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Identification and functional analysis of ammonium transporter family in potato

Wan LI[1,*], Zilin ZHANG[1], Ziyu ZHOU[2]

1. *School of biomedicine and food engineering, Shangluo university, Shangluo, Shaanxi, People's Republic of China.* ***2.*** *College of Agronomy, Northwest A&F University, Yangling, Shaanxi, People's Republic of China.* [*]*Corresponding author's email: liwan54926@126.com*

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ABSTRACT: In order to understand the characteristics and functions of potato ammonium transporters (AMT) family, bioinformatics and molecular biology research methods were used to systematically identify and analyze potato AMT family members (StAMTs) in this study, and preliminarily studied the functions of StAMTs in *Arabidopsis thaliana*. The results showed that there were 8 StAMTs in potato, which were divided into two subfamilies and distributed on different chromosomes. The amino acid residues of 8 StAMTs ranged from 460 to 512, the molecular weight ranged from 49.66 kDa to 55.11 kDa, the phosphorylation sites ranged from 34 to 55, the aliphatic indexes were all greater than 89, and the grand average of hydropathicity was all greater than 0. The 8 StAMTs had 11 transmembrane domains, and all of them were expressed on the cell membrane. The isoelectric point of most members was less than 7, the instability index was less than 40, and there was no signal peptide. The analysis results of the phylogenetic tree, motif composition, gene structure and GO annotation showed that 8 StAMTs had different functions. The results of expression pattern analysis under different treatments showed that StAMT1.1 may be a low affinity protein and StAMT2.5 may be a high affinity protein. In addition, the functions of StAMTs were studied in *Arabidopsis thaliana*. The results showed that different genes had different effects on survival rate, root length, fresh weight and ammonium nitrogen content. Under normal and NH_4^+ sufficiency conditions, the survival rate, root length and fresh weight of the transgenic lines overexpressing *StAMT2.3* or *StAMT2.5* were significantly lower than those of wild type *Arabidopsis thaliana* (Columbia-0) or had no significant change. However, under NH_4^+ deficiency condition, the survival rate of the transgenic lines overexpressing *StAMT2.3* or *StAMT2.5* showed no significant change, but the root length (about 33%-37% or 15%-27%) and fresh weight (about 87%-105% or 84%-97%) were significantly higher than those of Columbia-0, indicating that StAMT2.3 and StAMT2.5 may be high-affinity proteins. In summary, for StAMT2.5, the results of expression pattern analysis in potato and function analysis in *Arabidopsis thaliana* were consistent, it may promote the utilization efficiency of NH_4^+ in potato under NH_4^+ deficiency environment, so *StAMT2.5* can be used as a candidate gene for further study. The results of this study are conducive to further understanding the function of potato StAMTs, and provide a reference for studying the molecular mechanism and mechanism of potato absorption and utilization of NH_4^+.

KEY WORDS: Ammonium nitrogen, Ammonium transporters, *Arabidopsis thaliana*, *Solanum tuberosum*

INTRODUCTION

Potato (*Solanum tuberosum*) is currently the fourth largest food crop in the world after wheat (*Triticum aestivum*), rice (*Oryza sativa*) and corn (*Zea mays*) (Gong *et al*., 2024). In potato production, nitrogen is of great significance to the growth and development of potato, and its insufficient content is the main factor limiting the yield and quality of potato (Kolomiets *et al*., 2001; Nurmanov *et al*., 2019). In plants, there are two main types of nitrogen that can be absorbed and utilized, exogenous nitrogen (fertilizers, *etc*.) and endogenous nitrogen (the product synthesized from exogenous nitrogen in plants through a series of enzymatic actions). Exogenous nitrogen generally includes inorganic nitrogen and organic nitrogen. In inorganic nitrogen, nitrate nitrogen (NO_3^-) and ammonium nitrogen (NH_4^+) are the main nitrogen sources during plant growth and development, which are absorbed and transported by nitrate transporter family and ammonium transporter (AMT) family, respectively (Amtmann and Armengaud 2009). Due to the low energy required for absorption and assimilation, NH_4^+ is more absorbable source of nitrogen for plants (Setién *et al*., 2013). However, NH_4^+ is a paradoxical nutrient: plants growth can be promoted when moderate ammonium is supplied, but in excess ammonium causes a phytotoxic effect on plants (Xian *et al*., 2020).

There are two NH_4^+ absorption systems in plants, high affinity transport system (HATS) and low affinity transport system (LATS). When NH_4^+ concentration is less than 1 mmol/L or greater than 1 mmol/L, HATS or LATS is dominant, respectively (Wang *et al*., 2022; Xia *et al*., 2023). The first AMT gene in a plant was discovered in *Arabidopsis thaliana* (*A. thaliana*), and subsequently, the AMT family was identified in rice, tomato (*Solanum lycopersicum*) and several other plant species (Li *et al*., 2016; Sun *et al*., 2019; Hao *et al*., 2020; Wang *et al*., 2022). The AMT family of plants can be divided into AMT1 subfamily and AMT2 subfamily. There are 6 AMT genes in *A. thaliana*. The AMT1 subfamily contains 5 members (*AtAMT1.1~AMT1.5*), and the AMT2 subfamily contains only 1 member (*AMT2*)

(Lanquar *et al*., 2009). Characterization of *A. thaliana* mutants demonstrates that 3 genes of AMT1 subfamily (*AtAMT1.1*, *AtAMT1.2* and *AtAMT1.3*) contribute about 90% NH_4^+ uptake (Loqué *et al*., 2006; Yuan *et al*., 2007). In plants, the expression of AMT genes is regulated by nitrogen nutrition and has tissue expression specificity, and is also influenced by day and night alternation, sugar, soil pH and so on. For example, in *A. thaliana*, *AtAMT1.5* is only expressed under nitrogen deficiency condition, and the expression level of *AtAMT1.1* is increased significantly in root (Engineer and Kranz, 2007; Yuan *et al*., 2007). In rice, the expression levels of *OsAMT1.1*, *OsAMT1.2* and *OsAMT2.1* are increased under nitrogen deficiency condition, and the expression levels of *OsAMT1.1* and *OsAMT1.3* are inhibited when nitrogen content is enriched (Suenaga *et al*., 2003). In poplar (*Populus tremula*×*alba*), the expression of *AMT1.2* is significantly increased during root formation. The expression of *AMT3.1* gene increased significantly during leaf senescence. *AMT1.5* is specifically expressed in stamens, while *AMT1.6* is specifically expressed in female flowers (Couturier *et al*., 2007). The AMT1 subfamily members of pear tree (*Pyrus communis*) are expressed throughout the growth process, and *CoAMT1.1*, *CoAMT1.2* and *CoAMT1.3* are mainly expressed in the roots (Mota *et al*., 2011). In addition, in *A. thaliana*, the expression level of *AtAMT1.3* is increased threefold near the end of light exposure (Gazzarrini *et al*., 1999). In poplar, the expression levels of *PtAMT1.1*, *PtAMT1.2*, *PtAMT1.6* and *PtAMT2.1* are strongly influenced by diurnal variation and sugar content (Couturier *et al*., 2007). The expression levels of AMT genes at pH=7 are significantly higher than that at pH=4.0 in Prince Rupprecht's Larch (*Larix principis-rupprechtii* Mayr) (Meng *et al*., 2016).

Until now, studies on the potato AMT family have been rare. As one of the most important food crops in the world, the growth and development of potato can be promoted by applying nitrogen fertilizer, thereby improving the yield and quality, but the overuse of fertilizer has caused pollution to soil and water, and seriously damaged the ecological balance (Choudhury and Kennedy, 2005; Li *et al*., 2009; Yu *et al*., 2019a,b; Qu *et al*., 2023). For potato, it preferred NH_4^+ over NO_3^-. Therefore, AMT family may play an important role in potato growth and development. This study intends to use bioinformatics and molecular biology research methods to identify and analyze the characteristics and properties of AMT family members (StAMTs) in the whole genome of potato. Meanwhile, through overexpressing *StAMTs* in *A. thaliana*, the functions of StAMTs will be detected by analyzing the effects of different genes on plant growth, absorption and utilization of NH_4^+. The results of this study will provide experimental basis and scientific basis for improving the effective utilization rate of NH_4^+ in potato and improving the yield and quality.

MATERIAL AND METHODS

Plant materials and planting conditions

The ecotype of *A. thaliana* used in the study was wild-type Columbia (Columbia-0), and the variety of potato was tetraploid cultivated species "Desiree". The plants were cultured in light incubator with the photoperiod of 23℃, 8000 lux for 16 h and 22℃, 0 lux for 8 h in the normal environment without stresses.

Identification of AMT family members in potato

The amino acid sequences of 6 members of AMT family in *A. thaliana* (AtAMTs) were obtained from TAIR website (https://www.arabidopsis.org/). AtAMTs were used as queries to perform a protein search against the protein database of potato with a strict E value ($<1\times10^{-10}$) requirement by NCBI-Blast program (https://www.ncbi.nlm.nih.gov/) (Wang *et al*., 2010). All candidate AMT proteins of potato were screened by using the Conserved Domain Database (CDD) (https://www.ncbi.nlm.nih.gov/cdd/) for removing incorrect and redundant sequences, and the rest were StAMTs. The genome, coding region, amino acid and promoter sequences of StAMTs, as well as gene location information were obtained from Phytozome website (https://phytozome-next.jgi.doe.gov/).

