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Silver Nanoparticles Modulate Free Amino Acid and Metal Absorption in Rice Plants at the Seedling Stage

Mai Thi Phuong Nga*

University of Science and Technology of Hanoi, Vietnam Academy of Science and Technology, Hanoi 11300, Vietnam

Abstract

Rice (*Oryza sativa* L.) is a major staple crop whose production is increasingly constrained by environmental stresses. Therefore, there is a growing need for innovative strategies to enhance rice productivity sustainably. Silver nanoparticles (AgNPs) has emerged as a promising approach to improve stress tolerance and crop performance while potentially reducing fertilizer inputs and environmental impacts. In this study, IR64 rice seedlings were exposed to 0-100ppm AgNPs to assess their effects on free amino acid accumulation and metal uptake using high-performance liquid chromatography and inductively coupled plasma mass spectrometry. The responses were both concentration- and tissue-dependent, with increased total and non-essential amino acid levels but reduced essential amino acids at higher AgNP concentrations. The highest total amino acid concentration was obtained in rice plants grown in the 50 and 100ppm AgNP treatments, which reached approximately 22.8 mg g^{-1} plant dry weight. The highest amino acid content was detected in the serine family, which reached approximately 10 mg g^{-1}, while the aromatic family produced the lowest (approximately 0.76 mg g^{-1}). AgNP exposure reshaped metal uptake and partitioning in a metal- and tissue-specific manner, promoting metal sequestration in roots while suppressing root-to-shoot translocation. Calcium was the most abundant macroelement nutrient found in the roots of the plants. These findings provide insight into how AgNPs modulate amino acid metabolism and metal accumulation in rice, highlighting both their potential and limitations for agricultural applications.

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Correspondence to
Mai Thi Phuong Nga
mai-thi-phuong.nga@usth.edu.vn

ORCID
Mai Thi Phuong Nga
https://orcid.org/0000-0002-8492-4141

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Keywords

Amino acid metabolism, high-performance liquid chromatography, inductively coupled plasma mass spectrometry, mineral homeostasis, nutrient translocation

Introduction

Rice is the primary source of food for more than half of the global population and plays a central role in food security, particularly in

hydroponics, plants are grown without soil and are instead supplied with a nutrient-enriched Asia and developing countries (Mohidem *et al*., 2022). According to FAO (2025), the global rice output in the 2025-2026 season has been estimated at 556.4 million tonnes, of which Vietnam produces approximately 5% of the world's rice output. This places Vietnam among the top 5-6 rice producers worldwide..

Nanoparticles (NPs), which are defined as particles with at least one dimension ≤ 100nm, have rapidly emerged in agricultural science due to their unique physicochemical properties that allow them to interact with plant cells, tissues, and metabolic pathways in ways distinct from bulk materials (Fincheira *et al*., 2021). NPs have been used as nanofertilizers, stress ameliorants, and delivery vectors for agrochemicals (Pérez-de-Luque, 2017); however, their influence on plant primary metabolism, particularly free amino acid (FAA) and other metal absorption, remains complex and context-dependent.

Mechanisms of NP uptake and subsequent metabolic effects are influenced by factors such as NP composition, concentration, plant species, and environmental conditions (Silva *et al*., 2023). When plants are treated with NPs at low concentrations, NPs can stimulate the plant's development as well as its response to various stresses, and can be considered as biostimulators (Juárez-Maldonado *et al*., 2019).

Free amino acids serve as the primary bridge between carbon and nitrogen metabolism, and their fluctuations are among the most sensitive indicators of nanomaterial interaction. Studies on spinach have shown that a 0.25% nano-anatase (TiO) solution significantly enhanced the activities of the nitrate reductase, glutamine synthetase, and glutamate dehydrogenase enzymes, which accelerated the conversion of inorganic nitrogen into organic amino acids (Yang *et al*., 2006). NP-induced reactive oxygen species (ROS) trigger the accumulation of "stress-protective" amino acids, such as proline and gamma-aminobutyric acid (GABA**)**, which are frequently upregulated to serve as osmoprotectants and ROS scavengers when plants are exposed to metallic NPs like Ag or ZnO (Cao *et al.*, 2024). In wheat (*Triticum aestivum*), different metal oxide NPs have been shown to produce divergent effects: TiO_2 NPs at 500 mg kg^{-1} increased overall amino acid accumulation in the edible portion, Fe_2O_3 NPs at doses of 50 and 500 mg kg^{-1} selectively increased cysteine and threonine, while CuO NPs at doses of 50 and 500 mg kg^{-1} reduced the levels of several essential amino acids in grains (Wang *et al*., 2019). In water lettuce (*Pistia stratiotes* L.), plants incubated with 0.89 mg L^{-1} of zinc-NPs resulted in a decrease in the total content of amino acids (by 15%), and the contents of leucine, methionine, phenylalanine, proline, and tyrosine (by more than twice) (Olkhovych *et al.*, 2016). Meanwhile, when plants were treated with cadmium-NPs, a significant decrease in the total amino acids occurred due to respiratory inhibition (Zhang *et al*., 2024). This dichotomy suggests a dose, NP type, and plant species dependence. Understanding these interactions at mechanistic, physiological, and agronomic scales is critical for the sustainable use of nanotechnology in plant science.

