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Abstract

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Full Text

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Identification and functional analysis of the *CqAMTs* gene family in quinoa during arbuscular mycorrhizal symbiosis

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Abstract: Arbuscular mycorrhizal fungi (AMF) can assist plant roots in absorbing ammonium nitrogen through their mycelial networks; this uptake is mediated by ammonium transporters (AMTs). To reveal the molecular mechanism by which AMF regulate ammonium uptake in quinoa, this study cloned members of the quinoa *AMT* gene family and, using sequence alignment and phylogenetic analysis, yeast functional complementation, real-time fluorescence quantitative PCR, and subcellular localization techniques, systematically analyzed

the evolutionary characteristics, transport functions, tissue expression patterns, and protein localization of *CqAMTs*. The results showed that: (1) the quinoa genome contains 12 members of the *AMT* family, divided into two subfamilies, *AMT1* and *AMT2*. (2) *CqAMT1;1*, *CqAMT1;2*, *CqAMT1;4*, and *CqAMT1;8* exhibit ammonium-transport activity under low-ammonium conditions and are localized to the cytoplasmic membrane. (3) Members of the *CqAMTs* family show tissue-specific expression in roots, stems, leaves, and flowers; the expression levels of some *CqAMTs* are regulated by AMF colonization and ammonium nitrogen levels. This study clarifies the evolutionary classification, protein localization, and ammonium-transport functions of the quinoa *AMT* gene family, confirms that multiple *CqAMT* members participate in AMF-mediated quinoa ammonium uptake, and provides a theoretical basis for analyzing the mechanism by which AMF promote efficient ammonium absorption in quinoa.

Key words: quinoa; ammonium transporter (AMT); arbuscular mycorrhizal fungi (AMF); mycorrhizal induction; ammonium transport; gene family identification

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Abstract: Arbuscular mycorrhizal fungi (AMFs) facilitate plant root absorption of ammonium nitrogen through their mycelial networks, a process mediated by ammonium transporters (AMTs). To elucidate the molecular mechanisms by which AMFs regulate quinoa ammonium uptake, this study cloned members of the quinoa *AMT* gene family and employed sequence alignment, phylogenetic analysis, yeast functional complementation assays, real-time quantitative polymerase chain reaction, and subcellular localization. The results were as follows: (1) The quinoa genome

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contains 12 *AMT* family members, classified into two subfamilies: *AMT1* and *AMT2*. (2) *CqAMT1;1*, *CqAMT1;2*, *CqAMT1;4*, and *CqAMT1;8* exhibit ammonium transport activity under low-ammonium conditions and are localized to the plasma membrane. (3) *CqAMT* family members demonstrate tissue-specific expression in roots, stems, leaves, and flowers, with some *CqAMT* expression levels regulated by AMF colonization and ammonium nitrogen levels. This study clarifies the evolutionary classification, protein localization, and ammonium transport functions of the quinoa *AMT* gene family, confirming that multiple *CqAMT* members participate in AMF-mediated quinoa ammonium uptake, thereby providing a theoretical foundation for understanding the mechanism by which AMFs enhance efficient ammonium absorption in quinoa.
Key words: quinoa (*Chenopodium quinoa*), ammonium transporter (AMT), arbuscular mycorrhizal fungi (AMF), mycorrhizal induction, ammonium transport, gene family identification

Nitrogen is an indispensable mineral nutrient for plant growth and development. It is mainly absorbed and utilized by plants in the forms of ammonium nitrogen (NH_4^+) and nitrate nitrogen (NO_3^-) (Rivero-Marcos, 2025). Nitrogen deficiency leads to slow plant growth, reduced photosynthesis, and decreased yield (He et al., 2026). Therefore, efficient uptake and transport of ammonium nitrogen (NH_4^+) are crucial for plant nitrogen nutrition and utilization. Transmembrane uptake of ammonium nitrogen in plants is mainly mediated by ammonium transporters (AMTs), which are primarily divided into two subfamilies, *AMT1* and *AMT2* (Von et al., 2014). Among them, the *AMT1* subfamily is mostly composed of high-affinity transporters and mainly governs ammonium capture by roots under low-ammonium conditions; the *AMT2* subfamily is more often involved in long-distance ammonium transport and inter-tissue redistribution. Together, the two complete the supply of nitrogen throughout the plant life cycle. In recent years, the *AMT* families of plants such as *Arabidopsis thaliana* (Loque et al., 2006), rice (Konishi et al., 2021), maize (Gu et al., 2013), soybean (Yang et al., 2023), wheat (Wang et al., 2025), and cassava (Xia et al., 2023) have been identified.

