

Free amino acid production in response to selenium nanoparticles in rice plants

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Abstract. Rice (*Oryza sativa* L.) is the primary food source for nearly half of the world's population. Selenium nanoparticles (SeNPs) have emerged as promising agents in plant science because of their ability to mitigate both biotic and abiotic stresses, thereby enhancing plant productivity. Free amino acids (FAA) are key metabolites involved in plant stress responses and homeostatic regulation. The present study investigated the FAA profiles in response to SeNP treatment doses ranging from 0 to 25 ppm in two-week-old rice plants. The content of FAA was determined by means of high-performance liquid chromatography. The results demonstrated a dose-dependent increase in FAA accumulation, with the highest content when treated with 25 ppm of SeNPs. The maximum FAA content in shoots is approximately 33 mg/g dry weight, compared with 11–13 mg/g in roots. Moreover, increased concentration of SeNPs stimulated total FAA production more significantly. The aspartate family constituted the largest fraction of total FAA, comprising approximately 50% in the 25 ppm treatment. Additionally, essential amino acid levels increased dramatically with higher SeNP concentrations, reaching approximately 23 mg/g in shoots when treated with 25 ppm of SeNPs. The accumulation of lysine, threonine, and serine was notably enhanced in response to SeNP treatment, suggesting their involvement in protective or compensatory metabolic pathways under stress conditions. Among the FAAs, lysine exhibited the highest level of accumulation, indicating a potentially prominent role in the plant's adaptive response to SeNP-induced stress. These findings highlight the SeNP-induced modulation of FAA metabolism in rice, suggesting a potential mechanism for enhancing stress resilience through targeted amino acid biosynthesis.

Keywords: amino acid, high-performance liquid chromatography, rice, selenium nanoparticles, stress

1 Introduction

Free amino acids (FAAs) are not only the building blocks of proteins but also play diverse roles in plant growth, development, and stress responses [1]. They act as signaling molecules, osmotic protectants, precursors of stress-related metabolites, membrane stabilisation, and modulators of cellular redox status [2]. Moreover, plants also adjust the FAA profile in response to environmental stresses such as drought, salinity, cold, heat, and pathogen attack. Proline is the most studied FAA regarding drought stress. It

accumulates to stabilise proteins and membranes and scavenge reactive oxygen species [3], while salinity stress triggers the accumulation of proline, γ -aminobutyric acid (GABA), and branched-chain amino acids (BCAAs), such as valine, leucine, and isoleucine, which help in maintaining ion balance and mitigating oxidative damage [4]. Proline and GABA also contribute to membrane protection and reactive oxygen species scavenging under temperature stress [5].

Furthermore, FAAs are also involved in response to the biotic stresses of plants. Free

amino acids are crucial in plant defense against pathogens and herbivores, in which proline and GABA have been documented [6]. Free amino acids have been demonstrated to act as signaling molecules regulating gene expression [7]. Glutamate receptors were involved in long-distance signaling during wounding in *Arabidopsis thaliana* [8].

Numerous attempts have been made to target to FAA metabolism pathway to help plants have better adaptation to stresses. Overexpression of P5CS (Δ^1 -pyrroline-5-carboxylate synthase) enhanced proline production and salt tolerance in transgenic potato plants [9]. Manipulating GABA metabolism improves tolerance to salt stress [10].

Nanoparticles have received increased attention recently because of their wide applications, particularly in plant biotechnology. Several studies have explored the impact of nanoparticles on amino acid biosynthesis in plants. For instance, metal oxide nanoparticles have been shown to influence amino acid production in wheat grains (*Triticum aestivum*) [11]; silver nanoparticles have been studied in tomato [12], and copper and zinc nanoparticles have been evaluated in *Pistia stratiotes* L. (Araceae) [13]. In plant systems, SeNPs have been shown to play multifaceted roles, including the enhancement of growth, modulation of metabolic activities, and improvement of tolerance to environmental stresses. SeNPs can enhance the activity of antioxidant enzymes such as superoxide dismutase, catalase, and peroxidase, which help in scavenging reactive oxygen species generated under stress conditions [14]. Despite these findings, the role of SeNPs in modulating the production of FAAs in rice has not been previously investigated. Therefore, the present study aimed to understand how FAAs in IR64 rice plants were regulated in response to SeNPs.

2 Materials and methods

2.1 Plant materials

The IR64 rice accession, which has high nutrient value and a short life cycle, was used to determine its FAA content in response to SeNPs.

