



Article

Decreased Solution pH and Increased K⁺ Uptake Are Related to Ammonium Tolerance in Hydroponically Cultured Plants

Jinnan Song ¹ , Jingli Yang ¹ and Byoung Ryong Jeong ^{1,2,3,*}

¹ Department of Horticulture, Division of Applied Life Science (BK21 Four Program), Graduate School, Gyeongsang National University, Jinju 52828, Korea; jinnansong93@gmail.com (J.S.); yangmiaomiaode@gmail.com (J.Y.)

² Institute of Agriculture and Life Science, Gyeongsang National University, Jinju 52828, Korea

³ Research Institute of Life Science, Gyeongsang National University, Jinju 52828, Korea

* Correspondence: brjeong@gnu.ac.kr; Tel.: +82-55-772-1913

Abstract: The ammonium (NH₄⁺) tolerance of plants is an important issue in agriculture, associated with several plant characteristics. So far, plant tissue acidification has been shown as the primary cause of NH₄⁺ toxicity. Suppressed plant growth caused by excess NH₄⁺ can be counteracted by potassium (K⁺) application. However, the effects of NH₄⁺ tolerances on the differences regarding pH changes together with K⁺ uptake remain to be determined. Here, we performed an 84 h hydroponic cultivation of five species with different NH₄⁺ tolerances, subjected to three NH₄⁺:NO₃⁻ solutions (0:100, 50:50, or 100:0), to investigate the pH changes and ion uptakes. Consequently, the solution pH was lowered over time to varying extents in the presence of NH₄⁺. The NH₄⁺-tolerant ageratum and lettuce, shown to be tolerant to NH₄⁺ in this trial, rapidly lowered the solution pH, whereas extremely NH₄⁺-sensitive salvia and cabbage only gave a minor decrease in the solution pH when grown with 100:0 NH₄⁺:NO₃⁻. Additionally, the increased external NH₄⁺ level led to a substantial decline in the net cation influxes (K⁺, Ca²⁺, and Mg²⁺). As compared to solely NH₄⁺-fed salvia and cabbage, solely NH₄⁺-fed ageratum and lettuce ultimately showed a relatively greater net K⁺ influx. Taken together, this study discusses how the decreases in pH and K⁺ are related to NH₄⁺ tolerance in five hydroponically cultured species.

Keywords: hydroponics; acidification; calcium; magnesium; electrical conductivity (EC)



Citation: Song, J.; Yang, J.; Jeong, B.R. Decreased Solution pH and Increased K⁺ Uptake Are Related to Ammonium Tolerance in Hydroponically Cultured Plants. *Horticulturae* **2022**, *8*, 228. <https://doi.org/10.3390/horticulturae8030228>

Academic Editors: Tomo Milošević and Nebojša Milošević

Received: 7 February 2022

Accepted: 4 March 2022

Published: 7 March 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

As two predominant inorganic nitrogen (N) forms for higher plants absorption and assimilation, ammonium (NH₄⁺) and nitrate (NO₃⁻) not only show varying preferences by plants, but also affect the plant growth and physiology in a distinct manner [1–3]. Theoretically, the use of NH₄⁺ appeared to be superior to that of NO₃⁻, because NH₄⁺ was more metabolically efficient than NO₃⁻, requiring relatively less energy for assimilation [4,5]. Paradoxically, only a small number of plant species exhibited better growth when supplied with NH₄⁺ as the sole N source than when supplied with NO₃⁻ or a mixture of NH₄⁺ and NO₃⁻. Indeed, plants cultured exclusively with millimolar NH₄⁺ often developed ammonium toxicity symptoms, as characterized by stunted plant growth accompanied by leaf chlorosis and necrosis [6,7].

Several classical and current hypotheses have been advanced to elucidate the major NH₄⁺ toxicity causes: carbohydrate limitation for efficient detoxification; futile transmembrane NH₄⁺ cycling; disorders of photosynthesis and electron transport homeostasis; extracellular acidification shifting to an intolerable level; and disruption of the plant ion balance [8–12].

Fortunately, one consensus on the NH₄⁺ toxicity cause has been built recently: Hachiya and colleagues proposed that an elevation of the shoot acidity primarily caused NH₄⁺ toxicity in plants [13]. It has been well-established that the NH₄⁺ uptake is a proton-release

process, resulting in external acidification when NH_4^+ is present in excess [14]. In addition, the assimilation of NH_4^+ was identified to be a proton-generating process. A great deal of hydrogen (H^+) was secreted due to the NH_4^+ nutrition in the rhizosphere. As a consequence, the cell metabolism was interfered with, and the pH in the root environment decreased. In this regard, the root environment acidification and intracellular pH disturbance was accordingly related to the NH_4^+ toxicity symptoms. It was often observed that thrive plants grown under extremely acidic conditions where the N form was usually dominated by the existence of high NH_4^+ levels, such as lowland rice [15]. It seems that plants capable of high resistances or adaptations to NH_4^+ nutrition have low rhizosphere pH [16].

Furthermore, the reduction in the level of certain cations (i.e., K^+ in particular, Ca^{2+} and Mg^{2+}) inside plant tissues was recognized as a result of excessive NH_4^+ nutrition and therefore NH_4^+ toxicity [12,17]. The uptake of NH_4^+ was regarded to be coupled to the H^+ efflux and subsequently stimulated the K^+ efflux (in an antagonistic way) [18]. Therefore, K^+ channels should be a prime candidate for transporting considerable amounts of low-affinity NH_4^+ , thereby suppressing the NH_4^+ flux. On the other hand, the vital correlation between K^+ and NH_4^+ has shown that a high external K^+ level was able to deliver a protection strategy for plants against NH_4^+ toxicity [19,20]. For instance, an elevated supply of K^+ was found to have a pivotal role in attenuating the NH_4^+ toxicity by reducing the NH_4^+ influx [21]; the NH_4^+ entrance was eventually inhibited when imposed with a progressively increasing K^+ ranging from 0.02 mM to 40 mM in solely NH_4^+ -fed rice (NH_4^+ -tolerant plant) [22]. However, there is little research to date on how pH or K^+ metabolism is affected by NH_4^+ nutrition in hydroponically cultured plants.

Many studies have suggested that NH_4^+ tolerance is species-specific and most likely conferred by key NH_4^+ assimilation enzymes, such as GS and NADH-GDH [6,23–25], while largely neglecting the aforementioned effects from the pH and K^+ supply. Even though pH and K^+ were outlined to be related to NH_4^+ toxicity, the agreement towards the relationship between the root pH, K^+ transport, and plant NH_4^+ tolerance has yet to be established. Specifically, controversy remains regarding rhizospheric pH changes and K^+ metabolism in response to a prolonged, excessive NH_4^+ supply.

Accordingly, the experiment undertaken herein was designed, condensed, and conducted to investigate root pH changes and the kinetics of K^+ uptake by means of monitoring the hydroponic solutions. Five plant species underpinning contrasting NH_4^+ tolerances were hydroponically cultured in order to circumvent the interference of the growing media, since considerable amounts of Fe^{2+} were readily yielded from the inert medium [26]. The present study aims to evaluate the influence of different NH_4^+ tolerances of plant species grown with different forms of nitrogen (N) on hydroponic solution pH and ion uptake, while seeking a practical tool for selection of NH_4^+ -sensitive or -tolerant species.

