

Review

Ammonium Phytotoxicity and Tolerance: An Insight into Ammonium Nutrition to Improve Crop Productivity

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Abstract: Ammonium sensitivity is considered a globally stressful condition that affects overall crop productivity. The major toxic symptom associated with ammonium nutrition is growth retardation, which has been associated with a high energy cost for maintaining ion, pH, and hormone homeostasis and, eventually, the $\text{NH}_3/\text{NH}_4^+$ level in plant tissues. While certain species/genotypes exhibit extreme sensitivity to ammonium, other species/genotypes prefer ammonium to nitrate as a form of nitrogen. Some of the key tolerance mechanisms used by the plant to deal with NH_4^+ toxicity include an enhanced activity of an alternative oxidase pathway in mitochondria, greater NH_4^+ assimilation plus the retention of the minimum level of NH_4^+ in leaves, and/or poor response to extrinsic acidification or pH drop. Except for toxicity, ammonium can be considered as an energy-efficient nutrition in comparison to nitrate since it is already in a reduced form for use in amino acid metabolism. Through effective manipulation of the $\text{NH}_4^+/\text{NO}_3^-$ ratio, ammonium nutrition can be used to increase productivity, quality, and resistance to various biotic and abiotic stresses of crops. This review highlights recent advancements in ammonium toxicity and tolerance mechanisms, possible strategies to improve ammonium tolerance, and omics-based understanding of nitrogen use efficiency (NUE) in plants.

Keywords: ammonium toxicity; ammonium tolerance; crop productivity; nitrogen use efficiency; $\text{NH}_4^+/\text{NO}_3^-$ ratio; omics



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1. Introduction

Nitrogen (N) holds a prominent position in the metabolic system of plants, as it is part of amino acids, which are the building blocks of proteins and enzymes. Among all the mineral elements, N is regarded as one of the vital macronutrients for all living tissues in plants, including metabolism, resource distribution, growth, and development [1]. Nitrogen uptake is necessary for plant growth as it is crucial for the production of coenzymes, proteins, and photosynthetic processes. Although dinitrogen gas makes up about 78% of the atmosphere, plants cannot use it precisely. About 65% of the available N for the biosphere is produced via the biological N fixation process, which converts atmospheric N_2 to ammonium. Ammonium (NH_4^+) and nitrate (NO_3^-) are the two forms of accessible N that plants can take up from the soil and are assimilated into amino acids, either in the roots or shoots, depending on the plant species. In many plant species, the majority of the nitrate taken up by the roots is transferred to the shoot, where it is further assimilated into amino acids [2]. However, in a few species such as peas, amino acid synthesis primarily occurs in the roots, before being transported by the xylem to mature source leaves, where the amino-N is used in a variety of metabolic activities or loaded into the phloem to provide N to developing sinks, such as fruit and seeds [3]. Therefore, the main factors affecting soil nitrogen input for plant nutrition and agricultural yield are the decomposition of organic

matter, the use of synthetic fertilizers, and biological nitrogen fixation through nitrogenase enzyme activity. Due to low bioavailability and greater dependence of crop growth on nitrogen, there is a massive global market for N-based fertilizers, such as ammonium (NH_4^+), nitrate (NO_3^-), and urea ($\text{CH}_4\text{N}_2\text{O}$) [4]. Estimates from the FAO show that 107.7 Mt of elemental N from synthetic fertilizers were consumed globally for agriculture in 2018, with China, India, the United States, the EU28, and Brazil contributing 68% of the overall N use [5]. However, about 50% of nutrient losses from N-fertilizers, significantly contribute to low fertilizer use, which prevents crops from reaching their yield potential, increases the cost of farmers' investments in agricultural inputs, and worsens food and nutrition insecurity [6]. Moreover, N-fertilizer losses contribute to the eutrophication of surface waters, contamination of subsurface water, and atmospheric greenhouse gas emissions, all of which worsen the impact of agriculture on the environment [7]. Therefore, in order to achieve sustainable agriculture, it is imperative to enhance yield and nitrogen use efficiency (NUE), as well as reduce nitrogen application and losses to the environment.

Although inorganic nitrogen in the form of nitrate is primarily accessible to plants, ammonium can be the dominant N ion in some soils and hydroponic cultures, while amino acids can also be taken up by natural and agricultural crops [8]. The availability of ammonium (NH_4^+) in soils and subsequently the primary productivity of terrestrial ecosystems is controlled by two important soil microbial processes, known as gross ammonification (GA) and gross nitrification (GN) rates. Numerous factors, such as the pH, chemical makeup of the soil, temperature, the buildup of organic molecules, oxygenation, CO_2 , and light affect the accumulation of NH_4^+ in ecosystems. Typically, low pH, low temperature, the buildup of compounds with phenolic bases, and insufficient oxygen supply prevent many nitrifying bacteria, leading to higher rates of net ammonification than net nitrification [9]. Although ammonium toxicity may be pervasive, the point at which symptoms start to occur varies greatly among plant species. Generally, many domesticated plants such as potato, tomato, bean, pea, sugar beet, strawberry, and mustard are sensitive to NH_4^+ , while few crops such as rice, onion, cranberry, and blueberry are highly adapted to NH_4^+ [9,10]. Toxic quantities of ammonium build up in plant tissues when the net rate of converting ammonium into amino acids and amides is less than the rate of its intake and cellular synthesis, via amino acid catabolism, phenylpropanoid metabolism, nitrate reduction, and photorespiration. Therefore, plants must maintain a delicate balance between NH_4^+ absorption, production, and consumption in order to prevent toxicity. This review presents a brief overview of ammonium toxicity and its underlying tolerance mechanisms with a special emphasis on how ammonium nutrition can be potentially used to improve plant productivity, quality, and resistance to biotic and abiotic stresses. It also provides an update on recent advancements in nitrogen metabolism supported by omics research and the future perspectives to improve ammonium tolerance.

2. Nitrogen Metabolism and Ammonium Assimilation

N is a nutrient that plants can obtain from a variety of compounds, viz., nitrate, urea, ammonium, and amino acids and use for a variety of metabolic processes, including the synthesis of nucleic acids and proteins, while also as signaling and storage molecules. An essential physiological mechanism for the growth and development of plants is the assimilation of nitrogen into carbon skeletons. Under aerobic conditions, NO_3^- acts as the main source of N with rapid mobility and storability in vacuoles; however, its reduction to NH_4^+ is required for the synthesis of many organic compounds and proteins. All inorganic N is first converted to ammonium since it is the only reduced N form that plants can employ to assimilate into N-carrying amino acids [8]. A two-stage process is involved to convert nitrate into NH_4^+ ; in the first step, nitrate reductase converts NO_3^- into nitrite in the cytoplasm, and in the second step, nitrite is turned into NH_4^+ by nitrite reductase in the plastids [11]. Then, ammonia is assimilated into glutamine (Gln) and glutamate (Glu) through the glutamate synthase/ glutamine-2-oxoglutarate amino transferase (GS/GOGAT) cycle [12,13] (Figure 1). The enzyme, glutamine synthetase (GS) has a vital role in the assim-

ilation of NH_4^+ into glutamine, while it is also a rate-limiting step in the NH_4^+ assimilation. Additionally, GOGAT and GS have two isoforms and their location has been identified as being tissue specific. For instance, NADH-GOGAT (NADH-dependent glutamate synthase) and GS1 are implicated in nitrogen assimilation in the roots, while Fd-GOGAT (reduced ferredoxin-dependent glutamate synthase) and GS2 are primarily involved in nitrogen assimilation in leaves [13]. Among five *GS1* genes in *Arabidopsis* (GLN1;1–GLN1;5), GLN1;2 is the main isoenzyme responsible for GS1 activity in the roots. The *gln1;2* mutant exhibits poor GS activity, excessive ammonium buildup, and decreased plant growth when supplied with ammonia, indicating that GLN1;2 is crucial for both assimilation of ammonia and detoxification in roots [14]. NADH-GOGAT serves as the primary isoform in the roots of *Arabidopsis* and exogenous ammonium supply greatly induces the expression of this isoform. Under the conditions of ammonium availability, the *nadh-gogat* mutant exhibits lower glutamate formation and biomass buildup [15]. However, as endogenous NH_4^+ concentration rises, a different route regulated by the mitochondrial NAD(H)-dependent glutamate dehydrogenase (GDH) helps to lower this internal NH_4^+ concentration (Figure 1). The GDH enzyme catalyzes the reversible amination of 2-oxoglutarate (2-OG) to form glutamate, using ammonium as a substrate. Therefore, the GDH enzyme is theoretically able to either assimilate or release ammonium. Evidence suggests that under normal conditions, GDH operates in the direction of Glu deamination to form ammonium [16]. However, when ammonium concentration increases above a certain threshold, particularly under stress conditions [17], or when plants are grown under sole NH_4^+ N source, the GDH activity is greatly induced to lower the internal concentration of NH_4^+ [18]. The ability of GDH to release N from amino acids results in the creation of a keto-acid, 2-OG, and NH_3 , which can, then, be independently recycled for use in respiration and the synthesis of amides, respectively. The C-skeletons generated by other metabolic pathways, including respiration and photosynthesis, can also be used to integrate ammonia into the amino acids, glutamine, and glutamate. However, ammonium is thought to be toxic to plants at higher concentrations, as it can result in proton extrusion, which is linked to ammonium uptake, cytosolic pH alterations, and the dissociation of photophosphorylation in plants.

