BIO Web of Conferences **110**, 01005 (2024) https://doi.org/10.1051/bioconf/202411001005
ICRAHOR 2024

Ammonium sulphate induces dose-dependent ammonium stress in rice seedlings

Avani Maurya[1], *Tejasvita* Chaudhary[1], *Pallavi* Priya[1], *Vishakha* Sharma[1], *Garima* Balyan[1], *Adwithiya* Sharma[2], *Nidhi* Kandhol[1*], and *Durgesh Kumar* Tripathi[1*]
[1]Crop Nano Biology and Molecular Stress Physiology Lab, Amity Institute of Organic Agriculture, Amity University Uttar Pradesh, Sector-125, Noida-201313
[2] Amity Institute of Biotechnology, Amity University Uttar Pradesh, Sector-125, Noida-201313

*Corresponding author: nidhikandhol29@gmail.com dktripathiau@gmail.com

Abstract. Plants require nitrogen (N) in various forms to facilitate essential physiological functions. Nitrate (NO_3^-) is one of the most readily absorbed N forms by plants and is preferred in well-aerated soils because it can be easily transported within the plant. Ammonium (NH_4^+), on the other hand, is utilized especially in waterlogged or acidic soils, where it is directly absorbed by the roots and incorporated into amino acids. Urea (CH_4N_2O) is another significant N source found in many fertilizers; it is transformed into NH_4^+ and nitrate in the soil through microbial processes. These diverse forms of N are crucial for supporting photosynthesis, protein synthesis, and energy production in plants. The escalating use of ammonium sulphate ($(NH_4)_2SO_4$) as a N source in agriculture prompts a thorough examination of its impact on crop health and productivity. This study aimed to investigate the NH_4^+ toxicity on rice (*Oryza sativa*) plants by administering various dosages (0 mM, 5 mM, 7 mM, 10 mM, 12 mM, and 15 mM) and assessing their effects on plant growth parameters, particularly root-shoot lengths, root-shoot fresh biomass along with dry weight. Our research utilized a controlled experimental setup to monitor the growth responses of rice plants to these NH_4^+ concentrations. Results indicated a clear threshold of tolerance, with adverse effects becoming significant at concentrations starting from 7 mM. At this concentration and higher, there was a noticeable decline in root-shoot lengths, root-shoot biomass and dry biomass, marking the onset of toxicity symptoms in rice plants. These findings suggest a critical need for regulated application of $(NH_4)_2SO_4$ in rice cultivation to avoid detrimental effects on plant health and yield. The study underscores the importance of establishing safe usage guidelines for $(NH_4)_2SO_4$ in agriculture, ensuring sustainable farming practices while maintaining crop productivity.
Keywords: Rice, Ammonium sulphate, Toxicity, Root, Shoot, Biomass

1 Introduction

The rapid population growth and drastic climate change are two significant obstacles to achieving global food security [1]. Crops are significantly impacted by both biotic and abiotic stressors. Plants exhibit various biochemical, physiological, and morphological changes to adapt to stressful situations [2]. To achieve high crop yields, nitrogenous fertilizers like urea (CH_4N_2O), ammonium (NH_4^+), and nitrate (NO_3^-) are extensively used in crop cultivation. Overusing NH_4^+ as the sole N source can cause many morphological and physiological issues in plants, ultimately resulting in stunted growth and reduced production [3]. When NH_4^+ is administered in the optimum amount, it increases yield, but when it is applied in large dosages, it causes toxicity, resulting in changes of cytosolic pH, changes of root structure, alterations in protein, acidification of apoplast, accelerated absorption of ammonia, and changes in phytohormones and oxidative processes [4]. For instance, the toxicity of NH_4^+ rises with increasing pH levels in common hornwort (*Ceratophyllum demersum)*, leading to reduced plant development [5]. Higher levels of NH_4^+ also result in the increase of acidic stress. For example, NH_4^+ toxicity is mostly caused by the absorption of NH_4^+ by the enzyme GLUTAMINE SYNTHETASE 2 (GLN2) located in the cell plastid, apart from solely by the deposition of NH_4^+. *Arabidopsis thaliana* plants produce a high number of protons through the GLN2 enzyme in the shoots when exposed to excessive NH_4^+ levels, leading to increased cellular acidity. Elevated NH_4^+ levels not only increase acidity but also lead to disruption of the chloroplast membrane, causing enlarged compartments [6].