Arbuscular mycorrhizal fungi (AMF), as key symbiotic microorganisms in soil, form large numbers of hyphae within host plant roots and in rhizosphere soil. Their extraradical hyphae and plant roots jointly construct an extensive hyphal network, efficiently assisting plants in absorbing water and mineral nutrients and thereby establishing a symbiotic relationship (Fei et al., 2022; Duan et al., 2024). Numerous studies have shown that AMF can induce the specific expression of plant *AMT* genes and enhance the transport of NH_4^+ from the

symbiotic interface into the plant. For example, Hui et al. (2022) identified maize *ZmAMT3;1* as a key transporter in the mycorrhizal ammonium uptake pathway; Perez-Tienda et al. (2014) proposed that rice *OsAMT3;1* participates in mycorrhizal ammonium uptake; Koegel et al. (2013) found that sorghum *SbAMT3;1* and *SbAMT4* are localized to the periarbuscular membrane; Wang et al. (2022) found that *LjAMT2;2* of *Lotus japonicus* is upregulated in arbuscule-containing cells; Kobae et al. (2010) identified five AMF symbiosis-induced *AMT* genes in soybean; and Fang et al. (2023) confirmed that pepper *CaAMT2;1*, *CaAMT2;2*, and *CaAMT2;3* are upregulated by AMF induction.

Quinoa (*Chenopodium quinoa*) belongs to the Amaranthaceae and is a grain crop with both cereal-like characteristics and high nutritional value (Vilcacundo et al., 2017). It is native to the Andean region of South America and has extremely strong adaptability to adverse conditions such as salinity, alkalinity, and drought (Yazar et al., 2014). Nitrogen deficiency is one of the main factors limiting the improvement of quinoa yield, and ammonium nitrogen is a nitrogen source form suitable for quinoa utilization. AMF symbiosis can significantly promote quinoa growth and nitrogen accumulation (Yang Shifang et al., 2017). However, the molecular mechanism by which AMF regulates ammonium uptake in quinoa remains unclear. To clarify the roles of the *AMT* gene family in the direct NH_4^+ uptake pathway and the mycorrhizal uptake pathway in quinoa, this study used techniques including gene-family identification, yeast functional complementation experiments, qRT-PCR expression analysis, and subcellular localization to systematically analyze the evolutionary characteristics, transport functions, tissue expression, and AMF symbiotic response patterns of the quinoa *AMT* gene family. The aim is to clarify the functions of quinoa *AMT* genes in ammonium uptake and mycorrhizal symbiosis, and to provide a theoretical basis and genetic resources for revealing the molecular mechanism by which AMF promote efficient ammonium uptake in quinoa.

1 Materials and Methods

1.1 Plant Materials and Cultivation

Quinoa No. 3 was used as the material and cultivated in the greenhouse of the Institute of Biotechnology, Guangdong Ocean University. The cultivation conditions were 28 °C under light for 16 h and 20 °C in darkness for 8 h. After the culture soil was sterilized at 121 °C for 15 min, a non-inoculated group and an inoculated group were established; in the inoculated group, on the surface ···

Rhizophagus intraradice (laboratory-preserved arbuscular mycorrhizal fungus, AMF) was inoculated beneath the soil layer, and after spot-sowing the seeds, a thin layer of soil was used for covering. In the non-inoculated group, seeds were directly spot-sown and covered with a thin layer of soil.

The plants were cultured with Hoagland nutrient solution (purchased from Judongli Agricultural Technology) for 30 d. After successful inoculation in the

inoculated group, normal ammonium and low ammonium (0.1 mmol · L^{-1}) treatments were set up separately for the two groups. Before the low-ammonium treatment, the nutrient solution was withheld for 3 d, and nitrogen-deficient Hoagland nutrient solution supplemented with 0.1 mmol · L^{-1} NH_4^+Cl was used. Four treatment groups were ultimately obtained: normal ammonium without inoculation (NN−), normal ammonium with inoculation (NN+), low ammonium without inoculation (LN−), and low ammonium with inoculation (LN+). Each group was subjected to three biological replicates. Samples were collected after continued cultivation for 20 d and stored at −80°C.

1.2 Gene cloning and sequence analysis

Total RNA from quinoa was extracted using Transzol (Hunan Aikerui Bioengineering Co., Ltd., AG21102). Subsequently, cDNA was synthesized by reverse transcription using a reagent kit [Sangon Biotech (Shanghai) Co., Ltd., B639252] and stored at −20°C. Putative Arabidopsis AMT protein sequences were downloaded from the TAIR database (https://www.arabidopsis.org/index.Jsp), and quinoa reference genome sequences were downloaded from the Chenopodium DB database (https://www.cbrc.kaust.edu.sa/chenopodiumdb/) (Cq_PI614886_gene_V1_pseudomolecule.gff and Cq_PI614886_genome_V1_pseudomolecule.fas). Based on the putative Arabidopsis AMT protein sequences (AtAMT1;1 (CAA53473), AtAMT1;2 (AAD54639), AtAMT1;3 (AAD54638), AtAMT1;4 (CAB81458), AtAMT1;5 (NP_189072), and AtAMT2;1 (NP_181363)), quinoa AMT candidate proteins were obtained by BLASTP (with the e-value set to e^{-10}). The online tools NCBI CDD (https://www.ncbi.nlm.nih.gov/cdd/) and SMART (http://smart.embl-heidelberg.de) were used to analyze conserved structural domains of AMT proteins and to remove sequences lacking the characteristic domains of the *AMT* gene family. The genes were named according to their order on chromosomes. Primers were designed online using https://tool.vazyme.com:18002/cetool/singlefragment.html (Table 1).

