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Genome-wide identification and expression analysis of the *AhTrx* family genes in peanut

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Abstract

Thioredoxins (Trx) are small multifunctional redox proteins that contain thioredoxin conserved domain and active site WCXXC. The Trx family has an important role in multiple processes, including electron transport, seed germination, redox regulation, biotic and abiotic stresses resistance, *etc*. Although *Trx* genes have been extensively characterized in some plants, they have not been reported in peanut until now. The identification of *AhTrx* genes provides potential candidate genes for studying their effects and regulatory mechanisms in peanut (*Arachis hypogaea* L.) growth and development, especially under aluminium (Al) stress. It is also helpful to further analyze the Al resistance pathway in plants. Seventy *AhTrx* genes were identified using a genome-wide search method and conservative domain analysis. Then the basic physicochemical properties, phylogenetic relationship, gene structure, chromosomal localization, and promoter prediction were studied by the bioinformatic methods. Furthermore, the expressions of *AhTrx* genes under different Al treatment times in two peanut cultivars were tested using a real-time quantitative polymerase chain reaction. Seventy *AhTrx* genes were identified and characterized. Phylogenetic tree analysis showed that all *AhTrx* members could be classified into 9 groups with different conserved domains. Motif 1 was found to exist in every sequence, with an active site. Furthermore, the gene structures showed that the *AhTrx* family was complicated and changeable during evolution. The chromosomal localization indicated that the distribution and density of the *Trx* family on 20 peanut chromosomes were uneven. Predictive promoter analysis indicated that AhTrx proteins might play a role in phytohormones synthesis and stress response. Finally, the expression patterns of the *AhTrx* genes showed that every gene was differently expressed under Al treatment in different peanut cultivars, some were obvious, others had no significant difference, some were at a high level, while others were at a low level. This study systematically identifies the *Trx* gene family in peanut, providing some candidates for further study on its effects and regulatory mechanism under Al stress in peanut.

Keywords: aluminium stress, *Arachis hypogea*, chromosomal localization, expression analysis, gene structure, peanut, thioredoxins.

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Abbreviations: ABA - abscisic acid; AOX - alternative oxidase; A1 - aluminium; bp - base pair; CDS - coding sequnce; FBpase - fructose-2,6-bisphosphatase, GPX3 - glutathione peroxidase 3; GSH - glutathione, GRAVY - grand average of hydropathicity; MSRs - methionine sulfoxide reductase; NUDX6 - Nudix hydrolase 6; ox - oxidized; PCNA - proliferating cell nuclear antigen; PEP - plastid encoded RNA polymerase; PS - photosystem; red - reductive; ROS - reactive oxygen species; RT - reverse transcription; SBpase - sedoheptulose-1,7,-bisphosphatase; TBY-2 tobacco Bright Yellow-2; Trx - thioredoxin; UTR - untranslated region; ZH2 - Zhonghua NO.2.

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Introduction

Thioredoxins are small molecular proteins, that widely exist in animals, plants, and microorganisms. The thioredoxins are more complex in plants. At least 20 *Trx* genes have been identified in *Arabidopsis thaliana* (Meyer *et al*. 2005). In plants, several types of Trxs have been classified: the chloroplastic f, m, x, y, z and CDSP32, the mitochondrial Trxs o and h (Gelhaye *et al*. 2004, Balsera *et al*. 2014, Buchanan 2016) type is also located in the cytoplasm, mitochondria (Gelhaye *et al*. 2004), and nucleus (Serrato *et al*. 2003). These results reveal the extensive localization and functional diversity of the *Trx* family. To date, genome-wide analysis has identified various numbers of *Trx* genes in plants, such as poplar, rice, tomato, soybean, sunflower, rye, and others. However, there are no reports that they exist in peanut.

Conversion of thiol-disulfide bonds is the main mode of Trxs function in regulating the cellular redox environment. However, the main function of different members is still discrepant. *Trx* genes not only regulate redox but also play important roles in plant growth and stress response. For plant growth, *Trx f* (*Arabidopsis* coding sequence)-overexpression enhanced starch accumulation in tobacco leaves (Ancin *et al*. 2019). *Trx f* also played a central role in the redox regulation of enzymes in the Calvin-Benson cycle, such as fructose-2,6-bisphosphatase (FBpase), sedoheptulose-1,7-bisphosphatase (SBPase), and so on (Schurmann *et al*. 2008, Yoshida *et al*. 2015, Okegawa *et al*. 2020). It has also been reported that double mutant *trx f1f2* displays growth inhibition under short-day conditions (Naranjo *et al*. 2016), indicating that *Trx f* is essential for plant growth. Chloroplastic *Trx m* was also essential to regulate the redox of photosynthetic metabolism (Nikkanen *et al*. 2019). Inactivation of three *Trx m* (*m1*, *m2*, and *m4*) led to pale-green leaves and specifically reduced stability of the photosystem II (Wang *et al*. 2013). *Trx m4* could negatively regulate the NADH dehydrogenase-like complex-dependent plastoquinone reduction pathway and proton gradient regulation pathway to control a cyclic electron flow (Courteille *et al*. 2013). The *trx m3* mutation hampered meristem development, leading to a seedling-lethal phenotype (Benitez-Alfonso *et al*. 2009). Mutant *trx y2* specifically displayed a significantly reduced leaf methionine sulfoxide reductases (MSRs) capacity and reduced growth under high irradiance, so by inference, *Trx y2* played a physiological function in protein repair mechanisms and as an electron donor to MSRs (Laugier *et al*. 2013). Overexpression of *Trx CDSP32* could promote electron transfer, ATP synthase activity to alleviate cadmium-induced photoinhibition of photosystem (PS) II and PS I in tobacco leaves (Zhang *et al*. 2020). Chloroplastic *Trx z* was also found to regulate the plastid-encoded RNA-polymerase (PEP)-dependent transcription and chloroplast redox signalling pathway (Arsova *et al*. 2010). So many chloroplastic *Trx* members play role in the redox signalling and photometabolism of chloroplasts. Are there any differences among *Trxs*? What is the evolutionary relationship among *Trxs*? At present, there is no systematic research on peanut.

