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Genome-wide identification of the *AAT* gene family in quinoa and analysis of its expression pattern under abiotic stresses

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Abstract

Background Plant amino acid transporters play an important role in the absorption of soil amino acids by roots, the transport of amino acids between xylem and phloem, plant growth and development, and response to abiotic stress.

Result In this study, we identified 147 *AAT* genes in the quinoa genome sequence and categorized them into 12 subfamilies on the basis of their similarity and phylogenetic relationships with *AAT* found in Arabidopsis thaliana. Interestingly, these *AAT* genes are not evenly distributed on the quinoa chromosomes. Instead, most of these genes are centrally located on the outer edges of the chromosome arms. After performing motif analysis and gene structure analysis, we observed the consistent presence of similar motifs and intron-exon distribution patterns among subfamilies. Tissue expression analysis revealed that *CqAAT* gene was less expressed in fruits and more expressed in roots, stems, leaves and flowers. Meanwhile, expression analysis under four adversities of high temperature, low temperature, waterlogging, and drought and different treatments of nitrogen, phosphorus, and potash fertilizers found that two genes of the *CqGAT* subfamily, *AUR62031750* and *AUR62023955* were up-regulated expressed under abiotic stresses.

Conclusions In summary, there is a significant differentiation in the tissue expression and stress expression of the *CqAAT* gene, indicating that *CqAATs* play a role in regulating growth and development under abiotic stress.

Keywords Quinoa, *AAT*, Abiotic stresses , Bioinformatics, Gene expression

Introduction

Quinoa (Chenopodium quinoa Willd.) is an annual dicotyledonous plant of the chenopodium genus in the chenopodium subfamily of the Amaranthaceae family, with higher nutritional content of proteins, carotenoids, and vitamin C in its seeds than in most grains such as rice and wheat. Quinoa is considered by the Food and Agriculture Organization of the United Nations to be the only monocot plant that can meet the basic nutritional needs of the human body, and quinoa is officially recommended as the most suitable fully nutritious food for human beings [1,

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2]. Quinoa seedlings and young leaves are edible and, like spinach of the same family, are commonly used as a vegetable, providing important vitamins, nutrients and minerals to humans [3, 4].

Amino acids are not only necessary for protein formation, but also the main form of nitrogen absorbed by the plant root system, which plays an important role in the growth and development of plants. In highbrow plants, the absorption and transportation of amino acids mainly depend on amino acid transporter proteins (*AAT*), which are widely involved in important physiological processes such as amino acid uptake and transportation, root growth, floral organ development, seed germination, and abiotic stress in plants. The *AAT* gene family has been systematically identified and characterized in a variety of plant species. There are 63 *AAT* genes in Arabidopsis thaliana [5], 84 in Phaseolus vulgaris [6], 85 in rice [7], 189 in soybean [8], 296 in common wheat [9], 94 in cereal [10], 104 in Tartary buckwheat [11], and 72 in potato [12], indicating that the *AAT* gene family is widespread in higher plants. The *AAT* family in plants consists of the amino acid/Auxin permease (*AAAP*) family and the amino acid-polyamine-choline (*APC*) family of transporter proteins.The *AAAP* gene family is one of the largest gene superfamilies of *AAT*, and it can be divided into eight branches on the basis of sequence similarity and conserved structural domains, namely, the amino acid permease (*AAP*), the lysine and histidine transporter (*LHT*), the gamma- amino butyric acid transporter (*GAT*), Auxin permease transporter (*AUX*), proline transporter (*ProT*), aromatic and neutral amino acid transporter (*ANT*), and amino acid-like transporter (also classified as *ATLa* and *ATLb*) [13–15]. Members of the *AAAP* family belonging to different branches have low sequence similarity, but all *AAAP* proteins have the same conserved structural domain, PF01490. The *APC* family consists of four subfamilies: the cationic amino acid transporter protein (*CAT*), amino acid/polyamine transporter protein (*ACT*), and polyamine H^+ homotransporter protein (*PHS*), and the tyrosine-specific transporter protein (*TTP*) family, all *ACP* proteins have the same conserved structural domain, PF00324.

Amino acid transporter proteins have key roles in plant growth and development. *AAP6* in Arabidopsis may be responsible for the uptake of amino acids in xylem, and *AAP8* may be important in the uptake of amino acids in seeds [15]. *LHT1* is responsible for the uptake of soil amino acids by plants and the transport of amino acids by members of the *LHT* subfamily is also involved in coordinated interactions between pollen development and pollen gynoecium in plants [16–17]. Amino acid transporter proteins are not only responsible for the uptake and transportation of amino acids, but also for the transportation of non-amino acid substances. They may function as receivers of amino acid signals to regulate growth and development, modulate growth and yield traits, change plant shape and enhance disease resistance [18–20]. If amino acid transporter proteins are mutated or overexpressed in plants, some amino acids may act as signaling molecules to cause a series of complex signaling reactions to alter gene expression and ultimately lead to phenotypic changes [21]. In agricultural production, planted crops are often subjected to yield reductions due to abiotic stresses, such as high temperature, drought, low temperature, and waterlogging. Many *AAT* genes can also alleviate the damage of various abiotic stresses on plants. For example, the *ProT* gene can rapidly partition proline under water stress conditions, thus minimizing the damage caused by water deficit in plants [22]. *GmProT1* and *GmProT2* are stress-sensitive in roots, and transgenic plants can transport more proline after proline spraying [23]. Increased γ-aminobutyric acid significantly induced *AtGAT1* expression in mechanical injury, stress, or sensitive environments [24]. *OsAAP6*, *OsAAP11*, and *OsANT3* were significantly up-regulated under salt and drought stress, and they could regulate the plant's tolerance to adversity [7].

Systematic analysis of the *AAT* gene family in quinoa has not been reported. Quinoa is an important grain, feed and industrial raw material crop in China, as well as the largest crop grown in the world, so the study of quinoa *AAT* family genes has important theoretical and practical significance. In this study, based on the whole genome level of quinoa, we used bioinformatics to identify the members of quinoa *AAT* family, and analyzed their evolutionary relationship and tissue expression relationship to reveal the expression pattern of quinoa *AAT* family genes in different tissues and under adverse conditions.

Results

Identification and physicochemical properties analysis of *CqAAT* gene family members

Based on bidirectional Blast analysis and HMM (PF00324 and PF01490), a total of 147 *AAT* genes were identified in the quinoa genome. The physicochemical properties of the protein sequence encoded by the quinoa gene were analyzed on the ExPASy website, as shown in Table S1. The *CqAAT* protein contains an average of 466 amino acids, ranging in length from 141 (*AUR62023532*) to 1425 (*AUR62040229*) amino acids. The molecular weight ranges from 15,381 kD (*AUR62009229*) to 156543.52 kD (*AUR62040229*), with an average of 51085.53 kD. The maximum and minimum values differ by 141162.52 kD. The isoelectric points (PI) range from 5.19 (*AUR62000014*) to 10.12 (*AUR62001226*), with 105 members of the *CqAATs* gene family having isoelectric points exceeding 7, indicating that the majority of *CqAATs* belong to alkaline proteins. The instability index ranged

from 21.1 (*AUR62027056*) to 54.62 (*AUR62014264*), with 129 genes having an instability index less than 40, indicating that most *CqAATs* belong to stable proteins. The aliphatic index ranges from 81.86 (*AUR62043653*) to 129.3 (*AUR62027047*); The hydrophilicity ranges from − 0.312 (*AUR62043653*) to 0.923 (*AUR62018347*), indicating that most quinoa *AAT* family proteins belong to hydrophobic proteins, with only three hydrophilic proteins (*AUR62014264*, *AUR62043653*, *AUR62040228*). Subcellular localization prediction showed that most proteins were localized to the cell membrane, with 22 proteins localized to Golgi apparatus and 4 proteins localized to Chloroplast (Table S1).

Phylogenetic analysis of *AAT* gene family members in quinoa

To investigate the evolutionary relationship of the quinoa *AAT* gene family, 147 quinoa *AAT* protein sequences and 63 Arabidopsis *AAT* protein sequences were aligned. Through phylogenetic tree analysis, the 147 *CqAAP* proteins were divided into 12 subfamilies (Fig. 1): the *ATLb* subfamily contains 26 *AAT* members, the ANT subfamily

