

Research Article

Cloning and Functional Determination of Ammonium Transporter PpeAMT3;4 in Peach

Shuanghong You,^{1,2} Yuqing Wang,^{2,3} Yanju Li,^{2,4} Yuhe Li,^{2,3} Ping Tan,^{1,2} Zheng Wu,^{1,2} Wenjing Shi,^{1,2} and Zhizhong Song^{2,3} 

¹Chongqing Academy of Agricultural Sciences, Chongqing 401329, China

²Key Laboratory of Molecular Module-Based Breeding of High Yield and Abiotic Resistant Plants in Universities of Shandong (Ludong University), Yantai 264025, China

³College of Agriculture, Ludong University, Yantai 264025, China

⁴Yantai Academy of Agricultural Sciences, Yantai 264000, China

Correspondence should be addressed to Zhizhong Song; 3614@ldu.edu.cn

Received 20 July 2020; Revised 15 November 2020; Accepted 20 November 2020; Published 4 December 2020

Academic Editor: Hamid Mukhtar

Copyright © 2020 Shuanghong You et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Ammonium (NH_4^+) plays key roles in plant growth, development, fruit quality, and yield. In plants, NH_4^+ uptake and transport are facilitated by NH_4^+ transporters (AMT). However, molecular mechanisms and physiological functions of type-II AMT (AMT2) transporters in fruit trees are still unclear, especially in peach. In this study, we cloned and characterized an AMT2 family gene from peach, *PpeAMT3;4*, and determined its function in yeast mutant. Expression analysis showed that *PpeAMT3;4* was majorly expressed in peach roots and significantly decreased by NH_4^+ excess but had no response to NH_4^+ deficiency. Functional determination and ¹⁵nitrogen-labeled NH_4^+ uptake assay in yeast cells implied that *PpeAMT3;4* was a typical high-affinity transporter, with a K_m value of 86.3 μM , that can uptake external NH_4^+ in yeast cells. This study provides gene resources to uncover the biological function of AMT2 transporters and reveals molecular basis for NH_4^+ uptake and nitrogen (N) nutrition mechanisms in fruit trees.

1. Introduction

Ammonium (NH_4^+) is the preferred form of nitrogen (N) source absorbed by both annual and perennial plant species, especially in N-deficient soils [1, 2]. However, with the increasing soil N input and atmospheric deposition, plants have to deal with NH_4^+ stress from sources below and above the ground [1–6]. Thus, an optimal amount of NH_4^+ should be effectively absorbed from the soil via plant roots, to sustain basic growth demands.

Extensive studies in angiosperms [3–5] and in basal land plant liverwort [6] indicated that NH_4^+ acquisition and uptake were performed by NH_4^+ transporters (AMT) through the plasma membrane of root cells. The first plant AMT family gene was observed in *Arabidopsis* [7], and AtAMT1;1, AtAMT1;2, and AtAMT1;3 successfully

restored the normal growth of the yeast mutant that is deficient in NH_4^+ uptake systems. Subsequent studies of AMT transporters have been identified in diverse plant species, including *Lotus japonicus* (*L. japonicus*) [8, 9], *Lycopersicon esculentum* (*L. esculentum*) [10–12], *Populus trichocarpa* (*P. trichocarpa*) [13], *Oryza sativa* (*O. sativa*) [14, 15], *Triticum aestivum* (*T. aestivum*) [16], *Alternanthera philoxeroides* (*A. philoxeroides*) [17], and so on, which have been characterized in *Xenopus* oocytes or yeast mutant. The AMT family transporters can be divided into two subfamilies: AMT1 and AMT2 [3–5]. In particular, the phosphorylation of amino acid residues of AMT proteins is a recognized means, by which NH_4^+ uptake activities were regulated [18, 19]. In *Arabidopsis*, the phosphorylation of the threonine (Thr) residue in the C-terminal tail region of AtAMT1.1 (Thr⁴⁶⁰), AtAMT1.2 (Thr⁴⁷²), and AtAMT1.3

(Thr⁴⁶⁴) led to the loss of NH₄⁺ transport activity in response to increasing external NH₄⁺ supplies [18–21], providing a novel regulatory mechanism that NH₄⁺ transport can be rapidly shut-off under high NH₄⁺ supply conditions.

Functional studies of plant AMT members were mainly focused on the AMT1 family, especially of annual model plants. However, molecular mechanisms towards NH₄⁺ uptake and transport in fruit trees are largely rare but were just observed in citrus [22] and pear [23, 24]. The biological and physiological functions of AMT2 members remain unknown. Favorably, the knowledge on NH₄⁺ uptake and transport in model plants provides valuable insights into the investigation of their roles in fruit trees.