Trx o was mainly distributed in mitochondria (Laloi 1999), but *PsTrx o1* was both located in mitochondria and nucleus (Calderon *et al*. 2017). Further research found that proliferating cell nuclear antigen (PCNA) was the target of *PsTrx o1*, and *PsTrx o1* was involved in the cell cycle progression of tobacco Bright Yellow-2 (TBY-2) cell cultures (Calderon *et al*. 2017). The lack of mitochondrial *Trx o1* could affect alternative oxidase (AOX) activity and carbon metabolism under different irradiances in *Arabidopsis* (Florez-Sarasa *et al*. 2019). So *Trx o* has different functions due to different localization. The *Trx h* type is the largest subfamily, and 8 members were found in *Arabidopsis*. *AtTrx h1* has the active site WCGPC and was identified as a novel Al and proton tolerance gene (Nakano *et al*. 2020). Mutant *trx h2* showed a phenotype with delayed seed germination and reduced respiration, indicating the regulation of *Trx h2* for mitochondrial photorespiration in *Arabidopsis* (Da *et al*. 2020). The active site of *AtTrx h3* is WCPPC, and it was reported that AtTrx h3 has dual functions, acting as a disulfide reductase and molecular chaperone. Due to its chaperone function, *AtTrx h3* overexpression could enhance heat-shock tolerance (Park *et al*. 2009). *AtTrx h5* also has the active site WCPPC, which was stimulated by Nudix hydrolase 6 (AtNUDX6) to impact the plant immunity (Ishikawa *et al*. 2010). *AtTrx h7* and *h8* were responsive to glutathione (GSH) depletion with greatly increased mRNAs (Schnaubelt *et al*. 2015). The conservative active site of *AtTrx h9* was still WCGPC, and this member could react with glutathione peroxidase 3 **(**GPX3) through the formation and breakdown of disulfide bonds between Cys4 and Cys57 (Kuang *et al*. 2020). *Trx h9* was associated with the plasma membrane to move from cell to cell (Meng *et al*. 2010). In *Arabidopsis*, the five h members (h1, h2, h7, h8 and h9) contain the same WCGPC, and the other three h members contain WCPPC, but there is a big difference in their function. So, the diversity of their functions may result from the sequnces difference at their N-terminal or C-terminal ends.

Peanut is one of the most important oil and cash crops in the world. So far, more than 100 countries grow peanut. However, during their growth and development, peanut may be affected by various biotic or abiotic stresses, which may affect the growth of roots, mitochondrial function, respiration, and photosynthesis. Under these circumstances, the peanut itself will undergo different evolutionary mechanisms to reduce the damage, relying on its adaptation and defence. Studies had shown that once stressed, plants would show decreased ascorbic acid (AsA), ion imbalance, and osmotic disturbance at the initial stage leading to "the physiological drought" (Qian *et al.* 2014). The continuation or aggravation of stress is often accompanied by the production of reactive oxygen species (ROS) and the occurrence of secondary oxidative stress. Plants can remove extra ROS by their own enzymatic system. Trx family is defined as a new ROS scavenger because of its disulfide bonds participating in the reduction-oxidation reaction (Traverso *et al*. 2007), but the current research on this family was mainly concentrated on *Arabidopsis*, rice, and humans, and there are few studies on peanut. In this study, the nucleic acid and protein

sequences of the *Trxs* family were preliminarily analyzed by the bioinformatics method. It is useful to understand the structural characteristics of the proteins and their evolutionary relationship in the Trx system and provide a theoretical basis for further study on the function of *Trx* genes in peanut.

Materials and methods

Plants, growth conditions, and stress treatment: Two different peanut (*Arachis hypogaea* L.) cultivars, Zhonghua NO. 2 (ZH2, Al sensitive) and 99-1507 (Al resistant) provided by Oil Crop Research Institute, Chinese Academy of Agricultural Sciences were used in this study (Huang *et al*. 2014). The peanut seeds were cultured in moist *Perlite* for 4 d in dark. Germinated seeds with similar radicle lengths were transferred into modified Hoagland nutrient solution exchanged every 2 d. All peanuts were grown in a self-regulating incubator at a temperature of 26 °C, a 12-h photoperiod, the irradiance of 30 - 50 μmol m^{-2} s^{-1}, and air humidity of 70 %. For Al treatment, firstly, the seedlings were pretreated with 100 μM $CaCl_2$ (pH 4.2) for 24 h. And then, the seedlings were treated with 100 μM $AlCl_3$ (pH 4.2) containing 100 μM $CaCl_2$ for 8 and 24 h. The root tips about 1 cm were collected uniformly after treatment and immediately frozen in liquid nitrogen for RNA extraction.

Genome-wide identification of the *AhTrx* gene family in peanut: Peanut genome and protein sequences were obtained from the *Peanut Genome Resource* (http://peanutgr.fafu.edu.cn/index.php) (Zhuang *et al.* 2019) to query *Trx* genes. Basic *AhTrx* sequences were used to search for conserved *Pfam* from the *EMBL-EBI* website (http://pfam.xfam.org/search#tabview=tab1) to eliminate the sequence's lack of the thioredoxin domain. After manual correction, the protein sequences were checked through the National Center for Biotechnology Information (*NCBI*) Batch CD-Search Tool (https://www.ncbi.nlm.nih.gov/Structure/bwrpsb/bwrpsb.cgi) to identify the conserved domain with the default parameter settings. The results of CD searching were visualized through *TBtools* following the instructions (Chen *et al*. 2020). Finally, the definitive sequences were used for subsequent analysis.

