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Identification and Characterization of the *DREB2C* Drought-tolerant Gene in Peanut Cultivar L14 and Genetic Diversity Assessment of Some Peanut Cultivars based on SSR Markers Linked to Drought Tolerance

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ABSTRACT

Background: Peanut is a widely cultivated food crop of the legume family and is a major source of vegetable oil and protein in the global agricultural economy. However, the productivity of peanuts is severely affected by abiotic stress, particularly drought. Therefore, it is necessary to identify genes related to abiotic stress tolerance and analyze genetic diversity by SSR markers related to drought tolerance in peanut varieties.

Methods: PCR amplification was used for the isolation of the *DREB2C* gene. The *DREB2C* gene sequence was analyzed using bioinformatic tools to identify functional domains, 3D modeling structures and other important characteristics. RT-qPCR was performed to evaluate the expression level of the *DREB2C* gene in the different tissues of peanut cultivars. A phylogenetic tree was constructed based on SSR markers linked to drought tolerance to assess the genetic diversity among peanut cultivars.

Result: The present study identified the DREB2C gene in peanut cultivar L14. The gene encodes a polypeptide chain of 492 amino acids lacking transmembrane domains and signal peptides and with a conserved domain (AP2/ERF) and DNA binding site. The 3D protein structure was predicted with high confidence using various tools. The gene expression was investigated in different tissues and at different growth and developmental stages, as well as in various cultivars. Based on SSR markers linked to drought tolerance, the study revealed that peanut cultivars in Vietnam exhibit a medium level of diversity. It may be suggested that cross-breeding between different groups may increase variability among crops, allowing the generation of dominant varieties with improved drought tolerance.

Key words: *DREB2C*, Drought tolerance, Genetic diversity, Microsatellites, Peanut.

INTRODUCTION

Peanut (*Arachis hypogaea* L.), an essential food crop cultivated in semi-arid tropical and subtropical regions of the world, is a significant source of protein and vegetable oils (Zhao *et al.,* 2018). However, in recent years, the impacts of global warming have become more obvious and one of the most frequent phenomena that is predicted to get worse in the near future is drought (Burke *et al.*, 2006). As a result, environmental stress on crops in general and peanuts in particular will have a negative impact on them in many ways, resulting in decreased productivity and decreased economic output. However, there are currently few studies on the genes related to the ability of peanuts to tolerate drought (Bhogireddy *et al.*, 2020).

Transcription factors (TFs), as previously stated, are well-known proteins that regulate the expression of defense genes in plants (Chen *et al.,* 2004; Xu *et al.,* 2008). As a result, identifying and characterizing TFs associated with drought tolerance is critical, providing necessary information to aid in improving the drought tolerance of plant crops. Most TFs involved in responses to or regulation of environmental stresses are grouped into several large gene families, including AP2/ERF, bZIP, NAC, MYB, MYC, Cys2His2, zinc-finger and WRKY (Umezawa *et al.,* 2006). Dehydration-responsive element binding (DREB) factors, also known as C-repeat binding factors (CBFs), are ERF subfamily members that have piqued the interest of scientists in recent decades due to their importance in plant response to environmental stresses. DREB proteins in *Arabidopsis* are classified into six small subgroups called A-1 to A-6 (Sakuma

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et al., 2002). Among these, DREB2s (DREB2A, DREB2B, DREB2C and DREB2H), which belong to subgroup A-2, are significant proteins involved in plants' reactions to stress caused by heat, salt and drought (Trono *et al.,* 2022; Li *et al.,* 2014).

DREB2C has been demonstrated to enhance thermotolerance under drought or heat stress in transgenic *Arabidopsis* plants (Lim *et al.,* 2007; Chen *et al.,* 2010; Je *et al.,* 2014; Han *et al.,* 2022). In addition, studies also indicated that DREB2C regulates ABA biosynthesis related to drought tolerance during germination in *Arabidopsis* (Je *et al.*, 2014) or drought stress response during the flowering stage of mungbean cultivars (Ariharasutharsan *et al.*, 2022). These suggest that DREB2C has an important role in abiotic stress defense in plants.

In contrast to other subgroups like DREB2A or DREB2B, DREB2C was only slightly studied. As a result, more analysis is essential to identify, characterize and clarify the roles of this subgroup under environmental stresses. Furthermore, using genetic linkage and molecular markers such as microsatellites (SSR), it is possible to detect dominant genotypes that respond to environmental stresses, thereby improving crop yields (Varshney *et al.,* 2009; Mishra *et al.,* 2021).

In this study, we report on the identification, characteristics and transcriptional expression of the drought tolerance-related DREB2C gene in peanuts. We also look at the genetic diversity of some commonly grown peanut cultivars in central Vietnam by using SSR markers that are linked to drought tolerance.

MATERIALS AND METHODS

Plant materials

The current study used some peanut (*A. hypogaea*) cultivars that are commonly grown in central Vietnam, including local and hybrid or mutant cultivars with drought-tolerant capacities (Table 1).

