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Identification and Analysis of SOD Family Genes in Peanut (*Arachis hypogaea* L.) and Their Potential Roles in Stress Responses

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Abstract: Superoxide dismutases (SODs) are crucial in safeguarding plants against reactive oxygen species (ROS) toxicity caused by abiotic or biotic factors. Although recent research has revealed the involvement of the *SOD* gene family in plant biological processes, the understanding of the *SOD* gene family in peanut remains inadequate. This study comprehensively characterizes the *SOD* gene family in the peanut genome. A total of 25 *AhSOD* genes were identified and subsequently categorized into three subfamilies: sixteen AhCSDs, six AhFSDs, and three AhMSDs according to the phylogenetic tree. A comprehensive analysis revealed that the *AhSOD* genes underwent segmental duplications. The majority of *AhSOD* genes exhibited conserved exon–intron and motif structures within the same subfamily. The examination of *cis*-acting elements within the promoter regions of *SOD* genes revealed that the expression of *AhSOD* was subject to regulation by plant hormones, as well as responses to defense and stress. RNA-seq analysis showed expression diversity of *AhSOD* genes in various tissues and cold, drought, and salt stresses. Furthermore, the regulation of *AhSOD* gene expression is anticipated to involve numerous transcription factors. The gene ontology annotation results validate the role of *AhSOD* genes in various stress stimuli, SOD activity, reactive oxygen species metabolic processes, and cellular oxidant detoxification processes. This investigation serves as the initial genome-wide analysis of the *AhSOD* gene family, providing a basis for comprehending the function of the *AhSOD* gene family and enhancing plant tolerance to cold, drought, and salt stresses.

Keywords: peanut; SOD; cold; drought; salt; transcription factor; gene family

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1. Introduction

Abiotic stresses such as drought, salt, cold, and heavy metal poisoning negatively affect plant development and crop yield. These stresses induce the overproduction of toxic reactive oxygen species (ROS), which leads to oxidative stress and eventual cell death [1,2]. Plants have developed intricate enzymatic antioxidant defense systems, including the superoxide dismutase (SOD) system, peroxidase (POD) system, catalase (CAT) system, and ascorbate peroxidase (APX) system, in order to deal with these overwhelming toxic ROS [3]. The SOD enzymes have a significant function in protecting plants from oxidative stress. They are capable of catalyzing the dismutation of the harmful superoxide anion ($\cdot O_2-$) into molecular oxygen (O_2) and hydrogen peroxide (H_2O_2) within plant cells when exposed to oxidative stress [4–6].

Metalloenzymes called plant SODs have been found in different parts of plants, such as roots, leaves, fruits, and seeds. These enzymes play vital roles in protecting against

oxidative stress. Plant SODs can be classified into three subgroups depending on the metal ions they bind, which are Cu/Zn-SOD (CSD), Fe-SOD (FSD), and Mn-SOD (MSD) [7,8]. Arabidopsis thaliana has three members (CSD1-3, FSD1-3) in both the Cu/Zn-SOD and Fe-SOD subfamilies, while the Mn-SOD subfamily has two members (MSD1-2) [9]. Previous studies showed that SODs localized in various cellular organs. CSD primarily occurred in the cytoplasm, chloroplasts, peroxisomes, and also in the extracellular region [10]. The localization of FSD is mainly in the chloroplasts, while MSD is typically found in the mitochondria, although a small number of proteins can be found in the peroxisomes [11,12].

Exposure of plants to diverse environmental pressures, such as low temperatures, water scarcity, and salinity, triggers an elevation in the levels of reactive oxygen species (ROS), resulting in varied biochemical responses within plant cells. Scavenging ROS, SOD is commonly recognized as the initial component of the antioxidant defense system, serving a protective function. Multiple research studies have shown that the SOD individuals can participate in the reaction to non-living pressures and control the aging process of plants [13,14]. For example, an increase in SOD activity helps *Brassica juncea* plants exhibit high resistance to abiotic stress, including salt and drought stress [15], and cold caused oxidative damage to tomato (*Solanum lycopersicum*) [16]. Moreover, various environmental pressures can trigger the activation of the *SOD* genes. During periods of drought, the expression of the *MnSOD* gene in wheat (*Triticum aestivum*) was significantly increased [17]. It is worth mentioning that according to reports, the overexpression of *SikCuZnSOD3* has been found to enhance the resistance of cotton (*Gossypium hirsutum*) to drought, salt, and cold stresses [18]. In rice (*Oryza sativa*), transgenic plants' overexpression of *OsMSD1* displayed higher tolerance to heat, whereas knock-out *msd1* mutants was notably susceptible to the same stress [19]. Moreover, the overexpression of *MeCu/ZnSOD* and *MeCAT1* has been observed to significantly improve cold and drought tolerance in cassava (*Manihot esculenta*) [20]. Notably, overexpression of *AtHDG11* and *AhWRKY75* improved drought and salt tolerance in peanut by increasing the activity of SOD [21,22]. Collectively, these investigations have demonstrated that enhanced SOD activity and increased expression of genes encoding SOD can serve as mechanisms for enhancing plant resilience to various environmental stressors.

The complete determination of the *SOD* gene family has been conducted in different plant species, encompassing monocots like rice (*Oryza sativa*) [23], wheat (*Triticum aestivum*) [24], and sorghum (*Sorghum bicolor*) [25], as well as dicots such as cotton (*Gossypium hirsutum*) [26], alfalfa (*Medicago truncatula*) [10], and rapeseed (*Brassica napus*) [27]. Cultivated peanut (*Arachis hypogaea* L.) is a globally significant crop that plays a vital role in the economy. It serves as a valuable source of oils and proteins for human consumption. During the growing phase, peanut experienced various environmental pressures such as cold, high salinity, and drought. Numerous reports have been published on various gene families of peanuts since the release of the peanut genome database [28–30]. However, we have very limited knowledge about the *SOD* gene family. Considering significant roles of the *SODs* in participating in stress responses, a total of 25 peanut SOD members were identified from the peanut database in this study. The identified *AhSODs* were comprehensively analyzed for phylogenetic relationship, gene structure, *cis*-acting elements, chromosomal location, duplication event, and expression patterns. The findings indicate that peanut SOD members potentially fulfill significant functions in peanut development and in the adaptation to diverse environmental challenges.

