

# Genome-wide analysis and Stress-Responsive Expression Profiling of the LEA (late embryogenesis abundant) Gene Family in Wild Peanut

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## Research Article

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# Abstract

Late embryogenesis abundant (LEA) proteins generally accumulate in seeds during the later stages of maturation. However, LEA proteins also accumulate in response to abiotic stressors, particularly those involving cellular dehydration. LEA genes have been well-characterized in many plant species, but not in peanut. Here we studied the LEA genes in two wild peanut species (*Arachis duranensis* and *Arachis ipaensis*) in an effort to create a genetic resource for peanut crop improvement. Specifically, we examined AdLEA and AiLEA sequence characteristics, gene structure, evolutionary relationships, chromosomal localization, promoter cis-acting elements, and expression patterns in different tissues under low temperature, salinity, drought, and ABA stress. In all, we identified 65 *AdLEA* and 69 *AiLEA* genes representing all 8 LEA subfamilies, which were unevenly distributed across all 10 peanut chromosomes. The majority of LEA proteins were found to be highly hydrophilic. MEME analysis indicated that LEA gene motifs were conserved within groups, but not between groups. The LEA genes contained a diverse array of stress- and phytohormone-responsive *cis*-acting elements, with the *AdLEA2-20* and *AiLEA2-20* genes containing the greatest number of elements. Both *AdLEA2-20* and *AiLEA2-20* were upregulated in response to cold temperatures, drought, salinity, and ABA-exposure, although the dynamics were tissue-dependent. This study lays the foundation for future studies on the LEA gene family and abiotic stress in peanut, and our results will be invaluable for the genetic improvement of peanut by characterizing the genetic resources of wild peanut species.

## 1 Introduction

First discovered in cotton (*Gossypium hirsutum*), late embryogenesis abundant (LEA) proteins accumulate during the later stages of seed maturation and disappear following germination (Galau et al. 1981). Subsequent studies have found that plants also accumulate LEA proteins in response to stress, including drought, low temperature, salinity, and abscisic acid (ABA) treatment, among others (Zou et al. 2022). Furthermore, LEA proteins can be expressed in a variety of tissues, including roots, leaves, stems, and flowers (Cheng. 2021). LEA proteins have been detected in angiosperms, gymnosperms, non-vascular plants, and algae, suggesting that they may be ubiquitous across the plant kingdom (Campos et al. 2006). LEA proteins are highly hydrophilic, containing a high percentage of glycine and other small amino acids (e.g., alanine, serine, and threonine) and a low percentage of tryptophan and cysteine residues (Hundertmark et al. 2008). The molecular weight of LEA proteins generally ranges between 10 and 30 kD (Zhai et al. 2014). The majority of LEA proteins can be divided into 8 subfamilies based on sequence similarity, including the LEA1-6, dehydrin, and seed maturation protein (SMP) subfamilies (Artur et al. 2019). To date, LEA proteins have been comprehensively characterized in Japanese apricot (*Prunus mume*) (Du et al. 2013), mōsō bamboo (*Phyllostachys edulis*) (Huang et al. 2016), cassava (*Manihot esculenta*) (Wu et al. 2018), and Cavendish banana (*Musa acuminata*) (Piyatissa et al. 2021).

LEA proteins play key roles in plant abiotic stress response, acting as chaperones to mitigate damage to cellular structures (Raga-Carbajal et al. 2022). Wang et al. (Wang et al. 2018) demonstrated that 5 LEA proteins in the LEA1 subfamily are key drivers of drought stress resistance in foxtail millet (*Setaria italica*). LEA expression tends to exhibit variable temporal- and tissue-specific expression patterns depending on the type of stress experienced. Exogenous application or endogenous overexpression through genetic engineering has been found to increase stress resistance. For example, silencing *AtLEA4-5* in *Arabidopsis thaliana* leads to reduced drought tolerance (Olvera-Carrillo et al. 2010), and overexpression of *OsLEA3/2* leads to increased drought and salinity tolerance in both *Arabidopsis* and rice (*Oryza sativa*) (Duan et al. 2012). In fact, increasing LEA gene expression has resulted in improved abiotic stress tolerance in *Arabidopsis* (Acosta et al., 2015), rice (Yu et al., 2016), *Vigna glabrescens* (Singh et al. 2022), and cotton (Shiraku et al. 2022). LEA protein expression can also be regulated by ABA, as suggested by a study in maize (*Zea mays*), in which exogenous ABA application was found to upregulate expression of LEA4 subfamily members (Zamora-Briseno et al. 2016).

Peanut (*Arachis hypogaea*) is an economically important food and oil crop. In order to improve the abiotic stress tolerance of peanut, researchers and breeders are turning to the genetically-diverse wild peanut species (*A. duranensis* and *A.*

*ipaensis*) as genetic resources (Wang et al. 2010). However, to our knowledge, there have been no attempts to characterize the LEA gene family in wild peanut species. Recently, the whole genome sequences of both *A. duranensis* and *A. ipaensis* have been published (Bertioli et al. 2016), and these resources will expedite the study of wild peanuts as a source of novel genes for crop improvement. In order to characterize the structure and function of the LEA protein family genes in wild peanut, we used bioinformatics methods to identify the LEA proteins from both *A. duranensis* and *A. ipaensis*. Specifically, we analyzed the evolutionary relationships, localization, structure, and promoter elements of *LEA* genes. Furthermore, we studied the effect of several abiotic stressors, including salinity, drought, and ABA treatment, on *LEA* gene expression using quantitative real-time PCR (qRT-PCR). Our results provide a comprehensive, genome-wide survey of LEA proteins in two wild peanut species and an important genetic resource for peanut crop improvement.

## 2 Materials And Methods

### 2.1 Identification and classification of LEA gene family members in wild Peanut

The *A. duranensis* (Aradu.V14167.a1.M1) and *A. ipaensis* (Araip.K30076.a1.M1) genomic sequences were downloaded from the peanut genome database (PeanutBase, <https://www.peanutbase.org/>). The Hidden Markov Model (HMM) profiles of the LEA1 (PF03760), LEA2 (PF03168), LEA3 (PF03242), LEA4 (PF02987), LEA5 (PF00477), LEA6 (PF10714), dehydrin (PF00257), and SMP (PF04927) subfamilies were downloaded from the Pfam database (<https://pfam.xfam.org/>). The NCBI Conserved Domain Search (<https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi>) and Pfam databases were also used to identify LEA domains. The HMMER program (<http://hmmer.org/>) was used to match each LEA protein to the genomes. The molecular weight, isoelectric point (pI), and GRand AVerage of hydropathicity (GRAVY) for each protein were predicted using the Expasy tool (<https://www.expasy.org/>).

### 2.2 Sequence Characteristics And Chromosomal Localization Of Lea Genes

The LEA gene sequences of *A. duranensis* and *A. ipaensis* were stored in FASTA format. ClustalW software was used for multi-sequence alignment. The Neighbor-Joining (NJ) method was used to construct a phylogenetic tree. LEA gene chromosomal localization information was obtained from PeanutBase. MapInspect software (<http://mapinspect.software.informer.com/>) was used to create a genetic map.

### 2.3 Gene Structure And Conserved Motifs Of Lea Genes

The coding sequences (CDS) and genomic data of the *AdLEA* and *AiLEA* genes were downloaded from PeanutBase. The intron-exon maps were generated using the GSDS tool (<http://gsds.cbi.pku.edu.cn/>). Conserved motifs were analyzed using the MEME suite (<http://meme-suite.org/>), with the maximum value of discovery set to “5” and all other default settings.

### 2.4 Promoter cis-acting elements of LEA genes

TBtools (<https://bio.tools/tbtools>) was used to examine the 2000 bp sequences upstream of each gene. The PlantCARE online tool (<https://bioinformatics.psb.ugent.be/webtools/plantcare/html/>) was used to predict the *cis*-acting regulatory elements in the promoter region of each gene.

### 2.5 Analysis Of Abiotic Stress-induced Lea Gene Expression

Analysis of abiotic stress-induced *LEA* gene expression was conducted using three varieties of cultivated peanuts (HY23, GY3, and WT2016-6) from the Shandong Peanut Research Institute. Briefly, 20 healthy seeds from each variety were placed in petri dishes to soak. Peanut seeds were germinated at either 15°C or 25°C, or planted in potting soil with vermiculite (2:1) and grown in an incubator with a light/dark cycle of 16/8 h and a temperature cycle of 28°C/22°C. After the peanut seedlings had reached the three-leaf stage, each was subjected to 24 h of treatment with either NaCl (200 mmol/L), PEG6000 (20%), or ABA (100 µm/L). After treatment, the roots, stems, and leaves were frozen in liquid nitrogen and stored at -80°C for subsequent analyses. Each treatment consisted of three biological replicates (seeds). Total RNA was extracted using TRIzol and reverse transcribed into cDNA using either Oligo dT primers or a dNTP mixture. Relative gene expression was quantified using qRT-PCR, using the SYBR green dye, the  $2^{-\Delta\Delta C_t}$  method, and *ACTB* as the internal reference gene. All primers are listed in Table 1.

Table 1

Real-time fluorescence quantitative PCR primers and their base sequences

| Gene             | Forward primer              | Reverse primer              |
|------------------|-----------------------------|-----------------------------|
| <i>ACTB</i>      | 5'-GTCCATCAGGCAACTCGTAGC-3' | 5'-GCCCTCGACTATGAGCAAGAG-3' |
| <i>AdLEA2-20</i> | 5'-ATGAGCAATAGAGCGAAC-3'    | 5'-AAGAGAAGGAAGAGGCAG-3'    |
| <i>AiLEA2-20</i> | 5'-CCAAAATGGAGAGCAGAG-3'    | 5'-AGAGAAGGAAGAGGCAGC-3'    |

### 3 Results

#### 3.1 Identification and characterization of the LEA gene family in peanut

Using the HMMER software to confirm the LEA domain, a total of 65 *AdLEA* genes and 69 *AiLEA* genes from *A. duranensis* and *A. ipaensis*, respectively, were obtained. These genes were found to represent all 8 LEA subfamilies. The LEA2 subfamily had the highest representation, with 42 *AdLEA2* and 49 *AiLEA2* members. In all, the LEA1 subfamily was represented by 5 *AdLEA1* and 2 *AiLEA1* genes; the LEA3 subfamily was represented by 5 *AdLEA3* and *AiLEA3* genes; the LEA4 subfamily was represented by 3 *AdLEA4* and 4 *AiLEA4* genes; the LEA5 subfamily was represented by 5 *AdLEA5* and 4 *AiLEA5* genes; the SMP subfamily was represented by 3 *AdSMP* and *AiSMP* genes; and the LEA6 and dehydrin subfamilies were each represented by 1 gene. The physical and chemical properties of each LEA gene are listed in Tables 2 and 3. Overall, AdLEA proteins were characterized by a maximum molecular weight of 43,209.72 Da (AdLEA4-1), a minimum molecular weight of 9,659.06 Da (AdLEA3-2), and pls ranging from 4.78 (AdLEA2-1) to 10.51 (AdLEA2-30). The majority of AdLEA proteins had a pl > 7.0, indicating that they are primarily alkaline. AiLEA proteins were characterized by a maximum molecular weight of 43,388.9 Da (AiLEA4-1), a minimum molecular weight of 9,629.97 Da (AiLEA3-2), and pls ranging from 4.61 (AiSMP-2) to 10.38 (AiLEA2-50). The AdLEA family contained 15 hydrophobic proteins, with the remainder exhibiting high hydrophilicity, and a mean GRAVY ranging from 0.003 to -1.426. The AiLEA family contained 17 hydrophobic proteins, with the remainder exhibiting high hydrophilicity, and a mean GRAVY ranging from 0.023 to -1.477.

