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Genome-wide analysis of amino acid transporter genes reveals their potential roles in drought stress and drought-associated disease responses in peanut (*Arachis hypogaea*)

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ABSTRACT

Amino acid transporters (AATs) are central to nitrogen allocation, amino acid distribution, and metabolic adjustment in plants, but this gene family has not been systematically characterized in cultivated peanut (Arachis hypogaea). In this study, 30 AAT genes were identified from the peanut genome and analyzed for chromosomal distribution, protein properties, gene structure, phylogenetic relationships, and expression profiles. The identified ArahyAAT genes were unevenly distributed across 18 chromosomes and showed marked variation in exon-intron organization, which indicates structural diversification within the family. Transcriptome analysis revealed different expression patterns across vegetative and reproductive tissues, with several genes showing preferential expression in roots, nodules, and reproductive organs. To assess their stress responsiveness, five root low-expression genes were selected for RT-qPCR analysis under drought stress and combined drought stress plus charcoal rot infection. ArahyAAT04, ArahyAAT09, and ArahyAAT21 were induced under drought stress, while ArahyAAT07 showed strong induction under the combined treatment. ArahyAAT23 showed limited transcriptional change across the tested conditions. These results suggest that specific ArahyAAT genes may contribute to stress-associated amino acid transport and metabolic adjustment in peanut roots. This study provides a genome-wide characterization and expression analysis of the peanut AAT gene family and identifies candidate genes for future functional studies on drought adaptation and disease-related metabolic responses.

1. INTRODUCTION

Peanut (*Arachis hypogaea*) is a widely cultivated legume that originated in South America and has become an important crop in tropical and subtropical regions (Zhang et al., 2024). It provides a major source of edible oil, plant protein, and essential nutrients, and it contributes significantly to agricultural production and rural livelihoods (Mingrou et al., 2022). Peanut cultivation faces increasing challenges from environmental stress, with drought recognized as one of the most critical factors limiting yield, stability, and kernel quality,

particularly in rainfed production systems (Patel et al., 2022). Particularly, drought stress affects peanut growth, development, and productivity across multiple growth stages and also increases vulnerability to drought-associated diseases (Pokhrel et al., 2024). Charcoal rot, caused by the soil-borne fungus *Macrophomina phaseolina*, represents a major constraint under hot and dry conditions (Subedi et al., 2025). The pathogen colonizes root and stem tissues and disrupts water and nutrient transport, which accelerates senescence and promotes plant wilting and death (Martinez-Salgado et al., 2021). Evidence from field and controlled studies indicates that drought stress intensifies charcoal rot severity, with combined stress exerting stronger negative effects than single stress conditions (Martinez-Salgado et al., 2021). At the molecular level, drought and pathogen infection alter regulatory networks that control stress signaling, metabolic adjustment, and defense responses.

Under drought stress, plants often undergo changes in amino acid accumulation, osmotic balance, and nitrogen remobilization. These processes require coordinated transport of amino acids across membranes and between tissues. Of our interest, amino acid transporters (AATs), which belong to the amino acid-polyamine-organocation superfamily, play essential roles in amino acid uptake, long-distance transport, and source-sink distribution (Ortiz-Lopez et al., 2000; Yang et al., 2020; Yao et al., 2020). These functions link AATs to drought adaptation, nitrogen metabolism, and plant-pathogen interactions in several plant species (Ortiz-Lopez et al., 2000). In peanuts, drought stress also increases susceptibility to drought-associated diseases such as charcoal rot. Pathogen infection can further alter nutrient distribution and defense-related metabolism in root tissues. Therefore, AAT genes may contribute to peanut stress responses by regulating the movement and availability of amino acids needed for osmotic adjustment, cellular homeostasis, and defense-associated metabolic reprogramming. Recently, AAT families have been identified and characterized in several plant species, such as rice (*Oryza sativa*) (Zhao et al., 2012), wheat (*Triticum aestivum*) (Tian et al., 2020), common bean (*Phaseolus vulgaris*) (Nanjareddy et al., 2024), and quinoa (*Chenopodium quinoa*) (Li et al., 2025). Information on the AAT gene family in peanut remains limited, particularly regarding genome-wide identification and stress-responsive expression.

This study aims to conduct a comprehensive genome-wide identification and characterization of AAT genes in cultivated peanut and to analyze their structural features, evolutionary relationships, and tissue-specific expression patterns, with particular emphasis on transcriptional responses under drought stress and drought-associated charcoal rot infection, to elucidate their potential functional roles in stress adaptation and disease response and to provide a molecular foundation for future genetic improvement of peanut stress resilience.

2. MATERIALS AND METHODS

2.1. Screening of AATs in peanut

To identify AAT genes in peanut, known AAT protein sequences from *Arabidopsis thaliana* were retrieved from the TAIR database (Reiser et al., 2017) and used as reference queries. These sequences were employed in BLASTP searches against the peanut protein database (Zhuang et al., 2019) available in Phytozome (Goodstein et al., 2012), with an E-value threshold of 1×10^{-5} to obtain candidate homologs. All nonredundant hits were collected and further examined to confirm the presence of conserved AAT domains (Yao et al., 2020) using Pfam tools (Mistry et al., 2021). Two conserved AAT-related Pfam domains were used for confirmation: Aa_trans/transmembrane amino acid transporter protein domain (PF01490) and AA_permease/amino acid permease domain (PF00324). Redundant sequences were removed based on gene locus, protein sequence identity, and transcript annotation. When multiple protein entries corresponded to the same gene locus, only one representative sequence was retained. If splice variants were present, the longest transcript isoform with a complete open reading frame and conserved AAT domain was selected for further analysis. Identical protein sequences and sequences lacking PF01490 or PF00324 were excluded. Sequences with incomplete open reading frames or truncated conserved domains were also removed. The final nonredundant set of peanut AAT proteins was designated as the ArahyAAT gene family and was used for subsequent analyses.

