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Selenium agronomic biofortification and genotypic variability in physiological responses of cowpea plants under field conditions

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Abstract

Despite its importance as a vital nutrient for animals and humans, selenium (Se) deficiency in plants and human diets is a significant concern due to its low soil concentrations. Cowpea (*Vigna unguiculata*), a resilient crop widely cultivated in developing nations, shows potential for agronomic biofortification with Se. However, its genotypic diversity and ability to improve essential element uptake are often overlooked in breeding programs. This study aimed to evaluate the impact of Se biofortification in 20 cowpea genotypes, specifically examining the physiological responses related to photosynthetic pigments in leaves, nitrogen compounds, and sugar concentration in grains. Results revealed that Se application led to an increased cowpea yield. Additionally, all genotypes exhibited elevated sucrose and total sugar concentrations in grains, along with increased photosynthetic pigment levels in leaves upon Se supplementation. Notably, the application of Se resulted in increased allantoin, allantoic acid, and total ureide concentrations in all genotypes with highest yield, indicating enhanced nitrogen fixation in cowpea plants by Se in this genotype. These findings provide valuable insights into the potential of Se biofortification to improve the nutritional quality of cowpea grains by increasing Se and sugar concentrations, ultimately enhancing crop yields through improved nitrogen metabolism. This information can guide future breeding programs aimed at enhancing cowpea grain quality and nutritional value through biofortification strategies.

Keywords Vigna unguiculata · Total sugars · Nitrogen compounds · Grain quality · Photosynthetic pigments

Introduction

Selenium (Se) is an essential element for humans and performs a series of physiological functions, such as synthesis in the thyroid, action on the immune system, and displays antioxidant properties (Rayman 2000). In plant studies, it has been shown that Se promotes plant growth by strengthening

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defense mechanisms and imparting tolerance to both biotic and abiotic stresses (Gupta and Gupta 2017; Silva et al. 2020; Lanza et al. 2021; Brown et al. 2022). However, excessive Se application can lead to the overproduction of reactive oxygen species, inducing lipid membrane damage through peroxidation reactions and potentially resulting in plant toxicity (Mostofa et al. 2017; Mateus et al. 2021; Pereira et al. 2021).

The deficiency of Se in human is connected to a variety of health problems and disorders, such as thyroid dysfunction, brain damage, increased cancer risks, peripheral vascular diseases, and compromised immune response (Brigelius-Flohé 2018; Ligowe et al. 2020). The recommended daily intake of Se for adults typically falls within the range of $45-55 \mu g$ (Brigelius-Flohé 2018). Insufficient Se availability in arable soils can lead to the production of food with low Se concentration, affecting its nutritional value (Silva et al. 2020; Lanza et al. 2021; Mateus et al. 2021).

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Agronomic biofortification has emerged as an established strategy for combating hidden hunger. This approach entails fortifying the edible components of crops with essential nutrients such as Se, with the primary objective of augmenting these vital micronutrients in the human diet (White and Broadley 2009; White 2016). Extensive studies have already been conducted on agronomic biofortification, examining the enhancement of Se concentrations in many crops, such as soybean, rice, wheat, maize, barley, and cowpea grains (Bocchini et al. 2018; de Lima Lessa et al. 2019; Reis et al. 2020; Galić et al. 2021; Silva et al. 2022; Cunha et al. 2023). However, there is a strong genetic factor that limits Se accumulation in edible components of crops (White and Broadley 2009). Therefore, it is necessary to continuously search for plant genotypes that are easily accessible to the population and with a high potential for Se accumulation. The importance of work with agronomic biofortification in cowpea (Vigna unguiculata (L.) Walp.) genotypes is evident.

Cowpea is an important source of nutrients and protein, particularly for populations in low- and middle-income countries across Africa, Asia, and South America. It is highly recommended as a staple in the diets of vulnerable communities due to its exceptional nutritional value (Manzeke et al. 2017; Teka et al. 2020; Oliveira et al. 2024). The cowpea grain has important substances for human development such as protein, iron, calcium, magnesium, zinc, vitamins (mainly the B complex), and carbohydrates (Coelho et al. 2023; Martins et al. 2023). It is one of the main sources of income and food for family farmers in the Northeast region of Brazil, grown in small farms and marketed regionally, and it is essential to carry out research with the aim of increasing the yield and nutritional quality of the grain, mainly for human nutrition by Se (Silva et al. 2018; Martins et al. 2024).