Table 2  
Identification and structural analysis of the LEA gene family in *A. duranensis*

| Domain | Gene             | NGN       | Map position (bp)            | LR (bp) | LP (aa) | MW (Da)  | pI    | GRAVY  |
|--------|------------------|-----------|------------------------------|---------|---------|----------|-------|--------|
| LEA1   | <i>AdLEA1-1</i>  | 107485214 | Chr.A01:34563454–34564516    | 503     | 127     | 13706.44 | 9.41  | -0.963 |
|        | <i>AdLEA1-2</i>  | 107483079 | Chr.A01:25503735–25505110    | 707     | 127     | 13706.44 | 9.41  | -0.963 |
|        | <i>AdLEA1-3</i>  | 107492896 | Chr.A06: 12429509–12430847   | 923     | 205     | 21675.22 | 9.9   | -0.999 |
|        | <i>AdLEA1-4</i>  | 107464692 | Chr.A09:11498553–11499754)   | 700     | 137     | 14197.8  | 8.81  | -0.944 |
|        | <i>AdLEA1-5</i>  | 107494951 | Chr. 101051181–101052227     | 756     | 151     | 16154.13 | 5.47  | -0.819 |
| LEA2   | <i>AdLEA2-1</i>  | 107496667 | Chr.A07: 5225234–5227271     | 1419    | 324     | 36020.87 | 4.78  | -0.496 |
|        | <i>AdLEA2-2</i>  | 107485848 | Chr.A04:112862164–112864422  | 1037    | 195     | 20712.11 | 5.84  | 0.080  |
|        | <i>AdLEA2-3</i>  | 107472778 | Chr. A02: 2538142–2539536    | 586     | 156     | 17054.64 | 5.58  | -0.143 |
|        | <i>AdLEA2-4</i>  | 107468462 | Chr.A10: 3775207–3776059     | 853     | 196     | 22337.95 | 9.87  | -0.059 |
|        | <i>AdLEA2-5</i>  | 107494114 | Chr.A06: 77062165–77063330   | 1166    | 229     | 25477.62 | 9.59  | -0.025 |
|        | <i>AdLEA2-6</i>  | 107476157 | Chr.A02: 92281722–92282890   | 1169    | 256     | 28696.41 | 9.14  | 0.038  |
|        | <i>AdLEA2-7</i>  | 107458048 | Chr.A07: 16861040–16862273   | 1234    | 258     | 28700.29 | 10.1  | -0.338 |
|        | <i>AdLEA2-8</i>  | 107472891 | Chr.A02: 2549214–2549968     | 755     | 221     | 24414.52 | 9.69  | 0.003  |
|        | <i>AdLEA2-9</i>  | 107474407 | Chr.A01:8212174–8213384)     | 1211    | 255     | 28430.29 | 10.23 | -0.209 |
|        | <i>AdLEA2-10</i> | 107461516 | Chr.A08: 22825732–22826891   | 1268    | 257     | 27512    | 10.11 | -0.059 |
|        | <i>AdLEA2-11</i> | 107485620 | Chr.A04: 108768504–108771191 | 1047    | 193     | 20796.23 | 9.32  | 0.253  |
|        | <i>AdLEA2-12</i> | 107467148 | Chr.A09: 111988644–111989496 | 853     | 228     | 26002.74 | 10.24 | -0.358 |
|        | <i>AdLEA2-13</i> | 107471534 | Chr.A10: 104114798–104116038 | 1005    | 211     | 23660.48 | 9.8   | 0.090  |
|        | <i>AdLEA2-14</i> | 107476511 | Chr. A03:179385–183104       | 1008    | 290     | 31971.22 | 9.53  | 0.113  |
|        | <i>AdLEA2-15</i> | 107476155 | Chr.A02: 92270965–92272596   | 1112    | 210     | 23903.81 | 9.66  | 0.118  |

Note: NGM,NCBI gene number; LR, Length of mRNA; LP, Length of protein; MW, Molecular weight; pI, Isoelectric point.

| Domain | Gene             | NGN       | Map position (bp)            | LR (bp) | LP (aa) | MW (Da)  | pI    | GRAVY  |
|--------|------------------|-----------|------------------------------|---------|---------|----------|-------|--------|
|        | <i>AdLEA2-16</i> | 107477687 | Chr.A03:12488137–12489372    | 1029    | 215     | 23718.34 | 9.65  | 0.117  |
|        | <i>AdLEA2-17</i> | 107479837 | Chr.A03:96926309–96927519    | 1211    | 241     | 26461.42 | 9.32  | -0.095 |
|        | <i>AdLEA2-18</i> | 107481591 | Chr.A03: 129922422–129924178 | 1665    | 250     | 27359.99 | 9.5   | 0.161  |
|        | <i>AdLEA2-19</i> | 107488285 | Chr.A05: 15180113–15181582   | 1470    | 211     | 23525.2  | 10.21 | -0.135 |
|        | <i>AdLEA2-20</i> | 107468566 | Chr.A10: 6279943–6283387     | 1356    | 250     | 26828.85 | 9.39  | 0.110  |
|        | <i>AdLEA2-21</i> | 107490262 | Chr.A05: 91677980–91680390   | 1108    | 248     | 27746.78 | 8.26  | -0.031 |
|        | <i>AdLEA2-22</i> | 107465970 | Chr.A09: 78805518–78806442   | 925     | 260     | 29144.36 | 9.85  | -0.065 |
|        | <i>AdLEA2-23</i> | 107469522 | Chr.A10: 27205346–27206159   | 814     | 214     | 23692.28 | 9.47  | -0.127 |
|        | <i>AdLEA2-24</i> | 107491896 | Chr.A06: 1713575–1714821     | 1247    | 252     | 27753.53 | 9.72  | -0.078 |
|        | <i>AdLEA2-25</i> | 107488396 | Chr.A05: 16201639–16202991   | 1353    | 203     | 21691.18 | 9.6   | 0.428  |
|        | <i>AdLEA2-26</i> | 107459529 | Chr.A07: 71529179–71529797   | 390     | 130     | 14016.22 | 6.48  | 0.347  |
|        | <i>AdLEA2-27</i> | 107477326 | Chr.A03: 7947036–7950319     | 1222    | 304     | 34214.77 | 9.39  | -0.378 |
|        | <i>AdLEA2-28</i> | 107477798 | Chr. A03: 14443642–14444423  | 782     | 187     | 20466.4  | 9.41  | 0.443  |
|        | <i>AdLEA2-29</i> | 107490545 | Chr.A05: 96424889–96425954   | 1066    | 212     | 24236.95 | 9.8   | -0.036 |
|        | <i>AdLEA2-30</i> | 107479724 | Chr.A03: 90516372–90517722   | 1351    | 271     | 29462.26 | 10.51 | -0.134 |
|        | <i>AdLEA2-31</i> | 107480340 | Chr.A01: 13482390–13484266   | 1416    | 323     | 35323.28 | 9.93  | -0.369 |
|        | <i>AdLEA2-32</i> | 107496102 | Chr.A07: 1314549–1315962     | 1414    | 228     | 26216.78 | 9.9   | -0.031 |
|        | <i>AdLEA2-33</i> | 110281332 | Chr.A05: 96344403–96345532   | 1130    | 210     | 24282.13 | 9.65  | 0.048  |
|        | <i>AdLEA2-34</i> | 107488625 | Chr.A05: 20402723–20403934   | 1212    | 194     | 22011.27 | 9.31  | -0.426 |
|        | <i>AdLEA2-35</i> | 107473069 | Chr. A02 :4900876–4902095    | 1220    | 249     | 27958.65 | 9.46  | -0.154 |

Note: NGM,NCBI gene number; LR, Length of mRNA; LP, Length of protein; MW, Molecular weight; pI, Isoelectric point.

| Domain | Gene             | NGN       | Map position (bp)            | LR (bp) | LP (aa) | MW (Da)  | pI    | GRAVY  |
|--------|------------------|-----------|------------------------------|---------|---------|----------|-------|--------|
|        | <i>AdLEA2-36</i> | 107490686 | Chr.A05: 94867531–94869926   | 1554    | 316     | 34874.69 | 9.89  | -0.403 |
|        | <i>AdLEA2-37</i> | 107481916 | Chr.A03: 127783341–127785139 | 1630    | 317     | 35436.85 | 9.98  | -0.195 |
|        | <i>AdLEA2-38</i> | 110273779 | Chr.A07: 59399685–59400944   | 1260    | 253     | 28594.86 | 9.1   | -0.417 |
|        | <i>AdLEA2-39</i> | 107491812 | Chr. A06: 4599771–4600822    | 1052    | 232     | 26766.88 | 9.81  | -0.278 |
|        | <i>AdLEA2-40</i> | 107462096 | Chr.A08:28699897–28707501    | 1037    | 260     | 28923.97 | 9.8   | -0.289 |
|        | <i>AdLEA2-41</i> | 107471438 | Chr.A10: 104125428–104126386 | 959     | 226     | 26205.41 | 9.61  | -0.100 |
|        | <i>AdLEA2-42</i> | 107485390 | Chr.A04: 105054854           | 955     | 225     | 24750.37 | 9.41  | 0.154  |
| LEA3   | <i>AdLEA3-1</i>  | 107492478 | Chr.A06: 8170095–8170694     | 600     | 98      | 10189.49 | 10.06 | -0.494 |
|        | <i>AdLEA3-2</i>  | 107475974 | Chr.A02: 90798756–90800227   | 650     | 92      | 9659.06  | 9.93  | -0.359 |
|        | <i>AdLEA3-3</i>  | 107483578 | Chr.A04: 17452420–17454114   | 689     | 108     | 11873.36 | 10.09 | -1.252 |
|        | <i>AdLEA3-4</i>  | 107464042 | Chr.A01:96541679–96544211    | 488     | 96      | 10209.49 | 9.51  | -0.411 |
|        | <i>AdLEA3-5</i>  | 107475973 | Chr.A02: 90804235–90805959   | 493     | 103     | 10960.58 | 9.75  | 0.007  |
| LEA4   | <i>AdLEA4-1</i>  | 107493805 | Chr.A06 :57621052–57622969   | 1808    | 399     | 43209.72 | 6.08  | -1.121 |
|        | <i>AdLEA4-2</i>  | 107476234 | Chr.A02: 93238771–93240606   | 1274    | 340     | 36780.13 | 5.16  | -1.248 |
|        | <i>AdLEA4-3</i>  | 107470276 | Chr.A01: 100952551–100954058 | 1286    | 300     | 32371.46 | 5.21  | -1.136 |
| LEA5   | <i>AdLEA5-1</i>  | 107484967 | Chr. A04: 93820947–93822131  | 621     | 115     | 12598.93 | 6.85  | -1.383 |
|        | <i>AdLEA5-2</i>  | 107475431 | Chr.A02: 84227741–84228513   | 652     | 97      | 10427.24 | 5.28  | -1.417 |
|        | <i>AdLEA5-3</i>  | 107475320 | Chr.A02: 82181195–82182023   | 710     | 97      | 10487.48 | 6.61  | -1.395 |
|        | <i>AdLEA5-4</i>  | 107475321 | Chr.A02: 82184204–82185004   | 699     | 95      | 10140.11 | 5.91  | -1.377 |
|        | <i>AdLEA5-5</i>  | 107475319 | Chr.A02: 82129954–82130738,  | 676     | 97      | 10547.45 | 5.31  | -1.415 |
| LEA6   | <i>AdLEA6-1</i>  | 107487691 | Chr.A05: 6854251–6854781     | 531     | 76      | 11832.78 | 5.28  | -1.426 |