Analysis of StAMTs

The phylogenetic tree was constructed by MEGA7 software using the neighbour-joining method with the parameters of the Jones-Taylor-Tornton model and 1000 replicates for bootstrap analysis (Liu *et al*., 2017). The gene/protein features of StAMTs were analyzed by ProtParam website (http://web.expasy.org/protparam/). The transmembrane domains (TMDs) and signal peptides were analyzed by TOPCONS website (https://topcons.cbr.su.se/). The subcellular localization was predicted by CELLO website (http://cello.life.nctu.edu.tw/). The 10 motifs were analyzed by MEME website (http://meme-suite.org/tools/meme). The generic phosphorylation sites (GPSs) were analyzed by NetPho3.1 website (https://services.healthtech.dtu.dk/services/NetPhos-3.1/). The protein tertiary structure was predicted by PHYRE2 website (http://www.sbg.bio.ic.ac.uk/phyre2/html/page.cgi?id=index). The exon/intron structure was analyzed by GSDS website (http://gsds.cbi.pku.edu.cn/index.php). Gene Ontology (GO) annotation information was obtained from Spud DB website (http://spuddb.uga.edu/). The *cis*-acting elements (CAEs) of promoter regions (2000 bp upstream of start codon of a gene) were predicted by PlantCARE website (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/).

The gene duplication events were analyzed by NCBI-

Blast. If the length of an aligned sequence cover > 75% of the longer gene, and the similarity of the aligned regions is > 75%, the two genes were considered a gene duplication event (Jiang *et al*., 2013; Li *et al*., 2020). Non-synonymous mutation frequency (*Ka*) and synonymous mutation frequency (*Ks*) were calculated by DnaSP6 software.

Expression pattern analysis of *StAMTs* in different environments

Aseptic tissue culture potato seedlings with the same growth potential were transplanted into small pots containing vermiculite, and divided into 3 groups. The seedlings were cultured in normal Hoagland nutrient solution (Control), Hoagland nutrient (without NO_3^-) with 2 mM NH_4^+ (NH_4^+ sufficient) or 0.2 mM NH_4^+ (NH_4^+ deficient), respectively. The normal Hoagland nutrient solution and Hoagland nutrient (without NO_3^-) purchased from Qingdao Hope Bio-Technology Co., Ltd., China, NH_4^+ was derived from ammonium sulfate. After 3 d, the whole plants were sampled and stored at −80 °C prior to utilization.

The RNAprep Pure Plant Plus Kit (Tiangen, Beijing, China) was used to extract RNA. The *elongation factor 1-a* (*Ef1a*) gene (GenBank accession number: XM_006347752) was chosen as the reference gene (Nicot *et al*., 2005). Specific primers were designed using Primer Premier 6 software (Table S1). First-strand cDNAs were synthesized from 500 ng of RNA with the PrimeScript™ RT reagent Kit with gDNA Eraser (TaKaRa, Beijing, China) in a 20 μL reaction volume. Quantitative Real-time PCR (qRT-PCR) was set up on the basis of TB Green® Premix Ex Taq™ II FAST qPCR (Takara, Beijing, China) and was performed on the QuantStudio7 Flex Real-time PCR System (Applied Biosystems Inc., America) in a 10 μL reaction volume. The relative expression level of each gene was calculated using the $2^{-\Delta\Delta Ct}$ method (Liu *et al*., 2016).

Vector construction and *A. thaliana* transformation

Specific primers were designed by Primer Premier 6 software (Table S1), and *StAMTs* were isolated from potato cDNA by PCR. The overexpression vector pBI121-HA (pBI121 vector without GUS tag, but with some new restriction sites and HA tag) was digested by *Xba* I and *Xho* I. The PCR products and enzyme digestion products were detected and recovered on 1.5% agarose gel, and the recombinant vector of *StAMTs* and pBI121-HA were constructed using pEASY®-Basic Seamless Cloning and Assembly Kit (Transgen, Beijing, China), and were transformed into *Escherichia coli* chemically competent cell Dh5α. The recombinant plasmids of *StAMTs* and pBI121-HA were obtained through sequencing analysis and plasmid extraction. The recombinant plasmids were transformed into *Agrobacterium tumefaciens* chemically competent cell GV3101, and the positive transformants were obtained through PCR detection. The infective solution of *Agrobacterium tumefaciens* with recombinant plasmids (OD_{600}=0.8-1.0) was prepared. Columbia-0 was transformed three times in 3 weeks by *Agrobacterium*-mediated inflorescence infection method (Chen *et al*., 2017), and the T_0 transgenic seeds were harvested.

A reaction mixture containing templates, primers, buffer, dNTPs, and Taq DNA polymerase was subjected to the following procedure: pre-denaturation (94℃) for 5 min; followed by denaturation (94℃) for 30 s, annealing (55℃) for 30 s, extension (72℃) for 2 min for a total of 30 cycles. Final elongation step (72℃) was carried out for 10 min.

Screening of positive transgenic *A. thaliana* lines

Under sterile conditions, the T_0 seed surface was disinfected using 70% ethanol and 50% bleach (containing 0.05% Tween-20) for 5 minutes, and then washed three times in sterile ddH_2O for 10 minutes each time. The sterile seeds were placed at 4℃ for 3 d, then seeded on solid MS (Murashige and Skoog, Phytotech) medium with kanamycin (kana, 50 ng/μL) and placed in the light incubator for growth for about 10 d. The kana resistant plants with well grown were transplanted into small pots containing vermiculite for further growth for about 15 d. PCR was performed using genomic DNA isolated from leaves of the kana resistant plants. With Columbia-0 DNA and ddH_2O as the negative control and pBI121-HA plasmid DNA as the positive control, PCR analysis was carried out using the specific primer NPTIIF/R (Table S1).

The T_1 seeds of transformed plants identified by PCR were obtained and screened by MS medium containing kana, and the RNA was extracted from kana resistant plants and Columbia-0 for detecting relative expression levels of *StAMTs* by qRT-PCR. The *Atactin* gene (At1g49240) was chosen as the reference gene (Table S1). The positive transgenic plants identified by PCR and qRT-PCR were cultured and the seeds were harvested (T_2). T_2 seeds were screened on MS medium containing kana and cultured in light incubator for harvesting T_3 homozygous seeds.

Effect of StAMTs on growth of *A. thaliana*

After sterilization, T_3 homozygous and Columbia-0 seeds were placed at 4℃ for 3 d, then seeded on MS medium without antibiotics and vertically placed in the light incubator for 10 d. The seedlings of similar developmental stage and vigor were selected and cultured in Control, NH_4^+ sufficient or NH_4^+ deficient environments for 15 d, respectively. The nutrient solution was replaced every 3 d. The survival rate, root length, fresh weight and ammonium nitrogen content (ANC) were measured to analyze the effects of StAMTs on the plant growth. The ANC detection was assisted by Quanzhou Ruixin Biotechnology Co., LTD. The data was analyzed by SPSS 29.0 and Excel software.

Table 1. Features analysis of StAMTs

Gene number	Gene name	No. of AARs	Mole. Wt. (kDa)	*pI*	Instability index	Aliphatic index	GRAVY	TMD/ Direction	*Signal peptide	Subcellular Localization	No. of GPSs
Soltu.DM.03G006400.1	StAMT1.1	460	49.67	5.34	26.15	98.59	0.557	11/out→in	N	Cell membrane	45
Soltu.DM.04G019680.1	StAMT1.2	512	55.11	6.94	21.46	89.04	0.397	11/out→in	Y	Cell membrane	55
Soltu.DM.09G027420.1	StAMT1.3	491	52.60	6.45	24.30	89.27	0.435	11/out→in	N	Cell membrane	39
Soltu.DM.01G036550.1	StAMT2.1	476	51.84	5.76	29.22	106.55	0.527	11/out→in	N	Cell membrane	45
Soltu.DM.03G003430.1	StAMT2.2	469	51.76	7.73	35.01	96.91	0.350	11/out→in	N	Cell membrane	53
Soltu.DM.08G015590.1	StAMT2.3	485	52.70	6.74	37.01	101.57	0.530	11/out→in	N	Cell membrane	46
Soltu.DM.09G020440.1	StAMT2.4	475	52.29	6.45	36.98	105.14	0.496	11/out→in	N	Cell membrane	34
Soltu.DM.10G019400.1	StAMT2.5	483	52.05	7.71	40.45	102.88	0.515	11/out→in	N	Cell membrane	46

*: "N" indicated no signal peptide, "Y" indicated there is a signal peptide.