Selenium agronomic biofortification in cowpeas has been demonstrated as an effective strategy to address hidden hunger. By applying 10 g of selenate per hectare to cowpea plants, it is possible to enhance the Se content in the seeds, potentially resulting in an increase of daily human Se intake by 13 to 14 μ g/day. This application of Se has also led to elevated Se concentrations in the roots, leaves, stems, and grains across 29 commercially cultivated cowpea genotypes (Silva et al. 2021). Moreover, antagonistic effects of sulfur and synergetic effects of phosphorus on agronomic Se biofortification have already been observed in the cowpea grain (Silva et al. 2022; Santos et al. 2023). Multiple studies have shown that the application of low doses of Se not only elevates the Se concentration in edible plant parts but also enhances yield by triggering modifications in plant metabolism, including the biosynthesis of sugars and photosynthetic pigments, and nitrogen metabolism (Cunha et al. 2022; Silva et al. 2022; Mendes et al. 2023).

The application of Se at a concentration of 20 g ha^{-1} in soil resulted in increased chlorophyll and carotenoid contents in cowpea leaves (Santos et al. 2023). Therefore, the concentration of leaf pigments and photosynthetic activity may also be influenced by exogenous Se levels in soybeans (Cunha et al. 2023). The application of Se at concentrations of 80 mg L^{-1} led to an increase in total sugar content in coffee (Mateus et al. 2021). Further evidence of selenium's role in legumes was shown through its potential ability to potentiate nitrogen (N) compounds in cowpeas (Mendes et al. 2023). In groundnuts, Se application was related to an increase in nodulation, nitrate reductase activity, and ureide transport from nodules to leaves (Cunha et al. 2022). In legumes, N is primarily acquired through biological nitrogen fixation (BNF), a symbiotic interaction with root nodule bacteria (Moura et al. 2022). Selenium plays important roles during BNF, acting as a cofactor for hydrogenase, the enzyme responsible for recycling hydrogen (H_2) generated during BNF (Cunha et al. 2023). In the nodules, ammonia undergoes a conversion process resulting in the formation of ureides, specifically allantoin and allantoic acid. These ureides are then carried to the plant's shoots through the xylem sap. Once they enter the cellular cytosol, ureides are further converted into ammonium, amino acids, and proteins. These transformed compounds serve as valuable resources for chlorophyll synthesis, photosynthesis, and overall plant growth (Mendes et al. 2023).

There is an insufficient number of studies investigating the genotypic effects of Se fertilization in cowpea genotypes and its potential to improve the Se status of populations with a prevalence of deficiency in this element. Potential genotypic variation could be a valuable opportunity to explore the possible genotypic variation in terms of Se accumulation in grains and enhancement of grain quality, with the aim of addressing hidden hunger and improving human health. Furthermore, further elucidating the metabolic pathways, such as the biosynthesis of sugars, photosynthetic pigments, and nitrogen compounds, which form stable complexes with Se fertilization, could provide valuable insights. The hypothesis of this work is that Se biofortification will positively impact the physiological responses of cowpea plants, specifically by enhancing photosynthetic pigments in leaves, improving nitrogen metabolism, and increasing sugar concentration in grains. Therefore, the primary objective of this study was to evaluate the impact of Se biofortification on 20 cowpea genotypes, specifically examining the physiological responses related to photosynthetic pigments in leaves, nitrogen metabolism, and sugar concentration in grains.

Materials and methods

Experimental area

The study was conducted over two growing seasons (2020 and 2021) at the Federal Institute of Mato Grosso do Sul (IFMS) in Nova Andradina-MS, Brazil, on Typic Quartzipsamment soil (USDA 2014). On October 9 2020, a total of 30 soil samples were randomly collected from the uppermost 20 cm layer of the experimental site to assess the soil's chemical composition. Granulometric analysis revealed proportions of clay, silt, and sand at 100, 60, and 840 g kg⁻¹, respectively. The chemical attributes of the soil were measured as follows: pH (measured in a 0.01 M CaCl₂ solution) 4.6; levels of phosphorus (extracted with Mehlich-1 solution), boron (extracted with hot water), copper (extracted with DTPA [diethylenetriaminepentaacetic acid]), iron (extracted with DTPA), manganese (extracted with DTPA), and zinc (extracted with DTPA) at 0.9, 0.2, 0.1, 15, 13, and 0.75 mg dm⁻³, respectively; and potassium (resin), calcium (resin), magnesium (resin), H + Al (SMP buffer), and cation exchange capacity at 0.5, 8, 2, 14, and 24.5 mmolc dm⁻³, respectively. The total Se concentration and exchangeable Se in the unamended soil were found to be 32 μ g kg⁻¹ and 3 μ g kg⁻¹, respectively. The soil samples were analyzed according to Raij et al. (1997).