As one of the most popular *Rosaceae* fruit crops, peach (*Prunus persica*) has its genome sequenced [25, 26]. In this study, we isolated and characterized a putative AMT2 family gene from peach (entitled as *PpeAMT3;4*) and determined that *PpeAMT3;4* is a typical HATS-mediated AMT member responsible for NH₄⁺ uptake in peach roots. Nonetheless, our findings provide a molecular basis of NH₄⁺ uptake for fruit trees.

2. Materials and Methods

2.1. Plant Material and Growth Condition. ‘Feicheng’ peach seedlings grown in the Key Laboratory of Molecular Module-Based Breeding of High Yield and Abiotic Resistant Plants in Universities of Shandong in Yantai, China, were used throughout this study. Germinated seedlings with similar growth status were transferred into 1/2 Murashige and Skoog (MS) liquid solution ([17, 27], containing approximately 1 mM NH₄Cl), cultivated in a climate-controlled growth cabinet that was maintained under 28°C/23°C and 12/12 h light/dark, with 60% relative humidity.

For the NH₄⁺ deficiency treatments, NH₄⁺ was omitted from the 1/2 MS medium. For the NH₄⁺ excess treatments, germinated seedlings were grown in 1/2 MS solution containing 20 mM NH₄Cl (pH 5.8). Seedlings were exposed to treatment for 72 h before quantitative real-time PCR (qRT-PCR) determination.

2.2. Cloning of *PpeAMT3;4* Gene in Peach. Protein sequences of poplar AMT genes [13] were used as query to BLAST the Phytozome Peach Genome Database (<http://www.phytozome.net>) to identify putative homologues in peach. Information of *PpeAMT* genes were listed in Supplemental Table 1. Coding sequence (CDS) of the *PpeAMT3;4* gene was downloaded from the database, and the first 18 bp sequence from the ATG codon and the last 19 bp sequence from the TAA codon were chosen as the forward primer and reverse primer, respectively (Table 1). Total RNA of ‘Feicheng’ peach seedling was extracted using MiniBEST Plant RNA Extraction Kit (TaKaRa, Dalian, China) and reverse transcribed into the 1st cDNA using PrimeScript™ RT reagent Kit (TaKaRa, Dalian, China). Normal gene amplification was carried out to obtain the CDS region of *PpeAMT3;4* and then sequenced in Sanon Biotech (Shanghai, China).

2.3. Phylogenetic Analysis of *PpeAMT* Genes in Peach. Full-length amino acid sequences of *PpeAMT* transporters were aligned by ClustalX2.1 and imported into the Molecular Evolutionary Genetics Analysis (MEGA) package version 4.1. Phylogenetic analyses were conducted according to the description of Liang et al. [28]. Branch lengths indicate the corresponding phylogenetic distances. Sequence logos of AMT1 and AMT2 subfamilies were shown on the right, generated using WEBLOGO (<http://weblogo.berkeley.edu/logo.cgi>). The grand average of hydropathicity (GRAVY) and aliphatic index analyses of *PpeAMT3;4* were calculated using the ProtParam tool (<http://web.expasy.org/protparam/>), as described by Liang et al. [28].

2.4. qRT-PCR Assays. Specific expression primers for *PpeAMT3;4* and *Ubiquitin* (control gene) were designed using the NCBI/Primer-BLAST online server. Primer sequences were listed in Table 1. According to the description of Liang et al. [28], qRT-PCR analyses were carried out on 7500 Real-Time PCR System (Applied Biosystems, New York, USA), using SYBR Premix Ex Taq reaction kit (TaKaRa, Dalian, China). To calculate RT-qPCR efficiency and the starting template concentration for each sample, the linear regression of the log (fluorescence) per cycle number data was used according to the description of Gazzarrini et al. [29]. The relative expression levels were presented after normalization to the internal control *Ubiquitin* from three independent biological repeats.

2.5. Functional Determination of *PpeAMT3;4* in Yeast Mutant 31019b. The recombinant plasmid pYES2-*AMT3;4* was constructed by cloning the CDS region of the *PpeAMT3;4* gene into the pYES2 expression vector [17], using the primer pairs (Table 1, *Kpn* I site was introduced and underlined in the forward primer, *Not* I site was introduced and underlined in the reverse primer). The yeast strain 31019b (*MATa meplΔ mep2Δ::LEU2 mep3Δ::KanMX2 ura3*, [7, 13, 17]) was transformed with pYES2 harboring the CDS of *PpeAMT3;4*. Yeast complementation tests were carried out as described in previous reports [7, 13, 17]. Yeast strain 31019b was transformed with pYES2 or pYES2-*AMT3;4*, respectively. The growth of yeast cells were determined in the yeast nitrogen base (YNB) liquid medium, supplemented with 0.02, 0.2, or 2 mM NH₄⁺ as the sole N source (pH 5.8). Pictures were taken at the 3rd day after incubation at 30°C. The growth of transformed yeast cells in the YNB liquid medium was checked by determining absorbance at 600 nm [29].