2. Materials and Methods

2.1. Identification and Sequence Analysis of the AhSOD Gene Family

Two methods were carried out to identify the SOD family members in peanut. The peanut genome data and *Arabidopsis* genome data were obtained from Peanut Base (https://legacy.peanutbase.org/peanut_genome, accessed on 20 March 2023) and The Arabidopsis Information Resource (TAIR, https://www.arabidopsis.org/, accessed on 20 March 2023), respectively. First, the HMM profiles of CSD (PF00080) and FSD/MSD (PF00081, PF02777)

were employed to search for peanut protein sequences using the HMMER program (version 3.0; Robert D Finn, Ashburn, VA, USA, 2015), with a designated E-value threshold of 0.001. Second, the BLASTP program was utilized to search the peanut protein database using the published protein sequences of *Arabidopsis* SOD [9] as queries, with a cutoff E-value of 0.001. Subsequently, all candidate sequences of SOD protein were verified with CDD (https://www.ncbi.nlm.nih.gov/Structure/cdd/, accessed on 20 March 2023), SMART [31], and InterProScan databases [32]. Candidate protein sequences without SOD domains were manually deleted. Using the online tool ProtParam [33], the peanut SOD proteins were analyzed for their physical and chemical characteristics, such as the number of amino acids, molecular weight (MW), and theoretical isoelectric point (pI).

2.2. Construction of Phylogenetic Trees and Alignment of Multiple Sequences

Utilizing MAFFT [34] with default parameters, a multiple sequence alignment (MSA) of *Arabidopsis* SOD and recently found peanut SOD proteins was performed in the current investigation. The MEGA software (version 6.06) [35] was utilized to create a neighbor-joining (NJ) tree using pairwise deletion, Poisson model, and 1000 bootstrap tests, based on the obtained sequence alignment.

2.3. Conserved Motifs and the Structure of Exons-Introns

The structure of peanut *SOD* genes, including the 5′-UTR, CDS, intron, and 3′-UTR, was analyzed using the online software GSDS 2.0 [36], by examining their mRNA and genomic sequences. The preserved patterns of peanut SOD proteins were examined utilizing the MEME software (version 5.5.3) [37], employing particular settings such as distribution of sites, either zero or one instance per sequence (zoops), a maximum of 15 patterns, and a pattern width varying from six to 50 residues.

2.4. Chromosomal Distribution and Gene Duplication Event

Peanut Base provided the chromosomal location data for *SOD* genes in peanuts. The online tool MG2C v2.1 (http://mg2c.iask.in/mg2c_v2.1/, accessed on 25 March 2023) was used to create the physical map. Genes that exhibited a similarity of more than 70% and were situated within a 200 kb segment of a chromosome were recognized as tandem duplication genes and were visualized on the peanut chromosomes. MCScanX [38] and TBtools (version 1.120) [39] were utilized to examine and display the genes involved in segmental duplication. Subsequently, the duplication genes' Ka and Ks values were calculated using the DnaSP 5.0 program [40].

2.5. Analysis of cis-Elements within the AhSODs Promoter

To identify the *cis*-acting regulatory elements present in the promoter regions of the *AhSODs*, a segment of 2000 bp from the promoter regions, which are situated before the ATG start codon of the peanut *SOD* genes, was extracted and then sent to PlantCARE [41].

2.6. Tissue Expression Pattern Analysis of AhSOD Genes

The transcriptome (RNA-Seq) data (accession: PRJNA291488) for peanut *SOD* genes across various tissues were downloaded from the Peanut Base. A total of 22 tissues, including leaf 1, leaf 2, leaf 3, veg shoot, repr shoot, root, nodule, perianth, stamen, pistil, peg tip 1, peg tip 2, peg tip Pat. 1, fruit Pat. 1, fruit Pat. 3, pericarp Pat. 5, seed Pat. 5, and seed Pat. 7, were selected for expression analysis. TBtools (version 1.120) [39] was used to generate the heat map.

2.7. Prediction of Transcription Factors Involved in Controlling AhSOD Expression

To gain a deeper comprehension of the transcription factors that regulate *AhSODs* expression, the promoter sequences were utilized to examine potential binding sites for transcription factors. Specifically, the promoter sequences of *AhSOD* genes, with a length of 2 kb, were submitted to the PlantRegMap database, and the Binding Site Prediction tool

was employed to predict the transcription factors that regulate *AhSODs* expression with a *p* value of $\leq 10^{-5}$ [42].

2.8. GO Enrichment Analysis

To perform the Gene Ontology (GO) annotation analysis, the protein sequences of all AhSODs were submitted to the eggNOG website [43]. GO enrichment analysis was conducted using TBtools (version 1.120) [39].

3. Results

3.1. Identification and Characterization of AhSODs

The BLASTP method and HMM search were employed to identify *SOD* gene family members from the peanut genome. After checking the presence of SOD domains (PF00080, PF00081, and PF02777) in each candidate, a total of 25 *AhSOD* members were identified. The distribution of these genes was observed across 13 chromosomes in peanuts and were designated as *AhSOD01* to *AhSOD25*, based on their respective chromosomal locations. The characteristics of the peanut SOD members including the number of amino acids, Mw, and pI were analyzed (Supplementary Table S1). The amino acid count of the 25 AhSOD members varied between 103 (AhSOD04) and 414 (AhSOD05) in terms of protein length. The molecular weight of the AhSOD proteins varied between 11.02 kDa (AhSOD04) and 46.95 kDa (AhSOD05). The pI ranged from 5.04 (AhSOD01) to 9.63 (AhSOD05).

3.2. Phylogenetic Tree Construction

In order to examine the evolutionary relationships among the peanut *SOD* gene family members, a neighbor-joining tree was created utilizing the complete amino acid sequences of SOD from both peanut and *Arabidopsis* (Figure 1). The results of the phylogenetic analysis indicated that the peanut SOD proteins can be categorized into three subfamilies, along with the previously reported eight *Arabidopsis* SODs. Specifically, there are sixteen AhCSDs, six AhFSDs, and three AhMSDs. Furthermore, it is observed that the FSD subfamily and MSD subfamily exhibit distinct clustering, indicating that these subfamilies might have originated from a shared ancestor through early replication. It is noteworthy that all subfamilies encompass representatives from both peanut and *Arabidopsis*, implying that the divergence of peanut and *Arabidopsis* occurred subsequent to that of the *SOD* gene family.