2. Materials and Methods

2.1. Plant Materials and Environmental Conditions

Seeds of three bedding plants (*Salvia splendens* 'Vista Red', *Petunia hybrid* 'Madness Red', and *Ageratum houstonianum* 'Aloha Blue') and two leafy vegetables (Korean cabbage 'Ssamchu' and lettuce 'Caesar Green'), were ordered from PAN American Seeds Company (West Chicago, IL, USA), germinated in 200-cell plug trays containing the BVB medium (EN-12580, The Netherlands), and moistened with running tap water. The experiment was carried out in a glasshouse at Gyeongsang National University (35°90' N, 128°06' E, Jinju, Gyeongnam, Korea) from April to May and September to October of 2021. The temperature ranged from 25 ± 2 °C (light for 13 h) to 17 ± 2 °C (darkness for 11 h).

2.2. Hydroponic Cultivation Experiment

2.2.1. Preparation of the Plants

Subsequently, seedlings were placed on a metal bench and irrigated with a multipurpose nutrient solution (MNS) for 20 days, which contained macronutrients ($6.0 \text{ me}\cdot\text{L}^{-1} \text{ Ca}^{2+}$, $2.0 \text{ me}\cdot\text{L}^{-1} \text{ Mg}^{2+}$, $5.0 \text{ me}\cdot\text{L}^{-1} \text{ K}^+$, $2.0 \text{ me}\cdot\text{L}^{-1} \text{ NH}_4^+$, $11.0 \text{ me}\cdot\text{L}^{-1} \text{ NO}_3^-$, $2.0 \text{ me}\cdot\text{L}^{-1} \text{ SO}_4^{2-}$,

and $2.0 \text{ me}\cdot\text{L}^{-1} \text{ H}_2\text{PO}_4^-$) and micronutrients ($20 \mu\text{M B}$, $0.5 \mu\text{M Cu}$, $10 \mu\text{M Fe}$, $10 \mu\text{M Mn}$, $0.5 \mu\text{M Mo}$, and $4 \mu\text{M Zn}$) [24]. Juvenile plants with uniform growth and status were screened and taken out of the trays; the growth media on the plant roots were carefully washed off using distilled water and surface-blotting with absorbent paper.

2.2.2. Preparation of the Hydroponic System

Afterwards, three plants with massive root systems were anchored through the individual side holes in 450 mL hydroponic solutions using tissue culture vessels (PhytoCon™, Phyto Technology Laboratories, Lenexa, KS, USA). Three side-punched holes and one center-punched hole were designed for holding plants erect and providing air for the nutrient solutions, respectively (Figure 1). The air generated from a temporary immersion system (TIS) [27] used a pump (RITA type, Plantima, A-Tech Bioscientific Co., Taiwan). All the containers and lids were wrapped with cooking tin foil to avoid sunlight penetration.

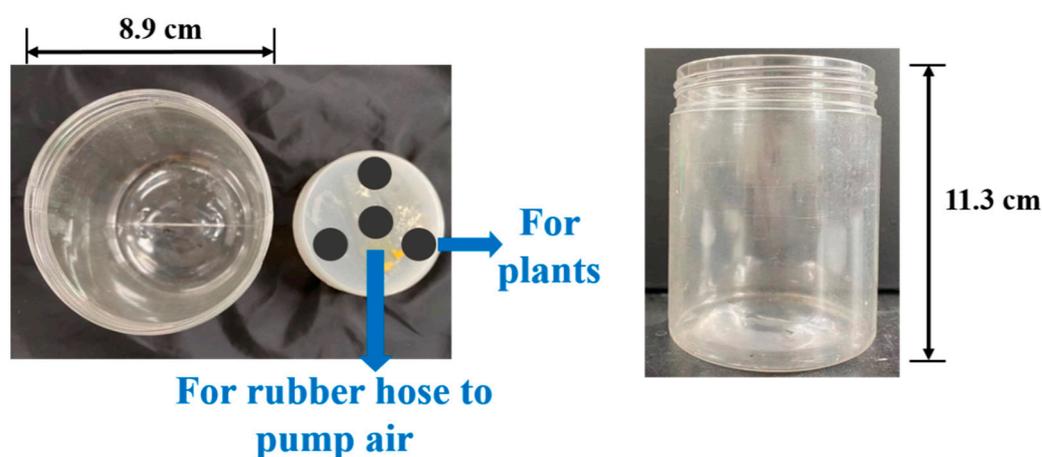


Figure 1. The container and lid retrofitted for the hydroponic system. Side holes were punched for the plants; the central hole was punched for a rubber hose linked to the air pump.

2.3. Treatment Solutions and Layout of the Plants

The multipurpose nutrient solution (MNS) was modified in order to supply a constant concentration of N ($13 \text{ me}\cdot\text{L}^{-1}$) at different $\text{NH}_4^+:\text{NO}_3^-$ ratios (0:100, 50:50, or 100:0). The details are shown in Table 1. Modifications as affected by $\text{NH}_4^+:\text{NO}_3^-$ ratio were made by balancing with the Cl^- level in order to keep an equal concentration ($\text{me}\cdot\text{L}^{-1}$) of cations and anions. However, three hydroponic solutions contained identical levels of other micronutrients, as described above. All the solutions' pH levels were initially adjusted to 6.0 with analytical grade 0.1 M HCl. The treatment solutions were not supplemented after the start of the experiment.

Table 1. Macronutrient composition ($\text{me}\cdot\text{L}^{-1}$) along with a constant concentration ($13.0 \text{ me}\cdot\text{L}^{-1}$) of N, but three different ammonium to nitrate ratios, used as the treatment solutions.

Nutrient Source	Ammonium to Nitrate Ratios in the Nutrient Solutions		
	0:100	50:50	100:0
$\text{Ca}(\text{NO}_3)_2\cdot 4\text{H}_2\text{O}$	6.9	5.9	-
KNO_3	4.8	-	-
$\text{Mg}(\text{NO}_3)_2\cdot 6\text{H}_2\text{O}$	1.3	0.6	-
$\text{MgSO}_4\cdot 7\text{H}_2\text{O}$	1.0	1.4	1.7
KH_2PO_4	1.0	-	2.0
$\text{NH}_4\text{H}_2\text{PO}_4$	-	2.0	-
$(\text{NH}_4)_2\text{SO}_4$	-	4.5	13.0
K_2SO_4	-	4.5	1.2
$\text{CaCl}_2\cdot 6\text{H}_2\text{O}$	-	-	4.9

Each biological replicate, randomly arranged with three $\text{NH}_4^+:\text{NO}_3^-$ ratios, was laid out as shown in Figure 2, and consisted of one pump for five species and one control (without plants). In total, 3 air pumps, 54 containers, and 27 plants per species were undertaken for 3 replicates in the experiment.

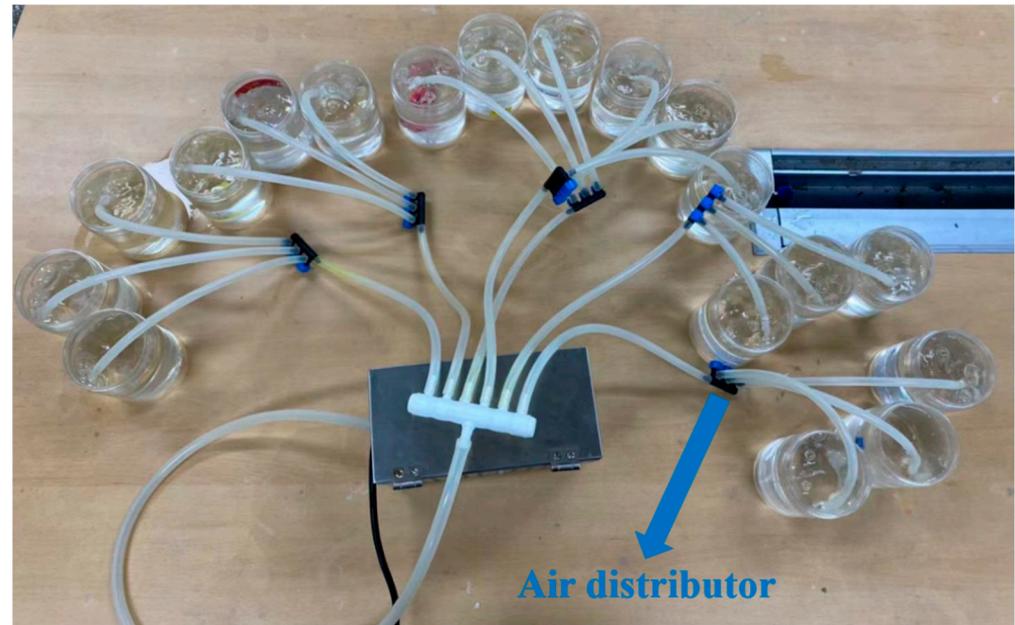


Figure 2. Distribution of the containers and species in the hydroponic system for one replicate. The air pump equally provides O_2 for the nutrient solutions via the air distributor.