Isolation of *DREB2C* gene

The genomic DNA from young leaves of peanut cultivar L14 was isolated using the GeneJET plant genomic DNA purification kit (Thermo Scientific, USA). The specific primers (Table 2) for PCR amplification of the *DREB2C* gene were designed by Primer 3 program based on the corresponding genes from the databases of PeanutBase and GenBank and then checked using the Primer-BLAST tool against the non-redundant cultivated peanut database. The PCR was carried out as follows: 94°C for 5 min, followed by 30 cycles of 94°C for 30 sec, 58°C for 45 sec, 72°C for 2 min and a final extension at 72°C for 10 min. The PCR product was then cloned into a pGEM T-easy vector (Promega, USA) and sequenced using the Sanger method.

Bioinformatic analysis

BLASTN searches were conducted for the PCR product (the putative *DREB2C* gene) against numerous databases, including NCBI, Phytozome, Peanutbase and PlantTFDB (v5.0), in an attempt to find close homologous sequences. Based on nrBLAST results, the CLC Sequence Viewer software (Qiagen) multi-aligned the strongest sequences and the predicted DREB gene to identify the presence of conserved motifs. The introns and exons were predicted by comparing their corresponding mRNA sequences from the Phytozome and PeanutBase databases. The deduced DREB2C protein was predicted for a variety of characteristics, including transmembrane helices predicted by the DeepTMHMM server, hydrophobicity assessed by ProtScale using the Kyte-Doolittle amino acid scale and structural and functional domains identified by the SMART and HMMSCAN tools. The 3D structure of the protein was built by various servers (PHYRE2, SWISS-MODEL, I-TASSER and AlphaFold 2.0) before being simulated by Pymol (v2.5.2). MEGA X generated the phylogenetic tree using the neighbor-joining method (NJ) with 1000 bootstrap replicates, which was then visualized by iTOL. The signal peptides and theoretical isoelectric point (pI) of these

Table 1: Some peanut cultivars commonly grown in central Vietnam.

Cultivar name	Abbre.	Cultivar type	Source
L14	L14	Hybrid	Field Crops Research Institute, Vietnam Academy of Agriculture Science (VAAS)
Cuc Ha Tinh	CucHT	Native	Plant Resources Center, VAAS
Ly Tay Nguyen	LyTN	Native	Cuong et al (2011)
L20	L20	Hybrid	Agriculture Sciences Institute of Northern Central Vietnam, VAAS
L27	L27	Hybrid	Field Crops Research Institute, VAAS
Se da ran	SeDR	Native	Ha Tinh province, Vietnam
Se Tay Nguyen	SeTN	Native	Plant Resources Center, VAAS
Sen Nghe An	SenNA	Native	Plant Resources Center, VAAS
Se Gia Lai	SeGL	Native	Plant Resources Center, VAAS
LDH01	LDH01	Mutant	Agricultural Sciences Institute for Southern Coastal Central of Vietnam, VAAS
LDH09	LDH09	Mutant	Field Crops Research Institute and Agricultural Sciences Institute for Southern Coastal Central of Vietnam, VAAS

proteins were analyzed by Signal IP 6.0 and Expasy's Compute pI/MW application, respectively.

Expression of *DREB2C* gene

Expression of the *DREB2C* gene in peanut cultivar L14 was analyzed by RT-PCR amplification. The total RNA of various explants (root, stem, leaf and young pod) was isolated by the GeneJET Plant RNA Purification Kit (ThermoFisher, USA). The first-strand cDNA was synthesized by the RevertAid First-Strand cDNA Synthesis Kit (Thermo Scientific, USA). The specific primers for PCR amplification are described in Table 3. Two microliters of the first-strand cDNA were used as a template and the PCR conditions were set up as follows: 94°C for 5 min; followed by 35 cycles of 94°C for 30 s, 58°C for 45 s and 72°C for 2 min; and a final extension at 72°C for 10 min. The actin gene was used as the internal control gene for measuring and reducing the errors between the samples in RT-PCR expression analysis Chi *et al.* (2012). The expression level of the *DREB2C* gene was visualized by GraphPad (v. 9) and measured by ImageJ software.

SSR analysis

The genomic DNA from young leaves of peanut cultivars (Table 1) was isolated by the GeneJet Plant Genomic DNA Purification Kit (Thermo, USA). A total of 7 SSR markers linked to drought tolerance were chosen based on published literature (Table 4). The PCR amplification was conducted at 94°C for 5 min, followed by 30 cycles of 94°C for 30 sec, 55°C for 40 sec and 72°C for 35 sec, with a final extension at 72°C for 10 min. The amplicons were analyzed on a 1.5% agarose gel. Each DNA band size was assigned a score of present (1) or absent (0) to analyze the polymorphism of SSR markers. The SSR marker data was analyzed by PowerMarker 3.25. The number of amplified alleles was used to calculate the polymorphic information content (PIC). The UPGMA method was utilized to generate the dendrogram of the polymorphic alleles and similarity coefficients by using the NTSYS pc 2.01 program.