Molecular mass, isoelectric point, grand average of hydropathicity, signal peptide, and subcellular localization: Basic physicochemical properties such as molecular mass, isoelectric point, and grand average of hydropathicity (GRAVY) were predicted on the *ExPASy* website (https://web.expasy.org/protparam/). Signal peptides of AhTrx proteins were analyzed using *SignalP 4.1* (http://www.cbs.dtu.dk/services/SignalP/). *WoLF PSORT* (https://wolfpsort.hgc.jp/) was used to predict the subcellular localization.

Phylogenetics, gene structure, conserved motifs, chromosomal localization, and promoter analyses: The sequences of the *Trx* family in *A. thaliana* were downloaded from the *TAIR* database (https://www.arabidopsis.org/). To investigate the relationships of *Trx* genes among *Arabidopsis* and peanut, the protein sequences were aligned using *MEGA 7.0* (Kumar *et al*. 2016). Based on the *BLAST* results, a phylogenetic tree was also constructed with the maximum likelihood method and 1 000 bootstrap replications.

According to the genomic gff3 files, the gene structures of peanut and *Arabidopsis* were also visualized through the "visualize gene structure tool" provided by *TBtools*. The conserved motifs of all sequences were determined using Multiple Expectation maximizations for Motif Elicitation (*MEME*) (https://meme-suite.org/meme/tools/meme) with 10 motifs to be found and other default parameters (the classic mode was selected in the motif discovery mode, and *in site* distribution, the option of zero or one occurrence per sequence was set). All motifs were also visualized through *TBtools*. The locations of *AhTrx* genes on the peanut chromosomes were investigated from the *gff3* file and visualized by the "amazing gene location from gff3 tool" on *TBtools*. Pattern maps of structure, motifs and chromosomal localization are shown individually.

To analyze the *cis*-acting elements of the *AhTrx* genes, the 2000-bp DNA sequences upstream of the transcription start sites were obtained from the genome file through *TBtools*. By uploading the promoter sequence on the website of *Plant CARE* (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/), prediction of all promoters was performed to obtain the *cis*-acting elements. The *cis*-acting elements of every gene were arranged by type in the *Excel* software and then visualized through *TBtools* following the instructions (Chen *et al*. 2020).

Expression analysis of *AhTrxs* under Al stress: Total RNA of peanut root tips was extracted using a Plant RNA kit (*Promega*, Madison, USA) following the manufacturer's instructions. Then, the RNA quality was tested by agarose gel electrophoresis and measured by ultramicrospectrophotometer *NanoDrop 2000c* (*Thermo Scientific*, Waltham, USA). The RT reactions were operated using *PrimeScript™ RT* reagent kit with *gDNA Eraser* (*Takara*, Dalian, China). Quantitative real-time PCR was performed following the instructions of *TB Green® Premix Ex Taq™* (*Takara,* Dalian, China) and completed by the *Bio-Rad CFX 96* (Hercules, USA). The *UBQ10R* was in constitutive expression in peanut and feasible as a reference gene (Yao *et al*. 2019) to normalize the transcription. All reactions were performed at least in three replicates. The data was analyzed in the way of $2^{-\Delta\Delta CT}$. The primers used in the study were listed in Table 2 Suppl.

Results

According to the comparison of conserved domains (Fig. 1 Suppl. and Fig. 2 Suppl.), candidate *Trx* genes in peanut contain at least one of two *Trx* domains: thioredoxin and TxlA. Seventy genes of the peanut *Trx* family were determined and named by the mRNA ID (Table. 1 Suppl.).