Elevated NH_4^+ levels not only harm plant shoots but also alter the root morphology, such as in *A. thaliana*. When a normal nitrate amount is applied, there is an increment in the lateral root length. However, with higher NH_4^+ concentrations, branching in lateral roots increases instead of length. Interestingly, the simultaneous application of nitrate and NH_4^+ enhances both the branching and elongation of lateral roots. The simultaneous application of nitrate and NH_4^+ has synergistic and complementary effects on the growth of lateral roots [7]. Visible symptoms of

NH_4^+ toxicity in plants include stunted root system, leaf chlorosis, decreased tissue accumulation of mineral cations, and increased root NH_4^+ fluxes in hydroponic trials [8]. Experiment conducted on transcriptome and physiological studies on common duckweed to investigate the effects of NH_4^+ toxicity. Their findings revealed that NH_4^+ toxicity can lead to the formation of reactive oxygen species (ROS), resulting in oxidative damage and ultimately cell death in *L. minor* [9].

Rice (*Oryza sativa*) is a fundamental food and cereal that supports two-thirds of the total population, being the crucial provider of livelihood for many due to its high nutritional and energy values. Rice is an important provider of fiber, energy, minerals, vitamins, and various other biomolecules, making it essential for human sustenance [10]. To get a high yield and increased output, nitrogen (N) is the essential fertilizer needed by rice plants [4]. Rice plants are particularly sensitive to excessive NH_4^+ concentrations (a form of N fertilizer). NH_4^+ toxicity involves complex physiological, biochemical, and molecular alterations in the rice plant. High quantities of NH_4^+ disturb cellular ionic equilibrium, causing an increase in reactive oxygen species (ROS) that harm cellular structures and hinder function [11]. Elevated NH_4^+ levels can impede plant growth, and various plant species have evolved unique approaches to enhance NH_4^+ uptake while mitigating its toxic effects by adjusting root growth. An increased supply of NH_4^+ limits the elongation of rice roots and causes them to grow in a spiral pattern. This change in growth pattern is linked to the acidification of the root environment, which occurs as a result of higher NH_4^+ absorption [12]. Higher doses of NH_4^+ also result in decrease in the fresh weight of the root and shoot of the rice plant [13].

This study examines the response of rice root and shoot to several dosages of $(NH_4)_2SO_4$ (a donor of NH_4^+) to assess its toxicity, so that sustainability of crops can be achieved by applying optimum doses of fertilizers such as NH_4^+ along with mitigating its toxic effects.

2 Materials and methods

2.1 Growth conditions of plant material:

Rice (*Oryza sativa* L.) seeds were acquired from a recognized seller in Allahabad, India, underwent sterilization using 10% (v/v) sodium hypochlorite for 10 minutes and then cleaned with distilled water. Then the seeds were kept on a damp muslin cloth and stored in darkness for 2 days to initiate sprouting. The germinated seedlings were transferred in petri plates and then to growth chamber (Impact model IIC 129D, New Delhi). The growing chamber maintained 65-70% of relative humidity, 300 µmol photons m-2 s-1 of photosynthetically active radiation (PAR), and 26 ±1ºC temperature, following a 12/12-hour day/night cycle. Within the growing phase, seedlings were provided with nutrients of Hoagland's solution. The Hoagland solution was composed of $MgSO_4.7H_2O$, $Ca(NO_3)_2.2H_2O$, KH_2PO_4, KNO_3 and Fe + EDTA ($FeCl_3$), H_3BO_3, $MnCl_3.4H_2O$, $ZnSO_4.7H_2O$, $Na_2MoO_4.2H_2O$ and $CuSO_4.5H_2O$ [18]