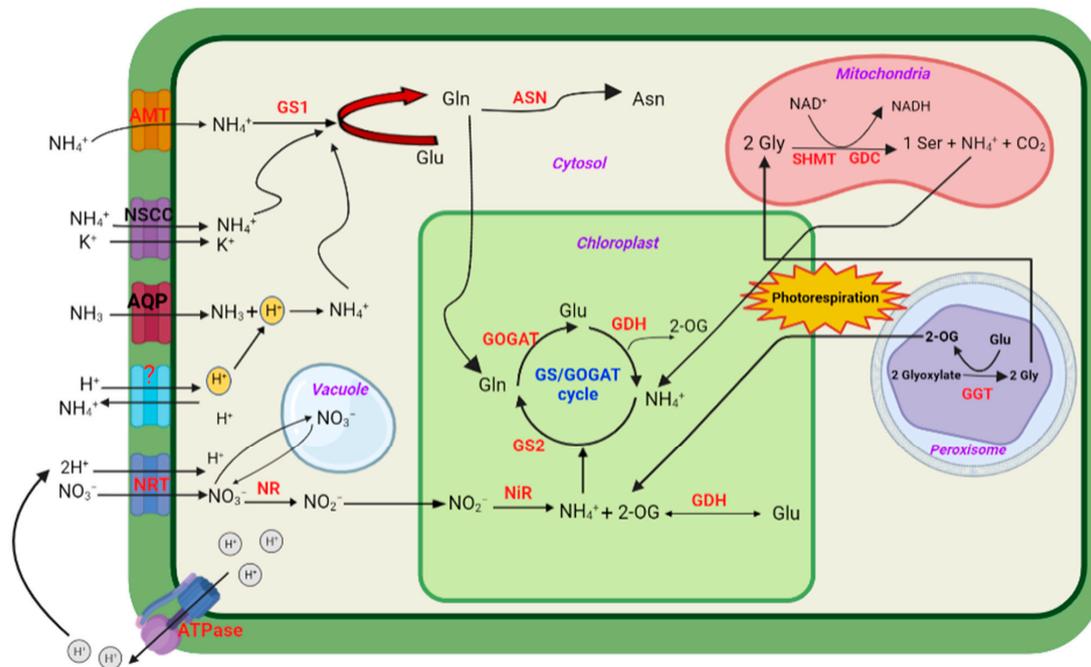


Figure 1. Mechanism of uptake and assimilation of nitrate and ammonium ions in plants. The transport of nitrate occurs through a nitrate transporter (NRT) followed by its conversion into nitrite

by nitrate reductase (NR) in the cytoplasm and its subsequent reduction into ammonium ions by nitrite reductase (NiR) in the chloroplast. The nitrate transporters act as H^+ / NO_3^- symporters that depend on the proton-motive force produced by the plasma membrane H^+ -ATPases. According to the hypothesis postulated by Coskun et al. [19], ammonium (NH_4^+) is taken up as the non-electrogenic flux of NH_3 through the plasma membrane, mediated by aquaporins (AQP). Ammonium is also transported through high-affinity ammonium transporters (AMT) and to some extent through the non-specific cation channels (NSCC) or K^+ channels. The ammonium ion that enters the cell directly goes to the GS/GOGAT cycle, where NH_4^+ is assimilated into glutamine (Gln) by glutamine synthetase and glutamate by glutamate synthase (GOGAT) in the chloroplast. Glutamine can also be converted into asparagine (Asn) via asparagine synthetase (ASN). The enzyme glutamate dehydrogenase (GDH) yields ammonium and 2-oxoglutarate (2-OG), a product that links nitrogen metabolism with photorespiration, which involves three cell organelles, such as chloroplast, peroxisome, and mitochondria. The photorespiratory product, glyoxylate from chloroplast is converted into glycine by the action of glutamate: glyoxylate aminotransferase (GGT) in peroxisome, while two molecules of glycine are converted into one molecule of serine by the combined action of glycine decarboxylase (GDC) and serine hydroxymethyltransferase (SHMT) in mitochondria.

3. Signs of Ammonium-Induced Toxicity

Ammonium toxicity often develops, when a plant is exposed to high levels of ammonium in the environment, or alternatively, when plant cells spontaneously overproduce ammonium due to enhanced proteolytic activity as a consequence of environmental stress. Widely varying documented NH_4^+ toxicity symptoms typically manifest at external NH_4^+ values over 0.1 to 0.5 mmol L^{-1} [20]. The primary phenotypic indicators of ammonium toxicity in plants are restricted root growth and chlorosis of the leaves [10]. Ammonium toxicity modifies the biochemical and physiological makeup of the plant, affecting its intracellular pH, osmotic balance, metabolism of phytohormones and polyamines, and ability to absorb nutrients (Figure 2). Ammonium toxicity can also cause a reduction in the amount of carbon availability, damage to the chloroplast's ultrastructure, an increase in proton efflux, ineffective transmembrane ammonium cycling, suppression of the enzyme GDP-mannose pyrophosphorylase, as well as oxidative stress [17,19]. Ammonium toxicity is also accompanied by lower levels of chlorophyll a and b and carotenoids, a decline in photosynthetic rates, and a rise in the production of ethylene [19,21]. In cucumber, higher levels of ammonium were found to be toxic, causing toxicity symptoms such as suppression of growth, chlorosis, curling of leaves, and low tissue accumulation of calcium and magnesium, while the addition of lesser amounts of NH_4^+ to NO_3^- has drastically improved the growth rate [22]. Increased ammonium levels have negatively impacted the uptake of cations, such as Ca, K, Mg, and Si, in papaya, while also reducing growth [23]. Excess ammonium has resulted in calcium deficit, restricted N uptake, altered N/Ca ratio as well as physiological changes in tomato plants [24]. Recently, the interactive role of ammonium nutrition with iron (Fe) homeostasis has been demonstrated in the model plant *Brachypodium distachyon* [25]. It was shown that plants that had been fed with ammonium showed heightened susceptibility to iron deficiency, indicating that ammonium nutrition resulted in decreased Fe uptake and root-to-shoot transfer. Thus, in order to better comprehend and enhance ammonium use efficiency and tolerance in plants, future studies are required to explain the mechanisms driving the interaction between Fe and NH_4^+ , particularly regarding the mechanism leading to Fe deficiency under ammonium stress.

Root development is more strongly impacted by excess NH_4^+ than any other plant organ. Higher NO_3^- concentrations promote lateral root growth, while higher NH_4^+ concentrations result in stunted roots with slow growth rates. Overabundance of NH_4^+ primarily shortens primary roots by preventing cell expansion and division in the meristems. Under these circumstances, GDP-mannose pyrophosphorylase (GMPase)-mediated NH_4^+ efflux increases in the meristematic zone, leading to a higher NH_4^+ influx in mature root cells [26]. Investigations in *Lotus japonicus*, employing split-root systems have revealed that root-specific NH_4^+ -induced reactions are regulated by NH_4^+ transporters that are more

closely related to NH_4^+ sensing than its assimilation. In both *L. japonicus* and *Arabidopsis*, *AMT1;3* mediates the short-root phenotype induced by NH_4^+ nutrition [27,28]. Ammonium suppresses root gravitropism in a similar way as it inhibits primary root growth. This phenomenon is linked to a considerable delay in the asymmetrical auxin distribution in the elongation zone of the root and a significant reduction in the expression of PIN2, the auxin exporter. ARG1 (Altered Response to Gravity 1) acts as an antagonist to PIN2 to re-establish root gravitropism through PIN3-mediated lateral auxin distribution and AUX1-induced shootward auxin reflux at the root apex under ammonium input [29]. The defective root gravitropism is greatly influenced by the antagonistic interaction between PIN2 and ARG1 in maintaining the asymmetric auxin fluxes under ammonium supply. Thus, PIN2 and ARG1 are the key targets of NH_4^+ -induced impairment of root gravitropism.