Moreover, NPs enable interactions with plant roots, soils, and metal ions in complex ways. These interactions can modulate the uptake of other metals by altering metal bioavailability, speciation, soil physicochemistry, and plant physiology (Zhang *et al*., 2024). NPs may either reduce or enhance metal absorption depending on their type, concentration, and application method, and are also impacted by the plant species and soil medium. NPs can modify soil physicochemical properties, increasing pH or interacting with soil organic matter, causing metals to become less mobile and thus less absorbable by roots (Rizwan *et al*., 2021). Within plant tissues, NPs have been shown to inhibit translocation from roots to shoots by altering gene expression related to metal transporters and enhancing plant antioxidant systems that mitigate metal stress responses (Rizwan *et al.*, 2021). On the other hand, nanoparticles hold great potential for enhancing nutrient uptake. NPs can be used to enhance mineral nutrients, particularly micronutrients (Zn, Fe, Mn, B, etc.), in plants, which may positively influence plant growth under certain conditions (Raliya *et al.*, 2017). In

rice plants, a foliar spray of 5~25mM SiO_2 NPs increased the translocation of potassium (K), magnesium (Mg), and Fe from the uppermost nodes to the rachises, whereas SiO_2 NPs had no significant effects on the translocation of calcium (Ca), zinc (Zn), and manganese (Mn) (Chen *et al*., 2017). However, this decrease or increase in uptake of Fe, Cu, and Mn by plants depended on the plant organ and nutrient type (Wang *et al.*, 2015).

Although previous studies have reported the effects of nanoparticles on amino acid production and metal uptake in plants, a systematic, concentration-dependent assessment of silver nanoparticles (AgNPs) on both free amino acid metabolism and metal uptake is still lacking, particularly in rice. Therefore, this study aimed to evaluate the effects of different AgNP concentrations on free amino acid profiles and metal uptake in rice at the seedling stage. The findings are expected to advance our understanding of AgNP-mediated metabolic modulation and its potential role in improving rice growth and stress responses.

Materials and Methods

Sample growth

The study used the IR64 rice variety, which was originally developed by the International Rice Research Institute and supplied by the Plant Resources Center of Vietnam. In the present study, the rice seeds were first dried in an oven at 50°C for 72 hours before being dehulled. The seed surfaces were then disinfected with 4% sodium hypochlorite and 0.05% Tween 80 for 25 minutes, followed by a 1-minute exposure in 70% ethanol. After that, the seeds were washed five times in sterilized water before being grown in a medium containing ½ Murashige and Skoog medium (MS), 30 g L^{-1} sucrose, 15 g L^{-1} agar, and a range of filtered AgNPs (size ≤ 15nm) with concentrations from 0 to 100ppm (Nguyen *et al.*, 2023). The rice plants were grown in a culture room at 28°C and a 16/8h photoperiod for two weeks.

AgNPs used for the study

The polyvinylpyrrolidone (PVP)-coated AgNPs, which were previously observed using transmission electron microscopy, were polydispersed and spherical in morphology, with average diameters ranging from a few nanometers to less than 50nm (Nguyen *et al.*, 2024). Cupric ions can significantly accelerate the dissolution rate of PVP-AgNPs when dispersed in a sodium chloride solution. The size of the AgNPs was characterized by dynamic light scattering (DLS), and their zeta potential was determined using a nanoparticle analyzer SZ100V (Horiba, Japan).

Plant sample collection

After two weeks, the rice seedlings were harvested and cleaned with milli-Q water. Then, they were dried at 60°C in an oven for 7 days until they reached a constant weight.

Sample preparation for FAA analysis

The dried samples were ground and mixed in a pH 2.0 acetate buffer. The mixture was stirred for an hour at room temperature and then centrifuged at 13,000rpm for 5 minutes. After that, the supernatant was filtered by using a 0.45 µm pore membrane (Sartorius AG, Germany), before being analyzed by high-performance liquid chromatography (HPLC) using a Biochrom 30+ system (Nguyen *et al.*, 2023).