2.4. Monitoring of the pH and EC Variations in the Hydroponic Solutions

Cultivations for a period of 84 h were performed for the plants with an uninterrupted air supply. The pH and EC values of the hydroponic solutions were individually monitored at 0, 12, 36, 60, and 84 h after treatment initiation with a portable microprocessor benchtop pH meter (Lab-SP-2300, Suntex Instruments Co., Ltd., Taiwan, China). To minimize the light influences on the plants during the research, this experiment started at 9:00 am, and the subsequent measurements were implemented after sunset.

2.5. Determinations of Potassium (K), Calcium (Ca), and Magnesium (Mg) Concentrations in Hydroponic Solutions

At the end of each experiment, the hydroponic solutions from 0 h and 84 h specimens were individually collected and passed through the filter paper to remove the residues, to obtain clear, impurity-free samples for the assay. Finally, the ion content was quantified three times and analyzed using an inductively coupled plasma (ICP) spectrometer (Optima 4300DV/5300DV, Perkin Elmer, Rodgau, Germany). The control ($t = 0$ h) was employed as the baseline for the analysis of the ion uptake. Changes in the ion content were expressed as a reduction after 84 h, compared to the control.

2.6. Ammonium Tolerances of *Salvia*, *Petunia*, *Ageratum*, *Cabbage* and *Lettuce*

The ammonium tolerances of the five selected plants species used in this study were determined on the basis of their responses, such as physiology and morphology, photosynthetic capacity, N-C distribution, and activities of major N assimilation enzymes, to high NH_4^+ concentration supply according to our pioneer research [6,24]. Korean cabbage ‘Ssamchu’ and salvia were extremely sensitive to high concentrations of NH_4^+ ; petunia was moderately sensitive to NH_4^+ ; ageratum was NH_4^+ -tolerant or NO_3^- -sensitive; and lettuce was also highly NH_4^+ -tolerant.

2.7. Statistical Analysis and Graphing

The statistical analysis was carried out with the SAS 8.2 statistical software (SAS Inst., Cary, NC, USA). All represented data are the mean \pm SD of at least three biological replicates. Significant differences among multiple groups were tested according to one-way ANOVA (Duncan's multiple range test) with a probability (p) of less than 0.05. GraphPad Prism 8.0 was employed for graphing.

3. Results

3.1. Major pH Changes of the Hydroponic Solutions during the 84 h Cultivation

When the comparisons were conducted on identical species, the pH of the hydroponic solutions were remarkably affected by the $\text{NH}_4^+:\text{NO}_3^-$ ratios, regardless of the species (Figure 3). In a whole, the pH of hydroponic solutions supplied with 50:50 $\text{NH}_4^+:\text{NO}_3^-$ and 100:0 $\text{NH}_4^+:\text{NO}_3^-$ substantially declined, whereas the pH of hydroponic solutions supplied with 0:100 $\text{NH}_4^+:\text{NO}_3^-$ were detected to show varying degrees of increase. The control group solutions maintained a 6.0 pH as initially set throughout the 84 h experimental period (Figure 3A). Notably, the significant pH changes caused by the different $\text{NH}_4^+:\text{NO}_3^-$ ratios were exhibited from 12 h after treatments were initiated (Figure 3B,D,F).

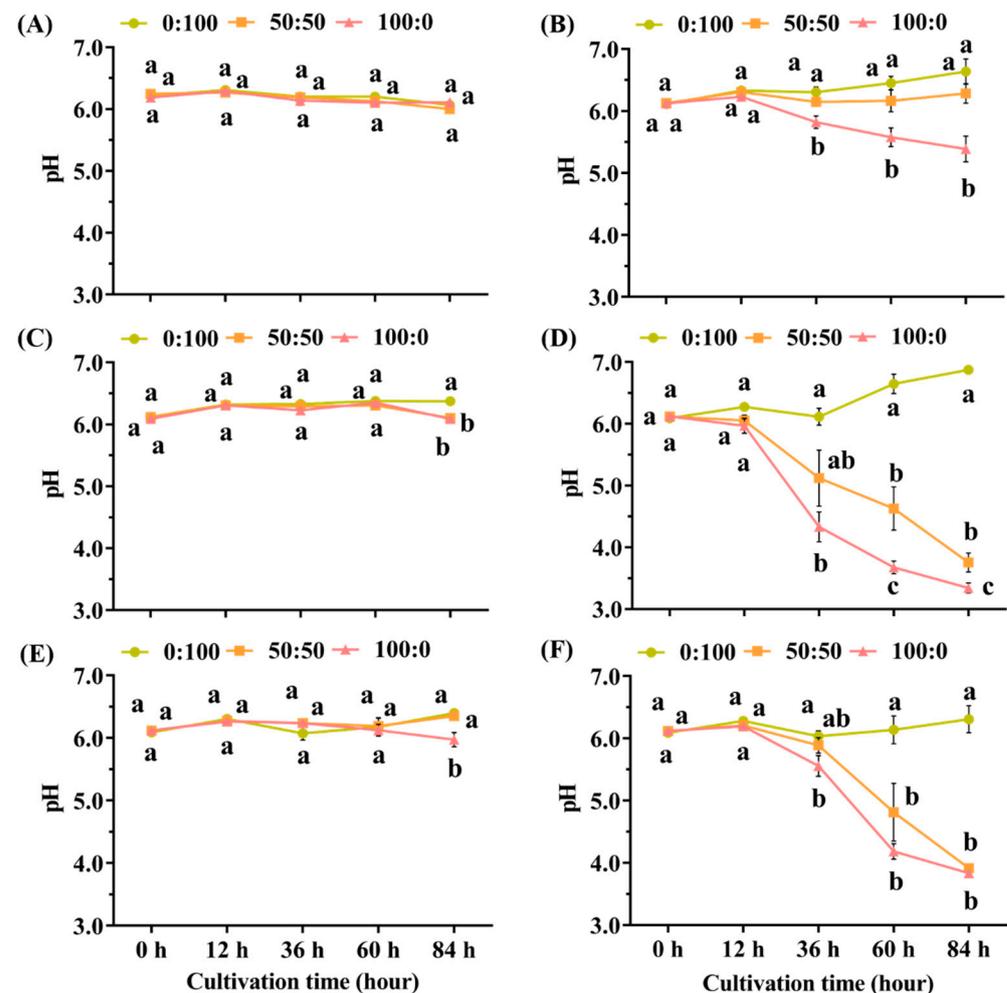


Figure 3. Time course changes in the pH of hydroponic solutions supplied with three $\text{NH}_4^+:\text{NO}_3^-$ ratios, as affected by 5 species during an 84 h cultivation. (A) Control without plants; (B) salvia; (C) petunia; (D) ageratum; (E) cabbage; and (F) lettuce. Data are expressed as means \pm SD of $n = 3$ biological replicates. p less than 0.05 was considered significantly different, indicated by different lower case letters according to the one-way ANOVA (Duncan's multiple range test).