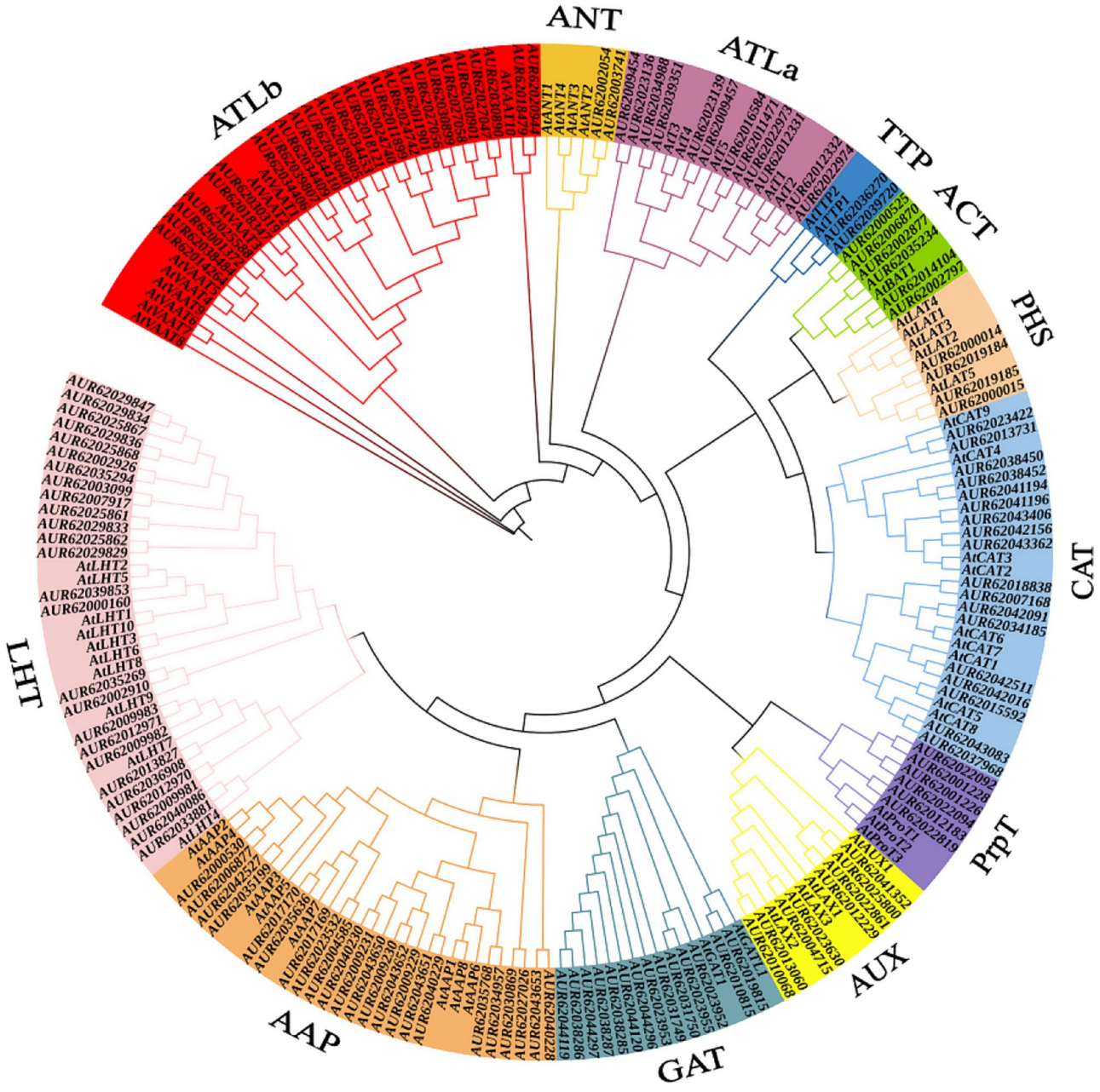

Fig. 1 Phylogenetic relationship constructed based on amino acid sequences of *AAT* protein in quinoa and Arabidopsis thaliana. Different subgroups are highlighted in different colors

contains 2 *AAT* members, the *ATLa* subfamily contains 12 *AAT* members, the *TTP* subfamily contains 2 *AAT* members, the *ACT* subfamily contains 6 *AAT* members, the *PHS* subfamily contains 4 *AAT* members, the *CAT* subfamily contains 18 *AAT* members, the *ProT* subfamily contains 6 *AAT* members, the *AUX* subfamily contains 8 *AAT* members, and the *GAT* subfamily contains 14 *AAT* members. There are 23 *AAT* members in the *AAP* family and 26 *AAT* members in the *LHT* family; In addition, there are significant differences in the number of branches at all levels and the number of genes in each branch among different subfamilies, indicating the existence of some short specific sequences between different subfamilies, which may endow the *CqAAT* family with diverse functions.

Gene structure and conserved domain analysis of *AAT* gene family members in quinoa

To further understand the structural characteristics of *CqAATs* proteins, 20 conserved motifs were identified from *CqAATs* using MEME online software (Table 1). Further analyze the gene structure of *CqAATs* using the GSDS2.0 gene structure display system, and finally draw the gene structure and conserved motif diagram based on the phylogenetic tree relationship (Fig. 2). Based on genome annotation, this study analyzed the intron exon structure of *CqAAT* family genes. Among the members of the *CqAAT* family, 10 gene sequences do not contain introns, while the number of introns in the sequences of other family members ranges from 1 to 20. *AUR62040229* contains the most exons and introns and 67 genes having no untranslated regions. Similar intron exon structures were observed in most *CqAAT* genes within the same subfamily, such as *CqACT*, *CqATLb*, *CqATLa*, *CqLHT*, *CqAUX*, *CqGAT* and *CqCAT*. Conservative motif analysis shows that the number of motifs in the *CqAATs* gene family members ranges from 1 to 35. Nine genes only contain Motif 11, while *AUR62040229*, *AUR62009231*, and *AUR62035636* have a higher number of motifs due to the presence of repetitive fragments. It is speculated that during the evolution of genes, certain sequence regions may remain highly conserved due to their importance to gene function. These conserved sequence regions (motifs) are preserved in gene family members and may appear multiple times in the genome through gene replication or evolution. The types and quantities of conserved motifs among members of the same subfamily are very similar, but there are also differences in the patterns of conserved motifs among members of the same subfamily (Fig. 2). Motif 12 only exists in the *AAP* subfamily; Motif 7 only exists in the *ACT*, *ATLa*, and *ATLb* subfamilies, while Motif 11 only exists in the *ACT*, *PHS*, and *CAT* subfamilies; Motif 10 only exists in the *GAT* subfamily; Motif 18 only exists in the *AUX* subfamily; Motif 19 only exists in the *AAP* and *LHT* subfamilies; Motif 14 only exists in the *ATLa* and *ATLb* subfamilies.

A B C.

Table 1 Motif sequences of *AAT* in Quinoa

Motif number	Consensus sequence	sites	E-value
Motif 1	HIITAIVGAGILSLPYAFAQLGW	96	4.1e-846
Motif 2	YFSVAFLGYLAFGNDVPSNIL	109	1.6e-707
Motif 3	KPFWLIALANIFVVJHVVGSYQVYAQPVFD-MLESFLVKKWK	57	3.7e-974
Motif 4	FIAIAFPFFGDJLSLLGALGFAPLTFILP	85	6.3e-778
Motif 5	LSQJPNFHSJAWLSLVAAIMSLGYSAIAW	51	3.8e-681
Motif 6	DKVFRFLSALGDIAFAYSGHNVVJEIQATLPS	41	3.8e-655
Motif 7	PGKRIDTYMDJGQAAFG	80	8.4e-359
Motif 8	HAQLGRRHLRFRDMAHDILGPRWGRYIVG-PVQFAVCYGAVVGACLJGGQC	10	5.7e-320
Motif 9	HNKGELLNWSGIPTAISLYGFCYSAHPVFPT-LYTSMQKKHQFSKVLLLCF	21	2.0e-575
Motif 10	LNLPTDKJSSKIAIYTTLITPLSKYALMLEPV-VISTESWFP	18	7.9e-367
Motif 11	ATVFFSYIGFDAVATLAEEVKNPQKDLPI	26	1.0e-324
Motif 12	WLCGPQQYIVLVGCDIGYTITAGISLMAV	34	1.5e-349
Motif 13	FGAIAIJATTFGNGIIPEIQATLAKPVKGKM-FKGLSTCYSV	23	4.6e-378
Motif 14	KIGRTIVSIFLYTELYLVVTGFLILEGDNLHN-LFPEMSLHA	21	3.3e-343
Motif 15	VEMYIAQRKIKKWSTKWIMLQLLSLLCLIVS-JAAAAGSIQG	17	9.7e-309
Motif 16	IITLYTSLLLADCYE	96	2.30E-302
Motif 17	FVJJIALILLPTVWLDBLSILSYISATGVLASVVI	22	6.40E-292
Motif 18	FYGFLGSWTAYLISVLYVEYRSRKEKEGVD-FKNHVIQWFEVLDGLLGPHW	8	1.70E-258
Motif 19	EPPENKPMWKGSLIAYIVTTL	42	1.20E-275
Motif 20	RSNCFHKKGHKASCKFSNNPYMIIFGIAE	18	7.50E-249

Chromosome localization and co linear analysis of *AAT* gene family members in Quinoa

Chromosome localization analysis revealed that 147 *CqAAT* genes were unevenly distributed on 18 chromosomes (Fig. 3A). The number of *AAT* genes distributed on different chromosomes ranged from 2 to 13, with chromosome 1 having the most genes and containing 13 *AAT* genes, followed by chromosomes 4, 8, and 15; Chromosomes 2, 11, and 13 contain the least number of *AAT* genes, with 2 *AAT* genes and 12 genes not located on chromosomes. In order to further investigate the evolutionary relationship between quinoa *AAT* genes and other species, we conducted collinearity analysis on quinoa with Arabidopsis and rice. The results showed that 147 quinoa *AAT* genes identified 34 pairs of collinear bases with Arabidopsis, involving all chromosomes of quinoa except for chromosome 9 and 18. Only 15 pairs of collinear gene pairs were identified between rice and Poaceae, suggesting a large-scale gene loss event in the *AAT* family after differentiation between Poaceae and