2.6. ¹⁵N Uptake Kinetics Assay. ¹⁵N labeled NH₄⁺ uptake assays were carried out according to the description of Guo et al. [17]. Yeast cells transformed with pYES2-*MT3;4* or pYES2 were grown in the YNB liquid medium, supplemented with ¹⁵N labeled NH₄Cl for 10 min. NH₄⁺ content was determined by Flash-2000 Delta VADVADTAGE Mass Spectrometer (Thermo Fisher, Waltham, Massachusetts, USA). Michaelis-Menten kinetic constants (*K_m* and *V_{max}*) were calculated as described by Guo et al. [17].

2.7. Statistical Analysis. All data were statistically analyzed using independent samples’ *t*-test in SPSS 13.0 software

TABLE 1: Primer sequences used in this work.

Purpose	Primer (5'-3')	Amplicon (bp)
Amplication of <i>PpeAMT3;4</i> CDS	F: ATGATGCGAGGTTGGTGC R: TTAGACCACCTGAGTGGCA	1065
Specific expression primers of <i>PpeAMT3;4</i>	F: TGTCGGAGCTTTCAGCTTGT R: TGAAACCTGCCCATCCCATC	224
Specific expression primers of <i>Ubiquitin</i>	F: AGGCTAAGATCCAAGACAAAGA R: CCACGAAGACGAAGCACTAAG	145
Construction of pYES2- <i>AMT3;4</i> plasmid	F: GAGCGGTACCATGATGCGAGGTTGGTGC R: GCGAGCGCCGCTTAGACCACCTGAGTGGC	1065