Experimental design and Se application

The experiment used a randomized complete block design with three blocks in a factorial scheme 20 (twenty cowpea genotypes) x 2 (two Se supply), totaling 120 plots. The cowpea genotypes used were sourced from the Brazilian Agricultural Research Corporation (EMBRAPA) germplasm bank (Supplementary Table 1). The plot comprised five rows of 3 m each, and the rows were spaced by 0.50 m. Twenty cowpea genotypes belonging to the color class with brown tegument grain were evaluated in response to absence (control) and 50 g ha^{-1} of Se applied as sodium selenite (Sigma-Aldrich, St. Louis, MO, USA) via foliar application (Silva et al. 2021). The choice to use selenite as a source was made because it is a low-cost option. Furthermore, previous studies conducted by our team used selenite, allowing for better comparability of results and the construction of a consistent data set.

The Se was applied at 45 days after sowing during both the first and second years, at the full bloom stage (Santos et al. 2023). The Se required for each treatment across all three replicates was weighed and diluted in 6 L of water to create a stock solution for each treatment. This stock solution was then divided into three portions of 2 L each. The solutions were applied via foliar spraying to each replicate, carefully distributed using a small flexible polyethylene bottle with a pierced cap.

Plant conduction

Sowing took place in November 2020 (first growing season) and February 2021 (second growing season), with a seeding density of 13 seeds per square meter. Fertilization along the planting furrow included 40 kg ha⁻¹ of potassium applied as KCl, 40 kg ha⁻¹ of phosphorus applied as single superphosphate, and 80 kg ha⁻¹ of nitrogen applied as urea. This fertilization process was mechanically executed simultaneously with sowing, and emergence began 8 days after sowing.

At 65 days after sowing in both growing seasons, during the full bloom stage, leaf sampling was conducted to analyze concentrations of nitrogen compounds, photosynthetic pigments, and nutrient analysis. The third trifoliate leaf (measured from the tip) was delicately harvested, dried until reaching a stable mass at 60°C, and finely ground using a Wiley mill (1 mm sieve). Fifteen trifoliate leaves were randomly collected from 15 uniformly growing plants in each plot.

Harvesting and plant height assessments were performed at 73 days after sowing in the first growing season and at 74 days after sowing in the second growing period. For harvesting, two uniform rows (2 m) were selected from each plot, and all pods were gently collected by hand; seeds were then carefully manually extracted from the pods. The seeds were subsequently dried to a constant mass at 60°C and ground in a Wiley (Marconi, MA 340, Piracicaba, Brazil) mill with a 1 mm sieve (Santos et al. 2017a, b) and subsequently used for Se analysis and total sugar and sucrose concentration.

Nitrogen compounds

The extraction of nitrogen compounds from leaves collected at 50 days after sowing was conducted according to Silva et al. (2023), including a MCW solution (composed of 60% methanol, 25% chloroform, and 15% water). Ureide content (allantoin and allantoic acid) was determined in leaves (500 mg) by method of Vogels and Van Der Drift (1970). This method involves a colorimetric assay where allantoin and allantoic acid react with specific reagents to produce a measurable color change, which is quantified using spectrophotometry. This reaction is based on the principle that ureides can be hydrolyzed and detected through their interaction with certain chemicals, resulting in a colored complex.

Photosynthetic pigment content

The total chlorophyll in fresh leaves (50 mg) was determined according by Lichtenthaler (1987). The leaf tissue was initially blended in an 80% acetone solution. Subsequently, a

200 μ L portion of the resultant extract was mixed with 1.8 mL of 80% acetone. Measurements were then taken using a spectrophotometer (SP-220, BioespectroTM) at specified wavelengths corresponding to the following absorbance values: 470, 647, 653, 663, and 665 nm.