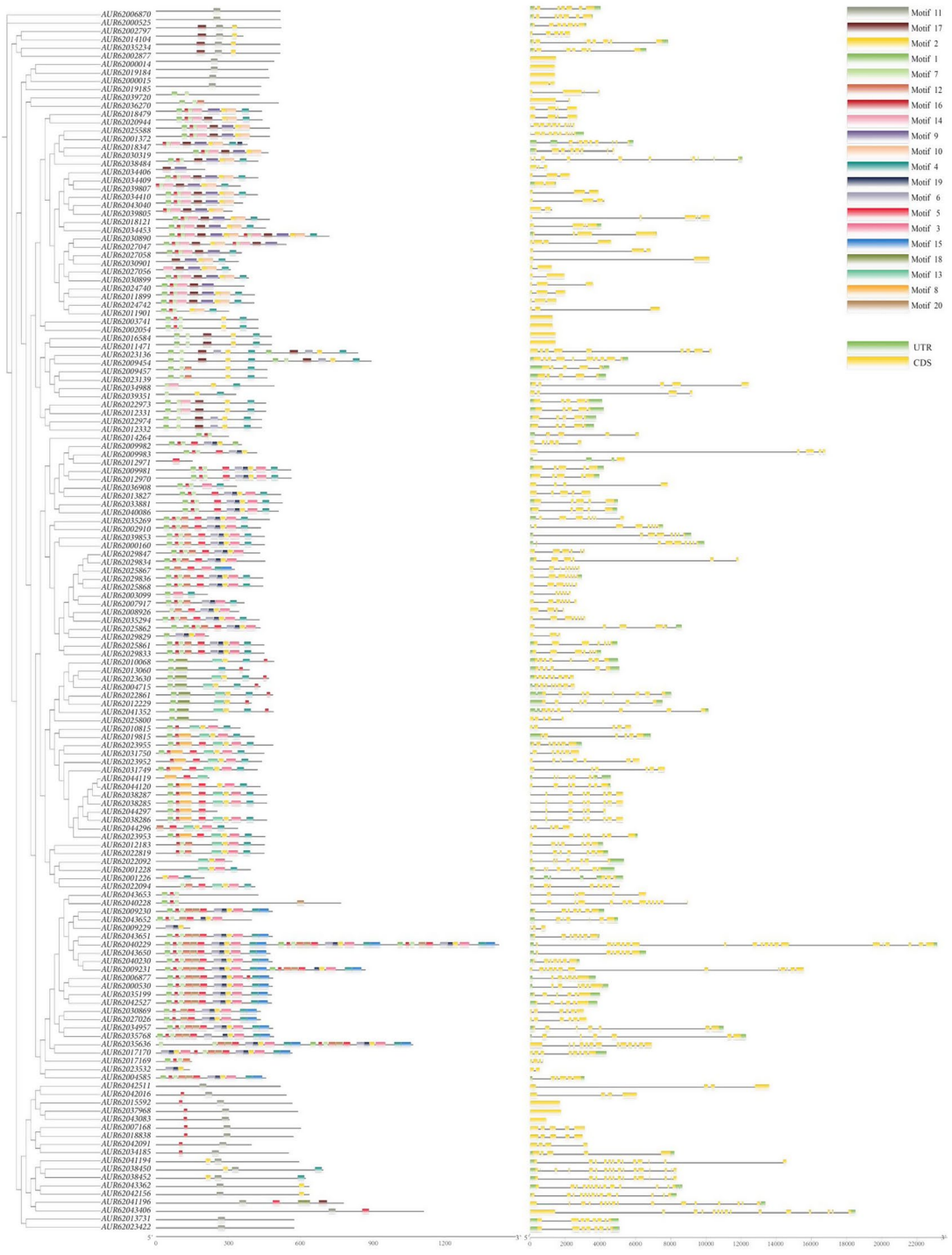

Fig. 2 Intraspecific clustering, conserved motifs, and gene structure analysis of the *AAT* gene family in quinoa. (**A**) Phylogenetic tree of the *AAT* gene family in quinoa; (**B**) 20 conserved *AAT* protein motifs, with different colored squares representing regions of conserved motifs and black lines representing regions without conserved sequences; (**C**) The exon/intron structure composition of the *AAT* gene, with green squares representing upstream/downstream regions, yellow squares representing exons, and black lines representing introns

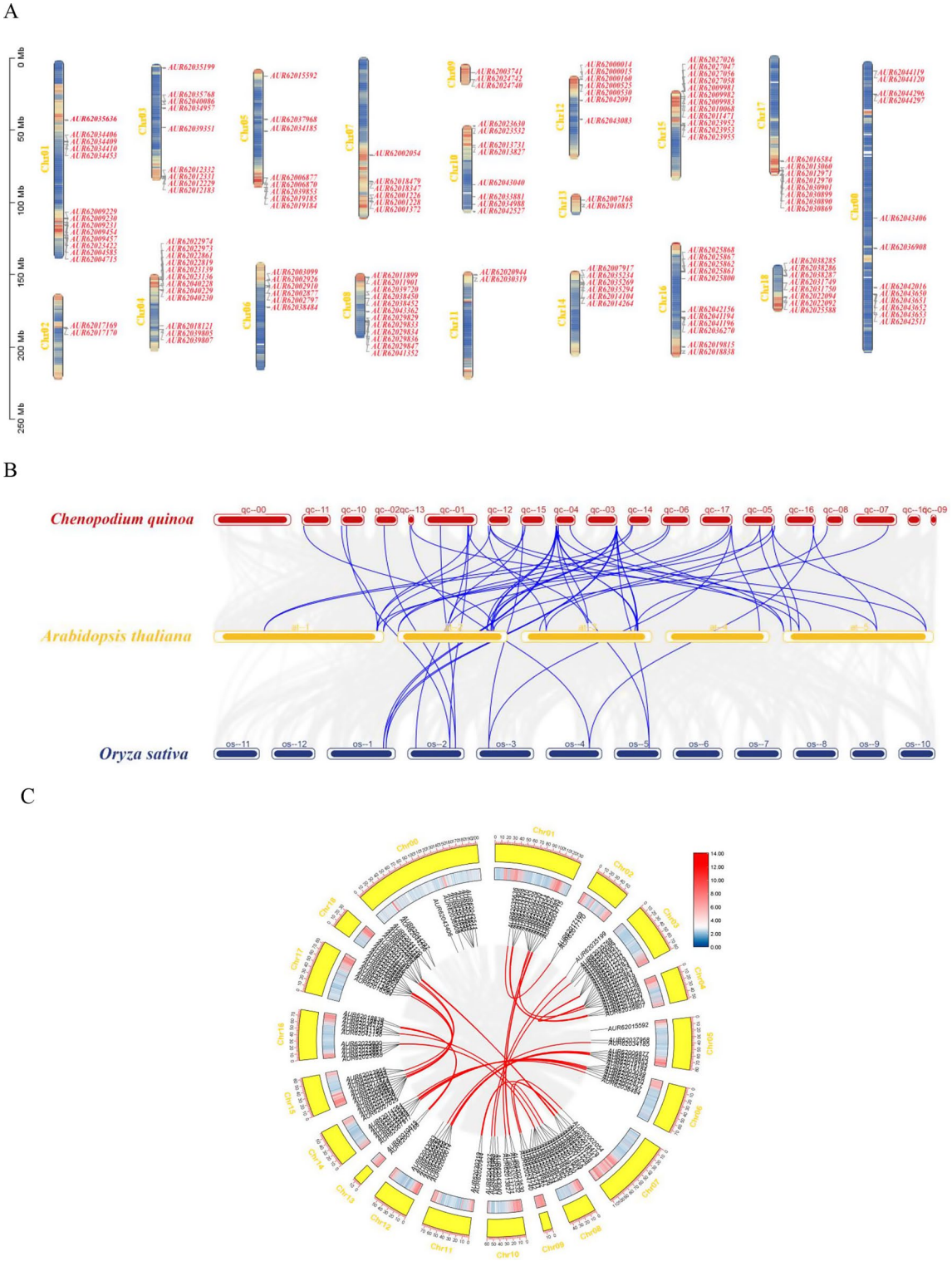

Fig. 3 (**A**) The distribution of 147 *CqAAT* genes identified on chromosomes. Red on chromosomes represents high density, while blue represents low density. (**B**) Inter species collinearity analysis of quinoa, rice, and Arabidopsis. The blue lines indicate collinearity between gene pairs. (**C**) The collinearity map of different *AAT* genes in quinoa. The red lines indicate collinearity between gene pairs. The first circle from the inner layer to the outer layer represents gene density, and the second circle represents chromosome coordinates

Chenopodiaceae. (Fig. 3B). In order to elucidate the evolutionary relationship between the *AAT* gene family in quinoa, we constructed a collinear map of the *AAT* genes in quinoa and found that 44 pairs of *AAT* genes had collinear relationships among the 18 chromosomes of quinoa (Fig. 3C).

Analysis of the cis acting components of the promoter

The cis acting regulatory elements are transcription factors that regulate gene expression. To further explore the potential biological functions of the *AAT* gene family genes in quinoa, PlantCARE was used to predict the promoter elements of 147 *AAT* gene family genes. Classify cis acting elements into five categories, namely phytohormone responsiveness (including ABRE, CGTCA-motif, CARE-motif, etc.), tissue-specific expression (including ARE, RY element), stress responsiveness (including MBS, LTR, GC motif, etc.), light responsiveness (including G-box, GT1 motif, MRE, etc.), and cell cycle regulation (CAT box, MSA like, etc.) (Fig. 4). We found that there are a large number of cis acting elements related to endogenous hormones and light response, and all genes contain cis acting elements related to endogenous hormone response and light response. The number of cis acting elements related to methyl jasmonate and salicylic acid is the highest, and the types of cis acting elements related to auxin gibberellin are the highest. Most genes contain at least one cis acting element related to stress response, with the highest number of cis acting elements involved in low-temperature response. These results indicate that *CqAATs* are not only involved in regulating plant growth, but also in various physiological processes related to hormone signaling responses and abiotic stress responses.

