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Review

# Overcoming ammonium toxicity

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ABSTRACT

Ammonia (ammonium ion under physiological conditions) is one of the key nitrogen sources in cellular amino acid biosynthesis. It is continuously produced in living organisms by a number of biochemical processes, but its accumulation in cells leads to tissue damage. Current knowledge suggests that a few enzymes and transporters are responsible for maintaining the delicate balance of ammonium fluxes in plant tissues. In this study we analyze the data in the scientific literature and the publicly available information on the dozens of biochemical reactions in which endogenous ammonium is produced or consumed, the enzymes that catalyze them, and the enzyme and transporter mutants listed in plant metabolic and genetic databases (Plant Metabolic Network, TAIR, and Genevestigator). Our compiled data show a surprisingly high number of little-studied reactions that might influence cellular ammonium concentrations. The role of ammonium in apoptosis, its relation to oxidative stress, and alterations in ammonium metabolism induced by environmental stress need to be explored in order to develop methods to manage ammonium toxicity.



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## 1. Introduction

Q2 Availability of nitrogen as a plant mineral nutrient is the strongest limiting factor (up to 50%) to plant growth and yield [1]. Nitrogen fertilizers for crops typically contain ammonium ($NH_4^+$) and nitrate ($NO_3^-$) salts (applied primarily as ammonium nitrate, ammonium sulfate and ammonium phosphate in solid form or in solution), urea ($CO(NH_2)_2$) or anhydrous ammonia (a gas produced by a modified Haber–Bosch process, injected under the soil surface). Following its application, anhydrous ammonia rapidly diffuses from the point of injection and dissolves in the soil solution in the form of ammonium ions. Unlike other nitrogen species used as fertilizers, only ammonium can be incorporated into amino acids and amides. [2]. Thus, urea, the worldwide leading nitrogen-fertilizer is converted to ammonium by urease enzymes of soil microorganisms and plants. Nitrate is transformed to ammonium by nitrate and nitrite reductase enzymes *in planta*, and atmospheric

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nitrogen ($N_2$) is also made available to the plants in the form of ammonium. This conversion is carried out by nitrogen-fixing bacteria (*e.g. Rhizobium*, *Frankia*, *etc.*). While both ammonium and nitrate are taken up readily by the plant roots from the soil, higher concentrations of ammonium are strongly phytotoxic [3,4]. It is interesting to note that the phenomenon of ammonium toxicity to plants was first demonstrated by Charles Darwin in 1882, when he described the inhibition of growth of *Euphorbia peplus* by the carbonate salt of this chemical [5]. As a result of climate change, ammonium as a nutrient will become increasingly important in the future, because under conditions of elevated carbon dioxide levels the assimilation of nitrate is inhibited in crop plants [6] and sea algae [7].

Concentrations of solvated ammonia [$NH_3$(sol)], solvated ammonium ion [$NH_4^+$(sol)], and ammonia gas [$NH_3$(gas)] in water–air systems are determined by the complex dynamic equilibria described in Eqs. (1) and (2). The p$K_a$ value for the deprotonation of the positively charged ammonium ion to uncharged ammonia in Eq. (1) is 9.24. Since the cytoplasmic pH of a plant cell is of 7.0 to 7.5, *ca.* 1% of the total cytoplasmic ammonia/ammonium is present in an uncharged form [8].

$$NH_4^+(sol) + OH(sol) \leftrightarrow NH_3(sol) + H_2O(liquid) \quad (1)$$

$$NH_3(sol) \leftrightarrow NH_3(gas) \quad (2)$$

Plant response to ammonium depends on the external and cellular concentrations of the components of these equations [9]. Toxic levels of ammonium in plant tissues accumulate when the overall rate of the conversion of ammonium into amino acids and amides becomes lower than the rate of its uptake and cellular production by amino acid catabolism, nitrate reduction, phenylpropanoid metabolism, and photorespiration [10]. The present paper discusses the fine balance between uptake, production, and detoxification of ammonia in crops and weeds under typical growth conditions and under environmental stress. The question addressed is: is it possible to reduce or eliminate ammonium phytotoxicity? In theory, toxic action of a chemical can be counterbalanced at different levels, beginning with its uptake and translocation. At later stages, the toxicant can be converted into less toxic or nontoxic derivatives (*i.e.* detoxification), and/or excreted out of the cell, the organism, or by compartmentation (sequestration into an organelle where it can cause no harm). There are well-demonstrated examples for all these processes, most of them discovered by studying the selective phytotoxic action of herbicides to crops and weeds [11].