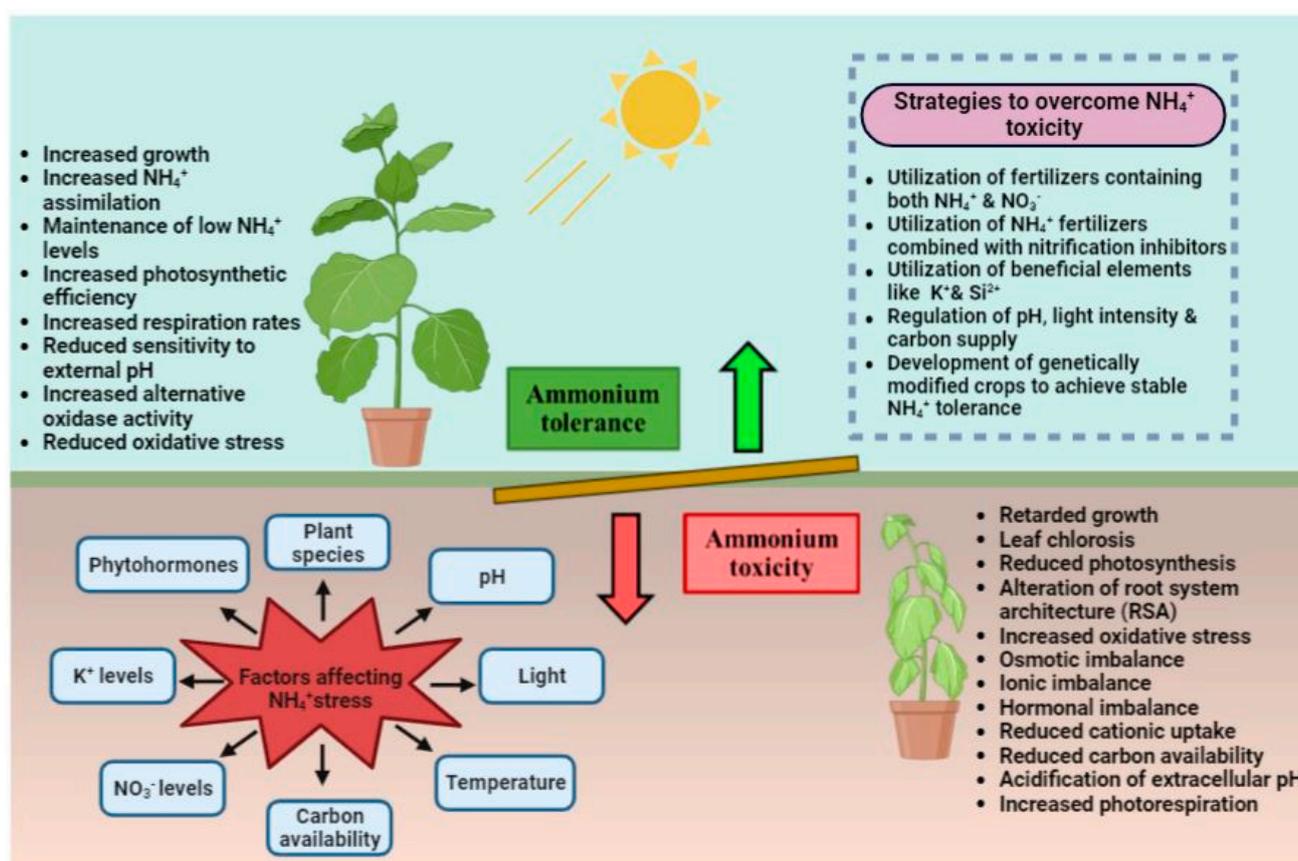


Figure 2. The major physiological changes and symptoms associated with ammonium toxicity and tolerance as well as the potential strategies to improve ammonium tolerance are illustrated. The ammonium toxicity is affected by various environmental factors and the extent of toxicity varies among species.

4. Postulated Theories of Ammonium Toxicity and Tolerance

The ability of a plant to tolerate ammonium largely depends on its capacity to assimilate ammonium. Shoots are typically the most affected area of the plant to NH_4^+ feeding, after roots. The early signs of NH_4^+ toxicity arise at the root level with a significant alteration of the root system architecture (RSA), as roots are the prime NH_4^+ sensor [30] (Figure 2). The exogenous NH_4^+ eventually reaches the leaves once the root system's storage capacity has been exhausted. The harmful effects of NH_4^+ on leaves are mainly associated with its impact on photosynthesis and its ability to cause oxidative stress. For instance, a higher concentration of NH_4^+ (5 mM) has affected photosynthesis by binding NH_3 in the oxygen-evolving complex (OEC) of photosystem II in the cyanobacterium, *Synechocystis* [31]. In *A. thaliana*, NH_4^+ nutrition did not alter the photosynthetic efficiency but raised the amounts of mitochondrial reactive oxygen species (ROS) in the leaf [32]. In

line with this, few other studies have confirmed that NH_4^+ nutrition caused enhanced lipid peroxidation and glutathione-ascorbate cycle enzyme activity, or alterations in the redox state, implying higher ROS production [33,34]. The tolerance mechanisms used by plants to deal with NH_4^+ toxicity have not been well described because the primary causes of NH_4^+ toxicity have not been determined clearly. According to the classical hypothesis of ammonium toxicity, some of the key traits contributing to NH_4^+ tolerance in plants include enhanced alternative oxidase pathway activity in mitochondria, greater NH_4^+ assimilation and retention of low levels of NH_4^+ in leaves, and/or poor sensitivity to extrinsic pH acidification [35]. Therefore, several factors, including root carbon metabolism and the capacity to maintain high respiration rates, the ability to limit NH_4^+ accumulation inside tissues, and tolerance to acidification of the root zone affect a plant's overall ability to withstand high NH_4^+ concentrations. The recent hypothesis on NH_4^+ toxicity, proposed by Coskun et al. [19], suggests gaseous NH_3 as the predominant N species transported across the cell membrane instead of NH_4^+ , as opposed to the conventional NH_4^+ futile cycle hypothesis [10] that has made significant advancements in the understanding of NH_4^+ uptake in plants. This result disproves the idea that excessive energy consumption is linked to NH_4^+ efflux and puts forth that NH_4^+ accumulation in cells and nutrient imbalance caused by lower cationic concentrations are the primary causes of NH_4^+ toxicity. The NH_4^+ is transported into cells through various transporters including (1) the MEP/AMT/Rh protein family that transports NH_3 by deprotonating NH_4^+ with high affinity under fewer NH_4^+ concentrations [36]; (2) the aquaporins (AQP) that aid in the non-electrogenic NH_3 flow through the plasma membrane at high NH_4^+ concentrations [37]; (3) the non-specific cation channels (NSCC) and K^+ specific channels that transport NH_4^+ ions in competition with K^+ , which are similar in size and surface charge density (Figure 1) [38]. One of the main causes of NH_4^+ toxicity is the impact of NH_4^+ nutrition on reducing the concentrations of cations (K^+ , Ca^{2+} , and Mg^{2+}) in plant tissue. The harmful effects of NH_4^+ nutrition can, however, be reduced by raising K^+ concentrations in the root media. K^+ can lessen the toxicity of NH_4^+ by enhancing the incorporation of NH_4^+ into organic N-compounds via the activation of enzymes such as glutamate dehydrogenase, glutamine synthetase, etc., as well as by limiting the absorption of NH_4^+ by low-affinity transporters [39]. K^+ concentrations in the root medium have the potential to obstruct the transport of NH_3 by specifically regulating the activity of AQP and the plant's water balance [40].

5. Signaling Responses Involved in Ammonium Sensing and Tolerance

Effective nutrient harvesting by plant roots requires a quick and sensitive assessment of nutrient availability in the environment. Nutrient transporters located at the plasma membrane are the key players in sensing nutrients in the external environment. For example, NRT1.1/NPF6.3 in *Arabidopsis* and Mep2 in yeast have been recognized as the major transporters of nitrate and ammonium, respectively, across the plasma membrane [41]. Numerous factors are engaged in the crosstalk signaling that controls plant growth and development under the conditions of NH_4^+ sensitivity and tolerance.

5.1. AMT-Mediated Extracellular Ammonium Sensing Responses

The extracellular sensing and transportation of ammonium are mediated through the phospho-dependent allosteric mechanism of activating the C-terminal domain of AMT1;1 in *Arabidopsis* [41,42]. The cytosolic C-terminus of each monomer of the trimeric AMT1 complex connects to the pore region of the adjacent subunit to transactivate the ammonium transport. The trans-inactivation of both the individual monomer as well as the entire trimeric complex is facilitated by the phosphorylation of threonine residue in the C-terminus of any monomer that leads to the relieving of contact between the C-terminus and the conducting pore. The critical threonine residue T460 in the C-terminal domain of AMT1;1 is dephosphorylated in conditions of low ammonium availability, whereas conditions of high ammonium supply result in phosphorylation and deactivation of the AMT1;1 trimer. Moreover, it has been demonstrated that glutamine, a product of ammonium assim-

ilation, and methionine sulfoximine (MSX), the glutamine synthetase inhibitor, are unable to induce T460 phosphorylation, suggesting that extracellular ammonium alone can specifically trigger the phospho-dependent inactivation of AMT1;1. Thus, in order to prevent excessive ammonium absorption, it has been postulated that AMT1;1 itself functions as a transceptor, whereby it attracts a kinase from the cytosol following extracellular ammonium binding, and confers either direct or indirect phosphorylation of AMT1;1. Intriguingly, trans-inactivation by C-terminal phosphorylation extends to heterotrimeric AMT1 complexes in addition to homotrimeric ones, as demonstrated for AMT1;1, AMT1;2 [43], and AMT1;3 [44]. This greatly increases the potential for regulating the exchange of signals between AMT isoforms via protein–protein interactions. In furtherance, AMT1;3 plays a crucial role in ammonium-mediated lateral root branching. Therefore, it appears that AMT1;1 and AMT1;3 are significant players in various signaling pathways, and their colocalization and physical interaction enable the coordination of both ammonium sensing and transport in response to the external availability of ammonium in the rhizosphere (Figure 3). Surprisingly as AMT1;3 seems to resist the C-terminal phosphorylation and inactivation caused by ammonium, an alternate feedback mechanism is activated in response to increased ammonium supply [45]. Following ammonium addition, AMT1;3 proteins aggregate and are subsequently removed from the plasma membrane through the endocytic pathway, as demonstrated in the glutamine synthetase mutant *gln1;2*. Hence this alternative shutoff mechanism helps to prevent ammonium toxicity in root cells.