Expression patterns of *CqAAT* gene family in different tissues

Display the tissue-specific expression pattern of *AAT* genes in quinoa using a heatmap (Fig. 5), including the transcription levels of *AAT* in roots, stems, leaves, flowers, and fruits. There are significant differences in the expression levels of *AAT* genes in various tissues of quinoa. Genes such as *AUR62012183*, *AUR62022819*, *AUR62042156*, *AUR62006877* and *AUR62000530* have high expression levels in roots, *AUR62012183*, *AUR62000530* and *AUR62006877* have high expression levels in stems, *AUR62009457*, *AUR62023139* and *AUR62003741* has high expression levels in leaves, and *AUR62011899* is not expressed in roots, stems, or leaves. The above indicates that *CqAATs* genes are expressed in a tissue-specific manner in quinoa. The expression patterns of the *AAT* gene in the flowers and fruits of two different colored quinoa are basically the same, indicating that the *AAT* gene is highly conserved in the evolutionary process. Through heatmap, it was found that *AUR62023955* had highest expression levels in white quinoa flowers, and *AUR62009457* had highest expression levels in yellow quinoa flowers; The genes with the highest expression levels in the fruits of white sweet quinoa and yellow bitter quinoa are *AUR62038452* and *AUR62043362*. *AUR62001226*, *AUR62025868*, and *AUR62009230* are not expressed in two types of quinoa fruits. We speculate that the *AAT* gene is also expressed in a tissue-specific manner in the flowers and fruits of quinoa (Table S2).

Expression pattern of *CqAATs* gene under drought stress

In order to investigate whether *CqAATs* gene expression is related to drought stress, based on transcriptome data from previous drought stress studies conducted by our research group, a gene expression heatmap was constructed. The results showed that compared to the control group, 13 genes were significantly down regulated and 9 genes were significantly up-regulated in the drought treatment group; Compared with the control group only the expression levels of *AUR62027047* and *AUR62018121* were significantly increased, while the expression levels of *AUR62040086* and *AUR62038450* were significantly decreased in rehydration treatment. We found that the expression levels of *AUR62035636*, *AUR62018121*, and *AUR62017170* increased after drought treatment, while the expression levels decreased after rehydration, which was similar to the control group; The expression levels of genes such as *AUR62027047*, *AUR62035199*, *AUR62023955*, *AUR62041352*, and *AUR62001226* decreased after drought treatment, while their expression levels increased after rehydration, similar to the control group; It is speculated that these genes may be key genes in response to drought stress in quinoa, and they belong to the *AAP*, *ATLb*, *GAT*, *AUX*, and *ProT* subgroups (Fig. 6A, Table S3).

Expression pattern of *CqAAT* gene under waterlogging stress

Based on transcriptome sequencing data, this study analyzed the expression characteristics of the *AAT* gene family in quinoa in response to waterlogging stress. The results showed that under 7 days of continuous waterlogging stress, three genes, *AUR62030901*, *AUR62027047*, and *AUR62040086*, showed a significant downregulation trend, while 13 genes including *AUR62009981*, *AUR62023955*, *AUR62031750*, and *AUR62025861* showed significant upregulation. Compared with the control group, 7 genes were significantly down regulated and 13 genes were significantly up regulated after rehydration treatment. Among them, 9 genes (including *AUR62023955*, etc.) continued to be up regulated during both waterlogging and rehydration periods, but

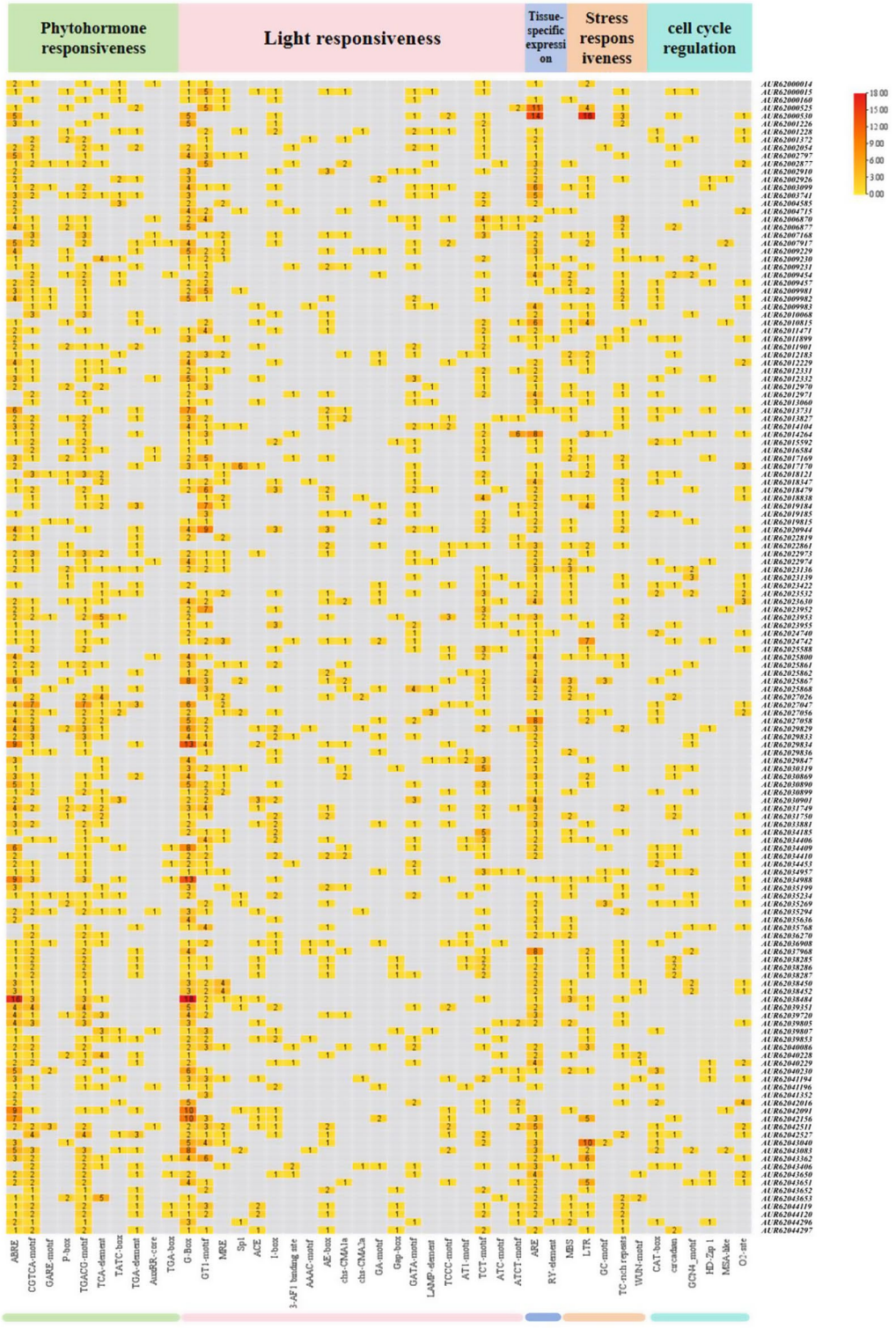

Fig. 4 Analysis of the cis-acting elements of the *CqAATs* genes. The horizontal axis represents different biological processes with different colors and is divided into different cis acting elements; The vertical axis represents the gene name; The numbers in the figure represent the number of cis acting components

