UNIVERSIDADE FEDERAL DE LAVRAS

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SELENIUM BIOFORTIFICATION OF SOYBEAN AND ITS EFFECT ON PLANT METABOLISM, STRESS RESISTANCE, AND GRAIN QUALITY

LAVRAS – MG
2023

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Thesis presented to the Federal University of Lavras, as part of the requirements of the Graduate Program in Soil Science area of concentration in Soil Fertility and Plant Nutrition, to obtain the title of Doctor.

Professor Luiz Roberto Guimarães Guilherme, Ph.D.
Advisor

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2023

Ficha catalográfica elaborada pelo Sistema de Geração de Ficha Catalográfica da Biblioteca Universitária da UFLA, com dados informados pelo(a) próprio(a) autor(a).

Silva, Maila Adriely.
Selenium biofortification of soybean and its effect on plant metabolism, stress resistance, and grain quality / Maila Adriely Silva. - 2023.
94 p. : il.

Orientador(a): Luiz Roberto Guimarães Guilherme.

Tese (doutorado) - Universidade Federal de Lavras, 2023.
Bibliografia.

1. Biofortification. 2. Selenium fertilizer. 3. Selenoaminoacids. I. Guilherme, Luiz Roberto Guimarães. II. Título.

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BIOFORTIFICAÇÃO DE SOJA COM SELÊNIO E SEU EFEITO NO METABOLISMO DE PLANTA, RESISTÊNCIA AO ESTRESSE E QUALIDADE DE GRÃOS

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APPROVED, April 06, 2023.

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LAVRAS – MG
2023

To God, who has always blessed me.
To my parents, Silvia and Eli (in memory).
To my sister, Thaisa, my brother, Júnior, my lovely grandmother, Geralda (in memory), and my aunt, Lourdes.
To my amazing and darling loverboy Gustavo
I dedicate!

ACKNOWLEDGMENTS

To God, who allowed me to live in this wonderful world and to be blessed, I am thankful. To my parents, Silvia and Eli, to be my support for my whole life. To Gustavo, my great love, who is always present and sharing with me all the details of this journey. I will be forever thankful. To my siblings, Thaisa and Júnior, for the moments shared. To the cutest little boy I have known, Moisés, I am thankful to make my life happier. My lovely grandmother Geralda for her encouragement. My exemplary aunt Lourdes and her family, especially Rogério and Rosana, for supporting me, encouraging me, and being my example.

To my friend Pedro, who was with me sharing all the moments. To friends at UFLA, especially Paula, Ana Paula Corguinha, Josimar Lessa, Filipe, Mariana, Éverton, Mari, and Fernanda for the shared knowledge and moments of distraction. To the unforgettable friends, I made during my journey abroad, Rita, Ricardo, Matt, Dani, Gustavo Brunetto, Angélica, and others, I am thankful for sharing the good times, expectations, and difficulties with me. To my other friends for their support, even from afar. To ICL South America (especially José Marcos, Guilherme Dinali, and Guilherme Amaral) and Elisana Baldissera, for their help with field experiments.

To Professor Patrick Brown, Douglas Amaral, Brown's Lab team, and UC Davis, for allowing me to live this experience. To researcher Gary Bañuelos and his lab, for the partnership. To the Laboratory of Biochemistry and Molecular Physiology/UFLA, which allowed me to develop part of the analyses. To the undergrad research team, especially Gustavo and Guilherme, who contributed so much to this work. To Professor Guilherme Lopes and Cynthia, for their help with the manuscripts. To Federal University of Lavras and the Department of Soil Science/UFLA, its technicians and staff, in special, Geila, Lívia, Mariene, Aline, José Roberto (Pezão), Roberto, and Alexandre, for their help throughout. To the Federal University of Lavras, for the openness and availability of the physical structure. To the funding agencies CAPES, CNPq, and FAPEMIG, for financial assistance and scholarship. This work was carried out with the support of the Minas Gerais Research Support Foundation (FAPEMIG).

To my advisor, professor Bebeto, for his friendship, for sharing his knowledge, and for encouraging and believing in my ability. Bebeto is an example of professionalism to me and I aspire to be as good as him. I will be eternally thankful.

THANK YOU!

“It always seems impossible until it is done.”

Nelson Mandela

GENERAL ABSTRACT

Selenium (Se) is an important nutrient for humans and animals. It comprises about 25 proteins and assists in thyroid regulation, fighting free radicals, and DNA synthesis. Selenium deficiency is common worldwide and crop biofortification is a strategy that can contribute to alleviating this problem. For plants, Se is not considered a nutrient, but it has beneficial effects. Due to the diversity of products made from soybean, its high protein content, and its great variability in planting, this plant is a good crop to be used in biofortification programs. To better understand Se management strategies for the biofortification of soybean grains with Se and to find out how they affect Se species in grains, three studies were carried out. The first study assessed physiological and agronomic responses of soybean plants exposed to soil-Se applications, using monoammonium phosphate fertilizer as a vehicle. The experiment was carried out in the field, for two crop seasons (2018/2019 and 2019/2020), applying conventional monoammonium phosphate and an enhanced efficiency monoammonium phosphate (fertilizer was coated with the humic and fulvic substances) combined or not with Se, in four soybean genotypes (M5917, 58I60 Lança, TMG7061, and NA5909). Fertilizers containing Se increased soybean yield in genotype TMG7061. Overall, the application of Se associated with conventional monoammonium phosphate increased the amino acid content in the grains and reduced lipid peroxidation of plants. The second experiment evaluated the effect of foliar-applied Se, associated or not with a multi-nutrient fertilizer (N, P, K, Mg, S, and B) in soybean, defining a critical Se threshold in grains to better understand the relationship between Se content and yield. Two experiments were carried out, under field conditions (2018/2019), separately, i.e., one with each soybean genotype (M5917 and 58I60 Lança). Selenium doses were sprayed, combined or not with multi-nutrient fertilizer, at the rates of 0, 10, 40, and 80 Se g ha^{-1}. The Se content in grains increased in both genotypes, according to the doses. The limit of Se in grains from which the yield was reduced is 1.0 mg kg^{-1} and 3.0 mg kg^{-1} in genotypes Lança and M5917, respectively. The third experiment aimed to determine the total Se content and Se speciation in soybean grains produced under different methods of Se application in the field. Treatments consisted of Se application, using organic (acetylselenide) or inorganic (sodium selenate) Se sources, at the rates of 10 g ha^{-1} and 80 g ha^{-1}, in two soybean genotypes. The application of inorganic Se via foliar spray at the dose of 80 g ha^{-1} favored the highest total Se content in the grains. Moreover, the application of 10 g ha^{-1} via foliar resulted in the same Se content in the grains as the soil application of 80 g ha^{-1}. Selenomethionine (SeMet) accounts for more than 80% of the Se found in grains. Finally, the highest levels of SeMet in grains were found when inorganic Se was applied to the soil or the leaves.

KEYWORDS: Sodium selenate. Phosphate fertilizer. Organic selenium. Selenium speciation. Selenoaminoacids.

