

Editorial

New Molecular Mechanisms of Plant Response to Ammonium Nutrition

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Ammonium (NH_4^+) and nitrate (NO_3^-) are two major inorganic nitrogen (N) forms for plants. In contrast to NH_4^+ , NO_3^- is highly mobile in soils and is easily leached into groundwater and open water bodies, contributing to eutrophication [1]. Furthermore, NO_3^- must be reduced to NH_4^+ in plant cells before it can be utilized by plants in metabolism. This process can consume 12–26% of photosynthetically generated energy [2]. Recent studies have shown that elevated carbon dioxide (CO_2) in the atmosphere can inhibit the assimilation of NO_3^- in crop plants and algae, potentially further decreasing the utilization efficiency for NO_3^- in future climates. However, it is puzzling that the theoretically preferred source of N is instead toxic to plants [3]. Therefore, it is important to study the molecular mechanisms of plant responses to NH_4^+ nutrient stress, and to coordinate NH_4^+ uptake and mitigate NH_4^+ toxicity. In addition, the effective mitigation of NH_4^+ toxicity and improvements in crop NH_4^+ tolerance are also crucial for the application of NH_4^+ -N fertilizers in agriculture. In recent years, many advances have been made in the study of plant responses to NH_4^+ nutrient stress.

(i) Which compound, NH_4^+ or glutamine (Gln), accumulates and leads to NH_4^+ toxicity?

High intracellular concentrations of free NH_4^+ can lead to NH_4^+ toxicity, which may also be exacerbated by metabolic disturbances from excessive NH_4^+ assimilation. A recent study indicates that the knockout of *AtGLN2* enhances tolerance to elevated NH_4^+ stress, despite the associated increase in free NH_4^+ accumulation [4]. Under high NH_4^+ conditions, the reaction of NH_4^+ to Gln catalyzed by shoot *AtGLN2* produced an abundance of H^+ within the cytosol, thereby inducing acidic stress in shoots. This observation suggests that acidic stress may serve as a principal contributor to NH_4^+ toxicity [4]. In rice, mutations in argininosuccinate lyase (ASL) render the roots more susceptible to NH_4^+ stress. Subsequent investigations reveal that ASL mitigates NH_4^+ toxicity by promoting the conversion of excess Gln to arginine in the presence of high NH_4^+ stress [5]. In summary, the data imply that the metabolic consequences of excess NH_4^+ , specifically Gln and H^+ accumulation, may pose a greater risk to plants under high NH_4^+ stress than the accumulation of free NH_4^+ alone.

(ii) How do plants modulate NH_4^+ uptake in response to elevated NH_4^+ concentrations?

Optimal regulation of NH_4^+ uptake from the environment plays a pivotal role in minimizing intracellular NH_4^+ concentrations, thereby alleviating potential NH_4^+ toxicity. A previous study has demonstrated that the protein kinase *AtCIPK23* can inhibit NH_4^+ uptake through the phosphorylation of a conserved threonine residue located in the C-terminus of *AtAMT1;1* and *AtAMT1;2* [6]. Subsequent studies have elucidated that the transcription factor *AtSTOP1* is capable of directly binding to the *AtCIPK23* promoter, leading to its transcriptional activation. This, in turn, suppresses the transcription of both *AtAMT1;1* and *AtAMT1;2* [7]. Furthermore, another protein kinase, *AtCIPK15*, has been identified to phosphorylate the C-termini of *AtAMT1;1*, *AtAMT1;2*, and *AtAMT1;3*, consequently inhibiting their NH_4^+ transport functions [8].



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(iii) How do phytohormones function in response to NH_4^+ toxicity?

Previous research has demonstrated that elevated NH_4^+ levels lead to a reduction in endogenous free IAA content, while the application of low concentrations of exogenous IAA can mitigate NH_4^+ toxicity [9]. Our recent studies indicate that elevated NH_4^+ diminishes free IAA content by enhancing IAA conjugation rather than by inhibiting IAA biosynthesis. Additionally, the transcription factor AtWRKY46 plays a pivotal role, as it directly suppresses the transcription of IAA-conjugating genes [10]. In addition, exogenous BR treatment significantly suppresses AtPIN2 expression and the nuclear auxin signal under high NH_4^+ stress, indicating that auxin operates downstream of the BR signaling pathway in response to elevated NH_4^+ stress [9].

Beyond IAA and BR, ABA is required for protecting chloroplast and root growth under high NH_4^+ stress [11]. Our recent study shows that high NH_4^+ induces ABA biosynthesis and activates the 'OsSAPK9-OsbZIP20' module, which in turn augments reactive oxygen species (ROS) scavengers and bolsters NH_4^+ assimilation, thereby minimizing ROS and free NH_4^+ accumulation in rice roots [11]. Intriguingly, the ABA signaling elicited by high NH_4^+ levels can also disrupt the interaction between AtABI1 and AtCIPK23 by inactivating AtABI1. As a consequence, the liberated AtCIPK23 is then able to further phosphorylate and inhibit AtAMTs in Arabidopsis [12].