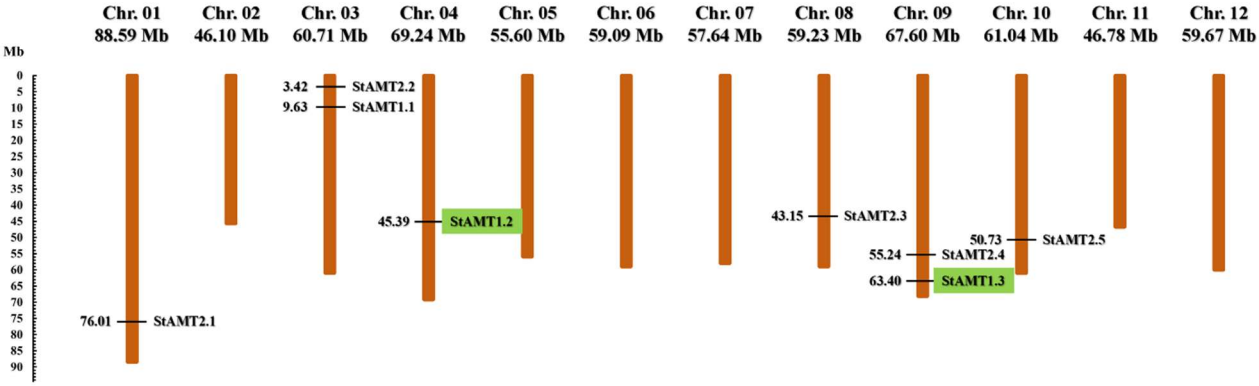

Fig. 1. Chromosome localization and gene duplication event analysis of 8 *StAMTs*. Green indicated gene duplication events. Chr.: Chromosome. Mb: million base pairs.

RESULTS

Identification of analysis of AMT family members in potato

A total of 8 StAMTs were screened from the potato genome. The 8 StAMTs were renamed according to gene ID and their location on chromosomes (Table 1, Fig. 1). The number (No.) of amino acid residues (AARs) of 8 StAMTs ranged from 460 to 512, the molecular weight ranged from 49.67 kDa to 55.11 kDa, and the No. of GPSs ranged from 34 to 55. The isoelectric points (*pI*) of StAMT2.2 and StAMT2.5 were greater than 7, and the *pI* of the other 6 proteins were all less than 7. The instability index of StAMT2.5 was greater than 40, and the other 7 proteins was less than 40. The 8 StAMTs were expressed on the cell membrane, all had 11 TMDs (out→in), the aliphatic index were all greater than 89, and the grand average of hydropathicity (GRAVY) were all greater than 0. However, except StAMT1.2, which had a signal peptide, the other 7 proteins had no signal peptides (Table 1).

Chromosome localization and phylogenetic tree analysis

The results of gene location showed that there were 12 chromosomes in potato, 6 of which contained *StAMTs*. Chromosomes 1, 4, 8 and 10 contained only one gene (*StAMT2.1*, *StAMT1.2*, *StAMT2.3* and *StAMT2.5*). Chromosomes 3 and 9 all had two genes, *StAMT1.1* and *StAMT2.2* were on chromosome 3, and *StAMT1.3* and *StAMT2.4* were on chromosome 9 (Fig. 1). The sequence similarity and coverage rate of 8 *StAMTs* were analyzed using NCBI-Blast program, and the results showed that only *StAMT1.2* and *StAMT1.3* had similarities and coverage rates exceeding 75%, indicating that the two genes were a pair of gene duplication event (Fig. 1). *Ka* and *Ks* values were 0.1438 and 2.9255 after calculation by DnaSP6 software, that is, *Ka*/*Ks*<1, indicating that the two genes were purifying selection (Li *et al*., 2019).

Based on the phylogenetic tree of AtAMTs and StAMTs, 8 StAMTs could be divided into two subfamilies: AMT1 and AMT2, which respectively had more than 65% consistency with the amino acid sequences of AMT1 and AMT2 members of *Arabidopsis thaliana* (Fig. **S1**). According to different distribution in the tree, AMT1 subfamily could be divided into two groups (Fig. **2**), and Group II contained only StAMT1.1, indicating that StAMT1.1 may have different sequence composition or gene function from other genes. The prediction results of the tertiary structure of StAMTs showed that the tertiary structure of StAMT1.2 and StAMT1.3 was similar, and 5 proteins of StAMT2 subfamily had similar structure (Fig. 3). However, the tertiary structure of StAMT1.1 was different from that of the other 7 proteins, which was consistent with the analysis results of phylogenetic tree, indicating that StAMT1.1 may have important and specific gene functions.

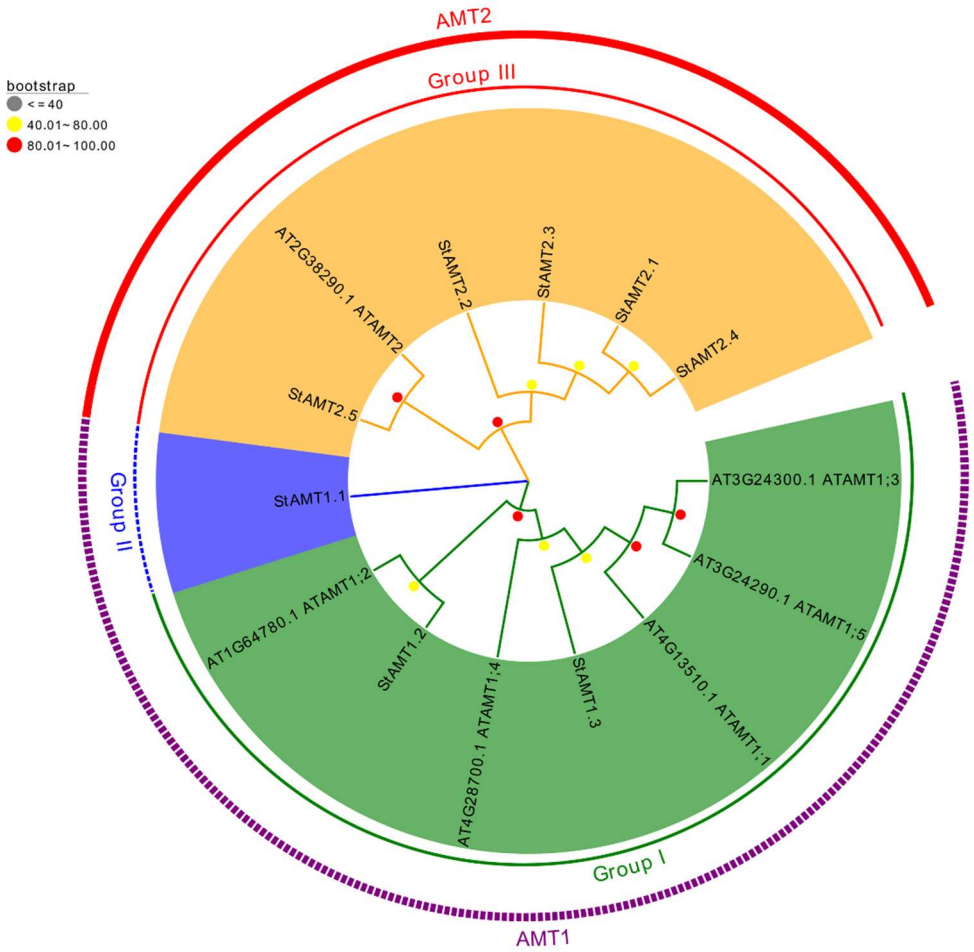

Fig. 2. Neighbor-joining tree for the AMT genes in potato based on characterized AMT genes in ***A. thaliana*.** Prefix "St" indicated potato, and "AT" indicated *A. thaliana*.

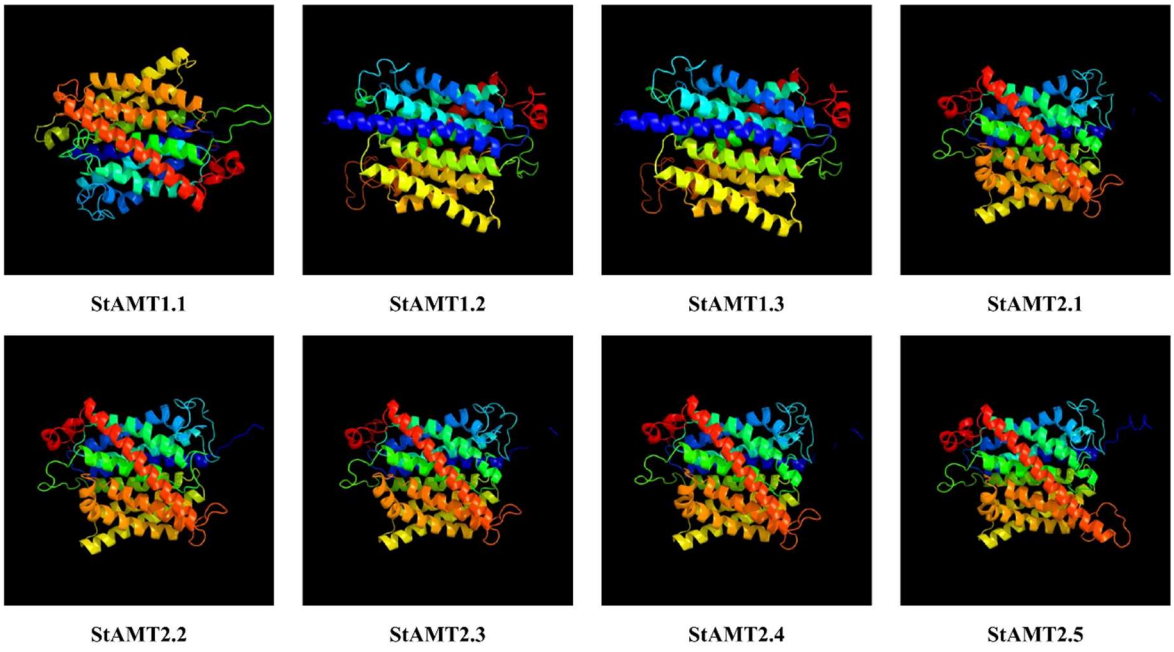

Fig. 3. Protein tertiary structure prediction of StAMTs.