MEGA11 was used to align AMT protein sequences from multiple species, and a phylogenetic tree was constructed using the maximum likelihood (ML) method (bootstrap = 1 000). The phylogenetic tree was visualized online using Evolview (https://evolgenius.info//evolview-v2/).

1.3 Yeast functional complementation assay

The CqAMTs-pESC-Ura expression vector was constructed, and the recombinant vector was transformed into the ammonium-transport-defective yeast mutant 31019b. Dilution spotting was performed under conditions in which different concentrations of NH_4Cl (0.01, 0.025, 0.05, 0.1, 0.5, 1, 5, and 10 mmol · L^{-1}) and 2 mmol · L^{-1} Arg served as the sole nitrogen source, and the growth phenotypes on the plates were observed.

1.4 Expression pattern analysis

1.4.1 Tissue specificity analysis

Quinoa roots, stems, young leaves, mature leaves, and flowers that had not been treated at the same developmental stage were selected. RNA was extracted using the transzol method, normalized to the same concentration, and reverse-transcribed to synthesize cDNA for qRT-PCR analysis. Tissue-specific primers were designed using Primer-Blast on the NCBI website (Table 1). Quantitative analysis was performed using the Bio-Rad CFX Real-Time PCR System. 2×SYBR Green Pro Taq HS Premix was purchased from Aikerui Bioengineering Co., Ltd. Relative gene expression was calculated using the $2^{-\ Ct}$ method. Graphs were drawn using GraphPad Prism 10.1.2, and data are presented as means ± standard deviations.

1.4.2 Detection of AM colonization and mycorrhiza-induced response

Trypan blue was used to stain the roots of the inoculated group. Specifically, a 1 cm cleaned quinoa root segment was taken and cleared in 5% KOH at 90°C in a water bath for 10 min, bleached with alkaline H_2O_2 for 15 min, acidified with 2% HCl for 5 min, and then stained with 0.05% trypan blue solution at 90°C for 60 min. The sample was destained at room temperature for 2 d, and AMF colonization of the roots was observed under an optical microscope.

Roots and leaves were selected from the four treatment groups, and RNA extraction and calculation of relative expression levels were performed using the method described in 1.4.1. Each group included three biological replicates.

1.4.3 Subcellular localization

WoLF PSORT (https://www.genscript.com/wolf-psort.html) online software was used for localization prediction. A CqAMTs-GFP fusion expression vector was constructed, and tobacco leaves were transiently infected via *Agrobacterium*-mediated transformation. GFP fluorescence signals were observed using a confocal microscope 48–72 h after infection. The excitation wavelength for GFP was 488 nm.

2 Results and Analysis

2.1 Systematic evolutionary analysis of the quinoa *CqAMT* gene family

Protein sequence alignment results showed that the AMT protein sequences of quinoa and *Arabidopsis* are highly conserved in regions such as FAM and CAGSVRAKN (Fig. 1), suggesting that these regions are key active centers for ammonium-ion binding or transport; *CqAMT1;4* contains relatively many sequence deletions, which may lead to changes in its function. The systematic

phylogenetic tree (Fig. 2) showed that quinoa AMTs are divided into two subfamilies: *CqAMT1;1*-*CqAMT1;10* belong to Cluster I (the *AMT1* subfamily), while *CqAMT2;1* and *CqAMT2;2* belong to Cluster II (the *AMT2* subfamily), and are relatively closely related to soybean *GmAMT3;1* and rice *OsAMT3;2*.

