G11432	<i>JrZFP55</i>	184	20.72	5.98	nucl:14
JreChr09G11433	<i>JrZFP56</i>	302	33.99	8.98	nucl:14
JreChr10G10800	<i>JrZFP57</i>	245	27.22	6.70	nucl:14
JreChr10G10922	<i>JrZFP58</i>	279	30.77	8.76	nucl:14
JreChr10G11227	<i>JrZFP59</i>	183	20.46	9.55	nucl:14
JreChr10G11228	<i>JrZFP60</i>	185	20.53	9.46	nucl:14
JreChr11G10412	<i>JrZFP61</i>	323	35.65	6.43	nucl:14
JreChr11G10751	<i>JrZFP62</i>	236	25.97	7.72	nucl:13,chlo:1
JreChr11G12142	<i>JrZFP63</i>	636	70.33	8.66	nucl:14
JreChr11G12534	<i>JrZFP64</i>	534	59.21	8.10	nucl:14
JreChr12G10255	<i>JrZFP65</i>	531	59.34	8.36	nucl:14
JreChr12G10530	<i>JrZFP66</i>	323	35.86	6.00	nucl:14
JreChr12G10641	<i>JrZFP67</i>	234	25.46	5.69	nucl:13,pero:1
JreChr12G11541	<i>JrZFP68</i>	578	64.33	8.72	nucl:14
JreChr13G10345	<i>JrZFP69</i>	359	38.95	8.30	nucl:14
JreChr13G10399	<i>JrZFP70</i>	304	33.03	8.12	nucl:14
JreChr13G10892	<i>JrZFP71</i>	450	49.59	9.15	nucl:14
JreChr13G10893	<i>JrZFP72</i>	476	53.51	7.61	nucl:14
JreChr13G11350	<i>JrZFP73</i>	496	54.66	5.94	nucl:14
JreChr13G11352	<i>JrZFP74</i>	368	41.21	5.58	nucl:14
JreChr13G12013	<i>JrZFP75</i>	204	22.79	5.69	nucl:14
JreChr13G12170	<i>JrZFP76</i>	157	17.30	9.57	nucl:14
JreChr14G10249	<i>JrZFP77</i>	369	40.27	6.00	nucl:14
JreChr14G11071	<i>JrZFP78</i>	520	57.70	6.58	nucl:14
JreChr14G11072	<i>JrZFP79</i>	373	41.77	5.76	nucl:14
JreChr14G11597	<i>JrZFP80</i>	248	28.11	5.33	nucl:14
JreChr14G11691	<i>JrZFP81</i>	163	18.03	10.11	nucl:14
JreChr15G10369	<i>JrZFP82</i>	304	32.97	8.44	nucl:12,extr:2

注:nucl. 细胞核;cyto. 细胞质;extr. 细胞外;pero. 过氧化物酶体;cysk. 细胞骨架;chlo. 叶绿体;cyto_nucl. 细胞质_细胞核。

Note: nucl. Nucleus; cyto. Cytoplasm; extr. Extracellular; pero. Peroxisome; cysk. Cytoskeleton; chlo. Chloroplast; cyto_nucl. Cytoplasm_nucleus.

