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**Research Article**

# Identification of Two Enzymes for Trehalose Synthesis and Their Potential Function in Growth and Development in Peanut (*Arachis hypogaea*)

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**ABSTRACT**

Plant trehalose has been regarded to play a key role in various biological processes during the growth and development stages. Trehalose-6-phosphate synthase (TPS) and trehalose-6-phosphate phosphatase (TPP) are two important enzymes for the synthesis of plant trehalose. Up till now, the *TPS* and *TPP* gene families have been identified and characterized in numerous higher plant species, but are rarely recorded in peanuts (*Arachis hypogaea*). In this study, a comprehensive search was performed to identify all putative TPS and TPP proteins in the peanut genome using *Arabidopsis* TPS and TPP proteins as queries. We then analyzed the characteristics of TPS and TPP members, including physic-chemical parameters, subcellular localization, phylogeny relationships, gene duplication, and expression patterns by various computational tools. As a result, a total of 17 *ArahyTPS* and 15 *ArahyTPP* genes were identified and annotated in the peanut genome, which was expanded by segmental duplication events. Our Neighbor-Joining based phylogenetic tree indicated that the ArahyTPS and ArahyTPP proteins could be categorized into three and two major branches. Gene structures and protein features analysis exhibited that the ArahyTPS and ArahyTPP proteins shared high structural and functional similarities. Based on previous RNA-Seq datasets, a majority of the *ArahyTPS* and *ArahyTPP* genes were found to specifically express in at least one major organ/tissue during the growth and development. This work will not only lead to a solid foundation on reveal the potential roles of *ArahyTPS* and *ArahyTPP* gene families in peanuts but also provide evidence to related trehalose research in other higher plant species.

*Keywords:* Gene identification, peanut (*Arachis hypogaea*), trehalose-6-phosphate synthase, trehalose-6-phosphate phosphatase,

## Introduction

Peanut, scientifically known as *Arachis hypogaea* is a legume crop that has been cultivated for over 3,500 years, with its origin traced back to South America [1]. Due to its high protein content, oil, and other nutrients, peanuts have been used as a valuable source of energy and nutrition [2]. In addition to being a popular food item, peanuts have been thought to apply in various industrial applications, such as animal feed, fuel, and organic fertilizer [3, 4]. However, climate change, like changes in temperature, rainfall patterns, and extreme weather events poses a significant risk to peanut cultivation [5]. Consequently, it would be important to develop adaptation strategies that can help safeguard the future of peanut cultivation.

Trehalose is a disaccharide that is widely dis-

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tributed in higher plant species, playing important roles in protecting cellular membranes and proteins from adverse environmental conditions, such as drought, extreme temperatures, and high salinity, and in regulating the growth and development of plants [6, 7]. Trehalose biosynthesis in plants is primarily mediated by two enzymes, including trehalose-6-phosphate synthase (TPS) and trehalose-6-phosphate phosphatase (TPP) [6, 8, 9]. TPS converts uridine diphosphate-glucose and glucose 6-phosphate to trehalose-6-phosphate, which is then dephosphorylated by TPP to form trehalose. More specifically, TPS in plant species consists of two domains, the N-terminal and C-terminal domains, which specifically bind to the nucleotide cofactor UDP-glucose and glucose-6-phosphate, respectively [10]. TPP comprises a catalytic domain containing a central beta-sheet surrounded by alpha-helices and a regulatory domain that modulates enzyme activity [7]. So, the investigation of the potential genes involving in the trehalose pathway could be an emerging strategy to increase the cultivation and yields of crops. Recently, these two important enzyme families have been identified and well-characterized in various higher plant species, like model plant *Arabidopsis thaliana* [11], rice (*Oryza sativa*) [12, 13], maize (*Zea mays*) [14], *Populus tomentosa* [15], *Medicago truncatula* [16], rapeseed (*Brassica napus*) [17], wheat (*Triticum aestivum*) [18], cotton (*Gossypium* spp.) [19], sweet orange (*Citrus sinensis*) [20], and tomato (*Solanum lycopersicum*) [21]. Unfortunately, there is no available information for the TPS and TPP families in peanut.

Therefore, the present study aimed to identify *TPS* and *TPP* genes in the peanut genome using bioinformatics tools. To get insight into the evolution of these two gene families in peanuts, the gene annotation, gene duplication, gene structure, and phylogeny relationship were initially analyzed. We also carried out a protein feature and subcellular localization analysis of the TPS and TPP proteins in peanut. Finally, the expression profiles of the *TPS* and *TPP* genes in major organs/tissues of peanut plants during the growth and development processes were explored.

## Material and Methods

### *Screening of TPSs and TPPs in peanut*

Well-characterized Arabidopsis TPS and TPP proteins collected from the previous study [11] were used to perform a TBLASTN search against the current assembly of peanuts (BioProject: PRJNA419393) [22] available from the Phytozome database [23]. All protein sequences, with ≥ 100 amino acid (aa) residues were then validated by the ClustalX tool [24] and the Pfam database [25]. The annotations, including coding DNA sequence, genomic DNA sequence, chromosomal distribution, GeneID, and ProteinID were consequently collected from these databases.

### *Sequence analysis of TPSs and TPPs in peanut*

All peanut TPS and TPP protein sequences were subjected to the ExPASy tool [26] following the previous studies [27-30]. We collected the basic physic-chemical properties of each protein, including protein length, molecular weight (mW), theoretical isoelectric point (pI), instability index (II), and total average hydrophilicity [26]. Next, the gene structure of the peanut *TPS* and *TPP* gene families was depicted with the Gene Structure Display Server [31] using both coding DNA sequence and genomic DNA sequence as previously described [27].