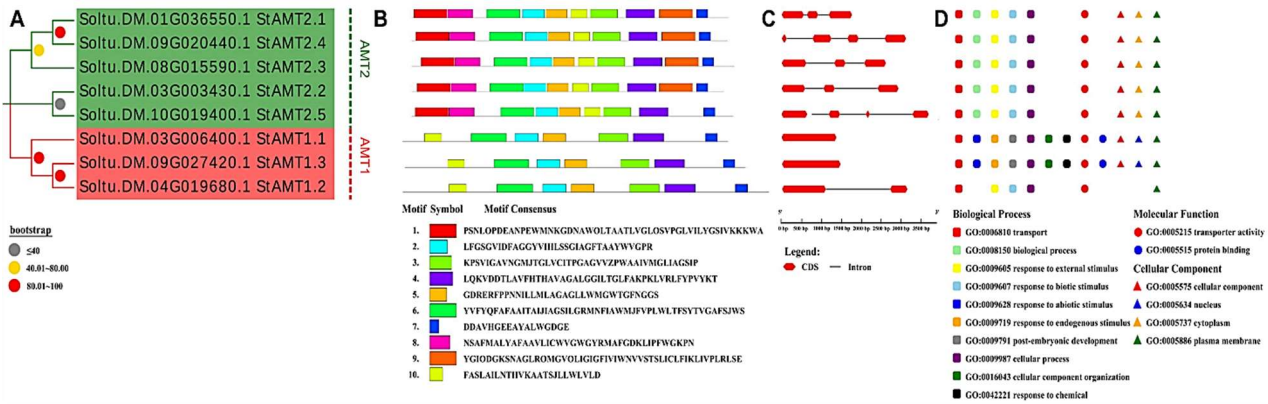

Fig. 4 Sequence analysis of StAMTs. **A.** Phylogenetic tree. **B.** Motif composition. **C.** Gene structure. **D.** GO annotation.

Analysis of gene structure, motif composition and GO annotation

Motif composition analysis results showed that members in the same subfamily had the same motif composition. AMT1 subfamily members were composed of motifs 2, 3, 4, 5, 6, 7 and 10, and 4 AMT2 subfamily members contained all 10 motifs, while StAMT2.5 lacked motif 9 (Fig. 4B). The results of gene structure analysis showed that only two proteins of StAMT1.1 and StAMT1.3 had no introns, while the other 6 proteins had 2-4 introns (Fig. 4C).

The GO annotation analysis results showed that 8 StAMTs had some functional similarities and differences (Fig. 4D). In Biological Process, AMT2 subfamily contained 5 functions, while StAMT1.2 lacked "biological process". StAMT1.1 and StAMT1.3 both had 7 functions, and only "transport" and "cellular process" were the same as the other 6 proteins. In Molecular Function, 8 StAMTs had "transporter activity", StAMT1.1 and StAMT1.3 had "transporter activity" and "protein binding". In Cellular Component, AMT2 subfamily members were the component of cell, cytoplasm and plasma membrane, StAMT1.1 and StAMT1.3 were the components of cell, nucleus and plasma membrane, while StAMT1.2 was only the component of plasma membrane.

CAEs analysis

Analysis results of CAEs in the promoter region of *StAMTs* showed that there were 73 different CAEs in the promoter sequence of 8 *StAMTs*, and the functions of 48 elements could be understood according to the information provided by PlantCARE website, which were related to abiotic stress response, growth and development, light and hormonal response, respectively (Table S2). The promoter region of 8 *StAMTs* contained 33, 30, 28, 25, 28, 34, 37 and 26 different elements, and the number and type of elements contained in each gene were different. AT~TATA-box, CAAT-box, MYB, MYC, TATA-box and Unnamed__4 were the common or core components of the promoter regions of 8 *StAMTs* (Zhang *et al*., 2023). CCAAT-box (MYBHv1 binding site) and MYB recognition site were unique to *StAMT1.1*. LAMP-element (part of a light responsive element), RY-element (CAE involved in seed-specific regulation) and WRE3 (wounding responsiveness (Yang *et al*., 2021)) were unique to *StAMT1.2*. AT-rich sequence (element for maximal elicitor-mediated activation), Box III (protein binding site), F-box (protein degradation related (Ramachandran *et al*., 2021)) and HD-Zip 3 (protein binding site) were unique to *StAMT1.3*. ACA-motif (part of gapA in (GAPA-CMA1) involved with light responsiveness) was unique to *StAMT2.1*. CTAG-motif (involved in the growth and development of plants (Gangwar *et al*., 2022), chs-CMA2a (part of a light responsive element), TCA-element (CAE involved in salicylic acid responsiveness) and TC-rich repeats (CAE involved in defense and stress responsiveness) were unique to *StAMT2.2*. AuxRE (part of an auxin-responsive element), CAT-box (CAE related to meristem expression), chs-Unit 1 m1 (part of a light responsive element) and MBSI (MYB binding site involved in flavonoid biosynthetic genes regulation) were unique to *StAMT2.4*. 3-AF1 binding site (light responsive element), A-box (sequence conserved in alpha-amylase promoters), AC-I (involved in the regulation of lignin biosynthesis (Zhu *et al*., 2023) and AuxRR-core (CAE involved in auxin responsiveness) were unique to *StAMT2.5*. In summary, these results suggested that 8 StAMTs may be involved in a variety of abiotic stress responses and plant growth regulation responses, with certain functional similarity and specificity.

Expression pattern analysis of StAMTs in different environments

The expression patterns of 8 *StAMTs* in Control, NH_4^+ sufficiency or NH_4^+ deficiency environments were detected by qRT-PCR (Fig. 5). The results showed that compared with the expression levels of 8 *StAMTs* in Control environment, under NH_4^+ sufficiency condition, the expression level of *StAMT1.1* was significantly increased, and the expression levels of other 7 genes were

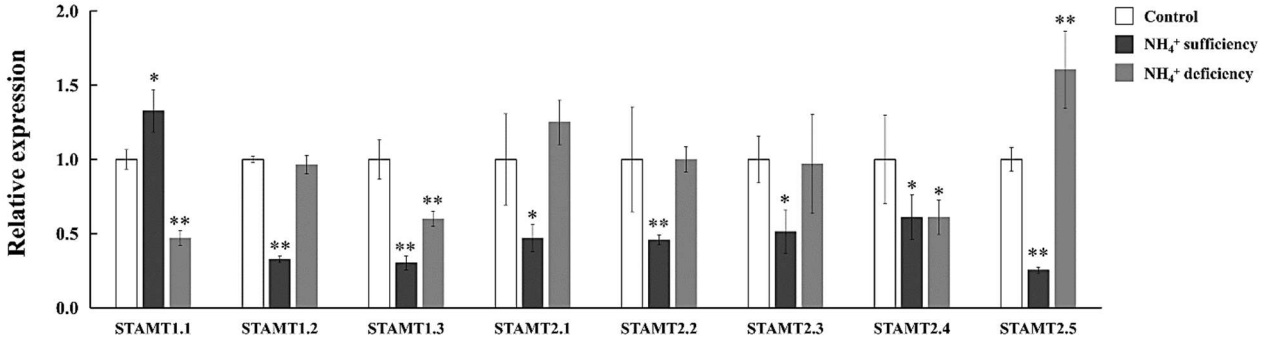

Fig. 5. Expression pattern analysis of StAMTs under Control, NH_4^+ sufficient and NH_4^+ deficient environments**.** Control, NH_4^+ sufficient and NH_4^+ deficient environments indicated normal Hoagland nutrient solution, Hoagland nutrient (without NO_3^-) with 2 mM NH_4^+, and Hoagland nutrient (without NO_3^-) with 0.2 mM NH_4^+, respectively. The quantitative data were detected by taking 3 biological replicates and 3 technical replicates, and the relative expression level of each gene was calculated using the $2^{-\Delta\Delta Ct}$ method. The data were analyzed by *t*-test of Excel software, and "*" and "**" indicated that the expression level of a gene under NH_4^+ sufficient or NH_4^+ deficient condition had significant and extremely significant differences compared with that under Control condition at $P<0.05$ and $P<0.01$ levels, respectively.

significantly decreased under NH_4^+ sufficiency condition. Under NH_4^+ deficiency condition, the expression levels of *StAMT1.1*, *StAMT1.3* and *StAMT2.4* decreased significantly, while the expression levels of *StAMT1.2*, *StAMT2.1*, *StAMT2.2* and *StAMT2.3* were not significantly changed, and the expression level of StAMT2.5 was significantly increased.

Effects of overexpressing StAMTs on the growth of *A. thaliana*

Recombinant vectors of 8 *StAMTs* and pBI121-HA were successfully constructed using homologous recombination technology, and transformed into GV3101 (Fig. S2). Transgenic *A. thaliana* lines overexpressing *StAMTs* were successfully obtained by *Agrobacteria*-mediated and the detection of PCR and qRT-PCR (Figs. S3, S4). Three lines were selected from the transgenic lines overexpressing different *StAMTs* for further study, and T_3 homozygous seeds were obtained.