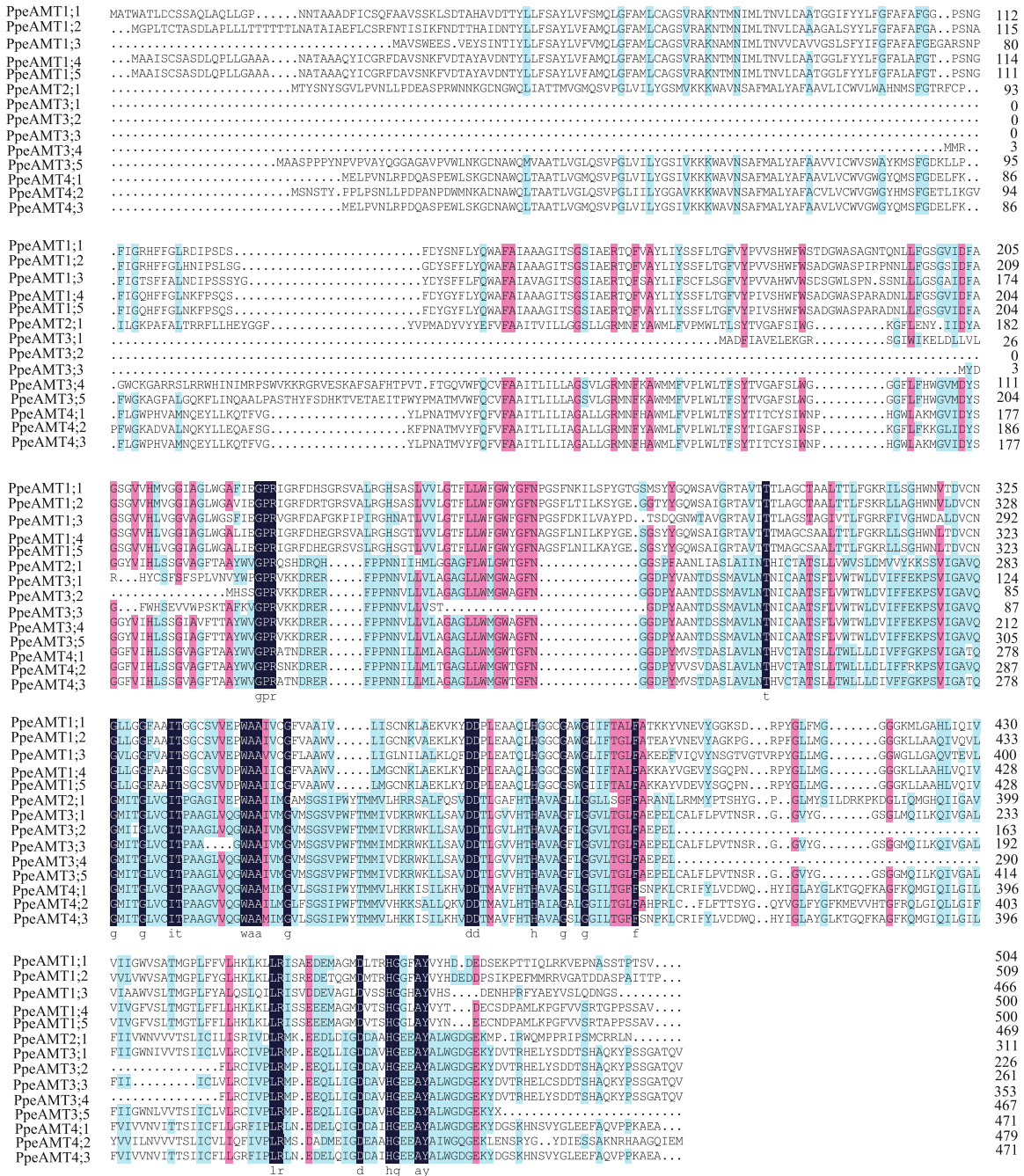


FIGURE 1: Amino acid sequence alignment of PpeAMT proteins in peach. Multiple sequence alignment of PpeAMT proteins were carried out via ClustalX2.1 software.

(SPSS Chicago, Illinois, USA). Details are described in figure legends. Asterisks indicate statistical differences between plants under control and stress treatment (* $P < 0.05$, ** $P < 0.01$).

3. Results

3.1. Characteristics and Phylogenetic Tree Construction of *PpeAMT* Transporters in Peach. By BLAST searching of the Phytozome Peach Genome Database, 14 putative *PpeAMT* genes were screened and identified [27]. Pr7 protein domain verification analyses showed that all *PpeAMT* transporters contain the NH_4^+ transporter transmembrane domain (PF00909). Information of these *PpeAMT* genes, including gene ID, gene location, CDS (coding sequence) length, and peptide length, are listed in Supplemental Table 1. Notably, the amino acid sequences of *PpeAMT* proteins shared an overall identity of 51.09% (Figure 1). To confirm the evolutionary relationships of *PpeAMT* proteins, a maximum likelihood (ML) phylogenetic tree was generated based on the alignment of the amino acid sequences of *PpeAMT* proteins. All peach AMT transporters were classified into 2 major subgroups: AMT1 and AMT2 (Figure 2), each with 5 and 9 members, respectively. The AMT2 subgroup in peach was further divided into three subclades, including *PpeAMT2* (1 member), *PpeAMT3* (5 members), and *PpeAMT4* (3 members) (Figure 2).

3.2. Cloning and Expression Analysis of *PpeAMT3;4*. As a member of the AMT2 subgroup in peach, the nucleotide sequence of *PpeAMT3;4* CDS was amplified from 'Feicheng' peach seedlings, using the specific primer pairs and the 1st cDNA template. According to the sequencing results, *PpeAMT3;4* possessed a coding sequence of 1065 bp, encoding a polypeptide of 355 amino acids with the deduced molecular weight of 39.09 kDa. Instability index assay implicated that *PpeAMT3;4* was stable and alkaline, with the theoretical PI value of 8.55. Moreover, both the GRAVY and aliphatic index analysis indicated that *PpeAMT3;4* was a hydrophilic protein.

3.3. Expression Profiles of *PpeAMT3;4*. We further performed reverse-transcribed PCR to determine the expression profiles of *PpeAMT3;4* in different tissues of 7-year-old 'Feicheng' trees. Results showed that *PpeAMT3;4* was majorly expressed in roots, followed by leaves, and hardly observed in stems, flowers, and fruits (Figure 3).

To investigate the role of *PpeAMT3;4* in maintaining NH_4^+ homeostasis in peach, we analyzed the expression profiles of *PpeAMT3;4* via the qRT-PCR analysis. Results showed that the expression of *PpeAMT3;4* was significantly decreased by NH_4^+ excess in roots of 'Feicheng' seedlings but had no response to NH_4^+ deficiency (Figure 4).

3.4. Functional Determination of *PpeAMT1;1* in Yeast Mutant. We further carried out functional determination of *PpeAMT3;4* via complementation of the yeast mutant 31019b [6, 13, 17, 30]. Results showed that yeast cells harboring pYES2 or pYES2-*PpeAMT3;4* grew well, with similar growth status, on the YNB medium that contains 2 mM

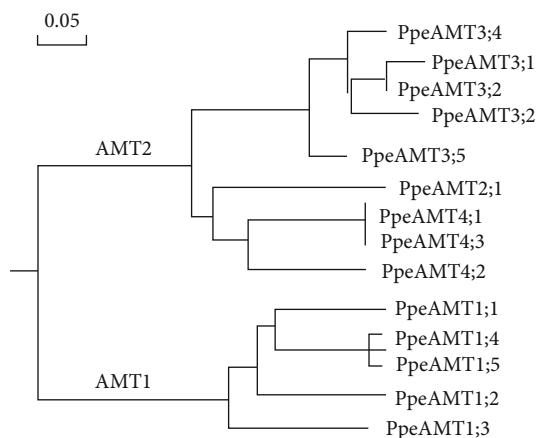


FIGURE 2: Phylogenetic tree of *PpeAMT* proteins in peach. A maximum likelihood (ML) tree was constructed by multiple alignment of *PpeAMT* proteins using ClustalX2.1 and MEGA7.0 software. The tree was based on 1000 bootstrap replicates neighbor-joining method. The *PpeAMT* family members were divided into two subgroups (AMT1 and AMT2, marked in blue).