### *Phylogenetic analysis of TPSs and TPPs in peanut*

The full-length TPS and TPP protein sequences in peanuts were initially used to generate the phylogenetic tree as previously described [27-29]. Particularly, we used ClustalX software, with the default parameters to perform multiple sequence alignment of all TPS and TPP proteins of peanut [24, 32]. The Neighbor-Joining algorithm of Molecular Evolutionary Genetics Analysis (MEGA X version) software was used to construct two unrooted phylogenetic trees of the TPS and TPP proteins in peanuts with 1000 bootstrap replications [33].

### *Subcellular localization of TPPs and TPPs in peanut*

The full-length TPS and TPP protein sequences in peanut were used to query against the Yloc database [34] as previously described [27-29]. Particularly, the organelle-specific signal peptide has been searched in each TPS and TPP protein sequence to predict the location (nucleus, cytoplasm, mitochondrion, plasma membrane, extracellular space, endoplasmic reticulum, peroxisome, Golgi apparatus, vacuole and chloroplast for the plant model) [34].

### *Gene duplication of TPSs and TPPs in peanut*

To predict the duplication events that occurred in the peanut *TPS* and *TPP* gene families, the corresponding coding DNA sequences of all peanut *TPS* and *TPP* genes were used as previously described [27]. Briefly, the similarity between all genes was calculated by using the ClustalX [24] and BioEDIT tools [35]. A duplicated pair was defined as at least two genes sharing a similarity of ≥ 70% [27]. Then, ratios between the non-synonymous substitutions per non-synonymous site (Ka) and synonymous substitutions per synonymous site (Ks) of the duplication events were counted by using DNAsp software [36] as previously reported [27]. The Ka/Ks value > 1 indicated positive selective pressure, whereas a ratio < 1 indicated the possibility of negative selective pressure [37].

### *Expression analysis of TPSs and TPPs in peanut*

To analyze the expression profiles of the peanut *TPS* and *TPP* genes during the growth and development, we re-analyzed the previous transcriptome atlas available in the Gene Expression Omnibus (GEO) NCBI [38] and PeanutBase tools [39]. Briefly, an expression atlas for major organs of the peanut plant (GEO accession: GSE71357) [40] in the Peanutbase [39] has been explored to assess the expression of the peanut *TPS* and *TPP* genes in 10 tissues, including lateral stem leaf, mainstem leaf, seedling leaf, vegetative shoot tip, reproductive shoot tip, root, nodule, perianth, stamen and pistil [40].

## Results and Discussion

### *Genome survey of the TPS and TPP families in peanut*

To survey all putative members in the TPS and TPP families, well-characterized Arabidopsis TPS and TPP proteins [11] were used to seek against the current assembly of peanuts (BioProject: PRJNA419393) [22] After validation by ClustalX tool [24] and the Pfam database [25], a total of 16 and 17 genes of the *TPP* and *TPS* families were identified from the peanut genome database (Table S1 and Table S2). According to the chromosomal positioning, the peanut *TPP* gene family was named from *ArahyTPP01* to *ArahyTPP15* (Table 1, Figure 1), while the peanut *TPS* gene family was named from *ArahyTPS01* to *ArahyTPS17* (Table 2, Figure 1). Consequently, the annotation, like GeneID, ProteinID, and chromosomal distribution of the *ArahyTPP* and *ArahyTPS* gene families were provided in Table 1, Table ,2 and Figure 1, respectively.

We found that all 17 *ArahyTPS* genes were unevenly distributed across 10 out of 20 chromosomes from the peanut genome (Figure 1). The amount of *ArahyTPS* genes per chromosome ranged from zero to four (Figure 1). Particularly, chromosomes Arahy.01, Arahy.05, Arahy.07, Arahy.09 Arahy.18 and Arahy.19 contained only one *ArahyTPS* member each, while four members of the *ArahyTPS* gene family, like *Arahy,TPS02*, *ArahyTPS03*, *ArahyTPS04,* and *ArahyTPS05* were realized to map onto chromosome Arahy.03 (Figure 1). Chromosomes Arahy.11, Arahy.13 and

Table 1. Attributes of the ArahyTPP protein family in peanut

| Gene name | GeneID | ProteinID | L | mW | pI | II | GRAVY | SL |
|---|---|---|---|---|---|---|---|---|
| *ArahyTPP01* | arahy.Tifrunner.gnm1.ann1.FJ2B1F | XP_025689815.1 | 355 | 39.95 | 9.22 | 31.75 | -0.39 | C |
| *ArahyTPP02* | arahy.Tifrunner.gnm1.ann1.51Y4LP | XP_025699131.1 | 386 | 43.29 | 6.22 | 51.52 | -0.38 | C |
| *ArahyTPP03* | arahy.Tifrunner.gnm1.ann1.J3QXZL | XP_025610286.1 | 355 | 40.18 | 9.36 | 35.32 | -0.36 | G |
| *ArahyTPP04* | arahy.Tifrunner.gnm1.ann1.0FY2NM | XP_025612766.1 | 373 | 42.40 | 9.22 | 43.72 | -0.33 | N |
| *ArahyTPP05* | arahy.Tifrunner.gnm1.ann1.B1753N | XP_025613103.1 | 309 | 34.89 | 9.07 | 32.31 | -0.35 | C |
| *ArahyTPP06* | arahy.Tifrunner.gnm1.ann1.SS1VDK | XP_025622743.1 | 391 | 43.91 | 9.04 | 32.28 | -0.35 | P |
| *ArahyTPP07* | arahy.Tifrunner.gnm1.ann1.8AAC5A | XP_025635806.1 | 273 | 31.18 | 5.70 | 43.83 | -0.15 | PM |
| *ArahyTPP08* | arahy.Tifrunner.gnm1.ann1.U769C2 | XP_025631680.2 | 330 | 37.69 | 5.39 | 38.15 | -0.35 | N |
| *ArahyTPP09* | arahy.Tifrunner.gnm1.ann1.FIK9JS | XP_029147297.1 | 388 | 43.21 | 6.57 | 35.26 | -0.27 | C |
| *ArahyTPP10* | arahy.Tifrunner.gnm1.ann1.5Q8BGE | XP_025653685.1 | 375 | 42.05 | 6.03 | 53.16 | -0.41 | C |
| *ArahyTPP11* | arahy.Tifrunner.gnm1.ann1.KW1U5A | XP_025668572.1 | 327 | 36.74 | 9.18 | 31.83 | -0.38 | C |
| *ArahyTPP12* | arahy.Tifrunner.gnm1.ann1.DP0G5T | XP_025669102.1 | 344 | 38.61 | 9.45 | 35.48 | -0.28 | N |
| *ArahyTPP13* | arahy.Tifrunner.gnm1.ann1.G2WE73 | XP_025668338.1 | 373 | 42.43 | 9.33 | 43.72 | -0.34 | G |
| *ArahyTPP14* | arahy.Tifrunner.gnm1.ann1.EKX8HF | XP_025684034.1 | 212 | 23.49 | 5.09 | 49.47 | -0.43 | C |
| *ArahyTPP15* | arahy.Tifrunner.gnm1.ann1.S15BQI | XP_025684774.1 | 374 | 41.95 | 9.12 | 30.90 | -0.47 | C |