2.2. Characterization of AATs in peanut

The physicochemical characteristics of the identified peanut AAT proteins were analyzed using the ExPASy ProtParam tool (Gasteiger et al., 2003). For each protein sequence, the total number of amino acids, molecular weight (kDa), and theoretical isoelectric point (pI) were calculated.

Protein stability was evaluated using the instability index (unitless), with values below 40 indicating stable proteins. The aliphatic index (unitless) was determined to estimate the relative volume occupied by aliphatic side chains and to infer thermostability. Protein hydrophobicity was assessed using the grand average of hydropathicity (GRAVY, unitless), with positive values indicating hydrophobic character and negative values indicating hydrophilic character.

2.3. Phylogenetic analysis of AATs in peanut

Phylogenetic analysis was conducted to examine the evolutionary relationships among AAT proteins in peanut, as previously described (Chu et al., 2025). Full-length amino acid sequences of all identified peanut AAT proteins were aligned using the ClustalX program (Larkin et al., 2007). The resulting alignment was used to construct a phylogenetic tree with MEGA software based on the Neighbor-Joining method and the Poisson correction model (Kumar et al., 2024). Bootstrap analysis with 1,000 replicates was carried out to assess the reliability of the inferred tree topology, as previously reported (Chu et al., 2025).

2.4. Gene organization analysis of AATs in peanut

The exon-intron organization of AAT genes was analyzed using the Gene Structure Display Server portal (Hu et al., 2015). Coding sequences and corresponding genomic DNA sequences of each AAT gene were obtained from the peanut genome database (Zhuang et al., 2019) and uploaded to the portal.

2.5. Transcriptomic analysis of AATs in peanut

To examine the expression profiles of AAT genes during different stages of growth and development in cultivated peanut, publicly available transcriptome datasets were reanalyzed. Expression data were retrieved from the GEO database of the National Center for Biotechnology Information (Clough et al., 2024). In particular, the expression atlas corresponding to the GEO accession number GSE71357 was used as previously reported (Bertioli et al., 2016). This dataset provided normalized Fragments Per Kilobase of transcript per Million mapped reads (FPKM) values, which were used to evaluate AAT gene expression across ten representative tissues and organs, including mainstem leaf, lateral stem leaf, seedling leaf, vegetative shoot tip, reproductive shoot tip, root, nodule, perianth, stamen, and pistil. Heat map visualization was performed using TBtools-II. Expression values were transformed as $\log_2$(FPKM + 1) before visualization. Hierarchical clustering was performed for both genes and tissues using Euclidean distance and the average linkage method. Genes with no detectable expression across all analyzed tissues were retained in the expression matrix but were interpreted as under the detection threshold.

2.6. Drought stress treatment and charcoal rot inoculation conditions

Cultivated peanut line L14, originating from the Agricultural Genetics Institute (https://agi.gov.vn/), was used for drought stress and charcoal rot inoculation experiments. Seeds were surface-sterilized and grown in plastic pots containing a uniform soil mixture of loam soil, peat, and sand at a ratio of 2:1:1. Plants were maintained in a controlled growth chamber under a 16 h light/8 h dark photoperiod, a light intensity of approximately 300 μmol m^{-2} s^{-1}, day/night temperatures of 28/22 °C, and relative humidity of 65-70%. All plants received identical irrigation and nutrient management until the four-leaf stage, approximately 20 days after sowing (Zhong et al., 2023). For RT-qPCR analysis, three experimental treatments, including control, drought stress alone, and combined drought stress and charcoal rot infection, were established. The experiment included 15 plants per treatment, with three biological replicates. Control plants were maintained at 75-80% field capacity and received sterile agar plugs near the root collar. Drought-stressed plants were subjected to water withholding from 20 days after sowing until soil moisture declined to approximately 30-35% of field capacity, as monitored with a soil moisture meter (Extech Mo750, Taiwan). For the combined drought stress and charcoal rot infection treatment, plants were inoculated with *M. phaseolina* at 20 days after sowing and simultaneously subjected to drought stress under the same water-deficit conditions described above. The pathogen isolate was obtained from the Agricultural Genetics Institute, Vietnam Academy of Agricultural Sciences (Hanoi city, Viet Nam), cultured on potato dextrose agar at 28 °C for 5-7 days, and applied by placing 5 mm mycelial plugs into the soil near the root collar at a depth of 2-3 cm. Root tissues from all treatments were collected at 30 days after sowing, immediately frozen in liquid nitrogen, and stored at -80 °C for subsequent RNA extraction. No separate charcoal rot-only treatment was included in the RT-qPCR experiment.