Note: NGM,NCBI gene number; LR, Length of mRNA; LP, Length of protein; MW, Molecular weight; pI, Isoelectric point.

| Domain                                                                                                              | Gene                | NGN       | Map position (bp)           | LR (bp) | LP (aa) | MW (Da)  | pI   | GRAVY  |
|---------------------------------------------------------------------------------------------------------------------|---------------------|-----------|-----------------------------|---------|---------|----------|------|--------|
| SMP                                                                                                                 | <i>AdSMP-1</i>      | 107467109 | Chr.A09:111268280–111269437 | 1067    | 257     | 26064.89 | 5.00 | -0.318 |
|                                                                                                                     | <i>AdSMP-2</i>      | 107473377 | Chr.A02: 8971264–8973732    | 1074    | 228     | 23560.34 | 4.72 | -0.402 |
|                                                                                                                     | <i>AdSMP-2-1</i>    | 107473377 | Chr.A02:8971264–8973732     | 1090    | 265     | 27275.43 | 4.85 | -0.402 |
| Dehydrin                                                                                                            | <i>AdDehydrin-1</i> | 107459078 | Chr.A07: 57016019–57017099  | 992     | 208     | 21045.99 | 7.23 | -0.926 |
| Note: NGM,NCBI gene number; LR, Length of mRNA; LP, Length of protein; MW, Molecular weight; pI, Isoelectric point. |                     |           |                             |         |         |          |      |        |

Table 3  
Identification and structural analysis of the LEA gene family in *A. ipaensis*

| Domain | Gene             | NGN       | Map position (bp)           | LR (bp) | LP (aa) | MW (Da)  | pI    | GRAVY  |
|--------|------------------|-----------|-----------------------------|---------|---------|----------|-------|--------|
| LEA1   | <i>AiLEA1-1</i>  | 107617559 | Chr.B01:31943122–31944403   | 732     | 127     | 13706.44 | 9.41  | -0.963 |
|        | <i>AiLEA1-2</i>  | 107645421 | Chr.B06:3646375–3647742     | 918     | 167     | 17232.75 | 9.65  | -1.092 |
| LEA2   | <i>AiLEA2-1</i>  | 107629071 | Chr.B03:4960532–4962558     | 1408    | 323     | 35905.79 | 4.81  | -0.486 |
|        | <i>AiLEA2-2</i>  | 107635106 | Chr.B04:122606460–122609473 | 1220    | 195     | 20684.06 | 5.84  | 0.068  |
|        | <i>AiLEA2-3</i>  | 107622459 | Chr.B02:1888747–1890614     | 843     | 156     | 17040.61 | 5.58  | 0.583  |
|        | <i>AiLEA2-4</i>  | 107622251 | Chr.B10:5617555–5618402     | 848     | 196     | 22337.95 | 9.87  | -0.059 |
|        | <i>AiLEA2-6</i>  | 107626350 | Chr.B02:106954346–106955286 | 941     | 256     | 28656.39 | 9.14  | 0.044  |
|        | <i>AiLEA2-7</i>  | 107608161 | Chr.B07:17834507–17835791   | 1285    | 258     | 28696.3  | 10.1  | -0.342 |
|        | <i>AiLEA2-8</i>  | 107628113 | Chr.B02:1893151–1894121     | 971     | 221     | 24451.6  | 9.78  | -0.013 |
|        | <i>AiLEA2-9</i>  | 107639295 | Chr.B01:1996862–1998033     | 1172    | 255     | 28378.17 | 10.1  | -0.205 |
|        | <i>AiLEA2-10</i> | 107613756 | Chr.B08:1250609–1251891     | 1283    | 257     | 27539.03 | 10.11 | -0.070 |
|        | <i>AiLEA2-11</i> | 107638037 | Chr.B04:117238928–117241652 | 1050    | 193     | 20812.23 | 9.32  | 0.239  |
|        | <i>AiLEA2-12</i> | 107626349 | Chr.B02:106934964–106943690 | 913     | 210     | 23827.75 | 9.66  | 0.147  |
|        | <i>AiLEA2-13</i> | 107624155 | Chr.B10:130772340–13077349  | 991     | 211     | 23688.53 | 9.8   | 0.102  |
|        | <i>AiLEA2-14</i> | 107634132 | Chr.B03:2141628–2142849     | 754     | 207     | 22604.14 | 7.09  | 0.244  |
|        | <i>AiLEA2-15</i> | 107619408 | Chr.B09:145196256–145197201 | 946     | 228     | 26018.74 | 10.05 | -0.357 |
|        | <i>AiLEA2-16</i> | 107629779 | Chr.B03:15209356–15210366   | 803     | 215     | 23704.32 | 9.65  | 0.116  |
|        | <i>AiLEA2-17</i> | 107630744 | Chr.B03:98515654–98516839   | 1186    | 241     | 26369.26 | 9.4   | -0.133 |
|        | <i>AiLEA2-18</i> | 107633872 | Chr.B03:130760138–130761881 | 1652    | 250     | 27402.07 | 9.5   | 0.179  |
|        | <i>AiLEA2-19</i> | 107640246 | Chr.B05:15983521–15985501   | 1981    | 212     | 23737.45 | 10.2  | -0.106 |

Note: NGM,NCBI gene number; LR, Length of mRNA; LP, Length of protein; MW, Molecular weight; pI, Isoelectric point.

| Domain | Gene               | NGN       | Map position (bp)            | LR (bp) | LP (aa) | MW (Da)  | pI    | GRAVY  |
|--------|--------------------|-----------|------------------------------|---------|---------|----------|-------|--------|
|        | <i>AiLEA2-20</i>   | 107623936 | Chr.B10:10571988–10575953    | 1357    | 249     | 26727.74 | 9.39  | 0.035  |
|        | <i>AiLEA2-20-1</i> | 107623936 | Chr.B10:10571988–10575953    | 1700    | 227     | 24491.09 | 9.25  | 0.035  |
|        | <i>AiLEA2-20-2</i> | 107623936 | Chr.B10:10571988–10575954    | 1700    | 242     | 26202.98 | 9.25  | -0.053 |
|        | <i>AiLEA2-21</i>   | 107644365 | Chr.B05:137149070–137151467  | 1109    | 249     | 27842.93 | 8.52  | -0.033 |
|        | <i>AiLEA2-22</i>   | 107618630 | Chr.B09:92573655–92575148    | 1494    | 260     | 29185.38 | 9.85  | -0.117 |
|        | <i>AiLEA2-23</i>   | 107621574 | Chr.B10:35648408–35649576    | 1169    | 214     | 23715.32 | 9.47  | -0.133 |
|        | <i>AiLEA2-24</i>   | 107646127 | Chr.B06:20211763–20213095    | 1333    | 252     | 27753.53 | 9.72  | -0.078 |
|        | <i>AiLEA2-25</i>   | 107641732 | Chr.B05:16792078–16793307    | 1230    | 203     | 21700.18 | 9.55  | 0.374  |
|        | <i>AiLEA2-26</i>   | 107610314 | Chr.B08:377155–379256        | 495     | 165     | 18081.43 | 4.97  | -0.079 |
|        | <i>AiLEA2-27</i>   | 107634172 | Chr.B03:10520172–10523472    | 1294    | 325     | 36643.43 | 9.58  | -0.455 |
|        | <i>AiLEA2-28</i>   | 107632693 | Chr.B03:17642232–17643060    | 829     | 197     | 21343.1  | 8.83  | 0.477  |
|        | <i>AiLEA2-30</i>   | 107630695 | Chr.B03:91396489–91397965)   | 1477    | 278     | 30137.98 | 10.28 | -0.125 |
|        | <i>AiLEA2-31</i>   | 107617004 | Chr.B01:18272804–18274776,   | 1440    | 323     | 35309.25 | 9.93  | -0.369 |
|        | <i>AiLEA2-32</i>   | 107608192 | Chr.B07:1009580–1010263)     | 684     | 228     | 26216.78 | 9.9   | -0.031 |
|        | <i>AiLEA2-33</i>   | 107641110 | Chr.B05:130152038–130153157  | 1120    | 210     | 24299.1  | 9.76  | -0.069 |
|        | <i>AiLEA2-33-1</i> | 107642077 | Chr.B05:129687475–129688325  | 851     | 212     | 24278.05 | 9.95  | -0.037 |
|        | <i>AiLEA2-33-2</i> | 107642082 | Chr.B05:130157667–130158576  | 910     | 212     | 24251.96 | 9.95  | -0.062 |
|        | <i>AiLEA2-34</i>   | 107640334 | Chr.B05:20443686–20444886)   | 1201    | 194     | 21966.32 | 9.52  | -0.388 |
|        | <i>AiLEA2-35</i>   | 107624430 | Chr.B02:6186551–6188071)     | 1521    | 249     | 28000.79 | 9.46  | -0.123 |
|        | <i>AiLEA2-36</i>   | 107644246 | Chr.B05:132811712–132814029) | 1482    | 316     | 34874.69 | 9.89  | -0.403 |
|        | <i>AiLEA2-37</i>   | 107633923 | Chr.B03:128424810–128426605, | 1630    | 289     | 32127.82 | 9.97  | -0.281 |

Note: NGM,NCBI gene number; LR, Length of mRNA; LP, Length of protein; MW, Molecular weight; pI, Isoelectric point.