2.2 Treatments:

$(NH_4)_2SO_4$ was used as a donor of NH_4^+. Different concentrations of $(NH_4)_2SO_4$ were given to rice plant such as T1 (0mM), T2 (5mM), T3 (7mM), T4 (10mM), T5 (12mM) and T6 (15mM). Seedlings were subjected to treatments for 7 days before subsequent analysis, following the methodology described by [19]. Each treatment solution, total 40 ml, was administered per cup. The cups were placed in plant growth chamber set with 300 µmol photons m−2 s−1 photon flux density, operating on a 12/12-day night cycle, with a temperature of 26 ± 1 ºC and 75–80% of relative humidity for 7 days. To prevent root hypoxia, the solutions for all treatments were renewed throughout the experimental period at every 3 days.

2.3 Growth parameters analysis*:* After harvesting, plant growth parameters were assessed, such as:

2.3.1: Root and shoot length: For measuring primary root length and shoot length of rice, measuring cm scale was used.

2.3.2 Root-shoot fresh-dry biomass: The root fresh weight of rice plants was calculated by separating the plant into shoot and roots, then measuring their weights with the digital weighing balance. The rice roots were dehydrated by wrapping them in butter paper and heating them in an oven at 80°C for 24 hours. Subsequently, their weight was determined using a digital weighing scale.

2.4 Statistical Analysis

We conducted statistical analyses using one-way analysis of variance (ANOVA) through SPSS 16.0 software, ensuring the data met the criteria for normal distribution. Duncan's multiple range test (DMRT) was applied at a significance level of $P<0.05$ to distinguish variations among treatments. The reported values represent the means derived from three independent biological replicates (n=3).

3 Results and discussion

Plant growth parameters were assessed during the harvest stage of the plant. Different parameters that were assessed during the experiment were root-shoot length, fresh biomass of shoot-root, shoot-root dry biomass. Morphological data show that there was immense decrease in growth characteristics with the increment in concentration of $(NH_4)_2SO_4$ (Fig 1). When the dose of 5mM is given, decrease in root and shoot length is observed. This decrease further rose with the increasing concentration of 7mM, 10mM, 12mM and 15mM respectively as compared to control.

Fig. 1. Morphological impact of different concentrations of $(NH_4)_2SO_4$ on root and shoot of rice plant. T1 (0mM), T2 (5mM), T3 (7mM), T4 (10mM), T5 (12mM) and T6 (15mM).

3.1 Root length:

Rice root length reduction was observed at rates of 2.7%, 13.5%, 18.9%, 24.3%, and 32.4% for NH_4^+ concentrations of 5 mM, 7 mM, 10 mM, 12 mM, and 15 mM, respectively, with respect to control (Fig 2). The findings revealed that up to a concentration of 7 mM, the decrease in rice root length was modest, but further increases in NH_4^+ concentration led to a substantial reduction in root length. $(NH_4)_2SO_4$ has been documented to exert a toxic impact on plant root length when used as the sole N source. A parallel investigation by Jia et al [12] revealed that NH_4^+ restricts the elongation of rice primary roots. Our results align with these findings, demonstrating the consistency of NH_4^+ effects across studies. Additionally, other NH_4^+ salts, such as those examined by Guo et al [14], also contribute to the reduction in root length. Specifically, a 72-hour exposure to NH_4Cl led to a notable dose-dependent decline in length of root in rice seedlings, further substantiating the patterns observed in our study. This accumulation of evidence underscores the consistent impact of NH_4^+ on root development across different studies.