HPLC analysis of free amino acid profiles

An HPLC method, which used ninhydrin derivatization and acetate buffers, was used to analyze the free amino acids (FAAs) in the rice plant samples. A 1-µL sample was injected per run, with a 60-minute separation time. The FAA contents were quantified by comparing peak areas to a standard amino acid curve (Thermo Scientific) (Jia *et al.*, 2018).

Metal measurement

The metal contents in the roots and shoots of the plants were analyzed using inductively coupled plasma mass spectrometry (ICP-MS), as described by Mozhayeva and Engelhard (2020). Briefly, after being dried to a constant weight, the inorganic matter in each 0.3g plant sample was removed by heating the sample at 550°C for 6h in a muffle furnace (Nabertherm, New Castle,

DE, USA). Then, wet ashing of the sample was performed by acid hydrolysis with HCl and a few drops of HNO_3. Next, the mixture was heated for 1h. After cooling, the samples were dissolved in 20mL of Milli-Q water, centrifuged at 13,000rpm for 5 minutes, filtered through a 0.45-µm pore membrane filter (Sartorius AG, Germany), and analyzed by ICP-MS (model: iCAP RQ). The concentration of metals in each plant sample was calculated by the formula:

$$\text{Metal concentration } (\frac{\text{mg}}{\text{kg}}) = \frac{\text{C} * \text{V} * \text{F}}{\text{W}}$$

in which C is the concentration of metal in the sample (mg L^{-1}); V is the volume of the sample (mL); W is the weight of the sample (g); and F is the dilution factor (if it exists).

Statistical analysis

The experiment was conducted using biological triplicates. For each condition, a minimum of 10 plants were analyzed. Statistical differences among parameters were assessed using one-way analysis of variance (ANOVA) and the post-hoc Tukey test in GraphPad Prism version 10, applying a significance threshold of P <0.05. Data are presented as mean ± standard deviation.

Results and Discussion

Characterization of AgNPs

The AgNPs were characterized by DLS and zeta potential. DLS analysis exhibited a relatively narrow particle size distribution within the nanometer range, indicating moderate uniformity and dispersion stability, and a Z-average of 47.1nm, which is suitable for biological applications (**Figure 1A**). The zeta potential was measured at -27.1mV, indicating that the AgNP suspension was relatively homogeneous and well dispersed without major subpopulations or extensive aggregation (**Figure 1B**). The absence of multiple peaks suggests that the nanoparticles maintained good colloidal uniformity in the dispersion medium. The smaller size observed by SEM in the previous study compared with DLS can be attributed to methodological differences: DLS measured the diameter of particles in suspension, which included the hydration shell and potentially small aggregates, whereas TEM measured the dry core diameter of individual nanoparticles.

The total FAA content in rice plants in response to varying concentrations of AgNPs

Figure 2 shows the total FAA content (mg g^{-1}) in the rice plants grown under various environmental conditions. The results clearly showed that AgNP application significantly affected the total FAA content in a concentration-dependent manner. The higher the AgNPs used, the higher the total FAA content obtained. In the control and the 12.5ppm AgNPs treatment, the total FAA content remained relatively low and statistically similar (P <0.05), indicating that very low AgNP concentrations did not substantially alter amino acid metabolism compared with the negative control. In the 25 ppm AgNPs treatment, a significant increase in the total FAA content was observed, which reached approximately 21 mg g^{-1}, suggesting that moderate AgNP exposure stimulated FAA accumulation. In the 50 and 100ppm AgNPs treatments, the highest FAA contents were statistically different from each other (P <0.05), indicating a strong activation effect that reached a plateau at higher concentrations. Overall, AgNPs at 25ppm and higher significantly enhanced the total FAA accumulation, with the maximum effect observed at 50-100ppm, reaching approximately 22.8 mg g^{-1}.

The distribution of total FAA in five families in response to AgNPs

Figure 3 presents the total FAA content (mg g^{-1}) in the rice plants grown under various environmental conditions supplemented with AgNPs and is grouped into five amino acid families, namely aspartate, pyruvate, serine, glutamate, and aromatic.