量分布在细胞质、过氧化物酶体和叶绿体中。

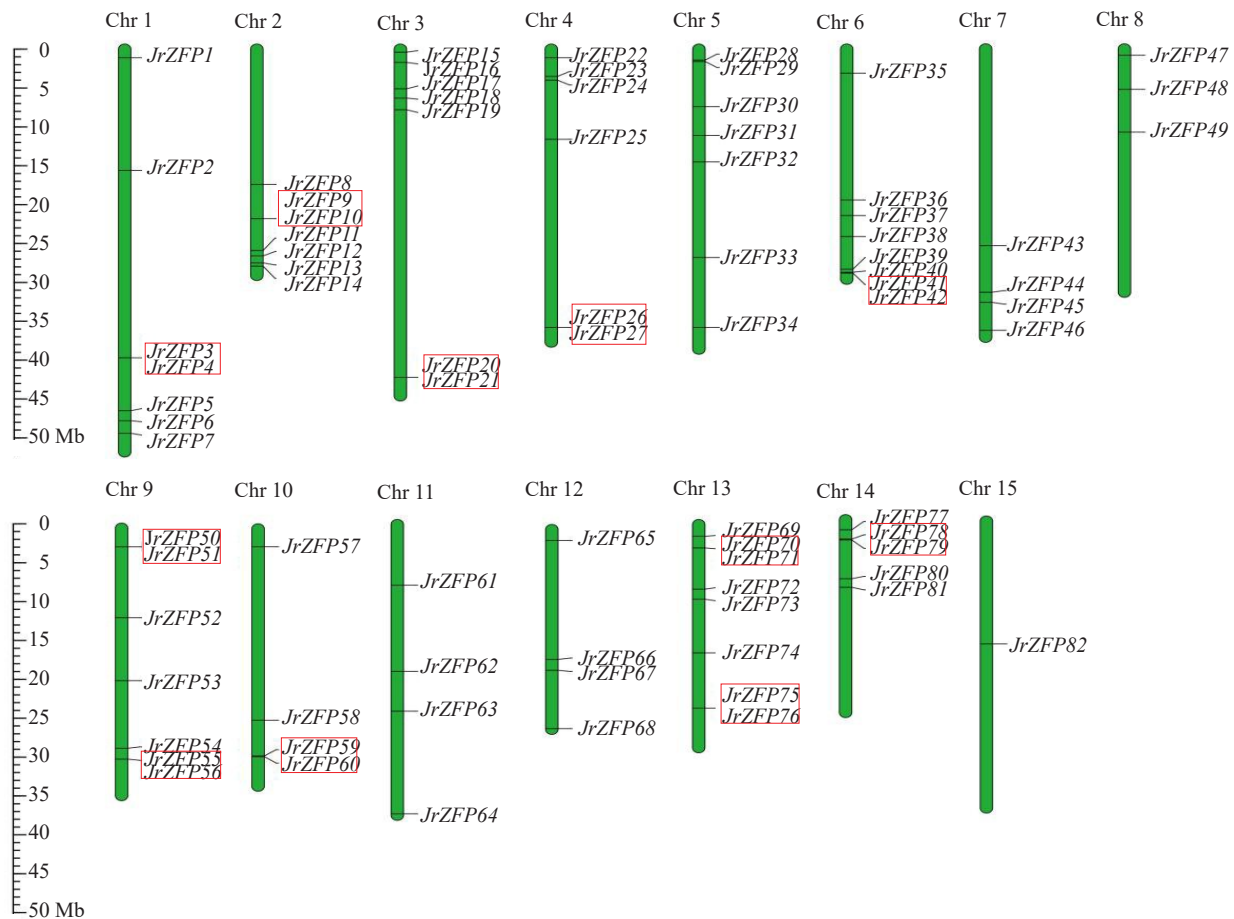
2.2 核桃Q型C2H2锌指蛋白基因家族染色体定位与基因复制

核桃Q型C2H2锌指蛋白基因家族成员不均匀分布在15条染色体上,其中15号染色体上只有1个基因*JrZFP82*。研究*JrZFPs*基因家族的串联重复和片段重复事件,以阐述核桃Q型C2H2锌指蛋白基因家族基因重复事件。共鉴定出11对(22/82,

26.83%)串联重复基因(图1),其中1、2、3、4、6、10和14号染色体各有1对串联重复基因,9和13号染色体中各有2对串联重复基因。除串联重复基因外,利用MCScanX鉴定出核桃基因组内关于*JrZFPs*基因家族发生节段重复事件基因共有63个(63/82, 76.83%)(图2)。

2.3 Q型C2H2锌指蛋白基因家族进化关系分析

为研究核桃和拟南芥Q型C2H2锌指蛋白基因



红色框中基因为串联重复基因。

The genes in the red box are tandem repeat genes.

图 1 核桃 Q 型 C2H2 锌指蛋白基因家族成员染色体定位

Fig. 1 Chromosomal location of members of walnut Q-type C2H2 gene family

之间的进化关系,对 140 个 Q 型 C2H2 锌指蛋白(包括 58 个拟南芥和 82 个核桃)氨基酸序列进行系统发育树分析(图 3)。根据序列相似性和拓扑结构,将进化树分为 7 个 ZFP 亚家族,分别为 C1、C2- I、C2- II、C2-III、C2-IV、C3- I 和 C3- II。C1 子类有 3 个 JrZFP 氨基酸序列和 2 个 ATZFP 氨基酸序列,C2 子类有 34 个 JrZFP 氨基酸序列和 27 个 ATZFP 氨基酸序列,C3 子类有 44 个 JrZFP 氨基酸序列和 30 个 ATZFP 氨基酸序列。通过图 3 中核桃和拟南芥的系统发育分析,82 个 JrZFP 氨基酸序列被划分为 7 个亚家族。构建的 *JrZFPs* 基因系统发育树如图 4-A 所示。

2.4 核桃 Q 型 C2H2 锌指蛋白基因家族基因结构

为深入了解 *JrZFPs* 基因结构,对其进行内含子和外显子及保守基序分析。结果显示,75 个 *JrZFP* 基因(91.46%)无内含子,其余成员中有 3 个含有 1 个内含子,4 个含有 2 个及 2 个以上的内含子(图 4-