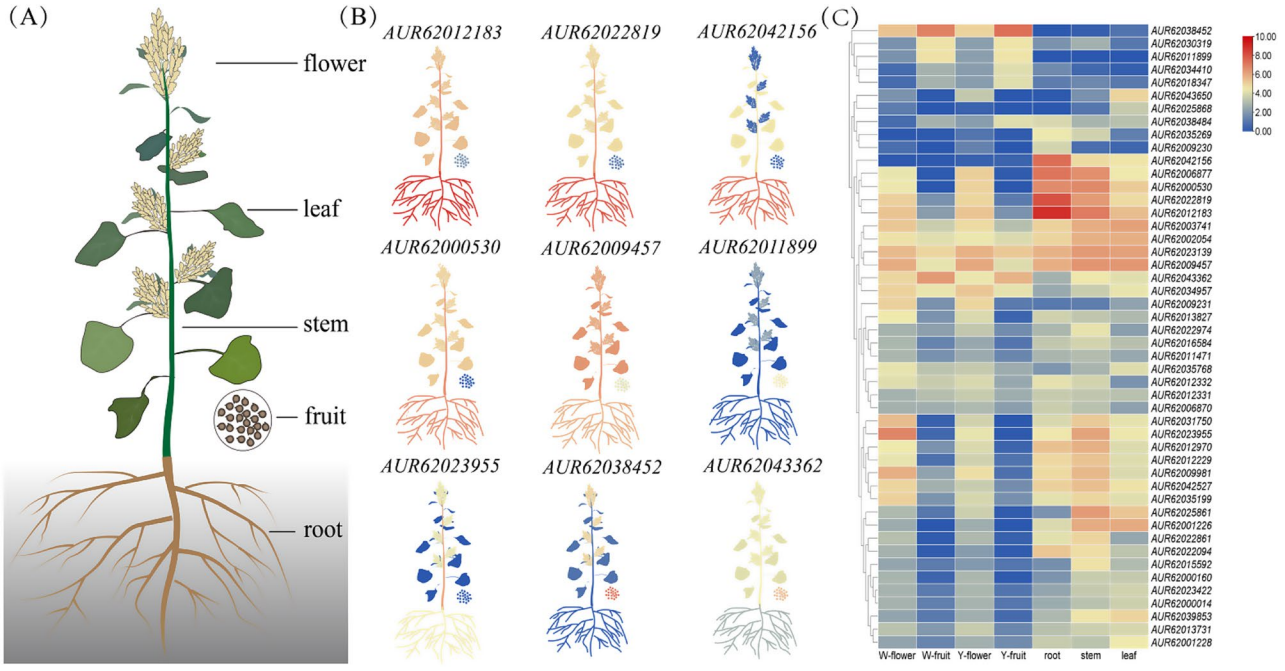

Fig. 5 Expression profiles of *CqAAT* gene in different tissues. (**A**) Schematic diagram of different tissues of quinoa. (**B**) Schematic diagram of the expression of 9 genes in yellow quinoa tissue. Different colors indicate the expression level of each tissue, with red indicating high expression level and blue indicating low expression level. (**C**) Heatmap of gene expression of *CqAATs* in different tissues. The horizontal axis represents different tissues; The vertical axis represents the gene name. Red indicates high expression level, while blue indicates low expression level

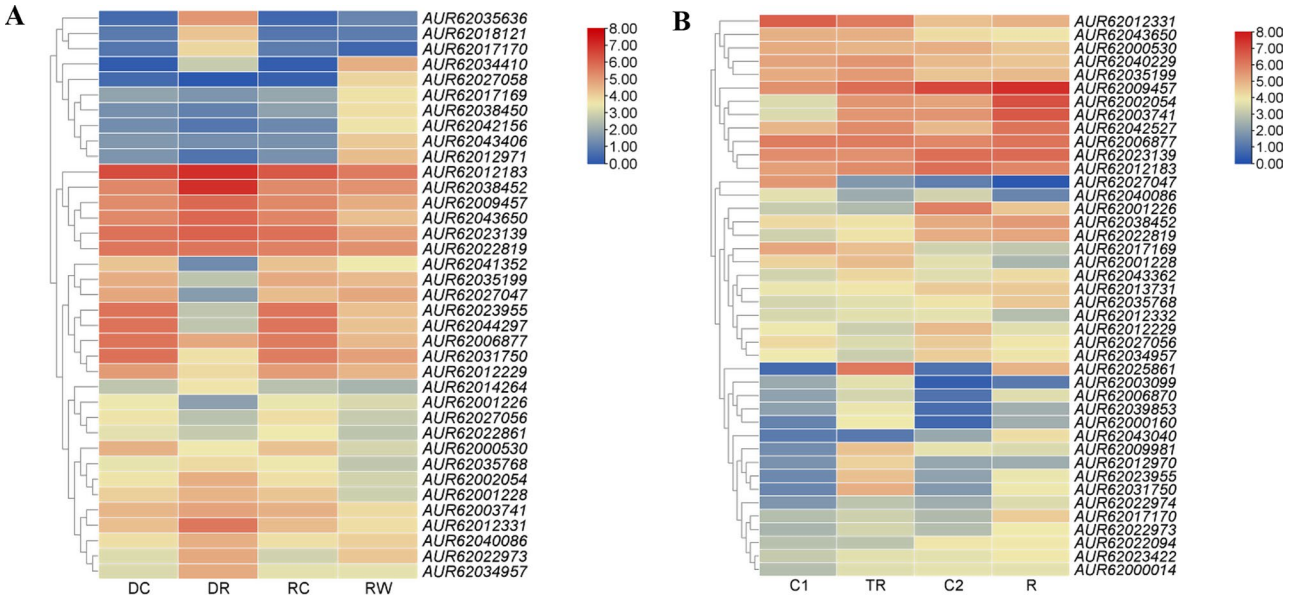

Fig. 6 Expression profile of *AAT* gene in quinoa under water stress. Red in the heatmap indicates high expression level, while blue indicates low expression level. (**A**) Expression profile of *AAT* gene in quinoa under drought stress. DC group represents 5 days drought control. DR group represents 5 days of drought. RC group represents 1 days of rewatering control. RW group represents 1 days of rewatering. (**B**) Expression profile of *AAT* gene in quinoa under waterlogging stress. C1 group represents 7 days waterlogging control; TR group represents 7 days of waterlogging. C2 group represents 7 days recovery control. R group represents 7 days of recovery

the difference in expression was reduced compared to the stress stage, indicating that these *CqAATs* may be involved in the rapid response mechanism of plants to waterlogging stress. From the heatmap, it can be seen that there are still significant differences between the experimental group and the original control group after 7 days of rehydration, indicating that short-term rehydration treatment is not sufficient to fully restore the metabolic network of the plants to steady state. Systematic evolutionary analysis shows that *AUR62031750* and *AUR62023955*, which are consistently highly expressed, belong to the *GAT* subfamily (Fig. 6B, Table S4).

Expression pattern of Cq*AAT* gene under high temperature stress

Based on transcriptome sequencing data, this study analyzed the response patterns of members of the quinoa *AAT* gene family to high temperature stress. Differential expression analysis showed that a total of 35 significantly differentially expressed genes were identified in the high emperature sensitive cultivars, of which 22 genes showed upregulated expression and 13 genes were downregulated; 32 DEGs were detected in the high temperature resistant cultivars, including 17 upregulated genes and 15 downregulated genes. It is worth noting that 29 genes in both cultivars showed the same expression pattern under high temperature stress, including 17 upregulated and 12 downregulated genes; However, the sensitive cultivars has five upregulated genes (*AUR62002054*, *AUR62003741*, *AUR62009231*, *AUR62009457*, *AUR62022819*), suggesting that these genes may play a negative regulatory role in heat tolerance (Fig. 7A, Table S5).

Expression pattern of *CqAAT* gene under low temperature stress

Based on transcriptome sequencing data, this study analyzed the dynamic regulatory characteristics of *AAT* gene family members in quinoa under cold stress (5℃) and freeze injury (-2℃). Among the low temperature sensitive cultivars, there were 10 significantly differentially expressed genes under cold damage, of which 8 were upregulated and 2 were downregulated; There are 12 significantly different genes under freeze injury, of which 7 are upregulated and 5 are downregulated. In the low-temperature tolerant cultivars, there are 16 significantly differentially expressed genes under cold damage, of which 4 are upregulated and 12 are downregulated; There are 13 significantly different genes under freeze injury, of which 7 are upregulated and 6 are downregulated. Both cultivars showed significant upregulation of *AUR62041196*, *AUR62043040*, and *AUR62043406* under cold and freeze conditions, indicating that these genes play a critical

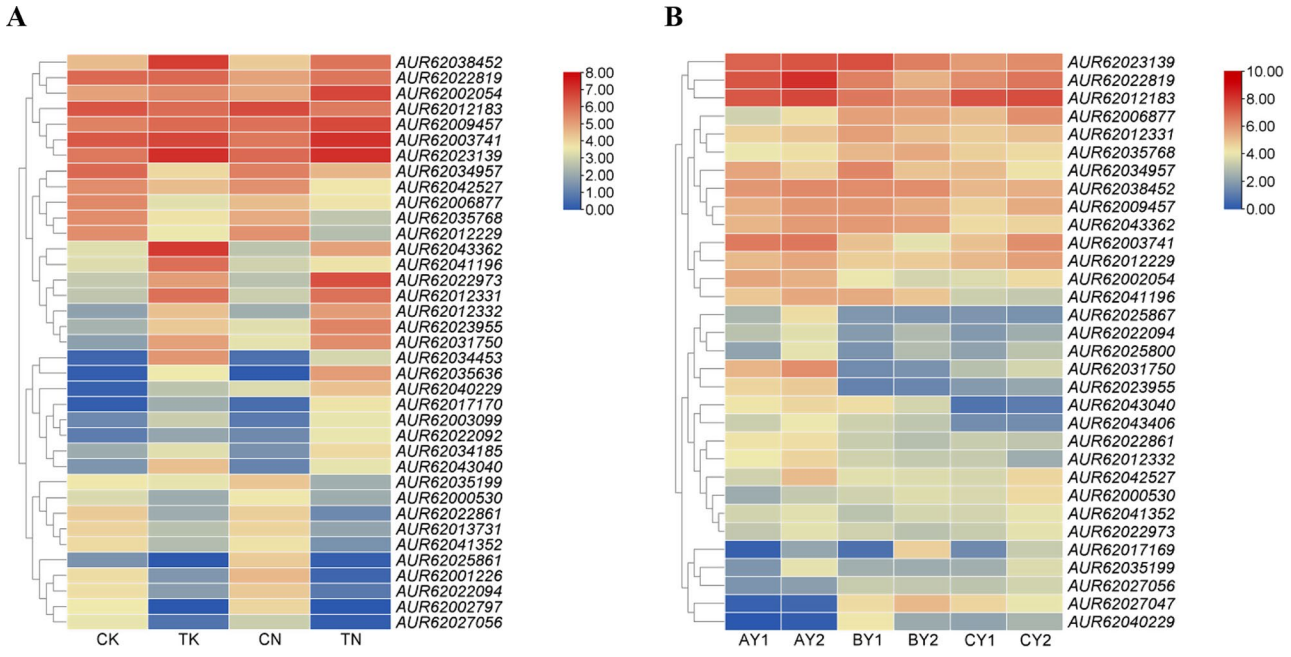

Fig. 7 Expression profile of *AAT* gene in quinoa under temperature stress. Red in the heatmap indicates high expression level, while blue indicates low expression level. (**A**) Expression profile of *AAT* gene in quinoa under high temperature stress. CK group represents normal temperature of heat resistant type. TK group represents heat damage of heat resistant type. CN group represents normal temperature of thermal sensitive type. TN group represents normal temperature of thermal sensitive type. (**B**) Expression profile of *AAT* gene in quinoa under low temperature stress. AY1 group represents freeze injury of low temperature sensitive type. BY1 group represents cold damage of low temperature sensitive type. CY1 group represents normal temperature of low temperature sensitive type. AY2 group represents freeze injury of low temperature resistant type. BY2 group represents cold damage of low temperature resistant type. CY2 group represents normal temperature of low temperature resistant type