The highest amino acid content was detected in the serine family, which reached approximately 10 mg g^{-1}, while the aromatic family produced the lowest (approximately 0.76 mg g^{-1}). Among the individual amino acids, serine was the most abundant, whereas tryptophan was not obtained in any sample, which could be explained by the stringent feedback regulation of its biosynthesis, rapid

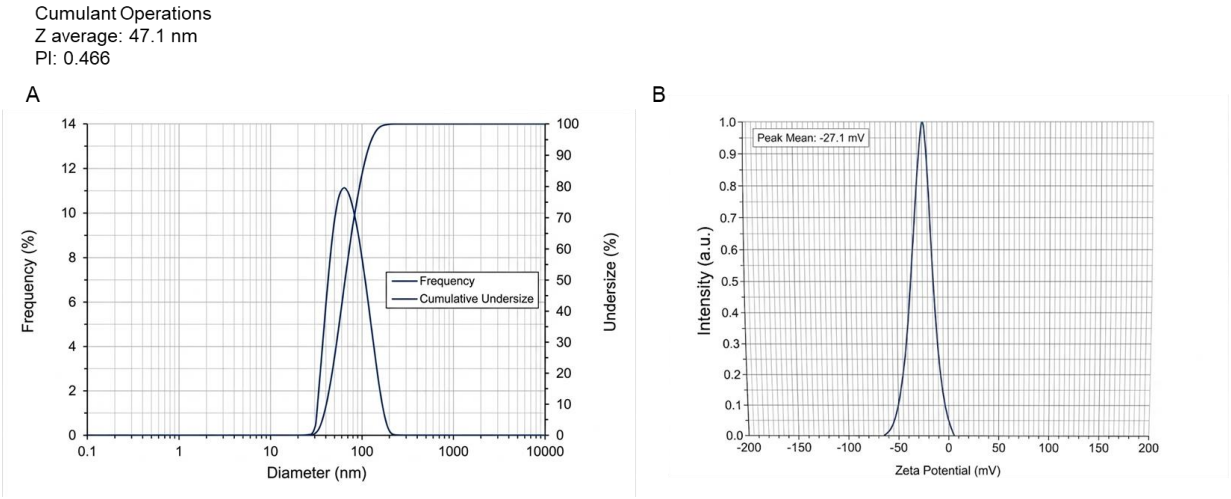


Figure 1. (A) The size distribution analysis of the AgNP solution by dynamic light scattering (DLS). (B) Zeta potential of AgNPs using a nanoparticle analyzer, HORIBA SZ-100.

metabolic channeling into secondary metabolite pathways, high energetic cost of synthesis, and preferential incorporation into proteins. Significant increases (P <0.05) in the contents of the glutamate and serine families were observed, whereas slightly decreasing or stable trends were obtained in the contents of the aromatic, pyruvate, and aspartate families, together with the increased concentration of the AgNPs used. These trends indicated that AgNP exposure promoted the accumulation of primary nitrogen assimilation–associated amino acids, while suppressing amino acid families associated with energy-intensive or secondary metabolic pathways.

Essential and non-essential amino acids in response t AgNPs

Figure 4 presents the essential (EAA) and non-essential amino acid (NEAA) contents (mg g^{-1}) in the rice plants grown under various environmental conditions supplemented with AgNPs. The data indicated that exposure to different concentrations of AgNP regulated the EAA and NEAA contents in the rice plants in a concentration-dependent manner. While the total FAA content increased progressively with increasing AgNP concentrations (from 18.50 mg g^{-1} at 0ppm to approximately 22.8 mg g^{-1} at 100ppm), this increase was driven predominantly by NEAAs, whereas EAAs showed a consistent decline across the AgNP gradient.

Specifically, the EAA content decreased from approximately 3.14 mg g^{-1} in the control to 2.35 mg g^{-1} at 100ppm, representing an approximate 25 % reduction. In contrast, the NEAAs increased substantially from approximately 15.36 mg g^{-1} (at 0ppm) to 20.60 mg g^{-1} (at 100ppm), accounting for nearly all of the observed increase in the total FAA content. Consequently, the EAA-to-FAA ratio declined with increasing AgNP concentrations, indicating a shift in amino acid allocation rather than a uniform enhancement of amino acid biosynthesis.

Effects of AgNPs on metal uptake and accumulation in rice plants

The data clearly demonstrated that the AgNPs significantly affected the accumulation and partitioning of essential mineral elements (Zn, Ca, Mn, Fe, and Cu) in the rice plants, with roots (R) acting as the primary site of metal accumulation across all AgNP concentrations used (0-100ppm). In contrast, metal translocation to shoots (S) was generally reduced or stabilized, particularly at higher AgNP doses (**Figure 5**).