RESULTS AND DISCUSSION

Isolation of *DREB2C* gene

The putative *DREB2C* gene from cultivar L14 was isolated by PCR amplification. The analysis revealed that this sequence was 2.0 kb long and contained two exons (nt 1-23 and 554-2009) and an intron (nt 24-553) encoding a polypeptide chain of 492 amino acids with a molecular weight (MW) of 55.31 kDa (GenBank: OQ685297.1). Our putative DREB2C sequence was longer and 84.8% similar to that of DREB2C from the Shitouqi peanut cultivar (GenBank: QHO54253.1, unpublished). Based on the BLASTN results in the Peanutbase database (https://peanutbase.org), the putative *DREB2C* gene has a high degree of similarity to its corresponding gene in some species of the *Arachis* genus, including *A. hypogaea* (99.3%), *Arachis ipaensis* (99.3%), *Arachis duranensis* (98.4%) and *Arachis cardenasii* (98.6%). Besides, the BLAST results in the Peanutbase database indicated *DREB2C* was present on chromosomes 2 (SE8WTS.1) and 12 (KD4831.1) of *A. hypogaea*. Whereas this gene only occurred on chromosome 2 of *A. ipaensis* (Araip.3JJ8N.1), *A. duranensis* (Aradu.GB4U4.1) and *A. cardenasii* (araca.K10017.gnm1.chr02).

Characterization of DREB2C protein

The results of the structural and functional domain analysis of the protein revealed that the isolated sequence was identified as *DREB2* gene with a conserved domain (AP2/ERF) from amino acids 203 to 580 and a DNA binding site with the following sequence: YRGVRQRIWGKWVAEIREP ISNHESKKNAKRLWLGTF. These findings suggested that *DREB2* gene encodes a nuclear protein containing one conserved AP2/ERF DBD and that DREB2 protein belongs to the DREB TF subfamily. According to InterProScan analysis, putative DREB2 protein is a member of the dehydration-responsive element-binding protein 2C family (PANTHER ID: PTHR31241:SF62). Plant Transcription Factor Database examination of the amino acid sequence of DREB2 also confirmed that it is the DREB2C protein (TF ID: AT2G40340.1). From the above bioinformatics analysis results, the putative *DREB2* gene was named *AhL14_ DREB2C*.

Data from the DeepTMHMM server showed that the AhL14_DREB2C protein lacks transmembrane domains and signal peptides. The presence of a nuclear localization signal at the N-terminus of the polypeptide chain in the *AhL14_DREB2C* gene with a probability of over 0.9516 suggests that this gene is involved in the regulation of transcription. Biological functional analysis of AhL14_ DREB2C based on annotation application through Gene Ontology (GO) revealed that AhL14_DREB2C is involved in

Table 2: Specific primers for PCR amplification of the *DREB2C* gene.

Primer name	Nucleotide sequence 5′-3′	Expected size of amplicon (bp)
DREB2C-F	ATGGCCACTGAAATCGTTTCTGG	2022
DREB2C-R	CACATTTAGGATCTTAGGAGTTCACAAATG	

Table 3: Specific primers for RT-PCR amplification of the *DREB2C* gene.

Primer name	Nucleotide sequence 5′-3′	Expected size of amplicon (bp)
DREB2C-F	GCAAAGAGGCTTTGGCTTGG	290
DREB2C-R	AGGAGCCTCACCACTACTCA	

biological processes (the regulation of DNA template transcription: GO:0006355) and molecular function (the activity of DNA-binding transcription factor: GO:0003700; and DNA binding: GO:0003677).

Also, the findings showed that the AhL14_DREB2C protein contains high-hydrophilic domains such as amino acids 45-46, 48-50, 109-118, 154-160, 239-241, 246-248, 270-272, 335-340, 376-378, 384-385, 455-458 and 483-488. The protein-protein interaction prediction revealed that AhL14_DREB2C interacted with some drought stress inducers such as ABF2 (abscisic acid responsive elements-binding protein 2), HSFA3 (heat shock transcription factor a3), ABF3 (abscisic acid responsive elements-binding factor 3), ABF4 (abscisic acid responsive elements-binding factor 4) and HSF1 (heat stress transcription factor A-1a) (Fig 1).

3D modeling of AhL4_DREB2C protein structure

The 3D protein structure of AhL14_DREB2C was predicted by PHYRE2, SWISS-MODEL, I-TASSER and AlphaFold-2. As analyzed by PHYRE2, AhL14_DREB2C was a DNA-binding protein and this program only modeled 17% of the AhL14_DREB2C sequence with 100% confidence using the single highest scoring template (PDB no. 5WX9). Whereas, I-TASSER modeled the protein structure with 26% coverage based on the best template of the ethylene-responsive transcription factor ERF096 of *Arabidopsis thaliana* (PDB no. 5WX9), which has the highest alignment score (Z-score: 4.43). The SWISS-MODEL was only modelled at DNA binding domains (amino acids 74-140) based on the template of ethylene-responsive element binding factor 1 (PDB no. 1gcc). The AlphaFold, which assesses the per-residue (amino acid) confidence score (pLDDT, from 0-100), found that 26% of the residues had a confidence of more than 70, whereas low-confidence regions (50-70), found at the N and T-termini, account for 19.3% of the residues.