Table 1 Primers used in this study

Primer name	Forward primer (5′(→)3′)	Backward primer (5′(→)3′)
pESC-Ura-CqAMT1;1	aagaagacctcgagtATGGCTT[illegible]	gctagcggtacc[illegible]ATACATTTGCTTGTCTACCGTC
pESC-Ura-CqAMT1;2	aagaagacctcgagtATGTCGT[illegible]	gctagcggtacc[illegible]CATACATTTCTTCCACCATTTG
pESC-Ura-CqAMT1;3	aagaagacctcgagtATGTCTT[illegible]	gctagcggtacc[illegible]AAGTTGACCCATATCCATC
pESC-Ura-CqAMT1;4	aagaagacctcgagtATGGCAG[illegible]	gctagcggtacc[illegible]CGCATCTGTATATAGTCA
pESC-Ura-CqAMT1;6	aagaagacctcgagtATGCAAG[illegible]	gctagcggtacc[illegible]ATGAAGTTGACCCATATCCATC
pESC-Ura-CqAMT1;7	aagaagacctcgagtATGTCGT[illegible]	gctagcggtacc[illegible]ATACATTTCTTCCACCGTTTG
pESC-Ura-CqAMT1;8	aagaagacctcgagtATGTCTT[illegible]	gctagcggtacc[illegible]CATTTGCTTGTCTACCATC
pESC-Ura-CqAMT2;1	aagaagacctcgagtATGGTAG[illegible]	gctagcggtacc[illegible]TGAGTAGCACCAAT
pESC-Ura-CqAMT2;2	aagaagacctcgagtATGGTAG[illegible]	gctagcggtacc[illegible]GCACCAGGAGTAAT
CqAMT1;1-GFP	taattaagacccgggacATGGCT[illegible]	agcaggatctaggactagt[illegible]CATACATTTGCTTGTCTAC
CqAMT1;2-GFP	taattaagacccgggacATGTCG[illegible]	agcaggatctaggactagtTCATACATTTCTTCCACCAT
CqAMT1;4-GFP	taattaagacccgggacATGGCA[illegible]	agcaggatctaggactagt[illegible]CTACGCATCTGTATATA
CqAMT1;8-GFP	taattaagacccgggacATGTCT[illegible]	agcaggatctaggactagt[illegible]ATACATTTGCTTGTCTAC
qPCR-CqAMT1;1	TGGGTTAGTGCCTTAAT[illegible]	[illegible]CCTTGTCATTGCAAAG
qPCR-CqAMT1;2	GTCAGTAGATGGATGG[illegible]	[illegible]ATGGACCACACCAGAC
qPCR-CqAMT1;3	CTTGGACTCCACCTTC[illegible]	[illegible]GTTGGTAAGCATGATGT
qPCR-CqAMT1;4	ATAGACTTTGCTGGGA[illegible]	[illegible]GAAACCAACCAAACCAG
qPCR-CqAMT1;6	ACATCATGCTTACCAAC[illegible]	[illegible]TGAAGGGGTTACTAGGT
qPCR-CqAMT1;7	GTGATAGATGTGTGCAA[illegible]	[illegible]ACAAACAATAGCAGCC
qPCR-CqAMT1;8	GGGGTCCGCTTTTCTTT[illegible]	[illegible]CCTTGTCATTGCAAAG
qPCR-CqAMT2;1	GGTCGGATGAATATAAG[illegible]	[illegible]AACAAGAACCCTCCAC
qPCR-CqAMT2;2	GGTCGGATGAATATAAG[illegible]	[illegible]TAATCCAACACACCCCA
qPCR-CqActin	GGTATTGGAACGGTGCG[illegible]	[illegible]TCGTGGTGCATCTCAA

Notes: pESC-Ura-CqAMTs is used to construct a yeast expression vector; CqAMTs-GFP is used to construct a plant fusion expression vector for subcellular localization; qPCR-CqAMTs and qPCR-CqActin are used for quantitative detection.

At. Arabidopsis thaliana; *Cq.* Chenopodium quinoa.

Fig. 1 Sequence alignment analysis of *Chenopodium quinoa* and *Arabidopsis thaliana* AMT proteins

Yellow circle indicates *Chenopodium quinoa*; white circle indicates *Sorghum bicolor*; blue star indicates *Arabidopsis thaliana*; cyan star indicates *Oryza sativa*; white star indicates *Capsicum annuum*; orange triangle indicates *Zea mays*; dark

Fig.3 Functional validation of *CqAMTs* in defective yeast 31019b

Figure 1: Fig.3 Functional validation of *CqAMTs* in defective yeast 31019b

Fig.4 Expression analysis of quinoa CqAMTs in different tissues

Figure 2: Fig.4 Expression analysis of quinoa CqAMTs in different tissues

purple triangle indicates *Triticum aestivum*; light purple square indicates *Manihot esculenta*; pink square indicates *Glycine max*; in the figure, red and green represent Group I and Group II, respectively.

Fig. 2 Phylogenetic tree of AMT proteins from other plants and *Chenopodium quinoa*

2.2 Yeast functional verification of quinoa *CqAMTs*

The results of yeast functional verification showed that, under low-ammonium ($NH_4^+ < 5$ mmol $\cdot L^{-1}$) conditions, yeast mutants transformed with the empty vector could not grow normally, whereas yeast transformed with *CqAMT1;1*, *CqAMT1;2*, and *CqAMT1;8* grew normally; among them, the ammonium transport activity of *CqAMT1;1* was weaker than that of the latter two (Fig. 3). Notably, transformants of *CqAMT1;4* grew more poorly at relatively high ammonium concentrations, suggesting that sequence loss led to functional differentiation (Fig. 3). These results indicate that *CqAMT1;1*, *CqAMT1;2*, *CqAMT1;4*, and *CqAMT1;8* can transport NH_4^+ ions under low-ammonium conditions, mediate the permeation of NH_4^+ across the plasma membrane of yeast cells, and belong to high-affinity ammonium transporter genes among the AMTs.