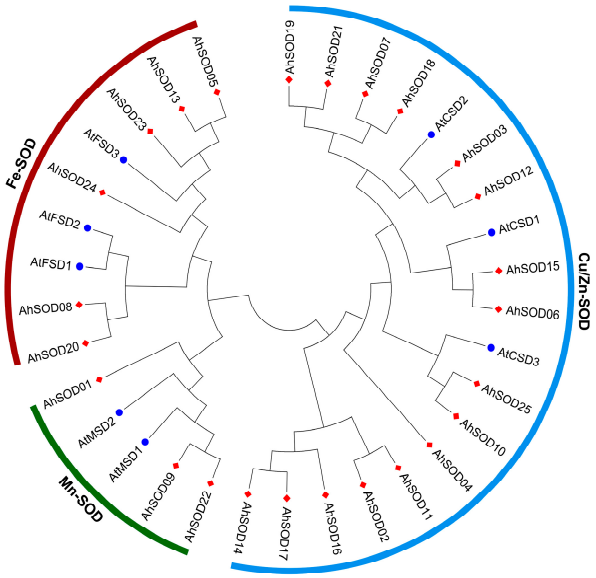


Figure 1. Phylogenetic tree construction of peanut SOD proteins. The phylogenetic tree was constructed by aligning the SOD proteins from peanut and *Arabidopsis*, followed by 1000 bootstrap replicates using the neighbor-joining (NJ) method. The resulting classification of the peanut SOD members and their *Arabidopsis* homologs revealed the existence of three subfamilies.

3.3. Conserved Motifs and the Structure Exon–Intron

The analysis of gene structure has the potential to yield significant insights into the evolutionary history of the SOD family in peanut. In order to generate a comprehensive understanding of the gene structures, both mRNA and genome sequences were utilized (Figure 2). The results indicated that the number of introns present in the peanut *SOD* gene family ranged from two (*AhSOD01/14/16/24*) to eleven (*AhSOD19*). It is noteworthy that the peanut *SOD* genes within the same subfamily exhibited a similar structure, thereby corroborating the results of our evolution analysis. The online software MEME (version 5.5.3) was subsequently employed to examine motif composition in the peanut SOD family. A total of ten unique motifs were identified (Figure 2, Supplementary Figure S1). Notably, motifs 2, 5, and 7 correspond to the SOD domain, and all SOD members identified possess at least one of these motifs. Additionally, members belonging to the same subfamily exhibit similar motifs.

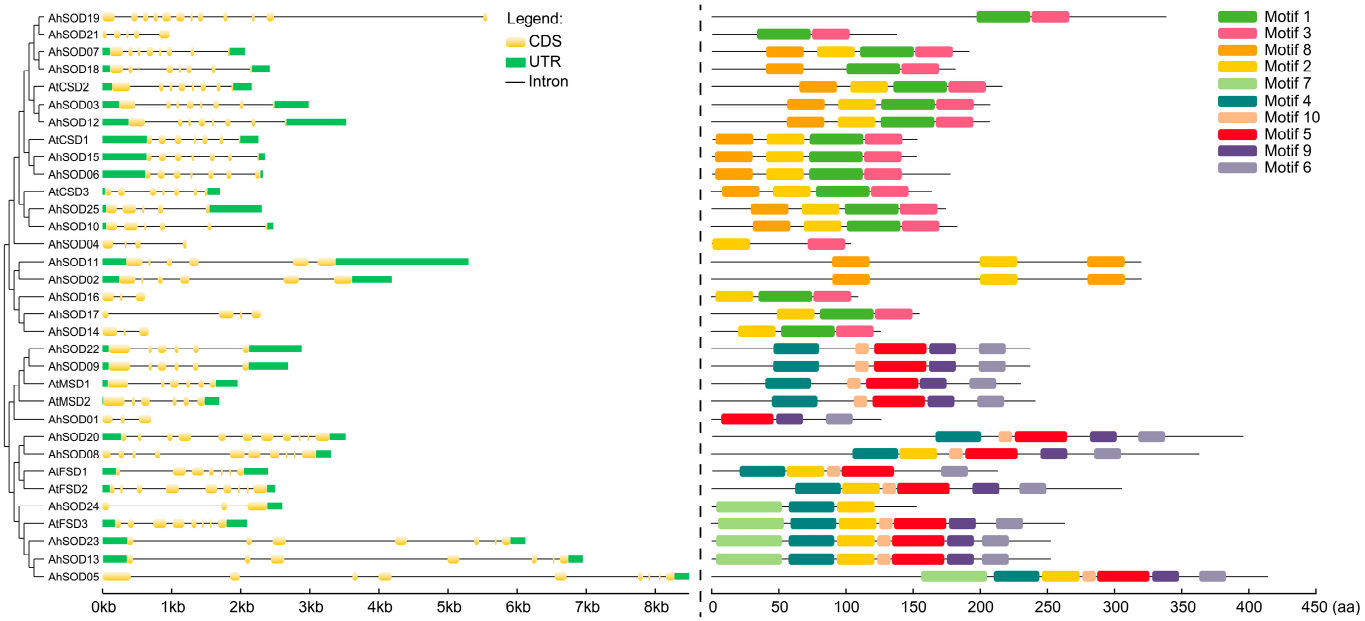


Figure 2. The exon–intron structure and conserved motif composition of AhSOD members.

3.4. Chromosomal Localization and Duplication Events

The result of chromosome localization showed that 25 peanut *SOD* genes were positioned on 13 peanut chromosomes (Figure 3). The peanut SOD genes were most abundant in Chr16 and Chr20, with a total of four genes. In contrast, Chr5, Chr6, Chr11, Chr13, Chr15, and Chr19 only had one peanut *SOD* gene each. It is interesting to note that most peanut *SOD* genes were close to the chromosome terminal. Furthermore, there were no instances of tandem duplication events detected within the *SOD* gene family of peanuts.