2.7. Quantitative reverse transcription PCR analysis of AATs in peanut

RT-qPCR analysis was performed to evaluate the expression levels of selected AAT genes under drought stress and following inoculation with charcoal rot. Gene-specific primers for *ArahyAAT04* (GeneID: arahy.Tifrunner.gnm1.ann1.4JM421), *ArahyAAT07* (GeneID: arahy.Tifrunner.gnm1.ann 1.1WCD0X), *ArahyAAT09* (GeneID: arahy. Tifrunner.gnm1.ann1.1G61GI), *ArahyAAT21* (GeneID: arahy.Tifrunner.gnm1.ann1.MMD2EH), and *ArahyAAT23* (GeneID: arahy.Tifrunner.gnm1. ann1.P1MSVW) were designed using the Primer-BLAST tool (Ye et al., 2012) based on their coding sequences. The primer sequences (forward and reverse) are provided in Table 1. Total RNA was extracted from frozen root tissues using a commercial plant RNA extraction kit (RNeasy Plant Mini Kit for RNA Extraction, Qiagen, Germany). RNA concentration and purity were assessed by using NanoDrop 2000 (Thermo Scientific, USA). First-strand cDNA was synthesized from 1 µg of total RNA using a reverse transcription kit. RT-qPCR reactions were conducted using a SYBR Green master mix on a PCR cycler Mx3000P (Stratagene, Germany). Each reaction was performed in a total volume of 20 µL containing diluted cDNA, gene-specific primers, and SYBR Green master mix. The amplification program consisted of an initial denaturation at 95 °C for 3 min, followed by 40 cycles of 95 °C for 10 s, 55-60 °C for 20 s, and 72 °C for 20 s. Each sample was analyzed using three biological replicates and three technical replicates. Relative expression levels of AAT genes were calculated using the $2^{-\Delta\Delta Ct}$ method. For normalization of gene expression levels, yellow-leaf-specific protein 8 (GeneID: NM_120912) was used as the internal reference gene (Wang et al., 2021). Data are presented as mean ± standard deviation. Statistical significance was assessed using one-way analysis of variance followed by Tukey's honestly significant difference test. Differences were considered statistically significant at p-value < 0.05.

3. RESULTS AND DISCUSSION

3.1. Identification of AATs in peanut

A total of 30 AAT genes were identified in the peanut genome through sequence similarity search and conserved domain verification. These genes were named *ArahyAAT01* to *ArahyAAT30* based on their physical positions on the chromosomes (Figure 1). The *ArahyAAT* genes showed an uneven distribution across 18 (out of 20) chromosomes from Arahy.01 to Arahy.20. Chromosome Arahy.04 contained three AAT genes, while chromosomes Arahy.06, Arahy.09, Arahy.13, Arahy.14, Arahy.16, Arahy.19, and Arahy.20 each harbored two or more members. The genomic locations ranged from 210,063 bp to 155,423,116 bp, which demonstrates a broad distribution across the peanut genome. Both the forward and reverse strands contained *ArahyAAT* genes, with more on the reverse strand. Several *ArahyAAT* genes occurred in close physical proximity on the same chromosome, such as *ArahyAAT05* and *ArahyAAT06* on Arahy.04, *ArahyAAT08* and *ArahyAAT09* on Arahy.06, *ArahyAAT12* and *ArahyAAT13* on Arahy.09, *ArahyAAT19* and *ArahyAAT20* on Arahy.14, *ArahyAAT22* and *ArahyAAT23* on Arahy.16, *ArahyAAT26* and *ArahyAAT27* on *Arahy.19*, and *ArahyAAT28* and *ArahyAAT29* on *Arahy.20*.

The size of the AAT family varies widely across plant species, which suggests lineage-specific expansion driven by genome architecture and duplication history. In the common bean, 84 AAT genes were identified genome-wide (Nanjareddy et al., 2024), which is close to the total reported for rice (Zhao et al., 2012) and indicates a comparatively compact AAT repertoire in diploid crops (Yao et al., 2020). In quinoa, 147 AAT genes were identified (Li et al., 2025), which places quinoa between diploid models and highly duplicated genomes. In wheat (*Triticum aestivum*), the family expands to 296 members, the largest among the species compared in these datasets and consistent with the extensive gene retention expected in polyploid cereals (Yao et al., 2020). Differences in AAT copy number are associated with polyploidization and whole-genome duplication, as well as post-duplication retention or loss, which varies by lineage. Wheat provides clear evidence that whole-genome duplication and tandem duplication contribute to AAT family expansion, and attributes its unusually large AAT complement to the allohexaploid genome and a complex evolutionary process (Tian et al., 2020). Quinoa is also polyploid, and its intermediate AAT count is consistent with partial retention after genome-scale duplication and subsequent fractionation. In contrast, common bean maintains an AAT count similar to other diploid crops, which supports a model in which fewer genome-doubling events, plus stronger purifying selection against redundant transport capacity, limit long-term expansion (Nanjareddy et al., 2024).

3.2. Analysis of properties of AATs in peanut

The physicochemical properties of the 30 identified ArahyAAT proteins were analyzed to assess their basic biochemical characteristics. Protein length ranged from 363 amino acid residues (ArahyAAT04) to 673 amino acid residues (ArahyAAT22), with most members exceeding 500 amino acid residues, which is consistent with typical amino acid transporters as previously described (Ortiz-Lopez et al., 2000; Yang et al., 2020; Yao et al., 2020). The predicted molecular weights ranged from 39.77 kDa to 71.97 kDa, closely matching the observed protein length variation. The theoretical pI values showed considerable diversity, ranging from 5.18 (ArahyAAT01) to 9.15 (ArahyAAT24), suggesting the presence of both acidic and basic AAT proteins in peanut. The instability index values ranged from 28.09 to 41.41, with most ArahyAAT proteins having values below 40, indicating predicted stability under physiological conditions. The aliphatic index ranged from 97.05 to 121.76, with most proteins showing high values, which suggests strong thermostability. All ArahyAAT proteins exhibited positive GRAVY values between 0.36 and 0.80, which indicates a predominantly hydrophobic nature that is consistent with membrane-localized transporter proteins.