2.3 Expression analysis of quinoa *CqAMTs* in different tissues

The qRT-PCR results (Fig. 4) showed that *CqAMT1;1*, *CqAMT1;2*, *CqAMT1;7*, and *CqAMT1;8* had the highest expression levels in roots; *CqAMT1;3*, *CqAMT1;4*, and *CqAMT2;2* had the highest expression levels in mature leaves; *CqAMT1;6* had the highest expression level in young leaves; and *CqAMT2;1* had the highest expression level in flowers, indicating that different AMTs in quinoa have tissue specificity.

Figure 3 Functional validation of *CqAMTs* in defective yeast 31019b

Fig.3 Functional validation of *CqAMTs* in defective yeast 31019b

S, F, YL, ML, and R represent stem, flower, young leaf, mature leaf, and root, respectively.

Figure 4 Expression analysis of quinoa *CqAMTs* in different tissues

Fig.4 Expression analysis of quinoa *CqAMTs* in different tissues

2.4 Analysis of colonization by arbuscular mycorrhizal fungi and their induced expression

Trypan blue staining showed that vesicles and hyphae could be observed in the root system of the inoculated group, indicating successful AMF inoculation (Fig. 5). The qRT-PCR results (Fig. 6) showed that, in leaves, under low-ammonium inoculation (LN+) treatment, the expression levels of *CqAMT1;2*, *CqAMT1;3*, *CqAMT1;4*, *CqAMT1;6*, *CqAMT1;7*, *CqAMT2;1*, and *CqAMT2;2* were significantly higher than those under low-ammonium non-inoculation (LN−); under normal-ammonium inoculation (NN+) treatment, only the expression level of *CqAMT1;3* was significantly higher than that under normal-ammonium non-inoculation (NN−), while the remaining genes showed no significant differences. The expression levels of *CqAMT1;1*, *CqAMT1;2*, *CqAMT1;7*, *CqAMT1;8*, *CqAMT2;1*, and *CqAMT2;2* under low-ammonium inoculation (LN+) were significantly higher than those under normal-ammonium inoculation (NN+). In roots, *CqAMT1;6* was not expressed; the expression levels of *CqAMT1;1*, *CqAMT1;7*, *CqAMT1;8*, and *CqAMT2;2* decreased after inoculation; the expression levels of *CqAMT1;2*, *CqAMT1;3*, *CqAMT1;4*, and *CqAMT2;1* under low-ammonium inoculation (LN+) treatment were higher than those under low-ammonium non-inoculation (LN−), but *CqAMT1;2* was not significant, whereas the expression levels of *CqAMT1;3* and *CqAMT1;4* under low-ammonium inoculation (LN+) and normal-ammonium inoculation (NN+) treatments were significantly higher than those in the corresponding non-inoculated groups; the expression levels of *CqAMT1;1*, *CqAMT1;8*, *CqAMT2;1*, and *CqAMT2;2* under low-ammonium inoculation (LN+) were also significantly higher than those under normal-ammonium inoculation (NN+). *CqAMT2;1* showed the highest expression level under low-ammonium inoculation (LN+) treatment, indicating that, under ammonium-deficient conditions, AMF mycorrhizae may play a dominant role.

H. Hyphae; **V.** Vesicles.

Fig.5 Observation of inoculation using trypan blue staining

NN- Normal ammonium concentration without inoculation; **NN+** Normal ammonium concentration with inoculation; **LN-** Low ammonium concentration without inoculation; **LN+** Low ammonium concentration with inoculation. Values $= \bar{x} \pm s$ $(n = 3)$. Different lowercase letters indicate significant differences between treatments $(P < 0.05)$.

Fig.6 Analysis of Induced Responses in Arbuscular Mycorrhizal Fungi

2.5 Subcellular localization of quinoa *CqAMTs*

Based on the yeast experimental results, *CqAMT1;1*, *CqAMT1;2*, *CqAMT1;4*, and *CqAMT1;8* were selected for localization prediction (Table 2). The results showed that all four identified CqAMTs proteins were predicted to be targeted to the plasma membrane. Subcellular localization also showed (Fig. 7) that

Figure 7. Subcellular localization of CqAMT1;1, CqAMT1;2, CqAMT1;4, and CqAMT1;8. Column labels: GFP, Bright Field, Merged. Row labels: GFP; CqAMT1;1-GFP; CqAMT1;2-GFP; CqAMT1;4-GFP; CqAMT1;8-GFP.

Figure 3: Figure 7. Subcellular localization of CqAMT1;1, CqAMT1;2, CqAMT1;4, and CqAMT1;8. Column labels: GFP, Bright Field, Merged. Row labels: GFP; CqAMT1;1-GFP; CqAMT1;2-GFP; CqAMT1;4-GFP; CqAMT1;8-GFP.