RESUMO GERAL

O selênio (Se) é um nutriente importante para os humanos e animais. A deficiência de Se ocorre ao redor do mundo e uma estratégia utilizada para reduzir esse problema é a biofortificação de culturas agrícolas. Para as plantas, o Se não é considerado um nutriente, mas apresenta efeitos benéficos. Devido à diversidade de produtos produzidos a partir da soja, ao seu alto teor de proteína e à grande variabilidade de plantio, ela é uma boa cultura para ser utilizada em programas de biofortificação. A fim de entender melhor sobre diferentes estratégias de manejo do Se para biofortificação de grãos de soja e compreender como elas afetam as espécies de Se nos grãos, foram realizados três estudos. O primeiro avaliou respostas fisiológicas e agronômicas das plantas submetidas à aplicação de Se via solo, utilizando como veículo, fertilizantes fosfatados. O estudo foi desenvolvido em condição de campo, durante duas safras, com a aplicação de Se associado ao fosfato monoamônio convencional e ao fosfato monoamônio de eficiência aumentada (fertilizante com substância húmica e fúlvica), em quatro cultivares de soja (M5917, 58I60 Lança, TMG7061 e NA5909). Os fertilizantes contendo Se aumentaram a produtividade da soja na cultivar TMG7061. A aplicação de Se associado ao fosfato monoamônio convencional aumentou os aminoácidos nos grãos e reduziu a peroxidacão lipídica. O segundo estudo avaliou a aplicação foliar de Se associada a um fertilizante multi-nutriente (N, P, K, Mg, S e B) e definiu um limite crítico de Se nos grãos para melhor entender a relação entre o teor de Se e a produtividade. Foram realizados dois experimentos, em condição de campo, sendo um com cada cultivar de soja (M5917 e 58I60 Lança). Doses de Se (0, 10, 40 e 80 g ha^{-1}) foram aplicadas via foliar, associadas ou não com o fertilizante multi-nutriente. Em ambas as cultivares, o teor de Se nos grãos aumentou a dose aplicada. A combinação do Se com o fertilizante multi-nutriente favoreceu o maior teor de Se nos grãos. O limite de Se nos grãos, foi 1.0 mg kg^{-1} e 3.0 mg kg^{-1} nas cultivares Lança e M5817, respectivamente. O terceiro estudo teve como objetivo avaliar o teor de Se total e a especiação de Se em grãos de soja produzidos sob diferentes métodos de aplicação de Se. Os tratamentos consistiram na aplicação de Se (via solo ou foliar), utilizando fonte orgânica (acetil selenido) ou inorgânica (selenato de sódio), nas doses de 10 g ha^{-1} ou 80 g ha^{-1}, em duas cultivares (M5917 e 58I60 Lança). A aplicação de Se inorgânico via foliar, na dose de 80 g ha^{-1} favoreceu o maior teor de Se total nos grãos e a aplicação de 10 g ha^{-1} via foliar se assemelha a aplicação de 80 g ha^{-1} via solo. A especiação química das formas de Se mostra que mais de 80% do Se encontrado nos grãos está na forma de selenometionina. Os maiores teores de selenometionina nos grãos foram encontrados com a aplicação de Se inorgânico, via solo ou via foliar.

PALAVRAS-CHAVE: Selenato de sódio. Fertilizante fosfatado. Selênio orgânico. Especiação de selênio. Seleno aminoácidos.

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FIRST PART

1 GENERAL INTRODUCTION

Humans require more than 22 essential chemical elements for growth and development, which can be obtained through their diet, yet the majority of the world population consumes a nutrient-deficient diet (WHITE; BROADLEY, 2009). It is estimated that more than 60% of people worldwide are deficient in iron (Fe), more than 30% in zinc (Zn) and iodine (I), and over 15% in selenium (Se). Moreover, calcium, magnesium, and copper deficiencies are also known to be widespread in developed countries (RUDE; GRUBER, 2004; WELCH; GRAHAM, 2002; WHITE; BROADLEY, 2009).

Consumption of low nutritional quality food may be attributed to crop growth in areas with low nutrient availability in soils, as this affects the element content in plant tissue. There are alternatives to minimize and reduce nutritional deficiency, including diet diversification, mineral supplementation, consumption of fortified foods, and crop biofortification (WHITE; BROADLEY, 2005, 2009). Biofortification is a process that aims to increase the content of minerals and vitamins in food through genetic and agronomic strategies (CAKMAK, 2008). In addition, biofortification is a form of solving problems related to food security of the population (SHIKUKU et al., 2019).

Genetic biofortification is performed via plant breeding whereas agronomic biofortification is made by fertilizing crops with supplementary doses of chemical elements necessary in human/animal diets. In soils with low contents of a target element, both approaches might be necessary, i.e., it is recommended to apply the fertilizer and cultivate a genotype capable of absorbing and accumulating that element in its tissues, preferably in the edible part (CAKMAK, 2008). Thus, using species with greater accumulative capacity in association with the application of mineral fertilizers is the adequate strategy for increasing not only the nutritional content of plants but also their yield in low-fertility soils (GRAHAM et al., 2007; PFEIFFER; MCCLAFFERTY, 2007). In summary, agronomic biofortification is required for maximizing the success of genetic biofortification and both should be used simultaneously.

Among several chemical elements targeted in biofortification programs, Se is definitely one that stands out. The recommended daily intake of this element is 60 $\mu g\ day^{-1}$ and 70 $\mu g\ day^{-1}$ for women and men adults, respectively (KIPP et al., 2015). Approximately 25 proteins of the human body have Se as a component (REEVES; HOFFMANN, 2009). It also aids in the regulation of thyroid hormone, DNA synthesis, and fighting free radicals (PEDRERO; MADRID, 2009; REEVES; HOFFMANN, 2009). Selenium deficiency in the body has been linked to a number of diseases, including neurological disorders, cancer, and heart disease (CARDOSO et al., 2015; COMBS, 2001; VINCETI et al., 2018).

For plants, Se is not considered a nutrient. Its effects on plants have been studied for over 70 years, but its essentiality has not yet been proven (LYONS et al., 2009). Despite this, a change in the concept of plant nutrients has recently been proposed, considering the inclusion of some chemical elements in this category, including Se (BROWN; ZHAO; DOBERMANN, 2022). Indeed, these authors discuss the importance of this element and propose a new definition as a starting point for future discussions. Several studies have reported the positive effect of Se application on tolerance to abiotic stresses, including the presence of heavy metals, excess cold, salinity, water deficit, and high temperatures (ANDRADE et al., 2018; FENG; WEI; TU, 2013; HASANUZZAMAN et al., 2022; LANZA; REIS, 2021; RAVELLO et al., 2021; SOUSA et al., 2022).

Selenium plays an important role in controlling stress and reducing reactive oxygen species (ROS). Reactive oxygen species are molecules naturally produced by plants, but they can increase when the plant is under stress conditions (FENG; WEI; TU, 2013). In general, two types of antioxidants are produced by plants to combat ROS, enzymatic and non-enzymatic (ASADA, 2006; BARBOSA et al., 2014; DINAKAR; DJILIANOV; BARTELS, 2012). Selenium can act as an antioxidant or as an inducer of antioxidative potential in plants (DJANAGUIRAMAN et al., 2005; DJANAGUIRAMAN; PRASAD; SEPPANEN, 2010; KELING et al., 2013). In the presence of Se, hydrogen peroxide (H_2O_2) is initially eliminated by glutathione peroxidase (GSH-Px) (TAKEDA; ISHIKAWA; SHIGEOKA, 1997). Although the superoxide dismutase (SOD) enzyme is not a selenoenzyme, this element can increase its gene expression and hence its concentration in the plant (JIANG et al., 2017).

The fertilization of soybean plants with Se (sodium selenate) during the pod-filling stage increased the Se content in grains and enhanced antioxidant enzyme activity (superoxide dismutase and glutathione peroxidase) (DJANAGUIRAMAN et al., 2005). The foliar application of 200 g ha^{-1} of Se (sodium selenite) in soybean increased the Se content by 20 times in the grains and 17 times in the protein (YANG et al., 2003). In soybean sprouts, biofortification with Se (sodium selenite) increased the concentration of carotenoids and isoflavonoids (LAZO-VÉLEZ et al., 2018). In addition, in a soybean protein isolate, Se acted to maintain the conformation of β-conglycinin (7S globulins) under AAPH-induced oxidative stress (ZHAO et al., 2019).

In the plant, uptake, translocation, and redistribution of Se vary according to plant species, physiological condition, the activity of membrane transporters, time of application, source of selenium, soil pH, redox potential, mineralogy, organic matter, and the presence of ions competing in soil solution (EL-RAMADY et al., 2014; LESSA et al., 2016; LI et al., 2017; LONGCHAMP et al., 2015; RENKEMA et al., 2012; TOLU et al., 2014). Analyzing selenate adsorption in tropical soils, Araujo et al. (2018) found a negative correlation between Se availability and clay content. In cultivated soils, Se sorption is lower compared to non-cultivated soils. This fact is justified by soil management and the application of fertilizers, especially those containing phosphorus and sulfur (LESSA et al., 2016).