Notes : L - length (aa residues), mW - molecular weight (kDa), pI - theoretical isoelectric point, II - instability index, GRAVY - total average hydrophilicity, SL - subcellular localization, C - cytoplasm, G - Golgi apparatus, N - nucleus. PM - plasma membrane, P - peroxisome

Table 2. Attributes of the ArahyTPS protein family in peanut

| Gene name | GeneID | ProteinID | L | mW | pI | II | GRAVY | SL |
|---|---|---|---|---|---|---|---|---|
| *ArahyTPS01* | arahy.Tifrunner.gnm1.ann1.G88L7W | XP_025607456.1 | 849 | 96.03 | 5.81 | 50.43 | -0.23 | C |
| *ArahyTPS02* | arahy.Tifrunner.gnm1.ann1.FP7P7G | XP_025690899.1 | 853 | 96.62 | 5.77 | 45.26 | -0.19 | C |
| *ArahyTPS03* | arahy.Tifrunner.gnm1.ann1.RIH8EG | XP_025687873.1 | 1003 | 113.23 | 6.22 | 54.36 | -0.27 | C |
| *ArahyTPS04* | arahy.Tifrunner.gnm1.ann1.CM68RF | XP_025690577.1 | 862 | 97.16 | 5.79 | 45.54 | -0.18 | C |
| *ArahyTPS05* | arahy.Tifrunner.gnm1.ann1.S5S9QI | XP_025640344.1 | 1022 | 116.17 | 8.31 | 40.76 | -0.30 | C |
| *ArahyTPS06* | arahy.Tifrunner.gnm1.ann1.PWAA2D | XP_025701609.1 | 829 | 94.05 | 5.82 | 48.55 | -0.24 | C |
| *ArahyTPS07* | arahy.Tifrunner.gnm1.ann1.E48PAY | XP_025610784.1 | 927 | 104.66 | 7.01 | 42.14 | -0.38 | C |
| *ArahyTPS08* | arahy.Tifrunner.gnm1.ann1.BL0RCS | XP_025620614.1 | 847 | 95.87 | 5.60 | 50.77 | -0.22 | C |
| *ArahyTPS09* | arahy.Tifrunner.gnm1.ann1.A0DAR9 | XP_025629152.1 | 848 | 96.61 | 5.91 | 48.57 | -0.22 | C |
| *ArahyTPS10* | arahy.Tifrunner.gnm1.ann1.1XW75L | XP_025630685.1 | 849 | 96.00 | 5.81 | 50.33 | -0.22 | C |
| *ArahyTPS11* | arahy.Tifrunner.gnm1.ann1.Q3GMD8 | XP_025639207.1 | 854 | 96.68 | 5.77 | 44.44 | -0.19 | C |
| *ArahyTPS12* | arahy.Tifrunner.gnm1.ann1.FTDL1P | XP_025643677.1 | 973 | 109.32 | 5.81 | 54.51 | -0.26 | C |
| *ArahyTPS13* | arahy.Tifrunner.gnm1.ann1.IE8W25 | XP_025638855.1 | 862 | 97.11 | 5.88 | 45.44 | -0.19 | C |
| *ArahyTPS14* | arahy.Tifrunner.gnm1.ann1.AKPI0I | XP_025654324.1 | 860 | 97.00 | 6.11 | 46.19 | -0.17 | C |
| *ArahyTPS15* | arahy.Tifrunner.gnm1.ann1.FZMU71 | XP_025655292.1 | 869 | 98.14 | 5.73 | 48.37 | -0.22 | C |
| *ArahyTPS16* | arahy.Tifrunner.gnm1.ann1.YY1VWG | XP_025673124.1 | 916 | 103.01 | 7.36 | 42.42 | -0.35 | C |
| *ArahyTPS17* | arahy.Tifrunner.gnm1.ann1.9VZ5EJ | XP_025675145.1 | 855 | 96.70 | 5.69 | 50.25 | -0.20 | C |

Notes : L - length (aa residues), mW - molecular weight (kDa), pI - theoretical isoelectric point, II - instability index, GRAVY - total average hydrophilicity, SL - subcellular localization, C - cytoplasm

Arahy.15 had two (*ArahyTPS09* and *ArahyTPS10*), three (*ArahyTPS11*, *ArahyTPS12* and *ArahyTPS13*) and two (*ArahyTPS14* and *ArahyTPS15*) genes, respectively (Figure 1). Additionally, 10 (out of 20) chromosomes, like Arahy.02, Arahy.04, Arahy.06, Arahy.08, Arahy.10, Arahy.12, Arahy.14, Arahy.16, Arahy.17, and Arahy.20 did not contain any *ArahyTPS* gene (Figure 1). Similarly, 15 members of the *ArahyTPP* gene family were found in 10 (out of 20) peanut chromosomes (Figure 1). Three chromosomes, like Arahy.08, Arahy.12 and Arahy.20 contained two *ArahyTPP* genes each, while six chromosomes, like Arahy.03, Arahy.05, Arahy.07, Arahy.10, Arahy.13, and Arahy.15 contained one *ArahyTPP* gene each (Figure 1). Three remaining *ArahyTPP* genes, including *ArahyTPP12*, *ArahyTPP13*, and *ArahyTPP14* were found to localize in chromosome Arahy.17 (Figure 1). It has been demonstrated that there was no significant correlation between chromosomal size and the amount of the *ArahyTPS* and *ArahyTPP* genes.