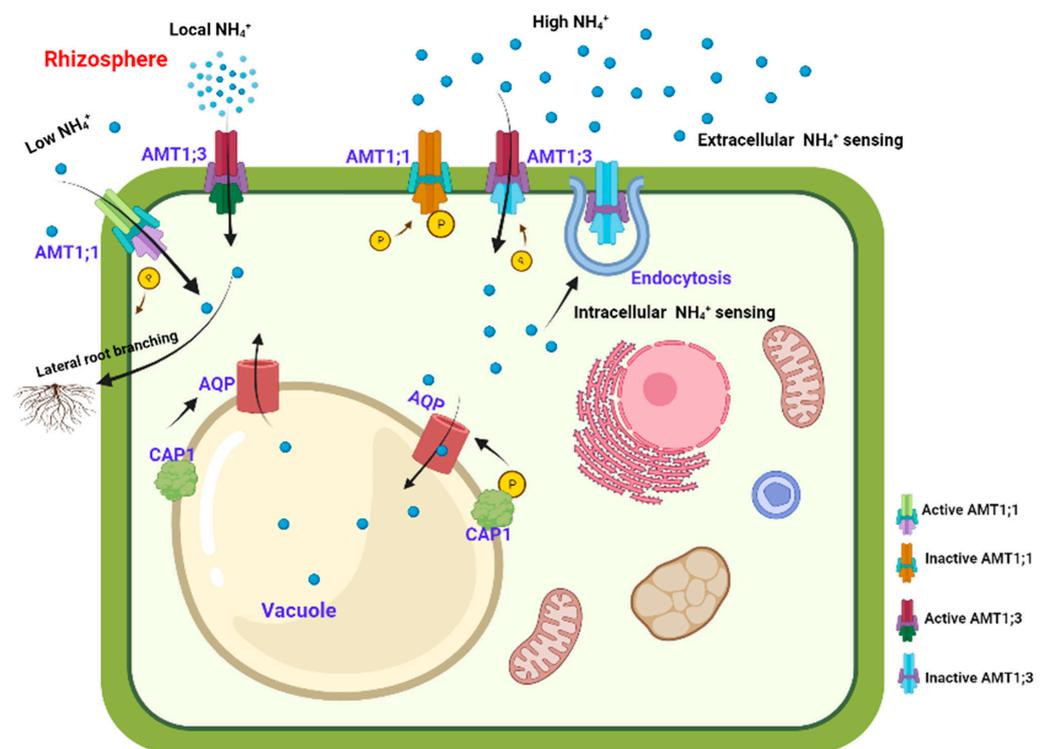


Figure 3. Extracellular and intracellular ammonium sensing events in plants. In low ammonium conditions, the crucial threonine residue T460 in the C-terminal domain of AMT1;1 is dephosphorylated allowing the ammonium transport pores to open and allows for ammonium uptake. AMT1;3 sends a signal to encourage lateral root development when roots are exposed to localized ammonium, which leads to ammonium scavenging. AMT1;1 is rendered inactive by phosphorylation of its C-terminal domain in response to increased ammonium availability. Elevated cytosolic ammonium levels cause AMT1;3 to be clustered and internalized into the cytoplasm by the endocytic pathway, which further stops the uptake of ammonia. The tonoplast-localized receptor-like kinase CAP1 facilitates the compartmentation of ammonium into vacuoles mediated through aquaporins (AQP), which protects cells from ammonium toxicity.

5.2. CAP1-Mediated Intracellular Ammonium Sensing Responses

In addition to ammonium assimilation, alternative mechanisms are also in operation to provide tolerance to ammonium toxicity. It has been shown in *Arabidopsis* that CAP1, a receptor-like kinase, mediates the compartmentation of ammonium in vacuoles under conditions of high intracellular ammonium [46] (Figure 3). The tonoplast localized unique regulator of the NH_4^+ homeostasis signal, CAP1 has been found to regulate the polar development of root hairs by maintaining tip-focused cytoplasmic Ca^{2+} gradients as well as ROS gradients. A CAP1 knockout mutant (*cap1-1*), exhibited an increased concentration of cytoplasmic NH_4^+ . In addition, on Murashige and Skoog media, the root hair growth of *cap1-1* was suppressed, while NH_4^+ depletion restored the Ca^{2+} gradient required for normal growth. The comparatively alkaline cytosolic pH of *cap1-1* root hairs and the reduced net NH_4^+ inflow across the vacuolar membrane suggested that CAP1 mutation promoted NH_4^+ accumulation in the cytoplasm. Moreover, ectopic expression of CAP1 in the yeast mutant *npr1* accomplished the missing function of NPR1, a crucial component of the ammonium-sensing pathway, and restored the phosphorylation as well as the ammonium transport activity of Mep2. This suggests that CAP1 may also function in plants as a regulatory component in intracellular ammonium-sensing events [46]. Autophosphorylation of CAP1 upon exposure to ammonium has been confirmed in *Arabidopsis* through a phosphoproteomics approach [46,47]. Moreover, the location of CAP1 in the tonoplast suggests that CAP1 may phosphorylate vacuolar ammonium transporters. The aquaporins, TIP2;1 and TIP2;3 that are located in the tonoplast and involved in ammonium compartmentation might be the potential putative targets for CAP1. However, so far only the transcriptional upregulation of the ammonia-conducting aquaporin TIP2;3 has been evidenced in ammonium treated *cap1-1* mutants [48]. Thus, the transcriptional regulators responsible for amplifying the ammonium-sensing event and orchestrating the subsequent ammonium responses are still unclear. Hence, future studies that examine the local integration of external and internal ammonium-dependent signals to control AMT-dependent ammonium transport are of utmost importance.

5.3. Nitrate, Auxins, NO, and Polyamines-Mediated Signaling of NH_4^+ Tolerance

The alleviating role of NO_3^- in ammonium toxicity has been mediated through the crosstalk signaling among NO_3^- , NRT1.1/NPF6.3, NO_3^- transporter, and auxin. Since NO_3^- induces the expression of the NRT1.1/NPF6.3 transporter gene, plants lacking NO_3^- in the root medium may experience disruptions in this signaling pathway—for example, short and less branched roots in the case of NH_4^+ and urea-fed *Medicago truncatula*, respectively [49]. The auxin-responsive reporter DR5:GUS-reporter has been used in a mutant strain of *Arabidopsis* to study the function of auxin signaling in the growth of primary roots fed with NH_4^+ . The study showed that the response of the reporter to auxins under NH_4^+ nutrition was dramatically reduced, whereas exogenous auxin only partially restored root growth [50]. It was determined that NRT1.1/NPF6.3 had a NO_3^- -independent function since NRT1.1/NPF6.3 defective mutants had a better tolerance for high concentrations of NH_4^+ and NO_3^- , which when administered did not increase this tolerance towards NH_4^+ . In NO_3^- -mediated reduction of ammonium stress, NO_3^- efflux channels, such as SLAH3, also played a significant role [51]. In the absence of NO_3^- , SLAH3-defective mutants are hypersensitive to high NH_4^+ concentrations as well as low pH.

Plants lack the essential enzyme, carbamoyl phosphate synthase (CPS)-type I of animals; nevertheless, they appear to possess a functional CPS-type II that utilizes glutamine as the NH_3 source for carbamoyl-P synthesis. As a result, plants are unable to produce urea directly from NH_4^+ , which would lower the internal NH_4^+ concentration under stress. The plant urea cycle comprises two phases: the biosynthetic phase, which produces arginine, and the catabolic phase, where arginine can be broken down by arginase to produce urea and ornithine or act as a substrate for arginine decarboxylase to make agmatine. Either agmatine or ornithine can further produce putrescine, spermidine, and other polyamines. Arginine has been proposed as an intermediate in NO synthesis and links the urea cycle

with NH_4^+ tolerance. It has been implied that nitric oxide (NO) derived from NO_3^- through nitrate reductase also plays a profound role in auxin-signaling by acting downstream of auxin. This was confirmed by arginase-negative mutants of *Arabidopsis*, through which increased NO builds up and effluxes, while promoting the development of lateral and adventitious roots [52]. The root tips, developing lateral roots, and hypocotyls of these mutants consistently displayed enhanced auxin signaling. However, the source of NO in plants fed with NH_4^+ or urea as the only N nutrition remains elusive. It was also found that the exogenous application of polyamines such as spermidine and spermine has led to the increased synthesis of NO in *Arabidopsis* [53]. It has been hypothesized that an excess of ammonium resulting either from NH_4^+ nutrition or from polyamine metabolism may operate as a feedback inhibitor of polyamine deamination, thereby reducing polyamine metabolism. Further, metabolic studies in peas and *Arabidopsis* have revealed that ammonium supplementation caused arginine accumulation, presumably indicating impaired polyamine production [44,54].