The DNA binding domain of *AhL14_DREB2C* with the GCC-box DNA motif was generated based on the ERF096 template of *A. thaliana* (PDB No.: 5WX9) (Fig 2). Additionally, we found an SNP marker (AX-147214668, underlined) in the DNA sequence of *AhL14_DREB2C* (TGCTCAAATCAC ATGATGATGTTAAACTTTTGGTCA/GATTCTTCGATTGG TACTTCTATATTCGCTACA).

Phylogenetics

Phylogenetic analysis showed that AhL14_DREB2C belongs to the same group as the corresponding genes of *A. hypogaea* and *A. duranensis* species (Fig 3). In particular, its deduced amino acid sequence shared a high degree of similarity to AhDREB2C of *A. hypogaea* (98.2% for Peanutbase ID: Arahy.KD4831; 97% for Peanutbase ID: Arahy.SE8WTS.1; and 84.8% for GenBank: QHO54253.1) and of *A. duranensis* (97% for Peanutbase ID: Aradu. GB4U4.1). These results suggest that the function of DREB2Cs in the aforementioned group may be relatively comparable.

Expression of *AhL14_DREB2C* gene

The transcriptional expression of the *AhL14_DREB2C* gene was found in different tissues and different growth and

Table 4: SSR markers related to drought tolerance-related traits.

Makers	Linkage/QTLs	Traits	Motifs	Forward 5'-3'	Reverse 5'-3'	References
pPGSseq9G5	T	T	(AC) 13 (CT) 22	CAAATTGTGCAGCCAAGAGA	CATATGCCCAGGAAGAGGAA	Ferguson *et al.* (2004)
pPGPSeq2C11	LG_AhXIII	SLA	(TAA) 17+(CAC)10	TGACCTCAATTTTGGGGAAG	GCCACTATTCATCGCGGTA	
pPGSseq9H8	A03/TE05_III	TE	(TAA) 20	CTGGATACATCGACGCTGAG	GCGGTCCAATACTAACAAAATC	
pPGSseq19H3	A03, A06, B01, B03 and B05/SLAHar04_IV	SLA	(ATT) 18	TGGCAGGCAGTAAACATCAG	TTGAGGACGTGATGAACTGG	
AHM689	AA10, A06, A10, B10/SLAPreTrt05_VI	SLA	GA) 20	GATGACAATAGCGACGAGCA	GTAAGCCTGCAGCAACAACA	Cuc *et al.* (2008)
IPAHM 105	AA05, A05 and B04/SLAHar05_XVII	SLA	CT) 18	CAGAGTTTGGGAATTGATGCT	GCCAGATCTGAGCAAGAACC	
pPGPseq2B9	B09/ SPADPreTrt04_VIII	SCMR	(ATT) 19	GCAACATGCTCTGAATTTTGAC	TGTGCAACCCAATTCAATAACTT	Zhao *et al.* (2012)

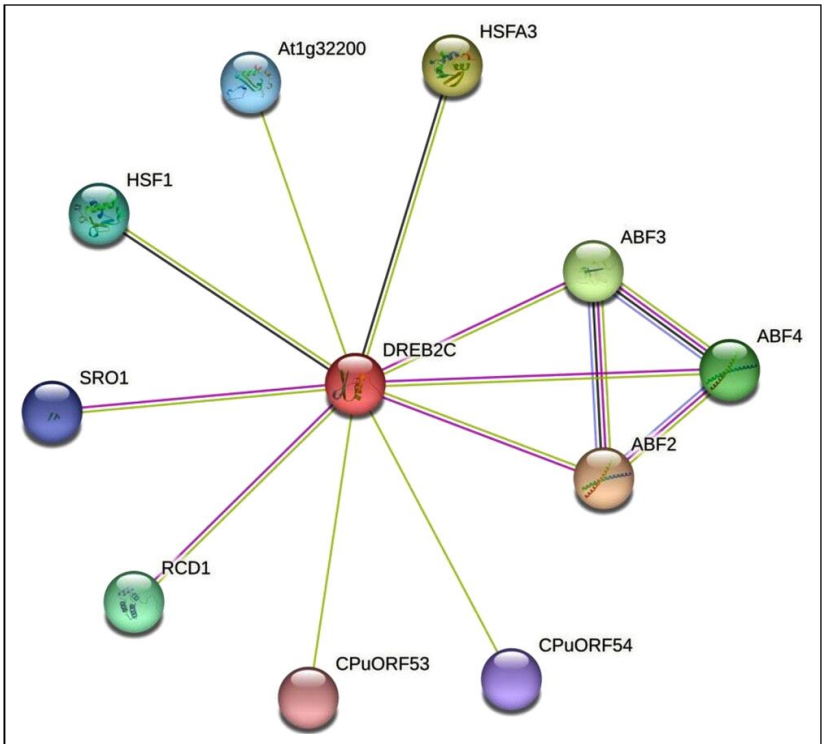
T: transpiration, TE: transpiration efficiency, SLA: specific leaf area, SCMR: SPAD chlorophyll meter reading.

development periods (Fig 4). Observations revealed that roots and leaves at the flowering stage had higher transcription levels than other tissues at the same stage (Fig 4A) and under similar growing conditions, the *AhL14_DREB2C* gene was more significantly expressed during the flowering stage than the 4-leaf stage (Fig 4B). Among the tested peanut cultivars, L14, L20, SeGL and LDH09 exhibited higher expression levels of the *AhL14_DREB2C* gene in roots compared to others (Fig 4C). Moreover, RT-PCR amplification indicated that hybrid cultivars (Table 1) had higher transcription levels than native cultivars.

