FILIPE AIURA NAMORATO

BIOFORTIFICATION TO ENHANCE NUTRITION: THE POTENTIAL OF RICE, COMMON BEANS, AND PAK CHOI

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Tese apresentada à Defesa de Doutorado, como parte das exigências do Programa de Pós-Graduação em Ciência do Solo, área de concentração em Fertilidade do Solo e Nutrição de Plantas.

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BIOFORTIFICAÇÃO PARA MELHORAR A NUTRIÇÃO: O POTENCIAL DO ARROZ, FEIJÃO E COUVE CHINESA

Tese apresentada à defesa de doutorado, como parte das exigências do Programa de Pós-Graduação em Ciência do Solo, área de concentração em Fertilidade do Solo e Nutrição de Plantas.

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2023

À minha família,
À minha namorada,
Aos meus amigos
Dedico.

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MEU SINCERO OBRIGADO!

“Coming together is the beginning. Keeping together is progress. Working together is success”

Henry Ford

GENERAL ABSTRACT

Biofortification is a promising strategy to improve the nutrient content in staple foods, aiming to tackle micronutrient deficiencies - hidden hunger - in the population. This document presents a compilation of studies aimed at investigating zinc (Zn), iron (Fe), and selenium (Se) biofortification in three important food crops: rice, common beans, and pak choi. Through agronomic and genetic biofortification approaches, such as the application of fertilizers and the study of promising genotypes, experiments were conducted to increase the content of these micronutrients in the selected crops. Four studies were conducted, involving different genotypes and/or application strategies to optimize the absorption and accumulation of Zn, Fe, and Se in the edible parts. The results demonstrated that biofortification was effective in increasing the content of these nutrients in rice, common beans, and pak choi crops using the selected strategies. Furthermore, it was observed that biofortification had an impact on the nutritional quality of the foods, as it affected the protein content in beans and rice, as well as amino acids in rice and phenolic compounds in pak choi. In pak choi, the accumulation capacity of Se was inversely related to the total content of phenolic compounds, which could be identified by the purple color of the genotypes. The San fan genotype was the most sensitive to Se application and also had the highest accumulation in the shoots. For common bean grains, the application of Se-enriched urea resulted in a higher Se content compared with ammonium sulfate, with the latter being also not recommended as it reduced the protein content in the grains. The Madrepérola genotype had the best response to biofortification with the use of Se fertilizers. Among the common bean genotypes studied for Fe, Zn, and protein, none reached levels above the average reported in the literature without supplementation. However, there was genetic variation in the content and accumulation of these elements, with Madrepérola, OV, and Supremo showing the best performance. In rice, a higher accumulation of Zn and Se was observed in whole grains compared with polished grains, indicating that the consumption of whole grains is the most efficient way to obtain the benefits of biofortification in this crop. Additionally, it was found that Zn was able to increase the accumulation of Se in whole grains. The genotypes CMG ERF 221-19 and CMG ERF 85-15 were the best genotypes for Zn and Se biofortification.

Keywords: Cereals. Fertilizers. Genotypes. Vegetables. Selenium. Zinc. Iron.

RESUMO GERAL

A biofortificação é uma estratégia promissora para melhorar o teor de nutrientes em alimentos básicos, visando combater a deficiência de micronutrientes - fome oculta - na população. Este documento apresenta um compilado de estudos que tiveram como objetivo investigar a biofortificação com zinco (Zn), ferro (Fe) e selênio (Se) em três culturas alimentares importantes: arroz, feijão e couve chinesa. Através de abordagens de biofortificação agronômica e genética, como a aplicação de fertilizantes e o estudo de genótipos promissores, foram realizados experimentos para aumentar o conteúdo desses micronutrientes nas culturas selecionadas. Foram quatro estudos, envolvendo diferentes genótipos e, ou, estratégias de aplicação visando otimizar a absorção e acumulação de Zn, Fe e Se em partes comestíveis. Os resultados demonstraram que a biofortificação foi eficaz em aumentar o teor desses nutrientes nas colheitas de arroz, feijão e couve chinesa, utilizando as estratégias selecionadas. Além disso, foi observado que a biofortificação teve impacto na qualidade nutricional dos alimentos, pois afetou o teor de proteínas no feijão e arroz, bem como aminoácidos no arroz, e compostos fenólicos na couve chinesa. Na couve chinesa, a capacidade de acumulação de Se encontrada foi inversamente relacionada ao maior teor total de compostos fenólicos, que puderam ser identificados pela coloração roxa dos genótipos. O genótipo San fan foi o mais sensível à aplicação de Se e também o maior acumulador na parte aérea. Para grãos de feijão, a aplicação de ureia enriquecida com Se resultou em maior teor de Se em comparação com o sulfato de amônio, sendo este último também não recomendado, pois diminuiu o teor de proteínas nos grãos. O genótipo Madrepérola teve a melhor resposta para biofortificação com o uso de fertilizantes de Se. Entre os genótipos de feijão estudados para Fe, Zn e proteína, nenhum atingiu níveis acima da média relatada na literatura sem suplementação. No entanto, houve variação genética no teor e acumulação desses elementos, sendo que Madrepérola, OV e Supremo apresentaram o melhor desempenho. No arroz, foi observada maior acumulação de Zn e Se em grãos integrais em comparação com grãos polidos, indicando que consumir grãos integrais é a forma mais eficiente de obter os benefícios da biofortificação nessa cultura. Além disso, constatou-se que o Zn foi capaz de aumentar a acumulação de Se nos grãos integrais. Os genótipos CMG ERF 221-19 e CMG ERF 85-15 foram os melhores genótipos para biofortificação de Zn e Se.

Palavras-chave: Cereais. Fertilizantes. Genótipos. Hortaliças. Selênio. Zinco. Ferro.

SUMMARY

FIRST PART

GENERAL INTRODUCTION

Food insecurity such as Se, Zn, and Fe deficiency are common in low and middle-income countries and are predicted to impact 2 billion people globally (MICHA et al., 2020; NATIONS, 2020). To revert, biofortification with Se, Zn, and Fe has gained prominence in agronomic research due to the fundamental role these micronutrients play in plants. These elements are essential (Zn and Fe) and beneficial (Se) for plant growth and development, directly influencing various physiological and biochemical processes. This is a strategy aimed at increasing the content of nutrients in agriculturally important crops (BUTURI et al., 2021). In this context, pak choi (*Brassica rapa* subsp. *chinensis*), rice (*Oryza sativa*), and beans (*Phaseolus vulgaris*) emerge as critical crops for the subsistence of millions of people worldwide.

Biofortification with Se, Zn, and Fe has been the subject of investigations to improve the nutritional quality of these foods, offering significant benefits to public health (Bailey et al. 2019). Selenium is a beneficial element for plants, being an enhancing factor of various antioxidant enzymes, such as glutathione peroxidase and thioredoxin reductase, which help combat oxidative stress caused by reactive oxygen species (ROS) during cellular metabolism (CHAUHAN et al., 2019; FENG; WEI; TU, 2013; RIZWAN et al., 2021; WAN et al., 2016). Additionally, Se also plays an essential role in the synthesis of sulfur-containing compounds, such as selenomethionine and selenocysteine, which are highly bioavailable forms of amino acids that are crucial for human health and nutrition when consumed through the diet (TRIPPE III; PILON-SMITS, 2021).

On the other hand, Zn is essential for the functioning of various enzymes involved in plant metabolism, such as superoxide dismutase (SOD) and carbonic anhydrase, which have antioxidant functions and play a crucial role in photosynthesis and plant growth (CHOI et al., 2018). Furthermore, Zn is also involved in the synthesis of phytohormones, such as auxins, gibberellins, and cytokinins, which regulate plant growth and development (NOULAS; TZIOUVALEKAS; KARYOTIS, 2018; TSONEV; CEBOLA LIDON, 2012).

Fe, in turn, is an essential component of the enzyme catalase, which plays a critical role in protecting plant cells against damage caused by hydrogen peroxide, a normal byproduct of photosynthesis. Additionally, Fe is an important cofactor for chlorophyll

synthesis, the molecule responsible for capturing sunlight during photosynthesis, and is also involved in the nitrogen fixation by symbiotic bacteria in the roots of some legumes (BRIAT; DUBOS; GAYMARD, 2015; CONNORTON; BALK; RODRÍGUEZ-CELMA, 2017; KOBAYASHI; NISHIZAWA, 2012; KOBAYASHI; NOZOYE; NISHIZAWA, 2019; MURGIA et al., 2022; RAMIREZ et al., 2011; VIGANI; MURGIA, 2018).

Pak choi, a popular vegetable in various cuisines, is an important source of antioxidants and bioactive compounds. Research has shown the potential of Se biofortification in this vegetable, revealing a significant increase in the contents of Se and beneficial compounds in the edible parts of the plant (ABDALLA; LENTZ; MÜHLING, 2022). This research highlights the feasibility of biofortification as a strategy to improve Se availability in plant-based foods, providing nutritional benefits to the population.

Regarding rice, which is a major source of calories for many communities worldwide, Zn and Se deficiency is a relevant concern. Several studies have demonstrated that Zn biofortification can increase the concentration of this mineral in rice grains. The proper application of Zn to the soil and/or via foliar sprays resulted in a significant increase in Zn absorption and concentration in rice grains, making it a promising option to combat Zn deficiency in the diet (BOONCHUAY et al., 2013; PROM-U-THAI et al., 2020; RAO et al., 2020). Moreover, research on Se biofortification in rice has shown encouraging results, with many findings indicating that Se biofortification increases the content of this mineral in rice, providing an efficient approach to improve Se availability in the diet of populations (FÉLIX et al., 2023; LESSA et al., 2019, 2020).

Beans, an essential component of the diet in many regions, are also a key target of biofortification research. Selenium, Zn, and Fe deficiency in common beans have direct implications for public health and nutrition and biofortification is an effective strategy for increasing the concentration of these minerals in beans. Indeed, several studies indicate that biofortification with Se, Zn, and Fe can make beans a richer source of these elements for the human diet (ARAÚJO et al., 2022; DE FIGUEIREDO et al., 2017; LIGARRETO, 2023).

Given the aforementioned, research focusing on improved absorption of Se, Zn, and Fe, while also increasing the content of these elements in food is of utmost importance to pursue global food security. Therefore, the studies presented in this thesis aim to advance the understanding of the response of pak choi, rice, and common bean varieties to these crucial nutrients, aiming to contribute to the implementation of sustainable public practices that reduce nutrient deficiencies worldwide.

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SECOND PART – MANUSCRIPTS

Manuscript 1: GENETIC VARIABILITY OF RESPONSE TO SELENIUM-ENRICHED UREA AND SELENIUM-ENRICHED AMMONIUM SULFATE APPLICATION IN COMMON BEAN

(Manuscript prepared to be submitted to Plant and Soil)

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ABSTRACT

The common bean is an important food source worldwide, particularly in developing countries, due to its high protein, vitamin, and mineral content. However, it is often grown in nutrient-poor soils, notably lacking in selenium (Se), an essential element for human health and disease prevention. Different Se application strategies can be used to improve its contents in the edible part of plants, and its addition in fertilizers commonly used in crops are promising techniques. However, there have been limited studies on Se-fertilizers' effectiveness in improving the Se content of bean plants. Thus, this study aimed to assess common bean grains' agronomic, physiological, and nutritional responses to different biofortification strategies involving Se application through the soil, using conventional nitrogen fertilizers as carriers. The experiment took place in a greenhouse, using a randomized block design with five top dressing sources: urea (devoid of S and Se), urea + Se, ammonium sulfate (devoid of Se), ammonium sulfate + Se, and without N-fertilizer (devoid of N, S, and Se), four bean genotypes (BRS 9435 Cometa, BRS Estilo, BRSMG Madrepérola, and Pérola), and three replicates. The applied dose of Se as sodium selenate was 0.94 mg of Se pot^{-1}. The dosage was obtained through previous tests. The application of Se increased grain yield in the Cometa and Pérola genotypes, but the effect depended on the source used. The Se content in the grains increased with Se application *via* urea and ammonium sulfate, regardless of the genotype. Overall, the urea + Se application resulted in the most significant increase in Se content. Ammonium sulfate + Se reduced grain protein synthesis in three genotypes, except for BRSMG Madrepérola, which showed the highest Se accumulation capacity. The composition of biomolecules and the physiological response were influenced by Se-induced stress, but the response varied depending on the fertilizer source and genotype tested.

Keywords: Fertilizers. SeO_4. Phaseolus vulgaris. Biofortification. Genotypes.

1 INTRODUCTION

The common bean (*Phaseolus vulgaris* L.) is the most consumed legume in grain (pulse) globally, standing out mainly in developing countries (Gregory et al. 2017; Rawal and Navarro 2019; Nassary et al. 2020). It is estimated that the consumption of beans has been responsible for meeting part of the protein intake in South America, Central America, Asia, and Africa (Schwember et al. 2019; Torres et al. 2020). Grain intake provides a balanced consumption of proteins, carbohydrates, lipids, vitamins, and micronutrients (*e.g.*, minerals) compared to cereals (Sá et al. 2020). Among the most relevant minerals in the grain are calcium, iron, and zinc, of which an average of 3 g, 40 mg, and 35 mg per kg of seed are provided, respectively (Grela et al. 2017). There are also other important minerals, but in lower concentrations, such as Se, found, on average, in cooked grains, at a concentration of 5.7 µg kg^{-1} (USDA 2019).

The common bean grain can be a valuable source of essential nutrients not abundant in its natural composition. To enhance its nutritional value, strategies like biofortification are necessary. Biofortification is a sustainable and cost-effective approach that efficiently addresses micronutrient deficiencies, providing a swift solution for improving overall nutrition (Natasha et al. 2018). Around the world, approximately 2 billion people suffer from undernutrition, predominantly in developing nations (Ritchie et al. 2018). Biofortification, through various agronomic practices, holds the potential to combat malnutrition on a global scale. These practices include foliar, soil application, and genetic and microbial biofortification (Danso et al. 2023).

Selenium (Se) is a micronutrient that has gained attention for its potential to enhance the nutritional content of food. In mammals, including humans, Se plays a vital role in the formation of selenocysteine (SeCys), a key component of enzymes like glutathione peroxidase (GPx) and thioredoxin reductase (TrxR) that are involved in essential physiological processes (Bela et al. 2015). A minimum of 1 billion people worldwide is estimated to have inadequate selenium (Se) intake (Combs 2001; Adadi et al. 2019).

Although Se is not essential for plants, it can provide benefits in specific scenarios where cellular health and plant metabolism remain unaffected. Selenium can enhance enzyme activity, such as GPx and TrxR while improving resistance against cold, drought, and metal stress in plants (Gupta and Gupta 2017; de Sousa et al. 2022; Silva et al. 2022). Despite its advantages, Se faces a limitation in that it may not be sufficiently available in food plants for absorption at adequate concentrations (Nothstein et al. 2016). Additionally, Se in the form of

selenate and sulfur (S) in the form of sulfate share similar transporters (SULTR) (El Mehdawi et al. 2018; Rao et al. 2021). The similarity extends to the unintentional inclusion of selenocysteine and selenomethionine in proteins, originally sulfur-based amino acids like cysteine and methionine. This similarity between these two compounds can result in antagonism or synergism, depending on the conditions. These amino acids are integral to the functioning of GPx and TrxR enzymes. (Kolbert et al. 2019). Besides, once Se is incorporated in the cited organic forms in comestible parts of plants, Se is more bioavailable to humans compared to mineral sources (Khanam and Platel 2016).

Around 40 countries possess soils with low Se levels (Wu et al. 2015). Brazil is one of the tropical countries facing this condition (Gabos et al. 2014; Matos et al. 2017; Carvalho et al. 2019). In Brazil, this condition arises from variable load soils with a high capacity to adsorb Se, particularly at low pH levels. Consequently, the availability of Se to plants is reduced (Lopes et al. 2017).

In consequence, it is necessary to employ strategies to enhance Se availability in soils. One approach is the utilization of enriched fertilizers, such as urea and ammonium sulfate, which can positively influence the nutritional availability of the soil (Araújo et al. 2022). Numerous studies on Se biofortification have been conducted on crops with significant food grain relevance in Brazilian soils, including rice (Boldrin et al. 2012; Reis et al. 2018; de Lima Lessa et al. 2019, 2020; Félix et al. 2023), common bean (Araújo et al. 2022; Ravello et al. 2022b), soybean (Silva et al. 2022) and wheat (Lara et al. 2019). Nevertheless, only a minority of these studies have specifically examined the efficacy of Se-enriched fertilizers, which are more commonly used than the direct application of Se salts on soil or leaves. Furthermore, no studies have been conducted to compare the efficiency of urea and ammonium sulfate in common bean genotypes. Consequently, further research is required to address these gaps and advance the understanding of biofortification methods.

Given the importance of selenium for both plants and humans, as well as the significance of common bean grain as a food source, this study aimed to assess the effectiveness and response of applying Se-enriched urea and Se-enriched ammonium sulfate fertilizers containing selenate as top-dressing fertilizers in various common bean genotypes, defining the best approach to produce biofortified bean, as well the Se effects on biomolecules, physiological response, and grain production.

2 MATERIAL AND METHODS

2.1 Experiment design and treatments

The experiment took place in a greenhouse at the Federal University of Lavras, Minas Gerais, Brazil, specifically at the Department of Soil Science.

For plant cultivation, pots containing 5 kg of soil were utilized. These pots were filled with air-dried soil samples obtained from the 0.00 - 0.20 m layer of an Oxisol, with clayey texture (sand: 28 g kg^{-1}, silt: 11 g kg^{-1}, clay: 61 g kg^{-1}) (Soil Survey Staff 1999). Based on chemical and textural analyses (Teixeira et al. 2017), the soil used in the experiment exhibited the following characteristics: pH of 4.6 in water, available P = 11.81 mg kg^{-1}, available K = 61.9 mg kg^{-1}, exchangeable Ca = 0.45 $cmol_c$ kg^{-1}, exchangeable Mg = 0.28 $cmol_c$ kg^{-1}, exchangeable Al = 1.18 $cmol_c$ kg^{-1}, H+Al = 11.62 $cmol_c$ kg^{-1}, P-rem = 18.34 mg L^{-1}, available Fe = 171.29 mg kg^{-1}, and organic matter (OM) = 3.27 dag kg^{-1}. The exchangeable/available contents were extracted by 1 M KCl/Mehlich-1 solutions. The total Se content, determined through soil digestion using the USEPA 3051A method, was 0.28 mg kg^{-1} (USEPA 2007).

Based on soil chemical analysis, lime was applied to increase the base saturation to 60%. Calcium carbonate ($CaCO_3$) and magnesium carbonate ($MgCO_3$) with 99% purity were used. This procedure was performed 30 days prior to the beginning of the experiment, when pots were maintained with a soil moisture ~70% of the total pore volume (TPV). After liming, the sowing was performed using six seeds per pot. Seven days after seedlings emergence, thinning was performed, leaving two plants in each pot. Planting fertilization was then conducted during bean plant sowing.

The experimental design consisted of randomized blocks arranged in a 5 x 4 x 3 factorial layout. There were five treatments: urea (U - positive control, without S and Se), urea + selenium (U + Se, without S) at a dose of 0.94 mg of Se per pot, ammonium sulfate (AS - positive control, without Se), ammonium sulfate + selenium (AS + Se) at a dose of 0.94 mg of Se per pot, and a Control (negative, without the application of N, S, and Se fertilizers). Additionally, four carioca bean genotypes (BRS 9435 Cometa, BRS Estilo, BRSMG Madrepérola, and Pérola) were included in the study. The experiment consisted of three replicates, resulting in sixty experimental units.

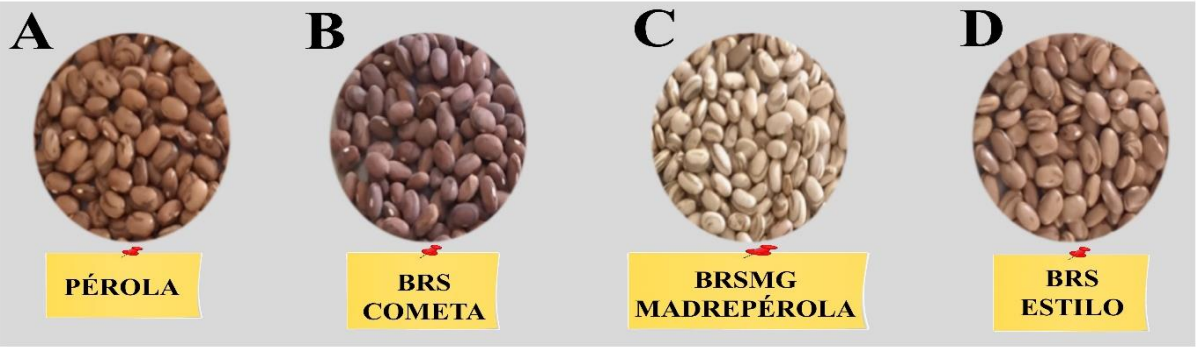

Figure 1 – Seeds of the carioca genotypes were used. Pérola (A), BRS 9435 Cometa (B), BRSMG Madrepérola (C), BRS Estilo (D).

The exact initial planting fertilization was applied to all treatments to ensure consistent starting conditions for all genotypes following the recommended values by (Malavolta 1981), using N - 150 mg kg^{-1}, P - 200 mg kg^{-1}, K - 75 mg kg^{-1}, S - 25 mg kg^{-1}, B - 0.5 mg kg^{-1}, Cu - 1.5 mg kg^{-1}, Fe - 5 mg kg^{-1}, Mo - 0.1 mg kg^{-1}, and Zn - 5 mg kg^{-1}. The macronutrient sources used were urea U(CH_4N_2O), potassium sulfate (K_2SO_4), potassium chloride (KCl), and triple superphosphate ($Ca(H_2PO_4)$ $2H_2O$). The micronutrient sources included copper sulfate ($CuSO_4$ $5H_2O$), iron sulfate ($FeSO_4$ $7H_2O$), zinc sulfate ($ZnSO_4 \cdot 7H_2O$), boric acid (H_3BO_3), and ammonium molybdate ($(NH_4)_6Mo_7O_{24}$).

At 20 days after seed emergence, the U and U + Se treatments received a top-dressing application based on the recommendation from (Malavolta 1981). These treatments included urea (CH_4N_2O), potassium sulfate (K_2SO_4), and potassium chloride (KCl) sources at rates of N - 150 mg kg^{-1}, K - 75 mg kg^{-1}, and S - 25 mg kg^{-1}. The sulfur content was extrapolated for the AS and AS + Se treatments due to fertilization with AS (21% N and 23% S), resulting in N - 150 mg kg^{-1}, K - 75 mg kg^{-1}, and S - 164 mg kg^{-1}. The sources used were ammonium sulfate ($(NH_4)_2SO_4$), potassium sulfate (K_2SO_4), and potassium chloride (KCl). The control treatment received only K - 75 mg kg^{-1} fertilization without nitrogen, sulfur, and selenium application in the top dressing. The experiment was conducted 90 days until grain harvest, maintaining soil moisture close to field capacity.

2.2 Method for preparing fertilizer and characterization of nitrogen fertilizers

To produce selenium-enriched fertilizers for the U + Se and AS + Se treatments, 1.15 mL of diethanolamine additive from Êxodo Científica Química Fina Indústria e Comércio Ltda, Sumaré, Brazil, was utilized. This additive facilitates the combination of sodium

selenate (Na_2SeO_4) with U (CH_4N_2O) or AS ($(NH_4)_2SO_4$). Additionally, twelve drops of liquid dye from Sherwin-Williams Brasil Indústria e Comércio Ltda, Taboão da Serra, Brazil, were added to ensure the mixture's uniformity between sodium selenate and the nitrogenous sources, thus ensuring successful blending and selenium adherence to the fertilization sources. Specifically, 260.8 mg of selenium (Table 1) were mixed with sodium selenate in 1 kg of AS containing 21% N. Considering its 46% N content, for U, 573.6 mg of selenium (Table 1) were mixed with sodium selenate in 1 kg of urea.

Table 1 – Result of Se (mg kg^{-1}) expected and obtained from the nitrogenous fertilizers studied.

Nitrogen fertilizers[a]	Expected	Obtained (n= 4)[b]
U	0.00	< LOD
U + Se	573.600	671.240
AS	0.00	< LOD
AS + Se	260.800	332.805

[a]Sources of nitrogen fertilization applied in top dressing. [b]Determination in Graphite Furnace Atomic Absorption Spectrometry (GFAAS). LOD: Limit of detection. U + Se: Se-enriched urea. U: urea. AS: ammonium sulfate. AS + Se: Se-ammonium sulfate.

2.3 Gas exchanges, fluorometry, and SPAD index

Gas exchange and fluorometry evaluations were conducted simultaneously using a portable infrared gas analyzer (IRGA, LI-COR Biosciences, model LICOR 6400) and Fluorometry (Mini-Pam II, Walz, Germany) after 14 days of top-dressing fertilization application. Gas exchange parameters measured included CO_2 assimilation rate (A - µmol CO_2 m^{-2} s^{-1}), stomatal conductance (g_s - mol H_2O m^{-2} s^{-1}), transpiration (E - mmol H_2O m^{-2} s^{-1}), and water use efficiency [WUE, (A/E - µmol CO_2 $mmol^{-1}$ H_2O)]. Fluorometry measurements focused on quantifying the photochemical efficiency of photosystem II [$\Delta F/F_m'$)], where ΔF represents steady-state fluorescence, and F_m' denotes the maximum fluorescence of a light-adapted sample after applying a saturation flash. Analysis were taken between 8:00 a.m. and 12:00 p.m. under optimal conditions. The true leaf, located in the middle region of the bean plant, was selected for measurement. The photosynthetically active radiation (PAR) was standardized at 1000 µmolm^{-2} s^{-1}, and the ambient CO_2 concentration was maintained at 420 mg kg^{-1}. The average relative humidity during the experiment was 70%, with a temperature range of 23 to 25° C. Additionally, chlorophyll levels were indirectly

measured in triplicate using the SPAD-502 portable chlorophyll meter (Konica, Minolta, Tokyo, Japan).

2.4 Grain production

After growing beans, the harvested grains were dried in a forced ventilation oven at 65° C for 72 hours to measure their dry mass and determine grain production. Subsequently, samples from each material were ground and stored appropriately for further analysis.

2.5 The total content of Se and S

For the analysis of selenium (Se) and sulfur (S) in the grains and Se in the nitrogen fertilizer applied in the top dressing, the extraction method employed was the 3051A methodology from the United States Environmental Protection Agency (USEPA 2007). The ground samples, weighing 500 mg each, were digested in Teflon® PTFE bottles with 5 mL of concentrated HNO_3 (≥ 65%) under a pressure of 0.76 MPa for 15 minutes using a microwave oven (CEM, model Mars 5). The pressure corresponded to a temperature of approximately 175° C. Subsequently, 5 mL of double-distilled water was added to the extract and filtered for elemental analysis. The elemental content in the digested solution was determined using graphite furnace atomic absorption spectrometry.

To ensure the accuracy and quality of the analysis, certified and blank standards were incorporated. The certified standard was White Clover (BCR 402, Institute for Reference Materials and Measurements, Geel, Belgium), with a known Se concentration of 6.7 mg kg^{-1}. The certified standard and the blank were included in each digestion batch to guarantee and monitor the analysis's quality. The average recovery of the reference standard used in the analysis was 91.72%.

2.6 Centesimal composition (biomolecules)

The centesimal composition (biomolecules) analysis of the ground grain samples followed the method outlined by the Association of Official Analytical Chemists - AOAC (AOAC 2016). The moisture content was determined by the drying method in an oven at a constant weight of 65° C. In comparison, ash content was determined through incineration in a muffle furnace at 550° C until a constant weight was achieved. Total nitrogen was quantified using the Kjeldahl method, and a conversion factor of 6.25 was applied to obtain

the total crude protein content. The lipid content was determined using the Soxhlet method with petroleum ether as the solvent for extraction. The total percentage of carbohydrates was calculated using the following equation:

$$Total\ carbohydrates(\%) = 100 - As - Lp - Cp$$

Where: Total carbohydrates (%) is the total carbohydrates (soluble and insoluble) in grain, As (%) is the total ashes in grain, Lp (%) is the total lipids in grain, and CP (%) is the total crude protein in grain.

2.7 Statistical analysis

The data obtained were submitted to analyze normality and variance, and the treatments were compared using the Scott-Knott test ($p \leq 0.05$) using the Speedstat 2.8® software (Carvalho et al. 2020)

3 RESULTS

3.1 Grain production

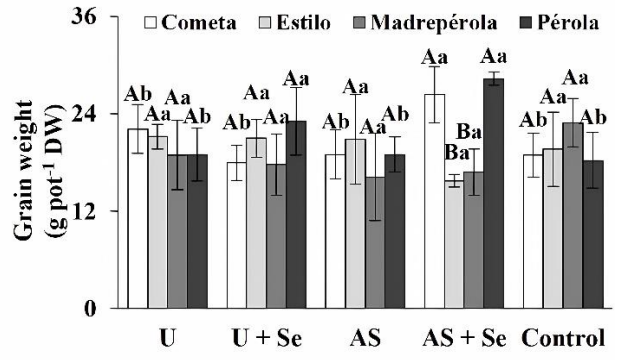

Figure 3 – Common bean genotypes grain production concerning sources of topdressing fertilization. Lowercase letters compare fertilizer sources in each genotype, and uppercase letters compare genotypes in each fertilizer source by the Scott-Knott test ($p < 0.05$).

Grain production varied between 15.76 g pot^{-1} (Estilo - AS + Se) and 28.31 g pot^{-1} (Pérola - AS + Se). Only for the AS + Se treatment, a significant difference in performance was observed among the genotypes, with Cometa and Pérola having higher means than the

others. Regarding the fertilization treatments, only Cometa and Pérola differed statistically from the other genotypes. For Cometa, AS + Se had a significantly higher mean, with differences of 32.82%, 20.52%, 33.17%, and 35.46% compared to U, U + Se, AS, and the Control, respectively. For Pérola, both AS + Se and U + Se applications showed higher averages but did not differ significantly. Amonium Sulfate (AS), U, and the Control had lower means, but no significant differences were observed. Applying AS + Se resulted in increments of 27.92%, 31.87%, and 28.19% for the treatments mentioned above, respectively (Fig. 3).