The growth of Columbia-0 and transgenic lines were analyzed under Control, NH_4^+ sufficiency or NH_4^+ deficiency conditions. The results showed that in terms of survival rate, compared with Columbia-0, the survival rates of transgenic lines overexpressing 8 *StAMTs* were all decreased by 36%-100% under Control condition. Under NH_4^+ sufficiency condition, the survival rates of the lines overexpressing *StAMT1.1* and *StAMT1.3* were increased significantly by 200%-300%, and the other lines died or had no significant change in survival rate. Under NH_4^+ deficiency condition, the lines overexpressing *StAMT1.1*, *StAMT1.3* and *StAMT2.4* and the line 21.1 overexpressing *StAMT2.1* were died or decreased significantly by about 57% in survival rate, while the survival rates of the lines overexpressing *StAMT1.2* were increased significantly by 57%-86%, and the survival rates of other lines did not change significantly (Fig. 6A).

In terms of root length, under Control condition, compared with Columbia-0, the root lengths of the lines 24.1 and 24.3 overexpressing *StAMT2.4* and the lines overexpressing *StAMT2.5* were not significantly different, while the root lengths of the other lines were significantly shorter than that of Columbia-0 with a reduction of 26%-61%. Under NH_4^+ sufficiency condition, there was no significant difference between the root lengths of the lines 11.3, 23.1, 24.1, 24.3, 25.1-25.3 and Columbia-0, and the other lines were either died or significantly shorter than Columbia-0 with a reduction of 13%-80%. Under NH_4^+ deficiency condition, the root lengths of the lines overexpressing *StAMT1.1*, *StAMT1.2*, *StAMT1.3*, *StAMT2.1* and *StAMT2.4* were significantly shorter than that of Columbia-0 with a reduction of 15%-58%, while the root lengths of the lines overexpressing *StAMT2.2*, *StAMT2.3* and *StAMT2.5* were significantly longer than that of Columbia-0 with an increase of 13%-37% (Figs. 6B, S5).

In fresh weight, under Control condition, the fresh weights of the lines 22.1, 23.3, 25.2 and 25.3 and the lines overexpressing *StAMT1.2* had no significant difference compared with Columbia-0, while the fresh weights of the lines overexpressing *StAMT2.4* and lines 23.1 and 23.2 were significantly higher than that of Columbia-0 with an increase of 64%-94%, and the other lines were either dead or significantly lower than Columbia-0 with a reduction of 22%-61%. Under NH_4^+ sufficiency condition, the fresh weights of all lines had no significant change or was significantly lower than that of Columbia-0. Under NH_4^+ deficiency condition, the fresh weights of lines overexpressing *StAMT2.3* and *StAMT2.5* were significantly higher than that of Columbia-0 with an increase of 84%-105%, while the other lines were dead or the fresh weight was significantly or insignificantly lower than that of Columbia-0 with a reduction of 11%-78% (Fig. 6C).

In terms of ANC, under Control condition, the ANCs of the lines overexpressing *StAMT1.3*, *StAMT2.3* and

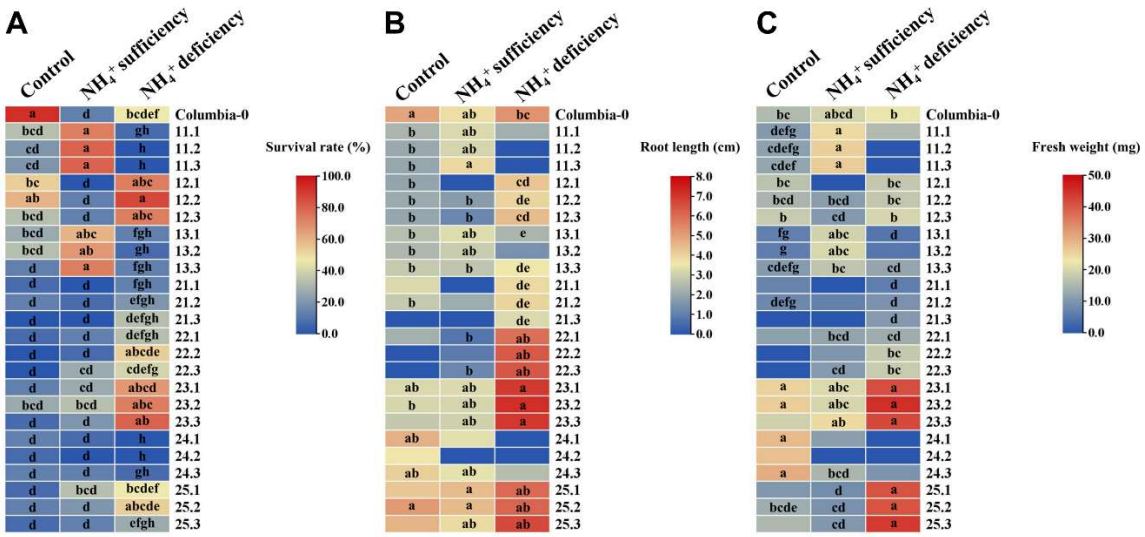

Fig. 6. Analysis of growth difference between transgenic ***A. thaliana*** and Columbia-0 under Control, NH_4^+ sufficiency and NH_4^+ deficiency conditions. **A.** Survival rate. **B.** Root length. **C.** Fresh weight. Control, NH_4^+ sufficient and NH_4^+ deficient environments indicated normal Hoagland nutrient solution, Hoagland nutrient (without NO_3^-) with 2 mM NH_4^+, and Hoagland nutrient (without NO_3^-) with 0.2 mM NH_4^+, respectively. 11.1-11.3, 12.1-12.3, 13.1-13.3, 21.1-21.3, 22.1-22.3, 23.1-23.3, 24.1-24.3, 25.1-25.3 were transgenic lines that overexpressing *StAMT1.1-StAMT2.5*, respectively. Each data was detected by taking 3 biological replicates and 5 technical replicates, and the data were analyzed by SPSS software (one-way analysis of variance, posterior comparisons, Tukey test). Different lowercase letters indicated significant differences at $P<0.05$. Some columns were not lettered because the survival rates of the corresponding lines resulted in only 0-2 measurable data, making it impossible to calculate the variance.

StAMT2.4 were increased (26%-100%) significantly compared with Columbia-0, and the other lines were died or the ANCs were significantly or insignificantly lower (0.8%-58%) than that of Columbia-0 (except for line 11.3) (Fig. 7). Under NH_4^+ sufficiency condition, the ANCs of the lines overexpressing *StAMT2.2* were significantly higher (3%-13%) than that of Columbia-0, while other lines were died or the ANCs were significantly lower (3%-81%) than that of Columbia-0 (Fig. 7). Under NH_4^+ deficiency condition, only the line 22.3 overexpressiong *StAMT2.3* had a significantly higher (4%) ANC than that of Columbia-0, while the other lines were died or the ANCs were significantly lower (5%-84%) than that of Columbia-0 (Fig. 7).

DISCUSSION

Through identification and analysis of potato AMT family members, 8 StAMTs were finally screened from potato genome. The No. of GPSs of 8 StAMTs ranged from 34 to 55, and the GPSs may promote the interaction of StAMTs with other proteins or bind to CAEs of other stress response genes, thus playing a regulatory role in gene expression and plant growth and development (Uçarli, 2021). StAMT2.2 and StAMT2.5 were basic proteins because the *pI* was greater than 7, while the other 6 proteins were acidic proteins. The instability indexes of StAMT2.5 was greater than 40, indicating poor stability, while the other 7 proteins all had a good stability. The aliphatic indexes of 8 StAMTs proteins were all greater than 89, indicating that they had good thermal stability. The GRAVYs of 8 StAMTs were greater than 0, they all were hydrophobic proteins (Drews *et al*., 2004; Li *et al*., 2022). TMDs and signal peptides are the hallmarks of secreted proteins, and the NO. of TMDs of AMT genes in various plant species ranges from 9 to 12 (von Wirén and Merrick, 2004). All 8 StAMTs contained 11 TMDs, and StAMT1.2 had a signal peptide which indicated that StAMT1.2 may be guided into suborganelles containing different membrane structures in cells under the action of the signal peptide to exert functions.

Based on the location distribution in the phylogenetic tree, gene function can be inferred (Li *et al*., 2019). For example, in *A. thaliana*, *AtAMT1.2* and *AtAMT2* are mainly expressed in the root or stem, and with the increase of time of nitrogen deficiency, the expression levels of *AtAMT1.2* and *AtAMT2* were changed significantly (Gazzarrini *et al*., 1999; Sohlenkamp *et al*., 2002). Therefore, according to the phylogenetic tree, *StAMT1.2* and *StAMT2.5*, which were closely related to *AtAMT1.2* and *AtAMT2*, may also have similar functions or roles. Previous studies considered these conserved motifs to play functional and/or structural roles in active proteins, and the exon/intron structure analyses could give an understanding of the gene functions, regulation and evolution (Yamasaki *et al*., 2013; Feng *et al*., 2016). Therefore, based on the analysis results of phylogenetic tree, gene structure, motif, CAEs of promoter sequence, GO annotation and protein tertiary structure prediction, the 8 *StAMTs* were both similar and different in structure and function.