Another important factor to be considered is the method of application. Selenium fertilizers are typically applied through foliar and/or soil applications. Foliar applications provide the benefit of improved use efficiency since the element can be absorbed directly through the leaves. In addition, foliar applications can be favorable by exploiting synergistic effects between different elements (BINDRABAN et al., 2015). For instance, the simultaneous foliar application of Zn, I, and Se increased the concentration of these elements in wheat grains (PROM-U-THAI et al., 2020; ZOU et al., 2019).

Plants can uptake Se as selenate, selenite, and organic Se (WHITE, 2018). Due to their chemical similarity, Se competes with S. In plants, it enters via sulfate or phosphate transporters present in the plasma membrane (LI; MCGRATH; ZHAO, 2008; SORS; ELLIS; SALT, 2005). In the form of selenate, Se is absorbed through sulfate transporters, while occurring as selenite, it is absorbed through phosphate transporters (LI; MCGRATH; ZHAO, 2008; ZHANG et al., 2003).

When Se is absorbed in the form of selenate, it is carried to the chloroplasts and is processed via the sulfur route. In chloroplasts, it is activated by ATP sulfurylase to form adenosine-5-phosphoselenate. Subsequently, this compound is reduced to selenite by the enzyme adenosine-5-phosphosulfate reductase and for this reaction to occur, reduced glutathione (GSH) is used as an electron donor. Selenite is further reduced to selenocysteine (SeCys) via enzymatic or non-enzymatic process (NG; ANDERSON, 1978). Both are incorporated into different selenoproteins and further transformation into other organic species is possible, which includes selenocysteine (SeCys), methyl selenomethionine (MeSeMet), methyl selenocysteine (SeMeCys) selenomethionine (SeMet), as well as elemental Se (Se^0) (LIMA; PILON-SMITS; SCHIAVON, 2018; PILON-SMITS; WINKEL; LIN, 2017). Furthermore, Se can be accumulated in plants in methylated forms or be volatilized (HAWKESFORD; ZHAO, 2007).

Overall, non-selenium-accumulating crops tend to accumulate most of the Se as organic Se compounds (e.g., SeMet, MeSeCys, and SeCys), with the remainder being inorganic forms (e.g., selenate and selenite) (HAUG et al., 2007). In cereal grains, for example, selenomethionine is the most abundant selenocompound, while MeSeCys is the major selenocompound in Se-enriched Cruciferae plants (i.e., garlic, onions, and broccoli) (BAÑUELOS; FREEMAN; ARROYO, 2022; HAUG et al., 2007). After applying Se via sodium selenite, selenospecies such as SeCys and SeMet accounted for approximately 74% of the total Se in soybeans (CHAN; AFTON; CARUSO, 2010).

The variability in genotype properties has an influence on Se content and chemical forms in edible parts of food (ŠINDELÁŘOVÁ et al., 2015; ZHANG et al., 2019). In addition to morphological attributes, element absorption efficiency is influenced by kinetic parameters (i.e., influx - I, maximum uptake rate - V_{max}, minimum concentration – C_{min}, and Michaelis-Menten constant - K_m) (MARTINEZ et al., 2015; YANG et al., 2007). The values of these kinetic parameters may vary according to the age and concentration of the nutrient in the plants, plant morphology, climate changes, and, mainly, between genotypes and plant species (BASSIRIRAD, 2000; PAULA et al., 2018; SACRAMENTO; ROSOLEM, 1997; WARNCKE; BARBER, 1974). Selenium biofortification attempts may take benefit from this variation among plant genotypes, selecting those with greater Se levels in edible parts. In this perspective, soybean has been proposed to be a potential alternative to providing biofortified cereal grains given its elevated protein content and therefore greater tendency to form organic Se forms (HU et al., 2014). In addition, soybean has high geographic variability of production, and many products are made from its grains.

As a result, studies are required to assess and complement information concerning the beneficial effect of Se on plant metabolism, stress reduction, and yield of soybean. Furthermore, field trials are needed for a better understanding of the role of different Se application forms on the quality of the soybean grains produced, as well as on how it affects the Se forms in grains.

2 GENERAL OBJECTIVES

- Assessing the efficiency of Se application via foliar and soil for biofortifying soybean grains with Se;
- Assessing the application of organic Se fertilizer in soybean grains;

- Assessing the use of conventional phosphate fertilizer + Se and enhanced-efficiency phosphate fertilizer + Se as vehicles to increase the Se content in soybean grains;
- Assessing the suitability of Se application associated with a multi-nutrient fertilizer (MNF) in soybean plants for biofortifying grains with Se;
- Assessing the effect of different vehicles for applying Se in soybean plants on the content of Se species in soybean grains.
- Defining Se critical threshold in soybean genotypes;
- Determining the recommended Se application dosage and method for the soybean crop under tropical conditions.

3 GENERAL HYPOTHESES

- Selenium application via foliar is more effective than soil application for biofortifying soybean grains with Se;
- The application of organic Se fertilizer favors a higher concentration of organic Se in soybean grains;
- Enhanced-efficiency monoammonium phosphate + Se (E-MAP + Se) is more effective for biofortifying soybean grains with Se when applied via soil than conventional monoammonium phosphate + Se;
- Selenium application associated with a multi-nutrient fertilizer (MNF) increases the Se content in soybean grains;
- The Se critical threshold is the same for soybean genotypes;
- Different vehicles of applying Se in soybean plant affects the content of Se species in soybean grains.

REFERENCES

ANDRADE, F. R. et al. Selenium protects rice plants from water deficit stress. **Ecotoxicology and Environmental Safety**, v. 164, p. 562–570, nov. 2018.

ARAUJO, A. M. et al. Soil management and ionic strength on selenite retention in oxidic soils. **Ciência e Agrotecnologia**, v. 42, n. 4, p. 395–407, aug. 2018.

ASADA, K. Production and scavenging of reactive oxygen species in chloroplasts and their functions. **Plant Physiology**, v. 141, n. 2, p. 391–396, 1 jun. 2006.

BAÑUELOS, G. S.; FREEMAN, J. L.; ARROYO, I. S. Selenium content and speciation differences in selenium enriched soups made from selenium biofortified plants. **Journal of Food Composition and Analysis**, v. 105, p. 104255, jan. 2022.

BARBOSA, M. R. et al. Geração e desintoxicação enzimática de espécies reativas de oxigênio em plantas. **Ciência Rural**, v. 44, n. 3, p. 453–460, mar. 2014.

BASSIRIRAD, H. Kinetics of nutrient uptake by roots: responses to global change: REVIEW Nutrient uptake kinetics and global change. **New Phytologist**, v. 147, n. 1, p. 155–169, jul. 2000.

BINDRABAN, P. S. et al. Revisiting fertilisers and fertilisation strategies for improved nutrient uptake by plants. **Biology and Fertility of Soils**, v. 51, n. 8, p. 897–911, nov. 2015.

BROWN, P. H.; ZHAO, F.-J.; DOBERMANN, A. What is a plant nutrient? Changing definitions to advance science and innovation in plant nutrition. **Plant and Soil**, v. 476, n. 1–2, p. 11–23, jul. 2022.

CAKMAK, I. Enrichment of cereal grains with zinc: Agronomic or genetic biofortification? **Plant and Soil**, v. 302, n. 1–2, p. 1–17, jan. 2008.

CARDOSO, B. R. et al. Selenium, selenoproteins and neurodegenerative diseases. **Metallomics**, v. 7, n. 8, p. 1213–1228, 2015.

CHAN, Q.; AFTON, S. E.; CARUSO, J. A. Selenium speciation profiles in selenite-enriched soybean (*Glycine max*) by HPLC-ICPMS and ESI-ITMS. **Metallomics**, v. 2, n. 2, p. 147–153, 2010.

COMBS, G. F. Selenium in global food systems. **British Journal of Nutrition**, v. 85, n. 5, p. 517–547, may, 2001.

DINAKAR, C.; DJILIANOV, D.; BARTELS, D. Photosynthesis in desiccation tolerant plants: energy metabolism and antioxidative stress defense. **Plant Science**, v. 182, p. 29–41, jan. 2012.

DJANAGUIRAMAN, M. et al. Selenium – an antioxidative protectant in soybean during senescence. **Plant and Soil**, v. 272, n. 1–2, p. 77–86, may, 2005.