The basic properties of peanut thioredoxins are shown

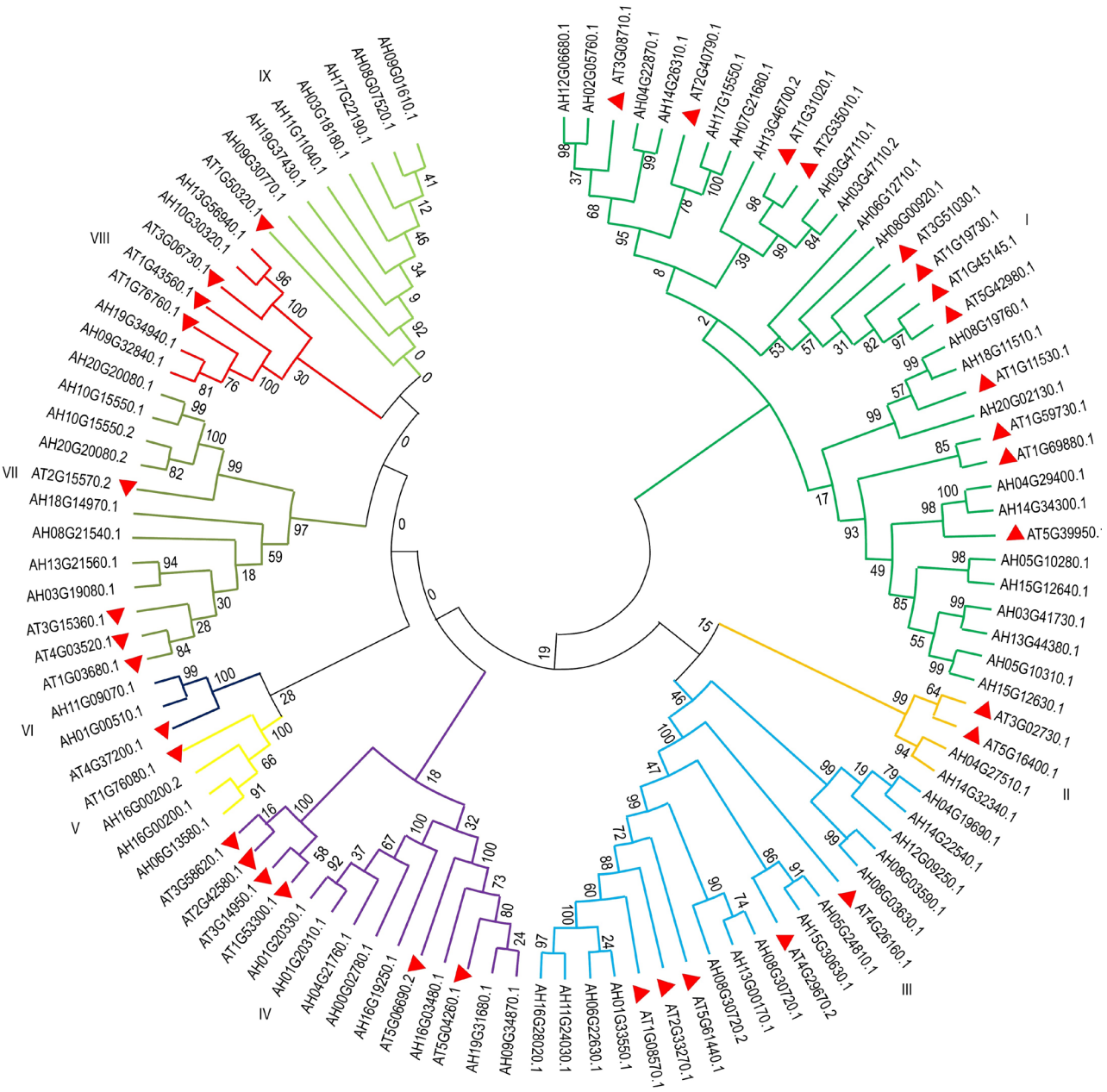

Fig. 1. Molecular phylogenetic analysis of Trx family in peanut and *Arabidopsis* done by maximum likelihood method. The number at the branch of the phylogenetic tree indicates the confidence of the branch. The higher value, the higher the confidence. Each member of the Trx family was marked with the *same colour*. The Trx members of *Arabidopsis* were marked with *red triangles*.

in Table. 1 Suppl. Among 70 *Trx* genes in peanut, the ORF length of AH13G46700.2 could be up to 1 089 bp, the longest member. Conversely, the shortest was AH04G21760.1 with 321 bp. The maximum molecular mass was 40.10 kD of AH13G46700.2, and that of AH04G21760.1 was only 11.76 kD. According to the theoretical pI, 36 Trx members were acidic, and the rest were alkaline. The largest isoelectric point was 9.81 in AH06G22630.1, but the smallest was 4.53 in AH20G02130.1. AH08G00920.1 and AH08G30720.2 had positive values in GRAVY, indicating that they are hydrophobic proteins. The other thioredoxins were hydrophilic proteins. The distribution of Trx proteins in organelles was also very different. In terms of subcellular location, chloroplast accounted for 64.29 % and this was the largest proportion. The second-largest proportion was in the cytoplasm. The next order from high to low was the nucleus, mitochondria, and extracellular space. In particular, the distribution possibility in chloroplast or nucleus of AH06G22630.1 and AH08G07520.1 was similar, with no clear subcellular location. Seventy Trx proteins in peanut are not secreted proteins and have no signal peptide.

To identify the subfamily members of Trx in peanut, a phylogenetic tree was constructed by aligning 35 AtTrx

Table 1. Specific information about the members of each group in peanut and *Arabidopsis*.

Group category	Number of AhTrxs	Number of AtTrxs	Name
I	22	12	h
II	2	2	f
III	14	5	ACHT
IV	8	6	WCRKC and TTL
V	3	1	CDSP32
VI	2	1	HCF164
VII	8	4	m
VIII	4	3	y and z
IX	7	1	x
Total number	70	35	