3.2 Gas exchanges, fluorometry, and SPAD index

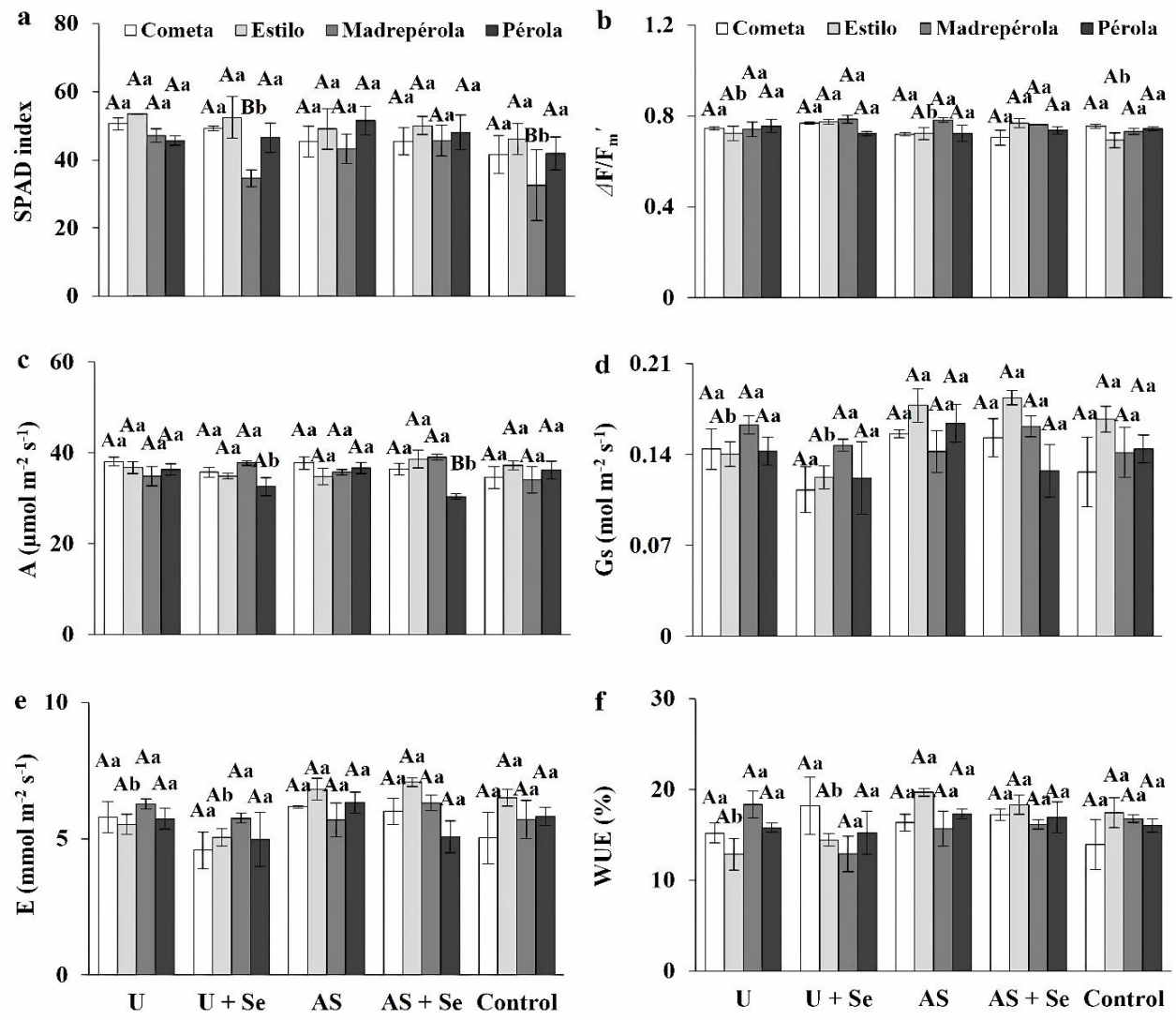

Figure 4 – SPAD index (a); maximum quantum efficiency of the photochemical activity of PSII (b); CO_2 assimilation rate (c); stomatal conductance (d); transpiration (e) and efficient

use of water (f). Lowercase letters compare fertilizer sources in each genotype, and uppercase letters compare genotypes in each fertilizer source by the Scott-Knott test ($p < 0.05$).

The SPAD index ranged from 32.53 (Madrepérola - Control) to 53.46 (Estilo - U). Significant differences in performance were observed among the genotypes only for the U + Se and Control treatments, with Madrepérola exhibiting the lowest average compared to the others. Regarding the fertilization sources, Madrepérola performed differently from the other genotypes. The U, AS, and AS + Se applications had the highest averages but no significant differences. The U + Se and Control treatments resulted in lower means, but no significant difference existed between them. Additionally, the U + Se treatment reduced the SPAD index by 26.65% compared to U (Fig. 4a).

The maximum quantum efficiency of the photochemical activity of PSII ranged from 0.69 (Estilo - Control) to 0.79 (Madrepérola - U + Se). No significant differences in performance were observed among genotypes for the different fertilization sources. However, the performance of Estilo alone was different between the fertilizer sources. In the applications with selenium, U + Se, and AS + Se, higher averages were observed, but they did not differ significantly from each other. The other fertilization sources also showed no significant differences (Fig. 4b).

The CO_2 assimilation rate varied between 30.37 (Pérola - AS + Se) and 39.06 (Madrepérola - AS + Se) µmol m^{-2} s^{-1}. A significant performance difference was observed among the genotypes only for the AS + Se source. Pérola had a lower average than the other genotypes and its performance significantly differed only between the fertilization sources. Pérola was affected by fertilization sources such as U, AS, and Control, which showed higher averages but did not differ significantly from each other. The fertilizer sources with added selenium resulted in the lowest averages, but there was no significant difference between them. The application of U + Se reduced the net photosynthesis values by 10.43% compared to U. The same trend was observed for ammonium sulfate, where the application with selenium (AS + Se) also resulted in a reduction of 17.14% compared to AS (Fig. 4c).

The other gas exchange parameters, *i.e.*, stomatal conductance (Fig. 4d), transpiration (Fig. 4e), and water use efficiency (Fig. 4f), varied within the ranges of 0.11 to 0.18 mol m^{-2} s^{-1}, 4.57 to 7.07 mmol m^{-2} s^{-1}, and 12.88 to 19.68%, respectively. No significant differences in performance were observed among the genotypes for these parameters within the same fertilization source. Only the Estilo genotype showed a different performance between the

fertilization sources. The treatments with AS with and without selenium and the Control exhibited higher averages, with no significant differences observed. Conversely, the applications with U resulted in the lowest averages, but there were no significant differences.

3.3 Accumulation of Se and S in grains

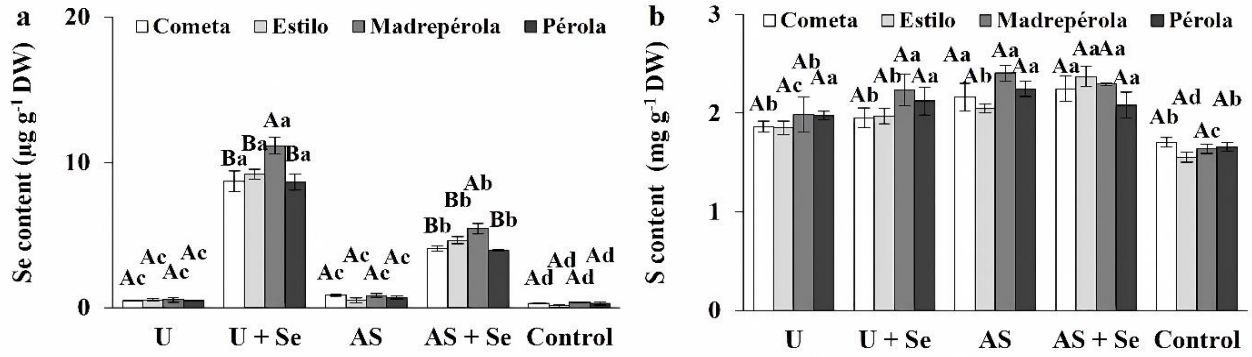

Figure 5 – Selenium and S content in grains of common bean genotypes subjected to sources of topdressing fertilization. Content of Se (a) and content of S (b). Lowercase letters compare fertilizer sources in each genotype, and uppercase letters compare genotypes in each fertilizer source by the Scott-Knott test ($p < 0.05$).

The selenium content in grains varied between 0.14 (Estilo - Control) and 11.17 (Madrepérola - U + Se) µg g^{-1}. A significant difference in performance was observed among the genotypes for the U + Se and AS + Se treatments, with Madrepérola exhibiting a higher average than the other genotypes for these two fertilization sources. The performance of the genotypes also differed between the fertilization sources. Applying U + Se resulted in statistically higher means regardless of the genotypes tested. Madrepérola had means that were 22.02%, 17.64%, and 22.47% higher than Cometa, Estilo, and Pérola, respectively. The second source with the highest average was AS + Se. Madrepérola had averages that were 25.32%, 14.68%, and 27.34% higher than Cometa, Estilo, and Pérola, respectively. No significant differences were observed among the genotypes in the fertilization sources where selenium was not added. However, when applying a nitrogen source in the coverage, the genotypes showed a higher selenium content than the Control. The increase was 45.97% for U and 61.74% for AS, with no statistical difference. The Control had the lowest selenium content (Fig. 5a).

Grain sulfur content varied between 1550.68 (Estilo - Control) and 2405.16 (Madrepérola - AS) mg g^{-1}. No significant performance differences were observed among the

genotypes for the specific fertilization source. However, the performance of the genotypes varied between the different fertilization sources. For Cometa, the highest averages were observed with the application of AS with and without selenium, and there was no statistical difference between them. The application of U with and without selenium also showed no significant difference. The Control treatment had the lowest average. On average, the applications with AS were 13.48% higher than those with U and 22.67% higher compared to the Control. Although urea-based fertilizers do not contain sulfur, they increased sulfur accumulation by 10.63% compared to the Control. For Estilo, the highest mean was observed in the application with AS + Se, followed by U + Se and AS. The treatment with U without selenium had the lowest average compared to the other treatments, except for the Control, which had the lowest average overall. When compared, the use of AS + Se resulted in increases of 21.94%, 17.04%, 13.69%, and 34.61% compared to U, U + Se, AS, and Control, respectively. Using U with selenium resulted in a percentage increase of 5.90% compared to U and 21.18% compared to the Control. For AS, the comparative increase was 9.55% compared to U and 24.24% compared to the Control.

For Madrepérola, applying U + Se and AS with and without selenium resulted in higher sulfur contents in this genotype, although they did not differ statistically. The U and Control treatments had the lowest averages, and applying U resulted in higher sulfur content. The U + Se treatment added 11.05% and 26.61% more sulfur content than U and the Control, respectively. For Pérola, only the Control differed significantly from the others, showing a lower average. The AS and U treatments, when compared to the Control, resulted in overall increases in sulfur content of 23.34% and 19.10%, respectively (Fig. 5b).

3.4 Centesimal analysis

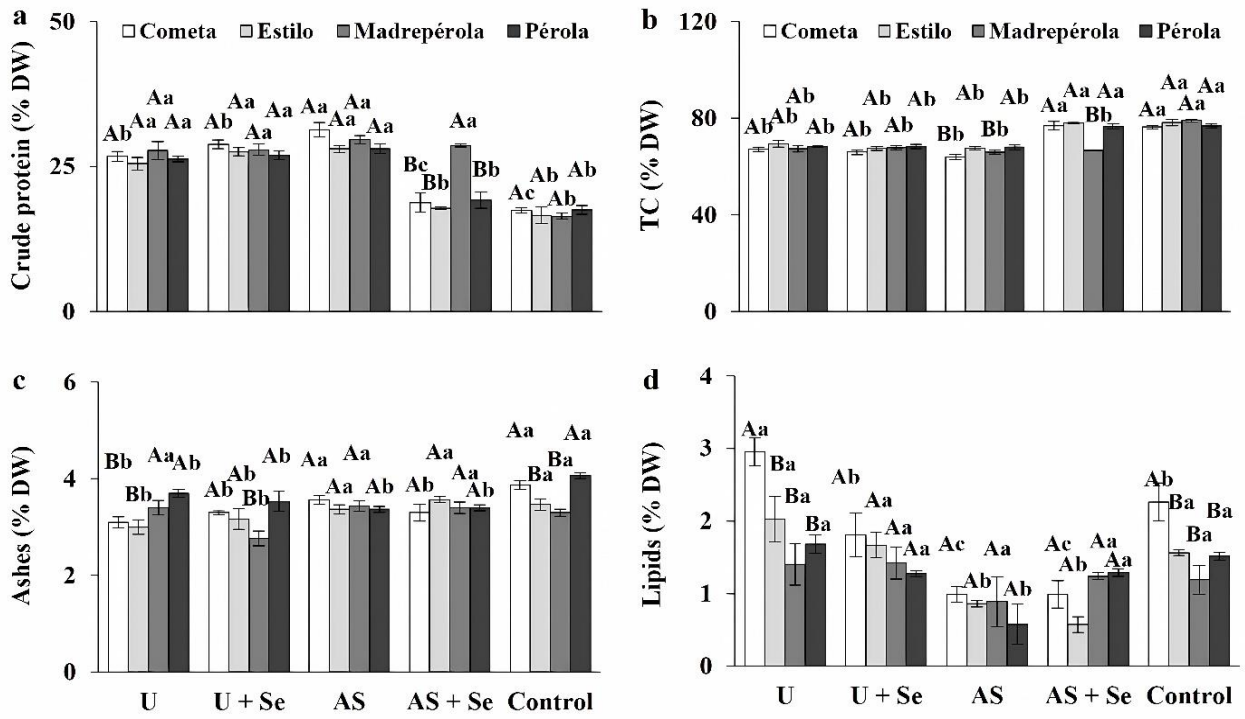

Figure 6 – Analysis of centesimal grain composition of common bean genotypes subjected to topdressing fertilization sources. Crude protein (a); TC (total carbohydrates) (b); ash (c) and lipids (d). Lowercase letters compare fertilizer sources in each genotype, and uppercase letters compare genotypes in each fertilizer source by the Scott-Knott test ($p < 0.05$).

The crude protein content in the grain varied between 16.45% (Madrepérola - control) and 31.43% (Cometa - AS). A significant difference in performance between the genotypes was observed only in AS + Se, with Madrepérola having the highest mean compared to the others. This difference was 34.43% (Cometa), 37.64% (Estilo), and 32.72% (Pérola). The performance of the genotypes was significantly different between the sources of fertilization. Cometa had the highest average percentage when AS was used. The treatments with U and U + Se showed statistically equal results, which were superior to the AS + Se and Control treatments. Estilo had its highest averages when U, U + Se, and AS were applied, with no significant differences observed. The AS + Se and Control applications resulted in the lowest averages, which were not significantly different from each other. Madrepérola was equally affected by the applications of U, U + Se, AS, and AS + Se, resulting in the highest averages. Control had the lowest average. The Pérola showed similar trends as the Estilo concerning fertilization sources. Compared to the treatment without selenium, AS + Se reduced the protein percentage for Cometa, Estilo, and Pérola by 40.24%, 36.30%, and 31.33%, respectively (Fig. 6a).

The total carbohydrate content in the grain varied between 64.02% (Cometa - AS) and 79.06% (Madrepérola - Control). A significant difference in performance between genotypes was observed only in treatments with AS without and with selenium. AS showed a difference between the studied genotypes, with Estilo and Pérola having superior averages compared to the others, and no difference was observed between them. In the application of AS + Se, only Madrepérola differed significantly from the others, with a lower mean compared to Cometa, Estilo, and Pérola by 15.30%, 16.90%, and 14.82%, respectively. No difference was observed among the other genotypes. The performance of the genotypes was different between the sources of fertilization. For the Cometa genotype, higher averages were observed with fertilizations with AS + Se and Control, with no significant differences. The treatments with U, U + Se, and AS showed lower means than the other fertilization sources, but they did not differ statistically. Madrepérola showed a higher mean with the Control treatment than the other fertilization sources. The other treatments did not differ statistically from each other. Pérola presented similar results to the Cometa and Estilo genotypes. The AS + Se treatment, compared to the AS treatment without selenium, increased the percentage of total carbohydrates for the Cometa, Estilo, and Pérola genotypes by 20.17%, 15.16%, and 12.68%, respectively (Fig. 6b).

The percentage of total ash in the grain ranged from 2.77% (Madrepérola - U + Se) to 4.07% (Pérola - Control). A significant difference in performance was observed between genotypes only in treatments with U, U + Se, and Control. The application of U resulted in a difference between the studied genotypes, with Madrepérola and Pérola having higher averages than the others, and no difference was observed between them. No differences were found among the other genotypes. In the U + Se treatment, only the Madrepérola genotype showed lower averages. For the Control treatment, Cometa and Pérola genotypes had higher averages compared to the others, with no significant difference. No difference was observed for the other genotypes. The performance of the genotypes was statistically different between the sources of fertilization. For Cometa, higher averages were observed with the applications of AS and Control, with no significant differences. The lowest averages were found for U, U + Se, and AS + Se treatments, with no significant differences. Estilo had the highest means in the treatments with AS without and with selenium and Control, showing no differences. U and U + Se treatments had the lowest averages. Madrepérola presented the highest averages when U, AS, AS + Se, and Control were applied, with no significant difference. U + Se treatment had a lower mean compared to the other treatments. For Pérola, only the Control

treatment differed significantly from the others, with the highest mean, and no differences were observed (Fig. 6c).

The percentage of total lipids in the grain ranged from 0.57% (Pérola - AS) to 2.96% (Cometa - U). A significant difference in performance between genotypes was observed only in U and Control treatments. Cometa showed a higher mean than the other genotypes in the U and Control treatments, while the other genotypes did not differ. The performance of the genotypes was significantly different between the fertilizer sources, except for Madrepérola. Cometa showed a higher average with the U application. The U + Se and Control treatments had the second-highest averages, but no significant difference existed between them. The applications with sulfate resulted in the lowest percentage of lipids, with no difference between them. For the Estilo genotype, higher averages were observed with U, U + Se, and Control treatments, with no difference. The AS and AS + Se treatments had the lowest averages, with no significant difference. No difference was observed between the sources of fertilization for Madrepérola. Pérola had the highest averages with U, U + Se, AS + Se, and Control treatments, with no significant difference observed (Fig. 6d).

4 DISCUSSION

In general, applying Se via U or AS in coverage did not harm the grain production of the tested genotypes. However, there was an increase in production in some cases, such as Cometa and Pérola (Fig. 3). Also, a difference in production response was observed between the tested genotypes when applied AS + Se. These results demonstrate that among the tested genotypes, there was genetic variability in the use of Se in the presence of a high concentration of S, which reflects on grain production. This effect happens because different genotypes for the same plant species have different gene expression patterns related to sulfate transporters, which influence the ability to control the absorption of S and Se (Coppa et al. 2023).

Biofortification considers enriching food without negatively affecting the plant cycle (Drahoňovský et al. 2016). Common beans showed potential for biofortification using the two fertilizers studied as Se carriers for plants. The increase in dry mass of grains in some genotypes, in both fertilizers enriched with Se, when compared to their ordinary form, collaborates with the reported benefits of Se. Previous studies on the use of Se in plants have reported increased plant peroxidation preventive activity, restoration of cell membrane

integrity and function, modification of antioxidant enzyme activity, and chloroplast repair and rebuilding (Mroczek-Zdyrska and Wójcik 2012; Ashraf et al. 2018; Ravello et al. 2022a).

A previous study carried out with common beans in tropical soils showed no increase in the dry mass of grains with Se application (selenate form) at doses of 0.25 to 2 mg dm^{-3} (Ravello et al. 2022). Araújo et al. (2022) also reported no increase in the dry mass of grains for common beans treated with Se via several strategies, *e.g.*, application of mono-ammonium phosphate enriched with Se, urea enriched with Se, foliar application of Se, mono-ammonium phosphate enriched with Se + enriched urea with Se, mono-ammonium phosphate enriched with Se + foliar application of Se, and foliar application of Se + urea enriched with Se. The Se (as selenate) doses used (without considering the control) ranged from 0.2 to 0.8 mg dm^{-3}. Notably, these studies did not include the possibility of a genotypic variation in the response to Se. Contrariwise, for soybean, the application of Se via MAP in the field at a dose of 80 g ha^{-1} increased the grain yield of the TMG7061 genotype (Silva et al. 2022).

Selenium can benefit plants in certain situations that the plant is exposed to. This condition is because the benefits of this element in plants are dose-dependent. It is also influenced by abiotic stresses that the plant may be exposed to (Khan et al. 2023). The control treatment, devoid of applying nitrogen, sulfur, and selenium in coverage, did not show a dry mass of grains more minor than the other treatments. The result showed that the fertilization used in planting recommended by (Malavolta 1981) was enough to meet the basic demands of N and S of the tested genotypes.

The application of U + Se decreased the SPAD index in the Madrepérola genotype (Fig. 4a), indicating a potential toxicity effect. This genotype also exhibited the highest concentration of Se in the grain (Fig. 5a), which could mean increased toxicity. Previous research conducted on lettuce in hydroponics, where varying doses of Se (selenate and selenite) ranging from 2 to 128 µM were evaluated without considering a control group, demonstrated that excessive Se damages the photosynthetic organs of lettuce and disrupts photosynthetic function (da Cruz Ferreira et al. 2020). The high concentration of Se in plants can have an antioxidant effect but may also become pro-oxidant, leading to membrane damage and the formation of non-specific selenoproteins. These changes can result in photosynthetic dysfunction due to the inhibition of plant photosystem reaction centers (Zhang et al. 2014; Gupta and Gupta 2017). Nitrogen (N) is an essential element for plant growth and development and a crucial chlorophyll component (Hawkesford et al. 2012). Sulfur (S) deficiency can affect nitrogen use efficiency and vice versa (Lee et al. 2016). Imbalances in

the supply of N and S to plants can reduce SPAD analysis, indicating potential disruptions in chlorophyll levels (Batista and Monteiro 2007).

The Estilo genotype showed a more significant increase in ΔF/Fm' when Se was applied (Fig. 4b). This could be due to the genotype's higher susceptibility to saline stress caused by salt-based fertilizers, similar to those used in this study. Previous research has demonstrated that exogenous Se did not alter the maximum quantum yield of photosystem II in tomatoes under non-saline conditions. However, in previous studies, Se application significantly increased ΔF/Fm' values by approximately 5% to 7% in saline stress conditions (Diao et al. 2014; Wu et al. 2023). This finding aligns with the overall increase of 7% observed in this experiment for the Estilo genotype. Despite the difference in ΔF/Fm', this did not result in a loss of grain production in the Estilo genotype (Fig. 3).

The Pérola genotype exhibited the lowest rate of CO_2 assimilation when Se was applied (Fig. 4c). Applying specific concentrations of Se can induce abiotic stress symptoms, which molecularly and physiologically affect plants. These effects can include rapid transcriptional and metabolic adjustments, regulation of osmotic potential, and a reduction in leaf expansion pressure (Liu et al. 2023). In the case of Se, one of the effects is the accumulation of carbohydrates, which can promote plant development and growth, such as grain production (Sager 2006). Non-stomatal limitations are known to down-regulate photosynthesis by reducing the amount and activity of Rubisco and causing carbohydrate accumulation (Torralbo et al. 2019).

Only the Estilo genotype showed a significant influence on the other gas exchange parameters studied, namely stomatal conductance (Fig. 4d), transpiration (Fig. 4e), and water use efficiency (Fig. 4f) when nitrogen fertilizers were applied. The higher values observed in Control, AS, and AS + Se treatments compared to U and U + Se may be attributed to the interplay between nitrogen, sulfur, and atmospheric CO_2 availability in plants. A study conducted by (Treml et al. 2022) on conifers in Central Europe, investigating the effects of atmospheric carbon, sulfur, and nitrogen on plant growth, found a strong synergistic relationship between CO_2, sulfur, and nitrogen in regulating stomatal function and optimizing water use efficiency.

The Madrepérola genotype exhibited the highest concentration of Se in its grains (Fig. 5a) compared to the other genotypes when Se was applied, indicating genetic variability. This characteristic, specific to this genotype, results in a more pronounced accumulation of Se in its grains. However, this accumulation is unrelated to grain production (Fig. 3a) or the overall

concentration effect. Interestingly, the Madrepérola genotype maintained protein content similar to other fertilization sources when AS + Se was applied (Fig. 6a). In contrast, protein synthesis was significantly reduced in different genotypes, leading to changes in the composition of the grain and alterations in the balance of biomolecules (Fig. 6a, b, c, and d), with a higher proportion of proteins and carbohydrates. These findings suggest a strong relationship between the genotype's ability to synthesize proteins and its high Se levels in grains.

The application of U + Se proved to be the most effective method for increasing Se contents in the grains of common bean genotypes (Fig. 5a). This finding is consistent with previous research conducted by (Araújo et al. 2022) in common beans, where urea was found to be the most effective in increasing Se contents compared to other forms of application, such as mono-ammonium phosphate and foliar application (Premarathna et al. 2012), also reported similar results in rice, where U + Se outperformed other application methods, such as soil preparation and foliar Se, in increasing Se levels. In a study conducted by (Félix et al. 2023) on upland rice, U + Se (applying 80 g of Se *via* selenate per hectare) efficiently increased Se content in polished grains across 20 different rice genotypes. This study further highlighted the existence of genetic variability among the tested genotypes concerning Se content in the grains.

The higher concentration of Se in grains when U is applied compared to AS can be attributed to various factors related to soil-plant interactions. Ammonium sulfate, composed of ammonium (NH_4^+), undergoes the ammonium nitrification reaction in the soil. This reaction releases two H^+ ions for every NH_4^+ molecule in the ammonium sulfate, containing two moles of NH_4^+. As a result, four moles of H^+ are released. On the other hand, urea is also subject to the same reaction, but due to its chemical composition, only two moles of H^+ are released. Another factor is that in urea, there are reactions at first, which cause the production of an unstable intermediate compound, which generates the volatilization of NH_3 in the soil, generating an increase in pH in the affected area (Pereira et al. 2021; Dai et al. 2023) and the increased of soil pH increases the content of Se water-soluble and available to uptake by plants (Hossain et al. 2021)

It is important to note that due to the lower nitrogen percentage in AS fertilizer (21%) compared to urea (46%), a higher amount of AS had to be applied to meet the nutrient requirement specified by (Malavolta 1981) for topdressing. This increased application of AS resulted in higher sulfur content in the soil, which may have hindered the uptake of Se by the

plant while promoting an increase in sulfur accumulation in the grains (Fig. 5b). Previous studies have shown that selenate primarily moves through sulfate transporters and is metabolized through the sulfur metabolic pathway in plants. The presence of excess sulfur in grains can induce sulfur starvation in plants, affecting their overall nutrient balance (White et al. 2004; El Kassis et al. 2007; Hsu et al. 2011; Boldrin et al. 2016; Schiavon et al. 2016; Drahoňovský et al. 2016; White 2017). Therefore, the specific characteristics and chemical reactions of each fertilizer influenced the soil conditions that Se was exposed to. The adsorption of Se is a significant factor controlling its concentration, and soil physicochemical properties, such as pH, play a crucial role in Se availability (He et al. 2018; Kushwaha et al. 2022).

The higher concentrations of Se and S in grains resulting from topdressing fertilization with nitrogen sources (U and AS) compared to the Control (Fig. 5a and 5b) are attributed to the nitrogen supply. Previous studies conducted in crops such as rice (Reis et al. 2018) and wheat (Chen et al. 2017; Klikocka et al. 2017) have reported that nitrogen can enhance the uptake and translocation of Se in plants. Nitrogen stimulates the production of O-acetyl serine, a key regulator of sulfur metabolism and cysteine synthesis (a sulfur-containing amino acid) in higher plants, leading to increased protein synthesis (Kim et al. 1999). This finding is consistent with the results of the present study (Fig. 6a). Another contributing factor for the absorption of S and Se, although not investigated in the present study, is root growth. Nitrogen promotes root development, thereby enhancing the uptake of phosphorus, potassium, sulfur, and other mineral elements, including Se (Chen et al. 2012).

5 CONCLUSIONS

The soil application of Se through Se-enriched fertilizers has shown potential for increasing Se contents in common bean grains. Additionally, Se-fertilizers can enhance grain production, and change physiological response depending on genotypes x Se-fertilizers interaction. The effectiveness of biofortification and the observed effects varied depending on the method of Se addition and the specific genotypes evaluated. It was found that Se contents in bean grains were higher when applied via Se-enriched urea, due to the initial alkalinization of this source in the soil zone around the fertilizer granule, and no S in composition. Using ammonium sulfate as a carrier of Se had a distinct effect, leading to a higher synthesis of carbohydrates over proteins in most of the tested genotypes. Among the genotypes, Madrepérola showed the best response to Se application, maintaining the balance between

biomolecules and demonstrating high efficiency in selenium accumulation in the grain. Additionally, top-dressing nitrogen fertilization played a role in increasing the Se content in grains. This study provides valuable insights into using fertilizers, particularly Se carriers, in tropical soils for the biofortification of food crops, leveraging agronomic knowledge of fertilization practices.