DJANAGUIRAMAN, M.; PRASAD, P. V. V.; SEPPANEN, M. Selenium protects sorghum leaves from oxidative damage under high temperature stress by enhancing antioxidant defense system. **Plant Physiology and Biochemistry**, v. 48, n. 12, p. 999–1007, dec. 2010.

EL-RAMADY, H. R. et al. Selenium and nano-selenium in agroecosystems. **Environmental Chemistry Letters**, v. 12, n. 4, p. 495–510, dec. 2014.

FENG, R.; WEI, C.; TU, S. The roles of selenium in protecting plants against abiotic stresses. **Environmental and Experimental Botany**, v. 87, p. 58–68, mar. 2013.

GRAHAM, R. D. et al. Nutritious subsistence food systems. **Advances in Agronomy,** v. 92, p. 1–74, 2007.

HASANUZZAMAN, M. et al. Selenium supplementation and crop plant tolerance to metal/metalloid toxicity. **Frontiers in Plant Science**, v. 12, p. 792770, 3 jan. 2022.

HAUG, A. et al. How to use the world's scarce selenium resources efficiently to increase the selenium concentration in food. **Microbial Ecology in Health and Disease**, v. 19, n. 4, p. 209–228, jan. 2007.

HAWKESFORD, M. J.; ZHAO, F.-J. Strategies for increasing the selenium content of wheat. **Journal of Cereal Science**, v. 46, n. 3, p. 282–292, nov. 2007.

HU, J. et al. Antioxidant activities of Se-SPI produced from soybean as accumulation and biotransformation reactor of natural selenium. **Food Chemistry**, v. 146, p. 531–537, mar. 2014.

JIANG, C. et al. Effect of exogenous selenium supply on photosynthesis, Na+ accumulation and antioxidative capacity of maize (*Zea mays* L.) under salinity stress. **Scientific Reports**, v. 7, n. 1, p. 42039, 7 feb. 2017.

KELING, H. et al. Influence of selenium on growth, lipid peroxidation and antioxidative enzyme activity in melon (*Cucumis melo* L.) seedlings under salt stress. **Acta Societatis Botanicorum Poloniae**, v. 82, n. 3, p. 193–197, 2013.

KIPP, A. P. et al. Revised reference values for selenium intake. **Journal of Trace Elements in Medicine and Biology**, v. 32, p. 195–199, oct. 2015.

LANZA, M. G. D. B.; REIS, A. R. DOS. Roles of selenium in mineral plant nutrition: ROS scavenging responses against abiotic stresses. **Plant Physiology and Biochemistry**, v. 164, p. 27–43, jul. 2021.

LAZO-VÉLEZ, M. A. et al. Effect of germination with sodium selenite on the isoflavones and cellular antioxidant activity of soybean (*Glycine max*). **LWT**, v. 93, p. 64–70, jul. 2018.

LESSA, J. H. L. et al. Adsorption-desorption reactions of selenium (VI) in tropical cultivated and uncultivated soils under Cerrado biome. **Chemosphere**, v. 164, p. 271–277, dec. 2016.

LI, H.; MCGRATH, S. P.; ZHAO, F. Selenium uptake, translocation and speciation in wheat supplied with selenate or selenite. **New Phytologist**, v. 178, n. 1, p. 92–102, apr. 2008.

LI, Z. et al. Interaction between selenium and soil organic matter and its impact on soil selenium bioavailability: A review. **Geoderma**, v. 295, p. 69–79, jun. 2017.

LIMA, L. W.; PILON-SMITS, E. A. H.; SCHIAVON, M. Mechanisms of selenium hyperaccumulation in plants: A survey of molecular, biochemical and ecological cues. **Biochimica et Biophysica Acta (BBA) - General Subjects**, v. 1862, n. 11, p. 2343–2353, nov. 2018.

LONGCHAMP, M. et al. Variations in the accumulation, localization and rate of metabolization of selenium in mature *Zea mays* plants supplied with selenite or selenate. **Food Chemistry**, v. 182, p. 128–135, sep. 2015.

LYONS, G. H. et al. Selenium increases seed production in Brassica. **Plant and Soil**, v. 318, n. 1–2, p. 73–80, may, 2009.

MARTINEZ, H. E. P. et al. Short-term water stress affecting NO_3^- absorption by almond plants. **Scientia Horticulturae**, v. 197, p. 50–56, dez. 2015.

NG, B. H.; ANDERSON, J. W. Synthesis of selenocysteine by cysteine synthases from selenium accumulator and non-accumulator plants. **Phytochemistry**, v. 17, n. 12, p. 2069–2074, jan. 1978.

PAULA, B. V. D. et al. Morphological and kinetic parameters of the uptake of nitrogen forms in clonal peach rootstocks. **Scientia Horticulturae**, v. 239, p. 205–209, set. 2018.

PEDRERO, Z.; MADRID, Y. Novel approaches for selenium speciation in foodstuffs and biological specimens: A review. **Analytica Chimica Acta**, v. 634, n. 2, p. 135–152, feb. 2009.

PFEIFFER, W. H.; MCCLAFFERTY, B. HarvestPlus: Breeding crops for better nutrition. **Crop Science**, v. 47, p. S-88-S-105, dec. 2007.

PILON-SMITS, E. A. H.; WINKEL, L. H. E.; LIN, Z.-Q. (EDS.). **Selenium in plants**. Cham: Springer International Publishing, 2017. v. 11

PROM-U-THAI, C. et al. Simultaneous biofortification of rice with zinc, iodine, iron and selenium through foliar treatment of a micronutrient cocktail in five countries. **Frontiers in Plant Science**, v. 11, p. 589835, 13 nov. 2020.

RAVELLO, R. A. V. et al. Selenium application influenced selenium biofortification and physiological traits in water-deficit common bean plants. **Crop and Pasture Science**, v. 73, n. 2, p. 44–55, 16 jul. 2021.

REEVES, M. A.; HOFFMANN, P. R. The human selenoproteome: recent insights into functions and regulation. **Cellular and Molecular Life Sciences**, v. 66, n. 15, p. 2457–2478, aug. 2009.

RENKEMA, H. et al. The effect of transpiration on selenium uptake and mobility in durum wheat and spring canola. **Plant and Soil**, v. 354, n. 1–2, p. 239–250, may, 2012.

RUDE, R. K.; GRUBER, H. E. Magnesium deficiency and osteoporosis: animal and human observations. **The Journal of Nutritional Biochemistry**, v. 15, n. 12, p. 710–716, dez. 2004.

SACRAMENTO L.V.S.; ROSOLEM, C.A. Cinética de absorção de potássio e seus ajustes em plantas de soja em função da idade e estado nutricional. R**evista Brasileira de Ciência do Solo**, v. 21, p. 213-219, 1997.

SHIKUKU, K. M. et al. Nutrition and food security impacts of quality seeds of biofortified orange-fleshed sweetpotato: Quasi-experimental evidence from Tanzania. **World Development**, v. 124, p. 104646, dec. 2019.

ŠINDELÁŘOVÁ, K. et al. The response of broccoli (*Brassica oleracea* convar. *italica*) varieties on foliar application of selenium: uptake, translocation, and speciation. **Food Additives & Contaminants: Part A**, p. 150928143022009, 28 sep. 2015.

SORS, T. G.; ELLIS, D. R.; SALT, D. E. Selenium uptake, translocation, assimilation and metabolic fate in plants. **Photosynthesis Research**, v. 86, n. 3, p. 373–389, dec. 2005.

SOUSA, G. F. de et al. Selenium enhances chilling stress tolerance in coffee species by modulating nutrient, carbohydrates, and amino acids content. **Frontiers in Plant Science**, v. 13, p. 1000430, 12 sep. 2022.

TAKEDA, T.; ISHIKAWA, T.; SHIGEOKA, S. Metabolism of hydrogen peroxide by the scavenging system in *Chlamydomonas reinhardtii*. **Physiologia Plantarum**, v. 99, n. 1, p. 49–55, jan. 1997.

TOLU, J. et al. Distribution and speciation of ambient selenium in contrasted soils, from mineral to organic rich. **Science of The Total Environment**, v. 479–480, p. 93–101, may, 2014.