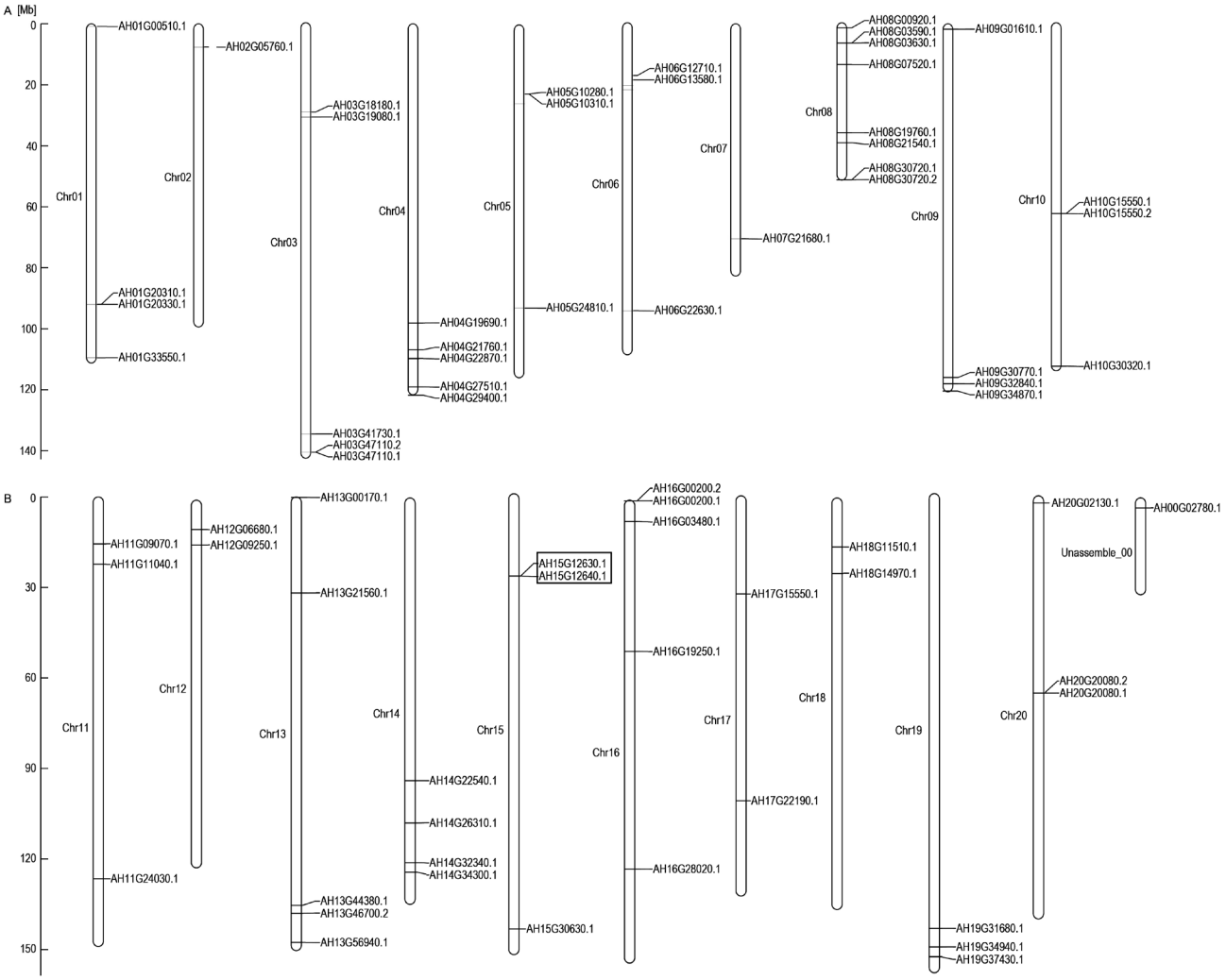

Fig. 2. Map of *AhTrxs* distribution in peanut chromosomes. The maps on chromosomes 1 to 10 were shown in the upper part, chromosomes 11 to 20 in the below part. Chr represents the chromosome number in the peanut chromosome map. *Rectangle* represents tandem repeat. Unassemble_00 means the chromosome with genes that were not mapped to the 20 chromosomes.

protein sequences with 70 AhTrx protein sequences in the *MEGA 7.0* (Fig. 1). The results indicated that the peanut Trx family could be classified into nine groups; Group I, Group II, Group III, Group IV, Group V, Group VI, Group VII, Group VIII and Group IX (Table 1). The specific number of each group is shown in Table 1. The majority of