The physicochemical characteristics of AATs show both conserved features and species-specific variation across plant genomes. In peanuts, the identified ArahyAAT proteins exhibit lengths ranging from 363 to 673 amino acids and molecular weights between approximately 40 and 72 kDa. This size range is comparable to that reported for AATs in other plant species, including wheat (Tian et al., 2020), quinoa (Li et al., 2025), and common bean (Nanjareddy et al., 2024), where most AAT proteins also exceed 500 amino acids and fall within a similar molecular weight interval. These similarities support the conserved structural framework of AATs, which typically function as multi-pass membrane proteins involved in amino acid transport across cellular membranes (Yao et al., 2020). The theoretical pI of peanut AATs spans a broad range from acidic to basic, a pattern that closely matches observations in wheat, quinoa, and common bean (Tian et al., 2020; Nanjareddy et al., 2024; Li et al., 2025). Studies in these species report AAT pI values distributed across acidic and alkaline ranges, which have been associated with functional diversification and differential localization within cellular compartments (Tian et al., 2020; Nanjareddy et al., 2024; Li et al., 2025). Such variation in pI may influence protein-protein interactions and transporter activity under different cellular and environmental conditions. Next, most ArahyAAT proteins show instability index values below 40, a trend also observed in wheat, quinoa, and common bean AATs (Tian et al., 2020; Nanjareddy et al., 2024; Li et al., 2025), which are generally predicted to be stable proteins. The high aliphatic index values recorded for peanut AATs align with those reported in other species and suggest favorable thermostability, which is important for transporter function under stress conditions such as drought and high temperature. In addition, positive GRAVY values across all peanut AATs indicate strong hydrophobic character, consistent with membrane localization. Similar hydrophobic profiles have been documented for AATs in wheat, quinoa, and common bean (Tian et al., 2020; Nanjareddy et al., 2024; Li et al., 2025), which demonstrates the conserved requirement for transmembrane domains in amino acid transport activity.

3.3. Categorization of AATs in peanut

In this study, we constructed a Neighbor-Joining-based phylogenetic tree of AAT proteins in peanut. The Neighbor-Joining method was selected because it has been widely applied in genome-wide gene family studies and allows consistent comparison of phylogenetic grouping among plant species. Previous studies on AAT gene families in rice and common bean also used the Neighbor-Joining method to classify AAT proteins into evolutionary groups (Zhao et al., 2012; Nanjareddy et al., 2024). Phylogenetic analysis divided the 30 peanut AAT proteins into several distinct groups with high bootstrap support. Closely related ArahyAAT members clustered into well-defined clades, which suggests shared evolutionary origins. One major group consisted of ArahyAAT18, ArahyAAT04, ArahyAAT19, and ArahyAAT05. Another group included ArahyAAT28, ArahyAAT16, and ArahyAAT03. ArahyAAT12, ArahyAAT26, ArahyAAT15, and ArahyAAT02 formed a separate clade, while ArahyAAT20 and ArahyAAT06 constituted an independent group. Additional clades included ArahyAAT22 with ArahyAAT08 and ArahyAAT23 with ArahyAAT09. ArahyAAT11 and ArahyAAT25 were grouped within the same clade, and ArahyAAT14 and ArahyAAT29 formed another distinct group. ArahyAAT10, ArahyAAT24, and ArahyAAT17 clustered into a single clade. ArahyAAT01, ArahyAAT30, ArahyAAT21, and ArahyAAT07 formed another

well-supported group, while ArahyAAT13 and ArahyAAT27 constituted a distinct lineage.

Phylogenetic classification of the AAT family in peanut revealed multiple well-defined evolutionary groups, which is consistent with reports from other plant species. In wheat, quinoa, and common bean, AAT proteins are also divided into several conserved clades that correspond to functional subfamilies within the amino acid-polyamine-organocation superfamily (Tian et al., 2020; Nanjareddy et al., 2024; Li et al., 2025). These shared classification patterns suggest that the major AAT lineages were established early during plant evolution and were retained across monocot and dicot species, including legumes such as peanut and common bean. Comparative studies in wheat have shown that AATs cluster into distinct phylogenetic groups that often display extensive expansion within individual clades, a feature attributed to whole-genome duplication and polyploidization (Tian et al., 2020). The presence of multiple wheat AAT members within the same clade contrasts with the more limited number of peanut AATs per group, which may indicate a more limited retention or expansion of AAT homologs in cultivated peanut than in wheat and quinoa. Quinoa shows an intermediate pattern, with several AAT clades containing multiple paralogs while maintaining overall clade structure similar to that observed in other dicots (Li et al., 2025). In common bean, AATs are distributed among comparable phylogenetic categories to those identified in peanut, with each clade typically containing fewer members than in wheat or quinoa (Nanjareddy et al., 2024). This similarity is consistent with the close evolutionary relationship between these two legume species. The presence of analogous AAT groups in peanut and common bean suggests the conservation of amino acid transport functions involved in nitrogen allocation, seed development, and stress responses. Overall, the phylogenetic categorization of peanut AATs aligns well with classification schemes reported for wheat, quinoa, and common bean. The conservation of major AAT groups across these species supports the functional importance of these transporters in plant growth and stress adaptation.