Moreover, we conducted a study on the duplication events of *SOD* genes in peanuts. A total of nine segmental duplication events were identified between 16 peanut *SOD* genes (Figure 4). These findings indicated that certain peanut *SOD* genes are possibly generated by gene duplication, and the segmental duplication events may have served as a significant driving force for peanut *SOD* evolution (Supplementary Table S2). In order to assess the potential impact of selective pressure on the *SOD* gene family, the Ka/Ks values were computed for the nine *SOD* gene pairs. Analysis revealed that nine segmental duplicated peanut *SOD* gene pairs exhibited Ka/Ks ratios below 1, indicating that a purifying selection pressure was imposed on these *SOD* genes throughout the course of evolution.

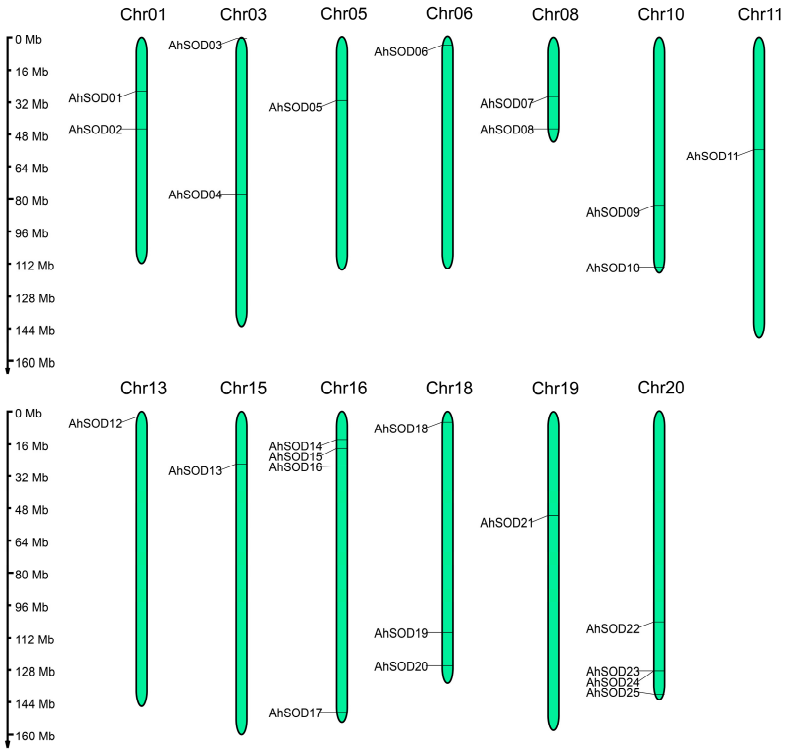


Figure 3. Chromosomal distribution of peanut *SOD* genes. A total of 25 *AhSOD* genes were positioned on 13 peanut chromosomes in peanut.

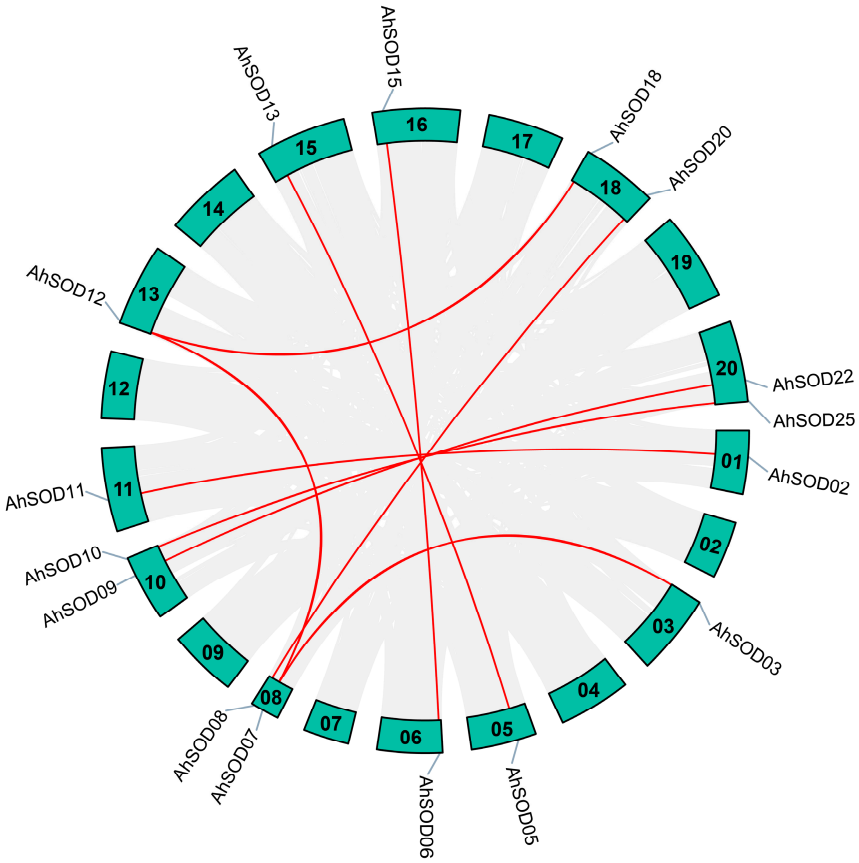


Figure 4. Duplication events of peanut *SOD* genes. MCScanX was used to investigate the nine presumed pairs of *AhSODs* that are segmental duplications, which are connected by the red lines.

3.5. Analysis of AhSOD Genes Syntenic

To better understand the phylogenetic relationship among the *SOD* genes in peanut, three syntenic maps were generated for peanut and three other plant species, including *Arabidopsis*, soybean, and tomato (Figure 5). The analysis revealed that 11 peanut *SOD* genes exhibited a syntenic relationship with the *SOD* genes in soybean, followed by *Arabidopsis* (eight), and tomato (six). The numbers of predicted orthologous pairs between the other three species (soybean, *Arabidopsis*, and tomato) were twenty-one, eight, and six, respectively. Moreover, it was discovered that certain peanut *SOD* genes exhibited a minimum of three collinear gene pairs with soybean, including *AhSOD03*, *AhSOD07*, and *AhSOD18*, suggesting their potential importance in the evolution of the *SOD* gene family in peanut. Moreover, it was discovered that there are four gene pairs that are in a straight line between peanut and the other three species. This suggests that these *SOD* genes already existed before the species diverged (Supplementary Table S3).