VINCETI, M. et al. Selenium for preventing cancer. **Cochrane Database of Systematic Reviews**, 29 jan. 2018.

WARNCKEAND, D. D. Root Development and nutrient uptake by corn grow in solution culture. **Agronomy Journal**, v. 66, jul, n. 5222, p. 514–516, 1974.

WELCH, R. M.; GRAHAM, R. D. Breeding crops for enhanced micronutrient content. In: ADU-GYAMFI, J. J. (Ed.). **Food Security in Nutrient-Stressed Environments: Exploiting Plants' Genetic Capabilities**. Dordrecht: Springer Netherlands, 2002. p. 267–276.

WHITE, P.; BROADLEY, M. Biofortifying crops with essential mineral elements. **Trends in Plant Science**, v. 10, n. 12, p. 586–593, dec. 2005.

WHITE, P. J. Selenium metabolism in plants. **Biochimica et Biophysica Acta (BBA) - General Subjects**, v. 1862, n. 11, p. 2333–2342, nov. 2018.

WHITE, P. J.; BROADLEY, M. R. Biofortification of crops with seven mineral elements often lacking in human diets – iron, zinc, copper, calcium, magnesium, selenium and iodine. **New Phytologist**, v. 182, n. 1, p. 49–84, apr. 2009.

YANG, F. et al. Effect of the application of selenium on selenium content of soybean and its products. **Biological Trace Element Research**, v. 93, n. 1–3, p. 249–256, 2003.

YANG, T. et al. Nitrate uptake kinetics of grapevine under root restriction. S**cientia Horticulturae**, v. 111, n. 4, p. 358–364, fev. 2007.

ZHANG, M. et al. Improving soil selenium availability as a strategy to promote selenium uptake by high-Se rice cultivar. **Environmental and Experimental Botany**, v. 163, p. 45–54, jul. 2019.

ZHANG, Y. et al. Uptake and transport of selenite and selenate by soybean seedlings of two genotypes. v. 253, p. 437–443, 2003.

ZHAO, X. et al. Distribution and effects of natural selenium in soybean proteins and its protective role in soybean β-conglycinin (7S globulins) under AAPH-induced oxidative stress. **Food Chemistry**, v. 272, p. 201–209, jan. 2019.

ZOU, C. et al. Simultaneous biofortification of wheat with zinc, iodine, selenium, and iron through foliar treatment of a micronutrient cocktail in six countries. **Journal of Agricultural and Food Chemistry**, v. 67, n. 29, p. 8096–8106, 24 jul. 2019.

SECOND PART – ARTICLES

ARTICLE 1: Selenium biofortification of soybean genotypes in a tropical soil via Se-enriched phosphate fertilizers

Published in Frontiers in Plant Science
(DOI - 10.3389/fpls.2022.988140)

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Abstract: Soybean is a major crop in Brazil and is usually grown in oxidic soils that need high rates of phosphate (P) fertilizers. Soybean is also very suitable for biofortification with Se, since its grains have high protein contents and are widely consumed worldwide (directly or indirectly). Few studies have addressed Se application under field conditions for soybean biofortification, especially in tropical soils. Here, we evaluated agronomic and physiological responses resulting from different strategies for biofortifying soybean grains with Se by applying this element via soil, using both conventional and enhanced-efficiency P fertilizers as Se carriers. The experiment was carried out at the Uva Farm, in Capão Bonito (São Paulo), Brazil. The experimental design was a randomized block split-plot design, with four fertilizer sources-conventional monoammonium phosphate (C-MAP), conventional monoammonium phosphate + Se (C-MAP+Se), enhanced-efficiency monoammonium phosphate (E-MAP), and enhanced efficiency monoammonium phosphate + Se (E-MAP+Se), and four soybean genotypes (M5917, 58I60 LANÇA, TMG7061, and NA5909). The selenium rate applied via C-MAP + Se and E-MAP + Se was 80 g ha^{-1}. The application of the tested fertilizers was carried out at the sowing of the 2018/2019 cropping season, with their residual effect being also assessed in the 2019/2020 cropping season. Selenium application increased grain yield for the TMG7061 genotype. For all evaluated genotypes, Se content in grains increased in the 2018/2019 harvest with the application of Se via C-MAP + Se and E-MAP + Se. In general, the application of Se via C-MAP favored an increase in amino acid contents in grains and decreased lipid peroxidation. In summary, the application of Se-enriched P fertilizers via soil increased soybean grain yield, leading to better grain quality. No residual effects for biofortifying soybean grains were detected in a subsequent soybean cropping season.

Keywords: biofortification; food security; cereal; nutritional quality; selenate

Introduction

Selenium (Se) is an essential element for humans and animals. It is a component of selenoaminoacids (e.g., selenocysteine), being necessary for the synthesis of more than 25 selenoproteins (Rayman, 2012; Oliver and Gregory, 2015). As a component of glutathione peroxidase, Se acts against oxidative stresses. In addition, Se also participates in thyroid metabolism and the immune system maintenance, reducing cancer and heart disease (Rayman, 2012; Avery and Hoffmann, 2018). It is estimated that about 1 billion people worldwide are Se deficient (Mora et al., 2015). Keshan and Kashin-Beck diseases are associated with Se deficiency in human organisms. Keshan is related to cardiomyopathy affecting children and young women and Keschin-Beck is related to osteoarthritis, promoting bone atrophy (Yao et al., 2011).

Selenium is not currently considered a plant nutrient though its beneficial effects on vegetables have been studied for over 70 years (Lyons et al., 2009; Feng et al., 2013). Several beneficial effects of this element for plants have been reported, such as improved rice growth (Boldrin et al., 2012), increased photosynthetic rate and wheat yield (Lara et al., 2019), reduced production of free radicals in lettuce (Ramos et al., 2011), increased protein content and total amino acids in soybean (Zhao et al., 2019), and reduced the damage caused by water stress in rice and common bean plants (Andrade et al., 2018; Ravello et al., 2021). For this reason, due to new trends in plant nutrient classification, Se and other beneficial elements (Na, Si, Al, Co, and I) may be considered plant nutrients in the future (Brown et al., 2021).

Selenium availability in soils depends on several factors, such as the Se source, soil mineralogy, redox condition, pH, and the presence of other anions (Lopes et al., 2017). Tropical soils are known for their high capacity to retain oxyanions-including selenite and selenate-with Se availability being decreased with increasing clay content. This is due to the high concentration of Fe/Al oxyhydroxides present in oxidic soils from tropical regions (Lopes et al., 2017; Araujo et al., 2018). Because of that, plants grown in soils with low Se concentration and availability show inadequate accumulation of this element in their edible parts (White and Broadley, 2009).

The adoption of biofortification practices is a suitable strategy to increase Se contents in food crops. Biofortification is a strategy that aims to increase the content of minerals and vitamins in crops via genetic (e.g., breeding) and/or agronomic (fertilization) practices (Cakmak, 2008; White and Broadley, 2009). Knowing the various constraints related to Se availability in Brazilian agroecosystems, the Brazilian Ministry of Agriculture, Livestock, and Supply approved a new legislation (normative N° 46/2016), which allowed the addition of Se in fertilizers marketed in Brazil (Brazil, 2016). A possible and relevant alternative to directly applying Se fertilizers in tropical agroecosystems could be its co-application via phosphate fertilizers, since the presence of competing anions, such as phosphate, reduces Se adsorption, increasing soil Se availability (Lessa et al., 2016; Mateus et al., 2021). Studies involving the biofortification of rice grown in tropical soils have reported the efficacy of the strategy of supplying Se to plants via its co-application with monoammonium phosphate-MAP (Lessa et al., 2020). Many P-fertilizer products are currently being used in oxidic soils with a technology to reduce phosphate retention (e.g., the so-called enhanced-efficiency products), it is thus relevant to determine if such technologies could improve Se use efficiency when selenium is soil-applied using enhanced-efficiency MAP as a carrier.