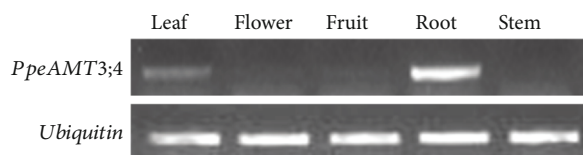


FIGURE 3: Expression profiles of *PpeAMT3;4* in peach. Reverse-transcribed PCR analysis was carried out using different cDNA templates from tissues of 7-year-old 'Feicheng' peach trees.

Arg as the sole N source (Figure 5). Yeast cells harboring pYES2 could not grow well on the YNB medium containing 0.02, 0.2, or 2 mM NH_4Cl under pH 5.8, while yeast cells harboring pYES2-*PpeAMT3;4* grew normally on the YNB medium supplemented under 0.2 and 2 mM NH_4Cl (Figure 5). These findings imply that *PpeAMT3;4* is involved in NH_4^+ in yeast, which may be responsible for NH_4^+ uptake in peach roots.

3.5. ^{15}N Uptake Kinetics Assay of *ApAMT3;4* in Yeast Cells. To verify the proposition that *PpeAMT3;4* really functions as an active NH_4^+ transporter, we determined its NH_4^+ uptake activity and the exact amount of ^{15}N -labeled NH_4^+ uptake in yeast cells via the ^{15}N isotope labeling method. Yeast cells were grown in the NYB liquid medium labeled with ^{15}N -labeled NH_4Cl , ranging from 0.02 mM to 2 mM. Affinity constant analysis showed that *PpeAMT3;4* exhibited a K_m value of 86.3 μM and a I_{max} value of 3.69 $\mu\text{mol}\cdot\text{min}^{-1}\cdot\mu\text{g}^{-1}$, respectively (Figure 6). These findings indicate that *PpeAMT3;4* was primarily involved in HATS-mediated NH_4^+ uptake in peach roots.

4. Discussion

NH_4^+ is the preferred form of N transport for lower energetic cost in assimilation [3–5, 29], while excess NH_4^+ is toxic to plant growth and development [30, 31]. In higher plants,

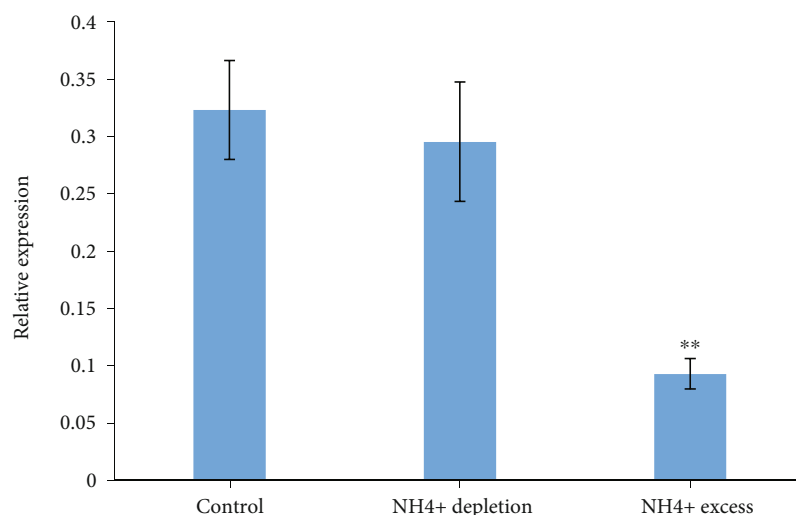


FIGURE 4: qRT-PCR analysis of *PpeAMT3;4* in roots of 1-month-old 'Feicheng' seedlings under different NH₄⁺ supplies. The relative expression level of *PpeAMT3;4* was presented after normalization to the internal control. Data are the means of values obtained from three independent biological repeats ± SE.

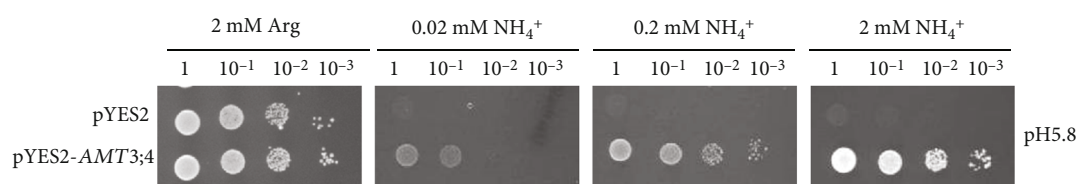


FIGURE 5: Functional determination of *PpeAMT3;4*-mediated NH₄⁺ uptake in yeast. Yeast cells grown on the YNB medium supplemented with 2 mM Arg as the sole N source was used as the positive control. Yeast strains 31019 b transformed with pYES2 or pYES2-*AMT3;4* were grown in the YNB medium, supplemented with different concentrations of NH₄Cl. Final diluted concentrations are indicated by 1, 10⁻¹, 10⁻², and 10⁻³.