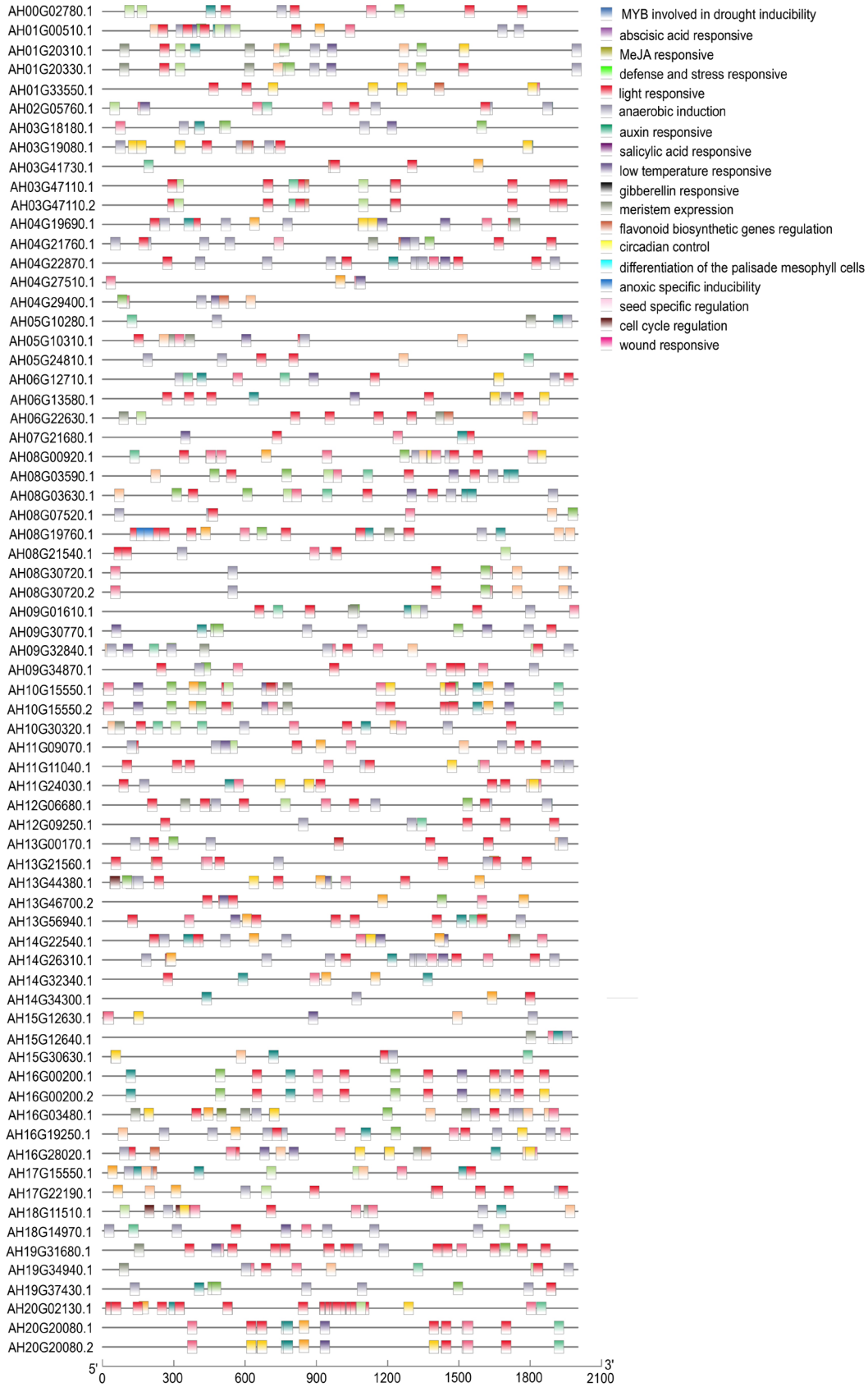

Fig. 3. Kinds and numbers of predicted promoter elements in the upstream regions of *AhTrxs*. *Rectangles* with different colours represent different types of promoter elements.

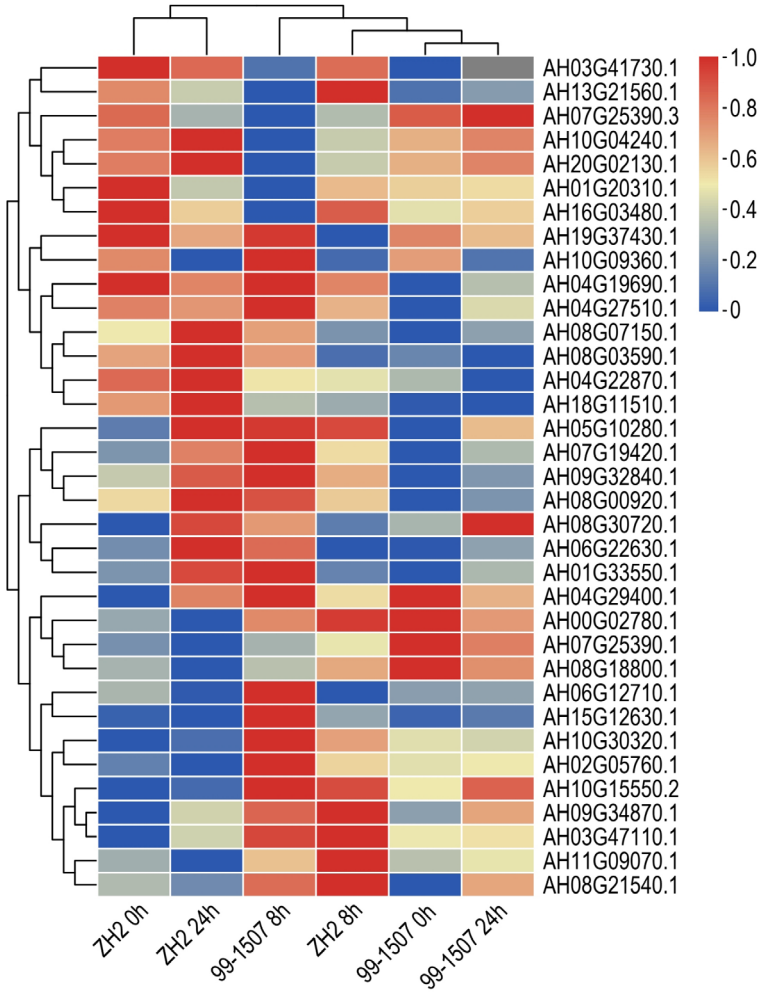

Fig. 4. *AhTrx* genes expression under Al stress as shown by RT-qPCR. ZH2 0 h, ZH2 8 h, ZH2 24 h, 99-1507 0 h, 99-1507 8 h, 99-1507 24 h denote the different Al treatment times in ZH2 and 99-1507 peanut cultivars. The colour histogram on the right of the heat map indicates the gene expression level. *Red* means high expression, *dark blue* means low expression. Note: A 2-based log function conversion is performed on the expression amount. The lines on the left and above of the figure represent clusters, one branch means more similar expression level.

Trxs in peanut belongs to group I, named as h-group.

According to the genome annotation file *Gff3* and candidate gene ID, the *AhTrx* and *AtTrx* gene family structures were analyzed and drawn with the *TBtools* software (Fig. 3 Suppl. and Fig. 4 Suppl.). Thirty-five *AtTrx* genes included entirely UTR, CDS, and introns (Fig. 3 Suppl.), 56 *AhTrx* genes also included the aforementioned three types of gene structural elements, 14 genes had only CDS and introns (Fig. 4 Suppl.). The number of introns in each open reading frame was different. The number of CDSs also varied, changing from one (AH12G09250.1) to ten (AH13G46700.2). The number of genes with three or four CDS is high, accounting for 31.43 % independently, those with two CDS are eighteen (25.71 %), with five CDS are three (4.29 %), with eight CDS are two (2.86 %), one gene has one CDS (1.43 %). Due to the CDS number and gene length change among genes, the introns were diverse with different number and length. AH03G19080.1 was the longest but contained only one intron and two CDS. The length of AH13G46700.2 was shorter, having nine introns and ten CDS. There was only downstream UTR in three genes structures. Eight genes had more than two UTRs, distributed upstream, in the middle part, and downstream. Fourteen genes had only intron and CDS without UTR. In the rest of the genes, in the middle of the gene structure were intron and CDS, and both ends were UTR.

The chromosomal localization of *AhTrx* genes was analyzed and visualized using *TBtools* (Fig. 2). It revealed that the distribution and density of the *AhTrx* genes on 20 peanut chromosomes were heterogeneous. All chromosomes had different numbers of *AhTrx* genes. Among them, chromosome 8 had the largest number with 8 *AhTrx* genes. Chromosomes 2 and 7 contained one gene respectively. AH00G02780.1 was not mapped to the 20 peanut chromosomes. Notably, AH15G12630.1 and AH15G12640.1 were tandem repeats on chromosome 15. As seen from the distribution, there were fewer *Trx* genes located in the middle regions of the chromosomes, while most of them were distributed on the ends of the

chromosomes.