3.4. Structural analysis of AATs in peanut

Gene structure analysis revealed substantial variation in exon-intron organization among the 30 *ArahyAAT* genes. The number of exons ranged from 1 to 16, which indicates pronounced structural diversity within the gene family (Figure 2). Four genes, *ArahyAAT01, ArahyAAT04, ArahyAAT17,* and *ArahyAAT30*, contained a single exon. Several *ArahyAAT* genes possessed two exons, including *ArahyAAT03*, *ArahyAAT10*, *ArahyAAT16*, *ArahyAAT21*, *ArahyAAT24*, and *ArahyAAT07*. Genes with moderate exon numbers were also common, such as *ArahyAAT12* and *ArahyAAT26* with four exons, *ArahyAAT15* and *ArahyAAT02* with five exons, and *ArahyAAT19* and *ArahyAAT05* with three exons. In contrast, a subset of *ArahyAAT* genes displayed complex structures with a high number of exons. *ArahyAAT22* contained the highest exon number at sixteen, followed by *ArahyAAT11*, *ArahyAAT29*, and *ArahyAAT09* with fourteen exons each, *ArahyAAT14* and *ArahyAAT25* with thirteen exons, and *ArahyAAT23* and *ArahyAAT08* with twelve and eleven exons, respectively. Genes with seven or eight exons, including *ArahyAAT13*, *ArahyAAT27*, *ArahyAAT20*, and *ArahyAAT06*, further contributed to the family's structural complexity.

3.5. Expression patterns of AATs in peanut during growth and development

The expression patterns of *ArahyAAT* genes were examined across 10 major peanut tissues and organs using transcriptome data (Figure 3). Among the 30 *ArahyAAT* genes, two genes, *ArahyAAT07* and *ArahyAAT23*, showed no detectable expression in any of the analyzed tissues and were classified as not detected. The remaining 28 genes showed detectable expression in at least one tissue, with 25 genes exhibiting broad expression across multiple tissues, suggesting potential roles in general growth and development. Several *ArahyAAT* genes exhibited relatively high expression levels, with FPKM values exceeding 50 in at least one tissue. Notably, a subset of genes showed tissue-preferential or tissue-exclusive expression with FPKM values exceeding 100. For example, *ArahyAAT11*, *ArahyAAT14*, *ArahyAAT28*, *ArahyAAT20*, *ArahyAAT29*, and *ArahyAAT06* displayed consistently high expression across vegetative and reproductive tissues, including leaves, roots, nodules, perianth, stamen, and pistil. *ArahyAAT14* and *ArahyAAT11* showed particularly high expression in reproductive organs such as the perianth and pistil, while *ArahyAAT28* exhibited strong expression in roots and nodules. Several genes, including *ArahyAAT12*, *ArahyAAT16*, and *ArahyAAT27*, showed enhanced expression in roots and nodules but limited expression in aerial tissues. This finding might suggest roles in belowground

nitrogen transport. The diverse expression profiles of *ArahyAAT* genes across tissues indicate functional differentiation within the gene family and suggest that both constitutively expressed and tissue-preferential AATs contribute to amino acid transport during peanut growth and development. Based on transcriptome analysis, five *ArahyAAT* genes, *ArahyAAT04*, *ArahyAAT07*, *ArahyAAT09*, *ArahyAAT21*, and *ArahyAAT23*, showed low expression levels in roots under normal growth conditions. These genes exhibited very low or undetectable transcript abundance in root tissue compared with other *ArahyAAT* members. Due to their low basal expression in roots, these genes were selected for further validation by RT-qPCR. Their expression patterns were examined under drought stress and drought-associated charcoal rot infection to evaluate potential stress-induced transcriptional responses.

3.6. Expression patterns of AATs in peanut under drought stress and drought-associated charcoal rot infection

RT-qPCR analysis was conducted to examine the expression patterns of five selected *ArahyAAT* genes in peanut roots under drought stress and combined drought stress plus charcoal rot infection (Figure 4). Under drought stress alone, *ArahyAAT04* and *ArahyAAT09* were markedly induced, reaching approximately 2.45- and 3.84-fold of the control, respectively. *ArahyAAT21* showed moderate upregulation with a 1.67-fold increase, whereas *ArahyAAT07* showed only a slight increase. *ArahyAAT23* showed no clear transcriptional response to drought stress. These results suggest that specific *ArahyAAT* members may contribute to stress-associated changes in amino acid transport, nitrogen redistribution, and osmotic adjustment in root tissues during water deficit. It should be noted that *AAT* genes encode membrane-associated transporter proteins and primarily function in amino acid uptake, intracellular transport, long-distance allocation, and metabolic regulation (Ortiz-Lopez et al., 2000; Yang et al., 2020; Yao et al., 2020). They should not be interpreted as direct receptors or primary signaling components in drought or pathogen-response pathways. Instead, changes in *ArahyAAT* expression may indicate downstream transcriptional regulation in response to altered metabolic demands under stress conditions. In this sense, the induction of selected *ArahyAAT* genes under drought stress and combined drought plus charcoal rot infection may support the redistribution of amino acids required for osmotic balance, nitrogen metabolism, cellular homeostasis, and defense-related metabolic adjustment. Similar drought-induced expression of AAT genes has been reported in other plant species, including wheat and quinoa, where specific AAT members respond to dehydration by modulating nitrogen transport and stress-related metabolism (Tian et al., 2020; Li et al., 2025).