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Manuscript 2: LEAF COLOR VARIATION IN PAK CHOI GENOTYPES AFFECTS THE INTERACTION OF SELENIUM AND PHENOLIC COMPOUNDS

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ABSTRACT

This study aimed to investigate the effect of selenium (Se) on phenolic compounds in different genotypes of pak choi (*Brassica rapa* L. *chinensis*). The experiment was conducted in a greenhouse using a hydroponic system. Ten genotypes of pak choi were evaluated, and subjected to three selenium treatments (control, selenate, and selenite at 25 µM), with three biological replicates. The analysis was performed only on the plant shoots. Both forms of selenium significantly increased the levels of phenolic compounds in most genotypes. The results showed that, under the studied conditions, selenite was more toxic than selenate, but the selenate group had the highest selenium levels. Physiological changes were observed in the plants, with negative responses being more frequent in selenite group at the tested dose. However, selenite toxicity induced a greater increase in shoots. The SF genotype showed the highest response to selenium application. Selenium content in the plant shoots was correlated with sulfur content. Exposure to the tested selenium forms resulted in toxic effects in some genotypes, leading to a reduction in phosphorus and sulfur content. Nevertheless, the presence of higher levels of phenolic compounds seemed to alleviate these symptoms in some cases. The intensely purple-colored genotypes had higher levels of phenolic compounds and lower levels of selenium. This suggests that phenolic compounds play a critical role in selenium tolerance in plants.

Keywords: *Brassica rapa* L.. Flavonoid. Secondary metabolism. Selenate. Selenite. Tolerance.

1 INTRODUCTION

Pak choi (*Brassica rapa* var *chinensis*) is a member of the Brassicaceae family and is widely farmed and eaten in Japan and China (HARBAUM et al., 2008; HUANG et al., 2022). Pak choi has a considerable number of useful antioxidants and nutrients (such as carotenoids, vitamins, phenolic compounds, and minerals) (BJÖRKMAN et al., 2011).

Brassicaceae crops are among the earliest cultivated plants (MAGGIONI et al., 2018). Brassica vegetables are known for their high protein and fiber content compared to other vegetables. Due to their high protein content and excellent dry matter digestibility, the use of Brassicas as crop foods is gaining increasing interest (FAVELA-GONZÁLEZ; HERNÁNDEZ-ALMANZA; DE LA FUENTE-SALCIDO, 2020; RABOANATAHIRY et al., 2021). Brassica species are commercially significant vegetables and their global output in 2017 was over 71 million tons (MARTÍNEZ et al., 2020). Cultivated species of Brassicaceae include oilseed rape, cauliflower, broccoli, Brussels sprouts, and green vegetables like kale, mustard, and pak choi.

All brassicas have significant amounts of secondary metabolites (*e.g.*, flavonoids), many of which are of considerable economic worth owing to their uses in the medical, pharmaceutical, agricultural, and cosmetic sectors (NEUGART et al., 2018). Among the secondary metabolites identified in brassicas that may be connected with these therapeutic benefits are phenolic substances, notably flavonoids, including quercetin and kaempferol (AKDAŞ; BAKKALBAŞI, 2017).

In humans, phenolic compounds are known to have high antioxidant as well as cardioprotective, immune system boosting, antibacterial, anti-cancer, and anti-inflammatory actions (BRIGUGLIO et al., 2020; SILVA; POGAČNIK, 2020; TUNGMUNNITHUM et al., 2018). In plants, these compounds are generated through the shikimic acid and phenylpropanoid acid pathways. These are found in the free form and as ester and/or glycosidic derivatives. Phenolic compounds include flavonoids and phenolic acids. It is worth mentioning that while these two categories are the principal polyphenols, many writers additionally include lignans, stilbenes, and tannins (AMAROWICZ; PEGG, 2019; FOSS; PRZYBYŁOWICZ; SAWICKI, 2022).

During particular stressful situations, phenolic compounds behave as poisons and antibiotics (D'ARCY, 2022). Abiotic stress examples include drought, heat, cold, ultraviolet radiation, high light intensities, and specific ion concentrations in the soil. These conditions

activate biosynthetic pathways. Biotic stimuli like viruses and herbivores may also induce the creation of specific defensive chemicals. Responses to both sorts of pressures are not mutually exclusive. In particular, flavonoid biosynthesis appears as an extremely universal stress response pathway that is activated by a wide variety of stress circumstances (ALBERT et al., 2018; BIAN et al., 2020a; FINI et al., 2011; JAAKOLA et al., 2004; LIANG et al., 2020; MA et al., 2019; PETRUSSA et al., 2013; WANG et al., 2022b; WINKEL-SHIRLEY, 2002; YIN et al., 2022).

One of the ions that has been extensively studied in plants that can activate phenolic compound pathways is selenium (Se). Most scientists believe that the impact of Se on plants' antioxidant defense mechanisms is what provides its health advantages. Studies have demonstrated that Se enhances the production of low molecular weight antioxidants (*e.g.*, phenolics), and increases the activity of antioxidant enzymes (ABDALLA; LENTZ; MÜHLING, 2022). Although higher plants do not need Se, they absorb inorganic Se forms (selenate and selenite) and organic Se compounds through different sulfate and phosphate transport mechanisms (SZŐLLŐSI et al., 2022).

To better understand the mechanisms of Se metabolism in plants, members of the Brassicaceae family have been used as model plants. Many of the Brassicaceae are classified as Se hyperaccumulators (some *Stanleya* species), and Se accumulators (*e.g.*, mustard, rapeseed, broccoli, and arugula) (SKRYPNIK et al., 2022). However, certain species like pak choi, have received limited research and require further study. A study that explored Se application to enhance beneficial substances in pak choi was conducted by Wang et al. (2022). This study showed that the application of Se nanoparticles (0.5 mg of Se kg^{-1}) to the soil resulted in a 264.9% increase in the plant's Se content. This also led to the accumulation of additional low molecular weight substances such as betaine, proline, glycine, norleucine, urocanic acid, and indole-3-acrylic acid. Another study conducted on pak choi in soil demonstrated the capacity of Se, as selenate (0.5 mg kg^{-1}), to neutralize the adverse effects of Pb stress (600 mg kg^{-1}) on photosynthesis, oxidative stress, and the AsA-GSH system (TAN et al., 2022). Furthermore, another study examined the effects of Se foliar applications ranging from 0.5 to 30 mg Se $plant^{-1}$. It was observed that plant growth was promoted at a concentration of 2 mg Se $plant^{-1}$, and there was an increase in isothiocyanates in fresh matter. Moreover, the sulfur content was enhanced in response to increasing Se concentrations (ABDALLA; MESCHEDE; MÜHLING, 2020).

Considering the necessity for further research on the application of Se in *Brassica rapa* L., especially those evaluating low molecular weight compounds relevant to human nutrition (*e.g.*, phenolic compounds - PCs) and understanding genotypic responses, this work aimed to assess the effect of Se upon PCs produced in different genotypes of pak choi.

2 MATERIAL AND METHODS

2.1 Plant materials

In this study, 10 pak choi genotypes (TABLE 1, and FIGURE 1) were used. Seeds were sown in trays with a commercial growing media (Metro-Mix 360, Sun Gro Horticulture) in a greenhouse with a photoperiod of 14 hours of light and 10 hours of darkness at a temperature of 23 to 25°C. The immature seedlings were carefully removed from the trails and cleansed free of soil after 12 days of germination, and then hydroponically grown for conditioning in a tray with 50% ionic strength of a Hoagland nutrient solution under continued aeration (HOAGLAND; ARNON, 1950).

After 7 days of conditioning, seedlings were transplanted into 2 L black polyethylene pots (3 seedlings per pot) using the 50% ionic strength Hoagland nutrient solution with the addition of the 3 treatments: 25 µM sodium selenate (Na_2SeO_4); 25 µM sodium selenite (Na_2SeO_3); and control (without Se). One week later, the shoots of 90 pots (10 genotypes × 3 treatments × 3 biological replicates) were harvested. In order to analyze the Se, S, P content, and dry weight. For further analyses, the other plant materials were placed in liquid nitrogen and kept at -80°C, and freeze-dried (-80°C, 5 mT) in an MCFD8508 freeze dryer (ilShinBioBase Co., Ltd., Dongducheon, Korea).

Table 1 – Pakchoi genotypes used in this study.

Genotypes	Color	Nickname
Extra Dwarf	Green	ED
Kosaitai	Green	K
Tatsoi	Green	T
San Fan	Green	SF
Garnet Giant	Green/purple	GG
Red Mizuna	Green/purple	RM

Red Choi	Green/purple	RC
Red Tatsoi	Green/purple	RT
Da Hong Summer	Purple	DHS
Purple Magic	Purple	PM

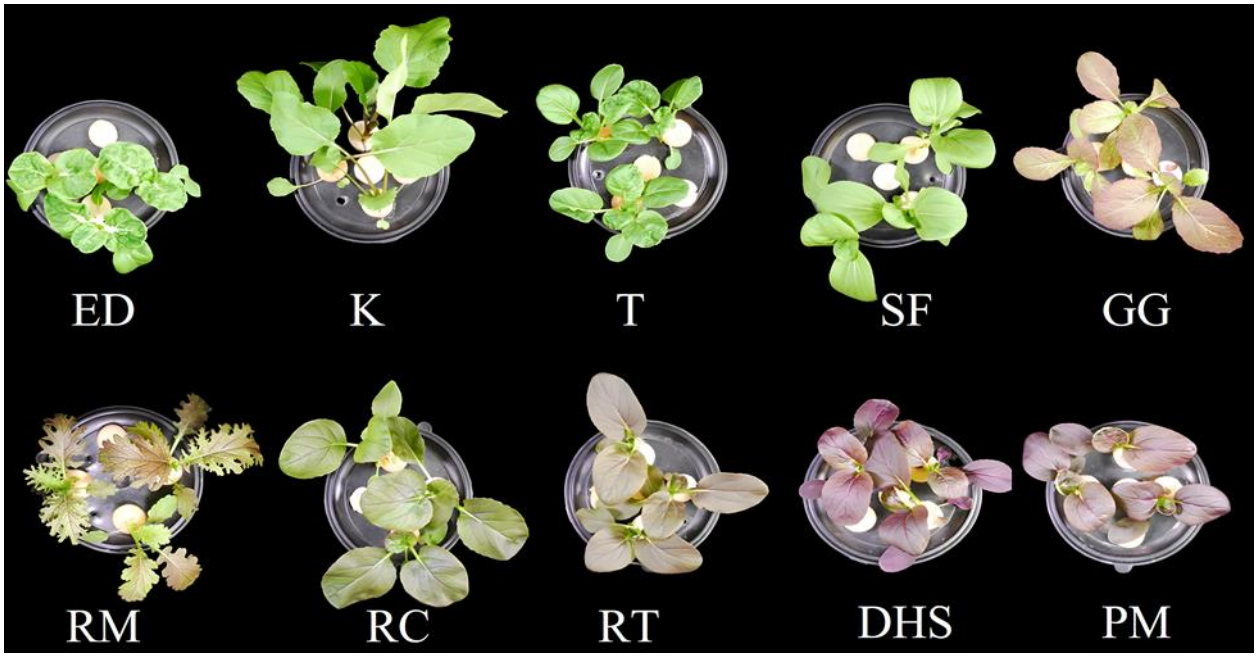

Figure 1 – Phenotypes of 10 pak choi genotypes at 26 days of growth.

2.2 Physiological responses

The relative chlorophyll content was measured before harvest using a chlorophyll meter (SPAD-502; Minolta Camera, Osaka, Japan). The average SPAD value of three randomly selected leaves from each plant was recorded. Net CO_2 assimilation rate and efficiency of photosystem II were recorded on a sunny day between 8 a.m. and 12 p.m., using a portable photosynthesis system (LI-6800, LI-COR Biosciences) equipped with a multiphase flash fluorometer chamber. Within the chamber, the density of photosynthetic photon flux was controlled at 1000 μmol m^{-2} s^{-1}. The concentration of carbon dioxide was fixed at 420 μmol mol^{-1}. One healthy and fully expanded leaf from the external canopy of each plant was randomly selected, and the evaluated parameters were recorded on the day of harvest when the leaf was placed in the chamber for approximately 1 minute (BUNCE, 2016).

2.3 Measurement of total Se, S, and P content by ICP

The total elements from the dried shoots were determined after acid digestion as detailed in our previous report (Figueiredo et al., 2017). The acid-digested samples were analyzed for element contents using an inductively coupled plasma (ICP) trace analyzer emission spectrometer (model ICAP 61E trace analyzer, Thermo Electron, San Jose, CA, United States).

2.4 Calculation of the Se efficiency indicator

Based on the calculation made by (DUCSAY et al., 2016), Se absorption efficiency in shoots was calculated (%) using the following equation:

$$SeE = \frac{[mg\ of\ Se\ absorbed]_{Se\ treatment} - [mg\ of\ Se\ absorbed]_{control}}{mg\ of\ Se\ applied} \times 100$$

2.5 Freeze-dried samples extraction

The precise weight of the freeze-dried macerated material was recorded when it was placed in a 1.5-mL polyethylene microtube (about 50 mg). Methanol of 80% UPLC grade was utilized for extraction in a 20-times weight-to-volume ratio. With ice added to the water, samples were sonicated in an ultrasonic cleaner (Model 150T, VWR Scientific-Aquasonic, West Chester, PA) for 30 minutes. Following centrifugation of the samples at 14000 rpm for 10 minutes at 4°C, the supernatant was transferred to a fresh 1.5-mL polyethylene micro tubes.

2.6 Measurement of total flavonoid

The determination of flavonoids concentration was carried out following the method from Djeridane et al. (2006) with adjustments. The method is based on the formation of an aluminum-flavonoid complex, resulting in a yellow-colored solution in the presence of soda. The developed color absorbs light in the visible spectrum at 430 nm. In each reaction, 50 µL of the appropriate plant extracts or standard rutin solution were mixed with 450 µL of aluminum trichloride solution ($AlCl_3$) at 2% w/v. The mixtures were incubated for 15 min. The flavonoid concentrations were quantified and expressed as milligrams of rutin equivalent per gram of dry weight (mg RE g^{-1} DW). All samples were analyzed in triplicate.

2.7 Measurement of total phenolic compounds

The determination of total polyphenol content was carried out using the Folin–Ciocalteu method adapted from Ainsworth; Gillespie (2007). The amount of polyphenols present in the plant extracts is directly proportional to the absorbance measured at 765 nm using a UV-visible spectrophotometer. Fifty µL of plant extracts were combined with 80 µL of Folin reagent (10% vol/vol). Following a 3-minute incubation at ambient temperature, 320 µL of a sodium carbonate solution, Na_2CO_3 (700 mM), was added. The mixture was then incubated for 15 minutes. The concentration of each phenolic component was determined through three separate experiments to ensure the validity of the findings. Gallic acid was used as the standard phenol. The total polyphenol concentrations were reported in terms of milligrams of gallic acid equivalents (GAE) per gram of dry weight (mg GAE g^{-1} DW).

2.8 Measurement of total quercetin, and kaempferol

A PCR tube was filled with 25 µL of extract and 25 µL of 2 M HCl. Subsequently, a thermal cycler (Model T100 Thermal Cycler, Bio-Rad, Singapore) was set to 90°C for 60 minutes to hydrolyze the sample. Following hydrolysis, 40 µL of the resulting mixture was transferred to a glass vial. The concentrations of quercetin, kaempferol, caffeic acid and p-coumaric acid were determined using an ACQUITY UPLC system equipped with a BEH C18 column (2.1 × 50 mm, 1.7 µm) (Waters in Milford, Massachusetts, USA) (Pic. 19). Using the mobile phases of solvent B (0.1% formic acid in acetonitrile) and solvent A (0.1% aqueous formic acid), the samples (4 µL) were fed into the column and eluted during four minutes at a flow rate of 0.6 mL min^{-1}. The column effluent was detected at 360 nm. The quantification of the obtained data was done using a standard curve, and the results were represented in mg g^{-1} DW.

2.9 Statistical analysis

Variance analysis was performed on the data ($p < 0.05$). Using the statistical program Speedstat software 2.8 (CARVALHO et al., 2020), the Scott-Knott test was used to compare the mean values. The cluster analysis using Ward's Method with Euclidean distance was

performed by the Software R Core Team (2023) using the "factoextra" package (KASSAMBARA; MUNDT, 2021).

3 RESULTS

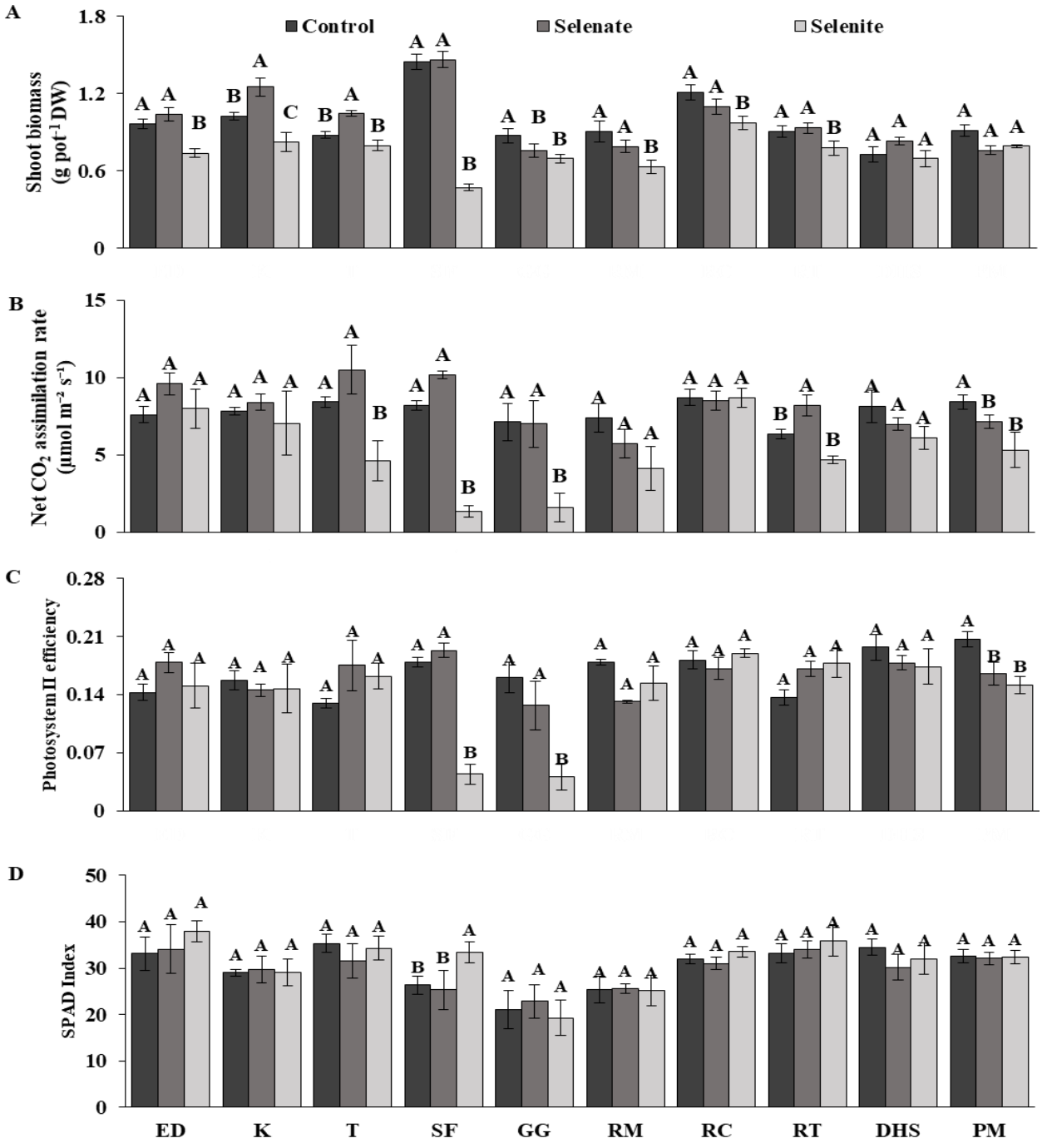

Figure 2 – Shoot biomass (A), net CO_2 assimilation rate (B), photosystem II efficiency (C), and SPAD index (D) of ten pak choi genotypes. The plants were subjected to different Se sources (Control, 25 µM Selenate, 25 µM Selenite) for one week. Data represent the means of

three biological replicates. Bars indicate standard deviation. Uppercase letters compare treatments in each genotype. Different letters above the columns show significant differences at $p < 0.05$ by the Scott-Knot test.

The results indicated that the interaction between genotypes and Se treatments generated significant differences ($p < 0.05$) for all variables evaluated in this study. Regarding the differences found in shoot biomass (FIGURE 2A), there was no significant difference between treatments in DHS and PM genotypes. The ED, SF, RM, RC, and RT genotypes showed a decrease in biomass in response to selenite treatment compared to control treatments (without Se) of 23.60%, 67.52%, 30.37%, 19.70%, and 14.17%, respectively.

The K genotype exhibited an increase of 22.36% in biomass due to selenate application, compared with the control. Selenite had the opposite effect on this genetic material, resulting in a decrease of 19.27%. In the case of T, there was an 18.93% increase in biomass in response to selenate treatment compared with the control, with no statistical difference between the control and selenite treatments. The application of Se salts resulted in a biomass decrease in the GG genotype, which was approximately 13.34% and 20.34% for selenate and selenite, respectively.

For the net CO_2 assimilation rate (FIGURE 2B), no significant differences were observed between the studied treatments in the ED, K, RC, and DHS genotypes. However, in the T, SF, and GG genotypes, selenite application led to a decrease of approximately 45.02%, 83.44%, and 77.87%, respectively, compared with the control. Selenate was not statistically different from the control in these genotypes. In the PM genotype, there was also a reduction. However, it was observed for both selenite and selenate treatments, with decreases of approximately 36.84% and 14.92%, respectively, compared with the control. In contrast with the observed in other genotypes, the RT exhibited an increase of 29.31% with selenate application compared with the control, while selenite was not statistically different from the control in this genotype.

The measurement of the efficiency of photosystem II (FIGURE 2C) indicated that the genotypes most affected by Se treatments in this variable were SF, GG, and PM. Among these, the first two genotypes showed a significant decrease of 75.42% and 74.45%, respectively, when selenite was applied, compared with the control. Selenate did not differ statistically from the control. The PM genotype showed a reduction of 26.62% and 20.35% with selenite and selenate treatments, respectively, compared with the control. The SPAD

index (FIGURE 2D) evaluated in the experiment showed statistical differences only in the SF genotype, where selenite application resulted in a 26.76% increase compared with the control. Selenate did not differ statistically from the control.

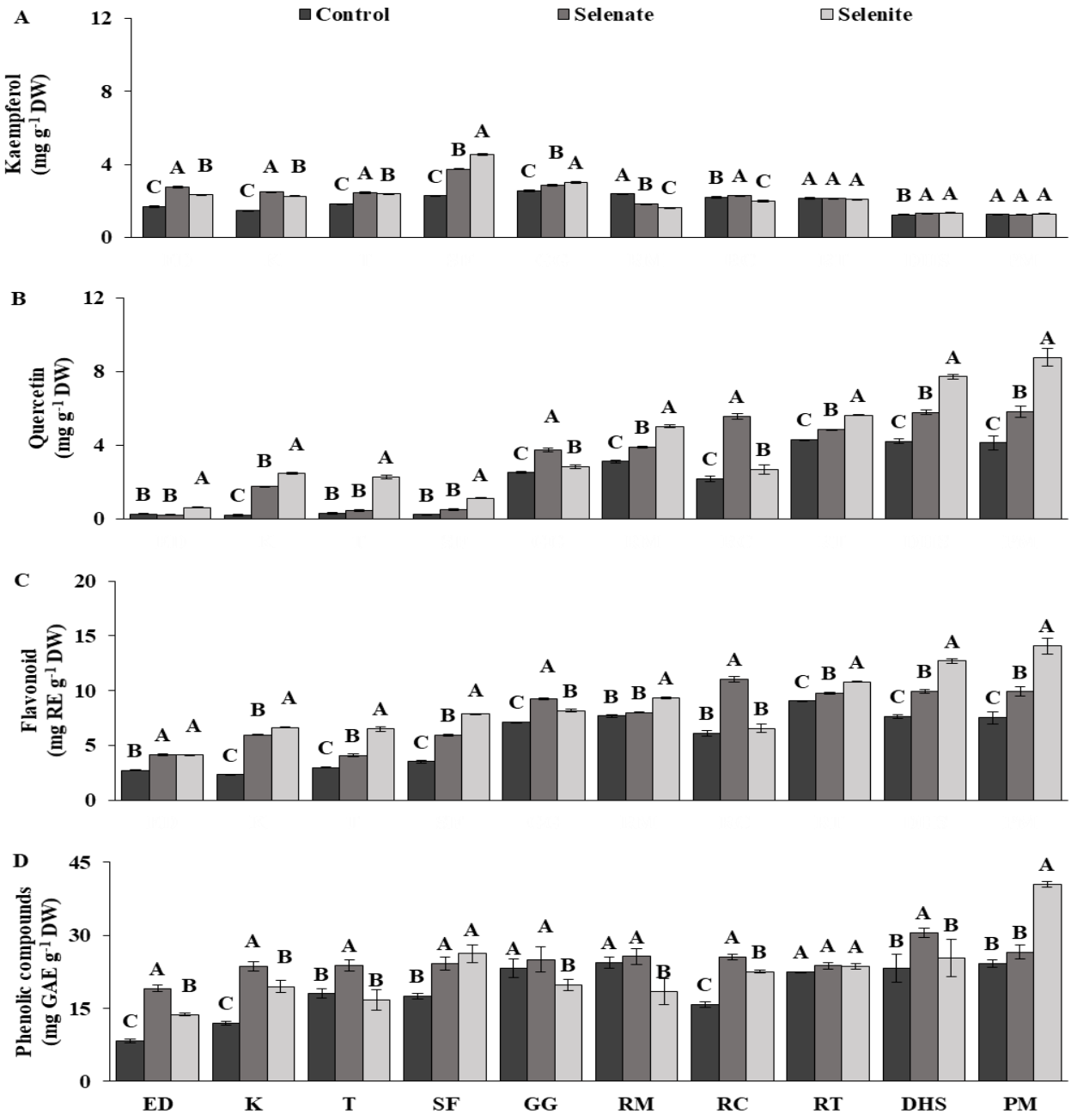

Figure 3 – Kaempferol (A), quercetin (B), flavonoid (C), and phenolic compounds (D) of ten pak choi genotypes. The plants were subjected to different Se sources (Control, 25 μM Selenate, 25 μM Selenite) for one week. Data represent the means of three biological replicates. Bars indicate standard deviation. Uppercase letters compare treatments in each

genotype. Different letters above the columns show significant differences at $p < 0.05$ by the Scott-Knot test.

Regarding the evaluated phenolic compounds in this study, the flavonoid kaempferol (FIGURE 3A) exhibited increased content in most of the evaluated genotypes, except for PM and RT, where no significant difference was observed between treatments. Genotypes ED, K, T, and DHS demonstrated an increase in kaempferol content by 63.63%, 69.59%, 34.49%, and 6.17%, respectively, when selenate was applied compared with the control. Similarly, selenite also contributed to elevated levels compared with the control, with approximately 38.61%, 54.77%, 30.34%, and 9.70% increments for ED, K, T, and DHS, respectively.

It is noteworthy that selenate showed a greater capacity for increasing kaempferol compared to selenite, except for DHS, where no difference was observed between selenate and selenite. For the SF and GG genotypes, an increase was also observed. However, selenite induced a greater increment than selenate, and both were higher than the control. Specifically, the increases were 98.27% and 16.97% for SF and GG, respectively, with selenite. In the case of selenate, the increases were 64.27% and 10.88%, respectively. In the RM genotype, Se treatments significantly decreased kaempferol levels compared with the control. Selenate exhibited a decrease of 22.83%, and selenite contributed to a decrease of 31.10%. The RC genotype also showed a decrease compared with the control of approximately 8.23%. However, the application of selenate led to a 5.31% increase when compared with the control.

The measured quercetin (FIGURE 3B) in this study was significantly affected by applied Se. Specifically, the K, RM, RT, DHS, and PM genotypes showed a significant increase compared with the control when exposed to selenate and selenite, respectively. The increases observed for selenate, in the order of previously mentioned genotypes, were 1211.37%, 61.71%, 30.70%, 83.16%, and 112.60%, while for selenite, they were 831.23%, 24.93%, 12.32%, 37.18%, and 41.44%. For GG and RC, there were also significant increases with Se treatments compared with the control. Notably, selenate induced greater increases of 50.01% and 157.55% compared to selenite (12.78% and 23.48%). In the cases of ED, T, and SF observed increases compared with the control were evident only in response to the selenite treatment, with increases of 148.81%, 668.61%, and 406.30%, respectively. Selenate did not differ statistically from the control.

For the total flavonoid content (FIGURE 3C), all genotypes exhibited a significant increase when treated with both selenate and selenite compared with the control. The

exceptions were RM and RC genotypes; for RM, no significant difference was observed between selenate and the control, while for RC, no significant difference was observed between selenite and the control. The increases observed for selenate, compared with the control, in the order of the genotypes ED, K, T, SF, GG, RC, RT, DHS, and PM, were 54.37%, 156.31%, 37.42%, 69.59%, 30.18%, 81.02%, 7.90%, 30.17%, and 31.59%, respectively. For selenite, the increases were 52.80%, 186.46%, 118.96%, 126.46%, 14.91%, 21.52%, 19.60%, 66.56%, and 87.20%. No significant difference was observed between treatments for the ED genotype. Selenite resulted in a larger increase than selenate for the genotypes K, T, SF, DHS, and PM. The opposite effect was found for the GG genotype, where selenate led to a larger increase than selenite, when compared with the control.

The response to selenate and selenite application differed among the studied genotypes for the total phenolic compound content (FIGURE 3D). Selenate significantly increased the total phenolic compound content compared with the control in all studied genotypes, except for GG, RM, RT, and PM, where no significant difference was observed between selenate and the control. The increases observed in the other genotypes were 129.27%, 97.25%, 32.69%, 38.44%, 62.40%, and 30.86%, respectively. Selenite application also induced an increase in the total phenolic compound content compared with the control in the genotypes ED, K, SF, RC, and PM, with increases of 64.78%, 62.63%, 50.23%, 42.99%, and 67.58%, respectively. No significant difference was observed between selenite and control for T, RT, and DHS. For ED, K, T, GG, RM, RC, and DHS, selenate was comparatively more efficient than selenite in increasing the total phenolic compound content. No significant difference was observed between selenate and selenite treatments for SF. However, in GG and RM, selenite application led to a decrease of 14.50% and 24.43%, respectively, compared with the control.