Overall, AgNP exposure induced a dose-dependent and organ-specific response. Among the five investigated metals, the concentration of calcium (Ca) was found to be the highest and increased with each increase in the concentration of AgNP used. It reached the maximum of approximately 1440 mg kg^{-1} dry roots sample of

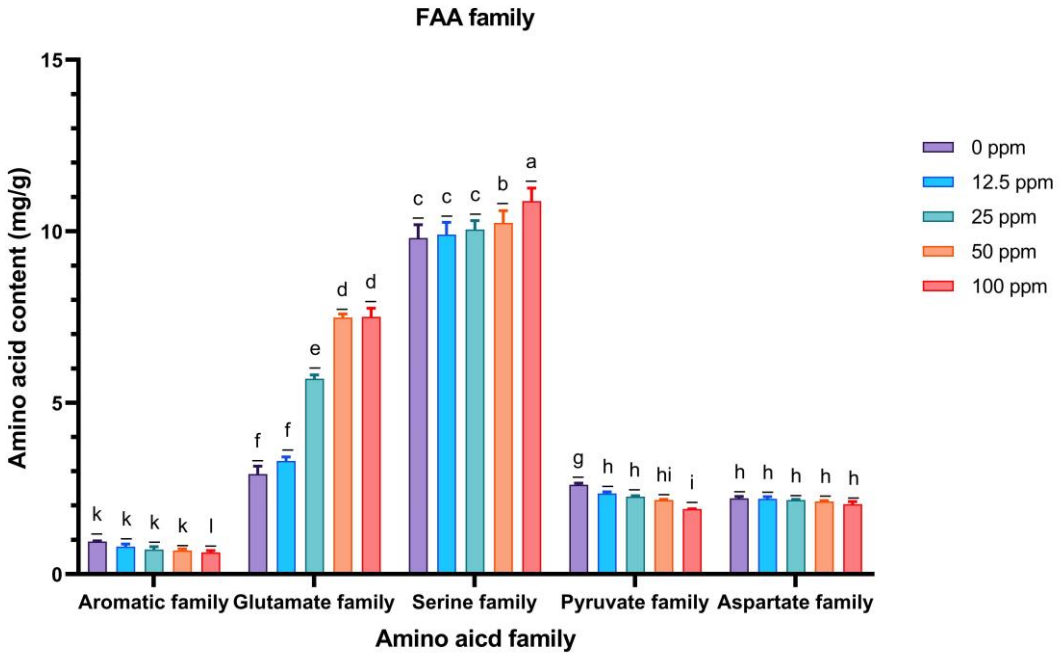


Figure 3. Production of free amino acid families in the rice plants in response to silver nanoparticles. Different letters indicate significant differences among FAA families in different AgNP conditions with *P* <0.05.

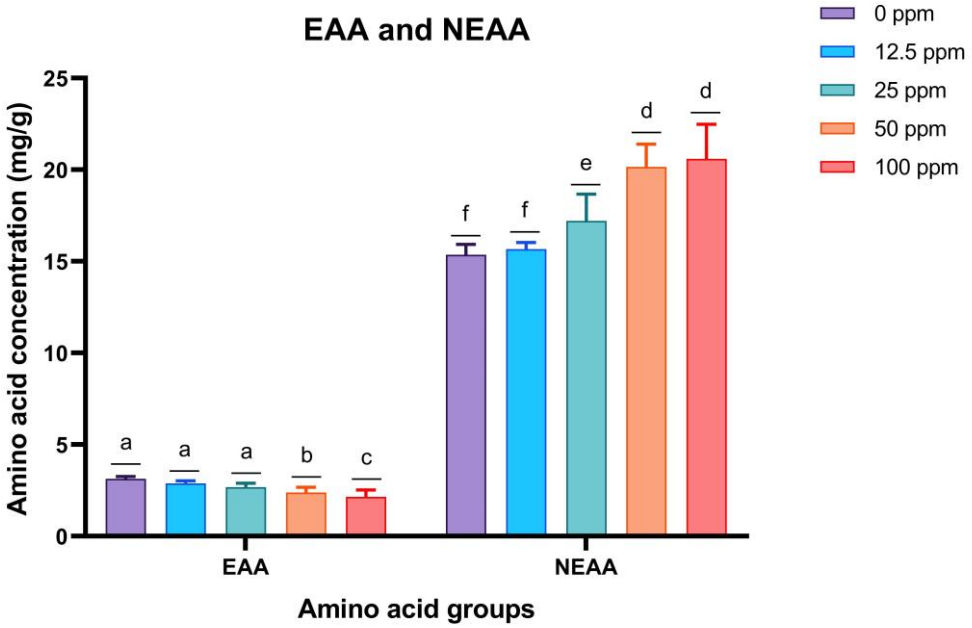


Figure 4. Production of amino acid families in the rice plants in response to silver nanoparticles. Different letters indicate significant differences between EAA and NEAA produced in different AgNP conditions with *P* <0.05.