Under combined drought stress and charcoal rot infection, *ArahyAAT07* showed the strongest induction, reaching approximately 7.44-fold that of the control. *ArahyAAT04* and *ArahyAAT21* also showed higher expression under the combined treatment than under drought stress alone, with expression levels approximately 3.62- and 3.56-fold relative to the control, respectively. This pattern indicates that *ArahyAAT07* may be associated with amino acid transport processes linked to pathogen-related metabolic adjustment under water-deficit conditions. Comparable pathogen-responsive induction of AAT genes has been observed in wheat and common bean, where certain AAT members show enhanced expression during fungal infection and are thought to contribute to defense-associated metabolic reprogramming (Tian et al., 2020; Nanjareddy et al., 2024). By contrast, *ArahyAAT09* showed lower expression under the combined treatment than under drought stress alone, although its expression remained above the control level. This result indicates treatment-specific regulation of this transporter. *ArahyAAT23* exhibited limited transcriptional changes across treatments, suggesting a minor or more stable transport-related role in peanut roots under the tested conditions. This lack of induction mirrors observations in other species, where certain AAT family members maintain stable expression and may function in housekeeping transport processes rather than stress adaptation (Ortiz-Lopez et al., 2000; Yang et al., 2020; Yao et al., 2020). Similar expression trends have been documented in quinoa, where several AAT genes show higher expression under combined abiotic and biotic stress than under single stress treatments (Li et al., 2025). These patterns support the view that AATs help maintain amino acid homeostasis under complex environmental conditions by adjusting transport capacity in response to both water limitation and pathogen pressure (Ku et al., 2022; Yao et al., 2025). Our findings show that selected *ArahyAAT* genes respond transcriptionally to drought stress and combined drought plus charcoal rot infection. Their expression patterns support their potential

involvement in stress-associated amino acid transport and metabolic adjustment. Further functional validation is required to determine their precise biological roles in peanut stress adaptation and disease-related metabolic responses.

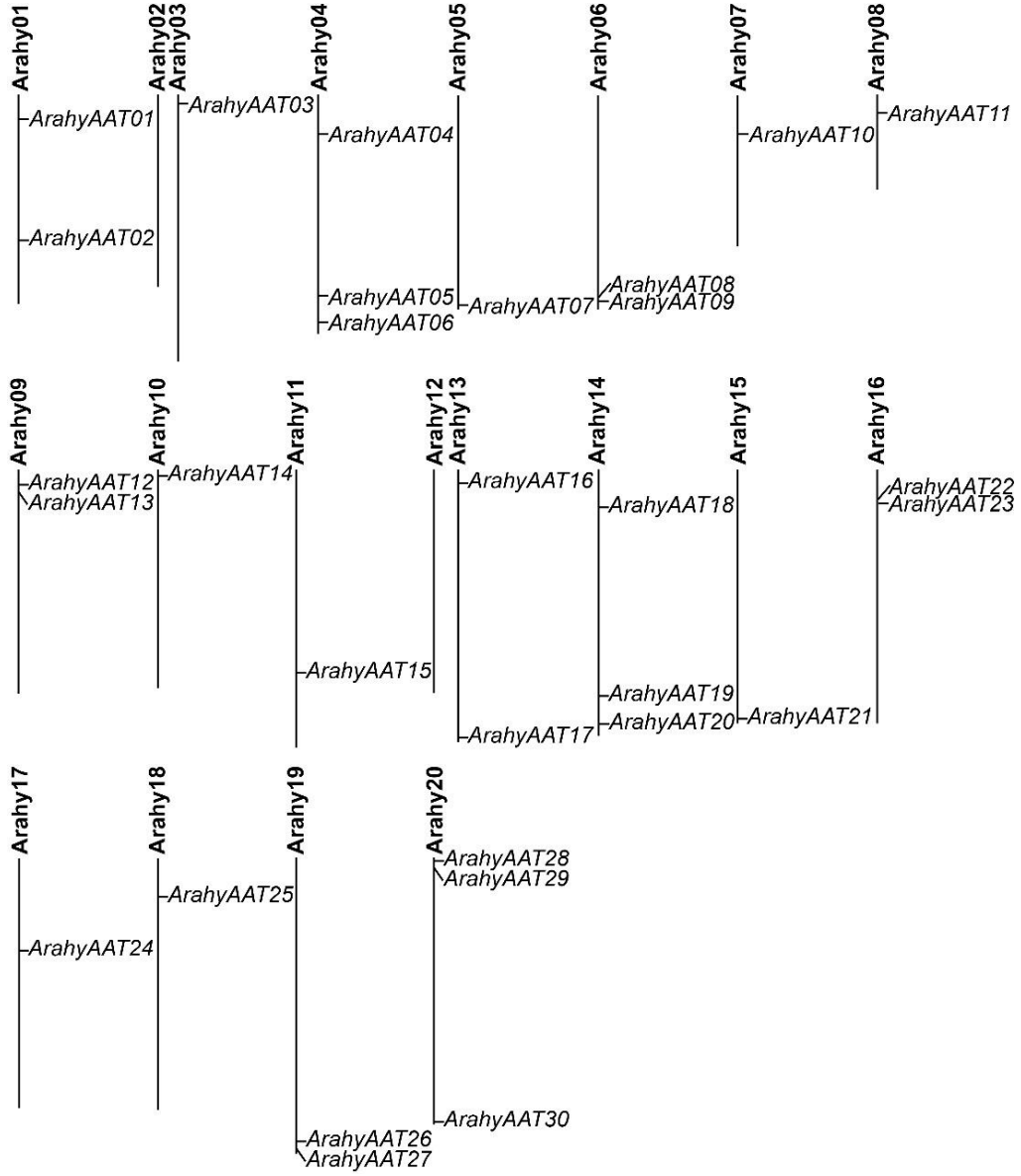

Figure 1. Chromosomal distribution of amino acid transporter genes in cultivated peanut