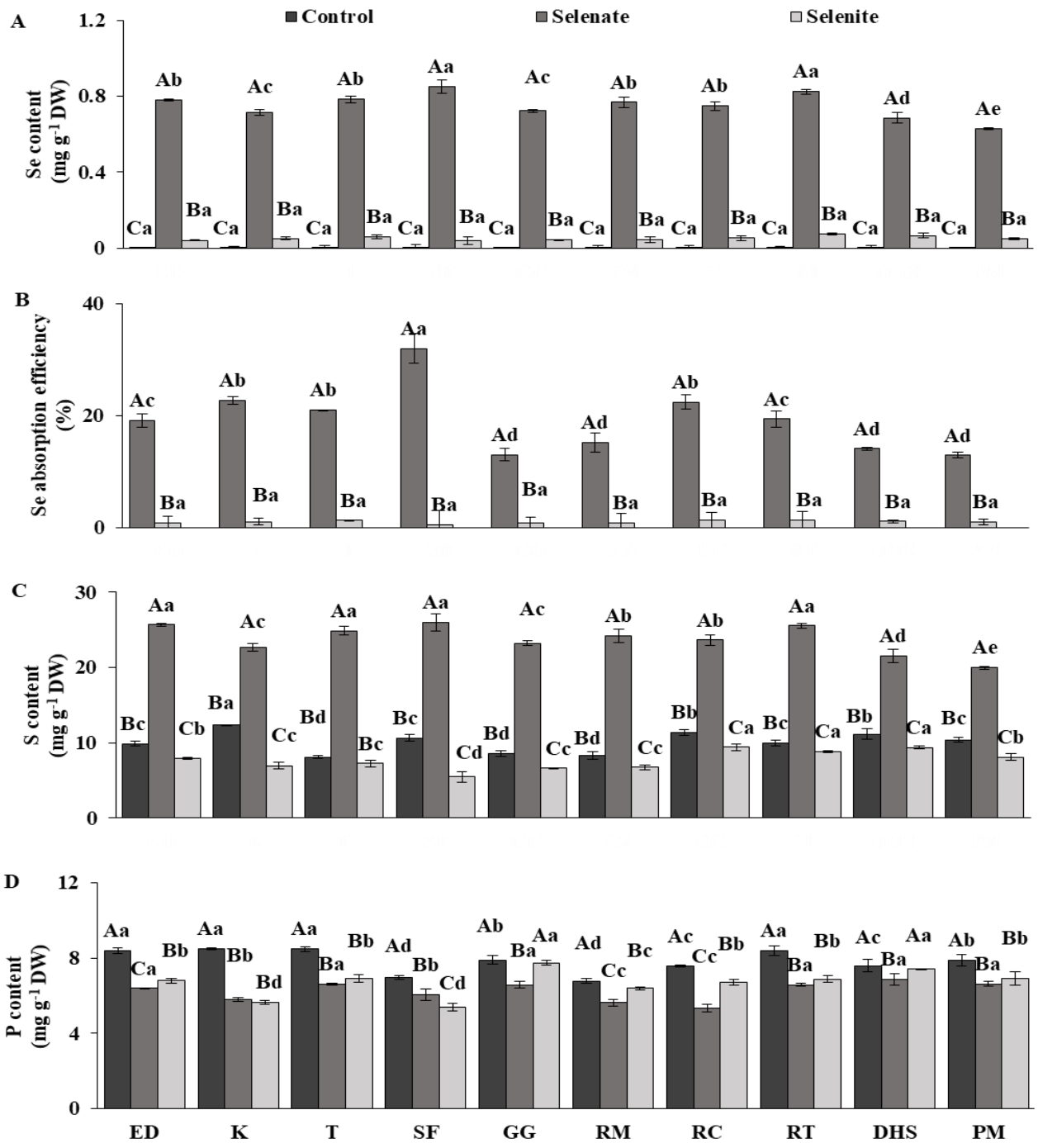

Figure 4 – Se content (A), Se absorption efficiency (B), S content (C), and P content (D) of ten pak choi genotypes. The plants were subjected to different Se sources (Control, 25 μM Selenate, 25 μM Selenite) for one week. Data represent the means of three biological replicates. Bars indicate standard deviation. Uppercase letters compare treatments in each genotype, and lowercase letters compare genotypes in each treatment. Different letters above the columns show significant differences at $p < 0.05$ by the Scott-Knot test.

Based on the elemental analysis of the dry shoot of the genotypes subjected to the treatments in this study, selenate application significantly increased the Se content (FIGURE 4A) compared with the control, increasing from 0.00056 mg g^{-1} to 0.75213 mg g^{-1} on a dry weight basis. The same effect was observed for selenite compared with the control, which increased dry weight Se content from 0.00056 mg g^{-1} to 0.05177 mg g^{-1}. However, for all studied genotypes, selenate treatment was more efficient in increasing the Se content than selenite. No differences were observed between genotypes for control and selenite treatments. In the selenate treatment, SF (0.85 mg g^{-1}) and RT (0.82 mg g^{-1}) exhibited the highest Se content among the genotypes. ED (0.77 mg g^{-1}), T (0.78 mg g^{-1}), RM (0.76 mg g^{-1}), and RT (0.82 mg g^{-1}) had the second-highest means, while K (0.71 mg g^{-1}) and GG (0.72 mg g^{-1}) had the third-highest means. Genotype DHS (0.68 mg g^{-1}) represented the fourth-highest mean, and PM (0.62 mg g^{-1}) displayed the lowest mean. Genotypes classified under the same Se content category did not exhibit significant differences.

The index calculated for Se absorption efficiency relative to shoot dry matter (FIGURE 4B) demonstrated that, on average, the application of selenate (19.14%) resulted in significantly higher values than that of selenite (0.96%) across all evaluated genotypes. In the selenite treatment, no statistically significant difference was observed among the genotypes. However, in the selenate treatment, SF (31.94%) showed the highest Se content compared to the other genotypes. K (22.70 mg g-1), T (22.95%), and RC (22.39%) had the second-highest averages, while ED (19.05%) and RT (19.35%) had the third-highest averages. Lower averages were observed for GG (12.97%), RM (15.14%), DHS (14.06%), and PM (12.86%). The genotypes grouped within the same classification of Se absorption efficiency did not show significant differences.

The S content (FIGURE 4C) in the shoot dry matter showed that selenate application significantly increased S levels compared with the control, increasing from 10.06 mg g^{-1} to 23.72 mg g^{-1} overall. The opposite effect was observed for selenite, resulting in a decrease in S levels from 10.06 mg g^{-1} to 7.65 mg g^{-1}, except for the SF genotype, which showed no significant difference. The results obtained with selenate application demonstrated a similar capacity of genotypes to accumulate S and Se, except for the T genotype, which was comparably equal to SF in this variable.

In both the control and selenite treatments, significant differences were observed among the evaluated genotypes. In the control treatment, the K genotype (12.35 mg g^{-1}) exhibited the highest S concentration, followed by RC (11.37 mg g^{-1}) and DHS (11.13 mg g^{-}

^{1}). ED (9.87 mg g^{-1}), SF (10.65 mg g^{-1}), RT (9.95 mg g^{-1}), and PM (10.06 mg g^{-1}) displayed the second-highest concentrations, while T (8.09 mg g^{-1}), GG (8.53 mg g^{-1}), and RM (8.27 mg g^{-1}) showed the lowest concentrations. For the selenite treatment, the results were different, with RC (9.38 mg g^{-1}), RT (8.79 mg g^{-1}), and DHS (9.36 mg g^{-1}) showing the highest levels, followed by ED (7.91 mg g^{-1}) and PM (8.10 mg g^{-1}) with the second-highest levels. K (6.94 mg g^{-1}), T (7.24 mg g^{-1}), GG (6.59 mg g^{-1}), and RM (6.72 mg g^{-1}) resulted in the third-highest levels, and finally, SF (5.49 mg g^{-1}) had the lowest level. The genotypes grouped in the same S content classification did not show significant differences.

The P content (FIGURE 4D) in the shoot dry matter was influenced by the treatments, with the application of selenate and selenite significantly decreasing P levels when compared with the control. This led to a reduction from 7.84 mg g^{-1} to 6.24 mg g^{-1} for selenate and from 6.68 mg g^{-1} to 6.24 mg g^{-1} for selenite overall. Exceptions were observed, with the genotypes GG and DHS showing no significant difference between the control and selenite. Moreover, ED, RM, and RC exhibited a greater response to selenate in terms of reducing P levels compared to selenite. In the case of selenate, SF (5.53 mg g^{-1}) had the lowest level, followed by GG (5.86 mg g^{-1}), T (5.88 mg g^{-1}), DHS (6.03 mg g^{-1}), RT (6.13 mg g^{-1}), PM (6.16 mg g^{-1}), RC (6.35 mg g^{-1}), K (6.36 mg g^{-1}), and ED (6.45 mg g^{-1}). For selenite, GG (6.66 mg g^{-1}) had the lowest level, followed by DHS (6.80 mg g^{-1}), T (6.81 mg g^{-1}), RT (6.90 mg g^{-1}), RC (6.92 mg g^{-1}), PM (7.05 mg g^{-1}), ED (7.18 mg g^{-1}), K (7.27 mg g^{-1}), and SF (7.30 mg g^{-1}). Similar to S content, the genotypes grouped into the same P content classification did not show significant differences.

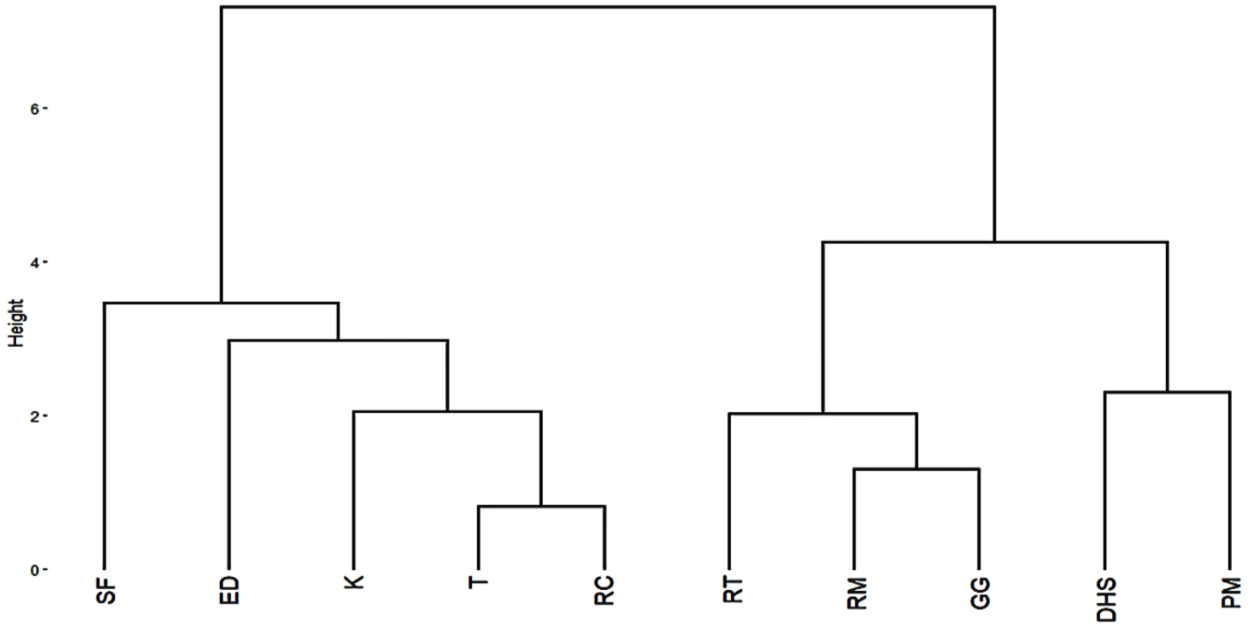

Figure 5 – Dendrogram of the cluster analysis of pak choi genotypes based on the kaempferol, quercetin, flavonoid, and phenolic compounds contents in the selenate and selenite treatments.

The dendrogram obtained from the cluster analysis using Euclidean distance showed two major and three minor clusters, while SF, GG, and ED did not fall in any cluster (FIGURE 5). One minor cluster comprised the genotypes PM and DHS, both intensely purple-colored and similar responses to Se. The second group comprised the genotypes RC, RM, and RT with intermediate purple coloring, and the last group comprised the genotypes K and T.

4 DISCUSSION

The trend of the studied parameters regarding the effect of Se sources varied among the studied genotypes, suggesting that the response to Se is mainly influenced by genetic factors under the evaluated conditions. The higher efficiency of Se accumulation in the shoot part in green-genotyped plants compared to those with higher purple pigmentation is evident (FIGURES 1, 4A, and B). The application of selenite (≤8 μM) during the seedling stage in purple lettuce promoted antioxidant capacities, particularly anthocyanin production (HUANG et al., 2023). The purple coloration in genotypes is associated with a higher base concentration of phenolic compounds (as seen in the control group of genotypes in Figure 3A, B, C, and D), especially anthocyanin, which is related to the purple coloration (KHOO et al., 2017). Phytochemicals, including phenolic compounds, have the function of protecting proteins, lipids, and nucleic acids from oxidative damage (REVELOU et al., 2022).

The connection between Se phytotoxicity and its benefits for plants is closely linked to the concentrations and types of species present in different plant tissues (LI et al., 2020). The increase in physiological response and growth in specific genotypes (FIGURE 2A and B) indicate the beneficial effect of Se. The benefit or harm of Se in plants can be attributed to various factors such as low doses, appropriate growth conditions, exposure duration, and genetic factors. Selenium can act as an antioxidant or pro-oxidant, increasing or reducing the plant's antioxidant activity. Key antioxidant mechanisms include both enzymatic and non-enzymatic compounds, such as phenolic compounds (RIZWAN et al., 2021; XIANG et al., 2022). This explains the results found in the genotypes concerning non-enzymatic antioxidant activity (FIGURE 3). In a previous study using green tea, it was observed that the presence of

10 mg L^{-1} of Se nanoparticles resulted in a significant increase in polyphenol levels. However, when the Se nanoparticle concentration was increased to 20 mg L^{-1}, inhibition of polyphenol accumulation in the tea was noted. The addition of Se can stimulate secondary metabolism in tea, leading to an increase in the accumulation of flavonoids such as apigenin, kaempferol, quercetin, myricetin, and rutin (LI et al., 2021). In another study, the results demonstrated that fortification of kale sprouts with organic Se compounds (15 mg Se L^{-1}), containing benzylselenoate scaffold, influences the production of isothiocyanates, phenolic acids, and enhances the antioxidant properties of fortified sprouts (PAŚKO et al., 2022). The addition of selenite and selenate (100 μmol L^{-1}) in the biofortification of broccoli sprouts increased flavonoid and anthocyanin levels, but there was a decrease in phenolic content (TIAN et al., 2016). A study conducted at different stages of maturity of Se-biofortified broccoli (50 μM in the form of selenate) resulted in higher levels of phenolics and antioxidant activity in seedlings (BACHIEGA et al., 2016).

The conflicting result observed in the SF genotype between the control group and the group treated with selenite regarding the SPAD index (FIGURE 2D) can be attributed to the dilution effect resulting from a significant reduction in shoot dry weight (FIGURE 2A). The dilution effect typically occurs when higher yields result in a dilution of minerals or other critical components within the biomass. However, the opposite can also occur (RENGEL; CAKMAK; WHITE, 2022). This effect increased the SPAD index (an indirect indication of chlorophyll concentration in leaves), along with a decrease in the efficiency of photosystem II and the net assimilation rate of CO_2. Despite this decrease in dry weight, the chlorophyll concentration showed a proportionally smaller decrease, resulting in a higher chlorophyll concentration in the leaf area of the SF genotype in the selenite-treated group, indicating high toxicity in this genotype.

The frequent and more intense reduction in shoots biomass, net assimilation rate of CO_2, and efficiency of photosystem II induced by selenite compared to selenate, as well as the higher Se accumulation in the shoots part with selenate compared to selenite, and a stronger trend of selenite to enhance the antioxidant response (*e.g.*, flavonoids and phenolic compounds) of the evaluated genotypes, are results attributed to the toxic potential of selenite under hydroponic conditions. The phytotoxic effect of Se, which impairs plant growth, can be attributed to imbalances in mineral nutrition. By modifying the absorption, accumulation, and transport of mineral nutrients, Se impacts various biochemical reactions and physiological

processes including growth, photosynthesis, respiration, gas exchange, water uptake, phloem unloading, and activation of protease-inhibiting genes (HASANUZZAMAN et al., 2020).

Under soil conditions, selenite tends to be strongly retained in soil colloids compared to selenate (SILVA et al., 2019), especially in soils with variable charges, where selenite is immobilized by iron and aluminum oxides/hydroxides, as well as to a lesser extent, by clays and organic matter. This competition between the soil and the plant for Se makes selenate more available to plants in such scenarios (BARKER; PILBEAM, 2015; FORDYCE, 2013).

Under hydroponic conditions, selenite is generally considered more toxic to plants than selenate. Since selenite is more soluble in water, it is thus, more easily absorbed by plant roots, potentially leading to excessive Se accumulation and consequently, higher toxicity. This indicates that exogenous selenate treatment was more efficient in facilitating Se translocation throughout the plant, whereas selenite treatment might have led to Se accumulation in the roots of pak choi, a parameter not evaluated in this study. This phenomenon can be explained by the differences in absorption and mobility mechanisms between selenite and selenate in plants. Selenate is taken up by plant roots through high-affinity sulfate transporters, while selenite is absorbed through diffusion (LI; MCGRATH; ZHAO, 2008; SORS; ELLIS; SALT, 2005). Following root absorption, selenate moves through the symplast to the stele and is loaded into the xylem for transport to the shoots. On the other hand, selenite is rapidly converted into low-mobility organic forms, leading to its accumulation in the roots (Li et al., 2008; Sors et al., 2005; C. Wang et al., 2022; White, 2004). Moreover, selenate is highly mobile in the xylem and is not easily assimilated into organic forms (ZAYED; LYTLE; TERRY, 1998).

At lower concentrations, selenite also induces more oxidative stress compared to selenate. In studies conducted on cucumber roots, the presence of selenate and selenite at concentrations > 80 μM and > 20 μM, respectively, intensified the accumulation of lipid peroxidation products, indicating a pro-oxidant and toxic effect. Additionally, selenite concentrations above 20 μM negatively affected the values of Fv/Fm, Fo, and Fm. In contrast, the presence of selenate at concentrations ranging from 2 to 80 μM did not influence chlorophyll fluorescence parameters (HAWRYLAK-NOWAK; MATRASZEK; POGORZELEC, 2015). In a study on lettuce, where selenate (10 mmol L^{-1}) and selenite (0.5 mmol L^{-1}) were applied under five different light spectra, selenite led to a more substantial reduction in shoots dry matter compared to treatments involving more stressful light spectra

for the plants (BIAN et al., 2020b). These findings demonstrate the higher toxicity associated with this inorganic form of Se.

The Se accumulation observed with selenate treatment is closely related to S accumulation (FIGURE 4A, B, and C) in the studied genotypes, as evidenced by results from the mentioned study below. In a previous study with pak choi, the increase of S content was observed in response to higher concentrations of Se foliar application, in addition, an increase in biomass and in the concentration of total glucosinolate hydrolysis products (ABDALLA; MESCHEDE; MÜHLING, 2020). A comparison of selenate and sulfate uptake by 39 plant species grown in hydroponic culture under identical conditions demonstrates that among the 37 species that did not accumulate Se, a positive and strong correlation existed between S and Se concentrations in the leaves, indicating a close association between selenate and sulfate accumulation. However, two Se hyperaccumulators (*Astragalus racemosus* and *Stanleya pinnata*) deviated from this relationship by exhibiting high Se concentration in their leaves. Generally, species from the *Brassicaceae* family have the ability to accumulate Se due to their ability to accumulate S (WHITE et al., 2007).

Selenium accumulation in the groups treated with selenate and selenite also induced some genotypes to decrease the P content in the shoots part. The reduction in growth caused by Se toxicity may result from imbalances in mineral nutrition. By affecting the absorption, accumulation, and transport of mineral nutrients, Se has an impact on various biochemical reactions and physiological processes including growth, photosynthesis, respiration, gas exchange, water uptake, phloem unloading, and activation of protease-inhibiting genes. Furthermore, Se can reduce or enhance the toxicity of essential or toxic elements, potentially limiting or exacerbating stresses induced by these elements (HASANUZZAMAN et al., 2020). A study on *Brassica oleracea* L. observed a decrease in foliar P concentrations, followed by an increase in S concentrations in plants treated with increasing doses of selenate (0 to 9 mg L^{-1}) (KOPSELL; RANDLE; MILLS, 2000). For the selenite-treated group, some studies have shown that this inorganic form of Se is inhibited by the presence of phosphate in the medium, and vice-versa, suggesting a possible involvement of phosphate transporters in selenite absorption. On certain occasions, these elements may compete for the same absorption sites in the roots (HOPPER; PARKER, 1999; LI; MCGRATH; ZHAO, 2008; ZHANG et al., 2014).

Selenium induces changes in plant metabolism, leading the plant to signal a stress situation through increased reactive oxygen species, thus requiring an antioxidant response

within the plant. Therefore, genotypes predisposed to having higher antioxidant compound concentrations possess a greater capability to detoxify the absorbed Se. Another relevant aspect is the genotypes' ability to accumulate S, which plays various essential roles in plants. Sulfur is present as Cys and Met in proteins and enzymes, as sulfolipids in membranes, and as sulfated lipooligosaccharides in biotic signaling. It provides thiol groups for redox control and detoxification of foreign substances and toxic elements, as well as acting as alliins and glucosinolates for defense against pests and pathogens (HAWKESFORD et al., 2012). Hence, genotypes with limited S accumulation capacity must generate alternative types of phytochemical compounds, like phenolic compounds, to protect themselves from abiotic stresses, such as Se.

The result of cluster analysis agreed with the genotypes' responses. The ten genotypes were clustered in two major groups with the same genotypes of similar color. The DHS and PM genotypes are clustered in one subgroup with an intense purple color and similar responses to Se/S accumulation and other parameters evaluated. Further, SF was the most diverse in all results and was isolated in the cluster analysis. The dendrogram is supported by the results in this study and was an efficient approach to show the relationship between the genotypes in response to Se treatments.

5 CONCLUSION

Based on the genetic diversity tested in pak choi, one of the Se-accumulating species, we obtained higher levels of phenolic compounds, associated with genotypes that exhibit purple color, lower S and Se content. This reduction in accumulation resulted in reduced toxicity symptoms. The phenolic compounds played a regulatory role in the accumulation of the studied elements, preventing greater damage to the plants. Overall, Se application promoted the studied non-enzymatic antioxidant activity, with selenite being the most potent promoter due to its greater toxic effect. The SF genotype proved to be extremely sensitive to Se application, being the most efficient in accumulating it in the plant shoot and suffering the most from negative effects. In some cases, Se application also had beneficial effects on the plants.

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Manuscript 3: ASSESSMENT OF POTENTIAL GENOTYPES FOR GENETIC BIOFORTIFICATION OF BRAZILIAN DRY BEANS WITH ZINC, IRON, AND PROTEIN LEVELS IN TROPICAL SOILS

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ABSTRACT

Studies that contribute to the knowledge about dry bean biofortification to increase levels of zinc (Zn), iron (Fe), and protein are important for combating global malnutrition. These nutrients are essential for a healthy diet and play fundamental roles in proper human body functioning. Deficiencies in Zn, Fe, and protein are public health issues in many regions worldwide, particularly affecting low-income populations with limited access to diverse diets. This study aimed to evaluate the levels of Zn, Fe, and crude protein in dry beans, as well as to assess the potential for biofortification in bean germplasms. The experiment was conducted in four tropical experimental areas: Lavras, Lambari, Patos de Minas, and Uberaba, using ten dry bean genotypes with four replications. The contents of Zn and Fe, their uptake, intake, and crude protein levels were assessed. The results showed that the levels of Zn, Fe, and crude protein in dry beans were consistent with values reported in the global literature. However, the different tropical soils evaluated, and the dilution effect directly impacted the accumulation of these nutrients. Based on the obtained levels, meeting the minimum requirements of Zn and Fe solely through the consumption of the evaluated genotypes was not possible. Therefore, agronomic practices are necessary to increase nutrient levels. Among the genotypes, ‘Supremo’, ‘OV’, and ‘Madrepérola’ showed the most promising potential for genetic biofortification to increase Zn, Fe, and crude protein concentrations. Particularly, the ‘Supremo’ genotype stands out in this study. Further research is needed to optimize the biofortification process for dry beans, taking into account the specific conditions of each experimental area.

Keywords: Common bean. Brazilian genotypes. Absorption efficiency. Fe. Genetic. Zn.

1 INTRODUCTION

Food security is a pressing global concern, and adequate nutrition is paramount for the health and well-being of populations worldwide. Almost 2.5 billion people, constituting one in three individuals worldwide, experience micronutrient deficiencies, commonly referred to as "hidden hunger". This phenomenon poses a significant health challenge for the global population (KHATIBZADEH et al., 2016). The deficiency of Zn and Fe is a major public health concern impacting millions of individuals, notably in low and middle-income countries (GUPTA; BRAZIER; LOWE, 2020; KUMAR et al., 2022). Another crucial concern is protein deficiency. Approximately half of the global population, roughly eight billion people, consistently experiences insufficient protein intake (DOLGANYUK et al., 2023).

In this context, the dry bean (*Phaseolus vulgaris*) stands out as a crucial dietary component, especially in regions where it serves as a significant source of plant-based proteins, carbohydrates, and fiber (SÁ; MORENO; CARCIOFI, 2020). A diet centered on protein-rich legumes offers a practical, sustainable, and wholesome approach to addressing malnutrition in underdeveloped nations. Despite the prevalent use of animal proteins in human diets, plant-based proteins are steadily gaining popularity. Due to their nutritional advantages, minimal carbon footprint, and environmental considerations, their demand has grown on a global scale (ASIF et al., 2013). Among the most notable legumes cultivated globally, dry beans rank as the second-most-consumed crop in Brazil, trailing only after soybean, which is mostly consumed by livestock (ALFARO-DIAZ et al., 2021; GOMES et al., 2020). Brazil cultivates various commercial bean varieties, including black, carioca, purple, mulatinho, rosinha, red, and manteigão. Among these, carioca and black beans are the most popular choices (BENTO et al., 2020).

Beans serve as a valuable alternative to enhance human dietary intake and counter nutrient deficiencies in resource-limited regions (*e.g.*, Zn and Fe). Additionally, due to their exceptional nutritional content, they act as a source of high-quality protein for vegetarians (MONK et al., 2019). Fe deficiency can result in severe health consequences, including anemia, fatigue, and compromised immune function (CHEN; KUNG; GNANA-PRAKASAM, 2022; DISTÉFANO et al., 2021; SEMBA, 2016). Zn deficiency inhibits protein production, leading to physiological issues such as growth retardation, heightened susceptibility to infectious illnesses, an impaired immune system, and compromised learning abilities (WESSELS; RINK, 2020). Inadequate protein intake results in suboptimal synthesis

and functioning of muscles, organs, enzymes, hormones, and the immune system (WU, 2009). Consequently, the development of effective biofortification strategies to elevate the levels of Zn, Fe, and protein in dry beans has emerged as a paramount focus in both agricultural and nutritional research.

Biofortification, defined as the improvement of element concentrations in food crops, presents a promising and sustainable solution for tackling prevalent nutritional deficiencies (BOUIS et al., 2011; SALTZMAN et al., 2013). Biofortification facilitates the increase of Zn, Fe, and protein levels in dry beans, elevating them to a more nutrient-dense source of both micronutrients and macronutrient for human consumption (TAKO et al., 2009; WHITE; BROADLEY, 2009). Furthermore, biofortification has the potential to improve the bioavailability and absorption of these minerals and proteins within the human body, thereby positively impacting the health of populations that heavily depend on dry beans as a primary nutritional source (HUERTAS et al., 2023).

However, biofortification for Zn, Fe, and protein in tropical soils faces challenges for adequate efficiency. The acidic pH of tropical soils favors the availability of Fe, Zn, and N (*e.g.*, ammonium). However, in excess, it can lead to toxicity. On the other hand, S is more available in soils with acidic or neutral pH. Brazilian tropical soils generally have variable levels of organic matter influenced by native vegetation and agricultural practices. Organic matter contributes to the formation of organic complexes of Fe and Zn, increasing their availability. Additionally, the decomposition of organic matter contributes to the release of N and S, making them available to plants. Tropical Brazilian soils with a high clay content generally exhibit greater retention of Fe and Zn, reducing their availability to plants. Conversely, sandy soils may be more susceptible to leaching of N and S, resulting in lower availability of these nutrients. Interactions with soil microorganisms are also relevant. For example, biological N fixation, carried out by symbiotic bacteria in association with legumes, is an important source of N for plants in Brazilian tropical soils. Furthermore, the presence of phosphate-solubilizing microorganisms can enhance the availability of nutrients, including Fe, Zn, and S (DE SOUSA; LOBATO, 2004; SARMAH; SARMA, 2023).

Understanding dry bean genotypes' responses to biofortification is critical for developing effective strategies that bolster food security and proper nutrition for vulnerable populations. Previous studies have highlighted a wide range of Fe levels in genotypes, varying from as low as 34 mg kg^{-1} to as high as 152 mg kg^{-1}. Similarly, Zn concentrations have been reported to span between 18 and 77 mg kg^{-1}, with an average of 28 or 31 mg kg^{-1}. Regarding

protein levels, observations have shown that landraces and modern varieties range from 16.54% to 25.23% (BEEBE; GONZALEZ; RENGIFO, 2000; CAPRONI et al., 2020; CELMELI et al., 2018; GLAHN; NOH, 2021; GUNJAČA et al., 2021; KATUURAMU et al., 2021; MURUBE et al., 2021).