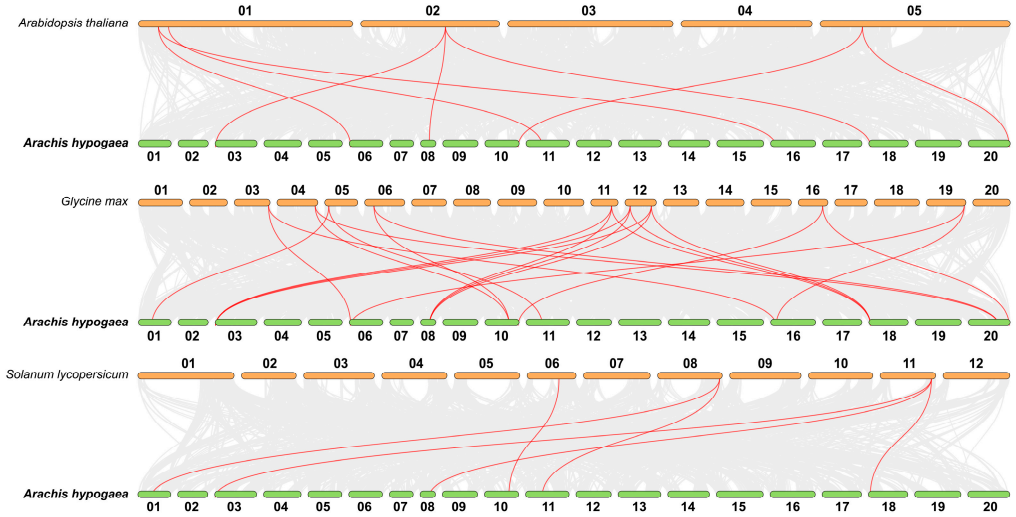


Figure 5. Comparative analysis of the arrangement of *SOD* genes in peanut and three additional plant species. The collinear blocks between peanut and three other plant species were represented by the gray line in the background, whereas the syntenic *SOD* gene pairs were exhibited by the red line.

3.6. Promoter Analysis of AhSOD Genes

The importance of *SOD* genes in the plant response to abiotic stress is supported by accumulating evidence. To gain insight into the potential regulatory mechanisms of *AhSOD* genes in abiotic stress or hormonal responses of peanut, the cis-acting elements in the promoter regions of the *AhSOD* genes were analyzed using plantCARE. We have chosen 11 *cis*-acting elements for the purpose of visualization (Figure 6). Four types of hormone-responsive elements, including ABRE, CGTCA-motif, GARE-motif, and TCA-element, were detected in two peanut *SOD* genes (*AhSOD01* and *AhSOD18*). These *cis*-elements are involved in abscisic acid (ABA) response, methyl jasmonate (Me-JA) response, gibberellin (GA) response, and salicylic acid (SA) response, respectively. The results suggested that ABA, Me-JA, GA, and SA might regulate the expression of these genes. Additionally, any hormone-responsive elements were not identified in two genes (*AhSOD14* and *AhSOD20*). Moreover, the promoters of peanut *SOD* genes were found to contain seven different types of elements associated with environmental stress. Various stress responses may involve these peanut *SOD* genes as the anaerobic induction elements (ARE) were found in twenty-one genes, low temperature responsive elements (LTR) in six genes, drought-inducibility elements (MBS) in eight genes, stress response elements (STRE) in fifteen genes, defense- and stress-responsive elements (TC-rich repeats) in seven genes, wound-responsive elements (WUN motif) in sixteen genes, and WRKY binding site (W-box) in eleven genes.