B)。亚家族中 C1 和 C2- I 所有成员均无内含子;C2- II、C2-III、C2-IV 和 C3- II 中均只有 1 个成员有内含子,其余成员均无内含子;C3- I 中 3 个成员内含子。使用 MEME 分析核桃 Q 型 C2H2 锌指蛋白基因家族成员保守基序分布情况(图 4-C),共鉴定出 10 个 Motif,主要的 3 个 Motif 如图 5 所示。其中,Motif 1 和 Motif 2 均含有 Q 型 C2H2 锌指蛋白核心序列 $CX_2-aCX_3FX_3QALGGHX_3-sH$,Motif 5 为 EAR-motif。每一亚族中大多数 *JrZFPs* 基因通常具有相似的母序构成。由图 4-C 可知,C1 和 C2 亚族中的 *JrZFPs* 基因包含 2 个锌指结构域,而 C3 亚族中的成员只包含 1 个锌指结构域。Motif 1 和 Motif 2 为 Q 型锌指结构域,广泛分布于 82 个 *JrZFP* 基因中。76 个 *JrZFP* 基因都含有 EAR-motif,其余成员无 EAR-motif。

2.5 核桃 Q 型 C2H2 锌指蛋白基因家族顺式调控元件分析

根据 82 个 *JrZFP* 基因家族成员上游 2000 bp 碱

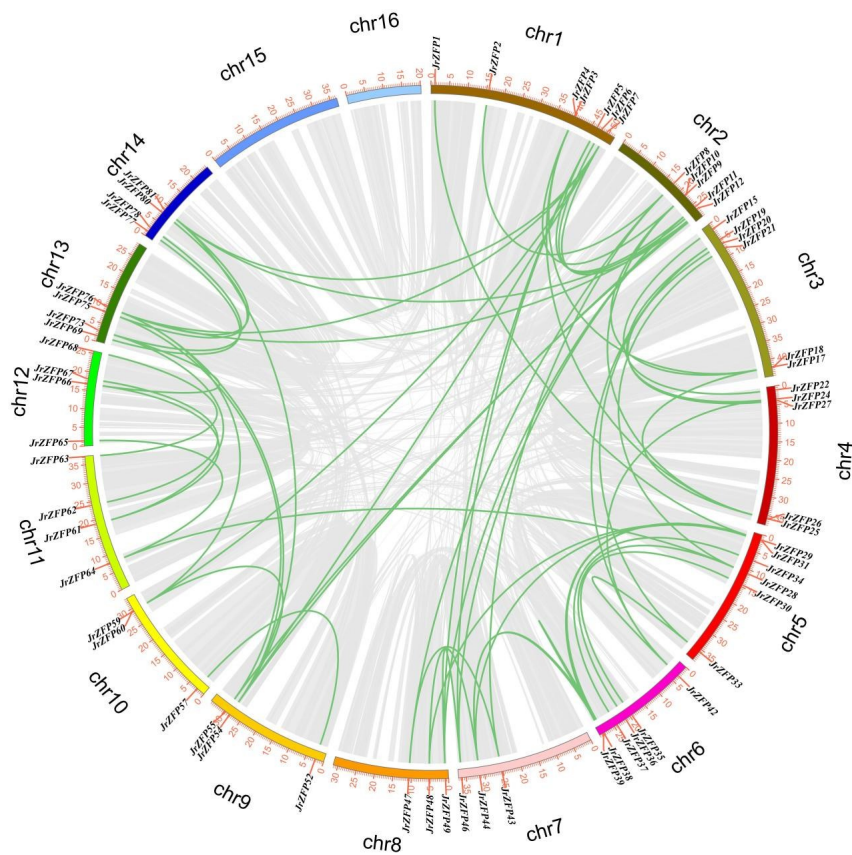


图2 核桃Q型C2H2锌指蛋白基因片段复制事件

Fig. 2 The segmental replication events of Q-type C2H2 genes in walnut

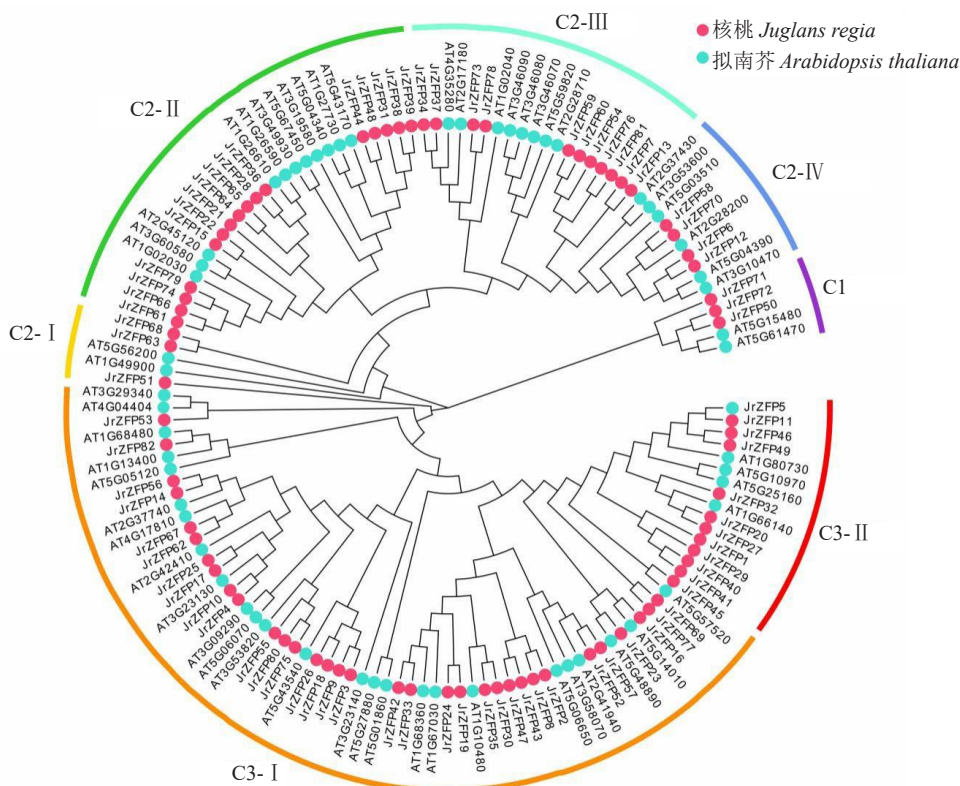


图3 拟南芥(AT)和核桃(Jr)Q型C2H2锌指蛋白进化树

Fig. 3 Phylogenetic tree of Q-type C2H2 in Arabidopsis thaliana (AT) and Juglans regia (Jr)