The 30 identified ArahyAAT genes are mapped to the 20 peanut chromosomes (Arahy01-Arahy20) based on their physical positions. Vertical bars represent individual chromosomes, and gene names indicate the approximate genomic locations of each ArahyAAT member. The distribution shows an uneven arrangement of ArahyAAT genes across chromosomes, with several chromosomes harboring multiple genes and others containing none.

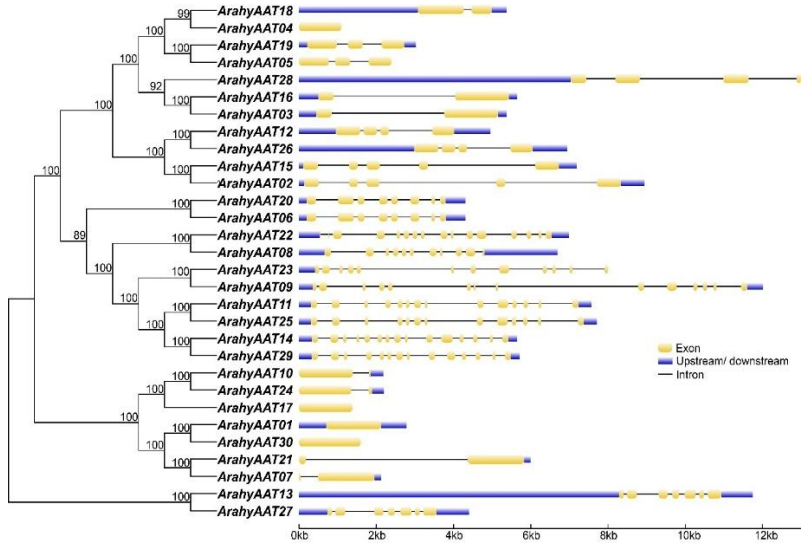

Figure 2. Phylogenetic relationships and gene structure organization of amino acid transporters in cultivated peanut

The phylogenetic tree was constructed from full-length AAT protein sequences, and bootstrap values are indicated at the branch nodes. The exon-intron structures of individual ArahyAAT genes are shown on the right, with yellow boxes representing exons, black lines representing introns, and blue boxes indicating upstream and downstream regions. Gene structures are drawn to scale according to genomic length (kb).

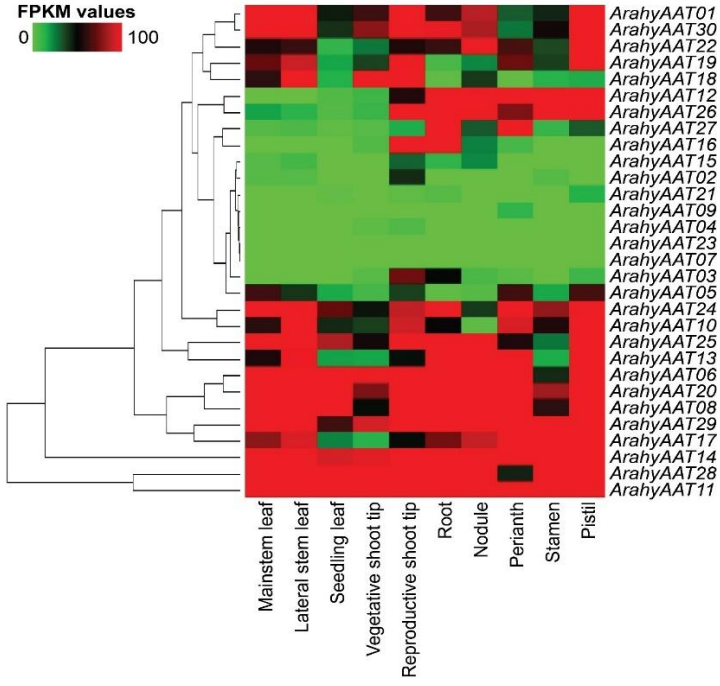

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Figure 3. Expression profiles of amino acid transporter genes in cultivated peanut across different tissues and organs

The heatmap represents transcript abundance based on normalized FPKM values derived from publicly available RNA-seq data. Tissues analyzed include mainstem leaf, lateral stem leaf, seedling leaf, vegetative shoot tip, reproductive shoot tip, root, nodule, perianth, stamen, and pistil. Color intensity indicates relative expression levels, with green representing low expression and red representing high expression. Hierarchical clustering was performed for both genes and tissues to reveal similarities in expression patterns among ArahyAAT members.