This study aims to evaluate the genetic biofortification of dry beans in terms of Zn, Fe, and crude protein, identifying Brazilian genotypes that are most responsive to biofortification.

2 MATERIAL AND METHODS

2.1 Experimental design

Dry bean genotypes (*Phaseolus vulgaris* L.) were simultaneously on field tests in four Brazilian towns: Lavras (LAV) (21°14'45"S 44°59'59"W; H; 919 m); Patos de Minas (PMS) (18°34'46"S 46°31'6"W; H: 832 m); Lambari (LAB) (21°58'32"S 45°21'32"W; H: 887 m); and Uberaba (UBE) (19°45'1" S 47°55'57" W; H: 823 m). These municipalities are classified according to the Köppen-Geiger climatic classification system as having a Cwa (Ube and PMS) and Cwb (LAB and LAV) climates, characterized by pleasant, wet summers and dry winters (KOTTEK et al., 2006). The American soil taxonomy and Brazilian soil taxonomy were used to classify the soils (SBCS, 2018; Soil Survey Staff, 1999). The tropical soils at each location were categorized as follows: dystrophic Red Latosol or Oxisol in Patos de Minas; dystrophic Red-Yellow Argisol or Ultisol in Lambari: eutrophic Red-Yellow Argisol or Ultisol in Lavras; and, dystrophic Red Latosol or Oxisol in Uberaba.

The studies were carried out using a split-plot design with four experimental regions (split-plots), ten genotypes (plots), and four replicates (blocks). The genotypes selected for this study are widely cultivated and consumed in Brazil (TABLE 1 and FIGURE 1).

Table 1 – Characteristics of dry bean genotypes used in the experiments.

Genotypes	Cycle	Growth habit	Plant architecture	Group	Nickname
BRS 9435 Cometa	SP	Ind. Type II	Erect	Carioca	Cometa
BRS FC402	N	Ind. Type III	Semi-erect	Carioca	FC402
BRSMG Madrepérola	SP	Ind. Type III	Prostrate	Carioca	Madrepérola
Pérola	N	Ind. Type II	Semi-prostrate	Carioca	Pérola
BRSMG Realce	P	Det. Type I	Erect	Carioca	Realce

Ouro Vermelho	N	Ind. Type II	Semi-prostrate	Red	OV
VR 20	SP	Det. Type II	Erect	Red	VR20
BRSMG Tesouro	N	Ind. Type II	Erect	Purple	Tesouro
BRSMG União	SP	Ind. Type III	Semi-prostrate	Jalo	União
BRS Supremo	SP	Ind. Type II	Erect	Black	Supremo

Cycle: Normal (N) 85-95 days from sowing to harvest; Semi-precocious (SP) 75-85 days from sowing to harvest; Precocious (P) < 75 days from sowing to harvest. Growth habit: ind (indeterminate) and det (determined).

Figure 1 – Visual characteristics of dry bean seeds used in the experiments. The genotypes seeds are: Cometa (A); FC402 (B); Madrepérola (C); Pérola (D); Realce (E); OV (F); VR 20 (G); Tesouro (H); BRSMG União (I); BRS Supremo (J).

Six rows were designated for each genotype, and experimental plots were sown with 15 seeds m^{-1} (3 meters in length), evenly spaced at 0.5 meters apart (split-plot design). The experiments received an application of 25 kg ha^{-1} of nitrogen, 150 kg ha^{-1} of phosphorus, applied as monoammonium phosphate (MAP), and 60.06 g ha^{-1} of molybdenum as sodium molybdate at sowing. Due to variations in soil results, potassium fertilization was administered at the following rates (kg ha^{-1} of K_2O): 40 (PMS); 20 (LAB); 40 (LAV); and 50 for each site (UBE). Top-dressing nitrogen fertilization (45 kg ha^{-1}) was applied 30 days after seedling emergence. The chemical and physical attributes of the soil according to Teixeira et al. (2017) are detailed in Table 2.

Table 2 – Soil chemical and physical properties before setting the experiments.

Depth (0-20 cm)	Experimental areas			
	LAB	LAV	PMS	UBE

pH ($CaCl_2$)	5.17	6.07	5.22	5.90
OM (dag kg^{-1})	1.85	2.64	3.11	1.61
P^e (mg dm^{-3})	5.25	21.86	48.74	31.96
K^e (mg dm^{-3})	96.48	69.63	63.41	56.53
Ca^e ($cmol_c$ dm^{-3})	2.07	3.94	1.20	1.27
Mg^e ($cmol_c$ dm^{-3})	0.44	1.42	0.39	0.32
Al^e ($cmol_c$ dm^{-3})	0.13	0.05	0.17	0.09
H+Al ($cmol_c$ dm^{-3})	3.97	3.58	6.95	1.86
CEC_T ($cmol_c$ dm^{-3})	6.73	9.12	8.69	3.59
CEC_t ($cmol_c$ dm^{-3})	2.89	5.59	1.92	1.82
Base saturation (%)	41.27	60.28	20.16	48.14
Al saturation (%)	4.59	0.89	9.01	5.13
Zn^e (mg dm^{-3})	2.43	2.14	2.69	3.90
Fe^e (mg dm^{-3})	81.65	49.16	48.29	47.09
Mn^e (mg dm^{-3})	7.31	30.32	117.76	8.66
Cu^e (mg dm^{-3})	0.57	0.81	13.61	0.64
B^e (mg dm^{-3})	0.11	0.08	0.10	0.05
S^e (mg dm^{-3})	5.38	3.21	46.97	2.30
Sand (dag kg^{-1})	33.33	40.33	27.17	78.00
Silt (dag kg^{-1})	7.67	17.33	39.67	7.00
Clay (dag kg^{-1})	59.00	42.33	33.17	15.00

^{e} = available; P: anion-exchange resin extraction; S: monocalcium phosphate in acetic acid extraction; H + Al: SMP extraction; B: hot water extraction; Other elements: Mehlich-I extraction; OM: soil organic matter extracted by sodium dichromate 4N + sulfuric acid 10N; CEC_t: effective cation exchange capacity; CEC_T: total cation exchange capacity.

2.2 Grain yield

Following a moisture content adjustment to 13%, grain yield (kg ha^{-1}) was assessed (MAPA, 2009). Only four rows from each plot were collected and weighed, and all borders that were within 0.5 m of another plot were excluded.

2.3 Determination of crude protein

After digestion with sulfuric acid, the nitrogen content was calculated using the macro-Kjeldahl technique. The nitrogen content was multiplied by 6.25 to determine the amount of crude protein (AOAC, 2016).

2.4 Total content, uptake, and intake of Zn and Fe in grains

The sample digestion procedure involved weighing approximately 500 mg of samples in triplicate. Subsequently, the samples were digested with 5 mL of $HNO_3 \geq 65\%$ in PTFE Teflon® tubes (CEM Corporation, Matthews, NC, USA). The resulting extract was left to stand overnight at room temperature, and the actual digestion process was performed the following morning. The vials were then hermetically sealed and placed in a microwave (CEM brand, model Mars-5) set at 175°C and a controlled pressure of 0.76 MPa for 15 minutes. After digestion, the extracts were allowed to cool down to room temperature and filtered using filter paper. To attain the final extract volume, an additional 5 mL of deionized water was added during the filtration process. Post-filtration, the extracts were transferred into smaller vials (30 mL) and stored at 5ºC until analysis (USEPA, 2007).

For quality control purposes, a sample of standard reference material (Peach Leaves-SRM 1547, obtained from the National Institute of Standards and Technology - NIST, Gaithersburg, USA) with certified Zn and Fe contents of 17.97 mg kg^{-1} and 219.8 mg kg^{-1}, respectively, was included in each digestion batch. Additionally, a blank sample was used to calculate the limits of detection and quantification. The mean recovery for Zn and Fe in the SRM 1547 were 95.13%, and 91.54% (n = 14), respectively. These values indicate reliable accuracy in the analytical data for elemental analysis in grains.

The grain element (Zn and Fe) uptake (EU, g ha^{-1}) was calculated based on the grain content, using the equation according to DUCSAY et al. (2016).

$$EU = [Gr]_{yield}\, x\, [E]_{content}$$

Where: $[Gr]_{yield}$ (kg ha^{-1}) is a grain yield and $[E]_{content}$ (g kg^{-1}) is an element content translocated to grain.

The elements intake (EI in mg ha^{-1}) in dry bean genotypes was also calculated, according to Lessa et al. (2020).

$$EI = [Gr]_{intake} \, x \, [E]_{content}$$

Where: EI (mg person^{-1} day^{-1}) is the daily element intake estimation per person; $[Gr]_{intake}$ (g person^{-1} day^{-1}) is the mean consumption of bean grains per person; $[E]_{content}$ (mg kg^{-1}) is an element content in dry beans grains verified for the studied treatments.

2.5 Statistical analysis

Employing the Speedstat 2.8 program, all data findings were subjected to analysis of variance (ANOVA). To compare treatments with significantly different means at the 0.05 level of significance, the Scoot-Knott test was used substantially (CARVALHO et al., 2020).

3 RESULTS

The dry bean yield varied, being affected by experimental areas and genetic diversity ($p < 0.05$). The grain yield values of ten bean genotypes and four areas ranged from 982.70 to 3398.53 kg ha^{-1} (TABLE 3). The 'OV' and 'FC402' genotypes had the higher mean yield for all areas, being 2758.84 and 2642.80 kg ha^{-1}, respectively, and the 'Cometa' bean reached the lowest mean grain yield (1475.91 kg ha^{-1}). However, within each area, the best production was not consistent across all genotypes. Regarding experimental areas, the highest ($p < 0.05$) mean grain yield for all genotypes was found in Patos de Minas (2978.45 kg ha^{-1}), followed by Uberaba (2604.41 kg ha^{-1}), Lambari (2155.45 kg ha^{-1}), and Lavras (1357.71 kg ha^{-1}), respectively (TABLE 3).

Table 3 – Grain yield (kg ha^{-1}) of dry bean grains according to genotypes and experimental areas.

Genotypes	Experimental areas					
	Lambari	Lavras	Patos de Minas	Uberaba	**Mean**	
Cometa	982.70	1078.49	1993.58	1848.86	**1475.91**	**d**
FC402	2430.86	1663.62	3228.01	3248.71	**2642.80**	**a**
Madrepérola	2537.77	1315.68	2964.26	3012.78	**2457.62**	**b**
Pérola	2763.80	1329.88	3398.53	2101.81	**2398.50**	**b**
Realce	1272.54	983.20	2968.29	2524.28	**1937.08**	**c**
OV	3362.75	1844.67	3163.98	2663.97	**2758.84**	**a**
VR 20	2013.57	1881.76	2954.56	2667.54	**2379.36**	**b**
Tesouro	1901.98	1092.13	2913.47	2096.06	**2000.91**	**c**
União	2256.80	1219.25	2850.73	2983.91	**2327.67**	**b**

Supremo	2031.74		1168.38		3349.07		2896.19		**2361.35**	**b**
Mean	**2155.45**	**C**	**1357.71**	**D**	**2978.45**	**A**	**2604.41**	**B**		

Capital letters, on the row, compare different studied sites. Lowercase letters, in the column, compare different genotypes. Means followed by the same letter do not differ from each other, by the Scott-Knott test at 5% probability.

Significant differences ($p < 0.05$), but no huge variability was observed among bean genotypes and experimental areas for mean crude protein percentage, which ranged from 21.58% to 24.51% (TABLE 4). The highest ($p < 0.05$) mean crude protein percentages were found in 'Cometa', 'VR20', and 'Supremo' genotypes, with values of 24.06%, 23.83%, and 23.81%, respectively (TABLE 4). Differences ($p < 0.005$) existed for all other genotypes, but the values were close. Regarding experimental areas, the highest mean value ($p < 0.05$) was found in Lavras (24.51%), and the lowest in Uberaba (21.58%).

Table 4 – Crude protein (%) of dry bean grains according to genotypes and experimental areas.

Genotypes	Experimental areas					
	Lambari	Lavras	Patos de Minas	Uberaba	**Mean**	
Cometa	23.13	26.26	24.94	21.91	**24.06**	**a**
FC402	21.55	23.97	23.88	22.99	**23.10**	**b**
Madrepérola	22.09	23.19	22.18	20.75	**22.05**	**c**
Pérola	22.58	24.31	21.94	21.18	**22.50**	**c**
Realce	22.73	24.10	23.92	18.52	**22.32**	**c**
OV	21.54	23.77	23.47	22.80	**22.90**	**c**
VR 20	23.76	23.48	24.77	23.31	**23.83**	**a**
Tesouro	23.23	25.89	23.21	21.23	**23.39**	**b**
União	21.13	24.48	23.29	20.18	**22.27**	**c**
Supremo	22.95	25.64	23.72	22.92	**23.81**	**a**
Mean	**22.47**	**C**	**24.51**	**A**	**23.53**	**B**	**21.58**	**D**		

Capital letters, on the row, compare different studied sites. Lowercase letters, in the column, compare different genotypes. Means followed by the same letter do not differ from each other, by the Scott-Knott test at 5% probability.

The average Fe content in dry beans grains was influenced by genetic diversity, with values ranging from 60.15 to 78.44 among the genotypes ($p < 0.05$) (TABLE 5). The 'Madrepérola', 'VR20', 'Supremo', and 'OV' genotypes had the highest values, 78.44, 78.13, 77.64, and 74.54 mg kg^{-1}, respectively, with no difference ($p > 0.05$) among them, according to Scott-Knott test. In contrast, the genotype 'Realce' displayed the lowest Fe content at 60.15 mg kg^{-1} ($p < 0.05$), which was statistically similar to 'Tesouro' and 'União' ($p > 0.05$). Across the assessed experimental areas, Uberaba emerged with the highest ($p < 0.05$) mean Fe content of 90.86 mg kg^{-1}, while the other areas exhibited similar Fe content ($p > 0.05$).

Table 5 – Iron content (mg kg^{-1}) of dry bean grains according to genotypes and experimental areas.

Genotypes	Experimental areas					
	Lambari	Lavras	Patos de Minas	Uberaba	**Mean**	
Cometa	76.76	63.12	73.60	64.26	**69.43**	**b**
FC402	61.13	65.62	58.66	93.29	**69.68**	**b**
Madrepérola	77.19	63.89	63.01	109.66	**78.44**	**a**
Pérola	66.37	57.62	64.61	80.51	**67.28**	**b**
Realce	57.37	60.07	54.51	68.66	**60.15**	**c**
OV	63.41	58.88	64.85	111.00	**74.54**	**a**
VR 20	66.97	58.78	63.42	123.35	**78.13**	**a**
Tesouro	62.71	65.25	57.58	67.71	**63.31**	**c**
União	56.01	55.14	59.49	76.70	**61.84**	**c**
Supremo	63.83	64.03	69.28	113.42	**77.64**	**a**
Mean	**65.18 B**	**61.24 B**	**62.90 B**	**90.86 A**		

Capital letters, on the row, compare different studied sites. Lowercase letters, in the column, compare different genotypes. Means followed by the same letter do not differ from each other, by the Scott-Knott test at 5% probability.

The mean of Fe uptake in bean grains tested showed a wide variability, ranging from 102.61 to 207.43 g ha^{-1} for genotypes and from 82.85 to 249.70 g ha^{-1} for experimental areas (TABLE 6). 'Supremo', 'OV', 'Madrepérola', and 'VR 20' showed the highest ($p < 0.05$) mean values for Fe uptake, similarly to Fe content (TABLE 5), with no difference among them ($p > 0.05$). The values recorded were 207.43, 204.27, 203.10, and 198.46 g ha^{-1}, respectively, whereas the least ($p < 0.05$) mean value for Fe uptake (102.61 g ha^{-1}) was noted for 'Cometa' genotype, which was statistically similar to 'Realce' and 'União' ($p > 0.05$) (TABLE 6).

Across the analyzed areas, the highest mean of Fe uptake content was found in Uberaba (249.70 g ha^{-1}), followed by Patos de Minas (186.24 g ha^{-1}), Lambari (142.13 g ha^{-1}), and Lavras (82.85 g ha^{-1}) ($p < 0.05$) (TABLE 6).

Table 6 – Iron uptake (g ha^{-1}) from dry bean grains according to genotypes and experimental areas.

Genotypes	Experimental areas					
	Lambari	Lavras	Patos de Minas	Uberaba	**Mean**	
Cometa	75.33	68.38	147.87	118.87	**102.61**	**d**
FC402	148.23	107.60	189.51	293.29	**184.66**	**b**
Madrepérola	216.48	84.87	186.65	324.40	**203.10**	**a**
Pérola	187.04	77.38	216.57	168.55	**162.39**	**b**
Realce	72.08	57.47	161.92	172.61	**116.02**	**d**
OV	209.33	108.61	204.65	294.48	**204.27**	**a**
VR 20	134.90	110.40	187.42	361.14	**198.46**	**a**

Tesouro	118.96		70.92		166.55		142.42		**124.71**	**d**
União	128.36		67.92		170.49		227.84		**148.65**	**c**
Supremo	130.55		74.99		230.74		393.43		**207.43**	**a**
Mean	**142.13**	**C**	**82.85**	**D**	**186.24**	**B**	**249.70**	**A**		

Capital letters, on the row, compare different studied sites. Lowercase letters, in the column, compare different genotypes. Means followed by the same letter do not differ from each other, by the Scott-Knott test at 5% probability.

Table 7 shows the results of Fe intake calculated based on average Brazilian bean consumption and table 8 displays the same parameter based on world bean consumption. The genotypes 'Madrepérola', 'OV', 'VR 20', and 'Supremo' that showed higher Fe content and uptake, also were responsible for the highest ($p < 0.05$) Fe intake, with mean values of 2.77, 2.63, 2.76, and 2.74 mg day^{-1}, respectively for Brazilian consumption (TABLE 7). For the same genotypes, the values of Fe intake based on the world bean consumption were 0.55, 0.52, 0.54, and 0.54 mg day^{-1}, respectively (TABLE 8). The reported lowest ($p < 0.05$) Fe intake was found for the genotypes 'Realce', 'Tesouro', and 'União' presenting mean values of 2.12, 2.23, and 2.18 mg day^{-1} for Brazilian bean consumption (TABLE 7), and 0.44, 0.43, 0.42 and 0.47 mg day^{-1}, respectively, for world bean consumption (TABLE 8). No significant differences in Fe uptake were observed among genotypes of the same group ($p > 0.05$). Regarding experimental areas, the same trend for Fe content was observed.

Table 7 – Iron intake (mg day^{-1}) from dry bean grains according to genotypes and experimental areas. Based on the average Brazilian bean consumption (35.28 g capita^{-1} day^{-1}) (FAO, 2020).

Genotypes	Experimental areas					
	Lambari	Lavras	Patos de Minas	Uberaba	**Mean**	
Cometa	2.71	2.23	2.60	2.27	**2.45**	**b**
FC402	2.16	2.32	2.07	3.29	**2.46**	**b**
Madrepérola	2.72	2.25	2.22	3.87	**2.77**	**a**
Pérola	2.34	2.03	2.28	2.84	**2.37**	**b**
Realce	2.02	2.12	1.92	2.42	**2.12**	**c**
OV	2.24	2.08	2.29	3.92	**2.63**	**a**
VR 20	2.36	2.07	2.24	4.35	**2.76**	**a**
Tesouro	2.21	2.30	2.03	2.39	**2.23**	**c**
União	1.98	1.95	2.10	2.71	**2.18**	**c**
Supremo	2.25	2.26	2.44	4.00	**2.74**	**a**
Mean	**2.30**	**2.16**	**2.22**	**3.21**		

Lowercase letters, in the column, compare different genotypes. Means followed by the same letter do not differ from each other, by the Scott-Knott test at 5% probability.

Table 8 – Iron intake (mg day^{-1}) from dry bean grains according to genotypes and experimental areas. Based on world bean consumption (6.96 g capita^{-1} day^{-1}) (FAO, 2020).

Genotypes	Experimental areas					
	Lambari	Lavras	Patos de Minas	Uberaba	**Mean**	
Cometa	0.53	0.44	0.51	0.45	**0.48**	**b**
FC402	0.43	0.46	0.41	0.65	**0.48**	**b**
Madrepérola	0.54	0.44	0.44	0.76	**0.55**	**a**
Pérola	0.46	0.40	0.45	0.56	**0.47**	**b**
Realce	0.40	0.42	0.38	0.48	**0.42**	**c**
OV	0.44	0.41	0.45	0.77	**0.52**	**a**
VR 20	0.47	0.41	0.44	0.86	**0.54**	**a**
Tesouro	0.44	0.45	0.40	0.47	**0.44**	**c**
União	0.39	0.38	0.41	0.53	**0.43**	**c**
Supremo	0.44	0.45	0.48	0.79	**0.54**	**a**
Mean	**0.45**	**0.43**	**0.44**	**0.63**		

Lowercase letters, in the column, compare different genotypes. Means followed by the same letter do not differ from each other, by the Scott-Knott test at 5% probability.

Zinc content in dry beans was also affected by genetic diversity and experimental areas ($p < 0.05$). Two of the genotypes with the highest Fe content also had the highest Zn content: 'Supremo' and 'Madrepérola', with similar mean values of 37.24 and 37.16 mg kg^{-1} (TABLE 9). The last mean value was recorded for the 'União' genotype with 31.56 mg kg^{-1}. For experimental areas, the highest ($p < 0.05$) value was 39.13 mg kg^{-1} for Lavras, followed by Uberaba (37.47 mg kg^{-1}), Lambari (31.86 mg kg^{-1}), and Pato de Minas (37.47 mg kg^{-1}).

Table 9 – Zinc content (mg kg^{-1}) from dry bean grains according to genotypes and experimental areas.

Genotypes	Experimental areas					
	Lambari	Lavras	Patos de Minas	Uberaba	**Mean**	
Cometa	35.62	41.64	29.81	33.33	**35.10**	**b**
FC402	31.33	36.62	29.23	39.76	**34.23**	**c**
Madrepérola	36.56	41.96	30.25	39.87	**37.16**	**a**
Pérola	32.25	35.90	28.61	36.60	**33.34**	**c**
Realce	29.44	39.08	28.21	35.18	**32.97**	**c**
OV	30.77	35.31	31.36	39.62	**34.26**	**c**
VR 20	31.11	37.27	33.43	41.84	**35.91**	**b**
Tesouro	31.36	43.48	29.85	35.35	**35.01**	**b**
União	28.26	36.61	29.30	32.06	**31.56**	**d**
Supremo	31.92	43.45	32.46	41.10	**37.24**	**a**
Mean	**31.86 C**	**39.13 A**	**30.25 D**	**37.47 B**		

Capital letters, on the row, compare different studied sites. Lowercase letters, in the column, compare different genotypes. Means followed by the same letter do not differ from each other, by the Scott-Knott test at 5% probability.

Zinc uptake was significantly affected by genotypes and experimental areas ($p < 0.05$). Interestingly, for Zn uptake, the highest mean value (95.99 g ha^{-1}) was observed in

genotype ‘OV’ (TABLE 10) that was not among the highest Zn content (TABLE 9). This value was followed by 89.39 and 89.34 g ha^{-1} in ‘Madrepérola’ and ‘FC402’, respectively. Results also showed a different trend for experimental areas, with the highest mean value of 97.91 g ha^{-1} recorded for Uberaba, followed by 91. 05 g ha^{-1} for Patos de Minas, 68.27 g ha^{-1} for Lambari, and 52.51 g ha^{-1} for Lavras.

Table 10 – Zn uptake (g ha^{-1}) from dry beans grain according to genotypes and experimental areas.

Genotypes	Experimental areas									
	Lambari		Lavras		Patos de Minas		Uberaba		**Mean**	
Cometa	35.17		43.97		59.55		61.60		**50.07**	**d**
FC402	75.73		59.60		94.33		127.70		**89.34**	**a**
Madrepérola	92.48		56.20		89.54		119.35		**89.39**	**a**
Pérola	89.70		47.69		96.14		76.92		**77.61**	**b**
Realce	37.02		38.51		84.26		88.53		**62.08**	**c**
OV	102.26		65.38		110.94		105.38		**95.99**	**a**
VR 20	62.71		70.41		98.66		110.87		**85.66**	**b**
Tesouro	59.10		48.25		86.97		74.46		**67.20**	**c**
União	63.65		44.41		82.44		95.62		**71.53**	**c**
Supremo	64.90		50.71		107.66		118.71		**85.50**	**b**
Mean	**68.27**	**C**	**52.51**	**D**	**91.05**	**B**	**97.91**	**A**		

Capital letters, on the row, compare different studied sites. Lowercase letters, in the column, compare different genotypes. Means followed by the same letter do not differ from each other, by the Scott-Knott test at 5% probability.

Table 11 shows the results of Zn intake calculated based on average Brazilian bean consumption and table 12 shows the Zn intake based on world consumption. As observed for the Zn content in table 9, the genotypes ‘Madrepérola’ and ‘Supremo’ showed the highest mean values for Zn intake ($p < 0.05$), with a mean value of 1.31 mg day^{-1}, both, for Brazilian consumption (TABLE 11). For the same genotypes, the value for Zn intake based on world bean consumption was 0.26 mg day^{-1} (TABLE 12). The reported lowest ($p < 0.05$) Zn intake was found for the genotype ‘União’ presenting a mean value of 1.11 mg day^{-1} for Brazilian bean consumption (TABLE 11), and 0.22 mg day^{-1}, for world consumption (TABLE 12). No significant differences in Zn uptake were observed among genotypes of the same group ($p > 0.05$). Regarding experimental areas, the Zn intake was correlated to Zn content results.

Table 11 – Zinc intake (mg day^{-1}) from dry bean grains according to genotypes and experimental areas. Based on Brazilian bean consumption (35.28 g $capita^{-1}$ day^{-1}) (FAO, 2020).

Genotypes	Experimental areas					
	Lambari	Lavras	Patos de Minas	Uberaba	**Mean**	
Cometa	1.26	1.47	1.05	1.18	**1.24**	**b**
FC402	1.11	1.29	1.03	1.40	**1.21**	**c**
Madrepérola	1.29	1.48	1.07	1.41	**1.31**	**a**
Pérola	1.14	1.27	1.01	1.29	**1.18**	**c**
Realce	1.04	1.38	1.00	1.24	**1.16**	**c**
OV	1.09	1.25	1.11	1.40	**1.21**	**c**
VR 20	1.10	1.32	1.18	1.48	**1.27**	**b**
Tesouro	1.11	1.53	1.05	1.25	**1.24**	**b**
União	1.00	1.29	1.03	1.13	**1.11**	**d**
Supremo	1.13	1.53	1.15	1.45	**1.31**	**a**
Mean	**1.12**	**1.38**	**1.07**	**1.32**		

Lowercase letters, in the column, compare different genotypes. Means followed by the same letter do not differ from each other, by the Scott-Knott test at 5% probability.

Table 12 – Zinc intake (mg day^{-1}) from dry bean grains according to genotypes and experimental areas. Based in world bean consumption (6.96 g $capita^{-1}$ day^{-1}) (FAO, 2020).

Genotypes	Experimental areas					
	Lambari	Lavras	Patos de Minas	Uberaba	**Mean**	
Cometa	0.25	0.29	0.21	0.23	**0.24**	**b**
FC402	0.22	0.25	0.20	0.28	**0.24**	**c**
Madrepérola	0.25	0.29	0.21	0.28	**0.26**	**a**
Pérola	0.22	0.25	0.20	0.25	**0.23**	**c**
Realce	0.20	0.27	0.20	0.24	**0.23**	**c**
OV	0.21	0.25	0.22	0.28	**0.24**	**c**
VR 20	0.22	0.26	0.23	0.29	**0.25**	**b**
Tesouro	0.22	0.30	0.21	0.25	**0.24**	**b**
União	0.20	0.25	0.20	0.22	**0.22**	**d**
Supremo	0.22	0.30	0.23	0.29	**0.26**	**a**
Mean	**0.22**	**0.27**	**0.21**	**0.26**		

Lowercase letters, in the column, compare different genotypes. Means followed by the same letter do not differ from each other, by the Scott-Knott test at 5% probability.

4 DISCUSSION

For breeding beans plants with high Fe and Zn in grains, genetic variability for Fe and Zn content should exist across the genotypes. In consideration of this, notable differences emerged across all assessed attributes. Every tested attribute revealed variations among the genotypes and regions. The behavior of the genotypes was not consistent across the evaluated areas, likely due to the many environmental factors they were exposed to. This underscores the fact that genotype performance can often sway with shifts in location, with the interaction

genotype × environment holding significant implications for the genetic improvement of plants.

The values found for grain yield in the areas ranged from 1357.71 to 2978.45 kg ha^{-1}. These results suppressed the global averages for different continents: Europe (1,806 kg ha^{-1}), Asia (783.6 kg ha^{-1}), Americas (1075.7 kg ha^{-1}), Oceania (857.7 kg ha^{-1}), Africa (893.4 kg ha^{-1}) (FAO, 2019). The grain yields observed in the experimental areas were influenced by the pre-existing physicochemical attributes of the soils. This influence was present despite the application of fertilization aimed at achieving uniform NPK nutrient levels. Soil fertility plays a crucial role in grain yield by dictating the soil's capability to provide nutrients and establish favorable growth conditions for plants. Diverse soil fertility factors impact grain yield, including the availability of key nutrients (such as nitrogen, phosphorus, potassium, and micronutrients), soil pH (which directly affects nutrient availability), presence of organic matter (essential for improving soil structure, water retention, and gradual nutrient release), and soil texture (which governs water and nutrient retention dynamics).