Polymorphism in SSR-linked drought tolerance markers

Eleven cultivars of peanuts commonly cultivated in Vietnam were employed in the present study to assess genetic diversity based SSR markers linked to drought tolerance-related traits (Table 5). The major allele frequency is the proportion of the most common allele in the population. The mean major allele frequency for all markers was 0.79, indicating that the majority of individuals in the population may carry the same allele for each marker. Besides, the mean allele number and gene diversity for all markers were 3 and 0.33, respectively, suggesting that each marker was reasonably informative and had a moderate degree of polymorphism. Further, the mean heterozygosity across all markers was 0.41, which is also of a moderate degree and suggests that the population is not highly inbred. The PIC value is a measure of the informativeness of a marker for genetic analysis. The mean PIC value for all markers was 0.30, indicating that the markers are moderately informative. Among studied SSR markers, IPAHM689, pPGSseq19H3 and PGSseq9G5 had the highest polymorphic values. (Fig 5). The SSR markers' polymorphic information content value ranged from 0.15 to 0.54. The result indicated genetic diversity analysis based on the SSR-linked drought tolerance markers in peanut varieties in Vietnam showed moderate polymorphism. The phylogenetic tree shows a similarity range of 73.6% to 94.7% among different peanut varieties in each group (Fig 6). The investigated peanut varieties were divided into two groups at various similar levels. Varieties L20 and LDH01 belong to group I. Group II is divided into 2 sub-clusters including CucHT, LyTN, L27, SenNA, SeGL, SeDR and seTN varieties. The other sub-cluster contains the varieties L14 and LDH09.

Peanuts (an allotetraploid species: 2n = 4x = 40) are an essential food source that provides nutritious oils and proteins and is farmed in tropical and subtropical semi-arid locations across the world (Zhao *et al.,* 2018). Peanuts, like many other plants, are subjected to a variety of abiotic stressors, including drought, heat, cold and salinization, which significantly affect their growth, development and yield (Agarwal *et al.*, 2017).



AhL14_DREB2C interacted with various drought stress inducers such as ABF2, HSFA3, ABF3, ABF4 and HSF1 .

Fig 1: Protein-protein interaction prediction by STRING v12.0.

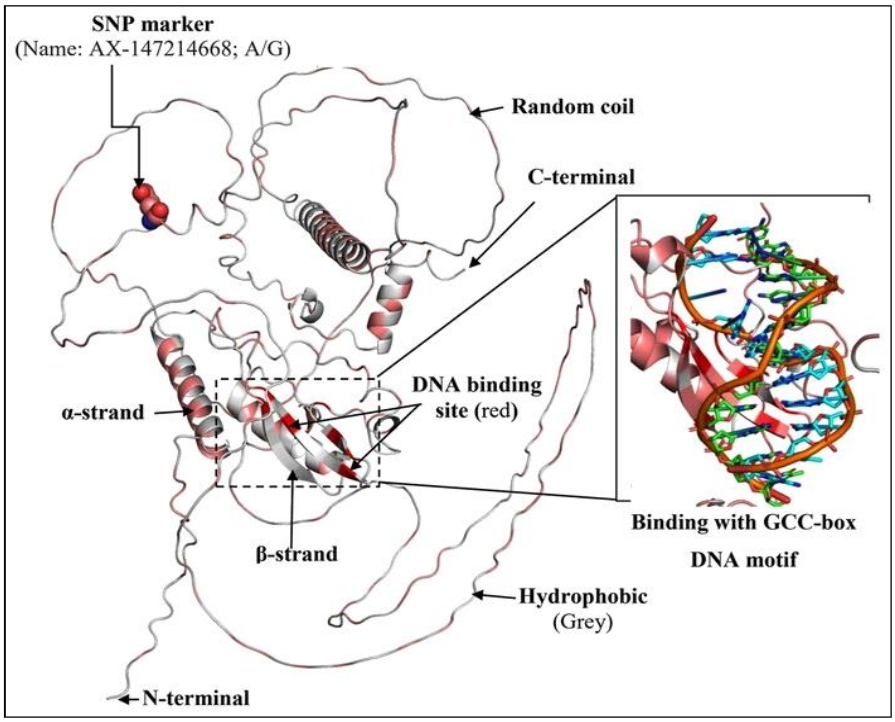
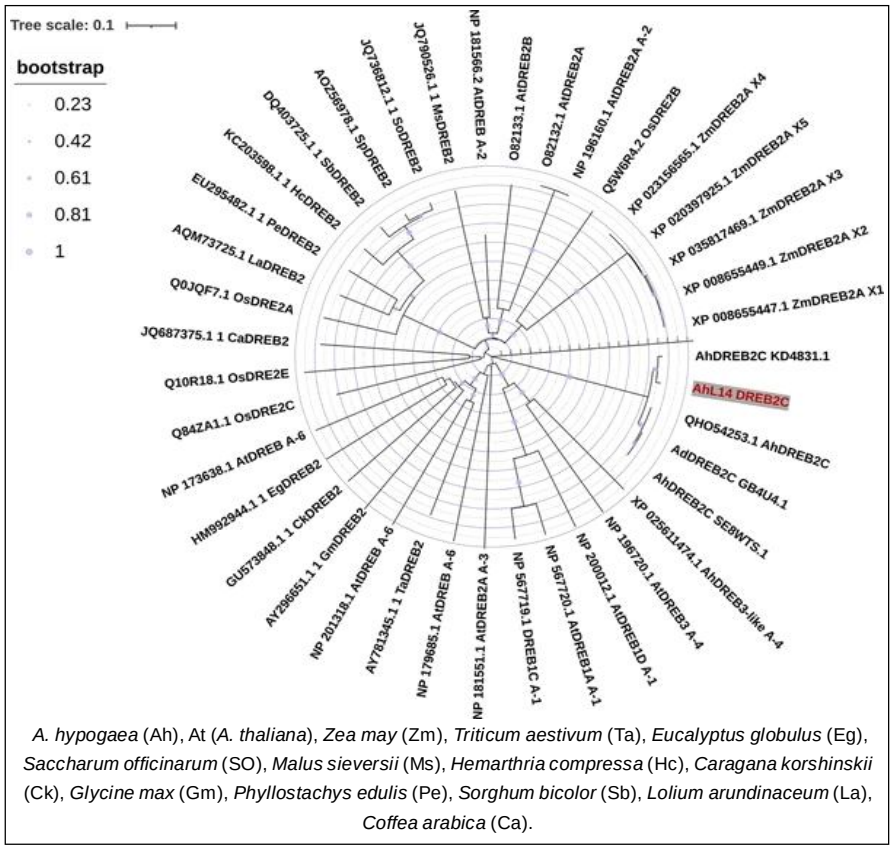