Additional studies evaluating Se application via soil associated with sources of phosphate fertilizers are still required. To the best of our knowledge, there are few studies in tropical soils assessing Se application, mainly focusing on the co-application of Se with phosphate fertilizers. Soybean is an interesting agricultural crop for biofortification with Se due to the large number of products generated from soybean grains, the high concentration of proteins, and the geographic distribution of soybean production. The present study aimed to evaluate the effectiveness of applying Se in association with phosphate fertilizers for soybean biofortification and its residual effect in the succeeding cropping season in tropical soils.

Materials and methods

Experimental area and treatments

The experiment was carried out with soybean crop (*Glycine max* L. Merril) grown under commercial field conditions during the cropping seasons of 2018/2019 (application of treatments with Se) and 2019/2020 (assessment of residual effects of Se previously applied) at the Uva Farm, located in Capão Bonito, State of São Paulo (SP), Brazil, at the following geographic coordinates: Lat: -24.040934, Lon: -48.262421 (Figure 1). The weather of the region is characterized as humid subtropical (Cfa), with an average rainfall of 1,628 mm and an average annual temperature of 18.8°C (Alvares et al., 2013).

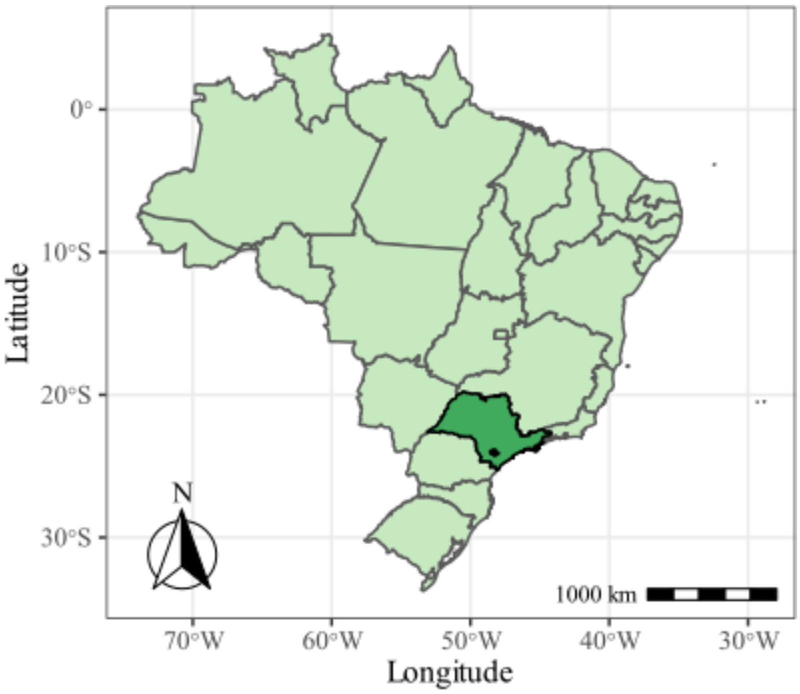


FIGURE 1. Location of the experimental area in Capão Bonito, SP, Brazil.

The soil of the experimental region-Oxisol-is classified as Typic Hapludox (Soil Survey Staff, United States Department of Agriculture Natural Resources Conservation Service, 2014) and the chemical and physical properties are as follows, according to the methodology suggested by Brazilian Agricultural Research Company (EMBRAPA) (1997) [pH (H_2O) = 6.0; H + Al = 2.96; Al = 0.06; P (Mehlich-1) = 34.8 mg dm^{-3}; K = 148 mg dm^{-3}; S = 4.11 mg L^{-1}; CEC = 9.83 cmolc dm^{-3}; Ca = 5.05 cmolc dm^{-3}; Mg = 1.44 cmolc dm^{-3}; P-rem = 28.10 mg L^{-1}; organic matter = 2.69 dag dm^{-3}; clay = 510 g kg^{-1}; silt =110 g kg^{-1}; and sand =380 g kg^{-1}].

The experiment was arranged in a randomized block split-plot design, with four replicates. The biofortification of soybean was tested applying four different fertilizers: (i) Conventional monoammonium phosphate (C-MAP); (ii) Conventional monoammonium phosphate + Se (C-MAP + Se); (iii) Enhanced-efficiency monoammonium phosphate (E-MAP); and (iv) Enhanced-efficiency monoammonium phosphate + Se (E-MAP + Se). Monoammonium phosphate was coated with the humic and fulvic substances. The C-MAP + Se and E-MAP + Se fertilizers were prepared by spraying Se to the fertilizer granule. For this purpose, the fertilizers were coated after the granulation with 500 mg kg^{-1} of Se (from a solution of sodium selenate-Na_2SeO_4, Sigma-Aldrich, Saint Louis, MO, United States).

Considering that 80 kg ha^{-1} of P_2O_5 were applied as MAP (~50% P_2O_5), the addition of Se-rich fertilizers (500 mg Se kg^{-1}) added a Se rate of 80 g ha^{-1}.

The aforementioned fertilizers were applied to four soybean genotypes, as follows: M5917 (maturity group = 5.9), 58I60 LANÇA (maturity group = 5.8), TMG7061 (maturity group = 6.1), and NA5909 (maturity group = 6.2); all of them presenting indeterminate growth type). Thus, the experiment had a total of 16 treatments, with four replicates, totaling 64 experimental plots. The fertilizers comprised the plots and the split-plots were represented by the genotypes. Each experimental split-plot was 30 m long by 3 m wide (soybean row spacing at 0.5 m, totaling 90 m^2). Planting was made with 14 seeds per meter and fertilization was carried out during the sowing at the soybean seeds line (localized placement) by applying 16 kg ha^{-1} of N, 80 kg ha^{-1} of P_2O_5, and 28 kg ha^{-1} of K_2O.

After the soybean harvest (described next), wheat was sown in the area but was not harvested for analysis. After wheat, soybean was sown in the succeeding summer crop to evaluate the residual effect of Se associated with the previously soil-applied phosphate fertilizer. Selenium treatments were not applied in this second season with all following the standard management carried out at the Uva farm.

Analysis of oxidative stress and antioxidant enzymes

The uppermost fully developed leaf (trifoliolate) from 10 plants during the first cropping season (2018/2019) were collected at the full pod stage (R4) to evaluate antioxidant enzymes and oxidative stress. The collected leaves were frozen immediately in liquid nitrogen and stored in a deep freezer at -80°C for subsequent analysis. After that, the frozen plant material (0.2 g) was macerated in a porcelain mortar with liquid nitrogen and polyvinylpolypyrrolidone (PVPP) and mixed with 1.5 ml of buffer solution (100 mM potassium phosphate at pH 7.8, 0.1 mM EDTA, and 10 mM ascorbic acid).

The extract was centrifuged at 13,000 g for 10 min at 4°C. The supernatant was collected for measuring the activities of the enzymes, as follows: superoxide dismutase (SOD; Giannopolitis and Ries, 1977), ascorbate peroxidase (APX; Nakano and Asada, 1981), and catalase (CAT; Havir and McHale, 1987). In addition to that, 0.3 g of macerated frozen material were homogenized with 1.5 ml of 0.1% trichloroacetic acid (TCA) and centrifuged at 12,000 g for 15 min at 4°C for hydrogen peroxide (H_2O_2; Velikova et al., 2000) and peroxidation lipid (MDA; Buege and Aust, 1978).

Soil Se content

For the determination of total Se content (partially available) in the soil, one composite soil sample (coming from five subsamples distributed around the experimental plot) was collected in each experimental plot at the full pod stage (R4). The samples were dried, homogenized, ground with a mortar and agate pestle, and passed through a 100-mesh nylon sieve. A sample mass of 0.5 g was mixed with 5 ml of aqua regia (a mixture of HNO_3 65% and HCl 37% - 1:3 v/v). The mixture/suspension was left to stand for 1 h, and the Teflon® vessels were hermetically sealed and heated in a Mars-5 microwave digestion oven (CEM Corp, Matthews, NC, United States) with a temperature set at 175°C and a controlled pressure of 0.76 MPa for 25 min. Next, the vessels were cooled to room temperature and the volume was completed to 40 ml with bidistilled water.