In this study we used publicly available information on biochemical metabolites, pathways, reactions, (and the enzymes catalyzing the reactions) in the plant metabolic and genetic databases Plant Metabolic Network (PMN) [12] and Genevestigator [13]. First we looked up "ammonia" and "ammonium" in the Compound Search of PMN that listed the reactions and pathways in which they participate. Then we tabulated the individual reactions and the enzymes (and the protein-coding genes in *Arabidopsis thaliana*) that catalyze them. Changes in expression of *Arabidopsis* genes under different N-nutritional and stress conditions were taken from Genevestigator for evaluation. Finally, we searched The Arabidopsis Information Resource (TAIR) [14] for phenotypical changes in mutants of the enzymes and transporters if available. Most of the enzymatic reactions of ammonia metabolism are reversible: the preferred directions (given in the table headings) were taken from PMN. Throughout the paper the enzyme nomenclature of PMN was used.

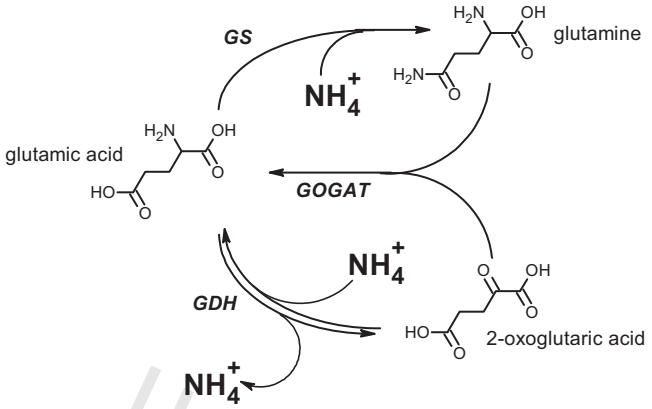


**Fig. 1.** Key detoxification pathways of ammonium in plants. GS: glutamine synthetase GOGAT: glutamate synthase; GDH: glutamate dehydrogenase.

## 2. Uptake and translocation of exogenous ammonium in plants

In plant tissues ammonium and non-charged ammonia may be transported *via* aquaporins, nonselective cation channels, potassium channels, simple osmotic diffusion, or with the participation of high-affinity ammonium transporters (AMTs) [4,15–17]. The relative importance of these mechanisms strongly varies with the external and internal ammonium/ammonia concentrations, the pH, and the presence of other nutrients (most importantly nitrate [18] and potassium [16]) in the growth medium. The uptake of ammonium from the soil solution and its translocation within the plant tissues are attributed to highly selective plasma membrane-located transporters of the AMT protein family [19]. AMTs are characterized by the presence of a conserved hydrophobic pore through which ammonium moves. However, uncertainties exist regarding the exact chemical species transported by these membrane proteins, which can be in the form of either uncharged ammonia molecule or charged ammonium ion and whether the process is active or passive [20,21].

In total, there are 6 AMTs in *Arabidopsis* (AMT1;1 to AMT1;5 and AMT2) [12]. Cellular ammonium concentrations in *Arabidopsis* are modulated at the uptake phase by a rapid regulatory mechanism mediated by the phosphorylation of AMTs. External ammonium levels as low as 50 μM triggered the phosphorylation of AMT1;1 at the conserved threonine residue (T460) in the C-terminus. As a result, active uptake of ammonium by the transporter stops as soon as its cellular level reaches the optimum [22]. In addition, expression of the ammonium transporter gene *amt*1 in *Arabidopsis* is down-regulated by glutamine (the first metabolite of ammonium assimilation, Fig. 1) rather than ammonium itself [23]. Further reduction of ammonium uptake may involve proteolysis of the transporters [22] or their clustering followed by internalization of the clusters in the cytoplasm [24]. CAP1, a tonoplast-localized receptor-like kinase was shown to mediate ammonium homeostasis in *Arabidopsis* [25]. Since AMT-mediated uptake of ammonium is shut down as soon as its cytosolic concentration reaches the optimum, toxic levels in plant roots exposed to high ammonium concentrations have to accumulate though other pathways, of which aquaporin-mediated transport could be particularly important [16]. Expression of the TIP2;3 aquaporin gene in *Arabidopsis* was down-regulated after 18 days exposure to 3 mM ammonium sulfate as compared to 3 mM potassium nitrate in the nutrient solution ([13], experiment no. AT-00417: fold-change 3.97, *p*-value 0.010; Supplement, Fig. A).

Bacterial and fungal AMTs are known to function as transceptors (proteins responsible for both transport and ammonium sensing)



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