CqAMT1;1, *CqAMT1;2*, *CqAMT1;4*, and *CqAMT1;8* were all localized to the plasma membrane, consistent with the prediction results, indicating that these genes play important roles in mediating the transport of ammonium ions from soil or the external environment into plant cells.

Table 2 Subcellular localization prediction

Gene ID	Gene name	Number of amino acids	Subcellular localization
AUR62006988-RA	*CqAMT1;1*	434	Plasma membrane
AUR62006987-RA	*CqAMT1;2*	499	Plasma membrane
AUR62001658-RA	*CqAMT1;4*	412	Plasma membrane
AUR62000635-RA	*CqAMT1;8*	495	Plasma membrane

Fig. 7 Subcellular localization of *CqAMT1;1*, *CqAMT1;2*, *CqAMT1;4*, *CqAMT1;8*

3 Discussion and Conclusion

NH_4^+ is the principal nitrogen source that plants can directly utilize. Ammonium transporter proteins (AMTs) are responsible for the uptake, transport, and distribution of ammonium, and play a core role in plant adaptation to low-nitrogen environments and in the exchange process of arbuscular mycorrhizal fungal (AMF) symbiotic nutrients. This study identified 12 *CqAMT* genes in quinoa. Sequence alignment showed that they are highly conserved with the AMT proteins of *Arabidopsis thaliana* in motifs such as FAM and CAGSVRAKN; these conserved motifs have been confirmed to be core functional regions for ammonium-ion binding and transport by AMT proteins (Loque et al., 2006). Sequence deletions exist in *CqAMT1;4*, and functional validation in yeast has shown that its transport activity is better under low-ammonium conditions, whereas growth is inhibited under high-ammonium conditions. This phenomenon suggests that structural loss may lead to functional

differentiation of this protein. Similar phenomena have been reported in fungi (*Geosiphon pyriformis*) and in millet *AMT* genes (Ellerbeck et al., 2013; Han et al., 2026). Phylogenetic analysis divided quinoa *AMT* into two subfamilies, *AMT1* and *AMT2*, among which *AMT1* contains 10 members, significantly more than *AMT2* (2 members). It is inferred that expansion of the *AMT1* subfamily is an evolutionary strategy by which quinoa adapts to the nitrogen-poor soils of its native Andean habitats (Yazar et al., 2014).

qRT-PCR results showed that quinoa *AMT* genes exhibit differentiated expression patterns in roots, stems, leaves, and flowers, suggesting that they undertake different physiological functions in nitrogen nutrition in quinoa. The genes highly expressed in roots (*CqAMT1;1*, *CqAMT1;2*, *CqAMT1;7*, *CqAMT1;8*) are mainly involved in direct absorption of ammonium from soil by the root system, which is consistent with the expression patterns of *Arabidopsis AtAMT1;1* and *AtAMT1;3* in root hairs and root epidermis (Loque et al., 2006). The genes highly expressed in mature leaves (*CqAMT1;3*, *CqAMT1;4*, *CqAMT2;2*) may participate in ammonium redistribution and reutilization in leaves, consistent with functional reports that birch *BpAMTs* participate in nitrogen redistribution in leaves (Yang Hai' e et al., 2023). The specifically high expression of *CqAMT1;6* in young leaves may meet the high nitrogen demand of rapidly growing tissues. *CqAMT2;1*, which is highly expressed in flowers, may play a key role in nitrogen supply during reproductive growth. The tissue-specific expression differentiation of quinoa is similar to the *AMT* expression patterns in spinach (Zhao Minhua et al., 2022), indicating that Chenopodiaceae plants may have formed unique nitrogen allocation strategies during evolution.

This study found that low ammonium and AMF inoculation significantly induced the upregulation of multiple *CqAMT* genes in quinoa roots and leaves, and that the response patterns differed between roots and leaves. In leaves, *CqAMT1;2*, *CqAMT1;3*, *CqAMT1;4*, *CqAMT1;6*, *CqAMT1;7*, *CqAMT2;1*, and *CqAMT2;2* responded significantly to AMF induction; in the root system, *CqAMT1;2*, *CqAMT1;3*, *CqAMT1;4*, and *CqAMT2;1* showed the highest expression under low-ammonium inoculation. In particular, *CqAMT2;1* was the most sensitive to low-nitrogen mycorrhizal signals. AMF extraradical hyphae can efficiently capture ammonium from the soil and transport it to the intraradical symbiotic interface, where release of NH_4^+ induces differential expression of host plant *AMT* genes, thereby forming a mycorrhizal pathway for ammonium uptake (Jean-Patrick et al., 2004; Hui et al., 2022). The response pattern of *CqAMT2;1* is highly consistent with that of validated symbiotic ammonium transporters such as rice *OsAMT3;1* (Perez-Tienda et al., 2014), indicating that the *CqAMT2;1* gene may also mediate ammonium transport from AMF to the host plant. In the root system, the expression of *CqAMT1;1*, *CqAMT1;7*, *CqAMT1;8*, and *CqAMT2;2* was downregulated after inoculation, suggesting a functional division of labor between the mycorrhizal pathway and the root direct-uptake pathway; that is, when AMF provide sufficient ammonium, plants downregulate their own uptake, thereby reducing energy consumption. This mechanism has been confirmed in *Lotus japonicus* roots