Selenium and nutrient concentration

Subsamples (0.50 g) of dried and milled leaves and grain materials were precisely weighed. These subsamples were then digested in digestion tubes made of perfluoroalkoxy (PFA) liner material containing 4 mL of HNO_3 , 2 mL of Milli-Q water, and 2 mL of H_2O_2 . Concentrations of Se, phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), boron (B), copper (Cu), iron (Fe), manganese (Mn), zinc (Zn) and nickel (Ni), were determined by inductively coupled plasma optical emission spectrometry (ICP-OES) as outlined by Santos et al. (2021).

Total sugars and sucrose concentration analysis

Total sugar and sucrose were analyzed by grinding seed subsamples of approximately 1.0 g dry weight, and measurements were taken for both sucrose and total sugar. The sample was extracted with a 5 mL solution of MCW (60% methanol, 25% chloroform, and 15% water v/v) and stored at 10°C to aid phase separation. The sucrose analysis was performed as described by van Handel (1968), while total sugar analysis was conducted according to the method outlined by Dubois et al. (1956). The sucrose analysis involves hydrolyzing sucrose into glucose and fructose using the



invertase enzyme. The glucose is then quantified with a glucose oxidase-peroxidase assay, which produces a colorimetric signal proportional to the sucrose concentration in the sample. The total sugar analysis, using the phenol-sulfuric acid method, treats carbohydrates with phenol and sulfuric acid, dehydrating them to form furfural or hydroxymethylfurfural. These compounds react with phenol to produce a yellow-gold color, measured spectrophotometrically, providing an estimate of the total sugar content.

Statistical analysis

The results were submitted to Anderson-Darling normality tests, and the homogeneity of variance was assessed through Leven's test and variance analysis (F test). Variations between treatments were compared using the Scott-Knott test at a significance level of 5%. To create graphical representations, we utilized SigmaPlot 12.5 software (Systat Software, San Jose, CA, USA).

Results and discussion

The yield of cowpea genotypes was affected by Se foliar fertilization (Fig. 1). However, it is noteworthy that the plants show similar reactions in the two agricultural years evaluated. The application of Se resulted in different yield responses of the genotypes. Without Se application, genotypes four, seven, eight, thirteen, and eighteen showed the highest yield compared to the other tested genotypes in the two agricultural years (approximately 200 kg ha⁻¹).



Fig. 1 Cowpea yield in the first (**a**) and second (**b**) year of twenty cowpea genotypes in response to application of Se. Error bars indicates the standard error of mean (number of replicates = 3). Different letters indicate difference between means according to Scott Knott test ($p \le 0.05$). Uppercase letters comparing genotypes with Se appli-

cation, and lowercase letters comparing genotypes without Se application. *Indicates difference between means of the same genotype under absence or presence of Se application and ns = not significantly according to Scott-Knott test ($p \le 0.05$)

However, the foliar adduction of Se allowed genotypes five, seven, eight, twelve, thirteen, fourteen and twenty to obtain the highest yield compared to the other genotypes (approximately 200 kg ha⁻¹). Fertilization with Se in the cowpea genotypes resulted in a yield approximately twice as high as that of the plants without Se application (control), except that the yield of genotypes one, four, seven, eight, thirteen, eighteen, and nineteen was not affected by the foliar application of Se in the two years evaluated.

Selenium concentration in cowpea leaves and seeds was positively increased in all genotypes with Se foliar fertilization (Fig. 2), except for genotypes one and seventeen, which did not show differences in Se accumulation in seeds (Fig. 2 c, d). The Se concentration in genotypes fertilized with Se was approximately three times higher in the leaves and seeds compared to genotypes without Se application. Notably, genotypes two, four, five, seven, eight, twelve, thirteen, fourteen, eighteen, and nineteen had the highest Se concentrations in the leaf in the 2 tested years. Genotypes two, four, five, seven, eight, twelve, thirteen, fourteen, eighteen, and nineteen showed the highest accumulation of Se in the cowpea grain. There were no differences in the concentration of nutrients (Supplementary Table 2).