6. Ameliorated Crop Quality and Productivity by Ammonium Nutrition

In agricultural settings, the two most common types of inorganic nitrogen taken by the roots of higher plants are ammonium (NH_4^+) and nitrate (NO_3^-). In the rhizosphere environment, the absorption of nitrate nitrogen is associated with the inorganic cation uptake and the release of OH^- , leading to an alkaline rhizosphere, while ammonium nitrogen absorption takes place by the uptake of inorganic anion and the release of H^+ , causing an acidic rhizosphere. An overabundance of free NH_4^+ in the cytosol is likely the primary source of ammonium toxicity and plants employ a number of rational ways to manage NH_4^+ levels, including absorption into organic compounds, storage of NH_4^+ in the vacuole, and outflow of NH_4^+ to the apoplast and rhizosphere. Despite ammonium stress affecting almost all plant species, there is considerable intra- and interspecific variation in response to ammonium feeding. While some species/genotypes exhibit severe sensitivity to ammonium when growing, other species/genotypes exhibit ammonium preference. The influence of ammonium and nitrate nutrition varied among selected bedding plants, such as *Salvia splendens*, marigold, *Ageratum houstonianum*, and *Petunia hybrida* [55]. *Salvia* and marigold were highly sensitive to NH_4^+ , while *ageratum*, being tolerant to NH_4^+ , was more sensitive to NO_3^- . The study also revealed that NH_4^+ -fed plants contained more anions, such as more Cl^- and fewer cations than NO_3^- -fed plants. NH_4^+ nutrition has significantly enhanced the chlorophyll contents of *ageratum* and *petunia*, while NO_3^- nutrition increased the chlorophyll contents of *Salvia* [56]. Upon investigating the effect of NH_4^+ and NO_3^- in the presence and absence of Cl^- , the uptake of NO_3^- was suppressed by NH_4^+ , while the uptake of NH_4^+ was unaffected by NO_3^- and the uptake of Cl^- was counteracted by both NH_4^+ and NO_3^- [57]. In *Arabidopsis*, ammonium as a predominant N source negatively impacted biomass production [58,59], while in another study, ammonium nutrition was found to be beneficial in the improvement of glucosinolate metabolism [60]. Ammonium as a sole N source has improved the grain quality in wheat by increasing the grain reserve proteins, such as gliadins and glutenins [61]. The effects of N nutrition in the form of NH_4^+ and NO_3^- have been extensively studied in many plant species. However, the requirement of N sources varied among species. For example, NO_3^- nutrition positively regulated the growth of wheat [62], sugar beet [63], tobacco [64], beans [65], and canola [66], whereas NH_4^+ nutrition was found suitable for the growth of larch and pine [67], tomato [68], and rice [69]. Nevertheless, employing NO_3^- as the exclusive source of nitrogen causes N leaching and nitrate buildup in plants [70,71]. For the majority of species, a combination of ammonium and nitrate is recommended as an excellent nutrient recipe than using nitrate or ammonium alone. Numerous studies have achieved high productivity and quality in many plant species by combining NH_4^+ and NO_3^- in a particular ratio (Table 1). For instance, optimum concentrations of NH_4^+ and NO_3^- have greatly influenced the growth and flowering of *Petunia hybrida*, grown in rockwool and peat-lite media. The highest shoot dry weight was obtained when equal concentrations

of NH_4^+ and NO_3^- (9 me. L^{-1} each) were added to the peat-lite media. Flowering was delayed at increased concentrations of both forms of nitrogen; however, increased NO_3^- highly affected flowering [72]. The japonica rice cultivars exhibited a high ammonium preference along with a partial requirement of nitrate ($\text{NH}_4^+:\text{NO}_3^-$, 75:25) for efficient photosynthetic activity, higher NUE and faster biomass accumulation [73]. In the case of cabbage, an equal ratio of ammonium and nitrate has greatly improved the seedling fresh weight, root length, and contents of phosphate (H_2PO_4^-), and mineral nutrients (K, Ca, and Mg), while also significantly reducing the nitrate content in leaves [74]. Upon investigating the effect of various $\text{NH}_4^+:\text{NO}_3^-$ proportions in strawberries, the ideal $\text{NH}_4^+:\text{NO}_3^-$ ratio for superior fruit development and quality in the semi-hydroponic fertigation system was found to be 29:71 [75]. In *Capsicum annuum* L, the ratio of ammonium to nitrate at 25:75 drastically improved root growth, and the accumulations of nutrient elements (N, P, and K) accumulation and dry matter [76]. The treatment also improved the fruit quality by raising vitamin C, contents of total phenols, flavonoids, soluble sugar and protein, dry matter, and pungency levels. The ammonium tolerance was further substantiated by greater levels of the enzymes, GS and GOGAT as well as higher relative expression of the genes encoding these enzymes. Similarly, the $\text{NH}_4^+:\text{NO}_3^-$ ratio of 25:75 has been shown to enhance N uptake and assimilation in flowering Chinese cabbage, mostly via controlling the pH of the nutrient solution [77]. It has been well established that NH_4^+ uptake and assimilation are associated with proton release processes leading to the acidification or pH drop of the rhizosphere. Thus, the interaction in the decrease in pH and K^+ uptake in NH_4^+ tolerance was investigated in hydroponically cultured ageratum, salvia, cabbage, and lettuce plants at three $\text{NH}_4^+:\text{NO}_3^-$ ratios (0:100, 50:50, and 100:0) [78]. The plants, such as ageratum and lettuce, were found to be more tolerant to NH_4^+ feeding, which exhibited a rapid decrease of solution pH as well as improved K^+ influx when compared to NH_4^+ sensitive, salvia, and cabbage. In another study, the effect of NH_4^+ and NO_3^- ratios (0:100, 50:50, and 100:0) on growth, photosynthesis, nitrogen, and carbohydrate concentrations alongside nitrogen assimilation enzymes were evaluated among NH_4^+ sensitive (cabbage) and tolerant (lettuce) species. The study reinforced that both plants benefitted from equal ratios of NH_4^+ and NO_3^- (50:50) with enhanced growth and quality. Moreover, in contrast to cabbage, the GS and GDH activities in lettuce were boosted in response to an increase in the level of external NH_4^+ leading to reduced NH_4^+ accumulation [79]. Thus, the co-provision of NH_4^+ with NO_3^- triggers a synergistic growth response that can outpace the maximum growth rates on either nitrogen source alone. For all of the above studies indicated, it is significant to note that the optimal ratio of $\text{NH}_4^+:\text{NO}_3^-$ is also influenced by the total N concentration and the source of NH_4^+ -N. The requirement of total N concentration varied among plant species, ranging between 5.0 and 15 mM L^{-1} , and in all the trials, the total N content was maintained constant, while the ratios of NH_4^+ to NO_3^- were altered (Table 1). Additionally, the source of NH_4^+ -N plays a vital role in determining the plant responses to nutrition, osmotic balance, stress, and overall plant development. Ammonium sulfate (AS) is one of the most widely used N fertilizers, which comprises 21% N and 60% sulfur (S). It is a great source of S for crops that demand huge supplies and offers a balanced supply of both N and S. In terms of nutrition, N and S play very close relationships with one another, particularly because both nutrients are necessary for the synthesis of chlorophyll and are components of amino acids. Sulfur is a structural element found in amino acids, protein disulfide bonds, cofactors, and vitamins. S deficiency impacts the biomass, general morphology, yield, and nutritional value of the plants [80]. Hence, the addition of a N source in the form of AS, also meets the S requirements of plants, promoting overall growth.

AS may have certain potential agronomic and environmental advantages over other N fertilizers, such as urea and ammonium nitrate (AN), including (1) minimal to no N loss via NH_3 volatilization upon surface application to acid or neutral soils, (2) a better source of N for saline soils as it lessens the detrimental effects of NaCl on plant growth and for saline-sodic calcareous soils, as it enhances soil structure, (3) minimal NO_3^- -N leaching from AS than AN, (4) improved N efficiency due to less denitrification than AN and reduced

emission of greenhouse gases (NO and N₂O), and (5) the formation of more acidic root rhizosphere by preferred absorption of NH₄-N of AS over NO₃-N of AN, which may boost soil phosphorus (P) and micronutrient availability [81]. Although AN has the highest proportion of N (33–34%), the use of AN is not recommended for flooded rice soils that are either rainfed or irrigated mainly due to the significant N losses from the denitrification of nitrate. Therefore, AS becomes the preferred choice of fertilizer for rice growers [81]. On the other hand, other N fertilizers, such as ammonium chloride, improved the yield of ratoon in sugarcane during the first cycle but decreased the yield in subsequent cycles. As sugarcane is moderately sensitive to soil salinity, the decrease in yield could be attributed to the saline effect caused by the residual Cl⁻ from NH₄Cl [82]. Similarly, the application of NH₄Cl has provoked the accumulation of cadmium in wheat due to the increased Cl⁻ concentration in the soil [83]. Hence, rationale utilization of the optimum concentration of NH₄Cl, especially in saline-sensitive crops is highly crucial, as Cl⁻ appears to be the preferable counter ion to NH₄⁺, the increased Cl⁻ ions exacerbate the symptoms of salinity stress, in addition to ammonium toxicity.