and alfalfa: in *L. japonicus*, expression of *LjAMT2;2* decreases significantly after mycorrhizal symbiosis is established (Boussageon et al., 2023), *LjAMT2;4* mutants show a de-repressed phenotype (Xie et al., 2025); and in alfalfa, *AMT2;3* and *AMT2;4* regulate symbiotic efficiency and are controlled by plant nitrogen status (Breuillin-Sessoms et al., 2015). Overall, under low-ammonium conditions, the induction effect of mycorrhizae on *CqAMTs* is stronger than under normal ammonium supply, indicating that "nitrogen starvation" signals can amplify the inductive effect of AMF on quinoa *AMT* genes; this regulation may be a key reason why quinoa can survive in nutrient-poor soils.

In summary, this study identified a total of 12 quinoa *AMT* family members, clarified their sequence characteristics, phylogenetic relationships, and the ammonium-transport functions of some members (*CqAMT1;1*, *CqAMT1;2*, *CqAMT1;4*, *CqAMT1;8*), and analyzed the tissue-specific expression of this family and the expression regulatory mechanism induced by AMF inoculation. Among them, *CqAMT2;1* responds significantly to AMF and may undertake the core function of ammonium transport in arbuscular mycorrhizal symbiosis, making it a key gene for quinoa adaptation to low-nitrogen environments. This study clarifies the role of the *CqAMT* family in nitrogen nutrient uptake during quinoa–arbuscular mycorrhizal symbiosis, provides a foundation for subsequent in-depth analysis of the molecular mechanism of ammonium transport in quinoa-arbuscular mycorrhizal symbiosis, and also offers an important theoretical basis for improving the nitrogen-use efficiency of quinoa through mycorrhizal symbiosis.

References:

Boussageon R, Serrano M, Formey D, et al., 2023. Knockdown of *Lotus japonicus ROP3* alters the root symbiotic phenotype and alters the expression of genes involved in nutrient acquisition during both rhizobial and mycorrhizal symbioses [J]. Symbiosis, 91: 167-178.

Breuillin-Sessoms F, Floss D S, Gomez S K, et al., 2015. Suppression of arbuscule degeneration in *Medicago truncatula phosphate transporter4* mutants is dependent on the ammonium transporter 2 family protein AMT2;3. Plant Cell, 27(4): 1352-1366.

Duan S, Feng G, Limpens E, et al., 2024. Cross-kingdom nutrient exchange in the plant-arbuscular mycorrhizal fungus-bacterium continuum [J]. Nature Reviews Microbiology, 22(12): 773-790.

Ellerbeck M, Schubler A, Brucker D, et al., 2013. Characterization of three ammonium transporters of the glomeromycotan fungus *Geosiphon pyriformis* [J]. Eukaryot Cell, 12(11): 1554-1562.

Fang L, Wang M, Chen X, et al., 2023. Analysis of the *AMT* gene family in chili pepper and the effects of arbuscular mycorrhizal colonization on the expression patterns of *CaAMT2* genes [J]. BMC Genomics, 24(1): 158.

Fei W, Lin Z, Jiachao Z, et al., 2022. Exploring the secrets of hyphosphere of arbuscular mycorrhizal fungi: processes and ecological functions [J]. Plant and Soil, 481(1/2): 1-22.

Gu R, Duan F, Aa X, et al., 2013. Characterization of AMT-mediated high-affinity ammonium uptake in roots of maize (*Zea mays* L.) [J]. Plant & Cell Physiology, 54(9): 1515-1524.

Han M, Yan J, Zhao K, et al., 2026. The Genome-Wide identification and expression analysis of the *AMT* gene family in foxtail millet [J]. Biology, 15(9): 710.

He J, Tan W L, Qin L, et al., 2026. Reduced nitrogen availability in hydroponically grown Chinese broccoli does not affect photosynthetic performance and yield while enhancing nitrogen use efficiency and nutritional quality [J]. Frontiers in Plant Science, 16: 1745794.

Hui J, An X, Li Z, et al., 2022. The mycorrhiza-specific ammonium transporter *ZmAMT3;1* mediates mycorrhiza-dependent nitrogen uptake in maize roots [J]. The Plant Cell, 34(10): 4066-4087.

Jean-Patrick T, Marc S, Christiane C, 2004. Nitrogen transfer and assimilation between the arbuscular mycorrhizal fungus *Glomus intraradices* Schenck & Smith and Ri T-DNA roots of *Daucus carota* L. in an *in vitro* compartmented system [J]. Canadian journal of microbiology, 50(4): 251-260.