Previously, TPP and TPS families have been identified and annotated in multiple higher plant species. For example, 10 and 12 members of the TPP families have been found in *Arabidopsis* and rice [11, 12]. A total of 10 and 13 members of the *TPP* and *TPS* genes were identified in the *Populus* genome, respectively [15]. Recently, a total of 31 genes has been recorded in the *TPP* family in wheat [18]. In cotton, 12, 17, 24 and 26 members have been reported in the *TPP* families in *G. raimondii*, *G. arboreum*, *G. hirsutum* and *G. barbadense*, respectively [19]. Eight *TPS* genes were found to be randomly distributed in sweet orange chromosomes [20], while 11 rice *TPS* genes encoding TPS proteins have been recorded [13]. Meanwhile, 11 and 26 *TPS* genes have been found in *M. truncatula* and *B. napus* [16, 17]. Taken together, our comparisons indicated that the *TPS* and *TPP* gene families in peanuts, perhaps in other plant species were multi-gene families.

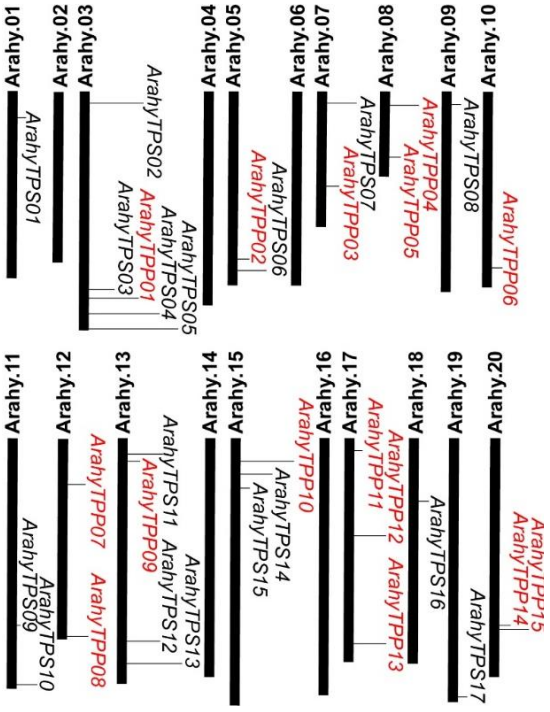


Figure 1. Physical localization of the ArahyTPS and ArahyTPP gene families in the peanut genome.

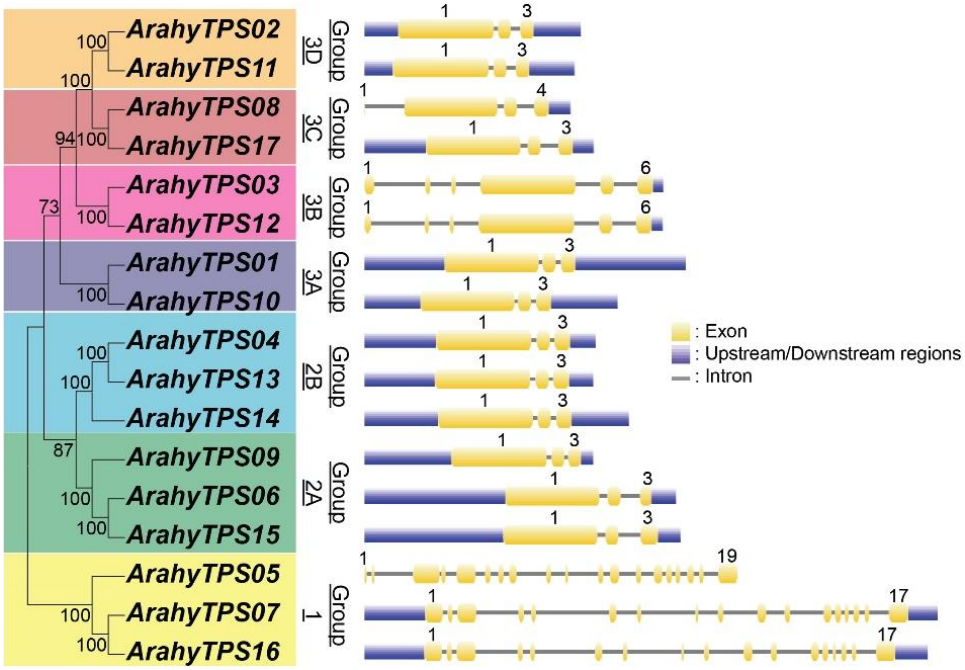


Figure 2. The phylogenetic tree and gene structure of the ArahyTPS family in peanut