the rice plants grown in the medium supplemented with 100ppm of AgNPs. In the shoots, the Ca concentration increased only moderately. The manganese (Mn) content showed moderate fluctuations, with no clear dose-dependent accumulation in the roots, while shoot Mn tended to decrease at higher AgNP concentrations. Iron (Fe) accumulation fluctuated in the range of the AgNP concentrations used, whereas Fe in the shoots declined sharply, especially at 100ppm, where it was recorded at 22.25 mg kg^{-1} dry shoot sample. Copper (Cu) showed a dose-dependent increase in the roots, reaching a maximum of approximately

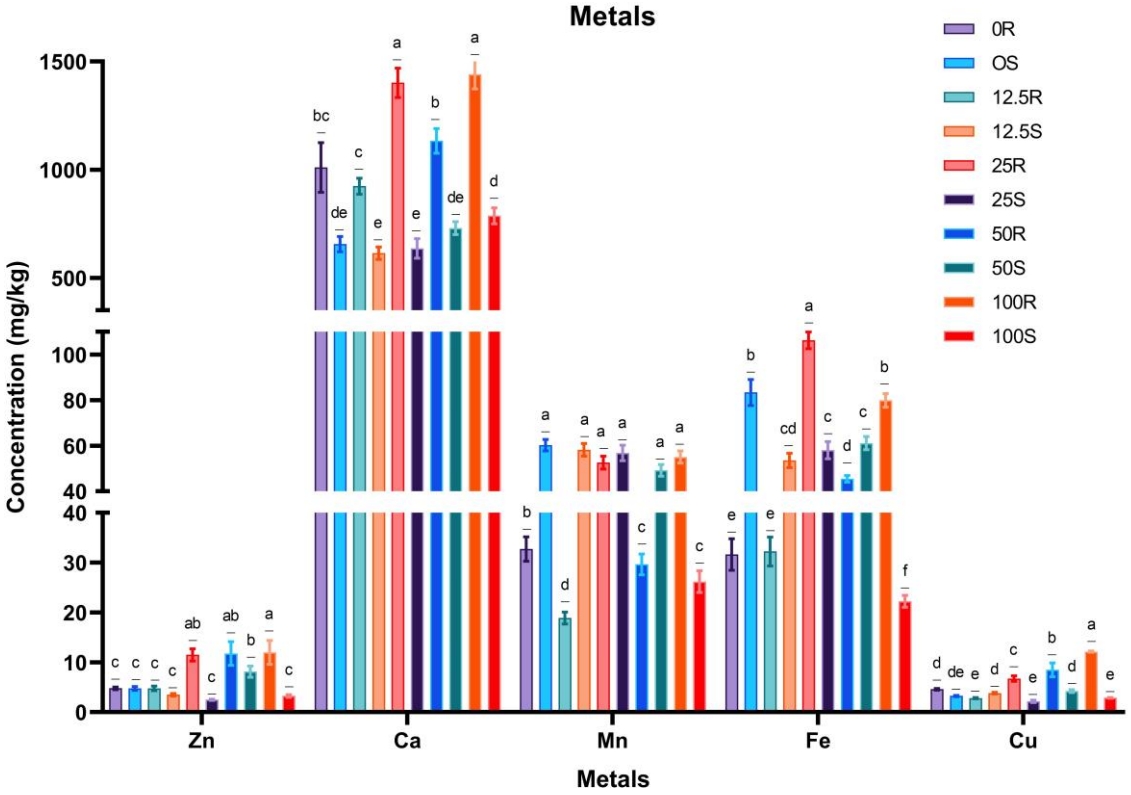


Figure 5. Metal accumulation in the rice plants in response to silver nanoparticles. R (roots), S (shoots). Different letters indicate significant differences between each metal accumulation in different AgNP conditions with P <0.05.

12.16 mg kg^{-1} dry sample at 100ppm, while Cu in shoots decreased substantially.

In summary, AgNP exposure from 0 to 100 ppm profoundly modified metal homeostasis in a metal-specific and tissue-specific manner in the rice plants, promoting root accumulation while generally reducing translocation to shoots at higher concentrations. These findings highlight the risk of high AgNP accumulation in agricultural systems, and the need for careful evaluation of nanoparticle concentrations in plant-soil environments is required.