Ammonium fertilizers are ineffective in cold climates and irrigated systems due to the significantly decreased activity of nitrifying microorganisms. Since NH₄⁺ is a cation, it greatly adheres to most soils, especially to clays and organic matter; thus, there is minimal leaching off with water. Hence, this causes more ammonium toxicity in irrigated systems where nitrification is greatly inhibited. Therefore, management of appropriate NH₄⁺-N source and concentration in irrigated fields is of prime requirement. Recently, several studies have examined the interaction effect between the NH₄⁺-N forms and irrigation systems in cultivated fields. For example, AS application has drastically improved the lint yield in a drip-irrigated, moderately saline cotton field by reducing rhizosphere soil pH and salinity as well as improving P nutrition [84]. In another study, AN fertilization at 120% crop evapotranspiration (ET_c), and drip irrigation conditions significantly improved the water use efficiency, chlorophyll, tuber quality, and yield of potatoes compared to AS and urea fertilization [85]. Eggplant is sensitive to NH₄⁺ nutrition in solution culture, while it has responded positively to an appropriate ratio (50:50) of AN fertilization with 75% irrigation level through improved vegetative growth, water use efficiency, and yield compared to AS fertilization [86].

Although nitrate fertilizers are less likely to be hazardous to plants than ammonium fertilizers, they contribute to significant N loss through NO₃⁻ leaching and higher NO emissions. Inhibitors of soil nitrification, including dicyandiamide (DCD), have been suggested as an efficient way to lessen soil N loss and N₂O emissions. It has been extensively demonstrated that using nitrification inhibitors in conjunction with ammonium fertilizers in agricultural fields can reduce the environmental impact of nitrogen fertilizers [87]. However, the maintenance of N in the NH₄⁺ form in soil by such compounds also raises concerns for crops that are sensitive to ammonium [88]. Hence, future research is needed to determine how nitrification inhibitors and the increased NH₄⁺-N concentrations in soil affect crop growth and yields.

Table 1. Influence of ammonium to nitrate ratio on growth, physiology, and nitrogen metabolism in plants.

Plant Species	NH ₄ ⁺ : NO ₃ ⁻ Ratios Investigated	Total N Concentration (mM L ⁻¹)	Source of the NH ₄ ⁺ ions	Optimum NH ₄ ⁺ : NO ₃ ⁻ Ratio	Effect on Growth, Photosynthesis, and Nitrogen Metabolism	Reference
<i>Chrysanthemum morifolium</i> var. <i>circus</i>	1:100, 40:60, and 80:20	5.0	(NH ₄) ₂ SO ₄	80:20 (at pH 7)	Highly vegetative, reproductive, and increased vesicular-arbuscular mycorrhizal (VAM) fungi growth and nutrient content (N, P, K) at pH 7	[89]
<i>Triticum durum</i> 'Barkay'	0:100, 25:75, and 50:50	1.4, 2.8, and 5.5, respectively	(NH ₄) ₂ SO ₄ and NH ₄ NO ₃	25:75	Increased grain yield and total yield	[90]

Table 1. Cont.

Plant Species	NH ₄ ⁺ : NO ₃ ⁻ Ratios Investigated	Total N Concentration (mM L ⁻¹)	Source of the NH ₄ ⁺ ions	Optimum NH ₄ ⁺ : NO ₃ ⁻ Ratio	Effect on Growth, Photosynthesis, and Nitrogen Metabolism	Reference
<i>Fragaria × ananassa Duch</i> cvs. 'Camarosa' and 'Selva'	0:100, 25:75, 50:50, and 75:25	3.26	NH ₄ H ₂ PO ₄ and (NH ₄) ₂ SO ₄	25:75	Greater vegetative growth (number of leaves, leaf fresh and weights, chlorophyll index, leaf area, and net photosynthetic and transpiration rates), reproductive growth (number of flowers and fruits, percentage of fruit set, fruit length, fresh weight and dry weights of fruit, and percent total soluble sugars and Ca content of fruit), and overall yield	[91]
<i>Persea Americana</i> Mill.	0:4, 1:3, 2:2, 3:1, and 4:0	3.0	(NH ₄) ₂ SO ₄	1:3	Increased dry matter accumulation and development of secondary shoots	[92]
<i>Lycopersicon esculentum</i> Mill. 'Xinpinbaoguan'	0:100, 25:75, and 50:50	5.0	(NH ₄) ₂ SO ₄	25:75 (both homogenous and heterogenous supply)	Higher shoot and root biomass and total N uptake	[93]
<i>Lycopersicon esculentum</i> Mill. 'Xi Nong 2011'	0:100, 25:75, 50:50, 75:25, and 100:0	8.0	NH ₄ Cl	25:75 (unstressed conditions) 50:50 (chilling stress conditions)	Increased biomass, relative growth rate (RGR), net photosynthetic rate, chlorophyll content, contents of soluble protein and free amino acids and higher activities of GS and NADH-GOGAT (under unstressed conditions)	[94]
<i>Gerbera jamesonii</i> L.	0:100, 20:80, 40:60, and 60:40	11.2	(NH ₄) ₂ SO ₄	20:80	Greater biomass, number of flowers, diameters of flower stalk and flower disk, vase life, fresh and dry weights of inflorescences, and relative water content of petals	[95]
<i>Phalaenopsis</i> 'Golden Peoker' and <i>Dendrobium</i> 'Valentine'	100:0, 75:25, 50:50, 25:75, and 0:100	3.0	(NH ₄) ₂ SO ₄	60:40 (<i>Phalaenopsis</i>) 50:50 (<i>Dendrobium</i>)	Bigger plants, greater dry matter, diameters of stem and pseudobulb, average number of pseudo bulbs and mineral nutrients	[96]
<i>Brassica chinensis</i> L.	0:15, 5:10, 7.5:7.5, and 10:5	15.0	(NH ₄) ₂ SO ₄	5:10	Greater biomass, photosynthetic activity, and low nitrate accumulation under water deficit and low light intensity conditions	[97]
<i>Saccharum officinarum</i> L.	0:100, 25:75, 50:50, 75:25, and 100:0	15.0	NH ₄ Cl	0:100 and 25:75	Greater biomass, leaf surface area, root growth, leaf gas exchange, and nutrient content	[98]
<i>Brassica oleraceae</i> L. var. <i>acephala</i>	0:100, 25:75, 50:50, and 75:25	10.0	(NH ₄) ₂ SO ₄ , NH ₄ NO ₃ , and (NH ₄) ₃ PO ₄	75:25	Higher biomass, leaf water content, nutrient content (N, P, K, Fe, Mn, Zn, and Cu), and lower nitrate content	[99]
<i>Brassica oleracea</i> var. <i>alboglabra</i> Coffea <i>arabica</i> L.	1:4, 1:8, and 1:12 0:100, 12.5:87.5, 50:50, and 100:0	8.0 12.0	(NH ₄) ₂ SO ₄ NH ₄ NO ₃	1:12 50:50	Increased leaf number, leaf area, and shoot and total fresh weights Highest dry mass yield, greater nutrient absorption, and photosynthetic ability	[100,101]
<i>Brassica napus</i> L. N-efficient genotype (D4-15) and N-inefficient genotype (D2-1)	0:100, 25:75, 50:50, 75:25, and 100:0	6.0	NH ₄ NO ₃	0:100 (N-efficient genotype) 75:25 (N-inefficient genotype)	Increased shoot and root biomass, well-developed root system, net photosynthetic rate, transpiration rate, stomatal conductance, nutrient content, better ammonium uptake, upregulation of ammonium transporter genes and greater tolerance to oxidative stress caused by NH ₄ ⁺ toxicity in N-inefficient genotype	[102]
<i>Vaccinium</i> spp. 'Emerald'	1:1, 2:1, and 4:1	7.5	(NH ₄) ₂ SO ₄	2:1	Greater plant height, crown width, chlorophyll content, root activity, and upregulation of nitrate transporter genes (<i>NRT1.5/NPF7.3</i> , <i>NRT2</i>)	[103]
<i>Lactuca sativa</i> var. <i>longifolia</i>	100:0, 20:80, 40:60, and 60:40	5.3	(NH ₄) ₂ SO ₄	20:80	Increased growth, head weight, and reduced nitrate accumulation in leaves Increased activities of nitrate reductase (NR) and glutamine synthetase (GS), high nitric oxide (NO) levels in leaves, upregulation of <i>NR</i> and <i>GS1</i> genes, and upregulation of proteins related to photosynthesis, carbon and energy metabolism, stress and defense, and protein folding and degradation responses	[104]
<i>Brassica Pekinensis</i> 'Jinwa no. 2'	0:100, 10:90, 15:85, and 25:75	5.0	(NH ₄) ₂ SO ₄	15:85 (normal light intensity) 10:90 (low light intensity)	Increased plant height, stem diameter, fresh and dry weights, length, surface area, volume and diameter of root, total N content, and higher NUE	[105]
<i>Brassica alboglabra</i> L. H. Bailey	0:100, 10:90, 25:75, and 50:50	5.0	NH ₄ Cl	25:75		[106]

Table 1. Cont.