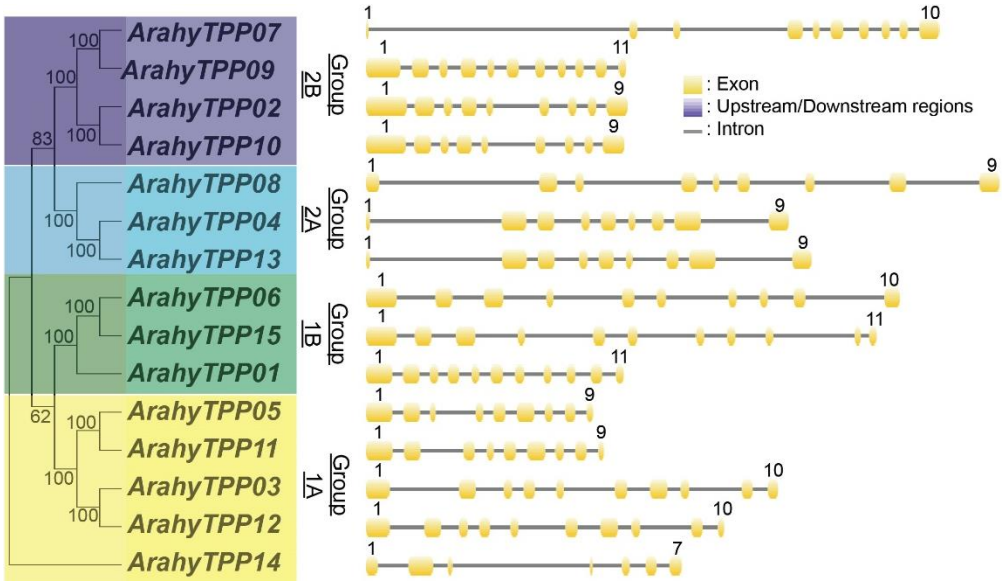


Figure 3. The phylogenetic tree and gene structure of the ArahyTPP family in peanut

***Analysis of basic physic-chemical properties of the TPS and TPP families in peanut***

To investigate the general features of the ArahyTPS and ArahyTPP proteins in peanuts, the ExPASy tool was used to analyze the full-length protein sequence of each member [26]. As a result, the aa sizes of the ArahyTPP proteins ranged from 212 (ArahyTPP14) and 391 aa residues (ArahyTPP06) (Table 1). The mass values of the ArahyTPP proteins were found to be varied from 23.49 (ArahyTPP14) to 43.91 kDa (ArahyTPP06) (Table 1). The pI scores of six (out of 15) ArahyTPP proteins, like ArahyTPP02, ArahyTPP07, ArahyTPP08, ArahyTPP09, ArahyTPP10, and ArahyTPP14 were < 7 (acidic), ranging from 5.09 (ArahyTPP14) to 6.57 (ArahyTPP09), while nine remaining ArahyTPP proteins were base (pI > 7), ranging from 9.04 (ArahyTPP06) to 9.45 (ArahyTPP12) (Table 1). Next, the II values of the ArahyTPP proteins were calculated to range from 30.90 (ArahyTPP15) to 53.16 (ArahyTPP10), respectively (Table 1).

Interestingly, the whole 15 members of the ArahyTPP family were exhibited to be hydrophilic proteins as the GRAVY scores were minus, ranging from -0.15 (ArahyTPP07) to -0.47 (ArahyTPP15) (Table 1). Similarly, the aa lengths of the ArahyTPS family varied from 829 (ArahyTPS06) to 1022 residues (ArahyTPS05) (Table 2). The mW of the ArahyTPS family ranged from 94.05 (ArahyTPS06) to 116.17 kDa (ArahyTPS05), and the average mW was 100.26 kDa (Table 2). The pI of the ArahyTPS family was from acid (pI = 5.60, ArahyTPS08) to alkaline (pI = 8.31, ArahyTPS05). Among them, a large number of members (14 out of 17) in the ArahyTPS family exhibited to be acidic (pI < 7.00), whereas only three ArahyTPS members, like ArahyTPS05, ArahyTPS07, and ArahyTPS16 were alkaline (Table 2). Next, the II scores of the ArahyTPS family were found to be more than 40.00, ranging from 40.76 (ArahyTPS05) to 54.51 (ArahyTPS12) (Table 2). Finally, the GRAVY score of the whole 17 members of the ArahyTPS family was < 0 (Table 2), indicating that these proteins were considered to be relatively hydrophilic.

Previously, the protein sizes of the TPP families in four cotton species varied from 134 to 422 amino acid residues, the average mass was 39.54 kDa, while the average pI score of these proteins was 8.41 [19]. In the case of wheat, the TPP proteins were reported to range from 249 to 584 amino acid residues in size, with an average of 386 amino acid residues, while their computed mW varied from 28.66 to 96.02 kDa [18]. Next, the theoretical pI score of the TPP proteins in wheat also ranged from acid (pI = 5.53) to alkaline (pI = 9.26) [18]. The full length of the TPS in *P. tomentosa* was counted to be 846 - 922 aa residues, while their II values varied from 84.55 to 90.46 [15]. The *P. tomentosa* TPP proteins were shorter than *P. tomentosa* TPS proteins, from 235 to 387 aa residues, and the II scores were from 75.10 to 83.08 [15]. All *P. tomentosa* TPS and TPP proteins were also relatively hydrophilic [15].