2.2 Rice plant's growth

The IR64 rice seeds were first dried for three days at 50 °C in an oven. The seeds were then sterilised [15] with 70% ethanol, TWEEN 80, and Javel (4%). Finally, the seeds were kept overnight at 28 °C in a dark place in sterilised water to stimulate germination. On the following day, 10 sterilised rice seeds were planted each in a 50 mL solid, half-strength Murashige and Skoog (MS) medium containing varying concentrations of SeNPs (Sigma-Aldrich): 0, 1.5625, 3.125, 6.25, 12.5, and 25 ppm. On the fourteenth day, the samples were collected and analysed for their FAA profile.

2.3 Sample preparation for analysis

The plants grown under different conditions were dried at 60 °C for a week, then ground and mixed in a pH 2.0 acetate buffer. The mixture was stirred for an hour, centrifuged, and the supernatant was filtered before analysis by means of HPLC on a Biochrom 30+ system (Biochrom, Cambridge, England) [16].

2.4 HPLC analyses of the free amino acid profile

A HPLC system with post-column ninhydrin derivatisation and acetate buffers was used to analyse FAAs. A 1 μ L sample was injected per run, with a 60-minute separation period. The FAA levels were quantified by comparing peak areas to a standard amino acid curve (Thermo Scientific) [17].

2.5 Statistical analysis

The experiment was conducted in triplicate. For each condition, a minimum of 10 plants were analysed. Statistical differences among parameters were assessed by using one-way analysis of variance (ANOVA) and post-hoc Tukey test in GraphPad Prism, with a significance threshold of $p < 0.05$. The data were presented as mean \pm standard deviation

3 Results

3.1 Total FAA content in rice plants in response to varying concentrations of SeNPs

Fig. 1 shows the total FAA content (mg/g) in rice plants grown under various conditions. The free amino acid content exhibited significant variation in response to different SeNP concentrations. It tends to be higher in shoots compared with their root counterparts at the same concentrations. Moreover, increased concentration of SeNPs stimulated more total FAA production. The highest FAA content was observed in the shoots of the plants supplemented with 25 ppm SeNPs, where it achieved approximately 33 mg/g sample, followed by 12.5 ppm ($p < 0.05$). In contrast, the root samples exhibited relatively constant FAA content at approximately 11–13 mg/g, regardless of the concentration of SeNPs. This might suggest that shoots have a dose-responsive accumulation of FAA, while roots possibly have a mechanism limiting FAA accumulation under increasing concentrations.

3.2 Distribution of total FAA in five families in response to SeNPs

Fig. 2 presents the total FAA content (in mg/g) in rice plants grown under various conditions supplemented with SeNPs and is grouped by amino acid family: aspartate, pyruvate, serine, glutamate, and aromatic. The aspartate family

consistently contributed the most to total FAA content under nearly all conditions, particularly under the 25 ppm SeNP condition, where it was dramatically high, accounting for about 50% of the total FAA production. The root samples tended to have a lower FAA content than the shoots at the same concentrations.

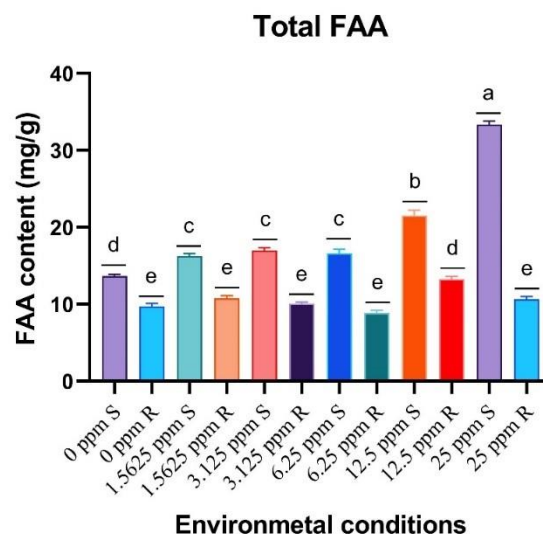


Fig. 1. Total free amino acid production in rice plants in response to selenium nanoparticles

Notes: R (roots), S (shoots). Different letters indicate significant differences between FAA in different conditions with p less than 0.05.