A. 基于邻接法构建的系统进化树; B. *JrZFP* 的基因结构; C. *JrZFP* 的保守基序。
 A. Phylogenetic tree was constructed using the Neighbor-joining method; B. The composition and position of Exon, introns and Intron in *JrZFP* genes; C. Conserved motifs in *JrZFP* proteins.

图 4 核桃 Q 型 C2H2 锌指蛋白基因家族的系统发育树、基因结构和保守基序

Fig. 4 Phylogenetic tree, gene structure and conserved motif in Q-type C2H2 gene family

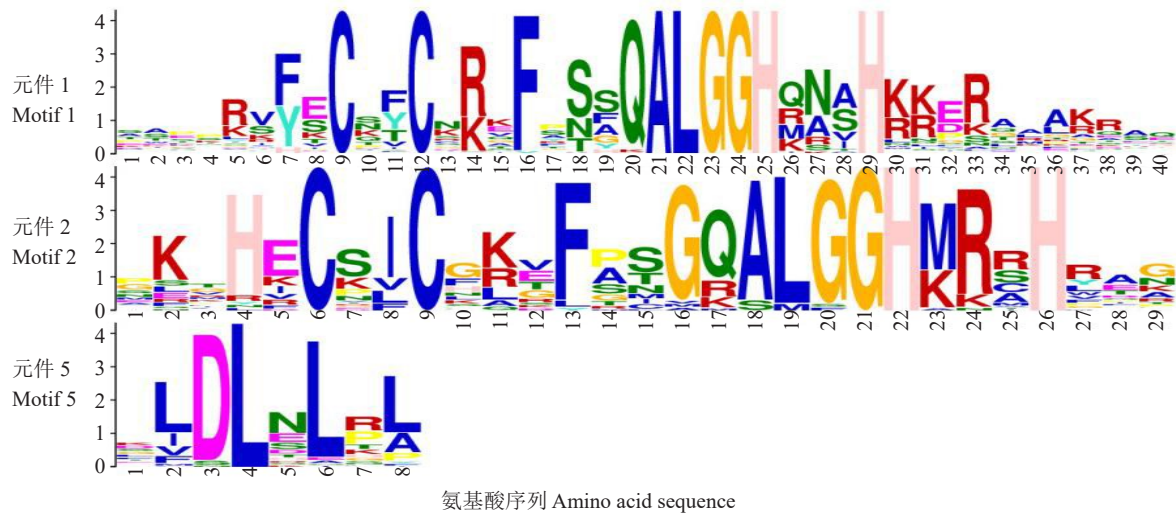


图5 核桃Q型C2H2锌指蛋白基因家族主要保守基序

Fig. 5 The conserved motifs of walnut Q-type C2H2 gene family

基序列预测其在启动子区顺式作用元件的种类和数量,研究该基因家族转录调控因子(图6)。核桃Q型C2H2锌指蛋白基因家族中存在与非生物胁迫响应相关的顺式作用元件,如82个*JrZFP*基因成员的启动子区共包含415个ACE(光)元件、261个ABRE(脱落酸)元件、87个CGTCA-motif(MeJA)元件、81个GARE-motif(赤霉素)元件、65个LTR(低温)元件、56个MBS(干旱)元件、46个TCA-element(水杨酸)元件、46个TGA-element(生长素)元件和30个TC-rich repeats(防御和压力)元件。该基因家族中98%的成员具有光响应元件,93%的成员具有脱落酸响应元件,50%的成员具有低温响应元件,44%的成员具有干旱响应元件。因此,推测*JrZFPs*基因家族在核桃树体响应干旱环境中起重要作用。