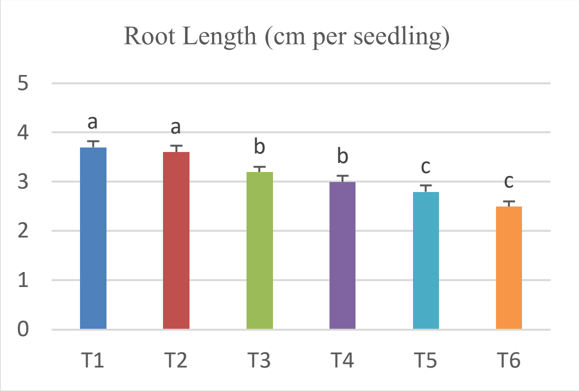

Fig. 2. Graphical representation of decline in the root length of rice at several concentrations of $(NH_4)_2SO_4$. T1 (0mM), T2 (5mM), T3 (7mM), T4 (10mM), T5 (12mM) and T6 (15mM).

3.2 Root Fresh Biomass:

The fresh biomass of rice root exhibited a reduction of 3.7%, 9%, 44.3%, 52.6%, and 81.2% in correspondence with escalating concentrations of 5 mM, 7 mM, 10 mM, 12 mM, and 15 mM, respectively, with respect to the control (Fig 3). The data indicate quite modest decrease in fresh weight at concentrations up to 7 mM, however, further exceeding concentrations led to a severe reduction. The toxic effects of $(NH_4)_2SO_4$ are manifested as a reduction in the fresh weight of roots, primarily due to the inhibition of primary root length [15] observed similar outcomes, noting a decrease in root biomass in seedlings exposed to two concentrations of NH_4^+ (10 mM and 80 mM), which aligns with the findings from our research. Additionally, a comparative study of N forms by Coleto et al [16] examined the impacts of nitrate versus NH_4^+ on root biomass. The results from this study highlight that NH_4^+exerts a more deleterious effect on root biomass compared to the nitrate form, further underscoring the differential toxicity of N sources on plant root development.

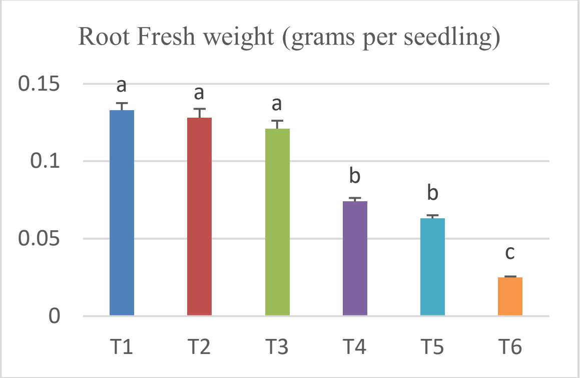

Fig. 3. Graphical representation of decline in fresh root biomass of rice at several concentrations of $(NH_4)_2SO_4$. T1 (0mM), T2 (5mM), T3 (7mM), T4 (10mM), T5 (12mM) and T6 (15mM).

3.3 Root Dry Biomass:

The root dry biomass of rice decreased by 4.2%, 14.2%, 42.8%, 50%, and 64.2% at concentrations of 5 mM, 7 mM, 10 mM, 12 mM, and 15 mM, respectively, relative to the control (Fig 4). The results demonstrate that the decrease in root dry weight was minimal at concentrations up to 7 mM, however, an increase beyond this concentration led to a substantial decline in the dry biomass of the rice roots. The harmful impact of $(NH_4)_2SO_4$ results in a decrease in root dry weight due to the suppression of primary root length and a loss in root dry weight. Studies conducted by Zhou et al [15] and Jia et al [12] show that higher levels of NH_4^+ (10 mM and 80 mM) result in a reduction in the root dry weight of rice plants.