Table 1. Primer sequences of five candidate AAT genes in peanut for RT-qPCR analysis

#	Gene name	Forward primer (5' → 3')	Reverse primer (5' → 3')	Expected amplicon size (bp)	PCR efficiency (%)
1	*ArahyAAT04*	ACCCCTCAGAGGC TGAGAAA	CAGACCTCTGC CTCACTTGG	142	97.6
2	*ArahyAAT07*	CCTGAAACAATAT AAATTGAGGGCT	CAGTCGAGACC CAAACGACA	118	96.9
3	*ArahyAAT09*	TGCGATTGAACAT GTAAATAACGA	ACAAAACCTCC AGAATGCCCA	154	99.1
4	*ArahyAAT21*	TGGTGATGATGAC ACAATCAGAGA	GGTGTGAGTTC TCCTTGGGG	136	98.4
5	*ArahyAAT23*	GTTGCTGGACGCA TGATAGC	GGCAGACAAAA CTTGCATCATCT	127	95.8

Table 2. Characteristics of the AAT proteins in peanut

Protein names	Protein size	Protein weight	pI	Instability index	Aliphatic index	GRAVY
ArahyAAT01	471	52.32	5.18	40.46	112.97	0.57
ArahyAAT02	591	65.78	8.58	35.65	109.86	0.56
ArahyAAT03	597	65.35	8.29	30.25	107.47	0.47
ArahyAAT04	363	39.77	5.31	35.41	97.05	0.42
ArahyAAT05	582	64.07	8.88	32.48	102.44	0.47
ArahyAAT06	563	59.35	6.74	38.60	121.76	0.80
ArahyAAT07	498	55.29	7.66	36.52	110.00	0.55
ArahyAAT08	503	53.12	6.35	28.09	109.92	0.63
ArahyAAT09	572	60.84	7.53	31.05	114.27	0.60
ArahyAAT10	477	52.76	8.44	32.94	113.84	0.62
ArahyAAT11	640	67.88	5.94	29.68	112.75	0.62
ArahyAAT12	591	63.73	8.76	36.34	99.66	0.65
ArahyAAT13	525	56.41	8.75	31.58	111.30	0.70
ArahyAAT14	669	71.14	5.56	33.36	112.96	0.69
ArahyAAT15	593	65.84	8.70	36.60	111.15	0.56
ArahyAAT16	597	65.31	8.47	30.59	107.15	0.48
ArahyAAT17	460	50.85	5.89	29.04	109.65	0.65
ArahyAAT18	570	62.65	8.81	37.07	102.68	0.55
ArahyAAT19	582	64.04	8.70	33.64	102.61	0.45
ArahyAAT20	563	59.32	6.50	38.01	120.89	0.79
ArahyAAT21	544	60.33	6.03	40.68	103.01	0.36
ArahyAAT22	673	71.97	7.47	35.76	116.05	0.65
ArahyAAT23	486	51.80	7.52	30.82	118.02	0.62
ArahyAAT24	476	52.91	9.15	35.77	114.29	0.63
ArahyAAT25	593	62.88	6.15	32.27	110.19	0.59
ArahyAAT26	591	63.73	8.76	36.34	99.66	0.65
ArahyAAT27	522	56.19	8.75	31.35	111.74	0.69
ArahyAAT28	600	66.23	8.01	35.5	106.78	0.44
ArahyAAT29	617	65.12	5.37	30.95	113.18	0.67
ArahyAAT30	531	59.17	5.45	41.41	109.92	0.50

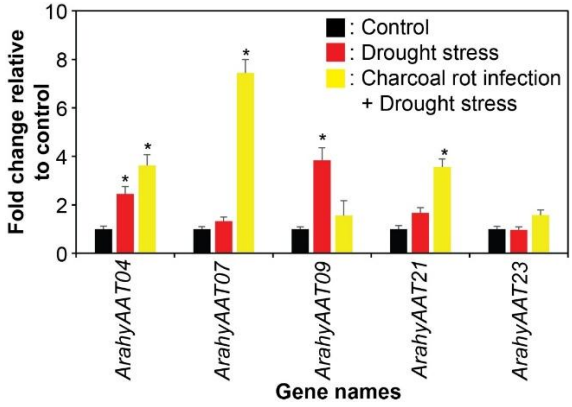

Figure 4. Relative expression levels of selected *ArahyAAT* genes in peanut roots under control conditions, drought stress, and combined drought stress plus charcoal rot infection.

Transcript abundance was determined by RT-qPCR and expressed as fold change relative to the control. Relative expression levels were calculated using the $2^{-\Delta\Delta Ct}$ relative quantification method. Black bars represent control plants, red bars represent drought stress treatment, and yellow bars represent combined drought stress plus charcoal rot infection. Data are shown as mean values ± standard deviation from three biological replicates. Asterisks indicate statistically significant differences compared with the control at $p < 0.05$.

4. CONCLUSION

This study systematically characterized the AAT gene family in cultivated peanut, defining their chromosomal distribution, structural features, phylogenetic relationships, and expression patterns across major tissues and developmental stages. The observed diversity in exon-intron organization and tissue-specific expression profiles indicates pronounced functional differentiation among ArahyAAT members. Expression analyses under drought stress and drought-associated charcoal rot infection revealed distinct transcriptional responses: *ArahyAAT04, ArahyAAT07, ArahyAAT09,* and *ArahyAAT21* showed clear stress-responsive regulation, whereas *ArahyAAT23* exhibited limited transcriptional change. These AAT genes represent promising candidates for further functional characterization to clarify their roles in amino acid transport, stress adaptation, and disease-related metabolic regulation. Taken together, the findings provide a valuable molecular resource for future functional genomics studies and support the identification of target genes for breeding and biotechnological approaches to enhance drought tolerance and disease resilience in peanut.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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