role in quinoa's response to low temperature stress. The expression levels of *AUR62017169* in the low temperature tolerant cultivar increased under cold damage conditions, showing significant differences compared to the low temperature sensitive cultivar. However, their expression levels were consistent under freeze injury conditions, indicating that this genes is key genes in regulating cold stress in quinoa. *AUR62017169* belongs to the *AAP* subfamily, *AUR62043040* belongs to the *ATLb* subfamily, and *AUR62041196* and *AUR62043406* belong to the *CAT* subfamily. The *GAT* subfamily genes *AUR62031750* and *AUR62023955* exhibit typical freeze injury specific activation patterns. Their expression levels did not show significant differences compared to the control group under cold damage stress, but were significantly upregulated under cold damage conditions. It is speculated that these two genes play a key role in quinoa's response to freeze injury stress (Fig. 7B, Table S6).

Analysis of the expression pattern of *CqAAT* gene in different fertilizers and different dosages

Expression pattern analysis of CqAAT gene under different nitrogen applications

To determine the role of *CqAAPs* in regulating quinoa's resistance to various nutritional stresses, we studied their transcriptional responses under these conditions. Nitrogen is a key element for plant growth, involved in the synthesis of proteins, nucleic acids, and chlorophyll, and is crucial for plant growth, development, and photosynthesis. In red quinoa, four nitrogen responsive genes (*AUR62012331*, *AUR62002054*, *AUR62038452*, *AUR62035199*) were significantly upregulated under low nitrogen stress; However, high nitrogen stress significantly upregulated *AUR62023955* and *AUR62035199*. It is worth noting that AUR62023955 shows high expression under both low and high nitrogen stress, suggesting that it may be a core regulatory hub for regulating nitrogen balance. In sharp contrast to red quinoa, no significantly differentially expressed genes were detected in white quinoa under nitrogen stress, indicating that red quinoa may be a high nitrogen sensitive strain, while white quinoa is a high nitrogen tolerant strain (Fig. 8A, Table S7).

Expression pattern analysis of CqAAT gene under different phosphorus applications

Phosphorus is a key element for plant growth and development, involved in energy metabolism, nucleic acid synthesis, and signal transduction. This study reveals the expression patterns of the *AAT* gene family in red and white quinoa under phosphorus stress. Under low phosphorus stress, three genes *AUR62002054*, *AUR62023955* and *AUR62035199* were significantly upregulated in red quinoa; There is a gene *AUR62043362* significantly downregulated in white quinoa. Under high phosphorus stress, there were no significant changes in the genes of both strains. *AUR62002054* belongs to the *ANT* subfamily, *AUR62023955* belongs to the *GAT* subfamily, *AUR62035199* belongs to the *AAP* subfamily, and *AUR62043362* belongs to the *CAT* subfamily (Fig. 8B, Table S8).

Expression pattern analysis of CqAAT gene under different potassium applications

Potassium is a key mineral for plant growth, essential for maintaining cell osmotic pressure, regulating stomatal

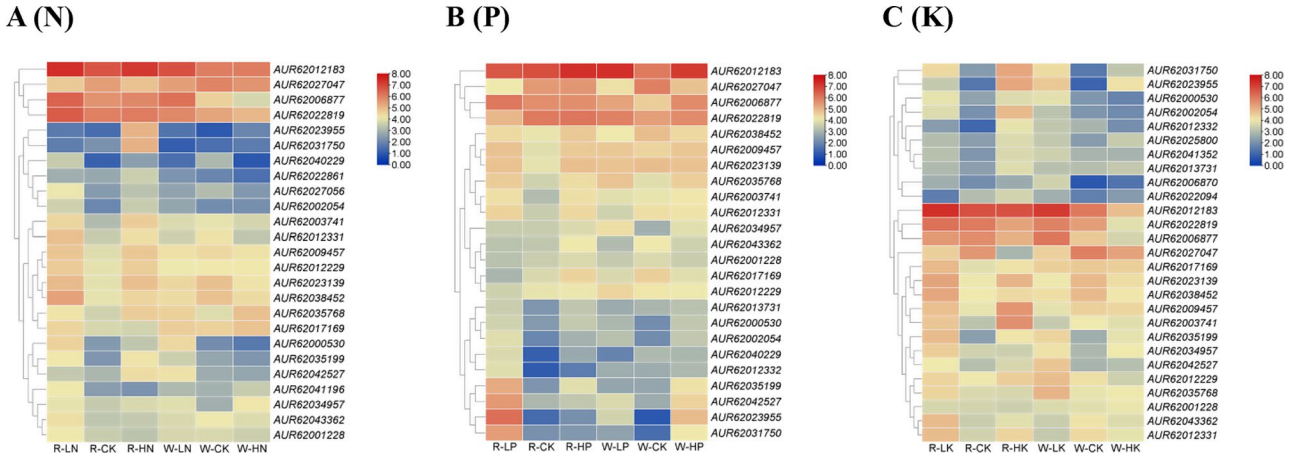

Fig. 8 Expression profile of *AAT* gene in quinoa under different fertilizers and at different dosages. R represents red quinoa and W represents white quinoa. Red in the heatmap indicates high expression level, while blue indicates low expression level. (**A**) Expression profile of *AAT* gene in quinoa under different nitrogen applications. LN represents low nitrogen; CK represents control group; HN represents high nitrogen. (**B**) Expression profile of *AAT* gene in quinoa under different phosphorus applications. LP represents low phosphorus; CK represents control group; HP represents high phosphorus. (**C**) Expression profile of *AAT* gene in quinoa under different potassium applications. LK represents low potassium; CK represents control group; HK represents high potassium

opening and closing, promoting photosynthesis, and enhancing plant stress resistance. Based on transcriptome sequencing results, this study found that there were 7 significantly upregulated genes in red quinoa under low potassium conditions, including *AUR62012331*, *AUR62002054*, *AUR62038452*, *AUR62023139*, *AUR62023955*, *AUR62035199*, *AUR62003741*; *AUR62027047* was significantly downregulated in expression; Under high potassium conditions, *AUR62002054* and *AUR62023955* were significantly upregulated, while *AUR62027047* was significantly downregulated. In white quinoa, *AUR62023955* was significantly upregulated and *AUR62009231* was significantly downregulated under high potassium conditions. There are significantly more genes in response to different potassium fertilizer application rates in red quinoa, indicating that this cultivar is more sensitive to potassium fertilizer (Fig. 8C, Table S9).

Discussion

Quinoa, as a fully nutritious crop, has important economic value, but the emergence of adverse environments often leads to a decrease in quinoa yield and quality. Therefore, it is of great significance to explore and study some key stress resistant genes for the future cultivation of excellent quinoa varieties, among which transcription factors, as one of the most studied transcription regulatory factors, play an important role in regulating plant response to external environmental stress. In recent years, due to the availability of the whole genome sequence of quinoa, research on the quinoa gene family has been reported, such as *VOZ* [25], *GST* [26], *WRKY* [27], *HAK* [28]、*ACS* [29]etc., but there have been no reports on the quinoa *AAT* gene family. Amino acid transporters (*AAT*s) play important roles in plants, responsible for transmembrane transport of amino acids and participating in multiple physiological processes of plant growth and development, including long-distance amino acid transport, absorption of amino acids from soil, and response to pathogens and abiotic stress [30]. So far, *AAT* has been recognized and thoroughly studied in many plant species, with 63 *AAT* genes in Arabidopsis [5], 84 in Phaseolus vulgaris [6], 85 in rice [7], 189 in soybean [8], 296 in common wheat [9], 94 in foxtail millet [10], 104 in Tartary buckwheat [11], and 72 in potatoes [12]. This study was based on the whole genome data of quinoa and used bioinformatics methods to screen 147 *CqAATs* genes, which were unevenly distributed on 18 chromosomes and divided into 12 subgroups, consistent with Arabidopsis. The overall average hydrophilicity index of the *AAT* gene in quinoa is 0.537, indicating that it belongs to a hydrophobic protein, which is similar to the hydrophobic properties of general transporters. Ten genes lack introns, which may be closely related to their specific regulation or function. The number of *AAT* genes on chromosome 1 is the highest, which may be related to gene density and chromosome size. Our analysis found that 121 genes are located on the plasma membrane, and so far all *AtAAPs* genes are located on the plasma membrane. The entry and exit of amino acids in various organelles are mediated by corresponding transporters and during the transport of amino acids, it is often accompanied by the transmembrane transport of hydrogen ions [31]. Quinoa is a heterozygous tetraploid [32]. Previous studies on orthologs and collinearity have found that it is not simply obtained by doubling the A and B subgenomes provided by donors, but involves complex chromosome fusion processes. The collinearity between quinoa chromosomes in this study also proves this.