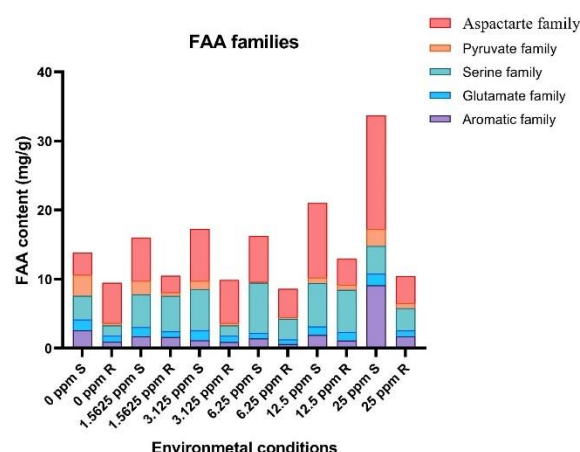


Fig. 2. Amino acid family's production of rice plants in response to selenium nanoparticles

Notes: R (roots), S (shoots)

The pyruvate family exhibited a slight increase in the FAA content under certain conditions (e.g., 12.5 ppm in roots and 25 ppm in shoots), but still represented a small fraction of the total FAA. The glutamate and aromatic families contributed the least under nearly all conditions, although the aromatic family presented an increasing content in the shoot at 25 ppm. The serine family showed moderate and consistent presence across most of the treatments and samples.

3.3 Individual amino acid composition in response to SeNPs

Table 1 presents the concentration of various FAAs produced by IR64 rice plants grown in the media supplemented with increasing concentrations of SeNPs. The data indicate that the response of amino acids to the increasing levels of SeNPs varied significantly and appeared to be amino-acid-specific. Notably, the lysine content in the rice plants exhibited a non-linear response to the increasing SeNP concentrations. At the lowest concentration (1.5625 ppm), the lysine level decreased compared with the control (0 ppm). However, a marked increase was observed at 3.125 ppm (11.86 mg/g), indicating a stimulatory effect of SeNPs at this concentration.

Although the lysine level declined slightly at 6.25 and 12.5 ppm, it remained higher than that of the control and most other amino acids. At 25 ppm, the lysine content increased again (10.5 mg/g), suggesting a possible second peak in the dose-response relationship. These results imply that SeNPs can enhance lysine biosynthesis in rice, particularly at moderate concentrations, although the response is dose-dependent and may involve complex regulatory mechanisms. The threonine content increased significantly at 12.5 ppm and 25 ppm of SeNPs, where it achieved a content of 3.98 and 4.91 mg/g, respectively. Serine also exists at an elevated level at higher SeNP concentrations, except 25 ppm. However, the arginine and histidine content tended to decrease or remained low with increasing SeNPs. Aspartic acid was undetectable at all concentrations of SeNPs except at 25 ppm, while the NLeucine and valine content remained low across all treatments.

Overall, the stimulation or suppression of amino acid synthesis may occur depending on the concentration of SeNPs, suggesting metabolic shifts or stress responses. Some amino acids (e.g., lysine, threonine, serine) might be upregulated as a protective or compensatory mechanism in response to SeNPs.

Table 1. Individual free amino acid production in rice plants in response to SeNPs

Item	0 ppm	1.5625 ppm	3.125 ppm	6.25 ppm	12.5 ppm	25 ppm
Tryptophan	1.62 ± 0.02 ^b	0.29 ± 0.03 ^d	0.23 ± 0.01 ^d	1.01 ± 0.01 ^c	0.08 ± 0.01 ^e	4.94 ± 0.58 ^a
Tyrosine	1.32 ± 0.1 ^b	0.90 ± 0.1 ^c	1.12 ± 0.1 ^b	0.32 ± 0.12 ^d	1.33 ± 0.05 ^b	2.58 ± 0.15 ^a
Phenylalanine	0.59 ± 0.08 ^d	2.13 ± 0.15 ^b	0.72 ± 0.04 ^d	0.68 ± 0.14 ^d	1.64 ± 0.12 ^c	3.27 ± 0.22 ^a
Proline	0.13 ± 0.01 ^d	0.00	0.71 ± 0.15 ^a	0.21 ± 0.02 ^c	0.49 ± 0.03 ^b	0.96 ± 0.13 ^a
Arginine	0.31 ± 0.02 ^b	0.61 ± 0.02 ^a	0.32 ± 0.02 ^b	0.09 ± 0.01 ^c	0.07 ± 0.01 ^c	0.03 ± 0.01 ^c
Histidine	0.33 ± 0.01 ^c	0.52 ± 0.02 ^b	0.27 ± 0.04 ^c	0.64 ± 0.02 ^a	0.72 ± 0.04 ^a	0.18 ± 0.05 ^c
Glutamic	1.71 ± 0.02 ^a	1.06 ± 0.03 ^c	1.04 ± 0.02 ^c	0.52 ± 0.03 ^d	1.18 ± 0.02 ^c	1.40 ± 0.06 ^b
Cysteic Acid	0.49 ± 0.03 ^a	0.48 ± 0.01 ^a	0.55 ± 0.06 ^a	0.31 ± 0.03 ^b	0.39 ± 0.03 ^b	0.36 ± 0.01 ^b
Serine	0.95 ± 0.06 ^d	4.36 ± 0.25 ^b	4.10 ± 0.51 ^b	6.24 ± 0.66 ^a	5.54 ± 0.46 ^a	2.89 ± 0.21 ^c