Selenium in the soil samples was analyzed by Graphite Furnace Atomic Absorption Spectrometry with Zeeman background correction and EDL lamp for Se (GFAAS; AAnalyst™ 800 AAS, Perkin Elmer). The calibration curve for Se measurements was obtained from a standard solution with 1 g L^{-1} of Se (≥98% of purity, Fluka, Buchs, Switzerland). The reference material used for soil Se concentration was SRM 2709a [San Joaquin Soil, from the National Institute of Standards & Technology (Gaithersburg, MD, United States)], which contains 1.5 mg kg^{-1} of Se. The mean recovery of Se in this certified material was 88%.

Harvest and yield determination

After R8 stage, when 95% of pods have attained maturity and have a variety-dependent color of brown or tan (134 and 495 days after the treatment application for the first and second season, respectively), grains from the useful area of the experimental plot were harvested and weighed to determine crop yield. Grain moisture was measured using a portable meter (model G650i, Gehaka®) and grain yield was corrected to 13%. A sample of each harvested plot was ground in a Willey mill for the determination of Se, N, protein, and total free amino acids.

Nitrogen and selenium content in grains

Nitrogen quantification was performed by the Kjeldahl method described by Bremner (1996). The extraction for determination of Se was obtained by acid digestion of 0.5 g of ground grain, in a microwave oven, following the USEPA 3051A method (Usepa, 2007). Selenium contents were performed using an inductively coupled plasma mass spectrometer (ICP-MS; PerkinElmer, model NexIon 2000 B, Waltham, United States).

To ensure the quality of the digestion process, a reference standard from the Institute for Reference and Measurement Materials (White Clover – BCR 402, IRMM, Geel, Belgium, with 6.70 mg Se kg^{-1}) and a blank sample were added to each digestion batch. The detection limit (LOD) was obtained using Se measurement in seven blank extracts and was calculated from the Equation 1:

$$LOD = (x + t \times s) \times d$$

Where:

$\bar{x}$ = mean content of the substance of interest in seven blank samples

t = Student value to 0.01 of probability

s = standard deviation

d = dilution

The fraction of the applied Se that was incorporated in soybean grains (Se recovery) was calculated using the Equation 2 described below:

$$\text{Se recovery (\%)} = \frac{\text{(Se treatment - Se control)}}{\text{Se rate}} \times 100$$

Where:

Se recovery (%) = use efficiency of the Se rates applied in the soil by soybean grains (Se utilization percentage);

Se treatment (g ha^{-1}) = Se contents in soybean grains from soybean plants grown in treatments that received Se applications, considering the yield obtained in each treatment;

Se control (g ha^{-1}) = Se contents in soybean grains from soybean plants grown in treatments without Se applications, considering the yield obtained in each treatment;

Se rate (g ha^{-1}) = Se rates applied in the soil.

Total free amino acids and protein

Total free amino acids were determined using the ninhydrin method (Yemm et al., 1954). The quantification of protein in the grains was determined by multiplying the value of the N content by 6.25.

Statistical analysis

The obtained data were primarily tested for their normality (Shapiro–Wilk's test) and homogeneity of variance (Bartlett's Test). Then, they were submitted to ANOVA, and when significant, mean values of variables found for each treatment were compared by the Tukey test at 5%. Principal component analysis (PCA) was performed for the dataset of conventional or enhanced fertilizer. The Pearson's linear correlation matrix ($p < 0.05$) was also carried out, aiming to validate clusters and potential relationships of Se application in soil and plant attributes as outcomes of PCA. The analyses were made using the R software (R Core Team, 2020).

Results

Soybean yield (cropping seasons of 2018/2019 and 2019/2020)

The tested factors (genotypes and fertilizer sources) affected soybean grain yield in the 2018/2019 season ($p < 0.05$). The fertilizer sources applied did not alter the yield of 58I60 LANÇA and M5917 genotypes. On the contrary, the genotype N5909 showed a statistical difference in yield by the Tukey's test ($p < 0.05$), between the application of C-MAP and E-MAP, with 92.08 and 76.36 bags ha^{-1}, respectively (Figure 2).

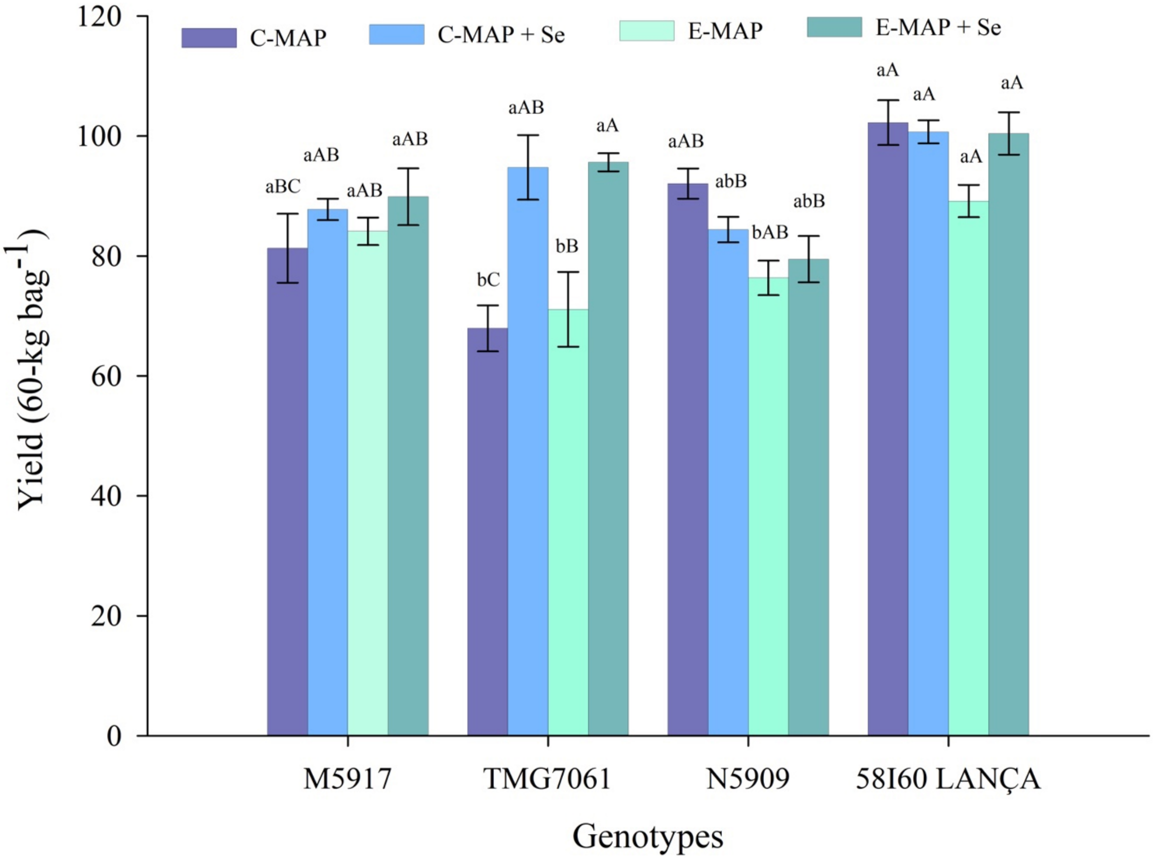


FIGURE 2. Yield (60-kg bags^{-1}) of soybean grains harvested from the 2018/2019 cropping season. Lowercase letters compare soybean yields among fertilizers in each genotype and capital letters compare soybean yields among genotypes in each fertilizer source at the level of 5% ($p < 0.05$) by the Tukey test. The vertical bars refer to the standard error ($n = 4$). C-MAP, conventional monoammonium phosphate; C-MAP + Se, conventional monoammonium phosphate + Se; E-MAP, enhanced-efficiency monoammonium phosphate; and E-MAP + Se, enhanced-efficiency monoammonium phosphate + Se.

Grain yield in the TMG7061 genotype was higher in treatments using C-MAP + Se and E-MAP + Se when compared to C-MAP and E-MAP, reaching yields of 94.77 and 95.62 bags ha^{-1}, respectively and gains of 24.51 and 26.85 bags ha^{-1} in yield, respectively. In the 2019/2020 cropping season, when the residual effect of Se applied in the soil was evaluated, the factors tested did not affect grain yield ($p > 0.05$; Supplementary Table 1).