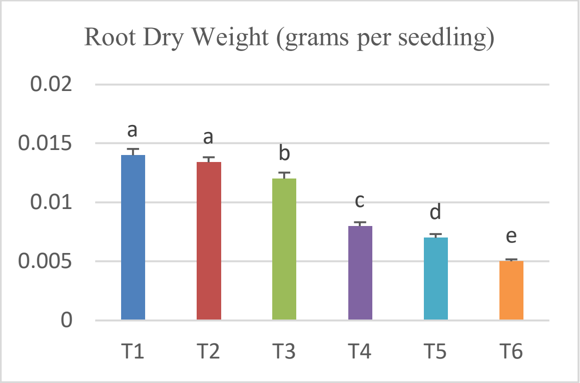

Fig. 4. Graphical representation of decline in dry root biomass of rice at several concentrations of $(NH_4)_2SO_4$. T1 (0mM), T2 (5mM), T3 (7mM), T4 (10mM), T5 (12mM) and T6 (15mM).

3.4 Shoot length:

Reductions in shoot length of rice plants were observed as 3.8%, 12.9%, 15.5%, 22% and 24.6% at concentrations of 5 mM, 7 mM, 10 mM, 12 mM, and 15 mM, respectively, in comparison to the control (Fig 5). The data indicates a slight decrease in shoot length at concentrations up to 7 mM; beyond this concentration, there was a significant decline in shoot length. Earlier research has demonstrated NH_4^+ toxicity, resulting in reduced shoot development and chlorophyll content, as well as increased leaf necrosis with higher concentrations of NH_4^+ (control, 30µM, and 120µM) [17].

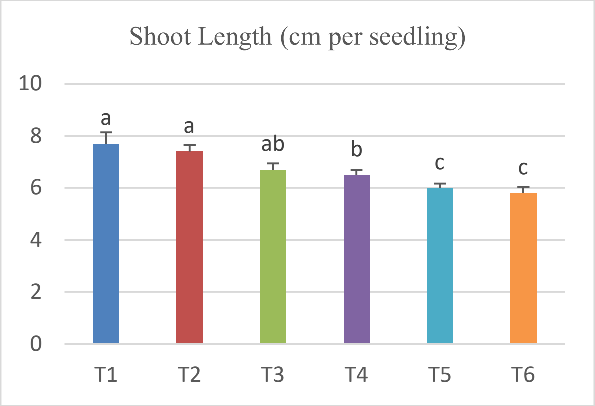

Fig. 5. Graphical representation of decline in the shoot length of rice at different concentrations of $(NH_4)_2SO_4$. T1 (0mM), T2 (5mM), T3 (7mM), T4 (10mM), T5 (12mM) and T6 (15mM).

3.5 Shoot Fresh Weight:

The shoot fresh weight of rice exhibited declines of 5%, 9.3%, 33.7%, 40.4%, and 43.4% at increasing concentrations of 5 mM, 7 mM, 10 mM, 12 mM, and 15 mM, respectively, compared to the control (Fig 6). The results demonstrated that the reduction in shoot fresh weight was negligible up to a concentration of 7 mM, whereas concentrations exceeding this threshold resulted in a severe decline in shoot fresh weight. Previous research has indicated negative effects of NH_4^+ in comparison to other N forms. A study by Coleto et al [16] found that NH_4^+ had a more detrimental influence on shoot biomass than nitrate when provided at varying amounts. Therefore, their findings align with our results in a comparable manner.

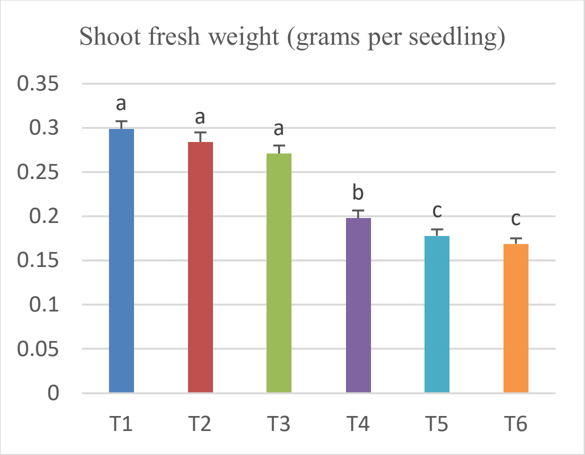

Fig. 6. Graphical representation of decline in fresh shoot biomass of rice at several concentrations of $(NH_4)_2SO_4$. T1 (0mM), T2 (5mM), T3 (7mM), T4 (10mM), T5 (12mM) and T6 (15mM).