2.6 核桃Q型C2H2锌指蛋白基因家族成员的表达分析

2.6.1 核桃Q型C2H2锌指蛋白基因家族在NaCl胁迫下的表达分析 对核桃组培苗进行NaCl胁迫(图7),15个*JrZFP*基因在不同时期表现出不同应答模式,均为显著上调。与S0相比,*JrZFP21*、*JrZFP44*、*JrZFP55*和*JrZFP65*能迅速响应NaCl胁迫,其相对表达量显著上调,在S1期就已达到峰值,其中*JrZFP55*上调最高,是S0的14.85倍;*JrZFP12*、*JrZFP27*、*JrZFP34*、*JrZFP35*、*JrZFP57*和*JrZFP71*应答较为缓慢,其相对表达量在S2达到峰值,其中*JrZFP71*上调最高,显著高于S0,是S0的13.97倍;*JrZFP4*、*JrZFP29*、*JrZFP59*、*JrZFP63*和*JrZFP77*应答最为缓慢,其相对表达量在S3达到峰值,其中

*JrZFP59*上调最高,显著高于S0,是S0的13.89倍。所有时期中,*JrZFP55*相对表达量和S0相比最高,推测*JrZFP55*在核桃NaCl胁迫中起正向调节作用。

2.6.2 核桃Q型C2H2锌指蛋白基因家族在干旱胁迫下的表达分析 对核桃组培苗进行PEG处理(图8),15个*JrZFP*基因在不同时期表现出不同的应答模式,既有显著上调又有显著下调。*JrZFP12*、*JrZFP27*、*JrZFP29*、*JrZFP35*、*JrZFP59*和*JrZFP63*均在S3中相对表达量最高,其中*JrZFP12*上调最高,是S0的13.97倍;与S0相比,*JrZFP4*、*JrZFP21*、*JrZFP34*、*JrZFP44*、*JrZFP55*、*JrZFP57*、*JrZFP65*、*JrZFP71*和*JrZFP77*均显著下调,其中*JrZFP4*、*JrZFP21*、*JrZFP44*和*JrZFP55*的相对表达量在S1最低;S2相对表达量最低的是*JrZFP77*; *JrZFP34*、*JrZFP57*、*JrZFP65*和*JrZFP77*的相对表达量在S3达到最低值。*JrZFP4*、*JrZFP55*和*JrZFP57*在盐胁迫中下调幅度最大,推测这3个基因在核桃干旱胁迫中起负调控作用。

3 讨论

Q型C2H2-ZFPs是植物特有的C2H2锌指蛋白亚家族^[6]。已有研究表明,Q型C2H2-ZFPs在拟南芥、水稻、小麦、马铃薯和杨树等植物中具有特异抗氧化活性^[20]。笔者在本研究中利用生物信息学方法在核桃全基因组范围内共鉴定出82个Q型C2H2锌指蛋白基因,该家族成员在核桃中的理论等电点介于5.33~10.23之间,其等电点范围与马铃薯^[20]和扫帚黍(*Dichantheium oligosanthes*)^[34]相似;核桃Q型