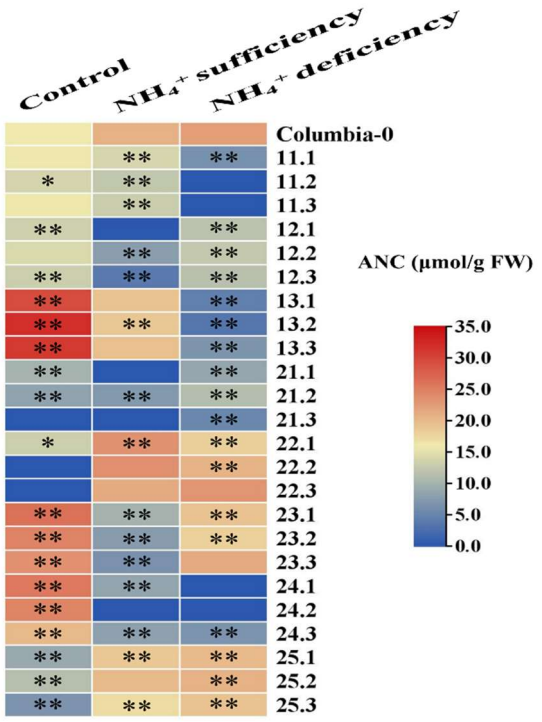

Fig. 7. ANC analysis of transgenic ***A. thaliana*** lines and Columbia-0 under Control, NH_4^+ sufficiency and NH_4^+ deficiency conditions. Control, NH_4^+ sufficient and NH_4^+ deficient environments indicated normal Hoagland nutrient solution, Hoagland nutrient (without NO_3^-) with 2 mM NH_4^+, and Hoagland nutrient (without NO_3^-) with 0.2 mM NH_4^+, respectively. 11.1-11.3, 12.1-12.3, 13.1-13.3, 21.1-21.3, 22.1-22.3, 23.1-23.3, 24.1-24.3, 25.1-25.3 were transgenic lines that overexpressing *StAMT1.1-StAMT2.5*, respectively. Each data was detected by taking 3 biological replicates and 3 technical replicates, and the data were analyzed by *t*-test of Excel software. "*" and "**" indicated that the index value of transgenic ***A. thaliana*** had significant and extremely significant differences compared with that of Columbia-0 at $P<0.05$ and $P<0.01$ levels, respectively.

HATS and LATS are two ammonium nitrogen absorption systems, and play key roles in the absorption and transport of NH_4^+ in plants when the concentration of NH_4^+ was <1 mM and >1 mM, respectively (Wang *et al*., 2022; Xia *et al*., 2023). In this study, by analyzing the expression patterns of 8 *StAMTs* under Control, NH_4^+ sufficient and NH_4^+ deficiency conditions, it was found that StAMT1.1 may be a low affinity protein and StAMT2.5 may be a high affinity protein.

AMT is closely related to the uptake and utilization of NH_4^+ and the growth and development of plants (von Wirén and Merrick, 2004; Wang *et al*., 2022). To investigate the functions of StAMTs in plant, 8 *StAMTs* were overexpressed in *A. thaliana* for analyzing the growth of transgenic lines and Columbia-0 under Control, NH_4^+ sufficient and NH_4^+ deficiency conditions. The results showed that overexpression of *StAMTs* had a certain effect on the growth of *A. thaliana*. It is worth noting that under Control and NH_4^+ sufficiency conditions, the survival rate, root length and fresh weight of the lines overexpressing *StAMT2.3* and *StAMT2.5* were significantly lower than those of Columbia-0 or had no significant changes. However, under NH_4^+ deficiency condition, the survival rate of the lines overexpressing *StAMT2.3* and *StAMT2.5* had no significant changes, but the root length and fresh weight were significantly higher than those of Columbia-0, indicating that *StAMT2.3* and *StAMT2.5* may be high-affinity proteins. Combined with the analysis results of *StAMTs* expression pattern in potato, the functional analysis results of StAMT2.5 in *A. thaliana* were consistent, indicating StAMT2.5 was worthy of further study. However, StAMT1.1 was predicted to be a low-affinity protein based on its expression pattern, which was inconsistent with the research results in *A. thaliana*. The analysis results of the phylogenetic tree and three-dimensional structure indicated that StAMT1.1 may have different functions from other proteins and may have special functions in potato, which may be the reason for the inconsistent results of expression pattern in potato and functional analysis in *A. thaliana*. In addition, the expression of AMT is related to NH_4^+ concentration. For example, PttAMT1.2 (*Populus tremula*×*tremuloides*) encodes a high-affinity protein, which is affected by NH_4^+ concentration, compared with the NH_4^+ concentration of 2 mmol/L, the transcription level was increased by four times when NH_4^+ concentration was 10 μmol/L and 100 μmol/L (Selle *et al*., 2005). Therefore, for StAMT2.3, it was predicted to may be a high-affinity protein based on the functional analysis in *A. thaliana*, and its expression level in potato decreased under NH_4^+ sufficiency condition, but under NH_4^+ deficiency condition, the upregulated expression of *StAMT2.3* may not be induced due to inappropriate concentration of NH_4^+, resulting in inconsistent results between quantitative analysis in potato and functional analysis in *A. thaliana*.

AMT is the main protein family involved in the absorption and transport of NH_4^+, and its expression level is affected by the nitrogen nutrition status and the NH_4^+ concentration in plants (Wang *et al*., 2022). When nitrogen nutrition is limited, the expression of *AtAMT1.1* in the roots of *A. thaliana* is increased (Gazzarrini *et al*., 1999). When nitrogen supply is sufficient, the expression of *AtAMT1.1* in leaves is significantly increased (Engineer and Kranz 2007). Under nitrogen deficiency condition, the expression of *LeAMT1.1* of tomato is significantly upregulated (von Wirén *et al*., 2000). *OsAMT1.1* dominates the uptake of NH_4^+ in rice roots, which is induced by low nitrogen and inhibited by high nitrogen. The expression of *BnAMT1.2* (*Brassica napus*) is increased with the increase of NH_4^+ concentration, but is downregulated under continuous high nitrogen condition (Pearson *et al*., 2002). These results suggest that AMT plays an important role in the uptake and utilization of NH_4^+ by plants. Through the ANC detection, the overexpression of *StAMTs* had significant effects on

the ANC in plants under different conditions. Combined with the analysis of plant growth phenotype, the lines overexpressing *StAMT2.3* may enhance the ability to absorb NH_4^+ under Control condition, but could not enhance the utilization of NH_4^+. Under the condition of only NH_4^+ supply, overexpression of *StAMT2.3* may be able to enhance the utilization ability of NH_4^+.Overexpression of *StAMT2.5* may reduce the ability of plants to absorb NH_4^+ under the Control and NH_4^+ sufficiency conditions, while may enhance the utilization of NH_4^+ under NH_4^+ deficiency condition.

In addition to NH_4^+ concentration, day and night alternation, light, plant hormones, protein phosphorylation, NO_3^-, CO_2, K^+, symbiotic bacteria, pH and environment factors all can regulate the expression and protein activity of AMT in plants (Gazzarrini *et al*., 1999; von Wirén *et al*., 2000; Sohlenkamp *et al*., 2002; DesRochers *et al*., 2003; D'Apuzzo *et al*., 2004; Selle *et al*., 2005; Chalot *et al*., 2006; Couturier *et al*., 2007; Loqué *et al*., 2007; Neuhäuser *et al*., 2007; Hoopen *et al*., 2010; Mota *et al*., 2011; Yuan *et al*., 2013; Zhang *et al*., 2014; Abouelsaad *et al*., 2016; Meng *et al*., 2016; Jia *et al*., 2017; Huang *et al*., 2018; Wu *et al*., 2019), indicating that the function of AMT is regulated by multiple factors, and its regulation of plant absorption and utilization of NH_4^+ may also be the result of collaboration of multiple proteins or factors, and the role of a single AMT may be regulated by other proteins. Therefore, the functions and molecular mechanism of *StAMTs* requires more studies to be elucidated. In the process of potato growth, due to microbial nitrification, both NH_4^+ in soil and NH_4^+ fertilizer will be converted into NO_3^-, so very little NH_4^+ can be absorbed and utilized by potatoes. Based on the results of this study, StAMT2.5 may improve the utilization of NH_4^+ in NH_4^+ deficiency environment, so it can be used as the first choice for further research.

CONCLUSION

In this study, 8 AMT family members were screened from potato genome, which can be divided into two subfamilies. These genes were distributed on different chromosomes, and *StAMT1.2* and *StAMT1.3* were a pair of gene duplication events (purification selection). The length of 8 proteins ranged from 460 to 512 AARs, the molecular weight ranged from 49.67 kDa to 55.11 kDa, and the No. of GPSs ranged from 34 to 55. All 8 StAMTs were hydrophobic proteins with good thermal stability and 11 TMDs. Among them, StAMT2.2 was a stable basic protein, StAMT2.5 was an unstable basic protein, and other proteins were stable acidic proteins, and only StAMT1.2 had a signal peptide. The functions of 8 StAMTs were inferred and analyzed by the analysis of phylogenetic tree, protein tertiary structure prediction, motif, gene structure, GO annotation and CAEs. By detecting the expression patterns under Control, NH_4^+ sufficient and NH_4^+ deficiency conditions, it was found that StAMT1.1 may be a low affinity protein and StAMT2.5 may be a high affinity protein. The transgenic *A. thaliana* lines overexpressing 8 *StAMTs* were constructed. The growth of transgenic lines and Columbia-0 under Control, NH_4^+ sufficient and NH_4^+ deficiency conditions were analyzed. The results showed that different genes had different effects on the growth of *A. thaliana*, and StAMT2.3 and StAMT2.5 may be high-affinity proteins. In addition, the ANC detection results showed that StAMT2.3 and StAMT2.5 may enhance the ability of plants to utilize NH_4^+ under NH_4^+ deficiency condition. In summary, StAMT2.5 may have a similar function in potato and *A. thaliana*, so it can be the next research focus.