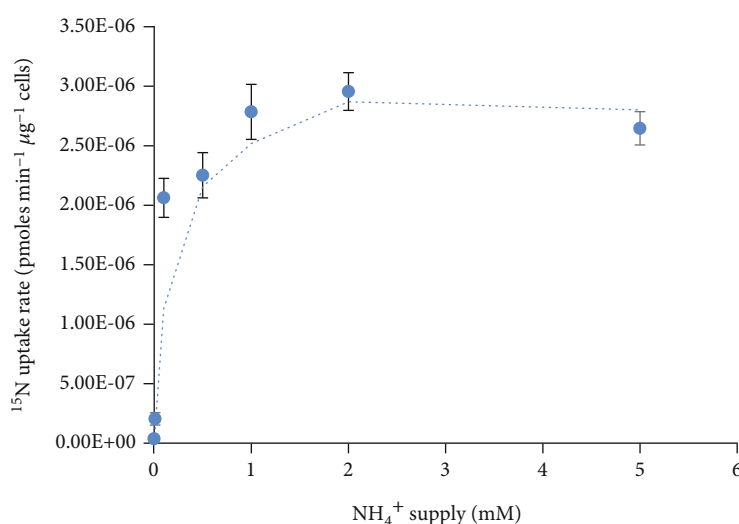


FIGURE 6: ¹⁵N-labeled NH₄⁺ uptake analysis of *PpeAMT3;4* in yeast. Yeast cells transformed with pYES2 or pYES2-*AMT3;4* were grown in the YNB liquid medium supplemented with different concentrations of ¹⁵N-labeled NH₄Cl. Values are presented as mean ± SE, n = 3. Error bars within the plot symbols were not visible.

high-affinity AMT1 subgroup genes encode NH₄⁺ transporters with a K_m value of micromolar grade involved in the HATS for NH₄⁺ uptake [12]. However, the molecular

mechanism towards the AMT2 subgroup genes is largely rare. In this present study, we initially isolated and determined a high-affinity AMT2 subgroup transporter gene in

peach, which helps to investigate the biological role of AMT2 family transporters in fruit trees.

Transcript levels of AMT1 genes are closely related to the N nutrition status of the plant, and NH_4^+ starvation mainly induced the expression levels of most AMT1 genes, may it be a clue of 'starvation response' for plants to grow under suboptimal N-source conditions [3, 16, 22]. However, *PpeAMT3;4* was mainly expressed in roots and was nonresponsive to NH_4^+ starvation in peach roots in this study but reduced significantly by excessive NH_4^+ stress (Figures 3 and 4). These findings implies that *PpeAMT3;4* may be an active NH_4^+ transporter at normal NH_4^+ supplies, even at extremely low NH_4^+ conditions, which is an indispensable AMT2 transporter responsible for NH_4^+ uptake in peach roots. If the NH_4^+ supply reached an excessive state, *PpeAMT3;4* began to stop its NH_4^+ uptake and transport capacity that are sufficient enough for internal N metabolism and usage.

Heterologous expression studies using NH_4^+ uptake defective yeast mutant or *Xenopus* oocytes indicate that AMT1 family genes encode high-affinity transporters [3, 4, 15], which play key roles in high-affinity NH_4^+ uptake from soils via the roots. While molecular mechanisms or biological functions of AMT2 subgroup members are extremely rare, in this present study, *PpeAMT3;4* could utilize the external NH_4^+ in yeast cells (Figure 5), and ^{15}N labeled NH_4^+ uptake kinetics analysis exhibited a K_m value of $86.3 \mu\text{M}$ (Figure 6). Nonetheless, *PpeAMT3;4* is a functional AMT2 transporter responsible for NH_4^+ uptake in peach roots, especially under normal and low external NH_4^+ conditions.

5. Conclusions

In this study, we isolated and characterized *PpeAMT3;4*, an AMT2 family gene from peach, and determined its function in yeast mutant. The *PpeAMT3;4* gene was majorly expressed in peach roots, whose expression was decreased under NH_4^+ excess but had no response to NH_4^+ deficiency. Functional determination and ^{15}N -labeled NH_4^+ uptake kinetics assay in yeast cells indicate that *PpeAMT3;4* was a typical high-affinity transporter, with a K_m value of $86.3 \mu\text{M}$ and a I_{\max} value of $3.69 \mu\text{mol min}^{-1} \mu\text{g}^{-1}$, which can uptake external NH_4^+ in yeast cells. Nonetheless, *PpeAMT3;4* might be a NH_4^+ transporter involved in NH_4^+ uptake in peach roots. This study not only provides a technological system to uncover the biological function of AMT2 transporters in fruit trees but also reveals molecular basis for NH_4^+ uptake and N nutrition mechanisms.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

We declare that we do not have any commercial or associative interest that represents a conflict of interest with the work submitted.