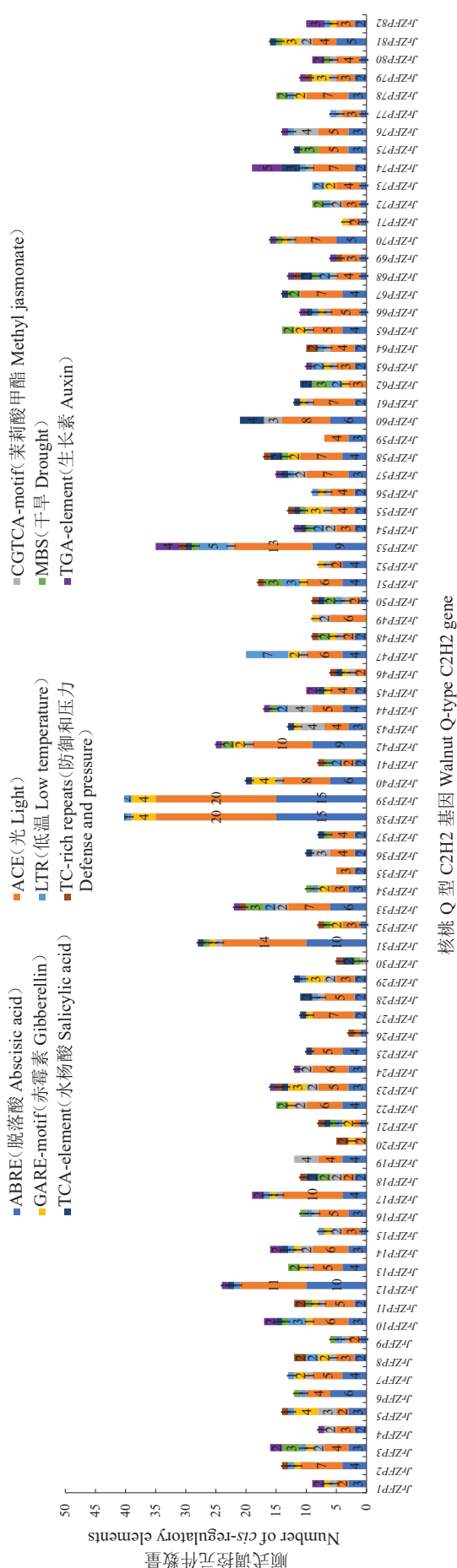
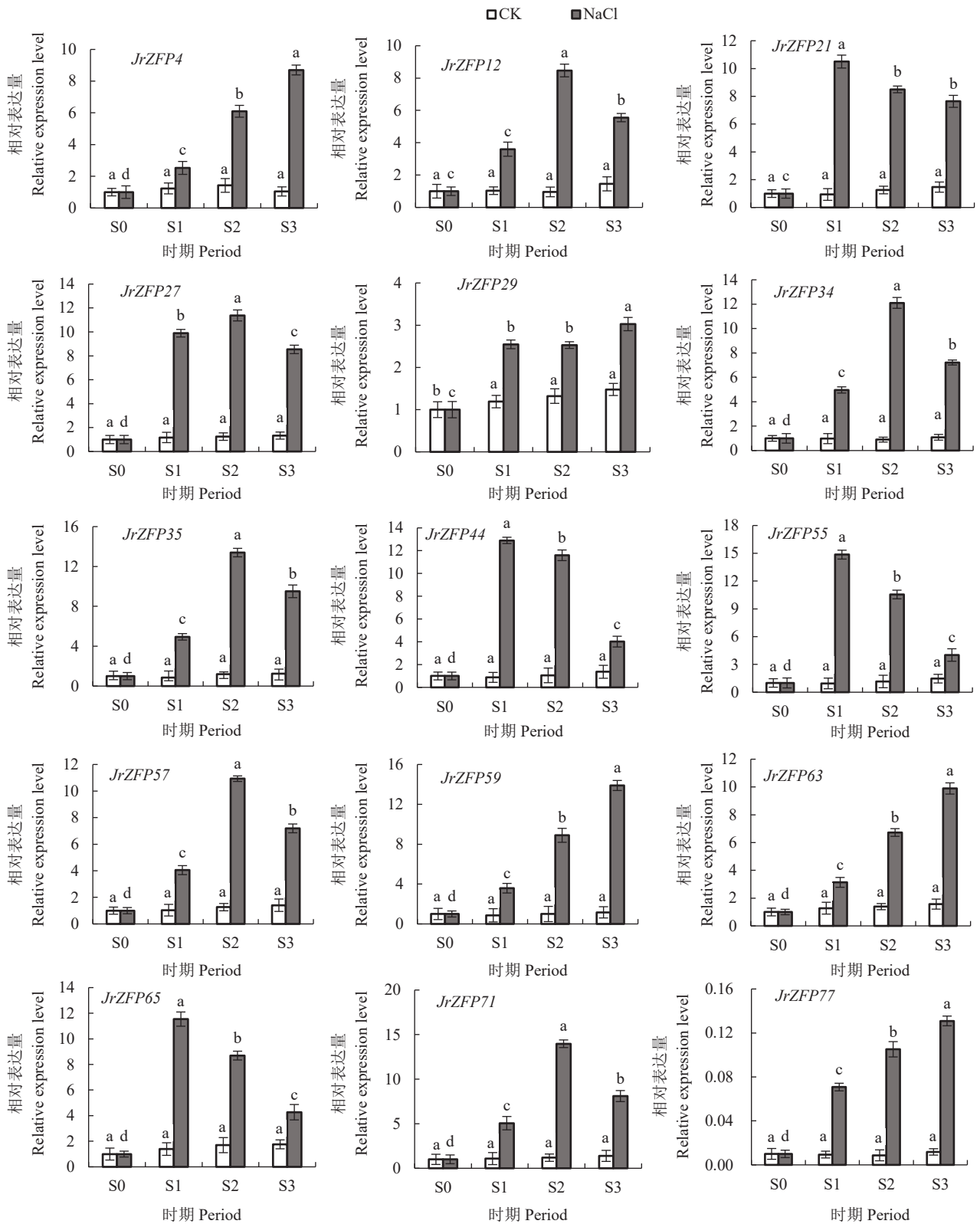