In this study, Patos de Minas had the highest yield. This might be due to higher P content in this area, in comparison to the others, since this nutrient is the second most growth limiting for agriculture. Additionally, this area also showed higher values for OM and micronutrients. The soil of the area in Patos de Minas also exhibited the intermediate clay content, and lowest sand content, despite the higher silt content. Clay-based soils exhibit higher water and nutrient retention capacities, while sandy soils present reduced retention potential (CHANDER et al., 2023; SCAVO et al., 2022).

The different grain yield capacities in the evaluated genotypes might be attributed to their distinct abilities to cope with the combined biotic and abiotic conditions inherent to the four evaluated areas. The two genotypes that showed the highest grain yield ('FC402' and 'OV') were characterized by a normal growth cycle, which proved to be the lengthiest among those evaluated in this study. A prolonged growth cycle in plants indicates an extended period for growth and development before reaching maturity and initiating fruit or seed production. Generally, plants with extended growth cycles have more time for resource accumulation, including nutrients and energy, which can potentially lead to heightened production. It is, however, vital to recognize that the growth cycle is just one of the several factors influencing yield.

Other factors such as sunlight availability, water, soil nutrients, proper pollination, and favorable weather conditions, also play a crucial role in plant production. Additionally, each

plant species has its specific characteristics and requirements. This means that the impact of a later cycle on yield may vary among different types of plants. In summary, although a later cycle may provide plants with more time to develop and accumulate resources, the final production will depend on a combination of factors, including the environment and the individual characteristics of the plant (CHO; YOON; AN, 2017).

In this study, among the ten evaluated genotypes, the Fe contents in beans ranged from 60.15 to 78.44 mg kg^{-1}, while Zn levels ranged from 31.56 to 37.24 mg kg^{-1}, and protein levels varied from 24.06 to 22.05%. These observed values corresponded to existing literature, where Fe levels generally range from 61 to 71 mg kg^{-1} for beans. Similarly, average Zn content varies from 28 to 31 mg kg^{-1}, while protein levels range from 16.54 to 25.23% (BEEBE; GONZALEZ; RENGIFO, 2000; CAPRONI et al., 2020; CELMELI et al., 2018; GLAHN; NOH, 2021; GUNJAČA et al., 2021; KATUURAMU et al., 2021; MURUBE et al., 2021).

The genotypes from the carioca group ‘Cometa’, red group ‘VR 20’, and black group ‘Supremo’ exhibited the highest crude protein levels among the evaluated genotypes. In the specific case of the ‘Cometa’ genotype, the high crude protein level seems to be mainly a result of the dilution effect, which is induced by the low grain yield. The dilution effect in plants occurs when the production or accumulation of a specific component, such as proteins, is concentrated due to lower plant biomass (RENGEL; CAKMAK; WHITE, 2022). In the case of crude protein, when a plant has a low grain yield, the total amount of crude protein produced is distributed in a smaller plant biomass. Wheat and maize studies have shown that the dilution effect has affected the nutrient content in edible parts (MINER et al., 2022; ZHANG et al., 2021).

The dilution effect also influenced the accumulation of crude protein in the evaluated areas. The Lavras area, which had a lower grain yield, also had the highest crude protein level. In the other areas, in addition to grain yield, the concentration of organic matter, sulfur, and phosphorus was critical. Organic matter is composed of plant and animal residues in different stages of decomposition. During decomposition, nutrients for plants, including nitrogen, are released. Nitrogen is crucial for protein synthesis in plants (BERNARD et al., 2022). Phosphorus is an essential element for plant growth and plays a fundamental role in ATP (adenosine triphosphate) synthesis, which is the main source of energy in plant cells. Protein synthesis requires energy, and ATP plays an important role in this process. Additionally, phosphorus is related to root growth, which provides a larger nutrient absorption

area for plants. Therefore, the adequate presence of phosphorus in the soil is crucial for protein accumulation in plant grains (RENGEL; CAKMAK; WHITE, 2022). Sulfur is another essential element for plants. It is involved in the synthesis of sulfur-containing amino acids, such as cysteine and methionine, which are key components of proteins. Additionally, sulfur is also important for the biological reduction of molecular nitrogen. Both sulfur and nitrogen are crucial for protein accumulation in plant grains (BARCZAK et al., 2013; SCHERER et al., 2008).

The content and uptake of Fe observed in the experimental areas were mainly influenced by the physical composition of the studied soils. The higher Fe content and uptake in the grains in Uberaba were primarily affected by pH and the lower clay content in that area. The soil analysis showed that the highest Fe content inherent to the soil was found in Lambari, an area that had the lowest pH. On the other hand, beans cultivated in Lavras presented the lowest content, uptake, and intake of Fe, even though in the soil, the Fe content was not different from the other areas, except by Lambari. This may be attributed to the highest pH recorded for this area, as pH is closely related to Fe availability in soils (COLOMBO et al., 2014; MARSCHNER, 1993).

The clay content in tropical soils can influence Fe content in plants. Clay is a mineral fraction of the soil composed of very small particles. It has physical and chemical characteristics that affect the availability of nutrients for plants, including Fe. In soils with lower clay content, higher proportions of sand and silt are usually found, which are larger particles compared to clay particles. These larger particles have a relatively lower capacity to retain water and nutrients compared to clay particles. Additionally, clay possesses negative charges on its surface, which enables it to retain positively charged nutrients such as Fe. In soils with lower clay content, the nutrient retention capacity, including Fe, can be reduced due to the smaller available clay surface area to adsorb and retain these nutrients (COLOMBO et al., 2014).

For the Zn content, in the case of Lavras and Patos de Minas, the lowest and highest grain yields respectively in these areas, the grain content was directly affected, causing a dilution effect, which was the opposite between the areas. This suggests that the highest Zn content in Lavras is correlated with lower production for this area, and in the same way, the highest Zn uptake is correlated with lower production for this area. Is noteworthy that across the analyzed areas, Lavras presented the highest Ca and Mg levels. These two macronutrients can displace Zn from complexes and adsorption sites on soil, impacting the concentration of

free Zn (MARSCHNER, 1993). Additionally, Patos de Minas had the highest P content, an important element that affects Zn uptake, as Zn uptake by plants is also reduced by high P levels in soils (MOUSAVI, 2011). In the other areas, the most influential factors were pH, organic matter, and clay content in the soil.

Soil pH plays a crucial role in nutrient availability for plants. For Zn, as a metal, the acidic pH of soil can be considered as one of the most crucial factors influencing their mobility and uptake by plants. A significant decrease in the Zn availability is observed in alkaline soils (pH above 7.0), and in very acidic pH levels (pH below 6.0) due to adsorption to soil (ABEDI; GAVANJI; MOJIRI, 2022). Thus, Zn is more available in slightly acidic to neutral soils (pH between 6.0 and 7.0). Therefore, appropriate soil pH is important for efficient Zn uptake by plants.

The presence of organic matter in the soil can increase Zn availability for plants, as organic matter is capable of complexing and solubilizing Zn, making it more accessible to plant roots. Additionally, organic matter can also improve soil structure and water retention capacity, indirectly influencing Zn uptake in grains. The clay content in the soil can influence nutrient availability and retention, including Zn. Soils with higher clay content tend to have a greater nutrient retention capacity, which can result in lower Zn availability for plants. Additionally, clay can form complexes with Zn, making it less accessible to plant roots. However, clay can also play a positive role as it has a higher capacity to store nutrients, including Zn, and gradually release them to plants as needed (NATASHA et al., 2022).

The performance of genotypes in terms of Zn content and uptake did not fully correspond, with only the ‘Madrepérola’ genotype ranking among the best in both parameters, like what was observed for Fe. The ‘FC402’ and ‘OV’ genotypes accumulated more Zn due to their higher grain yield. Several factors may be responsible for this response. As plants produce a larger quantity of grains, their nutritional demand also increases. This includes the demand for micronutrients such as Zn. To meet this increased demand, plants can enhance the absorption and transport of Zn from roots to plant tissues. During plant growth and development, various metabolic reactions that require the presence of Zn occur. Zinc plays a fundamental role in the synthesis of proteins, enzymes, and plant hormones. With a higher plant yield, there is an increase in metabolic activity and, consequently, a greater demand for Zn ((HÄNSCH; MENDEL, 2009; PALMER; GUERINOT, 2009; SALAMA et al., 2006; STURIKOVA et al., 2018).

Studies conducted in Turkey and Australia, using wheat and rice, have demonstrated that it is possible to combine higher levels of Zn with high grain yields (BOONCHUAY et al., 2013; CAKMAK et al., 2010). According to a review based on the analysis of 100 observations, it was found that 70% of Zn is retained in the plant roots, while the remaining 30% is transferred to the shoot. However, the amount of Zn stored in plant roots showed considerable variation, ranging from 10% to 99%. This variation reveals that plants with different genetic characteristics differ significantly in terms of Zn storage in root tissues and its transfer to the shoot (NATASHA et al., 2022)

The genotypes with red ‘OV’ and ‘VR 20’ and black ‘Supremo’ seed coat colors showed higher content, accumulation, and intake of Fe (both globally and in Brazil). These results are also consistent with those observed for crude protein accumulation in these genotypes and can be explained in the same way. The study conducted on 255 cultivated lines of dry beans showed that the lines producing colored seeds, such as black and red ones, demonstrated higher antioxidant capacities (MADRERA et al., 2021). Greater antioxidant capacity is related to better plant protection. Therefore, genotypes with higher antioxidant activity have a metabolism that is better protected against reactive oxygen species (ROS), which allows them to be more efficient in element absorption. However, in the case of the ‘Madrepérola’ genotype, which also showed high Fe content and accumulation, no dilution effect was observed. Possibly, the ability of this genotype to accumulate Fe is related to other genetic factors. One hypothesis is that its distinct architecture, being prostrate, may favor the uptake of Fe in the grains. Further studies are needed to understand the potential of this genotype.

The recommended minimum intake for adults of Fe and Zn is 8 and 11 mg per day, respectively (NATIONAL ACADEMIES OF SCIENCES, 2011). Despite the differences found among the genotypes in Fe and Zn levels, none of the studied genotypes were able to provide adequate amounts of Fe and Zn through the average consumption of their grains, both in Brazil and worldwide, without the addition of fertilizers. Although a varied diet that includes different complementary food sources could allow for obtaining all the essential nutrients necessary for human health (KENNEDY et al., 2017), legumes are a rich source of many nutrients. Additionally, unlike many cereals, grain legumes are not polished before consumption, which helps in preserving their total nutritional content.

5 CONCLUSIONS

The values found for Zn, Fe, and crude protein correspond to those reported in the global literature for dry beans. The evaluated soils and the dilution/concentration effect directly influenced the accumulation of these nutrients. Based on the levels obtained in the evaluated tropical soils, it is not possible to meet the minimum requirements of Zn and Fe in an adult solely through the consumption of the beans from the ten evaluated genotypes. Therefore, the use of agronomic practices is necessary to increase nutrient levels. The genotypes ‘Supremo’, ‘OV,’ and ‘Madrepérola’ are the most suitable for future breeding programs aiming at genetic biofortification of Zn, Fe, and protein. Among them, ‘Supremo’ (black bean) demonstrated the best stability in accumulating all three components. The insights gained will contribute to the development of cultivation and nutritional strategies that promote food and nutritional security for vulnerable populations.

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Manuscript 4: RESPONSES TO THE INTERACTION OF SELENIUM AND ZINC THROUGH FOLIAR APPLICATION IN PROCESSED GRAINS OF BRAZILIAN UPLAND RICE GENOTYPES

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ABSTRACT

This study aimed to evaluate the ability of upland rice genotypes to enrich grains with selenium (Se) and zinc (Zn) through foliar application and investigate the effects of these elements on agronomic traits, protein content, and amino acid composition of rice grains. Five pre-selected upland rice genotypes were evaluated in a randomized block design across two experimental areas. The experimental design consisted of two experimental areas, five genotypes, and four foliar applications of 5.22 g Se ha^{-1} (Na_2SeO_4), 1.42 kg Zn ha^{-1} ($ZnSO_4$), Se + Zn, and control. The measured parameters included hulling and milling yield, grain yield, Se and Zn content, uptake and intake, as well as protein and amino acid content in whole grain and polished grains. Foliar application of Zn and Se at specific conditions of this experiment resulted in an increase in both Se and Zn content in processed grains. The treatments of Zn and Se show promise for future biofortification programs. Consumption of whole-grain rice is recommended to maximize the benefits of this biofortification approach. Additionally, this study found that Zn combined with Se can change the content, accumulation, and intake of these elements, as well as protein and amino acid levels. However, the response is genetically dependent. This strategy has the potential to address global malnutrition by increasing daily intake of Se, Zn, and protein. However, selecting appropriate genotypes is crucial for achieving the desired benefits. Based on positive results observed in Zn and Se combination treatment, the genotypes CMG ERF 221-19 and CMG ERF 85-15 are recommended for foliar biofortification using these elements. These findings contribute to the understanding and application of Se and Zn biofortification, particularly in upland rice cultivation with nutrient-deficient soils.

Keywords: Selenate. Se IV. Zn + Se. $ZnSO_4$. Polished. Whole.

1 INTRODUCTION

One of the most important global current issues is food security. It refers not only to the adequate quantity and better distribution of produced food but also to improving their nutritional quality. However, when the latter is not considered, serious socioeconomic problems can arise on a global scale. According to global practices in combating malnutrition, there has been a reduction from 23% to 13% in the global population suffering from malnutrition between 1990 and 2015 (UN, 2015). However, this information was based solely on energy malnutrition and did not consider deficiencies related to micronutrients. It is estimated that over 2 billion people are affected by such deficiencies (MUTHAYYA et al., 2013).

Selenium (Se) and zinc (Zn) are among the most severe nutritional deficiencies related to human health (MORAES, 2008). Selenium is essential for maintaining human health since it is part of selenoproteins present in the human body, which regulate thyroid hormones and play a role in antioxidant and immune defenses (NAVARRO-ALARCON; CABRERA-VIQUE, 2008; NEWMAN et al., 2019; RAYMAN, 2008). Adequate intake of Se by humans can prevent various health problems, such as cardiovascular and degenerative diseases, and cancers (NEWMAN et al., 2019; RAYMAN, 2008). Regarding Zn, it is indispensable for both animals and plants. Zinc interacts with a large number of enzymes and proteins in the human body. Therefore, low Zn intake can lead to health issues such as growth retardation, impaired brain development, decreased immune system efficiency, pneumonia, diarrhea, stillbirths, reduced physical performance, skin lesions, alopecia, and impaired wound healing (BLACK et al., 2008; GIBSON, 2012; GIBSON et al., 2008; KREBS; MILLER; MICHAEL HAMBIDGE, 2014; TERRIN et al., 2015). Approximately 17.3% of the global population is at risk of inadequate Zn intake or consumption, and around 40 countries have soils with low Se levels, directly impacting the health of populations (WESSELLS; BROWN, 2012; WU et al., 2015). Therefore, actions are needed to reduce all forms of malnutrition worldwide, with the aim of improving global well-being.

Biofortification has been a good strategy to improve global well-being. This practice involves a set of techniques and practices, such as genetic improvement and agronomic management, aiming to increase nutrient content in edible parts of plants (BLOMBERG, 2008). Biofortification is an ideal strategy for improving nutrition in rural and poor

communities that rely on subsistence agriculture or lack access to fortified foods (BLANCQUAERT et al., 2017; BOUIS; SALTZMAN, 2017; CAKMAK, 2008).

Genetic biofortification and agronomic biofortification are two approaches aimed at enhancing the nutritional content of crops to address micronutrient deficiencies in human diets, particularly in regions where people have limited access to diverse and nutrient-rich foods. These strategies target improving the nutrient content of staple crops that are commonly consumed by large populations. Genetic biofortification involves the breeding and selection of crop varieties with naturally enhanced nutrient content through traditional breeding methods or modern biotechnological approaches. The goal is to increase the concentration of specific vitamins, minerals, and other nutrients in the edible parts of the plants. Agronomic biofortification involves altering the growing conditions and practices of crops to enhance their nutrient content. This approach does not involve genetic modification but rather focuses on optimizing the soil conditions, fertilization techniques, and agronomic practices to increase the uptake and accumulation of desired nutrients in crops (GARG et al., 2018).

Rice (*Oryza sativa* L.) is one of the most important crops in biofortification studies. According to the latest estimate, there are approximately 165 million hectares of rice worldwide in the 2022/2023 season, with an estimated grain production of 761 million tons (USDA ERS, 2023). Over half of the global population uses rice varieties/cultivars as a staple food, and the mean per capita consumption in 2020 was estimated at 78.91 kg per year (FAO, 2020; LENAERTS; DE MEY; DEMONT, 2018).

Biofortification through genetic improvement and agronomic practices is highly recommended, especially for rice cultivation. However, it is still an incipient practice worldwide. Furthermore, there is limited knowledge about the grain response of rice genotypes when biofortified simultaneously with Se and Zn, especially under specific regional conditions. Therefore, research development is necessary to understand these mechanisms and identify appropriate genotypes that can be subsequently recommended, along with improvements in biofortification efficiency.

The aim of this study is to assess the combination of foliar Se and Zn applications on grain biofortification and the effect of grain processing on grain quality.

2 MATERIAL AND METHODS

2.1 Experimental design

The experiment was carried out with five genotypes of upland rice (*Oryza sativa* L.): CMG 2188, ERF22116, ERF22119, and CMG ERF 85-15, during the 2019/2020 crop season from January to May, in the municipalities of Lavras (21° 14′ 45″S 44° 59′ 59″W; altitude: 919 m) and Lambari (21° 58′ 32″ S 45° 21′ 32″ W; altitude: 887 m), in the state of Minas Gerais, Brazil. The genotypes were selected in the study conducted by Felix et al. (2023), in which, among 20 genotypes that received the application of urea + Se as a top-dressing, the five genotypes that exhibited the highest levels of Se in polished grains were chosen. The climate in these municipalities is classified as Cwb according to the Köppen-Geiger climate classification system, characterized by mild and rainy summers and dry winters (KOTTEK et al., 2006). The soils in each location were classified as Dystrophic Red-Yellow Latosol - Lambari and Eutrophic Red-Yellow Argisol - Lavras (Soil Survey Staff 1999). The physicochemical attributes of the soils (TABLE 2) were determined according to the methodologies proposed by the Brazilian Agricultural Research Corporation and the United States Environmental Protection Agency, the latter for total soil Se content (TEIXEIRA et al., 2017; USEPA, 2007). Necessary corrections and fertilizations were performed (TABLE 3) according to the recommendations for the state of Minas Gerais (SOUSA; LOBATO, 2004).

Sodium selenate (Na_2SeO_4), heptahydrated zinc sulfate ($ZnSO_4.7\ H_2O$), and a mixture of both were diluted in a 0.5% surfactant solution (Assist®, BASF) to prepare the following Se and Zn doses: 5.22 g ha^{-1} and 1.42 kg ha^{-1}, respectively, divided into two applications. The first foliar application of foliar treatments was carried out at the heading stage, and the second at the grain-filling stage, respecting the cycle of each genotype. The control group received only deionized water containing the surfactant. The foliar applications were performed using a pressurized pump connected to a carbon dioxide container, and the plots were arranged in a randomized complete block design in a 2 x 5 x 4 scheme, with two locations, five genotypes, four application categories, and 3 repetitions. The plot size was 4 x 2 m, with a 0.5 m row spacing, totaling 5 rows per plot, with approximately 60 seeds per linear meter. The useful area of each plot was 4.8 m^2, resulting in a total of 120 plots.

Table 1 – Chemical and physical attributes of the studied soils.

Attributes (0-20 cm)	Lavras	Lambari
pH	4.9	4.7

$K_{available}$ (mg dm^{-3})	86.85	62.07
$P_{available}$ (mg dm^{-3})	20.64	12.49
$Ca_{available}$ (cmol dm^{-3})	2.26	1.55
$Mg_{available}$ (cmol dm^{-3})	0.59	0.21
$Al_{available}$ (cmol dm^{-3})	0.30	1.10
H+Al (cmol dm^{-3})	7.50	8
SB ($cmol_c$ dm^{-3})	3.07	1.92
t ($cmol_c$ dm^{-3})	3.37	3.02
T ($cmol_c$ dm^{-3})	10.57	9.92
V (%)	29.07	19.35
M (%)	8.90	36.42
O.M. (dag kg^{-1})	2.64	2.63
$Zn_{available}$ (mg dm^{-3})	2.70	1.20
$Fe_{available}$ (mg dm^{-3})	30.60	66.90
$Mn_{available}$ (mg dm^{-3})	12	17.10
$Cu_{available}$ (mg dm^{-3})	0.40	69.50
$B_{available}$ (mg dm^{-3})	0.08	0.01
$S_{available}$ (mg dm^{-3})	5.30	4.80
Clay (dag kg^{-1})	46	36
Silt (dag kg^{-1})	13	32
Sand (dag kg^{-1})	41	32
Se_{total} (mg kg^{-1})	0.29	0.18

pH = hydrogen potential; K = potassium; P = phosphorus; Mg = magnesium; Al = aluminum or exchangeable acidity; H+Al = total acidity; SB = sum of bases; t = effective cation exchange capacity; T = total cation exchange capacity; V = base saturation; M = aluminum saturation; O.M = organic matter; Mn = manganese; Cu = copper; B = boron.

Table 2 – Fertilizations and soil amendments in the studied soils.

Soil amendment before planting (60 days prior to sowing).			
Nutrients	Lavras	Lambari	Sources
Ca e Mg	V%=50	NA	$CaCO_3$/$MgCO_3$
Fertilization at planting			
N	20 kg ha^{-1}	20 kg ha^{-1}	8-28-16/U
P	35 kg ha^{-1}	70 kg ha^{-1}	8-28-16

K	20 kg ha^{-1}	40 kg ha^{-1}	8-28-16
Topdressing fertilization (40 days after sowing)			
N	50 kg ha^{-1}	50 kg ha^{-1}	SA
K	20 kg ha^{-1}	20 kg ha^{-1}	KCl

8-28-16 = commercial formulated with respective percentages of nitrogen, phosphorus, and potassium; CaCO3/MgCO3 = dolomitic limestone; NA = not applied due to operational issues; KCl = potassium chloride; N = nitrogen; U = urea; SA = ammonium sulfate.

2.2 Grain yield, hulling, and milling yield

The grain yield was obtained by harvesting the useful area of the plot and drying it in an oven at 60°C. The data were expressed in kg ha^{-1} with moisture corrected to 13%. The process of hulling and milling yield was carried out according to the Normative Instruction 06/2009 of the Ministry of Agriculture, Livestock, and Supply of Brazil, which establishes the official classification standards for rice grains (MAPA, 2009).

2.3 Processing of rice grains (polished and whole)

The production of processed grains was carried out using a mini-testing mill, which performed the process of hulling (whole rice) and milling (polished rice).

2.4 Measurement of total proteins, and total amino acids

The whole and polished grains were subsequently ground in a hand mill. The levels of total free amino acids were determined (COCKING, 1954). The protein content in each treatment was also determined (BRADFORD, 1976).

2.4 Measurement of Se and Zn total content, uptake, intake

The sample digestion procedure employed was based on the USEPA 3051a methodology (USEPA, 2007). Approximately 500 mg of ground samples of processed grains were weighed and digested in 5 mL of ≥ 65% HNO_3 in PTFE Teflon® tubes (CEM Corporation, Matthews, NC, USA). The extract was left overnight at room temperature, and the digestion was carried out the following morning. The vials were hermetically sealed and placed in a microwave (CEM brand, Mars - 5 model) with a controlled temperature of 0.76

MPa for 15 minutes. After digestion, the extracts were cooled to room temperature. Subsequently, the final extract volume was supplemented with an additional 5 mL of deionized water. After filtration, the extracts were transferred to smaller vials (30 mL) and stored at 5°C until analysis. Standard reference materials of Se and Zn (White Clover - BCR 402 and Tomato leaves - NIST SRM 1573a) for plant material were included in each batch for digestion quality control, along with a blank sample. The digested extract was used to measure the total content of Se and Zn in Graphite Furnace Atomic Absorption Spectrometry (GFAAS). The mean recovery for the Se and Zn in the standard reference materials were 90.31% and 97.27%, respectively for white clover and tomato leaves (n = 18).

Based on the Se and Zn content in the processed grains, the uptake of Se and Zn in the processed grains (EA) (mg) was calculated (DUCSAY et al., 2016). This was derived from multiplying the Se and Zn contents in the processed grains (mg kg^{-1}) and the yield in each processing (kg ha^{-1}). Also, the calculation of Se and Zn intake was performed, as carried out by (LESSA et al., 2020).

$$E = [Gr]_{intake}\ x\ [Se]_{content}$$

Where: EI (µg $person^{-1}$ day^{-1} for Se, and g $person^{-1}$ day^{-1} for Zn) is the daily Se and Zn intake estimation per person; $[Gr]_{intake}$ (g $person^{-1}$ day^{-1}) is the mean consumption of rice grains per person ; $[E]_{content}$ (µg kg^{-1} for Se, and g kg^{-1} for Zn) Se and Zn contents in rice grains (whole or polished) verified for the studied treatments.

According to the Statistical Database of the Food and Agriculture Organization, the mean consumption of rice in the world recorded for the last decade is 78.91 kg $person^{-1}$ $year^{-1}$ (216 g $person^{-1}$ day^{-1}) (FAO, 2020).

2.5 Statistical analysis

The obtained data were subjected to the ESD (Extreme Studentized Deviate) test to identify outliers, in addition to analysis of variance and the Scott-Knott mean test, using the statistical software Speedstat 2.8 (CARVALHO et al., 2020).

3 RESULTS

In terms of grain yield, there was an interaction between foliar treatments and genotypes. The administration of the control treatment and Zn to each genotype resulted in greater grain yield in genotype CMG ERF 221-16 (1205.32 and 1402.37 kg ha^{-1}, respectively). For genotype CMG ERF 221-19, besides the control and Zn treatments, a higher grain yield was also observed with the application of Zn + Se (1349.34, 1238.35, and 1216.77 kg ha^{-1}, respectively). No differences between foliar treatments were observed for the other genotypes. When comparing the genotypes within each foliar treatment, with the application of the control treatment, CMG ERF 221-16, CMG ERF 221-19, and ERF 85-15 exhibited higher grain yield than the others (1205.32, 1349.34, and 1361.19 kg ha^{-1}, respectively). With the application of Zn, all genotypes showed higher grain yield than the BRS ESMERALDA variety (CMG 2188 - 1301.16 kg ha^{-1}; CMG ERF 221-16 - 1402.37 kg ha^{-1}; CMG ERF 221-19 - 1238.35 kg ha^{-1}; and ERF 85-15 - 1415.60 kg ha^{-1}). With the application of Se, CMG 2188 and ERF 85-15 displayed higher grain yield (1181.32 and 1327.37 kg ha^{-1}, respectively). No differences between genotypes were observed with the application of the Zn + Se treatment. According to the overall mean, significant differences were found between foliar treatments and genotypes. The application of the control and Zn treatments resulted in higher grain yield (1194.62 and 1265.32 kg ha^{-1}, respectively). In terms of genotypes, all genotypes exhibited higher grain yield than the BRS ESMERALDA variety (CMG 2188 - 1226.18 kg ha^{-1}; CMG ERF 221-16 - 1135.14 kg ha^{-1}; CMG ERF 221-19 - 1176.60 kg ha^{-1}; and ERF 85-15 - 1316.89 kg ha^{-1}) (TABLE 3). No significant difference was observed in hulled whole grain (TABLE 4).

Table 3 – Grain yield (kg ha^{-1}) according to genotypes and foliar treatments.

Genotypes	General (All areas)									
	Control		Zn		Se		Zn + Se		Mean	
BRS ESMERALDA	936.94	bA	969.12	bA	1027.09	bA	886.99	aA	955.03	b
CMG 2188	1120.33	bA	1301.16	aA	1181.32	aA	1301.91	aA	1226.18	a
CMG ERF 221-16	1205.32	aA	1402.37	aA	906.73	bB	1026.13	aB	1135.14	a
CMG ERF 221-19	1349.34	aA	1238.35	aA	901.93	bB	1216.77	aA	1176.60	a
CMG ERF 85-15	1361.19	aA	1415.60	aA	1327.37	aA	1163.41	aA	1316.89	a
Mean	1194.62	A	1265.32	A	1068.89	B	1119.04	B		

Capital letters, on the line, compare different foliar treatments. Lowercase letters, in the column, compare different genotypes. Means followed by the same letter do not differ from each other, by the Scott-Knott test at 5% probability.

Table 4 – Hulling whole grain (%) according to genotypes and foliar treatments.

Genotypes	General (All areas)

	Control		Zn		Se		Zn + Se		Mean	
BRS ESMERALDA	77.60	aA	78.87	aA	79.10	aA	78.67	aA	78.56	a
CMG 2188	76.73	aA	78.53	aA	76.77	aA	77.70	aA	77.43	a
CMG ERF 221-16	75.97	aA	79.90	aA	80.23	aA	80.57	aA	79.17	a
CMG ERF 221-19	77.53	aA	79.20	aA	80.50	aA	79.93	aA	79.29	a
CMG ERF 85-15	79.17	aA	75.67	aA	79.43	aA	77.43	aA	77.93	a
Mean	77.40	A	78.43	A	79.21	A	78.86	A		

Capital letters, on the line, compare different foliar treatments. Lowercase letters, in the column, compare different genotypes. Means followed by the same letter do not differ from each other, by the Scott-Knott test at 5% probability.

Considering hulling polished grain, the only significant difference was found among genotypes, where BRS ESMERALDA, CMG ERF 221-16, and CMG ERF 221-19 exhibited a higher percentage of hulling polished grain with the application of Se compared to the other genotypes (64.73%, 64.20%, and 64.23%, respectively). According to the overall mean, a significant difference was found only among genotypes, with CMG ERF 221-16 and CMG ERF 221-19 showing a higher percentage of hulling polished grain with the applied foliar treatments, at 64.03% and 63.83%, respectively. No differences were observed between the treatments and the control. No significant differences were observed in foliar treatments or interaction between genotypes and foliar treatments (TABLE 5).