3.6 Shoot dry weight:

The dry weight of rice shoots declined by 5.5%, 16.6%, 33.3%, 33.3%, and 38.8% at concentrations of 5 mM, 7 mM, 10 mM, 12 mM, and 15 mM, respectively, compared to the control. The results indicate that up to a concentration of 7 mM, the decline in shoot dry weight was slight, however, a further increase in concentration resulted in a significant reduction.

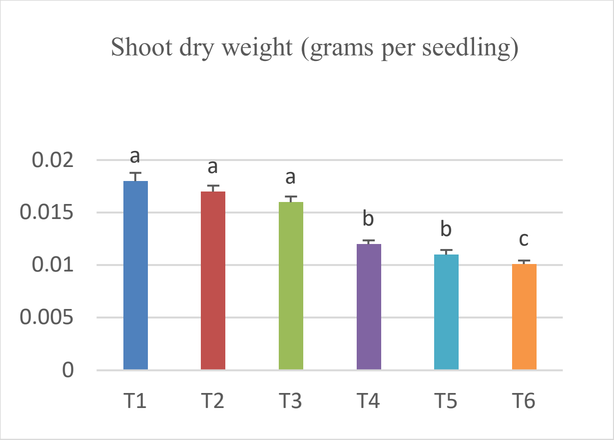

Fig. 7. Graphical representation of decline in dry shoot biomass of rice at several concentrations of $(NH_4)_2SO_4$. T1 (0mM), T2 (5mM), T3 (7mM), T4 (10mM), T5 (12mM) and T6 (15mM).

4 Conclusion

The conclusion of the study underscores the negative impacts of high doses of NH_4^+, specifically $(NH_4)_2SO_4$, on the development and growth of rice plants. It highlights that excessive NH_4^+ can inhibit various physiological and developmental processes within the plant, which are crucial for optimal growth and productivity. The recommendation to use an optimal dose of 7 mM of $(NH_4)_2SO_4$ in future experiments is based on observations from the study that this concentration begins to negatively impact plant development characteristics such as root length, shoot growth, and overall biomass. The choice of 7 mM is significant as it represents a threshold at which the initial signs of toxicity become apparent, making it a critical concentration for detailed examination in further studies. This approach facilitates a deeper understanding of how NH_4^+ interferes at cellular and molecular levels, which could lead to strategies for mitigating its negative effects in agricultural settings. The study's findings and recommendations are crucial for developing management practices that ensure the health and productivity of rice crops, particularly in regions where fertilizer application needs to be optimized to avoid detrimental levels of NH_4^+.

References

1. S. Fahad, A. A. Bajwa, U. Nazir, S. A. Anjum, A. Farooq, A. Zohaib, ... J. Huang, Crop production under drought and heat stress: plant responses and management options. Front. Plant Sci., **1147** (2017)
2. S. Saleem, N. U. Mushtaq, W. H. Shah, A. Rasool, K. R. Hakeem, R. U. Rehman. Morpho-physiological, biochemical and molecular adaptation of millets to abiotic stresses: A review. Phyton, **90**, 1363 (2021)
3. R. Esteban, I. Ariz, C. Cruz, J. F. Moran. Mechanisms of ammonium toxicity and the quest for tolerance. Plant Sci., **248**, 92-101 (2016)
4. S. T. Ata-Ul-Karim, X. Liu, Z. Lu, H. Zheng, W. Cao, Y. Zhu. Estimation of nitrogen fertilizer requirement for rice crop using critical nitrogen dilution curve. Field Crops Res., **201**, 32-40 (2017)
5. J. Gao, L. Li, Z. Hu, H. Yue, R. Zhang, Z. Xiong. Effect of ammonia stress on nitrogen metabolism of Ceratophyllum demersum. Environ. Toxicol. Chem., **35**, 205-211 (2016)
6. T. Hachiya, J. Inaba, M. Wakazaki, M. Sato, K. Toyooka, A. Miyagi, ... H. Sakakibara.