| Domain | Gene               | NGN       | Map position (bp)            | LR (bp) | LP (aa) | MW (Da)  | pI    | GRAVY  |
|--------|--------------------|-----------|------------------------------|---------|---------|----------|-------|--------|
|        | <i>AiLEA2-38</i>   | 107607332 | Chr.B07:56744364–56745626    | 1263    | 253     | 28826.25 | 9.24  | -0.431 |
|        | <i>AiLEA2-39</i>   | 107634840 | Chr.B03:17643163–17643967    | 805     | 200     | 21824.87 | 9.28  | 0.395  |
|        | <i>AiLEA2-40</i>   | 107628044 | Chr.B02:106948632–106949390) | 759     | 233     | 26759.12 | 10.02 | -0.199 |
|        | <i>AiLEA2-41</i>   | 107614367 | Chr.B08:5919921–5927007,     | 1255    | 253     | 28121.04 | 9.92  | -0.221 |
|        | <i>AiLEA2-41-1</i> | 107614267 | Chr.B08:5919921–5927007      | 1505    | 250     | 27777.57 | 9.83  | -0.253 |
|        | <i>AiLEA2-42</i>   | 107638001 | Chr.B04:113607369–113608385  | 1017    | 225     | 24839.42 | 9.41  | 0.142  |
|        | <i>AiLEA2-43</i>   | 107634744 | Chr.B03:129430563–129432478  | 1916    | 372     | 41938.45 | 10.17 | 0.023  |
|        | <i>AiLEA2-44</i>   | 107621320 | Chr.B10:130759543–130760169) | 627     | 209     | 23634.44 | 9.82  | 0.023  |
|        | <i>AiLEA2-45</i>   | 107644383 | Chr.B05:137709611–137711789  | 1296    | 322     | 35615.13 | 9.71  | -0.543 |
|        | <i>AiLEA2-46</i>   | 107635829 | Chr.B04:932806–935320)       | 1527    | 308     | 34019.67 | 4.75  | -0.318 |
|        | <i>AiLEA2-47</i>   | 107604665 | Chr.B06:94203295–94204441,   | 1147    | 229     | 25451.53 | 9.53  | -0.041 |
|        | <i>AiLEA2-48</i>   | 110271211 | Chr.B04:113620694–113621316  | 623     | 143     | 15791.52 | 9.17  | 0.144  |
|        | <i>AiLEA2-49</i>   | 107611820 | Chr.B08:5917567–5918730      | 1164    | 227     | 25139.91 | 8.94  | -0.001 |
|        | <i>AiLEA2-50</i>   | 107616387 | Chr.B01:7011800–7013852)     | 2053    | 257     | 29134.27 | 10.38 | -0.040 |
|        | <i>AiLEA2-51</i>   | 107638001 | Chr.B04:113607369–113608385  | 1017    | 225     | 24839.42 | 9.41  | 0.142  |
| LEA3   | <i>AiLEA3-1</i>    | 107645642 | Chr.B06:7912646–7913246)     | 601     | 98      | 10189.49 | 10.06 | -0.494 |
|        | <i>AiLEA3-2</i>    | 107626179 | Chr.B02:104614777–104616013) | 668     | 92      | 9629.97  | 9.7   | -0.302 |
|        | <i>AiLEA3-3</i>    | 107639040 | Chr.B04:17347795–17349700,   | 830     | 108     | 11760.24 | 10.09 | -0.536 |
|        | <i>AiLEA3-4</i>    | 107628585 | Chr.B02:104618486–104620690) | 966     | 103     | 10971.56 | 9.75  | -0.048 |
|        | <i>AiLEA3-5</i>    | 107638683 | Chr.B01:130162705–130163685) | 900     | 96      | 10333.68 | 9.84  | -0.469 |
| LEA4   | <i>AiLEA4-1</i>    | 107648779 | Chr.B06:62924915–62926845)   | 1822    | 400     | 43388.9  | 5.84  | -1.148 |

Note: NGM,NCBI gene number; LR, Length of mRNA; LP, Length of protein; MW, Molecular weight; pI, Isoelectric point.

| Domain                                                                                                              | Gene                | NGN       | Map position (bp)            | LR (bp) | LP (aa) | MW (Da)  | pI   | GRAVY  |
|---------------------------------------------------------------------------------------------------------------------|---------------------|-----------|------------------------------|---------|---------|----------|------|--------|
|                                                                                                                     | <i>AiLEA4-1-1</i>   | 107648779 | Chr.B06:62924915–62926845)   | 1827    | 400     | 43358.92 | 5.99 | -1.148 |
|                                                                                                                     | <i>AiLEA4-2</i>     | 107626431 | Chr.B02:107977216–107979056, | 1216    | 341     | 36853.44 | 5.55 | -1.233 |
|                                                                                                                     | <i>AiLEA4-3</i>     | 107635540 | Chr.B01:123329321–123330936  | 1311    | 300     | 32302.41 | 5.21 | -1.119 |
| LEA5                                                                                                                | <i>AiLEA5-1</i>     | 107637978 | Chr.B04:105851581–105852317  | 372     | 117     | 12747.99 | 5.8  | -1.375 |
|                                                                                                                     | <i>AiLEA5-2</i>     | 107625779 | Chr.B02:93958329–93961501,   | 1038    | 231     | 25286.96 | 5.62 | -1.173 |
|                                                                                                                     | <i>AiLEA5-3</i>     | 107625778 | Chr.B02:93955704–93956474,   | 670     | 97      | 10545.43 | 5.31 | -1.477 |
|                                                                                                                     | <i>AiLEA5-4</i>     | 107637129 | Chr.B04:121497458–121499834, | 831     | 187     | 20269.24 | 7.84 | -1.391 |
| LEA6                                                                                                                | <i>AiLEA6-1</i>     | 107641664 | Chr.B05:7108414–7109021      | 608     | 110     | 11832.78 | 5.28 | -1.426 |
| SMP                                                                                                                 | <i>AiSMP-1</i>      | 107619719 | Chr.B09:145741037–145742209  | 1082    | 257     | 26040.87 | 5.19 | -0.300 |
|                                                                                                                     | <i>AiSMP-2</i>      | 107624947 | Chr.B02:12132476–12134865,   | 1049    | 266     | 27369.58 | 4.74 | -0.370 |
|                                                                                                                     | <i>AiSMP-2-1</i>    | 107624947 | Chr.B02:12132476–12134865    | 1071    | 227     | 23364.13 | 4.61 | -0.293 |
| Dehydrin                                                                                                            | <i>AiDehydrin-1</i> | 107609923 | Chr.B07:64849982–64851061    | 993     | 208     | 21021.88 | 6.87 | -0.920 |
| Note: NGM,NCBI gene number; LR, Length of mRNA; LP, Length of protein; MW, Molecular weight; pI, Isoelectric point. |                     |           |                              |         |         |          |      |        |

## 3.2 Comparison Of Lea Gene Family In Peanut And Other Plants

To examine the evolutionary history of the LEA gene family in plants, we compared 69 *AiLEA* and 65 *AdLEA* genes with 51 LEA genes from *Arabidopsis*, 53 LEA genes from black cottonwood (*Populus trichocarpa*), 21 LEA genes from mōsō bamboo, and 32 LEA genes from maize (Table 4). Interestingly, it appears that peanut contains more LEA genes than any other species in our comparison and only *Arabidopsis* was found to contain members of the AtM subfamily. Overall, the phylogenetic results (Fig. 1) are consistent with the domain results, with LEA subfamilies clustered together. Furthermore, AdLEA1-5, which contains the LEA1 domain, is clustered together with *Arabidopsis*.

Table 4  
Number of LEA proteins identified in diverse plant species

| Species                                  | LEA1 | LEA2 | LEA3 | LEA4 | LEA5 | LEA6 | Dehydrin | SMP | AtM | Total |
|------------------------------------------|------|------|------|------|------|------|----------|-----|-----|-------|
| <i>Arachis duranensis</i>                | 5    | 42   | 5    | 3    | 5    | 1    | 1        | 3   | 0   | 65    |
| <i>Arachis ipaensis</i>                  | 2    | 49   | 5    | 4    | 4    | 1    | 1        | 3   | 0   | 69    |
| <i>Arabidopsis thaliana</i> <sup>a</sup> | 3    | 3    | 4    | 18   | 2    | 3    | 6        | 10  | 2   | 51    |
| <i>Populus trichocarpa</i> <sup>c</sup>  | 3    | 4    | 5    | 26   | 1    | 2    | 2        | 10  | 0   | 53    |
| Mōsō Bamboo <sup>b</sup>                 | 1    | 4    | 5    | 5    | 0    | 1    | 6        | 0   | 0   | 21    |
| Maize <sup>d</sup>                       | 4    | 6    | 7    | 3    | 2    | 1    | 5        | 4   | 0   | 32    |

a) LEA (Late Embryogenesis Abundant) proteins and their encoding genes in *Arabidopsis thaliana* (Hundertmark et al. 2008)

b) Genome-wide identification, characterization, and stress-responsive expression profiling of genes encoding LEA (late embryogenesis abundant) proteins in mōsō bamboo (*Phyllostachys edulis*) (Huang et al. 2016)

c) Data from genome-wide analysis of the LEA (late embryogenesis abundant) protein gene family in *Populus trichocarpa* (Lan et al. 2013)

d) Late embryogenesis abundant (LEA) gene family in maize: identification, evolution, and expression Profiles ( Li et al. 2016 )

### 3.3 Sequence Characteristics And Chromosomal Localization Of Lea Genes

The chromosomal localization of LEA genes is shown in Fig. 2. Overall, both *AdLEA* and *AiLEA* genes were found to be unevenly distributed across the 10 chromosomes, with the majority of genes located at the ends of each chromosome. *AdLEA* genes were primarily found on chromosome 2 (14 genes), with both chromosomes 3 and 5 containing 9 genes, and chromosome 8 containing 2 genes. *AdLEA* genes were also primarily found on chromosome 2 (13 genes), with chromosome 3 containing 11 genes, and chromosome 9 containing 3 genes. Because both *AdLEA* and *AiLEA* genes tended to be highly clustered on chromosome 2, it may be surmised that this chromosome is the origin locus. Gene duplication can lead to the evolution of new biological functions and adaptations. Here, we detected 6 pairs of orthologous homologs between *A. duranensis* and *A. ipaensis*. The majority of these orthologs shared the same chromosomal location in both species. However, the locations of some *AdLEA* genes (*AdLEA2-1*, *AdLEA2-39*, *AdLEA2-1*, *AdLEA2-21*, *AdLEA2-26*, and *AdLEA2-22*) were different from their *AiLEA* orthologs (*AiLEA2-10*, *AiLEA2-38*, *AiLEA2-1*, *AiLEA2-20*, *AiLEA2-25*, *AiLEA2-21*). Several LEA genes, including 21 *AdLEA* and 29 *AiLEA* genes, were found to be arranged as tandem repeats. Among these, the *AdSMP2/AdSMP2-1* gene pair and 4 *AiLEA* gene pairs (*AiSMP-2/2-1*, *AiLEA2-41/41-1*, *AiLEA2-31/31-1/31-2*, and *AiLEA2-39/39-1*) were found to be arranged as tandem repeats on a scaffold fragment (Tables 2 and 3). The densest distribution occurred on chromosome 2, with 11 LEA genes (*AdLEA5-5/AdLEA5-3/AdLEA5-4/AdLEA5-2*, *AdLEA3-2/AdLEA3-5/AdLEA2-15/AdLEA2-6/AdLEA2-2*, and *AiLEA2-11/AiLEA2-36/AiLEA2-5/AiLEA4-1*) clustered together.

### 3.4 Gene Structure And Conserved Motifs Of Lea Genes

Overall, the LEA genes exhibited a relatively simple structure, containing no more than 4 introns (Fig. 3). In *A. duranensis*, 19 genes contained 1 intron, 8 genes contained 2 introns, 3 genes contained 3 introns, and 2 genes contained 4 introns, with the remainder containing no introns. In *A. ipaensis*, 20 genes contained 1 intron, 12 genes contained 2 introns, 8 genes contained 3 introns, 3 genes contained 4 introns, with the remainder containing no introns. MEME analysis indicated that LEA gene motifs were conserved within groups, but not between groups. Motifs 4 and 20 were found only in LEA2 subfamily genes. Across both species, both LEA4 and LEA5 subfamily genes contained distinct conserved motifs. Interestingly *AdLEA6-1* was found to contain motif 32, but not *AiLEA6-1*. Motif 32 is repeated up to 8 times across LEA4 subfamily members from both species. Finally, motif 24 is shared across LEA5 subfamily members from both species.

### 3.5 Promoter cis-acting elements of LEA genes

In order to clarify the mechanism of stress-induced regulation of *AdLEA* and *AiLEA* genes, we identified the stress-induced *cis*-elements in the 2000 bp promoter region upstream of the initiation codon of each gene (Tables 5 and 6). Our analysis indicated that the promoter regions of peanut LEA genes contained multiple phytohormone-responsive regulatory elements, including ABA (ABRE), auxin (TGA-element), ethylene (ERE), gibberellin (GARE-motif), salicylic acid (TCA-element), and methyl jasmonate (CGTCA-motif) response elements. Additionally, several stress-responsive regulatory elements were also identified, including low temperature (LTRE, MYB, MYC), drought (MBS, MYB, MYC), light (G-box), and oxidative stress (ARE) response elements. Among these, the MYB and MYC transcription factors were the most common response elements across both *AdLEA* (95% and 87%, respectively) and *AiLEA* (92% and 97%, respectively) genes. Both the MYB and MYC transcription factors regulate gene expression in response to low temperatures, drought, or ABA exposure. Among all genes, *AdLEA2-20* was found to contain 31 *cis*-acting elements and *AiLEA2-20* was found to contain 32 *cis*-acting elements. These results confirm that LEA gene family members are crucial to plant stress response in peanut.