Kobae Y, Tamura Y, Takai S, et al., 2010. Localized expression of arbuscular mycorrhiza-inducible ammonium transporters in soybean [J]. Plant & Cell Physiology, 51(9): 1411-1415.

Koegel S, Ait Lahmidi N, Arnould C, et al., 2013. The family of ammonium transporters (*AMT*) in *Sorghum bicolor*: two *AMT* members are induced locally, but not systemically in roots colonized by arbuscular mycorrhizal fungi [J]. New Phytologist, 198(3): 853-865.

Konishi N, Ma J F, 2021. Three polarly localized ammonium transporter 1 members are cooperatively responsible for ammonium uptake in rice under low ammonium condition [J]. New Phytologist, 232(4): 1778-1792.

Loque D, Yuan L X, Kojima S, et al., 2006. Additive contribution of *AMT1;1* and *AMT1;3* to high-affinity ammonium uptake across the plasma membrane of nitrogendeficient *Arabidopsis* roots [J]. The Plant Journal, 48(4): 522-534.

Pérez-Tienda J, Corrêa A, Azcón-Aguilar C, et al., 2014. Transcriptional regulation of host NH_4^+ transporters and GS/GOGAT pathway in arbuscular mycorrhizal rice roots [J]. Plant Physiol Biochem, 75: 1-8.

Rivero-Marcos M, 2025. Functional crosstalk between nitrate and ammonium transporters in N acquisition and pH homeostasis [J]. Frontiers in Plant Science, 16: 1634119.

Vilcacundo R, Hernandez-ledesma B, 2017. Nutritional and biological value of quinoa (*Chenopodium quinoa* Willd.) [J]. Current Opinion in Food Science, 14: 1-6.

Von Wittgenstein N J, Le C H, Hawkins B J, et al., 2014. Evolutionary classification of ammonium, nitrate, and peptide transporters in land plants [J]. BMC Evolutionary Biology, 14(1): 11.

Wang Y, Li J, Liu X, et al., 2025. Genome-wide identification of the *AMT* gene family in wheat: expression profiles under ammonium nutrition and pathogen effects [J]. Genes, 16(12): 1451.

Wang Y, Zhou W, Wu J, et al., 2022. *LjAMT2;2* promotes ammonium nitrogen transport during arbuscular mycorrhizal fungi symbiosis in *Lotus japonicus* [J]. International Journal of Molecular Sciences, 23(17): 9522.

Xia J, Wang Y, Zhang T, et al., 2023. Genome-wide identification, expression profiling, and functional analysis of ammonium transporter 2 (*AMT2*) gene family in cassava (*Manihot esculenta* Crantz) [J]. Frontiers in Genetics, 14: 1145735.

Xie K, Ni Y, Bai L, et al., 2025. Functional characterization of ammonium transporter *LjAMT2;4* during *Lotus japonicus* symbiosis with rhizobia and arbuscular mycorrhizal fungi [J]. Journal of Fungi, 11(5): 340.

Yang H X, Liu X Y, Zhan Y G, et al., 2023. Genome-wide identification and expression analysis of *BpAMT* gene family in *Betula platyphylla* [J]. Forestry Research, 36(2): 133-143. [Yang Hai-ting, Liu Xiao-ying, Zhan Ya-guang, et al., 2023. Identification and expression pattern analysis of the *BpAMT* gene family in white birch [J]. Forestry Research, 36(2): 133-143.]

Yang W, Dong X, Yuan Z, et al., 2023. Genome-wide identification and expression analysis of the ammonium transporter family genes in Soybean [J]. International Journal of Molecular Sciences, 24(4): 3991.

Yang S F, Pang C H, Zhang Y Q, et al., 2017. Growth and physiological characteristics of quinoa inoculated with arbuscular mycorrhizal fungi under different nitrogen levels [J]. Acta Botanica Boreali-Occidentalia Sinica, 37(7): 1323-1330. [Yang Shi-fang, Pang Chun-hua, Zhang Yong-qing, et al., 2017. Effects of arbuscular mycorrhizal fungi on the growth and root physiological characteristics of quinoa under different nitrogen application levels [J]. Acta Botanica Boreali-Occidentalia Sinica, 37(7): 1323-1330.]

Yazar A, Kaya C, 2014. A new crop for salt affected and dry agricultural areas of turkey: Quinoa (*Chenopodium quinoa* Willd.) [J]. Turkish Journal of Agricultural and Natural Sciences, 1(2): 1440-1446.

Zhao M H, Liu J, Ge C H, et al., 2022. Identification and expression analysis of spinach *AMT* gene family [J]. Journal of Shanghai Normal University(Natural Sciences), 51(6): 769-780. [Zhao Min-hua, Liu Ji, Ge Chen-hui, et al., 2022. Identification and expression analysis of the spinach *AMT* gene family [J]. Journal of Shanghai Normal University: Natural Sciences Edition, 51(6): 769-780.]

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