Item	0 ppm	1.5625 ppm	3.125 ppm	6.25 ppm	12.5 ppm	25 ppm
Glycine	1.17 ± 0.02 ^e	2.64 ± 0.2 ^a	2.12 ± 0.02 ^b	1.67 ± 0.16 ^d	2.33 ± 0.03 ^a	1.74 ± 0.02 ^c
Cysteine	2.32 ± 0.23 ^b	2.37 ± 0.15 ^b	0.61 ± 0.03 ^c	2.00 ± 0.15 ^b	4.12 ± 0.23^a	2.29 ± 0.11^b
Alanine	2.75 ± 0.23 ^a	2.11 ± 0.18 ^b	1.12 ± 0.02 ^c	0.15 ± 0.01 ^d	1.29 ± 0.02 ^c	2.73 ± 0.15 ^a
NLeucine	0.21 ± 0.01 ^a	0.26 ± 0.03 ^a	0.15 ± 0.03 ^b	0.05 ± 0.01 ^c	0.06 ± 0.01 ^c	0.10 ± 0.01 ^b
Valine	0.38 ± 0.02 ^a	0.13 ± 0.01 ^b	0.30 ± 0.03 ^a	0.20 ± 0.01	0.20 ± 0.02 ^b	0.28 ± 0.03 ^a
Isoleucine	0.32 ± 0.02 ^c	0.87 ± 0.04 ^b	0.32 ± 0.01 ^c	0.00	0.03 ± 0.01 ^d	1.63 ± 0.15 ^a
Threonine	0.87 ± 0.03 ^c	0.77 ± 0.03 ^c	0.74 ± 0.04 ^c	0.44 ± 0.03 ^d	3.98 ± 0.19^b	4.91 ± 0.45^a
Methionine and derivatives	2.49 ± 0.15 ^b	3.59 ± 0.35 ^a	0.82 ± 0.05 ^e	1.40 ± 0.02 ^d	2.82 ± 0.12 ^b	2.21 ± 0.15 ^c
Lysine	5.32 ± 0.35^c	3.45 ± 0.14^d	11.86 ± 0.86^a	8.87 ± 0.55^b	7.75 ± 0.38^b	10.50 ± 0.85^a
Aspartic	n.d	n.d	n.d	n.d	n.d	1.08 ± 0.02 ^a

Notes: R (roots), S (shoots), n.d (not determined). The concentration of the amino acid is presented as mg/g sample. Different letters indicate significant differences between FAA under different conditions, with *p* less than 0.05.

3.4 Analysis of essential amino acid and non-essential amino acid biosynthesis in rice plants

Fig. 3 illustrates the essential amino acids (EAA) and non-essential amino acids (NEAA) produced by rice plants grown under the conditions supplemented with different SeNPs concentrations. Overall, under stress conditions, higher concentrations of SeNP significantly stimulated EAA accumulation, indicating a strong metabolic response possibly related to protein synthesis or stress signaling. At high concentrations of SeNPs starting from 6.25 ppm, the contents of EAA are higher than those of NEAA in shoots. The NEAA levels were more stable and less affected by environmental changes, suggesting they were maintained for cellular homeostasis. Moreover, shoots and roots showed differing patterns of EAA and NEAA accumulation. The shoots of plants supplemented with 25 ppm SeNPs produced approximately 23 mg/g of EAAs, which was the highest among all treatments.