Table 5 – Hulling polished grain (%) according to genotypes and foliar treatments.

Genotypes	General (All areas)									
	Control		Zn		Se		Zn + Se		Mean	
BRS ESMERALDA	62.75	aA	60.67	aA	64.73	aA	62.13	aA	62.57	b
CMG 2188	61.65	aA	61.67	aA	61.57	bA	61.28	aA	61.54	b
CMG ERF 221-16	63.23	aA	64.17	aA	64.20	aA	64.52	aA	64.03	a
CMG ERF 221-19	62.27	aA	63.80	aA	64.23	aA	65.02	aA	63.83	a
CMG ERF 85-15	62.22	aA	62.10	aA	60.00	bA	61.30	aA	61.40	b
Mean	62.42	A	62.48	A	62.95	A	62.85	A		

Capital letters, on the line, compare different foliar treatments. Lowercase letters, in the column, compare different genotypes. Means followed by the same letter do not differ from each other, by the Scott-Knott test at 5% probability.

Concerning milling yield, a significant difference was found only among genotypes, where BRS ESMERALDA and CMG 2188 exhibited a higher percentage of milling yield compared to the other genotypes with the application of all treatments (Control - 28.13% and 27.68%; Zn - 28.62% and 30.75%; Se - 32.22% and 27.93%; Zn + Se - 30.13% and 29.62%, respectively). According to the overall mean, a significant difference was found only among genotypes, with BRS ESMERALDA and CMG 2188 showing a higher percentage of milling yield with the applied foliar treatments, at 29.78% and 29.00%, respectively. No significant

differences were observed among the foliar treatments or in the interaction between genotypes and foliar treatments (TABLE 6).

Table 6 – Milling yield (%) according to genotypes and foliar treatments.

Genotypes	General (All areas)									
	Control	Zn		Se		Zn + Se		Mean		
BRS ESMERALDA	28.13	aA	28.62	aA	32.22	aA	30.13	aA	29.78	a
CMG 2188	27.68	aA	30.75	aA	27.93	aA	29.62	aA	29.00	a
CMG ERF 221-16	21.73	bA	22.87	bA	25.93	bA	23.93	bA	23.62	b
CMG ERF 221-19	19.67	bA	21.25	bA	23.08	bA	24.55	bA	22.14	b
CMG ERF 85-15	21.58	bA	20.17	bA	21.95	bA	22.65	bA	21.59	b
Mean	23.76	A	24.73	A	26.22	A	26.18	A		

Capital letters, on the line, compare different foliar treatments. Lowercase letters, in the column, compare different genotypes. Means followed by the same letter do not differ from each other, by the Scott-Knott test at 5% probability.

Observation of the interaction between foliar treatments and genotypes was noted for Zn content in whole grain. Regarding the foliar treatments for each genotype, the application of Zn and Zn + Se resulted in a higher content of Zn in the whole grain for all genotypes: BRS ESMERALDA (40.10 and 41.42 mg kg^{-1} DW, respectively), CMG 2188 (38.75 and 40.66 mg kg^{-1} DW, respectively), CMG ERF 221-16 (39.03 and 39.27 mg kg^{-1} DW, respectively), CMG ERF 221-19 (43.17 and 44.30 mg kg^{-1} DW, respectively), and ERF 85-15 (47.40 and 48.47 mg kg^{-1} DW, respectively). When comparing the genotypes within each foliar treatment, BRS ESMERALDA, CMG ERF 221-19, and ERF 85-15 exhibited the highest content of Zn in the whole grain in the control treatments (36.57, 35.30, and 37.44 mg kg^{-1} DW, respectively) and Se treatments (33.99, 34.43, and 36.89 mg kg^{-1} DW, respectively). With the application of Zn and Zn + Se, CMG ERF 221-19 and ERF 85-15 showed a higher content of Zn in the whole grain (Zn - 43.17 and 47.40 mg kg^{-1} DW, respectively; Zn + Se - 44.30 and 48.47 mg kg^{-1} DW, respectively). According to the overall mean, significant differences were found between the foliar treatments and genotypes. The application of Zn and Zn + Se resulted in a higher mean content of Zn in the whole grain (41.69 and 42.83 mg kg^{-1} DW, respectively). As for the genotypes, ERF 85-15 exhibited the highest mean (42.55 mg kg^{-1} DW), followed by BRS ESMERALDA (38.02 mg kg^{-1} DW) and CMG ERF 221-19 (39.30 mg kg^{-1} DW), while CMG 2188 (33.94 mg kg^{-1} DW) and CMG ERF 221-16 (34.53 mg kg^{-1} DW) had the lowest means (TABLE 7).

Table 7 – Zinc content in whole grain (mg kg^{-1} DW) according to genotypes and foliar treatments.

Genotypes	General (All areas)									
	Control	Zn		Se		Zn + Se		Mean		
BRS ESMERALDA	36.57	aB	40.10	bA	33.99	aB	41.42	bA	38.02	b
CMG 2188	27.97	bB	38.75	bA	28.39	bB	40.66	bA	33.94	c
CMG ERF 221-16	31.56	bB	39.03	bA	28.24	bB	39.27	bA	34.53	c
CMG ERF 221-19	35.30	aB	43.17	aA	34.43	aB	44.30	aA	39.30	b
CMG ERF 85-15	37.44	aB	47.40	aA	36.89	aB	48.47	aA	42.55	a
Mean	33.77	B	41.69	A	32.39	B	42.83	A		

Capital letters, on the line, compare different foliar treatments. Lowercase letters, in the column, compare different genotypes. Means followed by the same letter do not differ from each other, by the Scott-Knott test at 5% probability.

For Zn content in polished grain, an interaction between foliar treatments and genotypes was observed. Regarding the foliar treatments for each genotype, the application of Zn and Zn + Se resulted in a higher Zn content in the polished grain for the genotypes CMG 2188 (30.94 and 31.43 mg kg^{-1} DW, respectively), CMG ERF 221-16 (29.70 and 27.12 mg kg^{-1} DW, respectively), CMG ERF 221-19 (31.65 and 31.52 mg kg^{-1} DW, respectively), and ERF 85-15 (35.25 and 33.20 mg kg^{-1} DW, respectively). No difference between foliar treatments was observed for BRS ESMERALDA. When comparing genotypes under each foliar treatment, BRS ESMERALDA, CMG ERF 221-19, and ERF 85-15 showed the highest Zn content in the polished grain with the application of the control treatment (29.08, 26.94, and 27.33 mg kg^{-1} DW, respectively). With the application of Zn, ERF 85-15 exhibited a higher Zn content in the polished grain (35.25 mg kg^{-1} DW) compared to the other genotypes. With the application of Se, BRS ESMERALDA and ERF 85-15 showed a higher Zn content in the polished grain (30.15 and 27.26 mg kg^{-1} DW, respectively). With the Zn + Se treatment, all genotypes had a higher Zn content in the polished grain compared to CMG ERF 221-16 (BRS ESMERALDA - 33.06 mg kg^{-1} DW; CMG 2188 - 31.43 mg kg^{-1} DW; CMG ERF 221-19 - 31.52 mg kg^{-1} DW; ERF 85-15 - 33.20 mg kg^{-1} DW). According to the overall mean, significant differences were found between foliar treatments and genotypes. The application of Zn and Zn + Se resulted in a higher mean Zn content in the polished grain (31.72 and 31.26 mg kg^{-1} DW, respectively). Regarding genotypes, BRS ESMERALDA and ERF 85-15 showed a higher mean (30.83 and 30.76 mg kg^{-1} DW, respectively), followed by CMG ERF 221-19 (28.90 mg kg^{-1} DW), CMG 2188 (27.12 mg kg^{-1} DW), and CMG ERF 221-16 (26.00 mg kg^{-1} DW), with the latter two having the lowest means (TABLE 8).

Table 8 – Zinc content in polished grain (mg kg^{-1} DW) according to genotypes and foliar treatments.

Genotypes	General (All areas)									
	Control	Zn		Se		Zn + Se		Mean		
BRS ESMERALDA	29.08	aA	31.05	bA	30.15	aA	33.06	aA	30.83	a
CMG 2188	23.05	bB	30.94	bA	23.05	bB	31.43	aA	27.12	c
CMG ERF 221-16	24.14	bB	29.70	bA	23.03	bB	27.12	bA	26.00	c
CMG ERF 221-19	26.94	aB	31.65	bA	25.51	bB	31.52	aA	28.90	b
CMG ERF 85-15	27.33	aB	35.25	aA	27.26	aB	33.20	aA	30.76	a
Mean	26.11	B	31.72	A	25.80	B	31.26	A		

Capital letters, on the line, compare different foliar treatments. Lowercase letters, in the column, compare different genotypes. Means followed by the same letter do not differ from each other, by the Scott-Knott test at 5% probability.

Regarding Se content in whole grain, an interaction between foliar treatments and genotypes was found. Concerning the foliar treatments for each genotype, the application of Se and Zn + Se yielded a higher Se content in whole grain for the genotypes BRS ESMERALDA (0.26 and 0.28 mg kg^{-1} DW, respectively), CMG 2188 (0.24 and 0.26 mg kg^{-1} DW, respectively), and ERF 85-15 (0.35 and 0.34 mg kg^{-1} DW, respectively). For genotypes CMG ERF 221-16 and CMG ERF 221-19, the application of Zn + Se resulted in a higher Se content in whole grain (0.31 and 0.47 mg kg^{-1} DW, respectively), followed by the Se treatment (0.25 and 0.31 mg kg^{-1} DW, respectively), control (0.16 mg kg^{-1} DW), and Zn (0.17 and 0.21 mg kg^{-1} DW, respectively). When comparing genotypes under each foliar treatment, differences between genotypes were observed only with the application of Se and Zn + Se. CMG ERF 221-19 and ERF 85-15 exhibited the highest Se content in whole grain with the application of Se (0.31 and 0.35 mg kg^{-1} DW, respectively). In the case of the Zn + Se treatment, CMG ERF 221-19 showed the highest Se content in whole grain (0.47 mg kg^{-1} DW), followed by CMG ERF 221-16and ERF 85-15 (0.31 and 0.34 mg kg^{-1} DW, respectively), and finally, with the lowest contents, BRS ESMERALDA (0.28 mg kg^{-1} DW) and CMG 2188 (0.26 mg kg^{-1} DW). Significant differences were found between foliar treatments and genotypes according to the overall mean. The application of Zn + Se resulted in the highest mean Se content in whole grain (0.33 mg kg^{-1} DW), followed by the Se application (0.28 mg kg^{-1} DW), control (0.16 mg kg^{-1} DW), and Zn (0.17 mg kg^{-1} DW), with the latter two yielding the lowest means. Regarding the genotypes, CMG ERF 221-19 had the highest mean Se content in whole grain (0.29 mg kg^{-1} DW), followed by ERF 85-15 (0.26 mg kg^{-1} DW), BRS ESMERALDA (0.22 mg kg^{-1} DW), CMG 2188 (0.20 mg kg^{-1} DW), and

CMG ERF 221-16 (0.22 mg kg^{-1} DW), with the last three exhibiting the lowest means (TABLE 9).

Table 9 – Selenium content in whole grain (mg kg^{-1} DW) according to genotypes and foliar treatments.

Genotypes	General (All areas)									
	Control	Zn		Se		Zn + Se		Mean		
BRS ESMERALDA	0.16	aB	0.17	aB	0.26	bA	0.28	cA	0.22	c
CMG 2188	0.14	aB	0.15	aB	0.24	bA	0.26	cA	0.20	c
CMG ERF 221-16	0.16	aC	0.17	aC	0.25	bB	0.31	bA	0.22	c
CMG ERF 221-19	0.16	aC	0.21	aC	0.31	aB	0.47	aA	0.29	a
CMG ERF 85-15	0.19	aB	0.15	aB	0.35	aA	0.34	bA	0.26	b
Mean	0.16	C	0.17	C	0.28	B	0.33	A		

Capital letters, on the line, compare different foliar treatments. Lowercase letters, in the column, compare different genotypes. Means followed by the same letter do not differ from each other, by the Scott-Knott test at 5% probability.

Foliar treatments and genotypes exhibited an interaction that influenced the Se content found in the polished grain. The application of Se proved to be the most effective foliar treatment for increasing the Se content in the polished grain of the BRS ESMERALDA genotype (0.22 mg kg^{-1} DW). Conversely, for the CMG 2188, CMG ERF 221-19, and ERF 85-15 genotypes, both Se and Zn + Se treatments resulted in higher Se contents in the polished grain (CMG 2188 - 0.22 and 0.24 mg kg^{-1} DW, respectively; CMG ERF 221-19 - 0.21 and 0.24 mg kg^{-1} DW, respectively; ERF 85-15 - 0.25 and 0.26 mg kg^{-1} DW, respectively). In contrast, the application of Zn + Se exhibited the highest Se content in the polished grain for the CMG ERF 221-16genotype (0.23 mg kg^{-1} DW), followed by the Se treatment (0.18 mg kg^{-1} DW), while the control and Zn treatments yielded the lowest contents (0.13 mg kg^{-1} DW). When comparing genotypes within each foliar treatment, the BRS ESMERALDA genotype displayed a superior Se content in the polished grain when treated with the control (0.17 mg kg^{-1} DW). With the application of Zn, both BRS ESMERALDA and CMG 2188 genotypes showed higher Se contents in the polished grain (0.18 and 0.16 mg kg^{-1} DW, respectively). In the case of the Se treatment, except for ERF221-16, all other genotypes demonstrated higher Se contents in the polished grain (BRS ESMERALDA - 0.22 mg kg^{-1} DW; CMG 2188 - 0.22 mg kg^{-1} DW; CMG ERF 221-19 - 0.21 mg kg^{-1} DW; ERF 85-15 - 0.25 mg kg^{-1} DW). Similarly, when Zn + Se was applied, all genotypes exhibited higher Se contents in the polished grain compared to BRS ESMERALDA (CMG 2188 - 0.24 mg kg^{-1} DW; CMG ERF 221-16 - 0.23 mg kg^{-1} DW; CMG ERF 221-19 - 0.24 mg kg^{-1} DW; ERF 85-15 - 0.26 mg kg^{-1} DW). Overall, a significant difference was observed only between foliar

treatments, with the Se and Zn + Se applications resulting in higher mean Se contents in the polished grain (0.22 and 0.23 mg kg^{-1} DW, respectively) (TABLE 10).

Table 10 – Selenium content in polished grain (mg kg^{-1} DW) according to genotypes and foliar treatments.

Genotypes	General (All areas)									
	Control	Zn		Se		Zn + Se		Mean		
BRS ESMERALDA	0.17	aB	0.18	aB	0.22	aA	0.16	bB	0.18	a
CMG 2188	0.13	bB	0.16	aB	0.22	aA	0.24	aA	0.19	a
CMG ERF 221-16	0.13	bC	0.13	bC	0.18	bB	0.23	aA	0.17	a
CMG ERF 221-19	0.13	bB	0.14	bB	0.21	aA	0.24	aA	0.18	a
CMG ERF 85-15	0.14	bB	0.13	bB	0.25	aA	0.26	aA	0.20	a
Mean	0.14	B	0.15	B	0.22	A	0.23	A		

Capital letters, on the line, compare different foliar treatments. Lowercase letters, in the column, compare different genotypes. Means followed by the same letter do not differ from each other, by the Scott-Knott test at 5% probability.

The interaction between foliar treatments and genotypes affects Zn intake in whole grains. Regarding the foliar treatments for each genotype, the application of Zn and Zn + Se resulted in a higher Zn intake content in the whole grain for the genotypes BRS ESMERALDA (8.66 and 8.95 g day^{-1}, respectively), CMG 2188 (8.37 and 8.78 g day^{-1}, respectively), CMG ERF 221-16(8.43 and 8.48 g day^{-1}, respectively), CMG ERF 221-19 (9.33 and 9.57 g day^{-1}, respectively), and ERF 85-15 (10.24 and 10.47 g day^{-1}, respectively). Comparing the genotypes within each foliar treatment, BRS ESMERALDA, CMG ERF 221-19, and ERF 85-15 exhibited the highest Zn intake content in the whole grain under the control treatments (7.90, 7.62, and 8.09 g day^{-1}, respectively) and the Se treatments (7.34, 7.44, and 7.97 g day^{-1}, respectively). With the application of Zn and Zn + Se, CMG ERF 221-19 and ERF 85-15 showed higher Zn intake contents (Zn - 9.33 and 10.24 g day^{-1}, respectively; Zn + Se - 9.57 and 10.47 g day^{-1}, respectively). According to the overall mean, a significant difference was found between foliar treatments and genotypes. The application of Zn and Zn + Se resulted in a higher overall mean of Zn intake content in the whole grain (9.01 and 9.25 g day^{-1}, respectively). Among the genotypes, ERF 85-15 exhibited the highest overall mean (9.19 g day^{-1}), followed by BRS ESMERALDA (8.21 g day^{-1}) and CMG ERF 221-19 (8.49 g day^{-1}), while CMG 2188 (7.33 g day^{-1}) and CMG ERF 221-16(7.46 g day^{-1}) had the lowest overall means (TABLE 11).

Table 11 – Zinc intake in whole grain (g day^{-1}) according to genotypes and foliar treatments.

Genotypes	General (All areas)									
	Control	Zn		Se		Zn + Se		Mean		
BRS ESMERALDA	7.90	aB	8.66	bA	7.34	aB	8.95	bA	8.21	b
CMG 2188	6.04	bB	8.37	bA	6.13	bB	8.78	bA	7.33	c
CMG ERF 221-16	6.82	bB	8.43	bA	6.10	bB	8.48	bA	7.46	c
CMG ERF 221-19	7.62	aB	9.33	aA	7.44	aB	9.57	aA	8.49	b
CMG ERF 85-15	8.09	aB	10.24	aA	7.97	aB	10.47	aA	9.19	a
Mean	7.29	B	9.01	A	7.00	B	9.25	A		

Capital letters, on the line, compare different foliar treatments. Lowercase letters, in the column, compare different genotypes. Means followed by the same letter do not differ from each other, by the Scott-Knott test at 5% probability.

There was an interaction between foliar treatments and genotypes that affected Zn intake in polished grain. Concerning the foliar treatments for each genotype, the application of Zn and Zn + Se resulted in a higher Zn intake content in the polished grain for the genotypes CMG 2188 (6.68 and 6.79 g day^{-1}, respectively), CMG ERF 221-16(6.41 and 5.86 g day^{-1}, respectively), CMG ERF 221-19 (6.84 and 6.81 g day^{-1}, respectively), and ERF 85-15 (7.61 and 7.17 g day^{-1}, respectively). No difference between foliar treatments was observed for the BRS ESMERALDA genotype. Comparing the genotypes within each foliar treatment, BRS ESMERALDA, CMG ERF 221-19, and ERF 85-15 exhibited the highest Zn intake content in the polished grain under the control treatment (6.28, 5.82, and 5.90 g day^{-1}, respectively). With the application of Zn, ERF 85-15 showed a higher Zn intake content in the polished grain (7.61 g day^{-1}) compared to the other genotypes. With the application of Se, BRS ESMERALDA and ERF 85-15 showed a higher Zn intake content in the polished grain (6.51 and 5.89 g day^{-1}, respectively). In the case of the treatment with Zn + Se, except for ERF221-16, the other genotypes exhibited a higher Zn intake content in the polished grain (BRS ESMERALDA - 7.14 g day^{-1}; CMG 2188 - 6.79 g day^{-1}; CMG ERF 221-19 - 6.81 g day^{-1}; ERF 85-15 - 7.17 g day^{-1}). A significant difference was found between foliar treatments and genotypes based on the overall mean. The application of Zn and Zn + Se resulted in a higher overall mean of Zn intake content in the polished grain (6.85 and 6.75 g day^{-1}, respectively). Among the genotypes, BRS ESMERALDA and ERF 85-15 exhibited the highest overall mean (6.66 and 6.64 g day^{-1}, respectively), followed by CMG ERF 221-19 (6.24 g day^{-1}), while CMG 2188 (5.86 g day^{-1}) and CMG ERF 221-16(5.62 g day^{-1}) had the lowest overall means (TABLE 12).

Table 12 – Zinc intake in polished grain (g day^{-1}) according to genotypes and foliar treatments.

Genotypes	General (All areas)									
	Control	Zn		Se		Zn + Se		Mean		
BRS ESMERALDA	6.28	aA	6.71	bA	6.51	aA	7.14	aA	6.66	a
CMG 2188	4.98	bB	6.68	bA	4.98	bB	6.79	aA	5.86	c
CMG ERF 221-16	5.21	bB	6.41	bA	4.97	bB	5.86	bA	5.62	c
CMG ERF 221-19	5.82	aB	6.84	bA	5.51	bB	6.81	aA	6.24	b
CMG ERF 85-15	5.90	aB	7.61	aA	5.89	aB	7.17	aA	6.64	a
Mean	5.64	B	6.85	A	5.57	B	6.75	A		

Capital letters, on the line, compare different foliar treatments. Lowercase letters, in the column, compare different genotypes. Means followed by the same letter do not differ from each other, by the Scott-Knott test at 5% probability.

For Se intake in whole grain, an interaction between foliar treatments and genotypes was detected. Regarding the foliar treatments for each genotype, the application of Se and Zn + Se resulted in a higher content of Se intake in whole grain for the genotypes BRS ESMERALDA (56.58 and 59.98 µg day^{-1}, respectively), CMG 2188 (52.53 and 57.07 µg day^{-1}, respectively), and ERF 85-15 (75.17 and 73.13 µg day^{-1}, respectively). For genotypes CMG ERF 221-16and CMG ERF 221-19, the application of Zn + Se led to a higher content of Se intake in whole grain (67.18 and 100.77 µg day^{-1}, respectively), followed by the Se treatment (54.71 and 67.37 µg day^{-1}, respectively), control (34.53 and 34.25 µg day^{-1}, respectively), and Zn treatment (36.60 and 44.52 µg day^{-1}, respectively). When comparing the genotypes within each foliar treatment, differences were observed only with the application of Se and Zn + Se. CMG ERF 221-19 and ERF 85-15 exhibited the highest content of Se intake in whole grain with the Se treatment (67.37 and 75.17 µg day^{-1}, respectively). In the case of the Zn + Se treatment, CMG ERF 221-19 had the highest content of Se intake in whole grain (100.77 µg day^{-1}), followed by CMG ERF 221-16and ERF 85-15 (67.18 and 73.13 µg day^{-1}, respectively), and finally, with the lowest contents, BRS ESMERALDA (59.98 µg day^{-1}) and CMG 2188 (57.07 µg day^{-1}). According to the overall mean, a significant difference was found between foliar treatments and genotypes. The application of Zn + Se resulted in the highest overall mean of Se intake content in whole grain (71.63 µg day^{-1}), followed by the Se treatment (61.27 µg day^{-1}), control (35.07 µg day^{-1}), and Zn treatment (36.42 µg day^{-1}), with the latter two providing the lowest means. Among the genotypes, CMG ERF 221-19 had the highest overall mean of Se intake content in whole grain (61.73 µg day^{-1}), followed by ERF 85-15 (55.80 µg day^{-1}), BRS ESMERALDA (46.96 µg day^{-1}), CMG 2188 (42.74 µg day^{-1}), and CMG ERF 221-16(48.26 µg day^{-1}), with the last three exhibiting the lowest means (TABLE 13).

Table 13 – Selenium intake in whole grain (µg day^{-1}) according to genotypes and foliar treatments.

Genotypes	General (All areas)									
	Control	Zn		Se		Zn + Se		Mean		
BRS ESMERALDA	34.98	aB	36.30	aB	56.58	bA	59.98	cA	46.96	c
CMG 2188	29.65	aB	31.70	aB	52.53	bA	57.07	cA	42.74	c
CMG ERF 221-16	34.53	aC	36.60	aC	54.71	bB	67.18	bA	48.26	c
CMG ERF 221-19	34.25	aC	44.52	aC	67.37	aB	100.77	aA	61.73	a
CMG ERF 85-15	41.93	aB	32.97	aB	75.17	aA	73.13	bA	55.80	b
Mean	35.07	C	36.42	C	61.27	B	71.63	A		

Capital letters, on the line, compare different foliar treatments. Lowercase letters, in the column, compare different genotypes. Means followed by the same letter do not differ from each other, by the Scott-Knott test at 5% probability.

For Se intake in polished grain, there was an interaction between foliar treatments and genotypes. Regarding the foliar treatments for each genotype, the Se treatment resulted in a higher content of Se intake in polished grain for the BRS ESMERALDA genotype (47.32 µg day^{-1}). For the CMG 2188, CMG ERF 221-19, and ERF 85-15 genotypes, higher contents of Se intake in polished grain were observed with the application of Se and Zn + Se (CMG 2188 - 48.56 and 51.17 µg day^{-1}, respectively; CMG ERF 221-19 - 45.14 and 52.59 µg day^{-1}, respectively; ERF 85-15 - 53.34 and 56.47 µg day^{-1}, respectively). In the case of ERF221-16, the application of Zn + Se resulted in a higher content of Se intake in polished grain (49.47 µg day^{-1}), followed by the Se treatment (39.07 µg day^{-1}), and the control and Zn treatments (28.16 and 27.59 µg day^{-1}, respectively), with the latter two providing the lowest contents. When comparing the genotypes within each foliar treatment, with the control treatment, BRS ESMERALDA exhibited a higher content of Se intake in polished grain compared to the others (37.19 µg day^{-1}). With the Zn treatment, BRS ESMERALDA and CMG 2188 had the highest contents of Se intake in polished grain (38.32 and 35.09 µg day^{-1}, respectively). In the Se treatment, all genotypes showed a higher content of Se intake in polished grain compared to CMG ERF 221-16(BRS ESMERALDA - 47.32 µg day^{-1}; CMG 2188 - 48.56 µg day^{-1}; CMG ERF 221-19 - 45.14 µg day^{-1}; ERF 85-15 - 53.34 µg day^{-1}). With the application of Zn + Se, all genotypes exhibited a higher content of Se intake in polished grain compared to BRS ESMERALDA (CMG 2188 - 51.17 µg day^{-1}; CMG ERF 221-16 - 49.47 µg day^{-1}; CMG ERF 221-19 - 52.59 µg day^{-1}; ERF 85-15 - 56.47 µg day^{-1}). According to the overall mean, a significant difference was found only between foliar treatments, where the application of Se and Zn + Se resulted in higher contents of Se intake in polished grain (46.69 and 49.01 µg day^{-1}, respectively) (TABLE 14).

Table 14 – Selenium intake in polished grain (µg day^{-1}) according to genotypes and foliar treatments.

Genotypes	General (All areas)									
	Control	Zn		Se		Zn + Se		Mean		
BRS ESMERALDA	37.19	aB	38.32	aB	47.32	aA	35.36	bB	39.55	a
CMG 2188	27.47	bB	35.09	aB	48.56	aA	51.17	aA	40.57	a
CMG ERF 221-16	28.16	bC	27.59	bC	39.07	bB	49.47	aA	36.07	a
CMG ERF 221-19	27.43	bB	30.28	bB	45.14	aA	52.59	aA	38.86	a
CMG ERF 85-15	30.65	bB	28.96	bB	53.34	aA	56.47	aA	42.36	a
Mean	30.18	B	32.05	B	46.69	A	49.01	A		

Capital letters, on the line, compare different foliar treatments. Lowercase letters, in the column, compare different genotypes. Means followed by the same letter do not differ from each other, by the Scott-Knott test at 5% probability.

For Zn uptake in whole grain, an interaction between foliar treatments and genotypes was discovered. Regarding the foliar treatments for each genotype, the application of Zn resulted in a higher content of Zn uptake in whole grain for the CMG 2188 genotype (41.82 g ha^{-1} DW). For the CMG ERF 221-16genotype, the application of Zn and Zn + Se resulted in a higher content of Zn uptake in whole grain (41.36 and 32.29 g ha^{-1} DW, respectively). In the case of CMG ERF 221-19, the control, Zn, and Zn + Se treatments provided a higher content of Zn uptake in whole grain (37.37, 38.36, and 44.47 g ha^{-1} DW, respectively). No difference between foliar treatments was observed for the other genotypes. When comparing the genotypes within each foliar treatment, differences were observed only with the application of Zn + Se, where CMG ERF 221-19 and ERF 85-15 exhibited the highest content of Zn uptake in whole grain (44.47 and 43.91 g ha^{-1} DW, respectively). According to the overall mean, a significant difference was found between foliar treatments and genotypes. The application of Zn and Zn + Se resulted in a higher mean content of Zn uptake in whole grain, with 40.02 and 34.41 g ha^{-1} DW, respectively. Among the genotypes, CMG ERF 221-19 and ERF 85-15 had the highest mean content of Zn uptake in whole grain (36.23 and 40.72 g ha^{-1} DW, respectively), followed by CMG ERF 221-16 (29.41 g ha^{-1} DW), CMG 2188 (29.38 g ha^{-1} DW), and BRS ESMERALDA (29.14 g ha^{-1} DW) (TABLE 15).

Table 15 – Zinc uptake in whole grain (g ha^{-1} DW) according to genotypes and foliar treatments.

Genotypes	General (All areas)									
	Control	Zn		Se		Zn + Se		Mean		
BRS ESMERALDA	28.62	aA	32.41	aA	28.80	aA	26.74	bA	29.14	b

CMG 2188	24.19	aB	41.82	aA	26.85	aB	24.65	bB	29.38	b
CMG ERF 221-16	25.37	aB	41.36	aA	18.62	aB	32.29	bA	29.41	b
CMG ERF 221-19	37.37	aA	38.36	aA	24.70	aB	44.47	aA	36.23	a
CMG ERF 85-15	36.20	aA	46.13	aA	36.63	aA	43.91	aA	40.72	a
Mean	30.35	B	40.02	A	27.12	B	34.41	A		

Capital letters, on the line, compare different foliar treatments. Lowercase letters, in the column, compare different genotypes. Means followed by the same letter do not differ from each other, by the Scott-Knott test at 5% probability.