### *Phylogenetic analysis and prediction of the gene duplication in the TPS and TPP families in peanut*

To assess the relationship in the ArahyTPS and ArahyTPP families of peanuts, unrooted Neighbor-Joining phylogenetic trees of full-length protein sequences of whole 17 and 15 members were constructed, respectively. As provided in Figure 2, we found that the ArahyTPS family in peanuts could be categorized into seven different groups, namely group 1 to group 3D. Particularly, group 1 contained three ArahyTPS proteins, like ArahyTPS05, ArahyTPS07, and ArahyTPS16 (Figure 2). Group 2 had six members of the ArahyTPS family, of which were separated into two sub-groups, like group 2A (ArahyTPS06, ArahyTPS09 and ArahyTPS15) and group 2B (ArahyTPS04, ArahyTPS13 and ArahyTPS14) (Figure 2). Next, a total of four sub-groups has been recorded in group C, which exhibited the presence of eight members of the ArahyTPS family, including ArahyTPS01 and ArahyTPS10 (group 3A), ArahyTPS03 and ArahyTPS12 (group 3B), ArahyTPS08 and ArahyTPS17 (group 3C), and ArahyTPS02 and ArahyTPS11 (group 3D) (Figure 2). In the case of the ArahyTPP protein family, all 15 members could be clustered into two large groups, with four different clades (Figure 3). Among them, eight ArahyTPP members, including five (ArahyTPP03, ArahyTPP05, ArahyTPP11, ArahyTPP12, and ArahyTPP14) and three proteins (ArahyTPP01, ArahyTPP06, and ArahyTPP15) were in group 1A and 1B, respectively (Figure 3). Group 2A contained three ArahyTPP members, like ArahyTPP04, ArahyTPP08 and ArahyTPP13, while group 2B had four ArahyTPP proteins, including ArahyTPP02, ArahyTPP07, ArahyTPP09 and ArahyTPP10 (Figure 3). Our classification of the ArahyTPS and ArahyTPP families in peanuts was confirmed by previous reports. For example, an unrooted phylogenetic tree of 86 members of the TPP families in wheat, maize, rice, *Brachypodium distachyon*, *Arabidopsis*, and poplar demonstrated that these proteins could be classified into seven sub-families [18]. Recently, an unrooted phylogenetic tree of the TPP families in *Arabidopsis* and cotton was also constructed and divided these proteins into three major clades [19]. The phenomenon was also recorded in the phylogenetic tree of the *Populus* TPP proteins [15]. Recently, an unrooted phylogenetic tree of all TPS proteins in *P. trichocarpa*, rice, Arabidopsis, soybean, and *M. domestica* has been made and revealed the categorization as similar to the peanut TPS family [15]. Taken together, our comparisons strongly demonstrated that the TPS and TPP families in peanuts, perhaps in other higher plant species could be classified into three and two main groups, respectively.

Table 3. Duplication events found in the ArahyTPS gene family in peanut

| # | Duplicated pairs | Chromosome location | Similarity | Ka/Ks ratio | Mechanism |
|---|---|---|---|---|---|
| 1 | *ArahyTPS01/10* | Arahy.01/11 | 98.9 | 0.12 | Segmental duplication |
| 2 | *ArahyTPS02/11* | Arahy.01/13 | 98.9 | 0.31 | Segmental duplication |
| 3 | *ArahyTPS03/12* | Arahy.03/13 | 96.0 | 0.27 | Segmental duplication |
| 4 | *ArahyTPS04/13/14* | Arahy.03/13/15 | 99.2/84.3/84.2 | 0.29/0.31/0.30 | Segmental duplication |
| 5 | *ArahyTPS06/09/15* | Arahy.05/11/15 | 70.6/73.3/94.2 | 0.35/0.38/0.42 | Segmental duplication |
| 6 | *ArahyTPS07/16* | Arahy.07/18 | 97.8 | 0.50 | Segmental duplication |
| 7 | *ArahyTPS08/17* | Arahy.09/19 | 97.8 | 0.23 | Segmental duplication |

Table 4. Duplication events found in the ArahyTPP gene family in peanut

| # | Duplicated pairs | Chromosome location | Similarity | Ka/Ks ratio | Mechanism |
|---|---|---|---|---|---|
| 1 | *ArahyTPP01/06/15* | Arahy.01/10/20 | 70.4/92.0 | 0.26/0.64 | Segmental duplication |
| 2 | *ArahyTPP02/10* | Arahy.05/15 | 95.3 | 0.12 | Segmental duplication |
| 3 | *ArahyTPP03/12* | Arahy.07/17 | 84.3 | 0.60 | Segmental duplication |
| 4 | *ArahyTPP04/13* | Arahy.08/17 | 98.4 | 0.19 | Segmental duplication |
| 5 | *ArahyTPP05/11* | Arahy.08/17 | 91.1 | 0.27 | Segmental duplication |

As a main part of this study, we predicted the duplication events that occurred in the *ArahyTPS* gene family in peanuts, which could significantly explain the evolution of this important gene family in higher plant species. According to the similarity, a total of seven duplication events have been detected throughout the *ArahyTPS* gene family in peanuts (Table 3). The similarity scores of these duplicated events varied from 70.6 (*ArahyTPS06* and *ArahyTPS09*) to 99.2% (*ArahyTPS04* and *ArahyTPS13*) (Table 3). Whole seven duplicated gene pairs were detected as segmental duplication genes (Table 3). Particularly, a duplicated pair of two genes, like *ArahyTPS01* and *ArahyTPS10* was localized in chromosomes Arahy.01 and Arahy.11, while two duplicated pairs of two genes, including *ArahyTPS02* (localizing in the chromosome Arahy.01) and *ArahyTPS11* (localizing in the chromosome Arahy.13), and *ArahyTPS03* (localizing in the chromosome Arahy.03) and *ArahyTPS12* (localizing in the chromosome Arahy.13) were believed to distribute in the different chromosomes (Table 3). Next, two duplicated pairs of three genes, including a pair of *ArahyTPS04* (localizing in the chromosome Arahy.03), *ArahyTPS13* (localizing in the chromosome Arahy.13) and *ArahyTPS14* (localizing in the chromosome Arahy.15), and a pair of *ArahyTPS06* (localizing in the chromosome Arahy.05), *ArahyTPS09* (localizing in the chromosome Arahy.11) and *ArahyTPS15* (localizing in the chromosome Arahy.15) were also reported to be segmental duplications (Table 3). Two remaining duplicated pairs, including *ArahyTPS07* and *ArahyTPS16*, and *ArahyTPS08* and *ArahyTPS17* were initially localized in the chromosomes Arahy.07 and Arahy.18, and Arahy.09 and Arahy.19, respectively (Table 3). Additionally, the Ka/Ks scores of the whole seven duplication events were < 1 (Table 3), strongly suggesting that the repetitive *ArahyTPS* genes in peanuts were mainly constrained by purification selection pressure. This phenomenon was also observed in the *ArahyTPP* gene family in peanut. Briefly, a total of five duplication events of 11 *ArahyTPP* genes was predicted to be occurred from different peanut chromosomes (Table 4). The similarities of these duplicated *ArahyTPP* genes were varied from 70.4 (*ArahyTPP01* and *ArahyTPP06*) to 98.4% (*ArahyTPP04* and *ArahyTPP13*) (Table 4). The Ka/Ks values of whole five duplication events were minus (Table 4). Previously, nine tandemly duplication events have been reported in the *TPP* gene family in wheat [18]. Three segmental duplication events that occurred in the rice *TPP* gene family were recorded [12]. Taken together, our prediction hypothesized that the segmental duplication events played a key role in the expansion of the *ArahyTPS* and *ArahyTPP* gene families in peanuts.