Table 5  
LEA gene promoter *cis*-acting elements in *A. duranensis*

| Genes            | ABRE | TGA-<br>element | ERE | GARE-<br>motif | TCA-<br>element | CGTCA-<br>motif | LTR | MYB | MYC | MBS | G-<br>box | ARE |
|------------------|------|-----------------|-----|----------------|-----------------|-----------------|-----|-----|-----|-----|-----------|-----|
| <i>AdLEA1-1</i>  | 2    | 1               |     |                | 1               | 1               | 1   |     |     |     | 3         |     |
| <i>AdLEA1-2</i>  |      |                 |     |                |                 |                 |     |     |     | 3   |           |     |
| <i>AdLEA1-3</i>  |      |                 |     |                |                 |                 |     | 1   |     |     |           |     |
| <i>AdLEA1-4</i>  | 5    | 3               | 1   |                |                 | 1               |     | 1   | 3   |     | 6         | 2   |
| <i>AdLEA1-5</i>  | 5    |                 | 2   |                | 2               | 1               |     | 6   | 2   | 1   | 6         | 1   |
| <i>AdLEA2-1</i>  | 1    | 1               |     |                |                 | 1               | 1   | 4   | 7   |     | 7         | 1   |
| <i>AdLEA2-2</i>  | 3    | 1               |     |                |                 | 1               | 1   | 5   | 3   |     | 4         | 2   |
| <i>AdLEA2-3</i>  | 10   |                 | 4   |                | 1               |                 |     | 1   | 3   |     | 12        |     |
| <i>AdLEA2-4</i>  | 3    |                 |     |                | 1               | 1               |     | 8   | 4   |     | 4         | 4   |
| <i>AdLEA2-5</i>  | 2    |                 | 1   |                |                 | 1               |     | 1   | 1   |     | 4         | 3   |
| <i>AdLEA2-6</i>  | 1    | 2               | 3   |                | 1               | 6               |     | 1   | 2   |     | 2         | 2   |
| <i>AdLEA2-7</i>  | 5    |                 | 3   |                | 1               |                 |     | 4   | 5   |     | 7         | 2   |
| <i>AdLEA2-8</i>  | 4    |                 | 3   |                |                 | 2               |     | 1   | 3   |     | 4         |     |
| <i>AdLEA2-9</i>  | 2    | 1               | 2   |                |                 | 4               |     | 8   | 4   |     | 2         |     |
| <i>AdLEA2-10</i> |      |                 | 1   |                |                 | 1               |     | 3   | 2   |     | 1         | 1   |
| <i>AdLEA2-11</i> | 1    |                 | 1   |                | 1               |                 |     | 3   | 5   |     | 2         | 3   |
| <i>AdLEA2-12</i> | 1    | 1               |     |                |                 | 1               |     | 2   | 2   |     | 1         | 3   |
| <i>AdLEA2-13</i> | 1    |                 | 1   |                | 2               | 1               |     | 6   | 6   | 2   | 3         |     |
| <i>AdLEA2-14</i> | 1    | 1               | 1   |                |                 | 1               |     | 6   | 4   | 1   | 1         | 2   |
| <i>AdLEA2-15</i> | 1    |                 | 4   |                |                 | 1               |     | 2   |     | 1   | 1         | 2   |
| <i>AdLEA2-16</i> | 4    | 2               |     |                | 1               | 1               | 1   | 9   | 4   | 2   | 3         | 3   |
| <i>AdLEA2-17</i> | 3    |                 | 4   |                | 1               | 2               | 1   | 3   | 5   | 1   | 4         |     |
| <i>AdLEA2-18</i> | 3    |                 |     | 3              | 1               | 1               |     | 10  | 5   | 1   | 4         | 3   |
| <i>AdLEA2-19</i> | 2    | 1               |     |                | 2               | 2               |     | 6   | 3   | 1   | 6         | 1   |
| <i>AdLEA2-20</i> | 2    | 2               | 2   |                | 2               | 3               | 1   | 10  | 3   |     | 5         | 1   |
| <i>AdLEA2-21</i> | 9    | 1               | 3   | 1              | 2               |                 | 1   | 4   | 4   |     | 9         | 3   |
| <i>AdLEA2-22</i> | 2    | 1               | 1   |                |                 |                 |     | 4   | 5   |     | 3         | 1   |
| <i>AdLEA2-23</i> | 4    |                 | 1   | 1              | 2               | 1               |     | 2   | 3   |     | 7         |     |
| <i>AdLEA2-24</i> | 3    |                 |     |                |                 | 4               | 1   | 10  | 4   | 3   | 3         | 3   |
| <i>AdLEA2-25</i> | 1    | 2               | 4   | 1              |                 | 2               | 2   | 4   | 4   |     | 3         | 1   |

| Genes            | ABRE | TGA-<br>element | ERE | GARE-<br>motif | TCA-<br>element | CGTCA-<br>motif | LTR | MYB | MYC | MBS | G-<br>box | ARE |
|------------------|------|-----------------|-----|----------------|-----------------|-----------------|-----|-----|-----|-----|-----------|-----|
| <i>AdLEA2-26</i> | 4    |                 |     |                |                 | 3               | 1   | 5   | 1   |     | 5         | 1   |
| <i>AdLEA2-27</i> |      |                 |     |                | 1               | 2               |     | 1   | 1   |     | 1         | 1   |
| <i>AdLEA2-28</i> | 4    |                 | 1   |                |                 | 1               |     | 4   | 1   | 1   | 4         | 1   |
| <i>AdLEA2-29</i> | 4    | 1               | 2   |                |                 |                 |     | 1   | 6   |     | 1         | 1   |
| <i>AdLEA2-30</i> | 2    |                 | 4   | 1              | 1               | 3               |     | 3   | 2   |     | 3         | 1   |
| <i>AdLEA2-31</i> | 1    | 1               | 4   |                |                 |                 | 2   | 1   | 1   | 1   | 1         |     |
| <i>AdLEA2-32</i> | 1    |                 | 3   |                |                 | 1               | 1   | 2   | 2   |     | 1         | 2   |
| <i>AdLEA2-33</i> | 3    |                 | 1   |                |                 | 2               | 1   | 5   | 3   |     | 2         | 2   |
| <i>AdLEA2-34</i> | 3    |                 | 3   |                |                 | 2               |     | 7   | 9   | 1   | 3         | 2   |
| <i>AdLEA2-35</i> | 1    |                 |     |                |                 | 2               |     | 8   | 3   |     | 1         | 3   |
| <i>AdLEA2-36</i> | 1    | 1               | 5   |                |                 | 5               | 2   | 4   | 6   |     |           | 1   |
| <i>AdLEA2-37</i> | 3    |                 | 1   |                |                 | 3               |     | 9   | 5   | 2   | 5         | 3   |
| <i>AdLEA2-38</i> | 1    |                 | 1   |                | 1               | 2               |     | 3   | 1   |     | 2         |     |
| <i>AdLEA2-39</i> | 1    |                 | 3   |                |                 |                 |     | 2   | 2   |     | 2         | 1   |
| <i>AdLEA2-40</i> | 3    |                 | 2   |                | 1               | 1               |     | 3   | 3   | 1   | 6         | 2   |
| <i>AdLEA2-41</i> | 2    |                 |     |                | 1               |                 |     | 6   |     |     | 4         | 2   |
| <i>AdLEA2-42</i> |      | 1               | 2   |                |                 | 2               |     | 1   |     | 1   | 1         |     |
| <i>AdLEA3-1</i>  | 3    |                 | 4   |                |                 | 1               | 1   | 2   | 1   | 1   | 3         | 2   |
| <i>AdLEA3-2</i>  | 3    |                 |     |                |                 | 2               |     |     |     |     | 2         | 2   |
| <i>AdLEA3-3</i>  |      | 1               | 3   | 1              |                 |                 |     | 7   | 4   | 1   |           | 2   |
| <i>AdLEA3-4</i>  |      |                 |     |                | 1               |                 |     | 4   | 6   |     | 2         | 1   |
| <i>AdLEA3-5</i>  | 1    | 2               | 5   |                | 1               | 2               |     | 7   | 5   |     | 2         |     |
| <i>AdLEA4-1</i>  | 10   |                 |     |                | 1               |                 | 2   | 1   | 1   | 3   | 10        | 2   |
| <i>AdLEA4-2</i>  | 12   |                 | 2   | 1              |                 | 3               | 1   | 2   | 1   | 2   | 17        | 2   |
| <i>AdLEA4-3</i>  | 5    |                 | 4   |                |                 | 4               |     | 7   | 3   |     | 4         | 3   |
| <i>AdLEA5-1</i>  | 5    |                 |     |                |                 | 1               |     | 2   | 4   |     | 5         | 1   |
| <i>AdLEA5-2</i>  | 8    | 1               |     |                |                 |                 | 2   | 4   | 2   | 1   | 8         | 3   |
| <i>AdLEA5-3</i>  | 4    | 1               | 4   |                | 1               |                 | 1   | 4   |     | 1   | 4         |     |
| <i>AdLEA5-4</i>  | 4    |                 | 4   | 1              | 2               | 1               | 1   | 2   | 1   |     | 6         | 1   |
| <i>AdLEA5-5</i>  | 4    | 3               | 4   |                |                 |                 | 3   | 2   | 4   |     | 5         | 2   |
| <i>AdLEA6-1</i>  | 2    |                 | 2   |                |                 | 1               | 1   | 5   | 4   |     | 2         | 2   |
| <i>AdSMP-1</i>   | 6    |                 | 5   | 3              | 1               | 1               |     | 7   | 2   | 1   | 7         | 3   |

| Genes               | ABRE | TGA-<br>element | ERE | GARE-<br>motif | TCA-<br>element | CGTCA-<br>motif | LTR | MYB | MYC | MBS | G-<br>box | ARE |
|---------------------|------|-----------------|-----|----------------|-----------------|-----------------|-----|-----|-----|-----|-----------|-----|
| <i>AdSMP-2</i>      |      |                 | 2   |                |                 |                 |     | 2   | 2   | 1   |           | 2   |
| <i>AdSMP-2-1</i>    |      |                 | 2   |                |                 |                 |     | 2   | 2   | 1   |           | 2   |
| <i>AdDehydrin-1</i> | 4    |                 | 4   |                | 1               | 2               |     | 1   | 7   |     | 6         | 2   |