The Se concentration results showed that Se concentrations were highest in the leaves, followed by the seeds. Specifically. Se levels in the leaves ranged from 0.15 to 0.77 mg kg⁻¹, while in the seeds, they ranged from 0.05 to 0.11 mg kg⁻¹ in plants without Se application. In plants with Se application, Se concentrations in the leaves ranged from 1.15 to 2.80 mg kg^{-1} , while in the seeds, they ranged from 0.10to 1.33 mg kg⁻¹. These findings are consistent with previous studies on Se biofortification in cowpeas. For instance, Silva et al. (2019) reported Se concentrations ranging from 0.06 to 2.52 mg kg⁻¹ in leaves and from 0.04 to 2 mg kg⁻¹ in seeds of cowpeas treated with different Se doses, which aligns closely with our observed values. Similarly, Santos et al. (2023) found Se levels of 2 mg kg⁻¹ in biofortified cowpea grains, suggesting that our biofortification method achieves comparable Se enrichment. The similarity in Se uptake and accumulation patterns across different studies highlights





Fig. 2 Leaf and seeds Se concentration for the first $(\mathbf{a}-\mathbf{c})$ and second $(\mathbf{b}-\mathbf{d})$ year of twenty cowpea genotypes in response to application of Se. Error bars indicates the standard error of mean (number of replicates = 3). Different letters indicate difference between means according to Scott Knott test ($p \le 0.05$). Uppercase letters comparing

genotypes with Se application, and lowercase letters comparing genotypes without Se application. *Indicates difference between means of the same genotype under absence or presence of Se application and ns = not significantly according to Scott-Knott test ($p \le 0.05$)

the effectiveness of soil biofortification techniques. Additionally, the observed Se distribution among cowpea organs suggests potential pathways for Se translocation within the plant, which merits further investigation. Concentrations of photosynthetic pigments were higher in all cowpea genotypes that received Se fertilization (Fig. 3). Plants that received foliar Se showed a photosynthetic pigment concentration approximately twice as high as plants



Fig. 3 Concentration of chlorophyll a, chlorophyll b and carotenoids for the first (**a**–**c**–**e**) and second (**b**–**d**–**f**) year of twenty cowpea genotypes in response to application of Se. Error bars indicates the standard error of mean (number of replicates = 3). Different letters indicate difference between means according to Scott Knott test ($p \le 0.05$).

Uppercase letters comparing genotypes with Se application, and lowercase letters comparing genotypes without Se application. * Indicates difference between means of the same genotype under absence or presence of Se application and ns = not significantly according to Scott Knott test ($p \le 0.05$)

that did not receive Se. The application of Se also provided an increase in sucrose and total sugar concentrations in the grains in all cowpea genotypes (Fig. 4). Genotype twelve, when fertilized with foliar Se, showed the highest sucrose and total sugar concentrations in the grains compared to the other genotypes in the two growing seasons.

The nitrogenous compound concentrations presented a high positive correlation with the yield results in the two agricultural years. Likewise, when observing yield in plants without Se application, genotypes seven, eight, and thirteen showed the highest allantoin, allantoic acid, and total ureide concentrations (Fig. 5). When genotypes were fertilized with Se, it was noted that genotypes five, seven, eight, twelve, thirteen, fourteen, and twenty had the highest allantoin, allantoic acid, and total ureide concentrations. Furthermore, fertilization with Se in cowpea genotypes resulted in increases of approximately 20% in allantoin contents, 25% in allantoic acid concentrations, and 30% in total ureide concentrations compared to the same genotypes that did not receive foliar Se. Only genotypes seven, eight, thirteen, eighteen, and nineteen showed no effect of Se on nitrogenous compounds.

The precise functions of Se in biological nitrogen fixation (BNF) within legume plants are still not fully understood, as noted by Silva et al. (2018). However, recent research conducted by Cunha et al. (2023) revealed that Se can enhance the flavonoid accumulation in the roots of tropical legumes, which play a crucial role in nodulation (Bosse et al. 2021). This leads to an increased number of nodules and the synthesis of allantoin and allantoic acid, ultimately resulting in a greater production of Se had a greater impact on allantoin concentration compared to the allantoic acid concentration. The elevated ureide concentration, even at lower Se



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Fig.4 Concentration of sucrose and total sugar for the first (**a**–**c**) and second (**b**–**d**) year of twenty cowpea genotypes in response to application of Se. Error bars indicates the standard error of mean (number of replicates = 3). Different letters indicate difference between means according to Scott Knott test ($p \le 0.05$). Uppercase letters comparing

genotypes with Se application, and lowercase letters comparing genotypes without Se application. *Indicates difference between means of the same genotype under absence or presence of Se application and ns = not significantly according to Scott Knott test ($p \le 0.05$)





Fig. 5 Concentration of allantoin, allantoic acid and total ureides for the first (**a**–**c**–**e**) and second (**b**–**d**–**f**) year of twenty cowpea genotypes in response to application of Se. Error bars indicates the standard error of mean (number of replicates = 3). Different letters indicate difference between means according to Scott Knott test ($p \le 0.05$).