More importantly, the pH of solutions 50:50 $\text{NH}_4^+:\text{NO}_3^-$ and 100:0 $\text{NH}_4^+:\text{NO}_3^-$ were dramatically altered among species during the cultivation. Ageratum and lettuce grown with 50:50 $\text{NH}_4^+:\text{NO}_3^-$ sharply reduced the solution pH to 3.76 and 3.92, respectively, after 84 h cultivations, whereas salvia changed the solution pH to 6.29 after 84 h (Figure 3B,D,F). Likewise, in the absence of NO_3^- , the pH of hydroponic solutions supplied for ageratum and lettuce declined to as low as 3.34 and 3.83, respectively, whereas salvia treated with sole NH_4^+ nutrition decreased in solution pH to only 5.39 (Figure 3B,D,F). The solution pH changes were similar according to the species grown with 0:100 $\text{NH}_4^+:\text{NO}_3^-$. Moreover, it is noteworthy that ageratum displayed the biggest pH increase of 14.7% among all the species examined (Figure 3D).

Interestingly, the pH of solutions used to grow petunia exhibited minor changes in the pH, irrespective of the $\text{NH}_4^+:\text{NO}_3^-$ ratios (Figure 3C). In these, a highly conserved trend was observed from the baseline (Figure 3A) because of the relatively small root systems of petunia.

3.2. Ion Concentrations in the Hydroponic Solutions as Affected by the $\text{NH}_4^+:\text{NO}_3^-$ Ratios and Plant Species

To figure out the kinetics of major ion (K^+ , Ca^{2+} , Mg^{2+}) uptakes after 84 h cultivations, the ion concentrations in the hydroponic solutions were individually estimated and analyzed. As presented in Figure 4, on the basis of the control, ion uptake amounts were negatively correlated with the NH_4^+ level, regardless of the species. Specifically, as the external NH_4^+ concentrations increased from 0% to 50%, the greatest decrease in the K^+ uptake was observed in lettuce, with a 78.73% reduction, followed by petunia (71.78% reduction); on the other hand, the NH_4^+ -tolerant species ageratum showed the smallest decline (35.87%) (Figure 4C,D,F).

As the external NH_4^+ level was raised from 50% to 100%, salvia and cabbage plants treated with 100% NH_4^+ even excreted certain K^+ to the hydroponic solutions, resulting in a net efflux of K^+ from plant cells; by contrast, solely NH_4^+ -cultured ageratum absorbed 18-fold and 16.7-fold higher K^+ than salvia and cabbage grown with same solution, respectively (Figure 4B,D,E). Unlike the sharp reductions in the K^+ uptake observed in salvia and cabbage, moderately NH_4^+ -sensitive species petunia exhibited a gradual decline in the K^+ uptake in response to the 100% NH_4^+ treatment, which was in parallel with such trend shaped by ageratum and lettuce (Figure 4B,E).

Concomitantly, the changes in the Ca^{2+} and Mg^{2+} concentrations in hydroponic solutions were investigated and compared. Significant differences in the Ca^{2+} and Mg^{2+} concentrations were also observed in response to the different $\text{NH}_4^+:\text{NO}_3^-$ ratios (Figures S1 and S2), as well as presenting the same regulatory patterns as that of K^+ as described above. However, with the exception of Ca^{2+} changes in petunia, neither the Ca^{2+} nor Mg^{2+} showed a net influx when supplied with 100% NH_4^+ (Figures S1 and S2). Furthermore, approximately equal amounts, but the highest efflux of Ca^{2+} and Mg^{2+} were observed in the 100:0 $\text{NH}_4^+:\text{NO}_3^-$ hydroponic solutions supplied for NH_4^+ -tolerant ageratum and lettuce (Figures S1D,F and S2D,F).

Apparently, the NH_4^+ fluxes were strongly regulated by the K^+ , Ca^{2+} , and Mg^{2+} concentrations. Consistently, an addition of NH_4^+ to sole NO_3^- solutions rapidly decreased the cation concentrations in the hydroponic solutions. This decrease trend of cation levels differed according to the tested species.

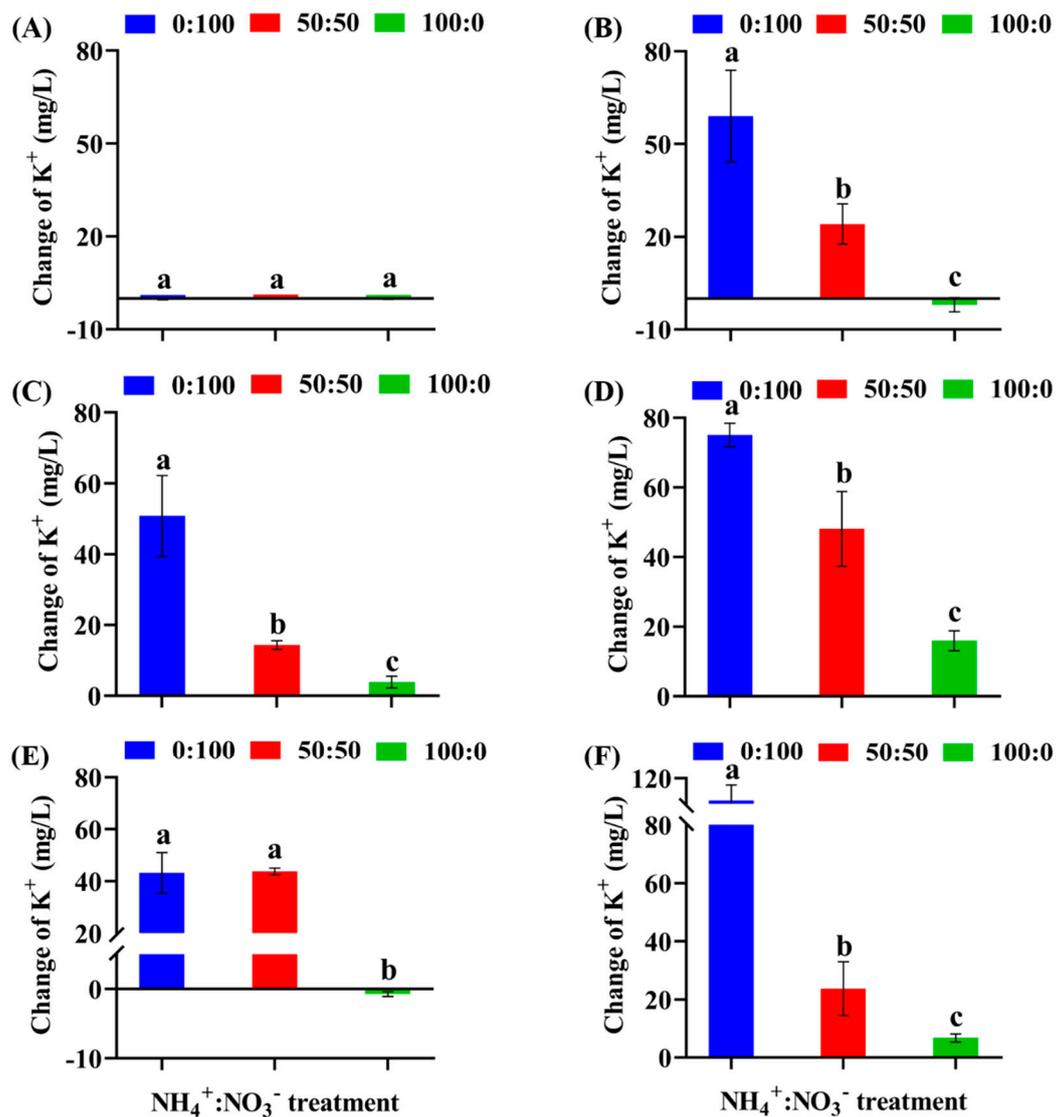


Figure 4. The K⁺ content changes in three NH₄⁺:NO₃⁻ hydroponic solutions supplied for (A) the control (without plants); (B) salvia; (C) petunia; (D) ageratum; (E) cabbage; and (F) lettuce. Data are means ± SD representing $n = 3$ biological replicates. Columns accompanied by different lower-case letters were regarded as significantly different, according to the one-way ANOVA following the Duncan's multiple range test at $p = 0.05$.