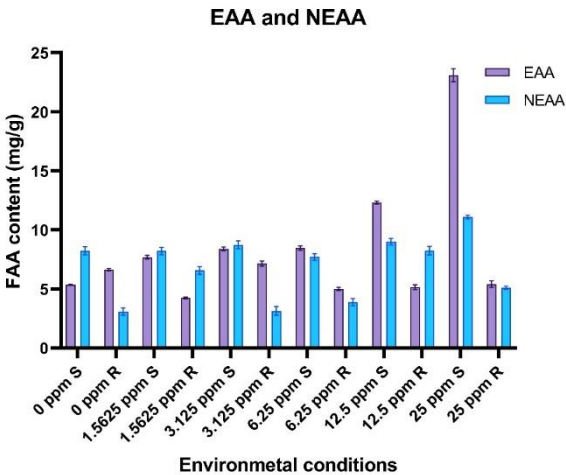


Fig. 3. Essential and non-essential amino acid biosynthesis in rice plants under varying environmental conditions in response to SeNPs
Notes: R (roots), S (shoots)

4 Discussion

4.1 Total FAA responded to SeNPs

In our study, the higher FAA content in shoots, produced by SeNPs, under increased stress could indicate a stress response mechanism, such as the accumulation of FAA as osmotic protectants. Conversely, roots may be employing different or

more efficient stress adaptation mechanisms that fail to trigger FAA accumulation. We obtained relatively stable FAA contents in the roots of plants at a level of approximately 11–13 mg/g, regardless of the concentration of SeNPs. This trend might be useful for discriminating between stress-sensitive and stress-resistant phenotypes, especially at higher SeNP ppm concentrations. Similarly, a study on tomato plants examined the effects of 20 nm AgNPs at concentrations of 0, 25, 50, 75, and 100 mg/L and demonstrated a linear increase in most free amino acids, correlating with higher AgNP concentration [18]. Other studies reported an increased FAA content when nanoparticles were applied to the plant culture media. For example, the application of ZnO nanoparticles resulted in an increase in total FAA at 80.7% and 77.5% under ambient and high-temperature conditions, respectively [19], and the exposure to cerium oxide and titanium oxide nanoparticles at a 500 mg/kg concentration increased the overall amino acid content in barley (*Hordeum vulgare* L.) [20].

4.2 FAA families responded to SeNPs

Overall, the aspartate family was the major driver of changes in the FAA content, especially in shoot samples, and appeared to respond the most to environmental conditions. The twenty-five ppm SeNPs concentration was a standout condition with dramatic increases in multiple families, especially aspartate and aromatic. Root samples generally showed a lower FAA content than shoots, suggesting possible resistance mechanisms limiting amino acid accumulation. Other families (serine, glutamate, pyruvate) remained relatively stable and might play supporting roles in the FAA profile in response to SeNPs. The glutamate family was the most abundant group in rice plants in response to nutrient starvation [21], while the aromatic family

increased response to TiO₂-nanoparticles in wheat [22].

4.3 Individual amino acid composition in response to SeNPs

Among the amino acids found in rice plants, lysine, threonine, and serine substantially increased the response to SeNPs. However, the highest increase was observed with the glutamine and asparagine content, 8 and 6 times as high as that of the control in tomato plants, respectively, elicited by AgNP, indicating an adaptive response to AgNP in tomato plants [23]. By contrast, the phenylalanine and tryptophan pathway was up-regulated in response to TiO₂-nanoparticles in wheat [22]. These results indicate the diverse responses of amino acids to different stresses.

4.4 Essential amino acid and non-essential amino acid biosynthesis responded to SeNPs

Under most of the environmental conditions, higher concentrations of EAA than those of NEAA were obtained in rice plants in response to SeNPs. Essential amino acids often show regulated biosynthesis because of their high-energy cost and involvement in stress responses. Stress induces specific genes encoding enzymes in amino acid biosynthetic pathways; therefore, the amino acid concentration often changes under stress conditions. Janmohammadi et al. [24] found cold stress accumulated in tryptophan and phenylalanine, which are EAAs in wheat. In other studies, the NEAAs like proline, alanine, and serine were likely to form more under salt and cold stresses [5, 25].

5 Conclusion

The present study examined the profile of FAAs in *Oryza sativa* L. cv. The IR64 plants were cultivated under different concentrations of

selenium nanoparticles. The findings revealed significant alterations in the total FAA content, amino acid family distribution, and individual amino acid composition. Notably, SeNP supplementation led to an increase in total FAA levels, with the most pronounced accumulation observed in the shoot tissues. Among the five amino acid families analysed, the aspartate family exhibited the highest concentration in response to SeNP treatment. Furthermore, the concentration of EAAs exceeded that of NEAAs under the same experimental conditions. These results contribute to a more comprehensive understanding of the modulation of free amino acid metabolism in rice plants in response to SeNP exposure, suggesting potential improvements in rice plant growth, stress tolerance, and nutritional quality, especially in shoot tissues when SeNPs are applied at appropriate concentrations.

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Conflict of interest

The author declares that there is no conflict of interest regarding the publication of this article.

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