Uppercase letters comparing genotypes with Se application, and lowercase letters comparing genotypes without Se application. *Indicates difference between means of the same genotype under absence or presence of Se application and ns = not significantly according to Scott Knott test ($p \le 0.05$)

levels, indicates a positive influence of Se on BNF. In tropical legumes, the export of fixed nitrogen primarily occurs through ureides, making them a reliable indicator of BNF (Baral et al. 2016).

In the case of alfalfa plants treated with Se spray at concentrations of 1 and 5 μ mol, increased protein synthesis as well as higher nodule count and fresh weight were observed (Hajiboland et al. 2015). While the exact role of Se in nitrogen fixation is still not fully understood, there is growing evidence suggesting its potential importance in this process. One proposed mechanism revolves around the presence of selenocysteine, a selenium-containing amino acid, in the active site of certain enzymes involved in nitrogen fixation, particularly hydrogenases. Hydrogenases are enzymes that play a crucial role in several metabolic pathways, including those related to nitrogen metabolism. Studies have indicated that selenocysteine may be present in the active sites of certain hydrogenases, suggesting a possible role for Se in optimizing their enzymatic activity (Baltazar et al. 2011; Ekanayake et al. 2017). By supplementing with Se, it is hypothesized that the presence of selenocysteine in hydrogenases could enhance their catalytic efficiency, potentially leading to increased rates of nitrogen fixation. This, in turn, could have positive effects on plant growth and nitrogen utilization. However, it is essential to note that research in this area is ongoing, and further studies are needed to fully elucidate the specific mechanisms by which Se influences nitrogen fixation in plants like peas (*Pisum sativum*) (Poblaciones and Rengel 2018). Nonetheless, these findings underscore the potential importance of Se in optimizing nitrogen metabolism and facilitating ureide biosynthesis (Baltazar et al. 2011; Ekanayake et al. 2017).

As previously mentioned, Se might also enhance flavonoid biosynthesis in legume plants, such as groundnuts and soybeans (Cunha et al. 2023). Daidzein is an isoflavone that plays a role in the expression of nod genes in rhizobia. These genes can enhance the synthesis of "nod factors," which, when released by rhizobia near the roots, lead to modifications in plant cells responsible for initiating nodule formation (Bosse et al. 2021). In the interaction between rhizobia and the legume plants, bacteria are accommodated in the nodules to reduce N for the plants, and the plants supply sugar as sucrose to the bacteria (Roy et al. 2020; Fonseca-García et al. 2021). In this exchange relationship, Se could be helpful, since previous reports demonstrate that the element increases sugar content in cowpeas (Santos et al. 2023). On the other hand, in mung beans, Se application increases the activity of sucrose synthase and β -amylase (Malik et al. 2011).

The concentrations of chlorophyll and carotenoids were influenced by different Se doses, as depicted in Fig. 4. With Se application, there was an increase in the concentrations of photosynthetic pigments. Plants treated with Se exhibited approximately twice the levels of chlorophyll a, chlorophyll b, and carotenoids compared to plants without Se fertilization. A study (Santos et al. 2023) also reported an initial increase in total chlorophyll and carotenoid concentrations followed by a decrease in these pigments when plants were exposed to different Se concentrations. The absorption of Se enhances electron donation in the respiratory chain, thereby accelerating chlorophyll (White 2016). This may explain the increase in pigment concentrations by Se application.

The application of Se resulted in the highest concentration of sucrose. Consistent with the findings on photosynthetic pigments, it was observed that plants treated with a dose of Se (50 g ha⁻¹) presented higher concentrations of photosynthetic pigments than those of untreated plants. Previous studies have shed light on the effects of Se on sucrose and total sugar concentration across various crops. Research has shown that Se application can lead to an increase in total sugar concentration in apples (Ren et al. 2021), cowpea leaves (Silva et al. 2020), coffee seedlings (Mateus et al. 2020), rice seeds (Lidon et al. 2018), and wheat (Lara et al. 2019). Ensuring appropriate nutritional management of Se is crucial for achieving cowpea grains with higher sugar concentrations, thereby enhancing the quality of agricultural products. However, similar to the observations on chlorophyll content, it was noted that high doses of Se reduced total sugar and sucrose concentrations. Elevated Se concentrations can lead to a decrease in photosynthetic rates (Lanza et al. 2021) and, consequently, sugar concentration in cowpeas (Silva et al. 2019).