图 6 核桃 Q 型 C2H2 锌指蛋白基因家族顺式调控元件
Fig. 6 Cis-regulatory elements of walnut Q-type ZFP gene family

C2H2 锌指蛋白基因家族 75 个(91.46%)*JrZFP* 基因无内含子,这一数量与水稻^[12]、杨树^[35]和马铃薯^[20]相比明显增加。顺式调控元件分析结果表明,核桃 Q 型 C2H2 锌指蛋白基因家族启动子包含多个对植物激素和应激信号响应的顺式调控元件,包括 ABRE (脱落酸)元件和 CGTCA-motif(MeJA)元件等。拟南芥^[16]中 C2H2-ZFPs 启动子中也发现类似的顺式元件,需要对这些调控区域进行进一步的分析,以验证其在逆境胁迫中的作用。在植物基因组进化^[36]中,基因重复事件有助于新基因家族成员的扩增。在本研究中共鉴定出 11 对(26.83%)串联重复 *JrZFPs* 基因,63 个(76.83%)*JrZFPs* 基因发生节段重复事件。重复事件的发生导致一些进化中的新成员可能会失去原有的功能,或获得新的功能以增强植物的适应性,或成为假基因^[37]。

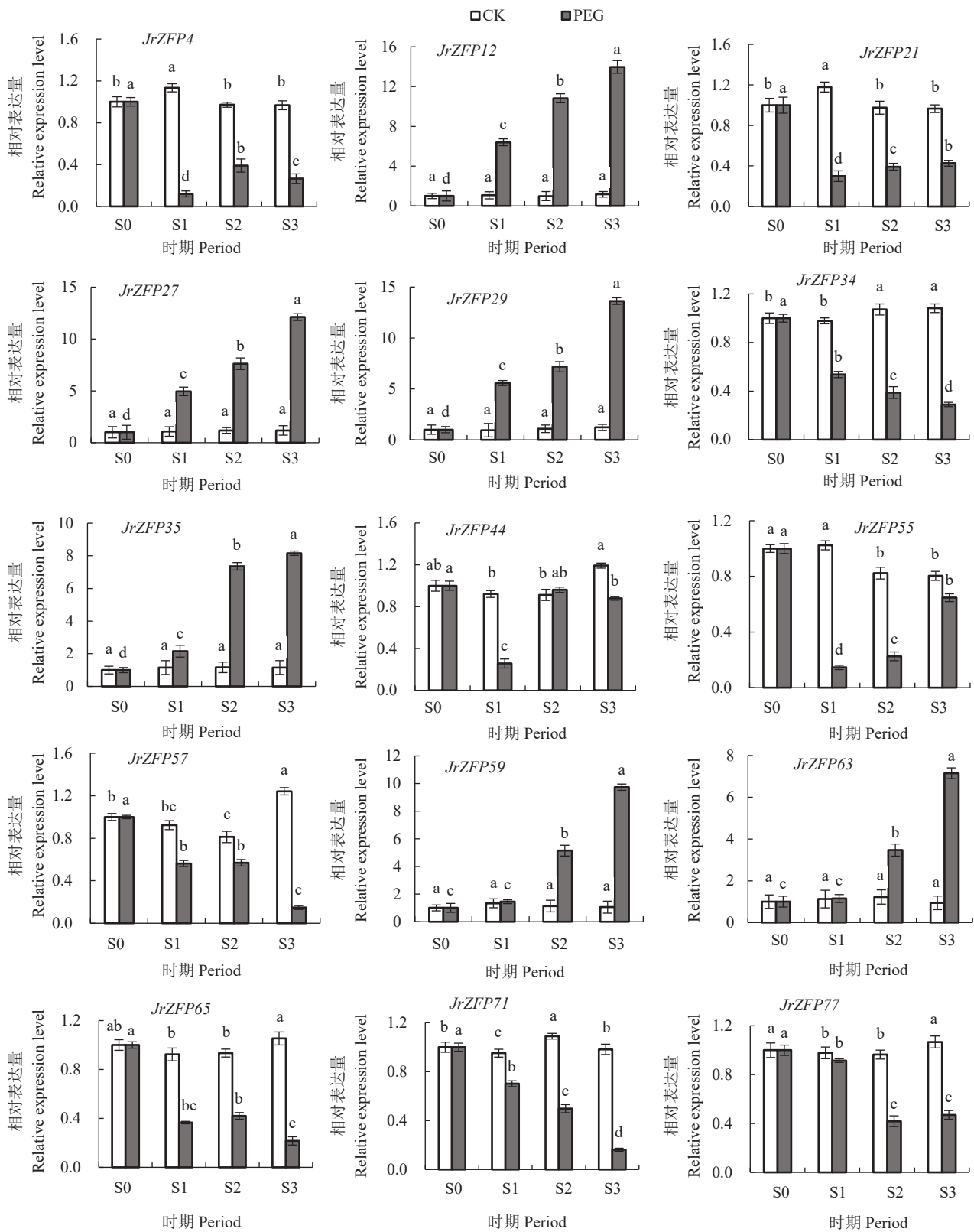
据前人报道,在植物中很多 ZFPs 特征都是转录抑制因子^[38]。例如,LATE(LATE FLOWERING)作为抑制开花的 C2H2 锌指蛋白,在所有组织中的异位表达均会导致植物出现晚开花、花器官特性改变和不育花的现象^[39];KNU(KNUCKLES)作为细胞增殖的锌指蛋白转录因子,沿着拟南芥雌核发育的近远轴调控花的确定性和基本模式元件的相对大小^[40];SUPERMAN 是活跃的抑制因子,其对拟南芥正常发育及开花至关重要^[41]。以上蛋白均含有乙烯响应元件结合因子相关的两亲抑制基序(EAR-motif),是植物中发现的最主要转录抑制基序,LxLxL 和 DLNxxP 是最常见的类型^[42]。蛋白基序是高度保守的氨基酸残基,被认为可能在活性蛋白中具有功能或结构作用^[43]。本研究中,Motif 5 包含 LxLxL 和 DLNxxP。82 个 *JrZFP* 中有 76 个(93%)含有 EAR-motif,其中有 69 个含 EAR-motif LxLxL,有 7 个含 EAR-motif DLNxxP。这表明核桃 Q 型 C2H2 锌指蛋白基因富集了潜在的转录抑制因子。