### *Subcellular localization and gene structure of the TPS and TPP families in peanut*

The subcellular localization of each protein molecule might suggest its potential role in the

cell. Thus, we performed a prediction of the distribution of the ArahyTPS proteins in the cell by using the Yloc tool [34]. As expected, a large number (eight out of 15) of the ArahyTPP family was localized in the cytoplasm, while three, two, one and one ArahyTPP proteins were found in the nucleus, Golgi apparatus, peroxisome and plasma membrane (Table 1). Next, all 17 members of the ArahyTPS family in peanuts were localized in the cytoplasm (Table 2). Previously, the most of TPP proteins in four cotton species were located in chloroplasts [19]. For example, nine, three and two (out of 17) members of the TPP family in *G. arboreum* were found to be localized in the chloroplast, mitochondrion and endoplasmic reticulum, respectively, while cytoplasm, cell membrane, peroxisome, nucleus contained one TPP protein each [19]. Next, five, three and two (out of 12) TPP proteins in *G. raimondii* were found in the chloroplast, mitochondrion and endoplasmic reticulum, respectively [19]. Two remaining TPP proteins in *G. raimondii* were predicted to localize in cytoplasm and nucleus [19]. Most of the TPS and TPP proteins in *Populus* were predicted to localize to the chloroplast, cytoplasm and nucleus [15]. A total of 17 TPP proteins found in *G. hirsutum* and *G. barbadense* were found in the chloroplast, whereas the remaining TPP proteins in these two cotton species were localized in different organelles [19]. By using the green fluorescent protein assay, four wheat TPP proteins, including TaTPP6, TaTPP7, TaTPP9 and TaTPP11 were demonstrated to be located in both the nucleus and cytoplasm [18]. To sum up, the subcellular localization of the TPP proteins in peanuts, perhaps in other plant species illustrated their functional diversity and complexity.

Next, to better get insight into the genetic features of peanut *ArahyTPS* gene family, we analyzed the exon/intron organization of each member by using the well-known web-based tool [31] as previously described [27]. The gene structure of 17 members of the *ArahyTPS* gene family in peanuts was well-described in Figure 2. We found that the number difference of exons, from three to 19 was obvious for the *ArahyTPS* gene family (Figure 2). For example, ArahyTPS05 protein was encoded by 19 exons which shared the largest amount of exons, while two members of the *ArahyTPS* gene family, like *ArahyTPS07* and *ArahyTPS16* contained 17 exons (Figure 2). Interestingly, most of the *ArahyTPS* gene family, 11 out of 17 genes harbored three exons. Additionally, *ArahyTPS08* and two *ArahyTPS* genes belonging to group 3B (*ArahyTPS03* and *ArahyTPS12*) contained four and six exons, respectively (Figure 2). In the case of the *ArahyTPP* gene family, the number of exons was varied from seven to 11 (Figure 3). Particularly, seven (out of 15) *ArahyTPP* genes contained nine exons, while four and three (out of 15) had 10 and 11 exons, respectively (Figure 3). Only *ArahyTPP14* contained seven exons (Figure 3). In recent years, increasing studies have been investigated the gene structure of the *TPP* gene families in higher plant species. For example, the common motif found in the structure of rice *TPP* genes contained 10 and 11 exons [12]. Recently, the number of exons found in the *TPP* gene family in wheat was varied from five to 13 [18]. Among them, the majority of the *TPP* genes in wheat had nine (14 out of 31 members) and 10 exons (10 out of 31 members) [18]. In four cotton species, the gene structures of the *TPP* gene family were recorded to be complex [19]. Particularly, 53 (out of 79) *TPP* genes in four cotton species contain at least 10 exons [19]. The exon number of the *TPS* genes in rapeseed was greatly varied, ranging from three to 18 [17]. Here, our results revealed that the *ArahyTPP* and *ArahyTPS* genes in the same branch typically shared similar exon/intron organization and the presences of three and nine exons were the most common motif found in the *ArahyTPS* and *ArahyTPP* gene families in peanuts, respectively.

### *Analysis of the expression profiles of the TPS and TPP families in peanut*

In order to get insight into the function of the *ArahyTPS* and *ArahyTPP* genes in peanuts, we checked the expression profiles of these genes in major organs/tissues under several conditions. In this study, we re-analyzed two transcriptome datasets to assess the expression levels of the *ArahyTPS* and *ArahyTPP* genes. According to the public transcriptome dataset GSE71357 [40], the expression profiles of the *ArahyTPS* genes in 10 major organs were extracted and provided in Figure 4A. Our re-analysis revealed that whole 17 members of the *ArahyTPS* gene family were diverse with differing levels of tissue/organ specificity. The overall expression patterns of two (out of 17) *ArahyTPS* genes, including *ArahyTPS05* and *ArahyTPS09* in both 10 organs were under the limit of detection (Figure 4A). In contrast, all 15