Plant Species	NH ₄ ⁺ : NO ₃ ⁻ Ratios Investigated	Total N Concentration (mM L ⁻¹)	Source of the NH ₄ ⁺ ions	Optimum NH ₄ ⁺ : NO ₃ ⁻ Ratio	Effect on Growth, Photosynthesis, and Nitrogen Metabolism	Reference
<i>Lycopersicon esculentum</i> Mill.	0:100, 8:92, 15:85, and 20:80	12.0	(NH ₄) ₂ SO ₄	15:85 (grafted tomato) 8:92 (non-grafted tomato)	Increased biomass production, number of trichomes, epidermal cell density, stomatal density, fruits per plant, fruit size, average fruit weight, and total soluble solids	[107]
<i>Brassica lee</i> ssp. <i>namai</i> Ssamchoo 'Chunssamhwang 51'	0:100, 16.7:83.3, 33.3:66.7, and 50:50 NH ₄ ⁺ :NO ₃ ⁻ :Urea 17:83:0, 50:50:0; 25:50:25, and 0:50:50	13.0	(NH ₄) ₂ SO ₄	16.7:83.3 (NH ₄ ⁺ : NO ₃ ⁻) and 25:50:25 (NH ₄ ⁺ :NO ₃ ⁻ :Urea) (combined with 10.7 mmol L ⁻¹ Si)	Increased germination rate, germination energy, mean germination rate, specific leaf weight, fresh weight, root activity, and chlorophyll and vitamin C contents	[108]
<i>Dracocephalum moldavica</i> L.	0:100, 25:75, 50:50, 75:25, and 100:0	7.7	(NH ₄) ₂ SO ₄ and NH ₄ NO ₃	0:100, 25:75, and 75:25	0:100—Highest growth parameters, biomass, and contents of total phenolics, total flavonoids, and carotenoids. 25:75—Highest nutrient (N, Ca, and Mg) and geraniol accumulation 75:25—Highest accumulation of phenolic compounds, viz., p-coumaric acid, rosmarinic acid, caffeic acid, quercetin, gallic acid and rutin and medicinal compounds, viz., geraniol and geranyl acetate	[109]

7. Ameliorated Crop Resilience to Biotic and Abiotic Stresses by Ammonium Nutrition

Ammonium stress can have beneficial effects on crop resilience, to biotic and abiotic stresses, and generate high-quality products, although it may have a modest detrimental influence on productivity in agricultural contexts. The synthesis of most defense-related secondary metabolites depends on nitrogen metabolism, which also serves as a key step in the production of nitric oxide (NO), whose function in plant-pathogen interactions has been extensively investigated [110]. Hence, nitrogen metabolism closely relates to plant immunity. Although plants that have been fed nitrate have generally shown enhanced resistance to pathogenic attacks [111,112], few species, particularly tomatoes, have shown notable resistance to pathogens when fed with ammonium. Ammonium-mediated repression of the virulence of *Fusarium oxysporum* has been demonstrated in tomatoes, mainly by inhibiting the penetration of virulence components through cellophane [113]. Another study demonstrated that the mild toxicity generated by ammonium stress has led to the buildup of H₂O₂ in tomato plants, which served as a signal to activate systemic acquired acclimation (SAA), and thereby provided resistance to the infections caused by *Pseudomonas syringae* [114]. Similarly, ammonium-mediated alterations in carbon and nitrogen metabolisms have been reported to increase the resistance against *P. syringae* in tomatoes [115]. In rice, the overexpression of the ammonium transporter *AMT1;2* improved NUE and resistance to sheath blight disease caused by *Rhizoctonia solani*, further indicating that ammonium uptake and assimilation are necessary for this defense [116].

In furtherance, it has been recognized that an optimum nitrate-to-ammonium ratio can enhance protein synthesis, decrease the lipid peroxidation rate, and boost the plant's ability to withstand various abiotic stresses. Ammonium feeding may cause a multitude of reactions, many of which are defense responses that are typical of various abiotic stress conditions. The significance of ammonium in alleviating heavy metal toxicity has been proven through some of the studies. The positive effect of NH₄NO₃ in improving manganese tolerance has been reported in *Trifolium subterraneum* L. genotypes [117]. Ammonium nutrition has been found to increase the aluminum (Al) tolerance in soybean [118], rice [119], and *Lespedeza bicolor* [120]. The primary symptom of Al toxicity is the inhibition of root elongation. Ammonium nutrition, however, dramatically increased root length and reduced Al buildup in roots under Al toxicity. The major mechanism of Al tolerance in the above-mentioned species was the restricted Al absorption, as a result of competition between positively

charged Al^+ and NH_4^+ for binding sites in the apoplast of root cell walls. Ammonium has been shown to have a strong mitigating effect on cadmium (Cd) toxicity by increasing antioxidant activity and glutathione-ascorbate cycle efficiency in rice [121]. The higher Cd detoxification effect of NH_4^+ compared to NO_3^- has been demonstrated in the hyperaccumulator, *Solanum nigrum* L [122]. Ammonium-mediated Cd toxicity in *S. nigrum* has been affected through decreased absorption and accumulation of Cd via downregulation of Cd transport-related genes, increased immobilization of Cd in the cell wall of roots by binding to pectin and cell wall components, and through the upregulation of *expansin*, (*SnExp*), a gene responsible for metal tolerance. In contrast to the report on the impact of NH_4^+ in worsening iron deficiency symptoms of the model plant, *B. distachyon* [25], the alleviating effects of NH_4NO_3 and NO in iron deficiency has been documented in pears [123]. The study showed that NH_4NO_3 significantly reduced the chlorosis of leaves caused by iron deficiency, mostly through promoting the activity of ferric chelate reductase and nitrogen assimilation enzymes. Ammonium has been found to improve the salinity tolerance of plants such as citrus [124], *Sorghum bicolor* [125], maize, [126], and tomato [127]. Ammonium has ameliorated the polyethylene glycol (PEG)-induced water stress tolerance in rice through improved regulation of NH_4^+ uptake, assimilation, and aquaporin activity [128,129]. An appropriate ratio of $\text{NH}_4^+ : \text{NO}_3^-$ (10:90) has effectively mitigated the low light intensity stress in *Brassica pekinensis* by modulating the expression of ammonium assimilatory enzymes, such as GS and NR [105]. In cucumber, the greater flux rate of NH_4^+ into vascular bundles of the lateral vein, midrib, and shoot tip, due to the increased conversion of NO_3^- to NH_4^+ , has substantially aided in overcoming low-temperature stress, as an energy conservatory mechanism [130]. An equal ratio of ammonium and nitrate (50:50) was found to improve the biomass, photosynthetic efficiency, protein content, and decrease lipid peroxidation in baby lettuce under heat stress conditions [131].

8. Omics Investigations in Comprehending NUE

Numerous key regulatory genes, such as transporters, reductase, synthase, glutamate dehydrogenase, and aminotransferase are involved in the various processes of nitrogen metabolism, including the uptake, translocation, assimilation, and remobilization. Until recently several “omics” studies have focused primarily on rapidly altering gene expression and metabolites in plants under various sources of nitrogen. For instance, a comparative transcriptomic analysis between the cultivated and wild lettuce revealed the tolerance mechanisms exhibited by wild under nitrogen starvation conditions [132]. The study evidenced an enhanced expression of nitrate transporter genes (*NRT2.1*, *NRT2.4*, and *NRT2.5*) and glutamate synthase genes (*GLN1* and *GLN2*) in wild lettuce compared to the cultivated one, suggesting high-efficiency N uptake under low nitrogen conditions. Hence, the study gave insights into improving the NUE of cultivated lettuce by the introgression of novel alleles from the wild species in the future. In perennial ryegrass, transcriptome profiling under various nitrogen levels has identified four crucially enriched pathways, such as the photosynthesis-antenna protein, carotenoid biosynthesis, steroid biosynthesis, and C5-branched dibasic acid metabolism, particularly at excessive nitrogen stress conditions [133]. Transcriptomic analysis was used to examine the processes of ammonium toxicity and tolerance in an aquatic macrophyte *Myriophyllum aquaticum* under situations of high NH_4^+ stress [134]. The study evidenced the transcriptional upregulation of chlorophyll a/b binding proteins genes contributing to high NH_4^+ resistance in the leaves. Moreover, the upregulation of nitrogen metabolism genes, along with the upregulation of catalase genes aided in combating NH_4^+ stress, by means of counteracting the reactive oxygen species.

A comparative transcriptome analysis between high NUE and low NUE cultivars of *Brassica juncea* showed that under low nitrate treatments, the high NUE cultivar had an upregulation of genes involved in N absorption, remobilization, and assimilation [135]. Furthermore, the co-expression network analysis has identified a number of nitrate regulatory modules that may be involved in the control of nitrate reductase activity, phenylpropanoid biosynthesis, and carbon:nitrogen interaction in *B. juncea*. The activation of

phenylpropanoid and ROS scavenging pathways to counteract NH_4^+ toxicity has also been confirmed by the transcriptional responses of the common duckweed *Lemna minor* under ammonium toxicity [136]. In *Camelia sinensis*, *NRT*, *AMT*, *AQP*, *GS*, and *GOGAT* have been identified as key differentially expressed genes (DEGs) regulating ammonium uptake and assimilation [137]. Analysis of the transcriptome of *Salicornia neei*, a halophyte grown in saline water containing 3 mM ammonium has led to the precise identification of the ammonium detoxification mechanism, mainly through the upregulation of glutamine synthetase and glutamate synthase genes involved in ammonium homeostasis [138].