Authors' Contributions

Shuanghong You, Yuqing Wang, and Yanju Li contributed equally to this work.

Acknowledgments

This work was jointly supported by the following grants: the National Key R & D Program of China (2019YFD1000500), the Sci-Tech Innovation Special Project for Social Undertakings and People's Livelihood Guarantee of Chongqing Science and Technology Bureau (cstc2019jscx-gksbX0138), the Improved Variety Innovation Project for Characteristic Benefit Agriculture of Chongqing (NKY20190030), the Agricultural Variety Improvement Project of Shandong Province (2019LZGC009), the Performance Incentive & Guidance Project Special for the Scientific Research Institutions of Chongqing (cstc2019jxjl80012), and the Key R&D Program of Shandong Province (2019GSF107095, 2018JHZ006).

Supplementary Materials

Supplemental Table 1: information of *PpeAMT* family genes in peach. (*Supplementary Materials*)

References

- [1] P. J. Syrett and I. Morris, "The inhibition of nitrate assimilation by ammonium in *Chlorella*," *Biochimica et Biophysica Acta*, vol. 67, pp. 566–575, 1963.
- [2] Q. Dortch, "The interaction between ammonium and nitrate uptake in phytoplankton," *Marine Ecology Progress Series*, vol. 61, pp. 183–201, 1990.
- [3] U. Ludewig, B. Neuhauser, and M. Dynowski, "Molecular mechanisms of ammonium transport and accumulation in plants," *FEBS Letters*, vol. 581, no. 12, pp. 2301–2308, 2007.
- [4] Y. Liu and N. von Wirén, "Ammonium as a signal for physiological and morphological responses in plants," *Journal of Experimental Botany*, vol. 68, no. 10, pp. 2581–2592, 2017.
- [5] T. R. McDonald and J. M. Ward, "Evolution of electrogenic ammonium transporters (AMTs)," *Frontiers in Plant Science*, vol. 7, p. 352, 2016.
- [6] H. Guo, N. Wang, T. R. McDonald, A. Reinders, and J. M. Ward, "MpAMT1;2 from *Marchantia polymorpha* is a high-affinity, plasma membrane ammonium transporter," *Plant Cell Physiology*, vol. 59, no. 5, pp. 997–1005, 2018.
- [7] O. Ninnemann, J. C. Jauniaux, and W. B. Frommer, "Identification of a high affinity NH_4^+ transporter from plants," *EMBO Journal*, vol. 13, no. 15, pp. 3464–3471, 1994.
- [8] F. Salvemini, A. M. Marini, A. Riccio, E. J. Patriarca, and M. Chiurazzi, "Functional characterization of an ammonium transporter gene from *Lotus japonicus*," *Gene*, vol. 270, no. 1–2, pp. 237–243, 2001.
- [9] E. D'Apuzzo, A. Rogato, U. Simon-Rosin et al., "Characterization of three functional high-affinity ammonium transporters in *Lotus japonicus* with differential transcriptional regulation and spatial expression," *Plant Physiology*, vol. 134, no. 4, pp. 1763–1774, 2004.
- [10] F. R. Lauter, O. Ninnemann, M. Bucher, J. W. Riesmeier, and W. B. Frommer, "Preferential expression of an ammonium transporter and of two putative nitrate transporters in root