remaining *ArahyTPS* genes were expressed at high levels in at least one (out of 10) major organ in peanut plants (Figure 4A). Among them, four *ArahyTPS* genes, including *ArahyTPS03*, *ArahyTPS06*, *ArahyTPS12* and *ArahyTPS15* were mainly detected in root and nodule tissues (Figure 4A). Four *ArahyTPS* genes, like *ArahyTPS07*, *ArahyTPS08*, *ArahyTPS16* and *ArahyTPS17* were highly expressed in reproductive shoot tip, root, nodule and pistil tissues (Figure 4A). We found that *ArahyTPS04* was specifically expressed in nodule and pistil, while *ArahyTPS13* was specific in the nodule, perianth and pistil (Figure 4A). Two *ArahyTPS* genes, like *ArahyTPS02* and *ArahyTPS11* were detected in only nodules, while *ArahyTPS01* and *ArahyTPS10* were highly expressed in both of four tissues, like root, nodule, perianth and pistil (Figure 4A). Finally, *ArahyTPS14* was specific in five organs, including the mainstem leaf, root, nodule, perianth and pistil (Figure 4A). In the case of the *ArahyTPP* gene family, *ArahyTPP05* was highly expressed in root tissues, while *ArahyTPP02* and *ArahyTPP15* was mostly found in perianth and nodule tissues, respectively (Figure 4B). Interestingly, *ArahyTPP01* was recorded to exclusively expressed in both of roots, nodules, perianths and stamen pistils, while *ArahyTPP09* was specific in lateral stem leaf, mainstem leaf, seedling leaf, vegetative shoot tip, vegetative shoot tip, root, nodule, perianth and stamen pistil tissues (Figure 4B). Expression profile of variation in expression of TPS and TPP genes in wheat which was wide changing in leaves, roots and reproductive tissue [41].

Trehalose 6-phosphate has been reported to regulate sucrose allocation which improves cereal yields [42]. Overexpressing *OsTPP1* gene improved yield of transgenic maize under different viabilities of water [43]. Additionally, *OsTPS8* enhanced salt stress tolerance by conferring suberin deposition in roots [44].

Previously, a loss-of-function mutant of *Ara-*

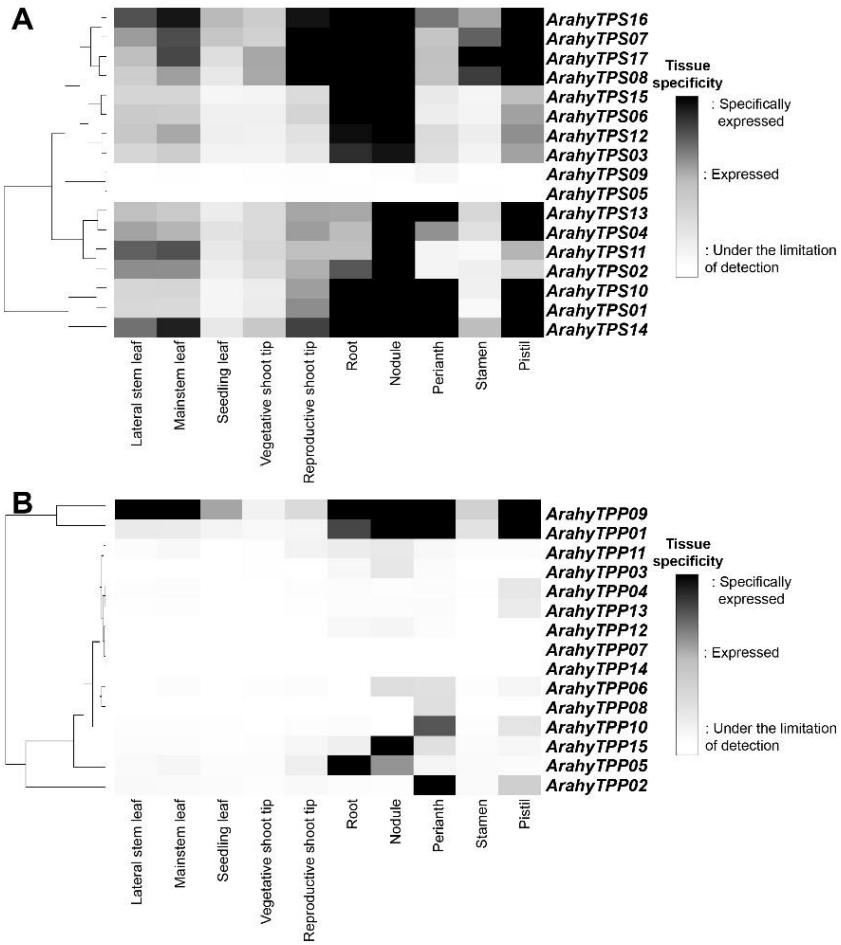


Figure 4. The expression patterns of the (A) ArahyTPS and (B) ArahyTPP gene families in 10 major organs/tissues during the growth and development of peanut plants.

*bidopsis AtTPPF* gene could result in a drought-sensitive phenotype, suggesting that *AtTPPF* gene regulated trehalose contents during drought stress [45]. Overexpression of *Arabidopsis AtTPPI* enhanced plant drought tolerance by regulating stomatal apertures [46]. Additionally, a large number of tomato *TPP* genes were recorded to be up-regulated by heat stress [21].

Tissue-specific expression of the *ArachyTPS* and *ArachyTPP* genes in roots and different parts of flower suggested that these genes play an important role in development of roots and flowers. This expression profile proposed that *ArachyTPS* and *ArachyTPP* genes are potential candidate genes to improve the peanut cultivation.

## Conclusion

In this study, we identified and annotated 17 and 15 members of the peanut *ArahyTPS* and *ArahyTPP* gene families which were randomly distributed across 20 peanut chromosomes. Segmental duplication events have been predicted as the main reason for the expansion of the *ArahyTPS* and *ArahyTPP* gene families in peanuts. Structural analysis revealed that the ArahyTPS and ArahyTPP proteins were variable in features. Our phylogeny analysis showed that the ArahyTPS family could be clearly classified into three distinct clades, while the ArahyTPP family was categorized into two groups. Expression analysis indicated that 15 *ArahyTPS* genes and five *ArahyTPP* genes were highly expressed in at least one major organ/tissue, respectively. Taken together, *ArahyTPS* and *ArahyTPP* gene families manipulation could be a promising approach to improving various biological processes in peanut plants.

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