To reveal the conservative domain and structural differences among different AhTrx proteins, 10 conserved motifs at each sequence were analyzed through the *MEME* online software (Fig. 5 Suppl.). Except for AH11G11040.1 and AH03G47110.2, the rest of the Trx proteins in *Arabidopsis* and peanut contained motif 1, which had active site (W)C(GP)C. There was no motif 2 on the sequences of AT5G06690.2, AH04G21760.1, AH08G03590.1, AH08G03630.1, AH12G09250.1, and AH05G10310.1. Motif 3 was nearly distributed in all sequences except AH08G03630.1 and AH12G09250.1. HCF164, m, y(or z) and x group members practically all contained motif 7 at the C-terminal. ACHT group had the conserved motifs 6, 1, 3, 2, and 4, distributing from the middle to the N-terminal of the sequences. The N-terminal of ACHT members contained other motifs. There were motifs 6, 1, 3, 2, and 5 in the sequences of the h group. CDSP32 group had the most number of motifs 6, 1, 3, 9, 1, 3, 2, and 5, ranging from N-terminal to C-terminal. So Trx subfamily was mostly conserved, but the type and number of motifs also had differentiation among groups. In general, the conserved Trx domain was generally located on the C-terminal, while the N-terminal was susceptible to change.

To reveal the *cis*-elements in the promoters of *AhTrxs*, we chose the 2 000 bp DNA sequence upstream of the transcription start sites from the peanut genome file and identified the promoter element on the online website. Visualization results are shown in Fig. 3. In a nutshell, the number of elements were varying from 5 (AH03G41730.1, AH05G10280.1, AH14G34300.1 and AH15G12640.1) to 75 (AH20G02130.1) on the corresponding sequence. There were 32 abscisic acid-responsive elements and 33 radiation responsive elements in the promoter sequence of AH20G02130.1, indicating an important role in ABA signalling and radiation responsive pathways. In terms of element types, the abscisic acid-responsive element was identified in 50 promoters of *AhTrxs*, auxin-responsive elements in 30 promoters, gibberellin responsive elements in 28 promoters, methyl jasmonate (MeJA) responsive elements in 54 promoters, and salicylic acid-responsive elements in 25 promoters. In addition to hormonal elements, 39 promoters were predicted to contain defence- and stress-responsive elements, 32 promoters have low-temperature responsive elements while 30 promoters with MYB were involved in drought response. It was worth mentioning that wound responsive elements were identified in the promoter of AH16G03480.1. These results showed that *AhTrxs* might participate in the hormone synthesis and stress response.

To obtain insight into the potential functional roles of *AhTrxs* in Al stress, 35 *AhTrxs* were selected based on the transcriptome (PRJ-NA525247) for detection of expression under Al stress through RT-qPCR (Fig. 4). The results showed that AH05G10280.1 achieved the highest expression at 24 h in cv. ZH2 and a stimulated expression by Al stress in cv. 99-1507. The trends of AH07G19420.1, AH08G00920.1, and AH09G32840.1 were the same as of AH05G10280.1. AH02G05760.1, AH10G30320.1, AH10G15550.2, AH09G34870.1, AH03G47110.1, AH08G21540.1, and AH11G09070.1 showed firstly an increased expression and then decreased trends after Al treatment in both cvs. ZH2 and 99-1507. The expressions of AH04G29400.1, AH00G02780.1, AH07G25390.1, and AH08G18800.1 decreased after an increase in cv. ZH2, having an opposite trend in cv. 99-1507. AH03G41730.1, AH04G27510.1, AH04G19690.1, AH08G07150.1, AH08G03590.1, AH18G11510.1, and AH04G22870.1 showed a decreased expression after 8 h of Al treatment and increased again in cv. ZH2, but it showed converse changes in cv. 99-1507. All the results indicated that different members might have a different role under Al stress in peanut.

Discussion

Plants have abundant Trx members localized in different subcellular compartments such as chloroplast, cytosol, mitochondria, and nucleus (Gelhaye *et al*. 2005). The Trx system is very complex in plants, in which f, h, and o types are closer to eukaryotic Trx evolution. However, m, x, and y types are more closely related to prokaryotic sequences (Meyer *et al*. 1999). Twenty *Trx* genes and about 30 Trx-like proteins (including TDX, CDSP32, and NTRC proteins) were found in *Arabidopsis* (Meyer *et al*. 2009, 2012), 61 in *Oryza sativa* (Nuruzzaman *et al*. 2012), and 45 in *Populus trichocarpa* (Chibani *et al*. 2009). Based on the *Pfam*, OsTrxs proteins were classified into 6 subfamilies, named Trx-A, Trx-B, Trx-TPR_1_2, Trx-ERp29, Trx-FeThRed_A, Trx-O (Nuruzzaman *et al*. 2012). In *Populus*, according to the sequences alignment, the Trx family is divided into 15 groups (Chibani *et al*. 2009). The classification method of the peanut Trx family is similar to that of *Populus*, and a total of 70 peanut AhTrxs are identified and classified into 9 groups based on the evolutionary relationship with AtTrx proteins and conserved motif. Hence, the total number of Trx families is variable, possibly due to many typical Trxs in different plants. But the typical Trxs with a single domain and a WCGPC or WCPPC active site are still relatively conserved in phylogenetic tree analysis, indicating similar functions.