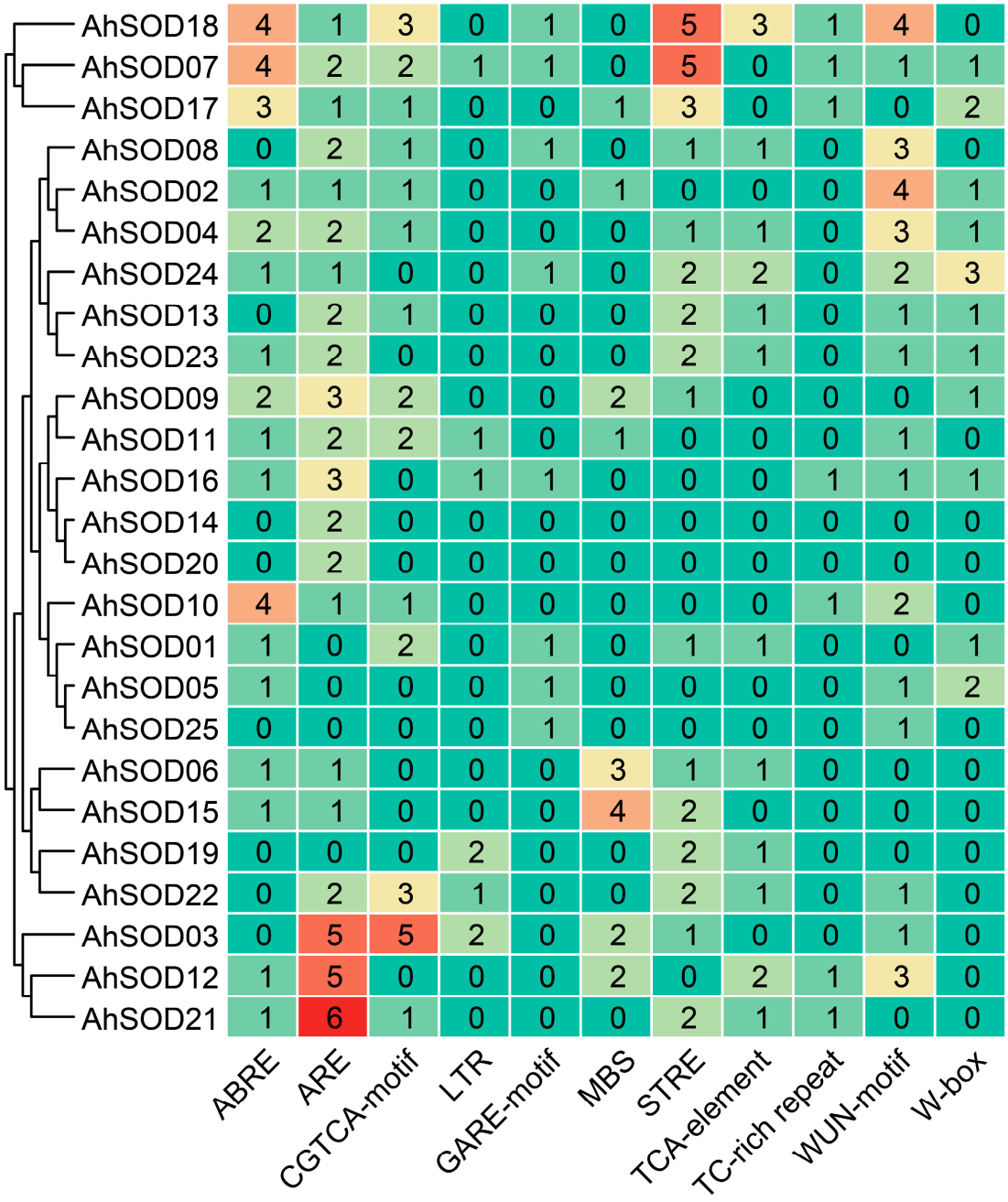


Figure 6. *Cis*-acting elements in the promoter regions of *AhSOD* genes.

3.7. Tissue Expression Pattern of AhSOD Genes

In order to comprehend the potential roles of the peanut *SOD* genes, an examination of their expression patterns in different tissues was conducted utilizing RNA-Seq data sourced from the Peanut Base. A total of 22 distinct tissues, including but not limited to the leaf, shoot, root, nodule, fruit, and seed, were selected for analysis. (Figure 7). Expression patterns showed that 84% (21 out of 25) of genes were expressed in most of these selected tissues, such as *AhSOD02*, *AhSOD10*, *AhSOD11*, *AhSOD13*, *AhSOD18*, and *AhSOD25*. Certain peanut *SOD* genes exhibited expression patterns specific to particular tissues. For instance, the expression of *AhSOD14* was only detected in the seed. Furthermore, *AhSOD08* and *AhSOD20* were highly expressed in leaves compared to the other tissues. It is worth mentioning that none of the tissues tested showed expression of four *SOD* genes, including *AhSOD01*, *AhSOD04*, *AhSOD16*, and *AhSOD17*.

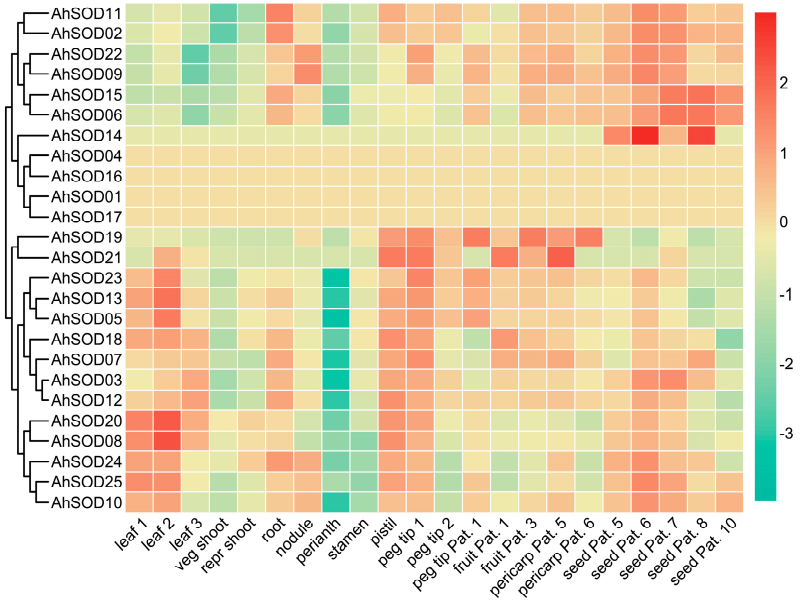


Figure 7. The expression patterns of *AhSOD* genes in 22 different tissues. TBtools was used to normalize and cluster the FPKM values of each *AhSOD* gene.

3.8. Expression Patterns of AhSOD Genes under Stress Treatments

To clarify the expression patterns of peanut *SOD* genes under abiotic stresses, we analyzed transcriptome data obtained from NCBI after exposure to cold (unpublished data), drought, and salt stresses [44,45]. Various expression patterns were observed in the majority of peanut *SOD* genes (Figure 8). Cold stress resulted in up-regulation of 68% (17 out of 25) genes. Furthermore, the expression of *AhSOD18* was found to be increased significantly under all three stresses and suggested that this gene may play a crucial role in response to these abiotic stresses. Various peanut *SOD* genes exhibited contradictory expression patterns in response to these stressful conditions. For instance, *AhSOD11*, *AhSOD24*, and *AhSOD25* were insensitive to salt and drought stresses, whereas they were significantly induced by cold stress. Furthermore, there were no notable alterations in the expression of certain *AhSOD* genes, such as *AhSOD14* and *AhSOD17*, following exposure to cold, drought, and salt stress treatments. The diverse patterns of expression observed in peanut *SOD* genes signify their distinct functions in response pathways to abiotic stresses.

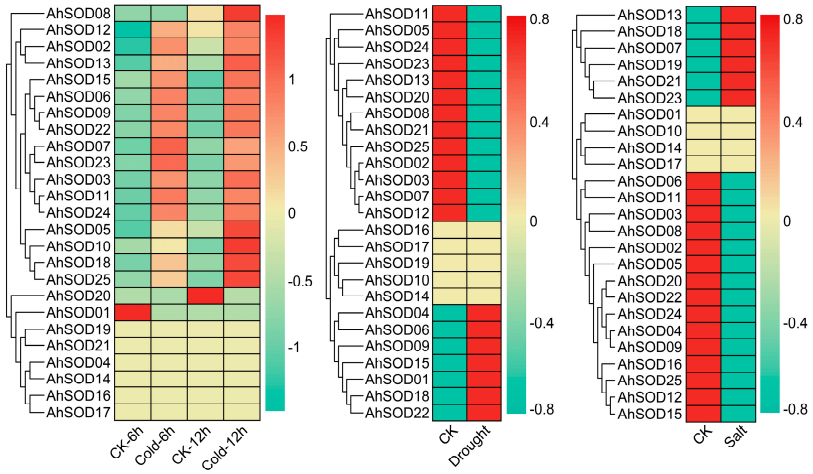


Figure 8. The gene expression profiles of *AhSODs* under cold, drought, and salt stress treatments. TBtools was used to normalize and cluster the FPKM values of each *AhSOD* gene.