As N is necessary for the synthesis of a wide range of metabolites, such as amino acids, proteins, lipids, nucleic acids, and chlorophyll, then, measuring metabolite levels using metabolomic techniques can reveal fundamental details about how biological processes react to physiological or environmental changes brought on by N status [139]. A clear discrimination of metabolic profiles of tea leaves and roots in response to N deficiency was observed by untargeted metabolomics using a GC-TOF/MS method [140]. Surprisingly, N deprivation elevated the concentrations of major phenylpropanoids, organic acids, and amino acids in roots, while in leaves there was a significant downregulation of amino acids. Thus, the differential metabolite accumulation in tea leaves and roots corresponds to their quality and stress response, respectively, under N deficiency conditions. An integrative metabolomic response of multiple species in response to ammonium nutrition showed that the accumulation of the essential amino acids varied depending on the species; however, the synthesis of Gln and/or asparagine (Asn) served as a more precise biomarker of ammonium nutrition [141]. The high NUE in maize fed with a mixture of urea and ammonium has been demonstrated with an integrated transcriptomics and metabolomics study [142]. In addition to a rapid elevation of the transcripts of nitrogen assimilation genes, including *ZmGLN1;2*, *ZmGLN1;5*, *ZmAMT1.1a*, *ZmGOT1*, and *ZmGOT3*, the combination of urea and ammonium also caused a significant buildup of amino acids, including tyrosine (Tyr), arginine (Arg), phenylalanine (Phe), and methionine (Met), in shoots. Quantitative proteomics analysis was carried out to investigate the ammonium-mediated growth inhibition of tomato roots. Even though the proteins (K4BPV5 and K4D9J3) corresponding to ammonium assimilation were upregulated, there was limited assimilation of ammonium leading to root growth inhibition. The reason for the higher accumulation of ammonium leading to toxicity could be explained by the downregulation of a protein, Q5NE21 corresponding to carbonic anhydrase, a key player in C metabolism [143].

9. Strategies to Overcome Ammonium Toxicity

The vast understanding of ammonium toxicity through extensive studies has led to the development of several strategies for overcoming ammonium toxicity, both at the laboratory and agricultural field levels (Figure 2). In laboratory conditions, ammonium toxicity can be reduced by increasing CO_2 concentrations, light intensities, and exogenous organic or inorganic carbon supplies [22,144,145]. The regulation of the pH of the nutrient solution has also been suggested as an efficient way to manage the symptoms of ammonium toxicity [146]. The beneficial role of silicon (Si) in mitigating ammonium toxicity and other abiotic stresses has been extensively studied [147,148]. Silicon application has effectively reversed the adverse effects of ammonium toxicity and enhanced the growth parameters, photosynthesis, and NUE of many plant species (Table 2). The ability of potassium and nitrate to counteract the harmful effects of ammonium on plants suggests that the strategic use of mineral fertilizers, including K^+ and NO_3^- , in the field could maximize nitrogen utilization and increase the safety of agricultural plants. It is a recommended practice to fertilize soils with ammonium and nitrate, in the form of NH_4NO_3 to balance the effects of NH_4^+ as NO_3^- absorption helps in the alkalization of the rhizosphere. However, the development of genetically modified (GM) plants is the potential strategy for achieving stable ammonium tolerance. In such cases, it may be beneficial to overexpress the transporters or enzymes involved in the assimilation, detoxification, and compartmentation of ammonia, whereas silencing the genes responsible for

ammonia uptake and translocation may result in nitrogen deficiency or other detrimental effects as it can block crucial metabolic pathways [149]. The role of hormones, such as ABA that are involved in AMOS1/EGY1-dependent retrograde signaling of ammonium stress and auxins at elevated concentrations have been reported to alleviate ammonium toxicity [26,50]. Table 2: alleviation of ammonium toxicity using silicon supplementation in various plant species.

Table 2. Alleviation of ammonium toxicity using silicon supplementation in various plant species.

PLANT SPECIES	Mode of Si Application in the Cultivation System	Form and Concentration of Si Used	Mitigating Effects of Si under Ammonium Toxicity	Reference
<i>Zea mays</i> L.	In the nutrient solution of the hydroponics system	10 mmol L ⁻¹ K ₂ SiO ₃	Increased N accumulation, shoot, and root dry mass production	[150]
<i>Lycopersicon esculentum</i> Mill.	In the nutrient solution of the hydroponics system	1 mmol L ⁻¹ monosilicic acid	Improved N and Si use efficiency, increased leaf area, root area, dry biomass, total chlorophyll, carotenoids, and reduced oxidative stress	[22]
<i>Cucumis sativus</i> L. var. Hokushin and Tsubasa	In the nutrient solution of the hydroponics system	0, 1, and 10 mmol L ⁻¹ K ₂ SiO ₃	Greater dry mass, N accumulation, green color index (GCI), and increased nitrate reductase activity	[151]
<i>Brassica oleracea</i> var. Botrytis cv Barcelona and <i>Brassica oleracea</i> var. Italica cv BRO 68	In the nutrient solution of the hydroponics system	2 mmol L ⁻¹ silicon using stabilized sodium and potassium silicate (114.9 g L ⁻¹ Si and 18.9 g L ⁻¹ K ₂ O).	Increased growth characteristics, physical integrity, total chlorophyll content, stomatal conductance, water use efficiency, accumulation of ions (N, K, Ca, Mg, and Si), and reduced electrolyte leakage	[152]
<i>Passiflora edulis</i> Sims f. flavicarpa O. Deg.	In the nutrient solution of the vermiculture cultivation	2 mmol L ⁻¹ K ₂ SiO ₃	Greater stem and root diameter, root length, dry matter, leaf green color index (GCI), increased accumulation of ions (N, Si, K, Ca, and Mg), improved NUE, and reduced electrolyte leakage	[153]
<i>Beta vulgaris</i> L.	In the nutrient solution of the vermiculture cultivation in growth chamber	28.5 g L ⁻¹ H ₄ SiO ₄	Improved photosynthesis, stomatal conductance, transpiration, dry mass production, and accumulation of N and Si in roots	[154]
<i>Raphanus sativus</i> L.	In the nutrient solution of the hydroponics system	2 mmol L ⁻¹ K ₂ SiO ₃	Improved photosynthesis, transpiration, stomatal conductance, water-use efficiency, and higher total dry biomass	[155]
<i>Eucalyptus urophylla</i> x <i>Eucalyptus grandis</i>	Fertigation of Si in vermiculture cultivation	2 mmol L ⁻¹ K ₂ SiO ₃	Increased dry mass production and improved the photosynthetic apparatus by decreasing fluorescence, and improving the quantum efficiency of photosystem II	[156]
<i>Brassica campestris</i> L. ssp. pekinensis	In the nutrient solution of the hydroponics system	0.0 and 1.0 me·L ⁻¹ K ₂ SiO ₃	Improved growth parameters, photosynthesis, stomatal conductance, cation accumulation (Ca, Mg, K, and Si) antioxidant enzyme activities, and N assimilation enzyme activities	[157]
<i>Salvia splendens</i> 'Vista Red'	In the nutrient solution of the hydroponics substrate system	0.0 and 1.0 me·L ⁻¹ K ₂ SiO ₃	Improved growth parameters, photosynthesis, stomatal conductance, cation accumulation (Ca, Mg, K, and Si) antioxidant enzyme activities, and N assimilation enzyme activities as well as reduced accumulation of ROS, and malondialdehyde (MDA)	[158]
<i>Brassica lee</i> ssp. namai 'Samchoo' 'Chunssamhwang51'	In the nutrient solution of the hydroponics system	0.0 and 10.7 me·L ⁻¹ K ₂ SiO ₃	Increased shoot fresh weight, chlorophyll content, leaf area, vitamin C, and antioxidant enzyme activities	[108]

10. Conclusions and Future Perspectives

Ammonium assimilation into plant metabolites requires limited energy than nitrate assimilation, as it is already in reduced form. Thus, ammonium nutrition can be considered an energy-saving option that can be readily utilized by plants. However, the toxic effect of ammonium demands the optimization of its use to minimize its detrimental effects. Utilizing high-efficiency N fertilizers that contain both NH₄⁺ and NO₃⁻ can significantly aid in managing ammonium toxicity issues and increase plant NUE in agricultural fields. To achieve high plant productivity at the laboratory level, different concentrations, and combinations of NH₄⁺ and NO₃⁻ must be investigated, because the ideal ratio of NH₄⁺ to NO₃⁻ probably varies for different species. Future research is highly needed to confirm the positive effects of ammonium in increasing plant quality, productivity, and resilience to biotic and abiotic stressors and their mechanisms behind. Moreover, the combined use of

physiological and multi-omics studies will allow the exploration of the molecular intricacies of ammonium toxicity and tolerance in the future.

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