AtTrxs were found on 5 chromosomes of *Arabidopsis*, Chr1 has the biggest number. Maybe due to the limitations of incomplete sequencing and assembly methods, AH00G02780.1 was not assembled into the chromosomes in the peanut, however, other *AhTrx* genes appeared clustered and scattered on 20 chromosomes. At the same time, we detected tandem repeats at two loci on chromosome 15, AH15G12630.1 and AH15G12640.1, indicating that tandem repeats played a role in the expansion of the gene family. In peanut, h type is still the largest group with 20 members, among them, AH02G05760.1, AH04G22870.1, AH14G26310.1, and AH12G06680.1 were closer to AtTrxh9 (AT3G08710.1) including the same number and type of motifs. About the function of AtTrx 9, it was reported that $AtTrxh9_{red}$ could interact with $AtGPX3_{ox}$ to act as an electron donor for

regulating reactive oxygen species (Kuang *et al*. 2020). AtTrxh9 was also found to be associated with the plasma membrane and move from cell to cell (Meng *et al*. 2010). It was predicted that AH02G05760.1, AH04G22870.1, AH14G26310.1, and AH12G06680.1 possess the same function as AtTrxh9. In *Arabidopsis*, h1, h3, h4, and h5 are classified into hII subgroup, h2, h7, and h8 are the hI subgroup, CXXS1 and CXXS2 are in the hIII subgroup (Meyer *et al*. 2012). In evolutionary tree analysis, a total of 8 members (AH03G41730.1, AH05G10280.1, AH05G10310.1, AH13G44380.1, AH15G12630.1, AH15G12640.1, AH14G34300.1, and AH04G29400.1) are closely related to hI subgroup, likely associated with seed germination, photosynthesis, redox regulation (Da *et al*. 2020), GSH depletion (Schnaubelt *et al*. 2015), and salt tolerance (Ji *et al*. 2020). AH08G00920.1 and AH06G12710.1 are clustered in the hII subgroup, most likely with the same roles, for example, molecular chaperone, plant immune response, and redox-imbalance control (Traverso *et al*. 2007). The sequence length and motif of the h group were the same between *Arabidopsis* and peanut, except for AH04G22870.1 and AH14G26310.1 with longer sequences. Although the sequence length difference was small, the hII subgroup was more variable in intron and CDS. Here, expressions of 11 h group members were detected in root tips under Al stress, in which the expression of 7 genes had significant changes. Hence, AH08G00920.1, AH05G10280.1, AH13G44380.1, AH15G12630.1, AH04G29400.1, AH20G02130.1, and AH18G11510.1 may play a great role in redox-imbalance control induced by Al stress.

According to the studies on *Trx* genes with known functions in *Arabidopsis*, the CDSP32 group including AH06G13580.1, AH16G00200.1, and AH16G00200.2, contain the same motif and similar gene structure as AtCDSP32 (AT1G76080.1). The group is involved in response to the drought and oxidative stress (Broin *et al*. 2000) and the protection of photosynthetic apparatus (Broin *et al*. 2002). The f group consists of AH04G27510.1 and AH14G32340.1, which may be associated with the redox regulation of enzymes in the Calvin-Benson cycle. The ACHT group contains AH14G22540.1, AH04G19690.1, AH12G09250.1, AH08G03590.1, AH08G03630.1, and so on, and localizes on different subcellular compartments predictably. This subfamily may be similar to AtACTH4 (AT1G08570.1) and is associated with the starch synthesis pathway. The ABA-responsive elements were also distributed in the promoter of AH14G22540.1, AH04G19690.1, AH12G09250.1. The expression of AH04G19690.1 also fluctuated with Al treatment in cv. 99-1507, which might be caused by ABA changes during Al stress (Hou *et al*. 2010). The fourth subfamily may be associated with the WCRKC proteins, which are involved in flowering regulation in upland cotton (Li *et al*. 2018). The HCF164 group contains AT4G37200.1, which is involved in the biogenesis of the plastid cytochrome *b* (Lennartz *et al*. 2001). The eighth subfamily is the same as Trx z (AT3G06730.1) and Trx y in motif and structure. Trx z was related to the chloroplast redox signalling pathway and autotrophic growth of *Arabidopsis* (Wimmelbacher *et al*. 2014). The function of the members may be similar to that of AT1G43560.1, which was related to protein repair mechanisms (Laugier *et al*. 2013). In peanut, the x group is formed by 8 members, including AT1G50320.1 and 7 members, meaning that it had more functions and complexity in peanut. Although the motif was similar, the UTR existed among CDS or not in peanut. Little research has reported that AtTrx x is modulated by C-2-Cys peroxiredoxin in chloroplasts (Ojeda *et al*. 2018). Peanut x subfamily may be the same as AtTrx x. Motif and gene structure were the same among 8 AhTrx m members and 4 AtTrx m members. The m group might be similar to that of AtTrx m proteins, which might act as redox regulators of photosynthetic metabolism.

In the subcellular localization of peanut Trx protein sequences, it was predicted that these genes were scattered in various subcellular compartments, indicating diversity in function. Diversity in function might be also reflected in the diversity of gene structure and sequence length. With a different time of Al treatment, the expression of some members was evidently changeable, particularly in h, ACHT, and WCRKC groups. These members might be associated with Al stress in peanut and could be important in Al responsive mechanisms. Although TTL members have not been studied with bioinformatics in this study, it has been previously reported that they have obvious changes in the detection of expression, and it must be a prospect in future research.

Conclusions

This study identified and characterized the Trx family in peanut, and 70 members were found. All members could be classified into 9 groups with corresponding conserved motifs and phylogenetic relationship. The gene structure, basic physicochemical properties, chromosomal localization, and promoter of *AhTrxs* candidates were further analyzed. Expression analysis of 35 *AhTrx* members under different Al treatment times showed that they can respond to Al stress and may have different effects on the physiology of two peanut cultivars. In brief, this study provides knowledge on the genetic background and molecular base of Trxs in peanut under Al stress.

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