3.3. Minor EC Changes of the Hydroponic Solutions during the 84 h Cultivation

The collections of EC were concomitantly performed with the pH monitoring. Despite substantial differences in each solution at the initial time point ($t = 0$ h), in comparison with the control, only minor variations in the EC of solutions were observed regardless of the species or treatment (Figure 5). Overall, apart from the ageratum treated with sole NO₃⁻, the EC of the hydroponic solutions progressively increased over certain cultivation time ranges.

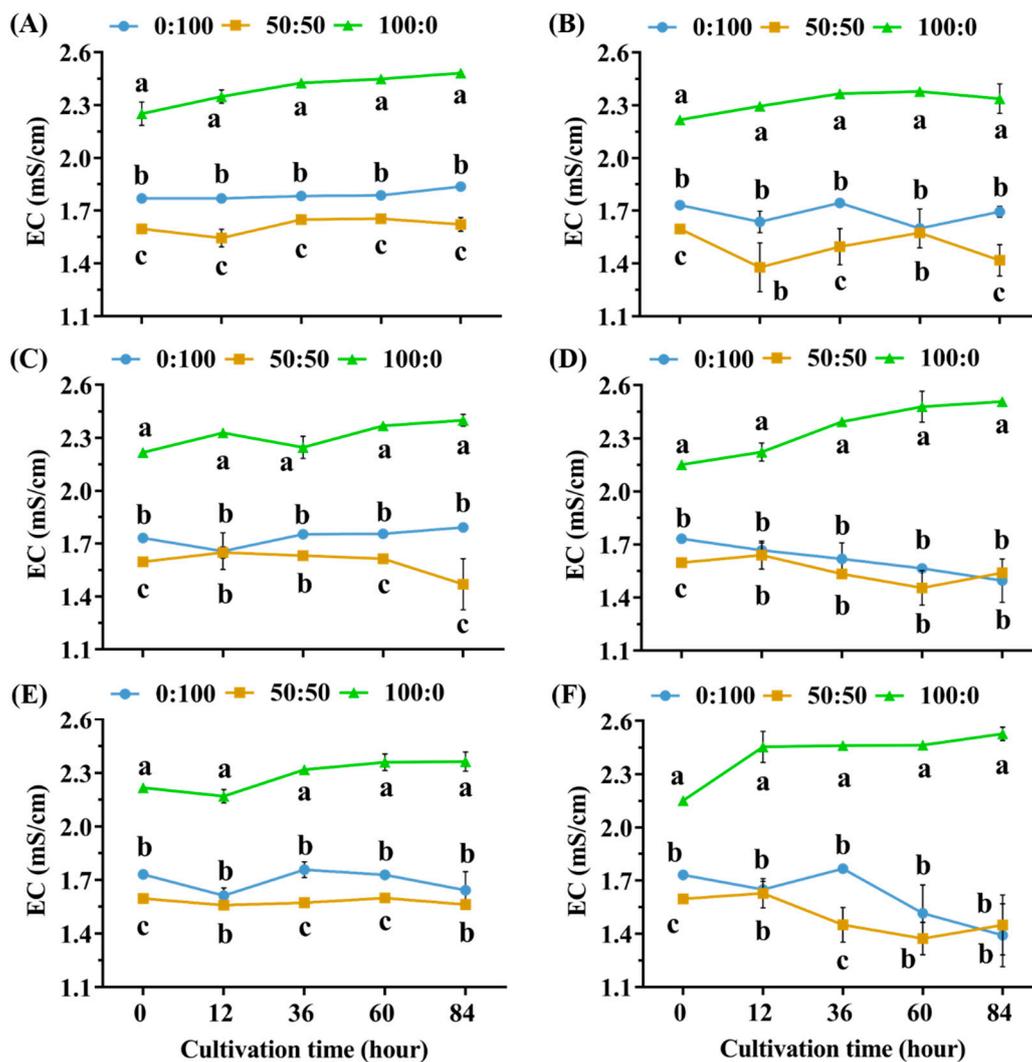


Figure 5. Kinetic variations of the EC of the three $\text{NH}_4^+:\text{NO}_3^-$ hydroponic solutions over an 84 h period cultivation on (A) the control without plants; (B) salvia; (C) petunia; (D) ageratum; (E) cabbage; and (F) lettuce. Data are means \pm SD of $n = 3$ biological replicates. p less than 0.05 was considered significantly different, indicated by different lower-case letters according to the one-way ANOVA (Duncan's multiple range test).

Additionally, NH_4^+ -tolerant ageratum and lettuce gradually reinforced the 100:0 $\text{NH}_4^+:\text{NO}_3^-$ solution EC from 2.15 to 2.51 $\text{mS}\cdot\text{cm}^{-1}$ and 2.15 to 2.53 $\text{mS}\cdot\text{cm}^{-1}$, respectively, whereas slight changes from 2.22 to 2.34 $\text{mS}\cdot\text{cm}^{-1}$ and 2.22 to 2.36 $\text{mS}\cdot\text{cm}^{-1}$ were found in extremely NH_4^+ -sensitive salvia and cabbage, respectively (Figure 5B,D–F). In the control, the EC of the 100:0 $\text{NH}_4^+:\text{NO}_3^-$ solution was observed to slightly increase (2.25–2.48 $\text{mS}\cdot\text{cm}^{-1}$) (Figure 5A).

4. Discussion

Ammonium tolerance is not a new subject and is directly related to the ammonium toxicity developed by plants. Few studies have elucidated which traits were mirrored towards NH_4^+ tolerance, even though extracellular acidification and cation inhibition were targeted to be the major factors leading to the NH_4^+ toxicity in plants [8,11,28]. This study accordingly investigated the relationships between the plant NH_4^+ tolerances and pH changes at the rhizosphere and uptake of certain cations. It is noteworthy that a closed hydroponic cultivation approach was employed in this experiment with a high feasibility for on-site measurement of the real-time pH [29]. Consequently, in our trials, distinct

differences were shaped regarding pH change and K^+ metabolism of hydroponic solutions over the cultivation time by the selected five plant species with different levels of NH_4^+ tolerance. Nevertheless, the net uptake of Ca^{2+} and Mg^{2+} by the test species failed to show a consistent tendency as K^+ metabolism with respect to the NH_4^+ tolerance.

Since chloride (Cl) was introduced in this study for balancing cation and anion (Table 1), it is worth noting the roles of chloride (Cl) in plant growth and development, as well as the impact of it on the results obtained in this study. The evidence that it functions as an essential nutrient in higher plants has been documented [30,31]. In particular, there is a widespread belief that the uptake of NO_3^- can be antagonized by increasing level of Cl, whereas the latter likely acts as a major companion ion for NH_4^+ uptake [31]. Furthermore, it has been well-established that Cl assists NH_4^+ uptake as a detoxification strategy, conferring a necessary role in the determination of NH_4^+ tolerance [32]. We therefore added more Cl in the 100:0 $NH_4^+ : NO_3^-$ solution because the manipulation capacity of Cl combined with NH_4^+ by plants was regarded as a vital indicator of NH_4^+ tolerance according to Jeong and Lee [32,33]. Additionally, the minor amount of additional Cl in form of HCl to 0:100 and 50:50 $NH_4^+ : NO_3^-$ solutions for the pH adjustments could be negligible for the acquired results. Equally importantly, Jeong and Lee [33] proposed that solution Cl level did not significantly influence the solution pH throughout the cultivation period. We therefore disregarded the effect of Cl on solution pH and target ions, although Cl level was initially different in three test solutions (Table 1).