(iv) How does NH_4^+ stress induce a burst of ROS?

High- NH_4^+ -triggered ROS accumulation has been reported in a previous study; however, the mechanisms underlying the NH_4^+ -induced ROS burst remain unclear [13]. Interestingly, two recent studies have proposed that iron (Fe) accumulation might be the primary driver of the NH_4^+ -induced ROS burst. When compared to NO_3^- , NH_4^+ induces greater Fe accumulation in the apoplast of the phloem, resulting in a ROS burst. This process appears to involve a cell wall-localized ferroxidase known as LPR2. Both the knockout of *AtLPR2* or reduction in Fe supplementation can significantly enhance root growth tolerance to high NH_4^+ stress [14]. Moreover, the acidification of the apoplast, caused by NH_4^+ uptake, results in Fe precipitation in the elongation and differentiation zones of the root tip, which, in turn, induces hydrogen peroxide (H_2O_2) accumulation and NH_4^+ toxicity. The knockout of *AtPDX1.1*, an enzyme involved in vitamin B6 biosynthesis, or application of exogenous vitamin B6 could quench ROS and partially rescue root growth under NH_4^+ stress [15].

(v) How do roots regulate NH_4^+/H^+ efflux in response to NH_4^+ uptake?

The capacity of NO_3^- to mitigate NH_4^+ toxicity has been documented in many species; however, the underlying molecular mechanism remains elusive. Earlier investigations have demonstrated that NH_4^+ uptake results in rhizosphere acidification, whereas NO_3^- uptake leads to its alkalization [16,17]. Thus, it is worth exploring whether NO_3^- counteracts the rhizosphere acidification instigated by NH_4^+ absorption and subsequently mitigates NH_4^+ toxicity. Recently, a team from China found that AtSLAH3 (a NO_3^- efflux channel) interacts with AtNRT1.1 (a NO_3^-/H^+ symporter) to form a 'transporter-channel' complex to manipulate NH_4^+ influx, NO_3^- influx/efflux, and H^+ influx through the membrane [18,19]. Consequently, the AtSLAH3/AtNRT1.1 complex enhances tolerance against NH_4^+ uptake-induced rhizosphere acidification.

Beyond the rhizosphere, futile NH_4^+ efflux, triggered by NH_4^+ absorption, is also a predominant factor contributing to NH_4^+ toxicity [10]. Our team recently identified a transcription factor, AtWRKY46, that curtails this futile NH_4^+ efflux in two distinct ways: Firstly, AtWRKY46 directly downregulates *AtNUDX9*, preserving the levels of N-glycosylated proteins and subsequently impeding futile NH_4^+ efflux. Secondly, AtWRKY46 directly suppresses the IAA-conjugating genes *AtGH3.1/GH3.6/UGT75D1/UGT84B2*, ensuring the maintenance of free IAA levels and inhibiting the futile NH_4^+ efflux from the roots [10].

As previously established, NH_4^+ uptake leads to an increase in H^+ efflux, and IAA also promotes H^+ efflux by phosphorylating the proton pump. Therefore, it is of interest to further identify the relationships between free IAA, H^+ and NH_4^+ efflux. Our recent

results further show that H⁺ secreted in the extracellular compartment can promote the NH₄⁺ efflux, while the PM H⁺-ATPase knockout mutants *Ataha1-7* and *Ataha2-5* exhibit lower NH₄⁺ efflux compared with wild-type. Intriguingly, a mutation in *AtPIN5*, an IAA transporter responsible for IAA translocation from the cytosol to the ER lumen, leads to enhanced cytosolic IAA accumulation, consequentially increasing H⁺ efflux and decreasing NH₄⁺ flux. The application of the PM H⁺-ATPase inhibitor, vanadate, in the medium further attenuates both H⁺ and NH₄⁺ efflux in *Atpin5*. This suggests that *AtPIN5* may play a pivotal role in synchronously modulating H⁺ and NH₄⁺ efflux by regulating free IAA levels in the cytoplasm [20].

Additionally, our study identified a transcription factor, *OsEIL1*, in rice, which has been demonstrated to directly associate with the promoter of *OsVTC1.3* (a homolog of *AtVTC1*). This binding promotes transcription, maintaining the levels of N-glycosylated proteins in roots, which in turn mitigates unnecessary NH₄⁺ efflux. Notably, this regulatory paradigm is absent in *Arabidopsis*, potentially offering an explanation as to why rice exhibits greater NH₄⁺ tolerance compared to *Arabidopsis* [21].

In summation, significant advancements have been made in discerning the molecular underpinnings of NH₄⁺ toxicity in recent times. This not only provides a theoretical groundwork for future explorations into plant NH₄⁺ response mechanisms but also holds promise for enhancing plant NH₄⁺ resilience in agriculture.

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