An interaction between foliar treatments and genotypes was observed for Zn uptake in polished grain. Regarding the foliar treatments for each genotype, the application of Zn resulted in a higher content of Zn uptake in polished grain for the CMG 2188 (25.50 g ha^{-1} DW) and CMG ERF 221-16(25.41 g ha^{-1} DW) genotypes. For the CMG ERF 221-19 genotype, the control, Zn, and Zn + Se treatments provided a higher content of Zn uptake in polished grain (22.48, 22.58, and 25.14 g ha^{-1} DW, respectively). No difference between foliar treatments was observed for the other genotypes. When comparing the genotypes within each foliar treatment, differences were observed only with the application of Se and Zn + Se. The BRS ESMERALDA and ERF 85-15 genotypes exhibited the highest content of Zn uptake in polished grain with the application of Se (20.68 and 20.57 g ha^{-1} DW, respectively), and CMG ERF 221-19 and ERF 85-15 had the highest content with the application of Zn + Se (25.14 and 23.65 g ha^{-1} DW, respectively). According to the overall mean, a significant difference was found between foliar treatments and genotypes. The application of Zn resulted in a higher mean content of Zn uptake in polished grain, with 24.04 g ha^{-1} DW. Among the genotypes, CMG ERF 221-19 and ERF 85-15 had the highest mean content of Zn uptake in polished grain (21.24 and 23.17 g ha^{-1} DW, respectively), followed by BRS ESMERALDA (18.48 g ha^{-1} DW), CMG 2188 (17.19 g ha^{-1} DW), and CMG ERF 221-16(17.86 g ha^{-1} DW) (TABLE 16).

Table 16 – Zinc uptake in polished grain (g ha^{-1} DW) according to genotypes and foliar treatments.

Genotypes	General (All areas)									
	Control		Zn		Se		Zn + Se		Mean	
BRS ESMERALDA	17.41	aA	18.93	aA	20.68	aA	16.88	bA	18.48	b
CMG 2188	15.12	aB	25.50	aA	14.21	bB	13.92	bB	17.19	b
CMG ERF 221-16	16.02	aB	25.41	aA	12.17	bB	17.83	bB	17.86	b
CMG ERF 221-19	22.48	aA	22.58	aA	14.75	bB	25.14	aA	21.24	a
CMG ERF 85-15	20.64	aA	27.80	aA	20.57	aA	23.65	aA	23.17	a
Mean	18.34	B	24.04	A	16.48	B	19.49	B		

Capital letters, on the line, compare different foliar treatments. Lowercase letters, in the column, compare different genotypes. Means followed by the same letter do not differ from each other, by the Scott-Knott test at 5% probability.

For Se uptake in whole grain, an interaction between foliar treatments and genotypes was observed. Regarding the foliar treatments for each genotype, the treatments with Se and Zn + Se application resulted in a higher content of Se uptake in whole grain for the BRS ESMERALDA (231.19 and 175.89 mg ha^{-1} DW, respectively) and ERF 85-15 (355.05 and 313.51 mg ha^{-1} DW, respectively) genotypes. For the CMG 2188 genotype, the application of Se provided a higher content of Se uptake in whole grain (235.45 mg ha^{-1} DW). For CMG ERF 221-16 and CMG ERF 221-19, only the Zn + Se treatment yielded a higher content of Se uptake in whole grain (258.99 and 455.43 mg ha^{-1} DW, respectively). When comparing the genotypes within each foliar treatment, differences were observed only with the application of Se and Zn + Se. The ERF 85-15 genotype had the highest content of Se uptake in whole grain with the application of Se (355.05 mg ha^{-1} DW), and CMG ERF 221-19 had the highest content with the application of Zn + Se (455.43 mg ha^{-1} DW), followed by CMG ERF 221-16 (258.99 mg ha^{-1} DW) and ERF 85-15 (313.51 mg ha^{-1} DW). The genotypes BRS ESMERALDA (175.89 mg ha^{-1} DW) and CMG 2188 (164.29 mg ha^{-1} DW) had the lowest contents. According to the overall mean, a significant difference was found between foliar treatments and genotypes. The application of Se and Zn + Se resulted in a higher mean content of Se uptake in whole grain, with 244.56 and 273.62 mg ha^{-1} DW, respectively. Among the genotypes, CMG ERF 221-19 and ERF 85-15 had the highest mean content of Se uptake in whole grain (256.19 and 255.03 mg ha^{-1} DW, respectively), followed by BRS ESMERALDA (165.68 mg ha^{-1} DW), CMG 2188 (168.24 mg ha^{-1} DW), and CMG ERF 221-16(185.53 mg ha^{-1} DW) (TABLE 17).

Table 17 – Selenium uptake in whole grain (mg ha^{-1} DW) according to genotypes and foliar treatments.

Genotypes	General (All areas)									
	Control	Zn		Se		Zn + Se		Mean	
BRS ESMERALDA	121.20	aB	134.45	aB	231.19	bA	175.89	cA	165.68	b
CMG 2188	121.46	aB	151.77	aB	235.45	bA	164.29	cB	168.24	b
CMG ERF 221-16	125.63	aB	181.74	aB	175.76	bB	258.99	bA	185.53	b
CMG ERF 221-19	166.16	aB	177.80	aB	225.36	bB	455.43	aA	256.19	a
CMG ERF 85-15	197.14	aB	154.43	aB	355.05	aA	313.51	bA	255.03	a
Mean	146.32	B	160.04	B	244.56	A	273.62	A		

Capital letters, on the line, compare different foliar treatments. Lowercase letters, in the column, compare different genotypes. Means followed by the same letter do not differ from each other, by the Scott-Knott test at 5% probability.

In terms of Se absorption in polished grains, an interaction between foliar treatments and genotypes was found. Regarding foliar treatments for each genotype, the Se application treatment resulted in a higher Se absorption content in polished grains for the BRS ESMERALDA genotype (155.53 mg ha^{-1} DW). In the CMG 2188 genotype, the application of Zn and Se resulted in a higher Se absorption content in polished grains (145.09 and 147.09 mg ha^{-1} DW, respectively). For the CMG ERF 221-16and CMG ERF 221-19 genotypes, only the Zn + Se treatment led to a higher Se absorption content in polished grains (150.56 and 200.57 mg ha^{-1} DW, respectively). As for the ERF 85-15 genotype, the highest contents were observed with the application of Se (180.03 mg ha-1 DW) and Zn + Se (188.28 mg ha^{-1} DW). When comparing genotypes within each foliar treatment, differences were observed only with the application of Se and Zn + Se. BRS ESMERALDA, CMG 2188, and ERF 85-15 exhibited the highest Se absorption contents in polished grains with the Se application (155.53, 147.09, and 180.03 mg ha^{-1} DW, respectively), while ERF221-16, CMG ERF 221-19, and ERF 85-15 showed the highest contents with the Zn + Se application (150.56, 200.57, and 188.28 mg ha^{-1} DW, respectively). According to the overall mean, a significant difference was found between foliar treatments and genotypes. The application of Se and Zn + Se resulted in a higher mean Se absorption in polished grains, with 139.31 and 139.47 mg ha^{-1} DW, respectively. Among the genotypes, CMG ERF 221-19 and ERF 85-15 exhibited the highest mean Se absorption in polished grains (131.79 and 144.42 mg ha^{-1} DW, respectively), followed by BRS ESMERALDA (111.07 mg ha^{-1} DW), CMG 2188 (111.56 mg ha^{-1} DW), and CMG ERF 221-16 (110.58 mg ha^{-1} DW) (TABLE 18).

Table 18 – Selenium uptake in polished grain (mg ha^{-1} DW) according to genotypes and foliar treatments.

Genotypes	General (All areas)									
	Control		Zn		Se		Zn + Se		Mean	
BRS ESMERALDA	105.97	aB	96.36	aB	155.53	aA	86.44	bB	111.07	b
CMG 2188	82.52	aB	145.09	aA	147.09	aA	71.52	bB	111.56	b
CMG ERF 221-16	85.43	aB	112.76	aB	93.58	bB	150.56	aA	110.58	b
CMG ERF 221-19	107.46	aB	98.80	aB	120.33	bB	200.57	aA	131.79	a
CMG ERF 85-15	102.37	aB	107.02	aB	180.03	aA	188.28	aA	144.42	a
Mean	96.75	B	112.01	B	139.31	A	139.47	A		

Capital letters, on the line, compare different foliar treatments. Lowercase letters, in the column, compare different genotypes. Means followed by the same letter do not differ from each other, by the Scott-Knott test at 5% probability.

Considering free proteins in whole grains, an interaction between foliar treatments and genotypes was observed. Regarding foliar treatments for each genotype, the control, Zn, and Se treatments resulted in a higher content of free proteins in whole grain for the BRS ESMERALDA genotype (5159.92, 6198.34, and 4990.87 µg protein g^{-1} DW, respectively). For the CMG ERF 221-16 genotype, Zn + Se treatment led to a higher content of free proteins in whole grain (5707.31 µg protein g^{-1} DW). As for ERF 85-15, Se and Zn + Se treatments resulted in a higher content of free proteins in whole grain (6979.17 and 7462.16 µg protein g^{-1} DW, respectively). No differences between foliar treatments were observed for the other genotypes. When comparing genotypes within each foliar treatment, with the control treatment, CMG 2188 exhibited a higher content of free proteins in whole grain compared to the others (6858.43 µg protein g^{-1} DW). With foliar application of Zn, BRS ESMERALDA and CMG 2188 showed a higher content of free proteins in whole grain (6198.34 and 7679.51 µg protein g^{-1} DW, respectively). For the Se foliar treatment, a higher content of free proteins in whole grain was observed in the genotypes CMG 2188 (7309.22 µg protein g^{-1} DW), CMG ERF 221-19 (5731.46 µg protein g^{-1} DW), and ERF 85-15 (6979.17 µg protein g^{-1} DW). For the Zn + Se treatment, all genotypes exhibited a higher content of free proteins in whole grain compared to BRS ESMERALDA (CMG 2188 - 6644.69 µg protein g^{-1} DW; CMG ERF 221-16 - 5707.31 µg protein g^{-1} DW; CMG ERF 221-19 - 5602.66 µg protein g^{-1} DW; and ERF 85-15 - 7462.16 µg protein g^{-1} DW). According to the overall mean, a significant difference was found only among genotypes, where CMG 2188 had the highest mean (7122.96 µg protein g^{-1} DW), followed by ERF 85-15 (6192.19 µg protein g^{-1} DW), BRS ESMERALDA (4916.41 µg protein g^{-1} DW), CMG ERF 221-16(4266.29 µg protein g^{-1} DW), and CMG ERF 221-19 (5236.39 µg protein g^{-1} DW), with the latter three genotypes exhibiting the lowest means (TABLE 19).

Table 19 – Free proteins in whole grain (ug protein g^{-1} DW) according to genotypes and foliar treatments.

Genotypes	General (All areas)									
	Control		Zn		Se		Zn + Se		Mean	
BRS ESMERALDA	5159.92	bA	6198.34	aA	4990.87	bA	3316.52	bB	4916.41	c
CMG 2188	6858.43	aA	7679.51	aA	7309.22	aA	6644.69	aA	7122.96	a
CMG ERF 221-16	3726.64	bB	4338.84	bB	3292.37	bB	5707.31	aA	4266.29	c

CMG ERF 221-19	4709.13	bA	4902.33	bA	5731.46	aA	5602.66	aA	5236.39	c
CMG ERF 85-15	5015.02	bB	5312.41	bB	6979.17	aA	7462.16	aA	6192.19	b
Mean	5093.83	A	5686.29	A	5660.62	A	5746.67	A		

Capital letters, on the line, compare different foliar treatments. Lowercase letters, in the column, compare different genotypes. Means followed by the same letter do not differ from each other, by the Scott-Knott test at 5% probability.

Significant differences were found only among genotypes regarding the content of free proteins in polished grain. CMG ERF 221-19 and ERF 85-15 exhibited a significantly higher content of free proteins in polished grain compared to the other genotypes under the control treatment (6504.24 and 5377.26 µg protein g^{-1} DW, respectively) and Zn treatment (6423.74 and 5731.46 µg protein g^{-1} DW, respectively). In the Se treatment, BRS ESMERALDA (5312.87 µg protein g^{-1} DW), CMG ERF 221-19 (5586.56 µg protein g^{-1} DW), and ERF 85-15 (6327.14 µg protein g^{-1} DW) displayed a significantly higher content of free proteins in polished grain compared to the other genotypes. No significant differences were observed among genotypes in the Zn + Se treatment. According to the overall mean, a significant difference was found only among genotypes, with CMG ERF 221-19 and ERF 85-15 exhibiting a higher content of free proteins in polished grain with the applied foliar treatments (6007.16 and 5697.24 µg protein g^{-1} DW, respectively), followed by BRS ESMERALDA (4618.57 µg protein g^{-1} DW), and finally, CMG 2188 (3306.71 µg protein g^{-1} DW) and CMG ERF 221-16(3634.48 µg protein g^{-1} DW), which had the lowest means. No significant differences were observed in the foliar treatments or the interaction between genotypes and foliar treatments (TABLE 20).

Table 20 – Free proteins in polished grain (ug protein g^{-1} DW) according to genotypes and foliar treatments.

Genotypes	General (All areas)									
	Control		Zn		Se		Zn + Se		Mean	
BRS ESMERALDA	3719.01	bA	4306.64	bA	5312.87	aA	5135.77	aA	4618.57	b
CMG 2188	3380.91	bA	3533.86	bA	2253.94	bA	4058.13	aA	3306.71	c
CMG ERF 221-16	3171.62	bA	3614.36	bA	3976.60	bA	3775.35	aA	3634.48	c
CMG ERF 221-19	6504.24	aA	6423.74	aA	5586.56	aA	5514.11	aA	6007.16	a
CMG ERF 85-15	5377.26	aA	5731.46	aA	6327.14	aA	5353.11	aA	5697.24	a
Mean	4430.61	A	4722.01	A	4691.42	A	4767.30	A		

Capital letters, on the line, compare different foliar treatments. Lowercase letters, in the column, compare different genotypes. Means followed by the same letter do not differ from each other, by the Scott-Knott test at 5% probability.

Regarding the content of free amino acids in whole grain, the only significant difference was found among genotypes, specifically in the control treatment, where CMG ERF 221-19 and ERF 85-15 exhibited a higher content of free amino acids in whole grain compared to the other genotypes (374.49 and 343.87 µmol amino acid g^{-1} DW). According to the overall mean, a significant difference was found only among genotypes, with CMG ERF 221-19 and ERF 85-15 showing a higher content of free amino acids in whole grain with the applied foliar treatments, measuring 342.11 and 312.03 µmol amino acid g^{-1} DW, respectively. No significant differences were observed in the foliar treatments or the interaction between genotypes and foliar treatments (TABLE 21).

Table 21 – Free amino acids in whole grain (µmol amino acid g^{-1} DW) according to genotypes and foliar treatments.

Genotypes	General (All areas)									
	Control	Zn		Se		Zn + Se		Mean		
BRS ESMERALDA	248.04	bA	253.80	aA	229.31	aA	217.60	aA	237.19	b
CMG 2188	258.31	bA	302.08	aA	286.41	aA	264.37	aA	277.79	b
CMG ERF 221-16	233.03	bA	289.11	aA	308.20	aA	317.57	aA	286.98	b
CMG ERF 221-19	374.49	aA	268.93	aA	383.68	aA	341.35	aA	342.11	a
CMG ERF 85-15	343.87	aA	284.61	aA	354.14	aA	265.51	aA	312.03	a
Mean	291.55	A	279.71	A	312.35	A	281.28	A		

Capital letters, on the line, compare different foliar treatments. Lowercase letters, in the column, compare different genotypes. Means followed by the same letter do not differ from each other, by the Scott-Knott test at 5% probability.

For the content of free amino acids in polished grain, an interaction between foliar treatments and genotypes was found. Regarding the foliar treatments for each genotype, the Zn + Se treatment resulted in a higher content of free amino acids in polished grain for the CMG 2188 genotype (340.41 µmol amino acid g^{-1} DW). For the ERF 85-15 genotype, in addition to the Zn + Se treatment (420.96 µmol amino acid g^{-1} DW), the application of Se also led to a higher content of free amino acids in polished grain (409.98 µmol amino acid g^{-1} DW). No differences between the foliar treatments were observed for the other genotypes. When comparing the genotypes, no differences were observed among genotypes with the control and Zn treatments. However, with the application of Se, the ERF 85-15 genotype exhibited a higher content of free amino acids in polished grain (409.98 µmol amino acid g^{-1} DW). With the application of Zn + Se, the CMG 2188 (340.41 µmol amino acid g^{-1} DW) and ERF 85-15 (420.96 µmol amino acid g^{-1} DW) genotypes showed a higher content of free amino acids in polished grain. According to the overall mean, significant differences were

found among the foliar treatments and the genotypes. The application of Se and Zn + Se resulted in a higher mean content of free amino acids in polished grain, measuring 239.86 and 261.14 µmol amino acid g^{-1} DW, respectively. Among the genotypes, ERF 85-15 exhibited the highest mean (313.02 µmol amino acid g^{-1} DW), followed by CMG 2188 (235.37 µmol amino acid g^{-1} DW), CMG ERF 221-16(195.89 µmol amino acid g^{-1} DW), CMG ERF 221-19 (199.90 µmol amino acid g^{-1} DW), and finally, BRS ESMERALDA, which had the lowest mean (141.53 µmol amino acid g^{-1} DW) (TABLE 22).

Table 22 – Free amino acids in polished grain (µmol amino acid g^{-1} DW) according to genotypes and foliar treatments.

Genotypes	General (All areas)									
	Control		Zn		Se		Zn + Se		Mean	
BRS ESMERALDA	123.17	aA	140.50	aA	144.64	bA	157.79	bA	141.53	c
CMG 2188	192.02	aB	188.60	aB	220.48	bB	340.41	aA	235.37	b
CMG ERF 221-16	217.06	aA	184.63	aA	225.16	bA	156.71	bA	195.89	b
CMG ERF 221-19	196.88	aA	173.83	aA	199.04	bA	229.85	bA	199.90	b
CMG ERF 85-15	176.89	aB	244.26	aB	409.98	aA	420.96	aA	313.02	a
Mean	181.20	B	186.36	B	239.86	A	261.14	A		

Capital letters, on the line, compare different foliar treatments. Lowercase letters, in the column, compare different genotypes. Means followed by the same letter do not differ from each other, by the Scott-Knott test at 5% probability.

4 DISCUSSION

This study focused on assessing the stability of genotype *x* foliar treatment results, using the experimental areas as a source of performance validation. A significant interaction between genotypes and foliar treatments was observed in all traits, except for the whole-grain hulling yield trait. This indicates that the behavior of genotypes varied across different foliar treatments, possibly due to phenotypic differences among the evaluated genotypes. This finding is of great relevance to plant breeding.

The hulling and milling characteristics of the grains were similar to the values found in other related studies (CRUSCIOL et al., 1999; FÉLIX et al., 2023). Regardless of the applied treatment, two genotypes showed the best hulling efficiency for polished grains, while another two genotypes exhibited the best milling efficiency for the grains. This demonstrates the existence of genotypic variability in the industrial processing efficiency of the grains. The application of Se and Se + Zn resulted in a reduction in grain yield for two genotypes in the case of Se application alone and one genotype in the combined application (Se + Zn). The response of rice to foliar application of Se can vary depending on the soil's Se availability, the

genetic characteristics of each rice variety, and specific agricultural practices. Scientific studies indicate that excessive use of Se in foliar application can lead to a decrease in plant yield. This can occur due to various factors, including nutritional imbalance, Se-related foliar toxicity, and complex interactions with other nutrients (CARDOSO et al., 2022; GUI et al., 2022; KOLBERT et al., 2019).

A study conducted with soybeans showed that the exclusive application of Se, at all tested doses, resulted in a reduction in grain yield, likely due to phytotoxicity and decreased leaf area. However, the mentioned study used four doses of Se (0,5; 1,0; 1,5; 2,0 kg ha^{-1}) (MARTINEZ et al., 2009). Additionally, a study conducted with rice cultivation revealed that soil application of Se, at doses ranging from 12 to 120 g Se ha^{-1}, was effective in translocating Se to the grains. However, no significant effects were observed on grain yield (LESSA et al., 2019). Another study on rice evaluated the application of urea enriched with Se via soil (80 g ha^{-1}), and no difference in grain yield was observed (FÉLIX et al., 2023). A foliar application of 30 g Se ha^{-1} in a Chinese rice variety, using different forms (organic and inorganic) such as selenite, fermented Se, and potassium selenocyanate, did not show a significant alteration in grain yield (YUAN et al., 2023). Another study conducted with a Chinese variety evaluated the foliar application of 75 g Se ha^{-1} using selenate and selenite sources, regardless of the growth stage at which the treatments were applied. The results of this study revealed a significant increase of 5.1% in rice grain yield (DENG et al., 2017).

The application of Zn in conjunction with Se alleviated the negative effects on grain yield in one of the genotypes. Zn plays essential roles in various physiological activities in plants, including the activation of enzymes, repair of damage to Photosystem II, and participation in DNA transcription (HÄNSCH; MENDEL, 2009; PALMER; GUERINOT, 2009). It is also reported that Zn promotes increased tolerance to various stresses such as drought, salinity, heavy metal stress, and biotic stresses (AL-ZAHRANI et al., 2021; HUSSEIN; ABOU-BAKER, 2018; MORKUNAS et al., 2018; UL HASSAN et al., 2017; UMAIR HASSAN et al., 2020).

The foliar application of the tested doses of Se and Zn in this study demonstrated effectiveness in increasing the content and accumulation of both whole grain and polished grains in all evaluated genotypes. However, overall, the content and accumulation of Zn and Se were higher in whole grains than in polished grains. The performance of these variables in grains was influenced by industrial processing, type of foliar application, and genotype. The combination of Zn + Se only favored the Se content. The dose of Se used in this study was

previously evaluated and resulted in similar Se content in polished grains (LESSA et al., 2020). Similar concentrations of Se were also found in previous studies on whole and polished rice grains. For rates of 1000 L ha^{-1} with 0.8% Zn, 1250 L ha^{-1} with 0.2% Zn, and 500-600 L ha^{-1} with 0.2% Zn applied during the tillering + heading, panicle initiation + flowering, and booting + milking growth stages, respectively (MABESA et al., 2013; PHATTARAKUL et al., 2012; WEI et al., 2012).

The main storage site of Zn in cereals is the protein storage vacuole of the embryo and the aleurone layer, where this mineral is stored along with phytate (BRINCH-PEDERSEN et al., 2007). A study observed that whole grains also showed a higher capacity to retain Zn compared to polished grains (BOONCHUAY et al., 2013). Additionally, in another study, there was a loss of 25 to 30% of Zn during the grain polishing process (RAO et al., 2020).

For Se, a study conducted on rice revealed that, in general, this element is present in all regions of rice grains, including the hull, aleurone layer, and endosperm, regardless of the method of Se application. This indicates a higher Se content in the endosperm, particularly near the edge of the polished grain. The central region of the grain, as well as the embryo, showed the lowest Se levels (LESSA et al., 2020). It was reported that there was a lower accumulation of Se in the endosperm of rice grains (which is the predominant part of the polished grain) when applying a dose of 1 mg Se kg^{-1} of soil, compared to other parts (FAROOQ; ZHU, 2019).

Therefore, during the polishing process, there is a loss of structures with high levels of Se present in the whole grains, resulting in average Se content reduction. These differences are associated with the role of Zn and Se in protein synthesis. In the case of Se, high accumulation in Se-sensitive plant species has been associated with the non-specific substitution of S-containing amino acids by their corresponding Se analogs. Consequently, Se-sensitive plants are characterized by higher levels of Se in proteins compared to Se-tolerant plants, where there is a restriction in the incorporation of this element into proteins. Additionally, it is important to note that the non-protein amino acids methylselenocysteine and selenocystathionine, which contain Se, are rarely found in Se-sensitive plant species (BROWN; SHRIFT, 1982). Zinc plays a central role in determining protein structure and catalytic function since it is a highly active Lewis acid that lacks redox activity under environmental and cellular conditions. This nutrient is involved in approximately 10% of protein functions in most eukaryotic proteomes (STANTON et al., 2022).

These aforementioned factors also explain the effect of foliar treatments on proteins and amino acids in the studied genotypes. Generally, a higher capacity to accumulate Zn and Se was associated with a higher content of proteins and amino acids. However, based on the presented results, it can be inferred that the studied genotypes exhibited different capacities to accumulate these compounds in their grain structures. This explains the variations observed in the results of foliar applications concerning genotypes and industrial grain processing.

For Zn, some genotypes showed a negative response in Zn accumulation in processed grains when the foliar application was combined with Se, compared to the isolated application of Zn. This resulted in an overall decrease in the Zn accumulation response in the Zn + Se treatment. The presence of Se in plants results in competition among cations. Several studies have demonstrated that the addition of Se has a significant impact on the absorption and translocation of metal cations such as Fe, Cu, Hg, and Cd (ISMAEL et al., 2019; ZHOU et al., 2020). Due to chemical or physical similarities, these cations compete with Zn for absorption at transporters and biotic binding sites. This can result in a reduction in Zn uptake when these cations are present simultaneously (BARWINSKA-SENDRA; WALDRON, 2017; HUANG et al., 2020; JANCSÓ et al., 2013; TIONG et al., 2015).

In the case of Se accumulation, both positive and negative effects were observed in processed grains with foliar application in conjunction with Zn. Zn can also have a positive effect on Se accumulation in plants. For example, Se acts as an enhancer of antioxidant activity through the biosynthesis of antioxidant enzymes such as superoxide dismutase, where Zn serves as a cofactor. Thus, the increase in Zn indirectly influences the increase in Se, and vice versa (FENG; WEI; TU, 2013; GEORGIADOU et al., 2018; SEPPÄNEN; TURAKAINEN; HARTIKAINEN, 2003; ULHASSAN et al., 2019; WU et al., 2020). In a conducted study, the combined application of zinc sulfate, sodium selenite, and ferrous sulfate was evaluated, and no interaction effect between Zn and Fe on Se content was observed (FANG et al., 2008). However, it is important to note that a different source of Se was used. In another study conducted in a greenhouse environment with rice, it was observed that at specific doses, soil application of Zn can promote an increase in Se content in the grains (EI et al., 2020). Indeed, previous studies have demonstrated antagonism between Se and Zn when foliar-applied or via soil in crops such as peas and wheat. This antagonistic relationship means that an increase in one element's concentration can lead to a decrease in the uptake or availability of the other element. Therefore, careful consideration should be given to the simultaneous application of Se and Zn to avoid potential negative interactions and ensure

optimal nutrient uptake by the plants (GERM et al., 2013; POBLACIONES; RENGEL, 2017).

It is estimated that the minimum recommended intake for adults is 11 mg day^{-1} for Zn and 55 μg day^{-1} for Se (MONSEN, 2000; TRUMBO et al., 2001). In the case of Zn, none of the types of industrial processing, treatments, or genotypes reached the minimum recommendation. However, it is important to note that the values were close to the recommendation. Regarding Se in polished grains, only the genotype CMG ERF 85-15, with the application of Zn + Se, was able to exceed the recommendation. For whole grains, all genotypes in which Se application was performed showed the capacity to exceed the minimum recommended intake. Two genotypes had their performance improved with the combined application of Se and Zn.

5 CONCLUSIONS

The foliar application of Se and Zn at specific dosages 5.22 g Se ha^{-1} and 1.42 kg Zn ha^{-1}, in combination and at different growth stages resulted in an increase in both Se and Zn content in polished and whole-grain rice for all studied genotypes. Based on elements intake, the treatments utilized are recommended for future biofortification programs. Despite the lower consumption, whole grain rice is highly recommended to maximize the benefits of this biofortification approach. Additionally, the results of this study demonstrated that Zn and Se can change the content and the uptake of each other. Also, the combined application of Se and Zn can change the content of protein and amino acids, suggesting that this strategy can be employed to increase the daily intake of Se, Zn, and protein to fight global malnutrition. However, it is crucial to utilize appropriate and efficient genotypes to obtain all the benefits evaluated in this study. Therefore, it can be concluded that there is genetic variability in upland rice to increase the content, accumulation, and response of these elements. The genotypes CMG ERF 221-19, and CMG ERF 85-15 are recommended for foliar biofortification with Zn and Se. These genotypes compared to the others in grains, when exposed to the application of Zn + Se, showed efficient metabolism and only synergistic and positive effects compared to the isolated application, when significant differences were observed. These findings contribute to defining the utility and application of Se and Zn through biofortification, promoting a significant increase in these elements in upland rice cultivation, particularly in soils with low nutrient availability.

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FINAL CONSIDERATIONS

Considering the results obtained in the studies compiled in this document, it can be concluded that biofortification approach shows promising potential to improve food and nutritional security, especially in regions where micronutrient deficiencies are prevalent. Through biofortification, it is possible to increase the content of these essential nutrients in the edible parts of plants, contributing to the combat of nutritional deficiencies and their negative impacts on human health. This strategy can be particularly relevant in low-income communities and developing countries, where access to a diverse and balanced diet is limited. In addition to improving nutrient intake, biofortification can also have a positive impact on agricultural sustainability. By strengthening the nutritional quality of staple crops such as rice, beans, and pak choi, the dependence on artificial food supplements can be reduced, promoting a more integrated and natural food production. However, it is important to emphasize that the effective implementation of biofortification requires a multidisciplinary approach that involves not only agronomic research and genetic improvement but also awareness and education of the population regarding the benefits of biofortification and the importance of a balanced diet. Considering the potential benefits of biofortification in terms of human health, food security, and agricultural sustainability, it is crucial for governments, research institutions, non-governmental organizations, and the private sector to work together to promote and support the implementation of this approach on a large scale. Ultimately, this thesis on the biofortification of Se, Zn, Fe, and protein in crops such as rice, beans, and pak choi highlights the importance of seeking to fill knowledge gaps and proposing innovative and sustainable approaches to improve the nutritional quality of food and address the challenges of malnutrition globally.