Table 6  
LEA gene promoter *cis*-acting elements in *A. ipaensis*

| Genes              | ABRE | TGA-<br>element | ERE | GARE-<br>Motif | TCA-<br>element | CGTCA-<br>Motif | LTR | MYB | MYC | MBS | G-box |
|--------------------|------|-----------------|-----|----------------|-----------------|-----------------|-----|-----|-----|-----|-------|
| <i>AiLEA1-1</i>    | 2    | 1               | 1   |                | 1               | 2               |     | 4   | 3   |     | 1     |
| <i>AiLEA1-2</i>    | 7    |                 | 4   | 1              |                 |                 | 1   | 5   | 3   |     | 7     |
| <i>AiLEA2-1</i>    |      |                 | 2   |                |                 | 1               |     | 3   | 4   |     | 3     |
| <i>AiLEA2-2</i>    | 2    | 2               |     |                | 2               | 1               |     | 9   | 6   | 1   |       |
| <i>AiLEA2-3</i>    | 9    |                 | 2   |                |                 |                 |     | 2   | 2   |     | 10    |
| <i>AiLEA2-4</i>    | 4    |                 | 1   |                |                 |                 |     | 8   | 4   |     | 5     |
| <i>AiLEA2-6</i>    | 1    | 2               | 1   |                |                 | 1               | 1   | 1   | 2   |     | 1     |
| <i>AiLEA2-7</i>    |      |                 |     |                |                 | 1               |     |     | 10  |     |       |
| <i>AiLEA2-8</i>    | 6    |                 | 3   |                |                 | 1               | 1   | 3   | 3   | 2   | 3     |
| <i>AiLEA2-9</i>    | 5    |                 |     |                | 1               | 3               |     | 8   | 3   | 1   | 6     |
| <i>AiLEA2-10</i>   | 1    |                 | 3   |                | 1               |                 |     | 4   | 6   |     | 1     |
| <i>AiLEA2-11</i>   | 2    |                 | 1   |                | 2               |                 |     | 3   | 3   |     | 2     |
| <i>AiLEA2-12</i>   | 3    |                 | 1   |                |                 | 1               |     | 3   | 1   |     | 3     |
| <i>AiLEA2-13</i>   | 1    | 1               |     |                | 2               | 1               |     | 5   | 4   | 2   | 1     |
| <i>AiLEA2-14</i>   | 1    |                 | 3   |                |                 |                 | 1   | 7   | 3   | 2   | 1     |
| <i>AiLEA2-15</i>   | 2    | 2               | 1   |                |                 | 2               |     |     | 2   |     | 2     |
| <i>AiLEA2-16</i>   | 2    |                 | 2   |                |                 | 1               | 1   | 5   | 3   |     | 2     |
| <i>AiLEA2-17</i>   | 1    | 1               |     |                |                 |                 | 1   | 2   | 2   |     | 2     |
| <i>AiLEA2-18</i>   | 3    |                 |     | 3              | 1               | 1               |     | 10  | 5   | 1   | 3     |
| <i>AiLEA2-19</i>   | 4    |                 | 2   |                | 2               | 2               |     | 6   | 2   | 1   | 7     |
| <i>1</i>           | 4    | 2               | 1   |                | 3               | 2               | 1   | 10  | 6   |     | 5     |
| <i>AiLEA2-20-1</i> | 4    | 2               | 1   |                | 3               | 2               | 1   | 10  | 6   |     | 5     |
| <i>AiLEA2-20-2</i> | 4    | 2               | 1   |                | 3               | 2               | 1   | 10  | 6   |     | 5     |
| <i>AiLEA2-21</i>   | 6    | 1               | 5   | 1              |                 | 2               |     | 2   | 4   |     | 5     |
| <i>AiLEA2-22</i>   | 1    |                 |     |                |                 |                 |     | 2   | 4   |     | 1     |
| <i>AiLEA2-23</i>   | 2    |                 |     | 3              |                 |                 |     | 8   | 2   |     | 1     |
| <i>AiLEA2-24</i>   | 3    |                 | 1   |                |                 | 3               | 1   | 9   | 5   | 3   | 3     |
| <i>AiLEA2-25</i>   | 3    |                 |     |                | 1               | 1               | 1   | 3   | 2   | 1   | 5     |
| <i>AiLEA2-26</i>   | 4    | 1               |     |                |                 | 3               | 1   | 5   | 3   |     | 5     |
| <i>AiLEA2-27</i>   |      |                 |     |                |                 | 2               |     | 3   | 1   |     |       |

| Genes              | ABRE | TGA-<br>element | ERE | GARE-<br>Motif | TCA-<br>element | CGTCA-<br>Motif | LTR | MYB | MYC | MBS | G-box |
|--------------------|------|-----------------|-----|----------------|-----------------|-----------------|-----|-----|-----|-----|-------|
| <i>AiLEA2-28</i>   |      |                 | 4   |                | 1               |                 |     | 4   | 4   |     |       |
| <i>AiLEA2-30</i>   | 1    | 1               | 1   | 2              | 1               | 1               | 4   | 5   | 4   |     | 2     |
| <i>AiLEA2-31</i>   | 1    | 1               | 3   | 1              |                 | 1               | 2   | 2   | 3   | 1   | 1     |
| <i>AiLEA2-32</i>   |      |                 | 1   |                |                 |                 |     |     | 3   |     | 1     |
| <i>AiLEA2-33</i>   | 2    |                 | 1   |                | 2               | 3               |     | 3   | 4   |     | 1     |
| <i>AiLEA2-33-1</i> | 1    |                 | 1   |                | 2               |                 |     |     | 1   |     | 1     |
| <i>AiLEA2-33-2</i> |      |                 | 2   |                |                 |                 |     | 3   | 2   |     | 1     |
| <i>AiLEA2-34</i>   | 1    |                 | 4   | 1              |                 |                 |     | 8   | 9   | 2   | 1     |
| <i>AiLEA2-35</i>   | 1    |                 |     |                |                 |                 |     | 7   | 3   |     | 1     |
| <i>AiLEA2-36</i>   |      |                 | 3   |                |                 | 1               | 1   | 7   | 6   | 2   |       |
| <i>AiLEA2-37</i>   | 2    |                 |     |                |                 | 3               | 1   | 8   | 6   | 2   | 4     |
| <i>AiLEA2-38</i>   |      |                 | 2   |                | 1               | 2               |     | 1   |     |     |       |
| <i>AiLEA2-39</i>   | 2    | 1               |     |                |                 | 1               |     | 3   | 3   | 2   | 2     |
| <i>AiLEA2-40</i>   | 1    |                 | 6   |                | 1               | 1               | 2   | 1   | 1   |     | 2     |
| <i>AiLEA2-41</i>   | 2    |                 | 1   |                |                 | 1               |     | 3   | 4   | 1   | 3     |
| <i>AiLEA2-41-1</i> | 2    |                 | 1   |                |                 | 1               |     | 3   | 4   | 1   | 3     |
| <i>AiLEA2-42</i>   |      | 1               | 1   |                |                 | 2               | 1   | 3   | 1   | 2   |       |
| <i>AiLEA2-43</i>   |      |                 | 1   |                |                 | 2               | 1   | 6   | 7   | 3   |       |
| <i>AiLEA2-44</i>   | 4    | 1               |     |                |                 | 1               |     |     | 1   |     | 5     |
| <i>AiLEA2-45</i>   | 1    |                 | 1   |                | 1               |                 |     | 2   | 1   | 1   | 2     |
| <i>AiLEA2-46</i>   |      | 1               |     |                |                 | 2               | 1   | 5   | 1   | 1   |       |
| <i>AiLEA2-47</i>   | 3    |                 | 1   |                |                 | 2               |     | 2   | 5   |     | 4     |
| <i>AiLEA2-48</i>   | 3    |                 | 2   |                | 1               |                 |     | 2   | 1   |     | 4     |
| <i>AiLEA2-49</i>   | 3    |                 | 2   | 1              | 1               | 1               |     | 7   | 2   | 1   | 4     |
| <i>AiLEA2-50</i>   |      |                 | 1   |                |                 |                 | 1   | 4   | 5   |     |       |
| <i>AiLEA2-51</i>   |      | 1               | 1   |                |                 | 2               | 1   | 3   | 1   | 2   |       |
| <i>AiLEA3-1</i>    |      | 1               | 1   |                | 1               | 2               | 2   | 3   | 3   |     |       |
| <i>AiLEA3-2</i>    | 4    |                 |     |                | 1               | 3               |     | 1   | 4   |     | 4     |
| <i>AiLEA3-3</i>    | 1    |                 | 1   |                |                 |                 |     | 6   |     |     | 1     |
| <i>AiLEA3-4</i>    | 2    | 2               | 1   |                | 1               | 3               | 1   | 6   | 3   | 1   | 2     |
| <i>AiLEA3-5</i>    |      |                 | 1   | 1              | 1               |                 |     | 7   | 6   | 1   | 1     |
| <i>AiLEA4-1</i>    | 13   |                 |     |                |                 |                 | 6   | 5   | 1   | 1   | 12    |

| Genes               | ABRE | TGA-<br>element | ERE | GARE-<br>Motif | TCA-<br>element | CGTCA-<br>Motif | LTR | MYB | MYC | MBS | G-box |
|---------------------|------|-----------------|-----|----------------|-----------------|-----------------|-----|-----|-----|-----|-------|
| <i>AiLEA4-1-1</i>   | 13   |                 |     |                |                 |                 | 6   | 5   | 1   | 1   | 12    |
| <i>AiLEA4-2</i>     | 11   |                 | 1   |                |                 |                 | 1   | 2   | 2   |     | 15    |
| <i>AiLEA4-3</i>     | 5    |                 | 6   |                | 1               | 1               | 1   | 5   | 2   |     | 4     |
| <i>AiLEA5-1</i>     |      |                 | 1   | 1              | 1               | 2               |     | 3   | 5   | 1   |       |
| <i>AiLEA5-2</i>     | 5    |                 | 2   |                | 2               | 2               |     | 1   | 4   |     | 4     |
| <i>AiLEA5-3</i>     | 4    |                 | 2   |                | 3               | 2               |     | 1   | 1   | 1   | 4     |
| <i>AiLEA5-4</i>     | 1    |                 | 1   |                |                 | 1               |     | 4   | 1   |     | 1     |
| <i>AiLEA6-1</i>     | 3    |                 | 1   |                | 1               | 1               |     | 5   | 2   |     | 3     |
| <i>AiSMP-1</i>      | 6    |                 | 5   | 2              | 1               | 2               |     | 4   | 3   | 1   | 7     |
| <i>AiSMP-2</i>      | 1    |                 | 3   |                | 2               | 3               |     | 3   | 5   |     | 1     |
| <i>AiSMP-2-1</i>    | 1    |                 | 3   |                | 2               | 3               |     | 3   | 5   |     | 1     |
| <i>AiDehydrin-1</i> | 6    |                 | 1   |                | 1               | 3               |     | 1   | 8   |     | 7     |

### 3.6 Abiotic Stress-induced Lea Gene Expression

Three cultivated peanut varieties (HY23, GY3, and WT2016-6) from the Shandong Peanut Research Institute were germinated for 5 days at 25 °C or 15 °C. The two genes with the highest number of regulatory elements (*AdLEA2-20* and *AiLEA2-20*) were selected for qRT-PCR analysis (Fig. 4). Expression of both genes tended to be upregulated in response to both low and high temperatures, although the magnitude of response tended to be gene- and variety-specific. Among all treatments, WT2016-6 exhibited the highest upregulation of *AdLEA2-20* under 25 °C, followed by *AiLEA2-20* under 15 °C. Using WT2016-6, the phytohormone- and abiotic stress-responsiveness of LEA genes in roots, stems, and leaves was also tested (Fig. 5). *AdLEA2-20* was significantly upregulated in response to ABA in roots and stems, while *AiLEA2-20* was upregulated in stems and leaves. Both *AdLEA2-20* and *AiLEA2-20* were significantly upregulated in response to drought in roots and stems. Both *AdLEA2-20* and *AiLEA2-20* were significantly upregulated in response to salinity only in stems.