The expression of amino acid transporters varies significantly in different plant tissues, and these differences are closely related to their functions in plant growth, development, and nutrient allocation. This study downloaded the gene expression data of various tissues of yellow quinoa and white quinoa from the NCBI database, and the results showed that the *AAT* gene was expressed in a tissue-specific manner in quinoa, indicating that different *AAT* proteins may have different functions, and the *AAT* gene expression was highest in the stem, followed by the roots and leaves, with less gene expression in the fruit. *AUR62023139* is highly expressed in all tissues, indicating that these genes are crucial for the overall growth and development of plants. Previous studies have found that tyrosine transporters of the *AAT* gene family in rice are highly expressed in black rice, but not transcribed in non black genotypes, which are related to regulating grain color formation [33]. Therefore, we analyzed the expression patterns of the *AAT* gene in the flowers and fruits of two different colored quinoa and found that the expression patterns were basically the same in both quinoa, indicating that the *AAT* gene was highly conserved in the evolutionary process. Through heat maps, we found that the expression level of *AUR62023955* in white quinoa flowers was significantly higher than that in yellow quinoa, and the expression level of *AUR62038452* in yellow quinoa fruits was significantly higher than that in white quinoa. The tissue specificity of gene expression may be due to the fact that these genes originate from different subgroups, and the *AAT* genes of different subgroups may mediate different physiological processes. For example, we found that genes with higher root expression belong to the *AAP* and *ProT* subgroups, and the first reported amino acid transporter protein in plants is encoded by the *AAP*s family genes [34]. *AAPs* are involved in processes such as root absorption of plant amino acids, xylem loading, phloem loading, exchange between xylem and phloem, and seed loading, affecting the growth and development of plant roots, stems, and leaves [19]. For example, *AtAAP1* and *AtAAP5* are responsible for root

amino acid absorption in Arabidopsis [35–36]. Arabidopsis thaliana At *AUX1* expression can promote lateral root formation in roots, while rice *OsAUX2* and *OsAUX5* are specifically expressed in young roots and may be involved in root growth and development processes [37]. In addition, proline as a free radical scavenger, can regulate the redox homeostasis of plant cells and participate in the defense response of plants against biological stress [38]. *AtProT2* is mainly expressed in the root epidermis and cortex, and plays a role in the absorption of compatible solutes proline and glycine betaine [39]. *AtProT1* is expressed in vascular tissues such as roots, stems, leaves, and flowers, mainly in stems, suggesting that it may play an important role in long-distance transport of proline. *AtProT2* is highly expressed in root epidermal and cortical cells, and is extensively induced in leaves under plant stress conditions, suggesting that it may play an important role in plant stress resistance [39–40]. Our research found that genes with high expression levels in the flower part of quinoa belong to the *ATLa* subfamily, while in Arabidopsis, the *AtLHT* subfamily is mainly responsible for the transport and distribution of lysine and histidine in flower organs, which in turn affects flower development [17, 41]. The genes *AUR62038452* and *AUR62043362* with the highest expression levels in quinoa fruit belong to the *CqCAT* subfamily. *CAT* genes such as *AtCAT2*, *AtCAT3*, and *AtCAT4* in Arabidopsis are highly expressed in leaves and seeds [42].

Abiotic stress can induce the expression of plant related genes, improve plant adaptability to external environments, enhance stress resistance, and ultimately affect plant quality and yield. The loss of function of amino acid transporters can lead to growth inhibition in plants under adverse conditions, such as reduced efficiency of amino acid allocation and utilization under drought or salt stress, which in turn affects the plant's stress resistance ability [36]. In Arabidopsis, the expression of the *AtAAPs* family is inhibited under osmotic pressure stress, while the expression levels of *AtAAP4* and *AtAAP6*, as well as *McAAT*2 in the ice leafed sunflower (Mesembryanthamum crystallized), are downregulated under salt stress [43]. In addition, the expression levels of *AtProT2*, *McAAT1*, and *HvProT* significantly increased under salt stress conditions, and some genes in the rice *OsAAT* family were significantly down regulated or up-regulated under salt and drought stress, including *OsANT3*, *OsAAP6*, and *OsAAP11*, indicating that amino acid transporters may play a key role in abiotic stress [7, 44, 45]. Studies have shown that *CAT* and *AAP1* are functionally conserved, and they participate in plant defense responses to abiotic stress by synergistically regulating the transmembrane transport of cationic amino acids. The promoter region of cotton *CAT* contains cis acting elements related to abiotic stress, and the expression of these genes is significantly affected by treatments such as drought, cold, and salt [46]. Overexpression of *TaAAP1* in wheat enhances salt tolerance by regulating ethylene production [47]. We found that the expression levels of *AUR62031750* and *AUR62023955* in the *GAT* subfamily of quinoa increased under different abiotic stresses and fertilizer treatments. γ-aminobutyric acid (GABA) is a ubiquitous four carbon non reducing amino acid that participates in regulating plant growth, development, and stress resistance as a signaling substance. When plants are subjected to abiotic stress, GABA accumulates in large quantities. *GAT* located on the cell membrane can transport GABA across the membrane and transport GABA from the extracellular matrix to the cytoplasm. When plants are subjected to abiotic stress, reducing the accumulation of reactive oxygen species (ROS) can alleviate the damage to cells caused by stress and help maintain the homeostasis of GABA in cells. Previous studies have found that under heavy metal aluminum stress, the *LcGAT* gene is upregulated and can specifically transport GABA. The accumulation of GABA in leaves promotes the closure of plant stomata, thereby improving plant tolerance [48–49]. Only one *GAT* was found in Arabidopsis, which can transfer GABA with high affinity. When subjected to stress or mechanical damage, plants strongly induce *AtGAT1* expression to accumulate more ABA [50–51]. Xu et al. studied Arabidopsis and demonstrated that the production of guard cell GABA can reduce stomatal opening and transpiration, thereby improving water use efficiency and drought resistance [52]. Overexpression of *PeuGAT3* increases the thickness of xylem cell walls in Arabidopsis and poplar, enhances lignin content in xylem tissues, and increases proline accumulation in poplar leaves, all of which may improve the tolerance of desert poplar to salt and drought stress [53].

AATs can promote the transport of amino acids from source organs to sink organs, thereby improving plant nitrogen absorption and utilization efficiency [54]. When *PsAAP1* in pea (*Pisum sativum* L.) is overexpressed in the phloem and embryo, the allocation of amino acid source pool is improved, significantly enhancing the nitrogen absorption efficiency and utilization efficiency of plants [55]. In Arabidopsis, *AtAAP2* encodes an amino acid osmolase that participates in the exchange of amino acids between the xylem and phloem, transporting the amino acids absorbed by the root system to the aboveground parts. When the *AtAAP2* gene is knocked out, more nitrogen is allocated to the leaves. At different nitrogen fertilizer levels, the chlorophyll content of leaves is generally higher, Rubisco enzyme activity is stronger, and photosynthetic nitrogen use efficiency (NUE) is also higher [56]. In wheat, the *TaATLa1* gene is significantly downregulated under nitrogen deficiency conditions, indicating its possible involvement in nitrogen metabolism and

source sink transport of amino acids [57]. The expression of *GmAAP6* and *GmAAP12* in soybeans is significantly upregulated under low nitrogen conditions, which may affect seed protein content by regulating amino acid transport efficiency [58]. In this study, *AUR62023955* and *AUR62031750* were significantly upregulated under high nitrogen conditions, suggesting their regulation of quinoa's response to nitrogen, providing targets for genetic improvement of quinoa nitrogen utilization and protein accumulation.

This study found that *AAT* in quinoa plays unique roles at different stages and parts of its growth and development, and is involved in quinoa's response to stress and adversity. Providing a research basis for the regulation mechanism of the *AAT* gene in quinoa under abiotic stress is of great significance for improving quinoa quality and stress resistance.

Materials and methods

Identification and physicochemical characterization of quinoa *AAT* gene family members

The whole genome sequence of quinoa was downloaded from NCBI (https://www.ncbi.nlm.nih.gov/). The Arabidopsis *AAT* family protein sequences were downloaded from TAIR (https://www.arabidopsis.org/). The quinoa family genes were characterized by 2 methods: first, the quinoa *AAT* genes were characterized by a bidirectional BLAST method, using the Arabidopsis protein sequences as seed files, and BLAST analysis was performed in TBTools [59]. Second, *AAT* protein Hidden Markov Model (HMM) seed files (PF00324 and PF01490) were obtained from the Pfam database (http://pfam.xfam.org/), and the NCBI-CDD (https://www.ncbi.nlm.nih.gov/cdd) database was used to analyze the candidate genes' Protein sequences were analyzed to exclude candidate proteins that did not contain the AA-permease structural domain and the Aa-trans structural domain and to finalize the quinoa *AAT* family members. Physicochemical properties of the self-identified protein sequences were analyzed using the ExPASy website (https://www.expasy.org/) [60]. Subcellular localization was performed using the Cell-PLoc2.0 website (http://www.csbio.sjtu.edu.cn/bioinf/Cell-PLoc-2/) [61].