Discussion

AgNPs mediate the total FAA produced in rice plants

Exposure to silver nanoparticles (AgNPs) markedly increased the total free amino acid (FAA) content in rice plants, indicating a substantial reprogramming of nitrogen and amino acid metabolism in response to nanoparticle-induced stress. Ullah & Ali (2025) suggested that this response is mediated by reactive oxygen species (ROS)-dependent signaling, which regulates nitrogen assimilation and amino acid biosynthesis under abiotic stress conditions.

AgNP-induced ROS generation has been shown to modulate key nitrogen-assimilating enzymes, including nitrate reductase (NR), glutamine synthetase (GS), and glutamate synthase (GOGAT) (Kishorekumar *et al.*, 2020), thereby expanding the primary nitrogen pool and explaining the elevated FAA levels observed at moderate AgNP concentrations (25-50ppm). Similarly, in the *Indica* rice (*Oryza sativa* L.) cultivar Huanghuazhan, AgNPs triggered ROS production and prompted plants to accumulate FAAs as compatible solutes and antioxidants. This response enhanced protein synthesis for repair and activated pathways like ammonia assimilation, boosting amino acid pools to buffer toxicity. At low doses (0.5-5 mg L^{-1}), such accumulation supported growth, whereas higher doses might inhibit it (Pan *et al.*, 2024). The increased FAA levels at 50-100ppm thus likely

reflect a balance between enhanced amino acid synthesis and protein turnover.

AgNPs redistributed the EAA and NEAA production in rice plants

Exposure to increased concentrations of AgNP increased NEAAs, whereas EAAs showed a consistent decline across the AgNP gradient. Differently, AgNPs alone upregulated EAA (e.g., phenylalanine, leucine) and some NEAAs (e.g., aspartic acid) in *Oryza sativa* L. cv. Chakhao Poireiton (Solanki *et al*., 2023), indicating redistribution toward higher production of specific profiles for better nutrition.

NEAAs such as glutamate, glutamine, aspartate, alanine, and proline are directly linked to the GS/GOGAT cycle and primary nitrogen assimilation (Mokhele *et al*., 2012). AgNP exposure has also been shown to modulate the activity of NR, GS, and GOGAT, leading to enhanced ammonium assimilation and expansion of the glutamate/glutamine pool (Kishorekumar *et al*., 2020). These amino acids act as nitrogen donors for multiple transamination reactions and accumulate rapidly under stress conditions. The strong increase in NEAAs observed at AgNP levels higher than 25ppm was therefore consistent with the enhanced nitrogen flux through GS/GOGAT, increased transamination activity, accumulation of stress-related amino acids functioning as osmoprotectants, and redox buffers.

In contrast, EAAs such as lysine, methionine, threonine, valine, leucine, isoleucine, phenylalanine, and tryptophan are synthesized through complex, tightly regulated, and energy-intensive pathways, including the aspartate-family pathway and the shikimate pathway. The observed decline in EAAs with increasing AgNP concentrations suggests metabolic prioritization, where plants favor the rapid accumulation of NEAAs that directly contribute to stress tolerance rather than the synthesis of EAAs required primarily for protein assembly (Luo *et al*., 2025). Similar shifts in amino acid profiles under nanoparticle treatments have been reported in multiple plant systems. For example, Pan *et al*. (2024) reported that AgNP-induced oxidative stress in rice activated ammonia assimilation and proline biosynthesis, while inhibiting growth-associated anabolic pathways. Likewise, Gupta (2020) showed that nanoparticle exposure increased the total FAA accumulation, dominated by NEAAs. Comparable patterns have also been observed under cadmium and zinc oxide nanoparticle stress, where NEAA enrichment was associated with enhanced stress tolerance, whereas EAA biosynthesis was suppressed due to metabolic cost and pathway sensitivity (Wang *et al.*, 2023).

AgNPs affected the production of amino acid families

The strong accumulation of the glutamate family suggested enhanced activity of the GS/GOGAT cycle, which assimilates ammonium into glutamine and glutamate. These amino acids serve as central nitrogen donors for transamination reactions, precursors for proline and arginine synthesis, and key metabolites in redox and osmotic stress responses. Similar results were also observed in wheat plants treated with various metal oxide nanoparticles (e.g., ZnO, TiO_2, Fe_3O_4) (Wang *et al*., 2019). The gradual increase in the serine family may be associated with enhanced photorespiratory flux, a common response to oxidative stress. Serine and glycine play critical roles in redox balancing and rapid nitrogen recycling (Misra, 2014). AgNP-induced stress might therefore stimulate photorespiration as a protective mechanism, leading to increased accumulation of serine-family amino acids. The decline in the aromatic family possibly indicated inhibition of the shikimate pathway, which is highly sensitive to oxidative stress and redox imbalance (Refaey *et al.*, 2015). Under AgNP stress, metabolic resources were likely diverted away from secondary anabolic pathways toward stress mitigation, resulting in reduced accumulation of free aromatic amino acids. The reduction in the pyruvate family suggested impaired branched-chain amino acid biosynthesis in rice plants, which is energetically costly and sensitive to mitochondrial dysfunction (Kim *et al.*, 2023). Until now, little information has been published about the effect of nanoparticles on the

redistribution of FAA families in plants; our study provides new insights into this subject.