As demonstrated by Das et al. (2018), Se application in rice has been shown to increase enzyme-related sugar activity and total sugar concentration. Hence, the presence of Se is crucial for enhancing plant sugar concentrations. In a study investigating the application of selenite to cowpeas, Silva et al. (2019) observed an increase in Se concentration in grains without compromising productivity, with doses of around 20 g ha⁻¹ considered more appropriate for specific conditions. However, our present study shows that the application of Se at a dose of 50 g ha⁻¹ can positively affect the synthesis of pigments and sugars, leading to higher grain quality. Selenium indirectly boosts sugar levels in tropical legumes by enhancing metabolic processes within the plant (Cunha et al. 2023). It improves photosynthesis by increasing enzyme activity, leading to more sugar production. Additionally, Se enhances the uptake of essential nutrients necessary for sugar metabolism and influences hormonal signaling, further promoting sugar accumulation in grains (Santos et al. 2023). By acting as an antioxidant, it reduces stress in plants, allowing them to allocate more resources to sugar synthesis (Alyemeni et al. 2018). Overall, while Se itself does not directly produce sugars, its positive effects on various plant processes indirectly contribute to higher sugar levels in cowpea grains.

The recommended dietary allowance for Se in adult humans is 55 μ g/day, with a tolerable upper intake level of 400 µg/day (Fairweather-Tait et al. 2011). Brazilian dietary habits vary significantly due to social, economic, and geographical factors, but staples like rice, cow milk, and eggs are widely consumed. The average Se concentrations in Brazilian rice, cow milk, and eggs are 0.31, 0.08, and 0.21 μ g/g, respectively. In the northeastern region of Brazil, the daily consumption of rice, cow milk, and eggs is approximately 142 g, 33 g, and 16 g, respectively (dos Santos et al. 2017a, b). By multiplying the Se concentration by the daily consumption of these foods, the total Se intake from them is about 25 µg/day per capita. Subtracting this value from the recommended dietary allowance of 55 µg/day, we can establish a guideline for the daily Se intake provided by cowpea seeds, which is approximately 30 µg/day.

Cowpea consumption varies greatly across regions. In the Northeast region of Brazil, the per capita consumption

of cowpea is 19.79 kg per year, which corresponds to 54.22 g per day (Freire Filho et al. 2017). The daily Se intake from cowpeas can be calculated by multiplying the daily cowpea intake by the Se concentration in each genotype with Se application. Genotypes two, four, five, seven, eight, twelve, thirteen, fourteen, eighteen, and nineteen, which showed a higher Se accumulation in cowpea seeds across two growing seasons (averaging 1.2 mg/kg), would provide an intake of 65 μ g Se/day for a daily consumption of 54.22 g of cowpea seeds.

Conclusions

The evaluation of twenty cowpea genotypes for Se biofortification revealed that genotypes five, seven, eight, twelve, thirteen, fourteen, and twenty are the most promising candidates for Se biofortification programs. These genotypes demonstrated superior performance in key areas, including yield, Se accumulation in leaves and grains, photosynthetic pigment concentrations, sugar content, and nitrogen metabolism. Notably, these genotypes exhibited the highest yield responses and Se concentrations, indicating their potential to enhance both productivity and nutritional quality through Se biofortification. Enhanced photosynthetic activity, evidenced by increased chlorophyll and carotenoid levels, along with elevated sucrose and total sugar concentrations, further supports their suitability for biofortification. Additionally, these genotypes showed improved nitrogen fixation and metabolism, as indicated by higher concentrations of nitrogenous compounds such as allantoin, allantoic acid, and total ureides. The integration of these genotypes into Se biofortification programs can significantly contribute to addressing hidden hunger by improving the nutritional value of cowpea grains while simultaneously boosting crop yields.

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Author contribution ECF, LEMFF, MAPS and AAO set up and conducted the experiment, performed the laboratory analyses, and conceptualized and wrote the manuscript. MMR and ARR provided support in the experiment conduction, laboratory analyses, and writing. GNS and VMS performed statistical analyses and aided in the results and discussion writing. EFS planned, conceptualized, and coordinated the project and validated the writing and analyses.

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Data availability All data generated or analysed during this study are included in this published article and its supplementary information files.

Declarations

Conflict of interest The authors have no relevant financial or non-financial interests to disclose.

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