## 4 Discussion

In plants, LEA proteins are involved in the abiotic stress response, and are known to be responsive to a wide array of stressors, including drought, low temperature, and salinity. LEA proteins also participate in diverse signal transduction and metabolic pathways. With the advent of whole genome sequencing techniques, the LEA gene family has been characterized in several plant species. For example, 51 LEA genes representing 9 subfamilies have been identified in *Arabidopsis* (Hundertmark et al. 2008), 34 LEA genes representing 7 subfamilies have been identified in rice (Wang et al. 2007), 72 LEA genes have been identified in sweet orange (*Citrus sinensis*) (Pedrosa et al. 2015), 11 LEA genes have been identified in tomato (*Solanum lycopersicum*) (Cao et al. 2015), 23 LEA genes have been identified in mōsō bamboo (Huang et al. 2016), and 108 LEA genes have been identified in rutabaga (*Brassica napus*) (Liang et al. 2016).

The gene structure and physicochemical properties of the LEA genes determine their functions (Rivera-Najera et al. 2014). Therefore, comprehensive analysis of gene structure and other properties should be coupled with expression profiling. Here, we conducted a genome-wide analysis of LEA genes and proteins in wild peanuts. In all, we identified 65 *AdLEA* and 69 *AiLEA* genes representing all 8 LEA subfamilies (Tables 2 and 3). MEME analysis indicated that LEA gene motifs were

conserved within groups, but not between groups. In general, the LEA proteins tended to be highly hydrophilic, with GRAVY ranging from 0.003 to -1.477. This makes sense because LEA proteins are known to protect plants against abiotic stress by acting as chaperones. The high hydrophilicity of the LEA proteins enables them to bind to misfolded proteins, preventing protein polymerization and protecting cells from damage. Additionally, hydrophilic proteins tend to capture and retain water, acting to prevent wilting and tissue damage through osmotic adjustment.

Stress-responsive genes exhibiting rapid expression level adjustment tend to contain few introns, as increasing numbers of introns leads to increasing transcript length, delaying effective cellular responses. In rutabaga, 85% of the *BnLEA* family members contain less than 3 introns (Liang et al. 2016), and in rice, less than 4% of *OsLEA* family members contain more than 2 introns (Jin et al. 2007). Here, we found that 92% of the *AdLEA* gene family members and 84% of the *AiLEA* gene family members contained less than 2 introns. These results indicate that the LEA genes in wild peanuts are closely linked to plant stress resistance. Gene replication is responsible for genetic diversification and the creation of novel biological functions. Here, we found 4 pairs of paralogous genes replicated by gene fragments and 3 tandem repeats of *AiLEA2*. Among the eight LEA subfamilies, LEA2 had the greatest representation in wild peanuts. MEME analysis indicated that sequence differentiation is great, suggesting that this gene subfamily may have experienced rapid evolution resulting in more complex physiological functions.

Drought, salinity, extreme temperatures, and other abiotic stressors negatively impact the growth and development of plants and can result in poor yields or crop failures. However, plants have evolved several metabolic response mechanisms to handle adverse environmental conditions. LEA genes are known to be both stress- and phytohormone-responsive due to the presence of a diverse array of response elements present in their promoter regions. Here, we found that wild peanut LEA genes contained several categories of *cis*-acting elements, including ABREs, TGA-elements, EREs, GARE-motifs, TCA-elements, CGTCA-motifs, LTREs, MYBs, MYCs, MBSs, G-boxes, and AREs. Previous studies have shown that members of the LEA gene family are some of the primary downstream targets in both the ABA-dependent and ABA-independent signaling pathways (Kobayashi et al. 2004; Espelund et al. 2010). In ABA-dependent signaling, ABA binds to the bZIP protein, or, by synthesizing MYC or MYB transcription factors, and activates the expression of downstream genes. In ABA-independent signaling, signals activate sensing proteins which are passed to DREB and then bind to the DRE elements to activate the expression of downstream genes.

LEA proteins generally accumulate at the last stages of embryogenesis under normal conditions. However, under stressful conditions, LEA proteins accumulate in other tissues and is closely associated with cellular dehydration. Here, we examined the tissue-specific gene expression profiles of LEA genes in response to low temperature, salinity, drought, and ABA exposure. Specifically, the two genes (*AdLEA20* and *AiLEA20*) containing the most regulatory elements were examined. Low temperature induced the expression of both *AdLEA20* and *AiLEA20*. *AdLEA2-20* was significantly upregulated in response to ABA in roots and stems, while *AiLEA2-20* was upregulated in stems and leaves. Both *AdLEA2-20* and *AiLEA2-20* were significantly upregulated in response to drought in roots and stems. Both *AdLEA2-20* and *AiLEA2-20* were significantly upregulated in response to salinity only in stems. These results suggest that LEA protein accumulation exhibits both stressor-specific and tissue-specific dynamics.

## Conclusion

Abiotic stress negatively impacts both the growth and development of plants. LEA proteins are known to increase plant resistance to abiotic stress by protecting cells from damage. Although this important gene family has been characterized in several plant species, there are no studies examining LEA genes or proteins in peanut. In all, we identified 65 *AdLEA* genes from *A. duranensis* and 69 *AiLEA* genes from *A. ipaensis* representing all 8 LEA subfamilies, which were unevenly distributed across all 10 peanut chromosomes. The majority of LEA proteins were found to be highly hydrophilic. MEME analysis indicated that LEA gene motifs were conserved within groups, but not between groups. The LEA genes contained a diverse array of stress- and phytohormone-responsive *cis*-acting elements, with the *AdLEA2-20* and *AiLEA2-20* genes

containing the greatest number of elements. Both *AdLEA2-20* and *AiLEA2-20* were upregulated in response to cold temperatures, drought, salinity, and ABA-exposure, although the dynamics were tissue-dependent. This study lays the foundation for future studies on the LEA gene family and abiotic stress in peanut, and our results will be invaluable for the genetic improvement of peanut by characterizing the genetic resources of wild peanut species.

## Declarations

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### Author Contribution

Jiang Huanhuan wrote the main manuscript text, Wang Tong designed the study, performed the research. Li sainan review and editing the manuscript, and acquisition of the financial support for the project leading to this publication. All authors reviewed the manuscript.

### Ethics declarations

The authors have not disclosed any competing interests

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## Figures

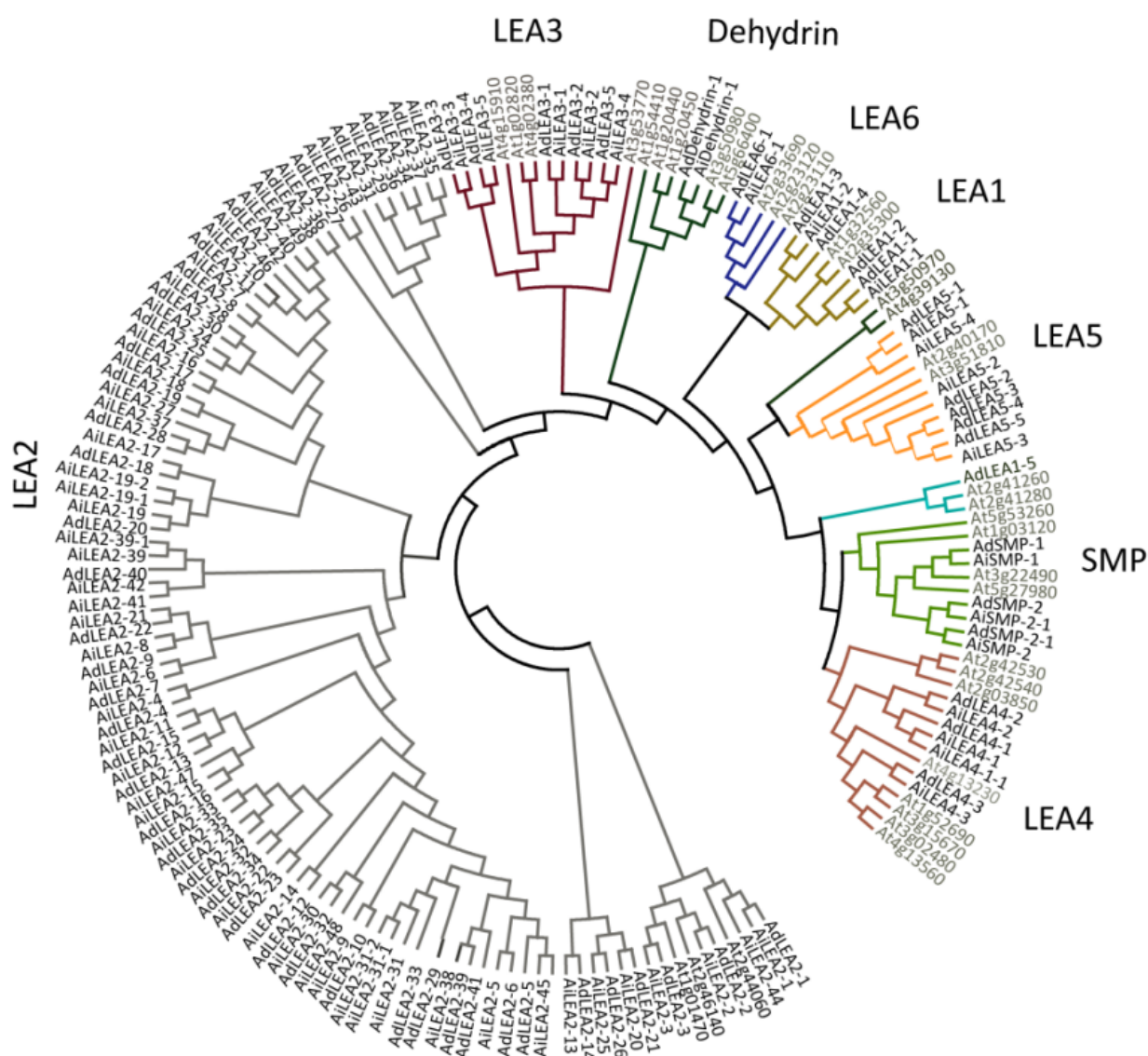


Figure 1

Phylogenetic relationships of the AdLEAs, AiLEAs and AtLEAs. LEA gene families are distinguished by different colors