AgNPs affect the metal accumulation

Among the five investigated metals, Ca was accumulated the highest under the AgNP treatments. Ca accumulation is commonly associated with stress signaling and membrane stabilization (Groenendyk *et al*., 2021). AgNP-induced oxidative stress likely activates Ca influx channels, leading to elevated cytosolic Ca^{2+} signaling, and enhanced deposition of Ca in root tissues to reinforce cell walls and membranes. However, limited Ca translocation to shoots suggested that the AgNPs interfered with transpiration-driven transport or damaged vascular functionality.

Mn is tightly regulated due to its dual role as an essential cofactor and potential toxin (Alejandro *et al*., 2020). The relatively stable Mn levels across the rice plant samples indicated the strong homeostatic control and the possible competition between Ag^{+} ions and Mn^{2+} at uptake sites. The suppression of Mn translocation could be because AgNP toxicity increases at high concentrations.

The Fe content in the shoots declined strongly according to the increasing concentrations of AgNPs used, which could be explained by the fact that AgNPs may disrupt Fe homeostasis by interfering with Fe(III)-chelate reductase activity, affecting Fe transporters, and inducing rhizosphere redox changes that limit Fe mobility. The reduced shoot Fe accumulation could impair chlorophyll synthesis up to 96.1 % and photosynthesis, consistent with reported NP-induced chlorosis in rice when high concentrations are used (Wang *et al.,* 2016).

Cu is highly redox-active and potentially toxic at elevated levels (Mir *et al.,* 2021). The reduction of Cu translocation from roots to shoots in the rice plants treated with AgNPs prevented oxidative damage to photosynthetic tissues (Pan *et al*., 2024). This pattern aligns with Cu immobilization observed under heavy metal and nanoparticle stress conditions. A recent study found that AgNPs reduced the root-to-shoot translocation of the heavy metal cadmium in tobacco, thereby mitigating cadmium-induced oxidative damage through improved antioxidant responses (Jabeen *et al.,* 2025).

Besides the beneficial effects on plants at low concentrations, nanoparticles can also have harmful effects at high concentrations. Mirzajani *et al.* found that AgNPs at 30 mg L^{-1} accelerated root growth of *Oryza sativa* L., while 60 mg L^{-1} of AgNPs restricted root growth (Mirzajani *et al.*, 2013). In our screening experiments, concentrations of AgNPs higher than 100 mg L^{-1} also inhibited rice plant growth. This dose–response effects seem like "hormesis" effects, in which low stress generated by ROS induces adaptive responses, while high stress by ROS inhibits plant growth. AgNPs at high concentrations can also pose significant environmental risks due to their persistence, transformation, and high biological reactivity (Lowry *et al.*, 2012). Taken together, the results from the present study indicate that AgNP-induced stress in rice operates through a systemic mechanism in which initial root exposure leads to nanoparticle uptake and translocation, metabolic reprogramming, and nutrient imbalance. These processes are tightly interconnected, affecting amino acid metabolism while modifying metal homeostasis and plant growth (Mai *et al*., 2025).

Conclusions

The present study, for the first time, investigated the effect of AgNPs on FAA, EAA, NEAA, and metal redistribution in rice plants, and indicated that the effect of AgNPs on free amino acid production and metal accumulation in rice plants is concentration- and tissue–dependent. Total FAA and NEAA production in plants increased when the AgNP concentration used increased, while the EAA contents decreased. The AgNPs differentially regulated metal uptake and partitioning, depending on the metal type and plant tissue, favoring root accumulation while restricting shoot translocation at higher concentrations. Overall, this study provides deeper insight into the modulation of FAA metabolism and metal accumulation in IR64 rice plants exposed to

varying AgNP concentrations, thereby contributing to a better understanding of the potential and limitations of nanoparticle application in future agricultural practices. Future studies should focus on elucidating Ag accumulation in rice tissues and the molecular mechanisms underlying AgNP-mediated alterations in nutrient uptake and translocation.

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