4.1. Declined pH of Hydroponic Solutions May Be Attributed to H^+ Extrusion from Roots

The declined pH of hydroponic solutions over the cultivation period when supplied with 50:50 $NH_4^+ : NO_3^-$ and 100:0 $NH_4^+ : NO_3^-$ stemmed from the fact that NH_4^+ uptake generated protons, whereas the pH of solutions supplied with 0:100 $NH_4^+ : NO_3^-$ slightly increased because the NO_3^- assimilation consumed protons (Figure 3) [34]. Concomitantly, we noticed varying degrees of pH decrease in solutions supplied with 100:0 $NH_4^+ : NO_3^-$ among species in response to an 84 h cultivation. The results well agreed with the previous findings performed on salvia and ageratum [33]. Specifically, the most likely explanation is that extremely NH_4^+ -sensitive species (salvia and cabbage) failed to secrete excessive hydrogen ions (H^+) from the root cells, or did so to a limited extent, resulting in a slight decrease in the solution pH (Figure 3B,E). In contrast, NH_4^+ -tolerant species (ageratum and lettuce) possessed a greater capacity in the hydrogen ion (H^+) extrusion, and thereby, the pH of ageratum and lettuce solutions was rapidly decreased to a very low level for a better maintenance of the H^+ homeostasis (Figure 3D,F). It is worth noting that lettuce showed a similar NH_4^+ tolerance with ageratum in the current study, even though, theoretically, it possessed a similar NH_4^+ tolerance as petunia [6,24]. Probable reasons for that could be as follows: lettuce had a relative bigger root system, which could detoxify high levels of NH_4^+ ; and this experiment was conducted in a short period of time (84 h), and NH_4^+ toxicity symptoms were not yet obvious in lettuce. Therefore, at least in this trail, lettuce was defined as an NH_4^+ -tolerant species as ageratum, and this was confirmed by the variation in its solution pH. Lettuce displayed a rapidly decreased pH curve over time, which was very similar to that of ageratum (Figure 3D,F).

Additionally, pioneering studies on the NH_4^+ / H^+ exchange mechanism implied that the uptake of NH_4^+ could be limited by the capacity of H^+ excretion [35,36]. For the similar hypothesis in our case, considerable amounts of hydrogen ions (H^+) were pumped out, which can be regarded as a detoxification strategy of excessive NH_4^+ by ageratum and lettuce. Nevertheless, the NH_4^+ -sensitive species salvia and cabbage were shown to be susceptible for NH_4^+ toxicity, which were probably attributed to the poor manipulation of the H^+ efflux [6,24,33,37]. Further explanation referred to these findings was the plasma membrane H^+ -ATPase, which is located in plant roots and is responsible for the H^+ transport [38,39]. Rhizosphere acidification was proven to promote the gene expression regarding the plasma membrane H^+ -ATPase activity under a constant supply of NH_4^+ [40]. Here, NH_4^+ -tolerant species (ageratum and lettuce) may have a more effective

plasma membrane H^+ -ATPase in the maintenance of H^+ -homeostasis than NH_4^+ -sensitive species do (salvia and cabbage).

4.2. Net Influx of K^+ by Ageratum May Help Prevent NH_4^+ Toxicity

Apart from the pH disorders, disruptions of the typical cation ion balance (including K^+ , Ca^{2+} , and Mg^{2+}) also constitute the integrated effect regarding excess NH_4^+ [8,11,17]. On average, plants grown with 0:100 $NH_4^+ : NO_3^-$ accumulated more cations from the hydroponic solutions, regardless of the cation type and species (Figures 4, S1 and S2), which were consistent with the results of previous studies [7,17,33,41]. The high uptake of cations for solely NO_3^- -fed plants were required for the maintenance of the 'kinetic cation-anion equilibrium' so that the N assimilation occurred normally [42]. Our findings revealed that the uptake of cations was inhibited by the uptake of NH_4^+ .

Outstandingly, the uptake of K^+ by solely NH_4^+ -fed ageratum ultimately appeared to be 18-fold higher than that by salvia cultured with 100% NH_4^+ (Figure 4B,D). Similarly to the discussion detailed above, and defined as an NH_4^+ -tolerant species, lettuce also displayed a nine-fold higher K^+ net influx than salvia with a 100% NH_4^+ nutrition (Figure 4B,F). It has been established that an adequate K^+ played a protective role against the detrimental aspects of NH_4^+ toxicity [7,43]. Large quantities of K^+ taken up by solely NH_4^+ -fed ageratum and lettuce possibly act to reinforce the NH_4^+ tolerance; a net K^+ efflux was observed in extremely NH_4^+ -sensitive species (salvia and cabbage) when the external NH_4^+ level was elevated to 100% (Figure 4B,E), highlighting the fact that salvia and cabbage were unable to mitigate the NH_4^+ toxicity posed by 100:0 $NH_4^+ : NO_3^-$ [6,24]. In explanation of this observation, a reciprocal inhibition role was found between K^+ and NH_4^+ , directly leading to ion competition [44]. This clearly demonstrates that NH_4^+ enters plant tissues primarily through K^+ channels, but also partly through other channels when the external NH_4^+ is abundant [22,45]. Unexpectedly, there were no major differences in the Ca^{2+} and Mg^{2+} variations among species in the absence of NO_3^- (Figure S1 '100:0 part' and Figure S2 '100:0 part'); in other words, NH_4^+ -tolerant species grown with 100:0 $NH_4^+ : NO_3^-$ ultimately did not introduce net influxes of Ca^{2+} or Mg^{2+} for detoxifying excess NH_4^+ , suggesting that neither Ca^{2+} nor Mg^{2+} contribute to the NH_4^+ tolerance.

4.3. Minor EC Changes of Hydroponic Solutions Is a Negligible Indicator of the NH_4^+ Tolerance

EC (electrical conductivity) was a direct estimator of the amount of dissolved salt content or ions present in the hydroponic solutions [46]. Usually, the EC of the culture medium increased over time in a closed hydroponic system because the nutrients were not rapidly removed by plants or run off via leaching [47]. Even though the initial EC of the hydroponic solutions were very different, the present study showed that except for solely NO_3^- -fed ageratum (Figure 5D), the ECs of all hydroponic solutions slightly increased as plants were cultivated, because ageratum is tolerant to NH_4^+ and sensitive to NO_3^- [6,33]. However, the EC of the hydroponic solution with 100:0 $NH_4^+ : NO_3^-$ in the control groups exhibited a highly similar increase to that in that supplied for ageratum and lettuce, which rendered the EC changes in the hydroponic solution for ageratum and lettuce supplied with 100:0 $NH_4^+ : NO_3^-$ as negligible. Succinctly, the enhanced EC of hydroponic solutions may not reflect the NH_4^+ tolerance in hydroponically cultured plants.