Phylogenetic analysis of quinoa *AAT* protein family members

Multiple sequence comparison of *AAT* protein sequences from Arabidopsis thaliana and quinoa was performed using MEGA11 software, and phylogenetic trees were constructed using the neighbor-joining (NJ) method with the parameter Bootstrap set to 1000 times [62]. The evolutionary tree was landscaped using the online tool iTOL (https://itol.embl.de/) [63].

Gene structure and conserved motif analysis of quinoa *AAT* gene family members

TBtools was utilized to identify introns and exons of the *CqAAT* gene [59]. Conserved motifs of quinoa *AAT* family protein sequences were analyzed using MEME online software, and the number of output conserved motifs was set to 20 [64]. The results of the above analysis were integrated and visualized by TBtools software.

Chromosomal localization and covariance analysis of quinoa *AAT* gene family members

Sequences of Arabidopsis and rice *AAT* family proteins were downloaded from TAIR (https://www.arabidopsis.org/) and Rice Genome Annotation Project (http://rice.uga.edu/index.shtml). Information on the distribution of the quinoa *AAT* gene on chromosomes was extracted from the gff3 file of the quinoa genome, and gene duplication events within quinoa species were analyzed by the Multiple Covariance Scanning Toolkit (MCScanX) and visualized by the Advanced Circos tool in TBtools. To explore the co-lineage relationship of *AAT* genes in quinoa and other selected species, co-lineage analysis maps were constructed using MCScanX and visualized using Multiple SyntenyPlot in TBtools [59].

Analysis of cis acting components

A sequence of 2000 bp upstream of the transcription start site of *AAT* gene family members was extracted from the quinoa genome database as the promoter region of the genes, and the cis acting elements were predicted and analyzed using the PlantCARE online website (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/) [65]. Use Excel to filter and analyze the types and quantities of cis acting elements, and use a heatmap to display the types and quantities of cis acting elements in the *AAT* gene [59].

Tissue expression analysis of *AAT* gene family members in quinoa

Download tissue expression data of various organs and tissues during the growth and development of quinoa from the GEO public database on the NCBI website (https://www.ncbi.nlm.nih.gov/sra)(Search numbers SRP278144, SRP226463). This data includes the roots, stems, leaves, flowers, and fruits of yellow quiona and the flowers and fruits of white quinoa. Obtain the corresponding expression level (FPKM) values of each member of the *AAT* gene family through screening. Then use the Heatmap tool in TBtool software to draw an organizational expression heatmap [59](Table S10).

Table 2 Samples information on waterlogging stress

Cultivar	Sampling time	Control	Treatment
Waterlogging resistant type Dianli-1844	7 Days	C1 (7 days waterlogging control)	TR (7 days of waterlogging)
	14 Days	C2 (7 days recovery control)	R (7 days of recovery)

Table 3 Samples information on drought stress

Cultivar	Sampling time	Control	Treatment
Drought resistant type Dianli-129	5 Days	DC (5 days drought control)	DR (5 days of drought)
	6 Days	RC (1 days of rewatering control)	RW (1 days of rewatering)

Table 4 Samples information on high temperature stress

Cultivar	Sample type	Treatment	Namber
Dianli–3101	Heat resistant type	40℃	TK (Heat damage)
		22℃	CK (normal temperature)
Dianli–3051	Thermal sensitive type	40℃	TN (Heat damage)
		22℃	CN (normal temperature)

Table 5 Samples information on low temperature stress

Cultivar	Material properties	Number Name	Treatment
Dianli-281	Low temperature resistant type	AY2	-2℃ (freeze injury)
		BY2	5℃ (Cold damage)
		CY2	22℃ (normal temperature)
Dianli-2324	Low temperature sensitive type	AY1	-2℃ (freeze injury)
		BY1	5℃ (Cold damage)
		CY1	22℃ (normal temperature)

Expression analysis of *AAT* gene in quinoa under different abiotic stress conditions

In order to investigate whether *CqAAT* gene expression is related to adversity stress, a gene expression heatmap was constructed to analyze the expression of *AAT* genes in quinoa leaves under different stresses based on quinoa transcriptome data from the previous high temperature, low temperature, drought, and waterlogging stress studies of our group [66–68] (Tables S11-S14). Perform differential expression analysis between sample groups using DESeq2 [69–70] to obtain a set of differentially expressed genes between two biological conditions. After differential analysis, it is necessary to use the Benjamin Hochberg method to perform multiple hypothesis testing correction on the hypothesis testing probability (P value) to obtain the False Discovery Rate (FDR). The screening criteria for differentially expressed genes are | log2Fold Change|>=1 and FDR < 0.05. The material is planted in a greenhouse at the Yunnan Agricultural University base in Xundian County, Kunming(102°41′E, 25°20′N). Samples were taken during the seedling stage (Six leaf stage) under high temperature, low temperature, and drought stress, while samples were taken during the grain filling stage under waterlogging stress. The sampling site is the leaf and the sample information for different stress treatments is shown in Tables 2, 3, 4 and 5.

Table 6 Sample information on different fertilization conditions

Number Name		Nitrogen (kg/hm$^{2)}$	phosphorus (kg/hm$^{2)}$	potassium (kg/hm$^{2)}$
Red quinoa Dianli-1299	White quinoa Dianli-71			
R1 (LN)	W1 (LN)	0	112.5	112.5
R2 (CK)	**W2 (CK)**	**112.5**	**112.5**	**112.5**
R3 (HN)	W3 (HN)	337.5	112.5	112.5
R4 (LP)	W4 (LP)	112.5	0	112.5
R5 (HP)	W5 (HP)	112.5	337.5	112.5
R6 (LK)	W6 (LK)	112.5	112.5	0
R7 (HK)	W7 (HK)	112.5	112.5	337.5

Expression analysis of quinoa *AAT* gene under different fertilization conditions

In order to investigate whether the expression of *CqAAT* gene responds to different fertilization conditions, a gene expression heatmap was constructed based on transcriptome data of red quinoa (Dianli-1299) and white quinoa (Dianli-71) leaves published in our research group. The expression of *AAT* gene in quinoa leaves under different nitrogen, phosphorus, and potassium supply conditions was analyzed [71–73](Table S15-S17). Perform differential expression analysis between sample groups using DESeq2 [69–70] to obtain a set of differentially expressed genes between two biological conditions. After differential analysis, it is necessary to use the Benjamin Hochberg method to perform multiple hypothesis testing correction on the hypothesis testing probability (P value) to obtain the False Discovery Rate (FDR). The screening criteria for differentially expressed genes are | log2Fold Change|>=1 and FDR < 0.05. The material is planted in a greenhouse at the Yunnan Agricultural University base in Xundian County, Kunming(102°41′E, 25°20′N). Sampling at the six leaf stage, with the sampling site being the leaves. The sample information for different fertilizers and dosages is shown in Table 6.

Conclusions

In this study, we identified 147 *AAT* genes in the quinoa genome sequence. Multiple analyses, including the construct phylogenetic tree, gene exon–intron structure and motif, indicated that the *CqAAT* gene family was divided into 12 subgroups. Organizational expression analysis revealed significant differences in the expression of the *CqAAT* gene, with less expression in fruits and more expression in roots, stems, leaves, and flowers.

The expression analysis of two genes *AUR62031750* and *AUR62023955* in the *CqGAT* subfamily was found to be upregulated under various abiotic stresses, including high temperature, low temperature, waterlogging, and drought, as well as different nitrogen, phosphorus, and potassium fertilizer application rates. This indicates that they play a role in the growth and development regulation of *CqAATs* under abiotic stress. Quinoa is rich in amino acids, and in-depth research on the function of *CqAATs* is needed in the future, which is of great significance for improving the quality and breeding efficiency of quinoa, as well as enhancing its stress resistance and adaptability.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12864-025-11491-3.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

Supplementary Material 5

Supplementary Material 6

Supplementary Material 7

Supplementary Material 8

Supplementary Material 9

Supplementary Material 10

Supplementary Material 11

Supplementary Material 12

Supplementary Material 13

Supplementary Material 14

Supplementary Material 15

Supplementary Material 16

Supplementary Material 17

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Author contributions

H.L. Writing-Original Draft, Methodology. C.J. Writing-Original Draft, Formal analysis. J.L. Writing-Original Draft, Formal analysis. P.Z. Conceptualization, Writing-Review & Editing. L.L. Formal analysis, Investigation. L.H. Methodology, Visualization. R.L. collected feld samples. X.W. Formal analysis, Investigation. G. J. Investigation. L.Z. Methodology, Visualization. Y. B. Investigation. P. Q. Supervision, Project administration, Funding acquisition.

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Data availability

The datasets generated and/or analyzed during the current study are available in the [NCBI] (National Center for Biotechnology Information) repository, [PRJNA939366]，[PRJNA1039692]，[PRJNA857812], [PRJNA946047], [PRJNA839110].

Declarations

Ethics approval and consent to participate

Experimental research on plants complies with relevant institutional, national, and international guidelines and legislation.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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