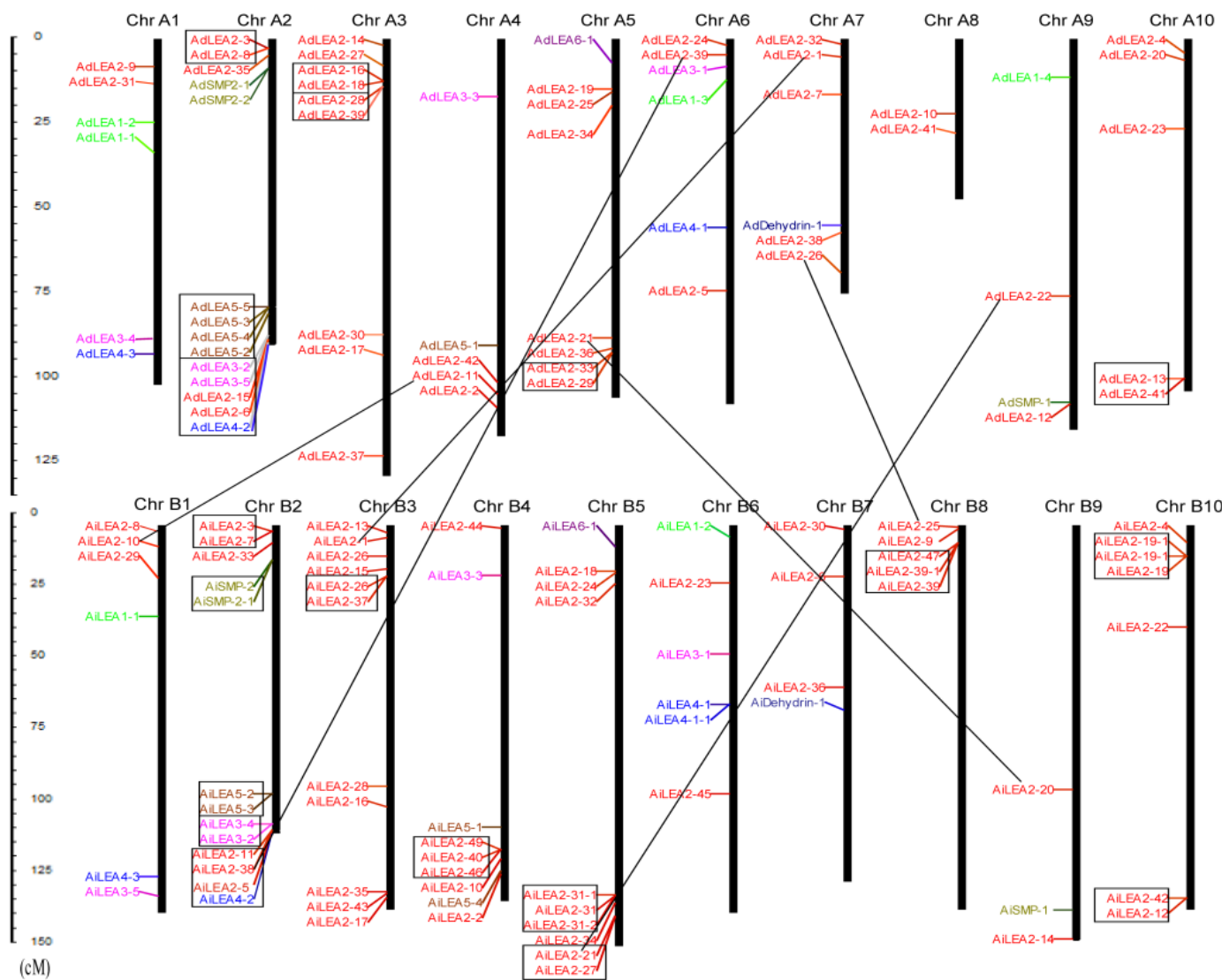
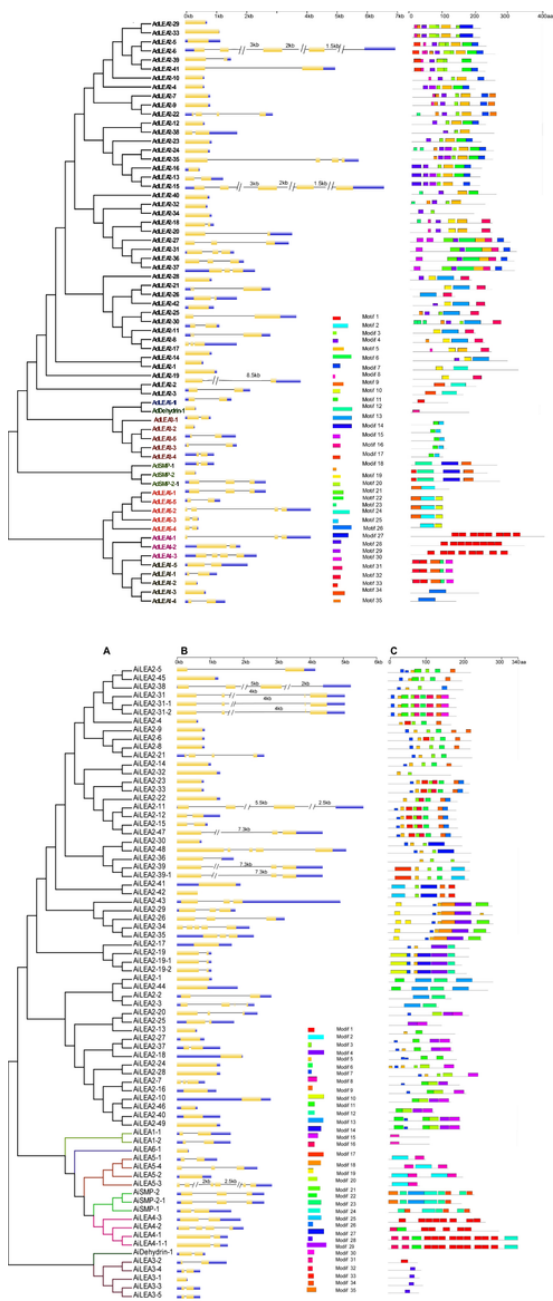


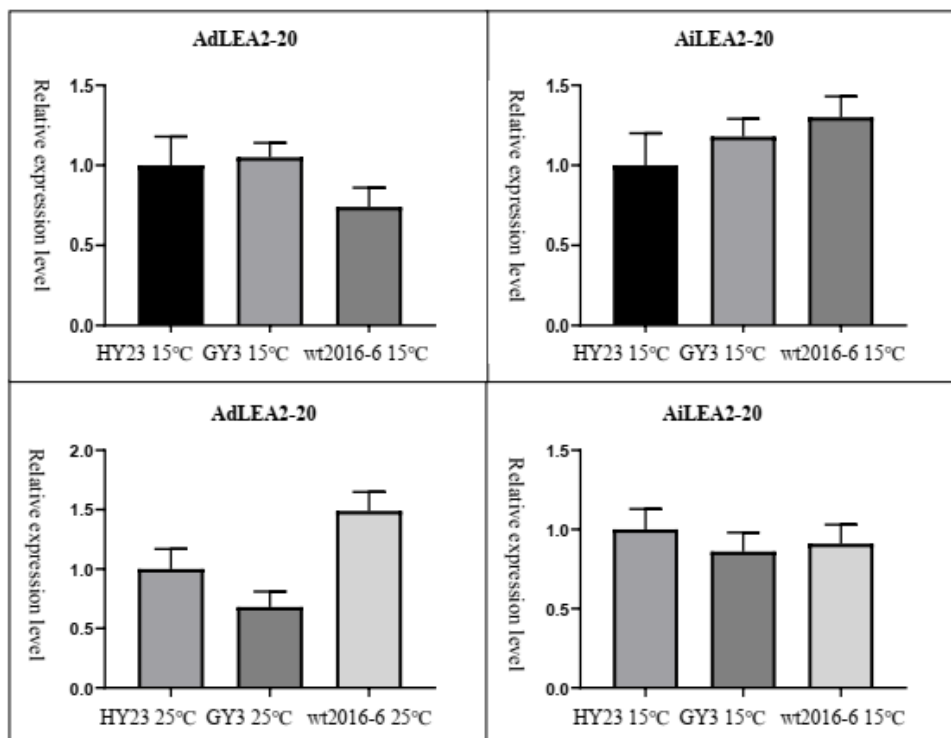
Figure 2

Chromosomal location of *AdLEA* and *AiLEA* genes. The chromosome numbers are shown at the top of each chromosome (black bars). The solid lines indicate the locations of orthologous genes. The black boxes indicate tandem repeats.



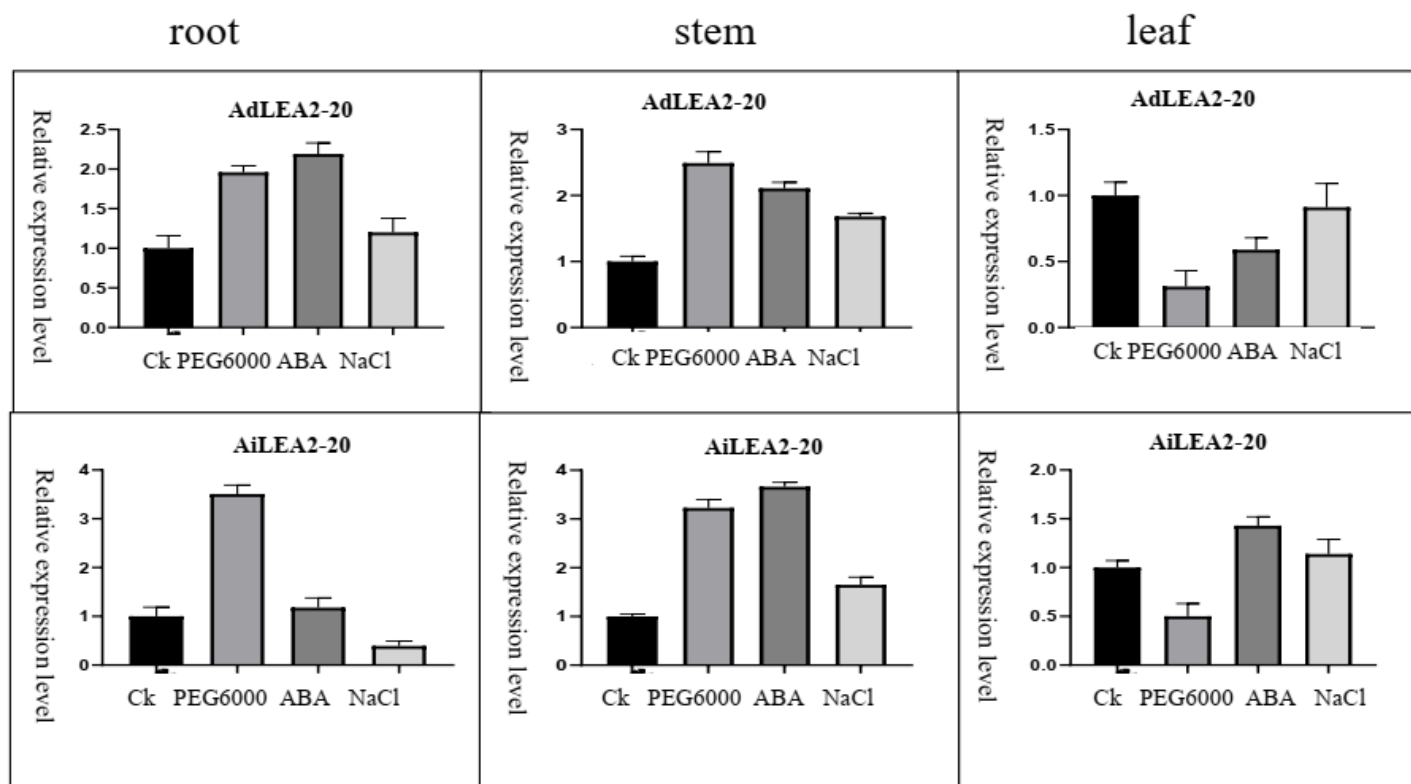
**Figure 3**

Phylogenetic relationships (a), gene structure (b), and motif structure (c) of LEA genes in *A. duranensis* and *A. ipaensis*



**Figure 4**

The expression of *AdLEA2-20* and *AiLEA2-20* of three peanut varieties (HY23, GY3, and WT2016-6) exposed to 25 °C and 15 °C.



**Figure 5**

The expression of *AdLEA2-20* and *AiLEA2-20* in roots, stems, and leaves of WT2016-6 in response to salinity, drought, and phytohormone stress.