3.9. Analysis of Possible Regulatory Interactions between Transcription Factors and AhSODs

To predict possible regulatory connections between transcription factors (TFs) and *AhSOD* genes, the PlantRegMap online software (version 5.0) was employed. A total of 17 TFs were identified as potential regulators of *AhSOD* gene expression. TBtools was used to determine and visualize the number of possible binding motifs for transcription factors (TFs) in the promoters of *AhSOD* genes (Figure 9). The regulatory patterns of *AhSOD07*, *AhSOD21*, and *AhSOD23* were found to be similar, as they were all regulated by ERF, MYB, and Dof. Furthermore, a distinct group comprising *AhSOD12*, *AhSOD20*, and *AhSOD25* exhibited comparable regulatory patterns, which were governed by AP2, MIKC_MADS, and TALE. It is noteworthy that the promoter of *AhSOD14* contains only one binding site for NAC transcription factors, indicating that *AhSOD14* may not be subject to regulation by transcription factors other than NAC.

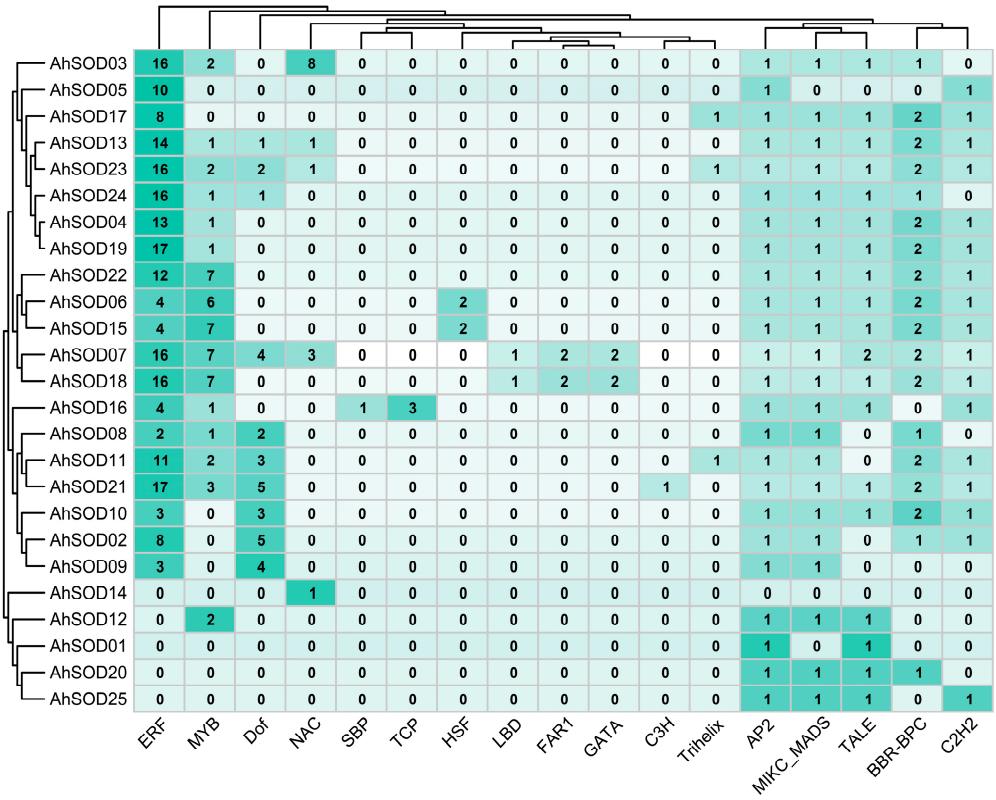


Figure 9. Predicted transcription factors controlling the expression of *AhSOD* genes.

3.10. GO Enrichment Analysis of AhSOD Genes

The *AhSODs* genes' functions were predicted via GO enrichment analysis, which encompassed the biological process (BP), molecular function (MF), and cellular component (CC) categories. The GO enrichment outcomes unveiled a multitude of significantly enriched terms. The analysis of BP annotations showed that these genes mainly participated in responses to superoxide (GO:0000303), responses to oxidative stress (GO:0006979), responses to stress (GO:0006950), responses to salt stress (GO:0009651), responses to abiotic stimulus (GO:0009628), response to toxic substance (GO:0009636), detoxification (GO:0098754), reactive oxygen species metabolic process (GO:0072593), etc. (Supplementary Figure S2). In summary, the GO enrichment analysis substantiated the involvement of *AhSODs* in various stress stimuli responses, cellular detoxification of oxidants, and SOD activity.

4. Discussion

Previous studies reported that *SOD* (superoxide dismutase) genes have been found to play crucial roles in both abiotic stress responses and plant development processes by scavenging ROS [46]. Given that cultivated peanut (*Arachis hypogaea* L.) is a crucial crop for the economy and oil production, it is also susceptible to various abiotic stresses such as cold, drought, and salt. The identification and analysis of *SOD* genes in peanut would hold significant implications for various stress responses. Thus far, various plant species have been found to possess SOD family genes, including *Zostera marina* with five genes [47], *Medicago truncatula* and barley (*Hordeum vulgare*) with seven genes [10,48], sorghum (*Sorghum bicolor*) with eight genes [25], tomato (*Solanum lycopersicum*) with nine genes [49], grape (*Vitis vinifera*) with ten genes [50], cotton (*Gossypium spp.*) with eighteen genes [26], banana (*Musa spp.*) with twenty-five genes [51], wheat (*Triticum aestivum*) with twenty-six genes [24], and *Brassica juncea* with twenty-nine genes [52]. However, a comprehensive analysis of the *SOD* gene family in peanut remains to be carried out. The *Arabidopsis* genome contains only eight *SOD* genes, whereas the present study identified a total of twenty-five *SOD* genes in peanut, which is likely attributed to its allopolytraploid nature. The classification of *SOD* genes in peanut is consistent with that of other plant species, such as tomato [49] and wheat [24]. Based on their domains and motifs, the peanut SOD members were divided into three subfamilies, which consisted of sixteen AhCSDs, six AhFSDs, and three AhMSDs (Figure 1).