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LITERATURE CITED

Abouelsaad, I., Weihrauch, D., Renault, S. 2016 Effects of salt stress on the expression of key genes related to nitrogen assimilation and transport in the roots of the cultivated tomato and its wild salt-tolerant relative. Sci. Hortic. **211**: 70–78.

Amtmann, A., Armengaud P. 2009 Effects of N, P, K and S on metabolism: new knowledge gained from multi-level analysis. Curr. Opin. Plant Biol. **12(3)**: 275–283.

Chalot, M., Blaudez, D., Brun, A. 2006 Ammonia: a candidate for nitrogen transfer at the mycorrhizal interface. Trends Plant Sci. **11(6)**: 263–266.

Chen, L.-G., Xiang, S.-Y., Chen, Y.-L., Li, D.-B., Yu, D.-Q. 2017 *Arabidopsis* WRKY45 interacts with the DELLA protein RGL1 to positively regulate age-triggered leaf senescence. Mol. Plant **10(9)**: 1174–1189.

Choudhury, A.T.M.A., Kennedy, I.R. 2005 Nitrogen Fertilizer Losses from Rice Soils and Control of Environmental Pollution Problems. Commun. Soil Sci. Plant Anal. **36(11-12)**: 1625–1639.

Couturier, J., Montanini, B., Martin, F., Brun, A., Blaudez, D., Chalot, M. 2007 The expanded family of ammonium transporters in the perennial poplar plant. New Phytol. **174(1)**: 137–150.

D'Apuzzo, E., Rogato, A., Simon-Rosin, U., Alaoui, H.E., Barbulova, A., Betti, M., Dimou, M., Katinakis, P., Marquez, A., Marini, A.M., Udvardi, M.K., Chiurazzi, M. 2004 Characterization of Three Functional High-Affinity Ammonium Transporters in *Lotus japonicus* with Differential Transcriptional Regulation and Spatial Expression. Plant Physiol. **134(4)**: 1763–1774.

DesRochers, A., van den Driessche, R., Thomas, B.R. 2003 Nitrogen fertilization of trembling aspen seedlings grown on soils of different pH. Can. J. For. Res. **33(4)**: 552–560.

Drews, O., Reil, G., Parlar, H., Görg, A. 2004 Setting up standards and a reference map for the alkaline proteome of

the Gram-positive bacterium *Lactococcus lactis*. Proteomics **4(5)**: 1293–1304.

Engineer, C.B., Kranz, R.G. 2007 Reciprocal leaf and root expression of *AtAmt1.1* and root architectural changes in response to nitrogen starvation. Plant Physiol. **143(1)**: 236–250.

Feng, K.-W., Liu, F.-Y., Zou, J.-W., Xing, G.-W., Deng, P.-C., Song, W.-N., Tong, W., Nie, X.-J. 2016 Genomewide identification, evolution, and co-expression network analysis of mitogen-activated protein kinase kinase kinases in *Brachypodium distachyon*. Front. Plant Sci. **7**: 1400.

Gangwar, H., Kumari, P., Gahlaut, V., Kumar, S., Jaiswal, V. 2022 Identification and comprehensive analysis of MIPSs in Rosaceae and their expression under abiotic stresses in rose (*Rosa chinensis*). Front. Plant Sci. **13**: 1021297.

Gazzarrini, S., Lejay, L., Gojon, A., Ninnemann, O., Frommer, W.B., von Wirén, N. 1999 Three Functional Transporters for Constitutive, Diurnally Regulated, and Starvation-Induced Uptake of Ammonium into *Arabidopsis* Roots. Plant Cell **11(5)**: 937–947.

Gong, H.-L., Ma, J.-X., Dusengemungu, L., Feng, Z.-P. 2024 Genome-wide identification and expression analysis of the cellulose synthase gene family in potato (*Solanum tuberosum* L.). Front. Plant Sci. **15**: 1457958.

Hao, D.-L., Zhou, J.-Y., Yang, S.-Y., Qi, W., Yang, K.-J., Su, Y.-H. 2020 Function and Regulation of Ammonium Transporters in Plants. Int. J. Mol. Sci. **21(10)**: 3557.

Hoopen, F.T., Cuin, T.A., Pedas, P., Hegelund, J.N., Shabala, S., Schjoerring, J.K., Jahn, T.P. 2010 Competition between uptake of ammonium and potassium in barley and *Arabidopsis* roots: molecular mechanisms and physiological consequences. J. Exp. Bot. **61(9)**: 2303–2315.

Huang, L.-L., Li, M.-J., Shao, Y., Sun, T.-T., Li, C.-Y., Ma, F.-W. 2018 Ammonium uptake increases in response to PEG-induced drought stress in *Malus hupehensis* Rehd. Environ. Exp. Bot. **151**: 32–42.

Jia, J.-B., Zhou, J., Shi, W.-G., Cao, X., Luo, J., Polle, A., Luo, Z.-B. 2017 Comparative transcriptomic analysis reveals the roles of overlapping heat-/drought-responsive genes in poplars exposed to high temperature and drought. Sci. Rep. **7**: 43215.

Jiang, S.-Y., González, J.M., Ramachandran, S. 2013 Comparative Genomic and Transcriptomic Analysis of Tandemly and Segmentally Duplicated Genes in Rice. PLoS One **8(5)**: e63551.

Kolomiets, M.V., Hannapel, D.J., Chen, H., Tymeson, M., Gladon, R.J. 2001 Lipoxygenase is involved in the control of potato tuber development. Plant Cell **13(3)**: 613–626.

Lanquar, V., Loqué, D., Hörmann, F., Yuan, L.-X., Bohner, A., Engelsberger, W.R., Lalonde, S., Schulze, W.X., von Wirén, N., Frommer, W.B. 2009 Feedback Inhibition of Ammonium Uptake by a Phospho-Dependent Allosteric Mechanism in *Arabidopsis*. Plant Cell **21(11)**: 3610–3622.

Li, H., Han, J.-L., Chang, Y.-H., Lin, J., Yang, Q.-S. 2016 Gene characterization and transcription analysis of two new ammonium transporters in pear rootstock (*Pyrus betulaefolia*). J. Plant Res. **129(4)**: 737–748.

Li, M., Wu, Y.-J., Yu, Z.-L., Sheng, G.-P., Yu, H.-Q. 2009 Enhanced nitrogen and phosphorus removal from eutrophic lake water by *Ipomoea aquatica* with low-energy ion implantation. Water Res. **43(5)**: 1247–1256.

Li, W., Chen, Y., Ye, M.-H., Lu, H.-B., Wang, D.-D., Chen, Q. 2020 Evolutionary history of the C-repeat binding factor/dehydration-responsive element-binding 1 (CBF/DREB1) protein family in 43 plant species and characterization of CBF/DREB1 proteins in *Solanum tuberosum*. BMC Evol. Biol. **20**: 142.

Li, W., Cheng, M., Zhao, Y.-P. 2022 Identification and characterization of potassium (K^+) transporters in potato (*Solanum tuberosum* L.). Taiwania **67(3)**: 422–434.

Li, W., Dong, J.-Y., Cao, M.-X., Gao, X.-X., Wang, D.-D., Liu, B.-L., Chen, Q. 2019 Genome-wide identification and characterization of HD-ZIP genes in potato. Gene **697**: 103–117.

Liu, B.-L., Zhang, G.-D., Murphy, A., de Koeyer, D., Tai, H., Bizimungu, B., Si, H.-J., Li, X.-Q. 2016 Differences between the Bud End and Stem End of Potatoes in Dry Matter Content, Starch Granule Size, and Carbohydrate Metabolic Gene Expression at the Growing and Sprouting Stages. J. Agric. Food. Chem. **64(5)**: 1176–1184.

Liu, B.-L., Zhao, S., Wu, X.-F., Wang, X.-Y., Nan, Y.-Y., Wang, D.-D., Chen, Q. 2017 Characterization of phosphate transporter genes in potato. J. Biotechnol. **264**: 17–28.

Loqué, D., Lalonde, S., Looger, L.L., von Wirén, N., Frommer, W.B. 2007 A cytosolic *trans*-activation domain essential for ammonium uptake. Nature **446(7132)**: 195–198.

Loqué, D., Yuan, L.-X., Kojima, S., Gojon, A., Wirth, J., Gazzarrini, S., Ishiyama, K., Takahashi, H., von Wirén, N. 2006 Additive contribution of AMT1;1 and AMT1;3 to high-affinity ammonium uptake across the plasma membrane of nitrogen-deficient *Arabidopsis* roots. The Plant Journal **48(4)**: 522–534.