5. Conclusions

To sum up, the pH of the hydroponic solutions declined when NH_4^+ was served as the exclusive N source or as a combination with NO_3^- ; more importantly, the pH of hydroponic solutions supplied with 100:0 $NH_4^+ : NO_3^-$ remarkably decreased when culturing the NH_4^+ -tolerant species ageratum and lettuce, but only slightly declined when culturing extremely NH_4^+ -sensitive species salvia and cabbage. Comparisons of the ion uptake between 0 and 84 h after culture initiation showed that, in general, the external NH_4^+ level is negatively correlated with the cation uptake level; outstandingly, NH_4^+ -tolerant species ageratum and lettuce ultimately took up drastically greater amounts of K^+ when cultured with a 100%

NH_4^+ supply, whereas the extremely NH_4^+ -sensitive species salvia and cabbage presented a net K^+ efflux regulatory pattern in response to high concentrations of NH_4^+ . The data regarding the plants with contrasting NH_4^+ tolerances tested in this study revealed that the declined pH of hydroponic solutions, together with greater net K^+ influx by plants, was linked to NH_4^+ tolerance. To the best of the authors' knowledge, this research is the first to identify a promising tool for practically selecting NH_4^+ -tolerant or -sensitive species using hydroponics.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/horticulturae8030228/s1>, Figure S1: The Ca^{2+} content changes in three $\text{NH}_4^+:\text{NO}_3^-$ hydroponic solutions supplied for (A) the control (without plants); (B) salvia; (C) petunia; (D) ageratum; (E) cabbage; and (F) lettuce. Data are means \pm SD representing $n = 3$ biological replicates. Columns accompanied by different lower case letters were regarded as significantly different, according to the one-way ANOVA following the Duncan's multiple range test at $p = 0.05$; Figure S2: The Mg^{2+} content changes in three $\text{NH}_4^+:\text{NO}_3^-$ hydroponic solutions supplied for (A) the control (without plants); (B) salvia; (C) petunia; (D) ageratum; (E) cabbage; and (F) lettuce. Data are means \pm SD representing $n = 3$ biological replicates. Columns accompanied by different lower case letters were regarded as significantly different, according to the one-way ANOVA following the Duncan's multiple range test at $p = 0.05$.

Author Contributions: Conceptualization, B.R.J.; methodology, B.R.J. and J.S.; software, J.S.; validation, B.R.J. and J.S.; formal analysis, B.R.J. and J.S.; investigation, J.S. and J.Y.; resources, B.R.J.; data curation, J.S.; writing—original draft preparation, J.S.; writing—review and editing, B.R.J. and J.S.; supervision, B.R.J.; project administration, B.R.J.; funding acquisition, B.R.J. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding. Jinnan Song and Jingli Yang were supported by BK21 Four Program, Ministry of Education, Korea.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

- Buchanan, B.B.; Gruissem, W.; Jones, R.L. *Biochemistry and Molecular Biology of Plants*; John Wiley & Sons: Hoboken, NJ, USA, 2015.
- Miller, A.; Cramer, M. Root nitrogen acquisition and assimilation. *Plant Soil* **2005**, *274*, 1–36. [[CrossRef](#)]
- Coruzzi, G.; Bush, D.R. Nitrogen and carbon nutrient and metabolite signaling in plants. *Plant Physiol.* **2001**, *125*, 61–64. [[CrossRef](#)]
- Middleton, K.; Smith, G. A comparison of ammoniacal and nitrate nutrition of perennial ryegrass through a thermodynamic model. *Plant Soil* **1979**, *53*, 487–504. [[CrossRef](#)]
- Guerrero, M.G.; Vega, J.M.; Losada, M. The assimilatory nitrate-reducing system and its regulation. *Annu. Rev. Plant Physiol.* **1981**, *32*, 169–204. [[CrossRef](#)]
- Song, J.; Yang, J.; Jeong, B.R. Root GS and NADH-GDH Play Important Roles in Enhancing the Ammonium Tolerance in Three Bedding Plants. *Int. J. Mol. Sci.* **2022**, *23*, 1061. [[CrossRef](#)]
- Roosta, H.R.; Schjoerring, J.K. Effects of ammonium toxicity on nitrogen metabolism and elemental profile of cucumber plants. *J. Plant Nutr.* **2007**, *30*, 1933–1951. [[CrossRef](#)]
- Britto, D.T.; Kronzucker, H.J. NH_4^+ toxicity in higher plants: A critical review. *J. Plant Physiol.* **2002**, *159*, 567–584. [[CrossRef](#)]
- Britto, D.T.; Siddiqi, M.Y.; Glass, A.D.; Kronzucker, H.J. Futile transmembrane NH_4^+ cycling: A cellular hypothesis to explain ammonium toxicity in plants. *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 4255–4258. [[CrossRef](#)]
- Ariz, I.; Cruz, C.; Moran, J.F.; González-Moro, M.B.; García-Olaverri, C.; González-Murua, C.; Martins-Loução, M.A.; Aparicio-Tejo, P.M. Depletion of the heaviest stable N isotope is associated with $\text{NH}_4^+/\text{NH}_3^-$ toxicity in NH_4^+ -fed plants. *BMC Plant Biol.* **2011**, *11*, 83. [[CrossRef](#)]
- Bittsánszky, A.; Pilinszky, K.; Gyulai, G.; Komives, T. Overcoming ammonium toxicity. *Plant Sci.* **2015**, *231*, 184–190. [[CrossRef](#)] [[PubMed](#)]
- Kronzucker, H.J.; Britto, D.T.; Davenport, R.J.; Tester, M. Ammonium toxicity and the real cost of transport. *Trends Plant Sci.* **2001**, *6*, 335–337. [[CrossRef](#)]
- Hachiya, T.; Inaba, J.; Wakazaki, M.; Sato, M.; Toyooka, K.; Miyagi, A.; Kawai-Yamada, M.; Sugiura, D.; Nakagawa, T.; Kiba, T. Excessive ammonium assimilation by plastidic glutamine synthetase causes ammonium toxicity in *Arabidopsis thaliana*. *Nat. Commun.* **2021**, *12*, 4944. [[CrossRef](#)] [[PubMed](#)]