The gene structure and conserved motifs have a significant impact on the evolution of the *SOD* gene family. The *Arabidopsis ERECTA* gene, for instance, requires the presence of multiple introns for proper expression [53]. By examining the gene structure, it was found that the peanut *SOD* genes exhibited variations in the quantity of exons and introns (Figure 2). Specifically, the Fe-SOD subfamily exhibited a range of seven to eleven exons and six to ten introns, with the exception of *AhSOD24,* which contained three exons and two introns. The Mn-SOD subfamily, on the other hand, consistently exhibited six exons and five introns, with the exception of *AhSOD01* that possessed three exons and two introns. Within the Cu/Zn-SOD subfamily, ten genes exhibited a range of six to twelve exons and five to eleven introns, while six genes contained three to five exons and two to four introns. Seven *TaSOD* genes were identified to contain seven introns in wheat [24], while the number of introns in sorghum ranged from five to seven [25]. Notably, the *SOD* genes within each cluster exhibited comparable exon–intron associations and conserved motifs (Figure 2). Additionally, the protein structure of AhSOD members is highly conserved within a same subfamily. The findings suggested that these genes may be involved in similar functions related to various abiotic stresses. Similar results have been reported in sorghum (*Sorghum bicolor*) [25], tomato (*Solanum lycopersicum*) [49], cotton (*Gossypium spp.*) [26], and wheat (*Triticum aestivum*) [24].

The expansion of gene families in plant genomes is attributed to segmental duplication and tandem duplication events [54,55]. The present study reveals that the *AhSOD* genes expansion in peanut is primarily due to segmental duplication, no tandem duplication. Nine pairs of segmental duplication were identified (Figure 4), and all of the Ka/Ks ratios associated with these duplication pairs were less than one (Supplementary Table S2). This finding suggested that the duplicated *SOD* genes in peanut may have undergone purifying selective pressure during the course of peanut evolution. Furthermore, the duplicated *SOD* genes in peanuts were found to be part of the same subfamily, such as *AhSOD02/11* in Cu/Zn-SOD subfamily, *AhSOD08/20* in Fe-SOD subfamily, and *AhSOD09/22* in Mn-SOD subfamily. Notably, *AhSOD05* and *AhSOD13*, as a segmental duplication pair, displayed uniform expression patterns across various organs and tissues (Figure 7).

In order to gain a deeper comprehension of the function of *AhSOD* genes in response to various environmental stresses, prophesying *cis*-acting elements in the promoter regions were conducted. The findings of this study revealed the identification of two distinct types of *cis*-acting elements, including stress- and hormone-responsive. The majority of the identified *cis*-acting elements were found to be associated with ABA, MeJA, GA,

SA, drought, low temperature, and anaerobic induction. It was reported that *cis*-acting elements contribute to plant stress responses [56,57]. These consequences were corroborated by the results of the GO enrichment analysis (Supplementary Figure S2). Additionally, various researchers have reported similar results in various agricultural crops, where *SOD* genes were observed to play a crucial role in diverse stress conditions [24,52]. These findings can enhance our comprehension of *AhSOD* genes under varying environmental conditions. Furthermore, the regulation of *SOD* gene expression in plants in response to stress is mediated by transcription factors. Overexpression of *MuWRKY3* in transgenic peanut resulted in significant up-regulation of *SOD* gene expression and subsequently improved drought stress tolerance in the transgenic plants [58]. Compared to wild-type plants, the expression levels of *SOD*, *POD*, and *CBF* genes in *BpERF13* transgenic lines were dramatically up-regulated under cold stress [59]. Under salt treatment, the up-regulation of stress-related genes, including *SOD*, *POD*, *APX*, and *P5CS*, was observed in *IbMYB308* transgenic plants [60]. Soybean (*Glycine max*) NAC2 transcription factor negatively regulates drought resistance in transgenic tobacco through the suppression of plant *SOD* genes expression, resulting in the accumulation of ROS [61]. Notably, the promoter region of the *AhSOD* genes was found to contain multiple predicted transcription factor binding sites, such as ERF, MYB, Dof, and NAC (Figure 9). This finding suggests that the *AhSOD* genes possess diverse regulatory mechanisms to adapt to the intricate external environment.

Previous studies have indicated that the expression of *SOD* genes differs across tissues [51,62]. Therefore, we utilized RNA-seq data to evaluate the tissue-specific expression levels of *AhSOD* genes in 22 distinct developmental tissues (Figure 7), which corroborated previous findings [26]. Notably, certain genes exhibited higher expression across all tested tissues, suggesting their potential involvement in the growth and development of peanut. In addition, crops are negatively impacted by three primary abiotic stresses: cold, drought, and salt stresses, which hinder their growth, development, and yields [63]. Under conditions of these abiotic stresses, plant cells will accumulate reactive oxygen species (ROS), and an excess of ROS will induce oxidative stress [1]. It should be emphasized that several SOD members have been documented to participate in the response to plant environmental pressures by scavenging ROS [46]. In the current study, the RNA-seq data of peanut under abiotic stresses showed that several genes were induced by cold, drought, and salt treatments (Figure 8). These findings are consistent with previous reports, which demonstrated elevated expression of multiple *SOD* genes in response to stresses. For example, some *SOD* genes were found to be up-regulated in response to cold, heat, salinity, and drought stresses in grape [64]. All *SOD* genes exhibited significant up-regulation in tomato when subjected to salt and drought treatments [49]. Similarly, the activity of SOD was notably enhanced in rapeseed when exposed to cold stress [65]. These results offer compelling evidence that *SOD* genes exhibit a conserved function in mitigating abiotic stresses across various plant species.

5. Conclusions

In this study, a total of 25 AhSOD members were identified from peanut, which were categorized into three subfamilies based on their *Arabidopsis* homologs. Our investigation of the *SOD* genes in both peanut and *Arabidopsis* demonstrated their variability in terms of member count, evolutionary connections, gene structure, chromosomal positions, gene duplication, collinearity, tissue-specific expression patterns, and responses to cold, drought, and salt stresses. The findings suggest that the *AhSOD* genes play a crucial role in modulating peanut response to abiotic stresses and growth.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy13081959/s1, Figure S1: The putative conserved motifs in AhSOD proteins; Figure S2: GO enrichment analysis of AhSOD genes; Table S1: The detail information of identified peanut SOD family members; Table S2: The detail information of segmental duplication gene pairs; Table S3: The syntenic pairs between peanut and other three plant species.

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