Fig 2: 3D structure prediction of AhL14_DREB2C.

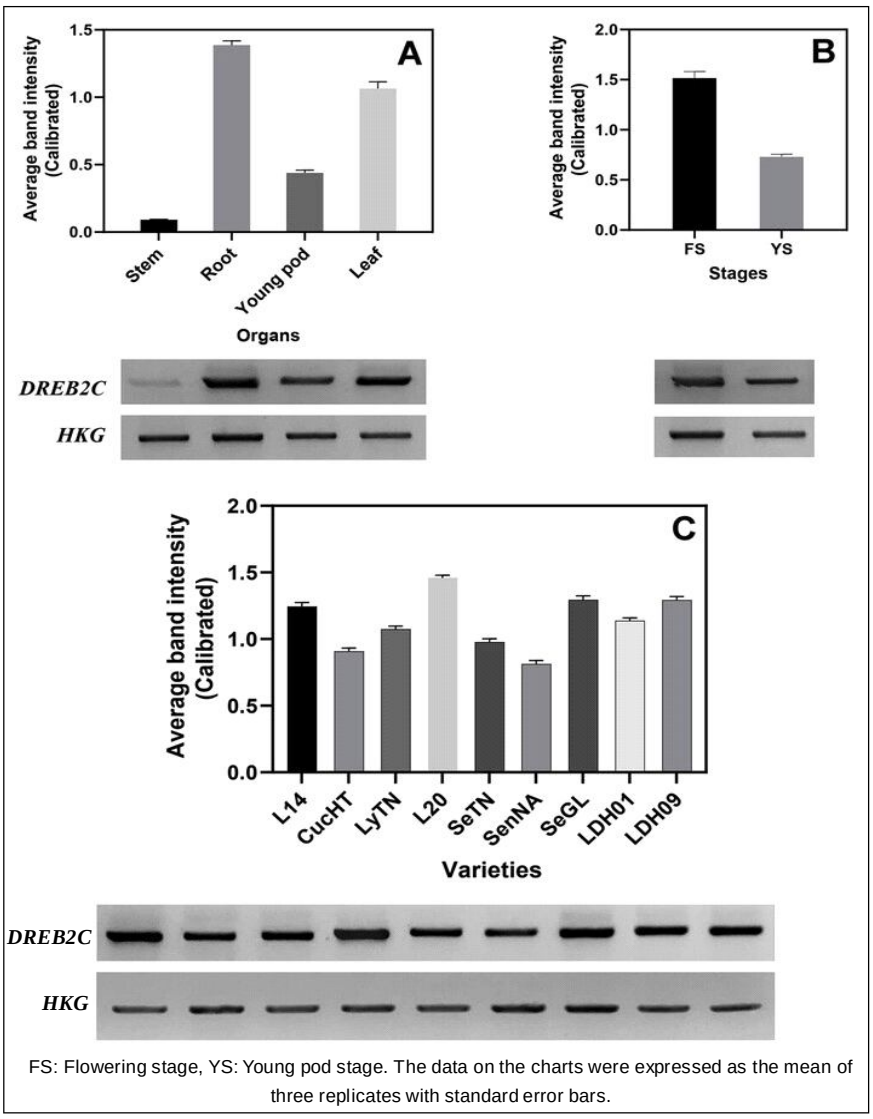


A. hypogaea (Ah), At (*A. thaliana*), *Zea may* (Zm), *Triticum aestivum* (Ta), *Eucalyptus globulus* (Eg), *Saccharum officinarum* (SO), *Malus sieversii* (Ms), *Hemarthria compressa* (Hc), *Caragana korshinskii* (Ck), *Glycine max* (Gm), *Phyllostachys edulis* (Pe), *Sorghum bicolor* (Sb), *Lolium arundinaceum* (La), *Coffea arabica* (Ca).

Fig 3: Phylogenetic tree of DREB2 genes from various plant species.

Plant stress tolerance is dependent on regulatory genes that promote the overexpression of stress-tolerance genes, which improves the ability of plants to adapt to different environmental stresses (Zhang *et al.,* 2018; Mizoi *et al.,* 2012). The DREB2C protein, a member of the AP2/ERF superfamily of plant transcription factors, has been demonstrated to play a significant role in the regulation of abiotic stress responses as well as plant growth and development. Several studies have been conducted on the identification of the DREB2C gene (Lee *et al.