14. Schubert, S.; Yan, F. Nitrate and ammonium nutrition of plants: Effects on acid/base balance and adaptation of root cell plasmalemma H⁺-ATPase. *Z. Pflanz. Bodenkd.* **1997**, *160*, 275–281. [[CrossRef](#)]
15. Kirk, G.; Bajita, J. Root-induced iron oxidation, pH changes and zinc solubilization in the rhizosphere of lowland rice. *N. Phytol.* **1995**, *131*, 129–137. [[CrossRef](#)]
16. Brix, H.; Dyhr-Jensen, K.; Lorenzen, B. Root-zone acidity and nitrogen source affects *Typha latifolia* L. growth and uptake kinetics of ammonium and nitrate. *J. Exp. Bot.* **2002**, *53*, 2441–2450. [[CrossRef](#)]
17. Ariz, I.; Artola, E.; Asensio, A.C.; Cruchaga, S.; Aparicio-Tejo, P.M.; Moran, J.F. High irradiance increases NH₄⁺ tolerance in *Pisum sativum*: Higher carbon and energy availability improve ion balance but not N assimilation. *J. Plant Physiol.* **2011**, *168*, 1009–1015. [[CrossRef](#)]
18. Ohmori, M.; Kanda, J. H⁺ and K⁺ efflux associated with NH₄⁺ uptake in *Anabaena cylindrica* cells in the dark. *J. Gen. Appl. Microbiol.* **1987**, *33*, 311–319. [[CrossRef](#)]
19. Szczerba, M.W.; Britto, D.T.; Kronzucker, H.J. Rapid, futile K⁺ cycling and pool-size dynamics define low-affinity potassium transport in barley. *Plant Physiol.* **2006**, *141*, 1494–1507. [[CrossRef](#)]
20. Zou, N.; Li, B.; Dong, G.; Kronzucker, H.J.; Shi, W. Ammonium-induced loss of root gravitropism is related to auxin distribution and TRH1 function, and is uncoupled from the inhibition of root elongation in *Arabidopsis*. *J. Exp. Bot.* **2012**, *63*, 3777–3788. [[CrossRef](#)]
21. Santa-Maria, G.E.; Danna, C.H.; Czibener, C. High-affinity potassium transport in barley roots. Ammonium-sensitive and-insensitive pathways. *Plant Physiol.* **2000**, *123*, 297–306. [[CrossRef](#)]
22. Balkos, K.D.; Britto, D.T.; Kronzucker, H.J. Optimization of ammonium acquisition and metabolism by potassium in rice (*Oryza sativa* L. cv. IR-72). *Plant Cell Environ.* **2010**, *33*, 23–34.
23. Xian, L.; Zhang, Y.; Cao, Y.; Wan, T.; Gong, Y.; Dai, C.; Ochieng, W.A.; Nasimiyu, A.T.; Li, W.; Liu, F. Glutamate dehydrogenase plays an important role in ammonium detoxification by submerged macrophytes. *Sci. Total Environ.* **2020**, *722*, 137859. [[CrossRef](#)]
24. Song, J.; Yang, J.; Jeong, B.R. Growth, Quality, and Nitrogen Assimilation in Response to High Ammonium or Nitrate Supply in Cabbage (*Brassica campestris* L.) and Lettuce (*Lactuca sativa* L.). *Agronomy* **2021**, *11*, 2556. [[CrossRef](#)]
25. El Omari, R.; Rueda-López, M.; Avila, C.; Crespillo, R.; Nhiri, M.; Cánovas, F.M. Ammonium tolerance and the regulation of two cytosolic glutamine synthetases in the roots of sorghum. *Funct. Plant Biol.* **2010**, *37*, 55–63. [[CrossRef](#)]
26. Rupp, L.; Dudley, L. Iron availability in rockwool may affect rose nutrition. *HortScience* **1989**, *24*, 258–260.
27. Thi, L.T.; Park, Y.G.; Jeong, B.R. Growth and development of carnation ‘Dreambyul’ plantlets in a temporary immersion system and comparisons with conventional solid culture methods. *Vitr. Cell Dev. Biol.-Plant* **2019**, *55*, 539–548. [[CrossRef](#)]
28. Esteban, R.; Ariz, I.; Cruz, C.; Moran, J.F. Mechanisms of ammonium toxicity and the quest for tolerance. *Plant Sci.* **2016**, *248*, 92–101. [[CrossRef](#)]
29. Son, J.E.; Kim, H.J.; Ahn, T.I. Hydroponic systems. In *Plant Factory*; Elsevier: Amsterdam, The Netherlands, 2020; pp. 273–283.
30. Xu, G.; Magen, H.; Tarchitzky, J.; Kafkafi, U. Advances in chloride nutrition of plants. *Adv. Agron.* **1999**, *68*, 97–150.
31. Colmenero-Flores, J.M.; Franco-Navarro, J.D.; Cubero-Font, P.; Peinado-Torrubia, P.; Rosales, M.A. Chloride as a beneficial macronutrient in higher plants: New roles and regulation. *Int. J. Mol. Sci.* **2019**, *20*, 4686. [[CrossRef](#)]
32. Jeong, B.R.; Lee, C.W. Growth suppression and raised tissue Cl⁻ contents in NH₄⁺-fed marigold, petunia, and salvia. *J. Am. Soc. Hortic. Sci.* **1992**, *117*, 762–768. [[CrossRef](#)]
33. Jeong, B.R.; Lee, C.W. Influence of ammonium, nitrate, and chloride on solution pH and ion uptake by ageratum and salvia in hydroponic culture. *J. Plant Nutr.* **1996**, *19*, 1343–1360. [[CrossRef](#)]
34. Pearson, J.; Stewart, G.R. The deposition of atmospheric ammonia and its effects on plants. *New Phytol.* **1993**, *125*, 283–305. [[CrossRef](#)]
35. Roos, W.; Luckner, M. Relationships between proton extrusion and fluxes of ammonium ions and organic acids in *Penicillium cyclopium*. *Microbiology* **1984**, *130*, 1007–1014. [[CrossRef](#)]
36. Hackette, S.L.; Skye, G.; Burton, C.; Segel, I.H. Characterization of an ammonium transport system in filamentous fungi with methylammonium-14C as the substrate. *J. Biol. Chem.* **1970**, *245*, 4241–4250. [[CrossRef](#)]
37. Bar-Yosef, B.; Mattson, N.; Lieth, H. Effects of NH₄⁺: NO₃⁻: Urea ratio on cut roses yield, leaf nutrients content and proton efflux by roots in closed hydroponic system. *Sci. Hortic.* **2009**, *122*, 610–619. [[CrossRef](#)]
38. Michelet, B.; Boutry, M. The plasma membrane H⁺-ATPase (A highly regulated enzyme with multiple physiological functions). *Plant Physiol.* **1995**, *108*, 1–6. [[CrossRef](#)]
39. Yan, F.; Feuerle, R.; Schäffer, S.; Fortmeier, H.; Schubert, S. Adaptation of active proton pumping and plasmalemma ATPase activity of corn roots to low root medium pH. *Plant Physiol.* **1998**, *117*, 311–319. [[CrossRef](#)]
40. Zhu, Y.; Di, T.; Xu, G.; Chen, X.; Zeng, H.; Yan, F.; Shen, Q. Adaptation of plasma membrane H⁺-ATPase of rice roots to low pH as related to ammonium nutrition. *Plant Cell Environ.* **2009**, *32*, 1428–1440. [[CrossRef](#)]
41. Hachiya, T.; Watanabe, C.K.; Fujimoto, M.; Ishikawa, T.; Takahara, K.; Kawai-Yamada, M.; Uchimiya, H.; Uesono, Y.; Terashima, I.; Noguchi, K. Nitrate addition alleviates ammonium toxicity without lessening ammonium accumulation, organic acid depletion and inorganic cation depletion in *Arabidopsis thaliana* shoots. *Plant Cell Physiol.* **2012**, *53*, 577–591. [[CrossRef](#)]
42. Kirkby, E.A.; Knight, A.H. Influence of the level of nitrate nutrition on ion uptake and assimilation, organic acid accumulation, and cation-anion balance in whole tomato plants. *Plant Physiol.* **1977**, *60*, 349–353. [[CrossRef](#)]

43. Zhu, J.K.; Liu, J.; Xiong, L. Genetic analysis of salt tolerance in *Arabidopsis*: Evidence for a critical role of potassium nutrition. *Plant Cell* **1998**, *10*, 1181–1191. [[CrossRef](#)]
44. Hoopen, Ft.; Cuin, T.A.; Pedas, P.; Hegelund, J.N.; Shabala, S.; Schjoerring, J.K.; Jahn, T.P. Competition between uptake of ammonium and potassium in barley and *Arabidopsis* roots: Molecular mechanisms and physiological consequences. *J. Exp. Bot.* **2010**, *61*, 2303–2315. [[CrossRef](#)]
45. Zhang, L.; Song, H.; Li, B.; Wang, M.; Di, D.; Lin, X.; Kronzucker, H.J.; Shi, W.; Li, G. Induction of S-nitrosoglutathione reductase protects root growth from ammonium toxicity by regulating potassium homeostasis in *Arabidopsis* and rice. *J. Exp. Bot.* **2021**, *72*, 4548–4564. [[CrossRef](#)]
46. Samarakoon, U.; Weerasinghe, P.; Weerakkody, W. Effect of electrical conductivity (EC) of the nutrient solution on nutrient uptake, growth and yield of leaf lettuce (*Lactuca sativa* L.) in stationary culture. *Trop. Agric. Res.* **2006**, *18*, 13–21.
47. James, E.C.; van Iersel, M.W. Fertilizer concentration affects growth and flowering of subirrigated petunias and begonias. *HortScience* **2001**, *36*, 40–44. [[CrossRef](#)]