植物 Q 型 C2H2 锌指蛋白转录因子在植物对干旱、寒冷、渗透胁迫、伤害和机械负荷等各种环境胁迫的耐受性中发挥重要作用^[17]。本研究中,NaCl 处理下 *JrZFPs* 基因均为上调基因,其中 *JrZFP55* 相对表达量显著上调,推测 *JrZFP55* 参与植物 NaCl 胁迫并起正向调节作用。*TaZFP33* 在水稻中同源率最高的蛋白是 *ZOS8-13*,主要在种子中表达,盐胁迫下在叶片中表达上调^[21],这与 *JrZFP34* 表达结果一致。已有研究报道,过表达 *ZAT18* 提高了拟南芥的



15 个 *JrZFPs* 基因在 0(S0)、6(S1)、12(S2)和 24 h(S3)NaCl 胁迫下的表达分析。不同小写字母表示不同时期差异显著($p < 0.05$)。下同。
 The expression analyses of fifteen *JrZFPs* under NaCl stress 0(S0), 6(S1), 12(S2) and 24 h(S3). Different small letters represent significant difference among different periods ($p < 0.05$). The same below.

图 7 在 NaCl 处理下核桃 Q 型 C2H2 锌指蛋白基因家族表达分析
 Fig. 7 Expression analysis of Q-type C2H2 gene family under NaCl treatments in walnut



15 个 *JrZFPs* 在 0(S0)、6(S1)、12(S2)和 24 h(S3)PEG 胁迫下表达分析。

The expression analyses of fifteen *JrZFPs* under PEG stress 0(S0), 6(S1), 12(S2) and 24 h(S3).

图 8 在 PEG 处理下核桃 Q 型 C2H2 锌指蛋白基因家族表达分析

Fig. 8 Expression analysis of Q-type C2H2 gene family under PEG treatments in walnut

耐旱性,说明 *ZATI8* 在拟南芥的耐旱性中发挥了积极作用^[44]。在本研究中, *JrZFP12* 相对表达量显著上调,而且随着干旱胁迫时间的延长不断增加,说明其可能与干旱响应相关; *JrZFP4*、*JrZFP55* 和 *JrZFP57* 显著下调,推测其在干旱胁迫中起负调控作用。

4 结 论

笔者在本研究中采用生物信息学、实时荧光定量等方法在核桃中鉴定了82个 *JrZFP* 基因,并对其进行了理化性质、染色体定位、进化关系、基因结构分析以及干旱胁迫和盐胁迫处理的 qRT-PCR 表达。研究表明, *JrZFP55* 在核桃 NaCl 胁迫中起正向调节作用, *JrZFP4*、*JrZFP55* 和 *JrZFP57* 这3个基因在核桃干旱胁迫中起负调控作用, *JrZFP12* 在核桃干旱胁迫中起正调控作用。初步获得这些参与盐胁迫和干旱胁迫响应的成员,为下一步解析这些响应基因的功能奠定了基础。

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