*, 2009) and its expression in plants subjected to various abiotic stress conditions, such as drought (Han *et al.,* 2022; Ariharasutharsan *et al.,* 2022), heat (Lee *et al.,* 2010; Chen *et al.,* 2010; Lim *et al.,* 2007), salt stress (Song *et al.,* 2014) and cold stress (Lim *et al.,* 2007; Lee *et al.,* 2010). Here, we isolated the AhL14_DREB2C gene, which contains an intron and has a CDS length of 1479 bp and an AP2/ERF domain, which implies that AhL14_DREB2C maybe has an important role in the ABA signaling system and the response of plants to drought as other DREBs.

Besides, the expression of DREB2C in the flowering stage was differently induced in various organs, such as the root, stem and leaf. where the gene expression was higher in root tissue compared to other tissues. The expression of DREB2C in root tissues also showed differences between flowering and earlier stages, suggesting that perhaps the presence of DREB2C would support plant growth at the water-demanding stage. Moreover, the expression of DREB2C in root tissues differs among peanut cultivars cultivated in Vietnam. The peanut varieties with good drought tolerance in previous studies, such as L14, L20 and LDH09, showed higher DREB2C expression than



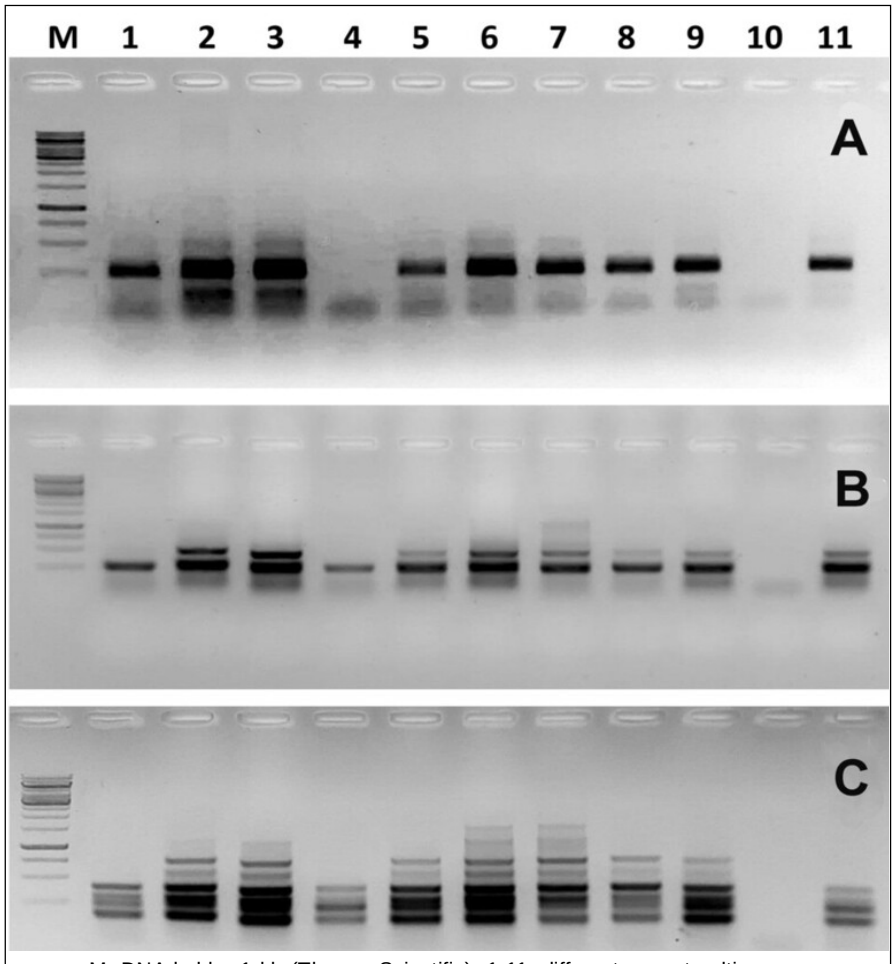
FS: Flowering stage, YS: Young pod stage. The data on the charts were expressed as the mean of three replicates with standard error bars.