- hairs of tomato,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 93, no. 15, pp. 8139–8144, 1996.
- [11] U. Ludewig, N. von Wirén, and W. B. Frommer, “Uniport of NH_4^+ by the root hair plasma membrane ammonium transporter LeAMT1;1,” *Journal of Biological Chemistry*, vol. 277, no. 16, pp. 13548–13555, 2002.
- [12] N. von Wirén, S. Gazzarrini, A. Gojon, and W. B. Frommer, “The molecular physiology of ammonium uptake and retrieval,” *Current Opinion in Plant Biology*, vol. 3, no. 3, pp. 254–261, 2000.
- [13] J. Couturier, B. Montanini, F. Martin, A. Brun, D. Blaudez, and M. Chalot, “The expanded family of ammonium transporters in the perennial poplar plant,” *New Phytologist*, vol. 174, no. 1, pp. 137–150, 2007.
- [14] Y. Sonoda, A. Ikeda, S. Saiki, N. . Wirén, T. Yamaya, and J. Yamaguchi, “Distinct expression and function of three ammonium transporter genes (OsAMT1; 1–1; 3) in rice,” *Plant Cell Physiology*, vol. 44, no. 7, pp. 726–734, 2003.
- [15] A. Suenaga, K. Moriya, Y. Sonoda et al., “Constitutive expression of a novel-type ammonium transporter OsAMT2 in rice plants,” *Plant Cell Physiology*, vol. 44, no. 2, pp. 206–211, 2003.
- [16] T. Li, K. Liao, X. Xu et al., “Wheat ammonium transporter (AMT) gene family: diversity and possible role in host-pathogen interaction with stem rust,” *Frontiers in Plant Science*, vol. 8, p. 1637, 2017.
- [17] X. T. Guo, Y. T. Sheng, S. Y. Yang et al., “Isolation and characterization of a high-affinity ammonium transporter ApAMT1;1 in alligatorweed,” *Plant Growth Regulation*, vol. 89, no. 3, pp. 321–330, 2019.
- [18] L. Yuan, R. Gu, Y. Xuan et al., “Allosteric regulation of transport activity by heterotrimerization of Arabidopsis ammonium transporter complexes in vivo,” *Plant Cell*, vol. 25, no. 3, pp. 974–984, 2013.
- [19] T. Straub, U. Ludewig, and B. Neuhäuser, “The kinase CIPK23 inhibits ammonium transport in Arabidopsis thaliana,” *Plant Cell*, vol. 29, no. 2, pp. 409–422, 2017.
- [20] D. Loqué, S. Lalonde, L. L. Looger, N. von Wirén, and W. B. Frommer, “A cytosolic trans-activation domain essential for ammonium uptake,” *Nature*, vol. 446, no. 7132, pp. 195–198, 2007.
- [21] L. Yuan, D. Loqué, S. Kojima et al., “The organization of high-affinity ammonium uptake in Arabidopsis roots depends on the spatial arrangement and biochemical properties of AMT1-type transporters,” *Plant Cell*, vol. 19, no. 8, pp. 2636–2652, 2007.
- [22] G. Camañes, M. Cerezo, E. Primo-Millo, A. Gojon, and P. García-Agustín, “Ammonium transport and CitAMT1 expression are regulated by N in citrus plants,” *Planta*, vol. 229, no. 2, pp. 331–342, 2009.
- [23] H. Li, J. L. Han, Y. H. Chang, J. Lin, and Q. S. Yang, “Gene characterization and transcription analysis of two new ammonium transporters in pear rootstock (*Pyrus betulaefolia*),” *Journal of Plant Research*, vol. 129, no. 4, pp. 737–748, 2016.
- [24] H. Li, Y. Cong, Y. H. Chang, and J. Lin, “Two AMT2-type ammonium transporters from *Pyrus betulaefolia* demonstrate distinct expression characteristics,” *Plant Molecular Biology Reporter*, vol. 34, no. 4, pp. 707–719, 2016.
- [25] D. R. Layne and D. Bassi, *The Peach: Botany, Production and Uses*, CABI, London, 2008.
- [26] S. Jung, M. Staton, T. Lee et al., “GDR (Genome Database for Rosaceae): integrated web-database for Rosaceae genomics and genetics data,” *Nucleic Acids Research*, vol. 36, no. Database, pp. D1034–D1040, 2007.
- [27] M. Tang, Y. Li, Y. Chen, L. Han, H. Zhang, and Z. Song, “Characterization and expression of ammonium transporter in peach (*Prunus persica*) and regulation analysis in response to external ammonium supply,” *Phyton-International Journal of Experimental Botany*, vol. 89, no. 4, pp. 925–941, 2020.
- [28] M. Liang, Y. Gao, T. Mao et al., “Characterization and expression of KT/HAK/KUP transporter family genes in willow under potassium deficiency, drought, and salt stresses,” *BioMed Research International*, vol. 2020, Article ID 2690760, 12 pages, 2020.
- [29] S. Gazzarrini, L. Lejay, A. Gojon, O. Ninnemann, W. B. Frommer, and N. von Wirén, “Three functional transporters for constitutive, diurnally regulated and starvation-induced uptake of ammonium into Arabidopsis roots,” *Plant Cell*, vol. 11, no. 5, pp. 937–947, 1999.
- [30] D. T. Britto, M. Y. Siddiqi, A. D. M. Glass, and H. J. Kronzucker, “Futile transmembrane NH_4^+ cycling: a cellular hypothesis to explain ammonium toxicity in plants,” *Proceedings of the National. Academy of Sciences of the United States of America*, vol. 98, no. 7, pp. 4255–4258, 2001.
- [31] D. T. Britto and H. J. Kronzucker, “ NH_4^+ toxicity in higher plants: a critical review,” *Journal of Plant Physiology*, vol. 159, no. 6, pp. 567–584, 2002.