Meng, S., Zhang, C.-X., Su, L., Li, Y.-M., Cao, Y., Zhao, Z. 2016 Distinct effect of pH on N uptake and assimilation in two conifer species. Trees-Struct. Funct. **30(5)**: 1607–1618.

Mota, M., Neto, C., Monteiro, A., Oliveira, C. 2011 Preferential ammonium uptake during growth cycle and identification of ammonium transporter genes in young pear trees. J. Plant Nutr. **34(6)**: 798–814.

Neuhäuser, B., Dynowski, M., Mayer, M., Ludewig, U. 2007 Regulation of NH_4^+ Transport by Essential Cross Talk between AMT Monomers through the Carboxyl Tails. Plant Physiol. **143(4)**: 1651–1659.

Nicot, N., Hausman, J.F., Hoffmann, L., Evers, D. 2005 Housekeeping gene selection for real-time RT-PCR normalization in potato during biotic and abiotic stress. J. Exp. Bot. **56(421)**: 2907–2914.

Nurmanov, Y.T., Chernenok, V.G., Kuzdanova, R.S. 2019 Potato in response to nitrogen nutrition regime and nitrogen fertilization. Field Crops Res. **231**: 115–121.

Pearson, J.N., Finnemann, J., Schjoerring, J.K. 2002 Regulation of the high-affinity ammonium transporter (*BnAMT1;2*) in the leaves of *Brassica napus* by nitrogen status. Plant Mol. Biol. **49(5)**: 483–490.

Qu, C.-Y., Li, L.-S., Feng, F., Jiang, K.-N., Wu, X., Qin, M.-C., Tang, J., Tang, X., Xiao, R.-Y., Wu, D., Tang, C.-J. 2023 Enhancement of extracellular Cr(VI) reduction for anammox recovery using hydrazine: performance, pathways, and mechanism. Front. Env. Sci. Eng. **17(9)**: 115.

Ramachandran, P., Joshi, J.B., Maupin-Furlow, J.A., Uthandi, S. 2021 Bacterial effectors mimicking ubiquitin-proteasome pathway tweak plant immunity. Microbiol. Res. **250**: 126810.

Selle, A., Willmann, M., Grunze, N., Geßler, A., Weiß, M., Nehls, U. 2005 The high-affinity poplar ammonium importer PttAMT1.2 and its role in ectomycorrhizal symbiosis. New Phytol. **168(3)**: 697–706.

Setién, I., Fuertes-Mendizabal, T., González, A., Aparicio-Tejo, P.M., González-Murua, C., González-Moro, M.B., Estavillo, J.M. 2013 High irradiance improves ammonium tolerance in wheat plants by increasing N assimilation. J. Plant Physiol. **170(8)**: 758–771.

Sohlenkamp, C., Wood, C.C., Roeb, G.W., Udvardi, M.K. 2002 Characterization of *Arabidopsis* AtAMT2, a high-affinity ammonium transporter of the plasma membrane. Plant Physiol. **130(4)**: 1788–1796.

Suenaga, A., Moriya, K., Sonoda, Y., Ikeda, A., von Wirén, N., Hayakawa, T., Yamaguchi, J., Yamaya, T. 2003 Constitutive expression of a novel-type ammonium transporter *OsAMT2* in rice plants. Plant Cell Physiol. **44(2)**: 206–211.

Sun, Y.-C., Sheng, S., Fan, T.-F., Liu, L., Ke, J., Wang, D.-B., Hua, J.-P., Liu, L.-H., Cao, F.-Q. 2019 Molecular identification and functional characterization of GhAMT1.3 in ammonium transport with a high affinity from cotton (*Gossypium hirsutum* L.). Physiol. Plant. **167(2)**: 217–231.

Uçarli, C. 2021 Genome-wide identification and in silico gene expression analysis of the related to ABI3/VP1 (RAV) transcription factor family in barley (*Hordeum vulgare* L.). Biocell **45(6)**: 1673–1685.

von Wirén, N., Lauter, F.R., Ninnemann, O., Gillissen, B., Walch-Liu, P., Engels, C., Jost, W., Frommer, W.B. 2000 Differential regulation of three functional ammonium transporter genes by nitrogen in root hairs and by light in leaves of tomato. The Plant Journal **21(2)**: 167–175.

von Wirén, N., Merrick, M. 2004 Regulation and function of ammonium carriers in bacteria, fungi, and plants. In: Molecular Mechanisms Controlling Transmembrane Transport. Topics in Current Genetics, vol 9. Springer, Berlin, Heidelberg.

Wang, Y.-J., Deng, D.-X., Bian, Y.-L., Lv, Y.-P., Xie, Q. 2010 Genome-wide analysis of primary auxin-responsive *Aux/IAA* gene family in maize (*Zea mays*. L.). Mol. Biol. Rep. **37(8)**: 3991–4001.

Wang, Y., Xuan, Y.-M., Wang, S.-M., Fan, D.-M., Wang, X.-C., Zheng, X.-Q. 2022 Genome-wide identification, characterization, and expression analysis of the ammonium transporter gene family in tea plants (*Camellia sinensis* L.) Physiol. Plant. **174**: e13646.

Wu, X.-Y., Liu, T., Zhang, Y.-J., Duan, F.-Y., Neuhäuser, B., Ludewig, U., Schulze, W.X., Yuan, L.-X. 2019 Ammonium and nitrate regulate NH_4^+ uptake activity of *Arabidopsis* ammonium transporter AtAMT1;3 via phosphorylation at multiple C-terminal sites. J. Exp. Bot. **70(18)**: 4919–4930.

Xia, J.-Z., Wang, Y., Zhang, T.-T., Pan, C., Ji, Y.-Y., Zhou, Y., Jiang, X.-Y. 2023 Genome-wide identification, expression profiling, and functional analysis of ammonium transporter 2 (AMT2) gene family in cassava (*Manihot esculenta* crantz). Front. Genet. **14**: 1145735.

Xian, L., Zhang, Y.-Z., Cao, Y., Wan, T., Gong, Y.-B., Dai, C., Ochieng, W.A., Nasimiyu, A.T., Li, W., Liu, F. 2020 Glutamate dehydrogenase plays an important role in ammonium detoxification by submerged macrophytes. Sci. Total Environ. **722**: 137859.

Yamasaki, K., Kigawa, T., Seki, M., Shinozaki, K., Yokoyama, S. 2013 DNA-binding domains of plant-specific transcription factors: structure, function, and evolution. Trends Plant Sci. **18(5)**: 267–276.

Yang, J., Xie, M.-X., Wang, X.-F., Wang, G.-N., Zhang, Y., Li, Z.-K., Ma, Z.-Y. 2021 Identification of cell wall-associated kinases as important regulators involved in *Gossypium hirsutum* resistance to *Verticillium dahliae*. BMC Plant Biol. **21**: 220.

Yu, C., Li, L.-S., Jiang, C.-K., Tang, X., Feng, F., Tang, J., Tang, C.-J., Chai, L.-Y. 2019a Insights into Anammox activity inhibition under trivalent and hexavalent chromium stresses. Biochem. Eng. J. **147**: 118–125.

Yu, C., Tang, X., Li, L.-S., Chai, X.-L., Xiao, R., Wu, D., Tang, C.-J., Chai, L.-Y. 2019b The long-term effects of hexavalent chromium on anaerobic ammonium oxidation process: Performance inhibition, hexavalent chromium reduction and unexpected nitrite oxidation. Bioresour. Technol. **283**: 138–147.

Yuan, L.-X., Gu, R.-L., Xuan, Y.-H., Smith-Valle, E., Loqué, D., Frommer, W.B., von Wirénd, N. 2013 Allosteric Regulation of Transport Activity by Heterotrimerization of *Arabidopsis* Ammonium Transporter Complexes in Vivo. Plant Cell **25(3)**: 974–984.

Yuan, L.-X., Loqué, D., Kojima, S., Rauch, S., Ishiyama, K., Inoue, E., Takahashi, H., von Wirénd, N. 2007 The organization of high-affinity ammonium uptake in *Arabidopsis* roots depends on the spatial arrangement and biochemical properties of AMT1-type transporters. Plant Cell **19(8)**: 2636–2652.

Zhang, C.-X., Meng, S., Li, Y.-M., Zhao, Z. 2014 Net NH_4^+ and NO_3^- fluxes, and expression of NH_4^+ and NO_3^- transporter genes in roots of *Populus simonii* after acclimation to moderate salinity. Trees **28(6)**: 1813–1821.

Zhang, H.-J., Zhu, X.-X., Xu, R.-Z., Yuan, Y.-S., Abugu, M.N., Yan, C.-S., Tieman, D., Li, X. 2023 Postharvest chilling diminishes melon flavor *via* effects on volatile acetate ester biosynthesis. Front. Plant Sci. **13**: 1067680.

Zhu, Y.-S., Wang, Y.-C., Jiang, H.-Y., Liu, W.-J., Zhang, S.-H., Hou, X.-K., Zhang, S.-S., Wang, N., Zhang, R., Zhang, Z.-Y., Chen, X.-S. 2023 Transcriptome analysis reveals that PbMYB61 and PbMYB308 are involved in the regulation of lignin biosynthesis in pear fruit stone cells. The Plant Journal **116(1)**: 217–233.

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