Fig 4: Transcriptional expression analysis by RT-PCR was performed in the various tissues (A), in roots of growth and developmental stages (B) and in roots of various peanut varieties (C).

the local varieties, except for the SeGL variety. This suggests that the DREB2C gene can be used as a molecular marker to aid in the screening of drought-tolerant peanut varieties. In addition to identifying genes associated with drought tolerance, the use of gene-linked microsatellite markers is an important research tool to assist molecular breeding in order to generate better drought-resistant cultivars. In this study, SSRs associated with drought tolerance traits such as transpiration, transpiration efficiency, specific leaf area and SPAD chlorophyll meter reading were used to analyze genetic diversity among peanut varieties commonly grown in Vietnam. The results of genetic diversity analysis using SSR markers showed that 11 types of peanuts were separated into many sub-branches with various degrees of

Table 5: Different parameters analyzed with drought-linked SSR markers of peanut varieties in Vietnam.

SSR marker	Major allele frequency	Allele no	Gene diversity	Heterozygosity	PIC value
pPGSseq9G5	0.86	3.00	0.24	0.30	0.23
pPGPseq2C11	0.91	2.00	0.17	0.19	0.15
pPGSseq9H8	0.86	3.00	0.24	0.27	0.23
pPGSseq19H3	0.59	3.00	0.54	0.82	0.46
IPAHM689	0.59	5.00	0.59	0.82	0.54
IPAHM 105	0.77	3.00	0.38	0.46	0.34
pPGPseq2B9	0.91	2.00	0.17	0.18	0.15
Mean	0.79	3.00	0.33	0.41	0.30



M: DNA ladder 1 kb (Thermo Scientific), 1-11: different peanut cultivars as listed in Table 1.

Fig 5: DNA band polymorphisms from peanut genotypes were amplified by PCR with SSR markers such as PGSseq19H3 (A), PGSseq9G5 (B) and IPAHM689 (C).

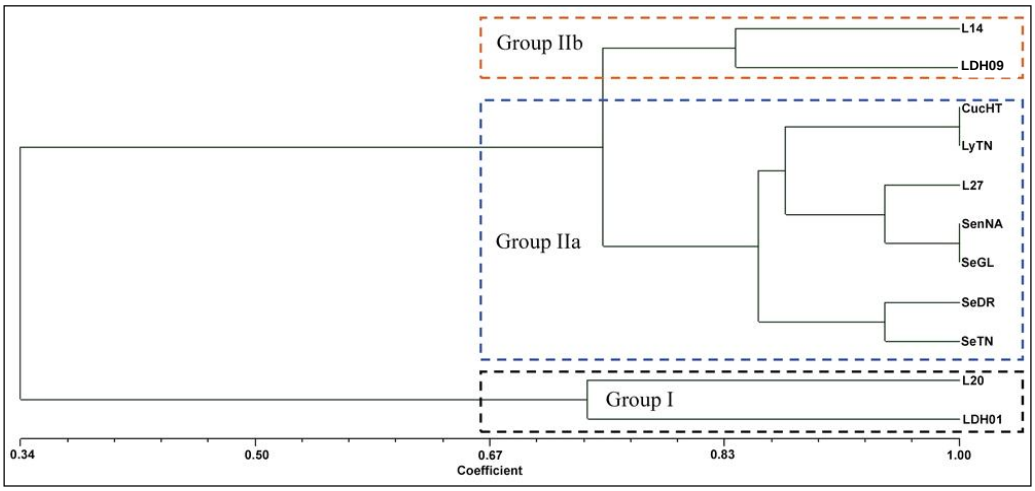


Fig 6: A dendrogram of SSR markers linked to drought tolerance among genotypes of peanut cultivars in Vietnam.

similarity. The findings of the SSR analysis revealed that the peanut varieties used in the study had a medium level of diversity; this conclusion is similar to other previous studies (Cuc *et al.,* 2013; Ren *et al.,* 2014). The phylogenetic tree based on SSR-related drought tolerance marker results may suggest that cross-varieties between different groups can increase the variability among the cultivated varieties for selecting the dominant varieties with improved drought tolerance.

CONCLUSION

This work sheds light on the characterization and expression of a drought-related *DREB2C* gene in peanut cultivar L14 and the potential of generating hybrid dominants with drought-tolerant traits in the future.

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Author contributions

Loc NH and Tien NQD designed and performed the overall study. Tien NQD and Kha H conducted gene cloning, gene expression and bioinformatic analysis. Tien NQD and Loc NH wrote and approved the final manuscript.

Research involving human and animal rights

This study did not involve human and animal subjects.

Conflict of interest

All authors declared no conflict of interest.

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