Excessive ammonium assimilation by plastidic glutamine synthetase causes ammonium toxicity in Arabidopsis thaliana. Nat. Commun., **12**, 4944 (2021)

7. T. Hachiya, H. Sakakibara. Interactions between nitrate and ammonium in their uptake, allocation, assimilation, and signaling in plants. J. Exp. Bot., **68**, 2501-2512 (2017)
8. J. Shi[2]lpha, J. Song, B. R. Jeong. Ammonium Phytotoxicity and tolerance: An insight into ammonium nutrition to improve crop productivity. Agronomy, **13**, 1487 (2023)
9. W. Wang, R. Li, Q. Zhu, X. Tang, Q. Zhao. Transcriptomic and physiological analysis of common duckweed Lemna minor responses to NH4+ toxicity. BMC Plant Biol., **16**, 1-13 (2016)
10. S. Sen, R. Chakraborty, P. Kalita. Rice-not just a staple food: A comprehensive review on its phytochemicals and therapeutic potential. Trends Food Sci. Technol., **97**, 265-285 (2020)
11. S. Yang, D. Hao, M. Jin, Y. Li, Z. Liu, Y. Huang, ... Y. Su. Internal ammonium excess induces ROS-mediated reactions and causes carbon scarcity in rice. BMC Plant Biol., **20**, 1-15 (2020)
12. L. Jia, Y. Xie, Z. Wang, L. Luo, C. Zhang, P. M. Pélissier, ... W. Xuan. Rice plants respond to ammonium stress by adopting a helical root growth pattern. The Plant J., **104**, 1023-1037 (2020)
13. E. G. Pereira, L. M. Ferreira, E. D. C. Fernandes, B. R. D. Lima, L. A. Santos, M. S. Fernandes. Root morphology and ammonium uptake kinetics in two traditional rice varieties submitted to different doses of ammonium nutrition. J. Plant Nutr., **44**, 2715-2728 (2021)
14. K. Guo, G. An, N. Wang, B. Pang, Z. Shi, H. Bai, ... W. Xu. Thymol ameliorates ammonium toxicity via repressing polyamine oxidase-derived hydrogen peroxide and modulating ammonium transporters in rice root. Food Prod. Process. Nutr., **3**, 1-9 (2021)
15. H. Zhou, Y. Zhou, F. Zhai, T. Wu, Y. Xie, G. Xu, C. H. Foyer. Rice seedlings grown under high ammonia do not show enhanced defence responses. Food Energy Secur., **11**, e331 (2022)
16. I. Coleto, M. de la Peña, J. Rodríguez-Escalante, I. Bejarano, G. Glauser, P. M. Aparicio-Tejo, ... D. Marino. Leaves play a central role in the adaptation of nitrogen and sulfur metabolism to ammonium nutrition in oilseed rape (Brassica napus). BMC Plant Biol., **17**, 1-13 (2017)
17. Y. Ontoria, A. Cuesta-Gracia, J. M. Ruiz, J. Romero, M. Perez. The negative effects of short-term extreme thermal events on the seagrass Posidonia oceanica are exacerbated by ammonium additions. *PLoS One*, **14**, e0222798 (2019)
18. D. R. Hoagland, D. I. Arnon. The water-culture method for growing plants without soil. Circular Calif. Agric. Exp. Stn., **347**, 2-13 (1950)
19. D. K. Tripathi, P. Rai, G. Guerriero, S. Sharma, F. J. Corpas, V. P. Singh (2021). Silicon induces adventitious root formation in rice under arsenate stress with involvement of nitric oxide and indole-3-acetic acid. *J. Exp. Bot.,* **72**, 4457-4471 (2019)