Selenium content in soybean grains and soil

Selenium content analyzed in the reference material was 7.37 mg kg^{-1}, indicating a recovery of 110%. The Se content in soybean harvested in the first season was influenced by the genotypes and sources of fertilizers applied ($p < 0.05$; Figure 3A). In all tested genotypes, the application of C-MAP + Se and E-MAP + Se increased the Se content in grains. In the genotype TMG7061, the increase in Se content was 2.90 and 3.31 times greater with the application of C-MAP + Se and E-MAP + Se, compared with their respective fertilizers without Se. In the other genotypes, the application of C-MAP + Se and E-MAP + Se presented values higher than two times the Se content accumulated into grains when the fertilizers C-MAP and E-MAP were applied.

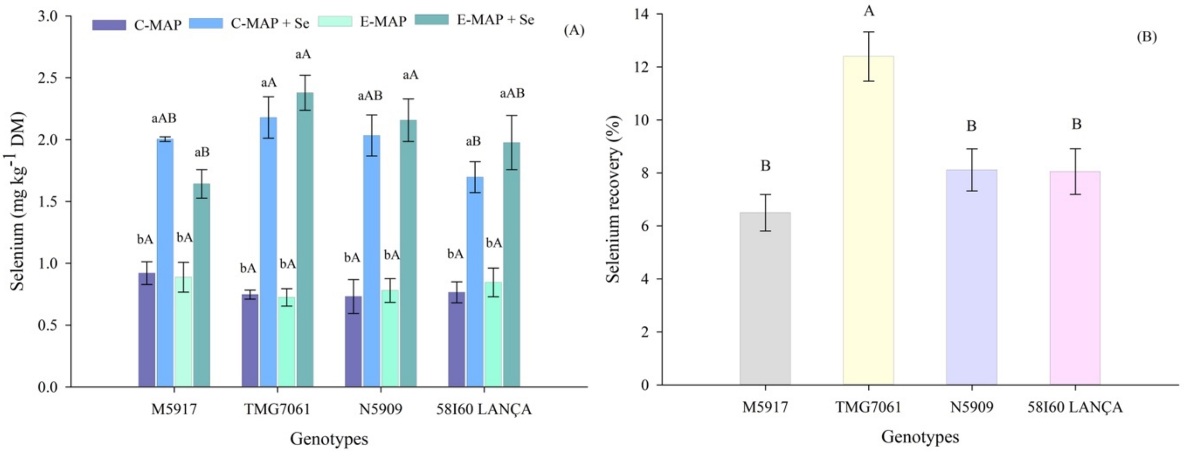


FIGURE 3. Selenium content (mg kg^{-1}) and Se recovery (%) (B) in soybean grains harvested from the 2018/2019 cropping season. Lowercase letters compare Se contents and Se recovery among fertilizers in each genotype and capital letters compare Se contents and Se recovery among genotypes in each fertilizer source at the level of 5% ($p < 0.05$) by the Tukey test. The vertical bars refer to the standard error ($n = 4$). C-MAP, conventional monoammonium phosphate; C-MAP + Se, conventional monoammonium phosphate + Se; E-MAP, enhanced-efficiency monoammonium phosphate; and E-MAP + Se, enhanced-efficiency monoammonium phosphate + Se. The vertical bars refer to the standard error (A-n = 4; B-n = 8).

Observing the content of Se in grains, with the use of C-MAP + Se and E-MAP + Se, the genotype TMG7061 presented the highest content, being however statistically different only from the genotype 58I60 LANÇA for C-MAP + Se and from the genotype M5917 for E-MAP + Se. The Se recovery by soybean grains was different among the tested genotypes ($p < 0.05$; Figure 3B), with the genotype TMG7061 showing the highest value (close to 12.4%).

The Se content in soybean grains harvested in the 2019/2020 crop was not influenced by the variables analyzed ($p > 0.05$; Supplementary Table 1). The average grain contents as a function of the fertilizers applied were 0.48 mg kg^{-1} (E-MAP), 0.52 mg kg^{-1} (C-MAP), 0.55 mg kg^{-1} (E-MAP + Se), and 0.62 mg kg^{-1} (C-MAP + Se). In the soil, the Se content did not differ statistically among treatments. The overall average Se content found in the soil in phase R4 was 0.73 mg dm^{-3}, which justifies the low Se concentration in soybean grains of the crop carried out in the 2019/2020 cropping season (Supplementary Table 2).

Nitrogen, protein, and amino acids

Nitrogen content, proteins, and total free amino acids were affected by the interaction between the tested genotypes and fertilizers. Following the application of C-MAP, the genotypes M5917 and 58I60 LANÇA showed higher N and protein contents compared with other treatments (Figure 4A; Supplementary Table 1). The total free amino acid content was higher with C-MAP + Se than with the other fertilizer sources for genotypes N5909 and 58I60 LANÇA (Figure 4B). Total free amino acid contents did not change due to the fertilizer sources applied for genotype M5917, whereas for genotype TMG7061, the highest and lowest values were verified after the application of C-MAP + Se and E-MAP and E-MAP + Se, respectively.

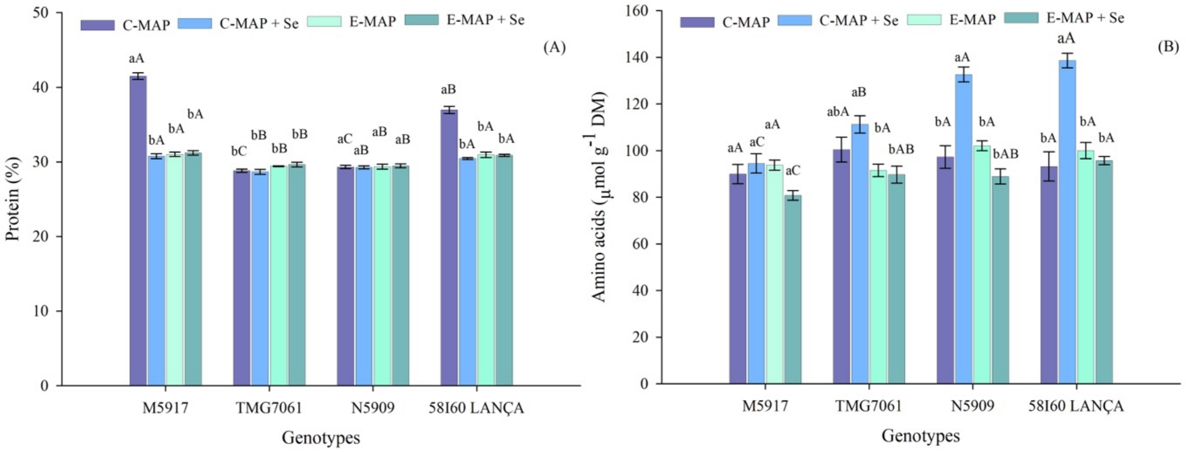


FIGURE 4. Protein (%) (A) and amino acids (μmol g^{-1} DM) (B) in soybean grains harvested from 2018/2019 cropping season. Lowercase letters compare protein and amino acids among fertilizers in each genotype and capital letters compare protein and amino acids among genotypes in each fertilizer source at the level of 5% ($p < 0.05$) by the Tukey test. The vertical bars refer to the standard error ($n = 4$). C-MAP, conventional monoammonium phosphate; C-MAP + Se, conventional monoammonium phosphate + Se; E-MAP, enhanced-efficiency monoammonium phosphate; and E-MAP + Se, enhanced-efficiency monoammonium phosphate + Se.

Antioxidative metabolism

Overall, the activity of enzymes was not affected by the different fertilizers sources (Table 1). Superoxide dismutase and CAT had different activities among the genotypes, while APX was not affected by any of the factors under study. The genotype TMG7061 showed lower SOD activity and lower H_2O_2 concentration. Among the sources of fertilizers applied, C-MAP presented higher H_2O_2 content (2.09 μmol H_2O_2 g^{-1} MF), yet it differed only from the treatment with the application of E-MAP + Se (1.54 μmol H_2O_2 g^{-1} MF).

TABLE 1. Effect of Se application via soil on the activities of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), lipid peroxidation by the MDA, and hydrogen peroxide (H_2O_2) with SEs (n = 4).

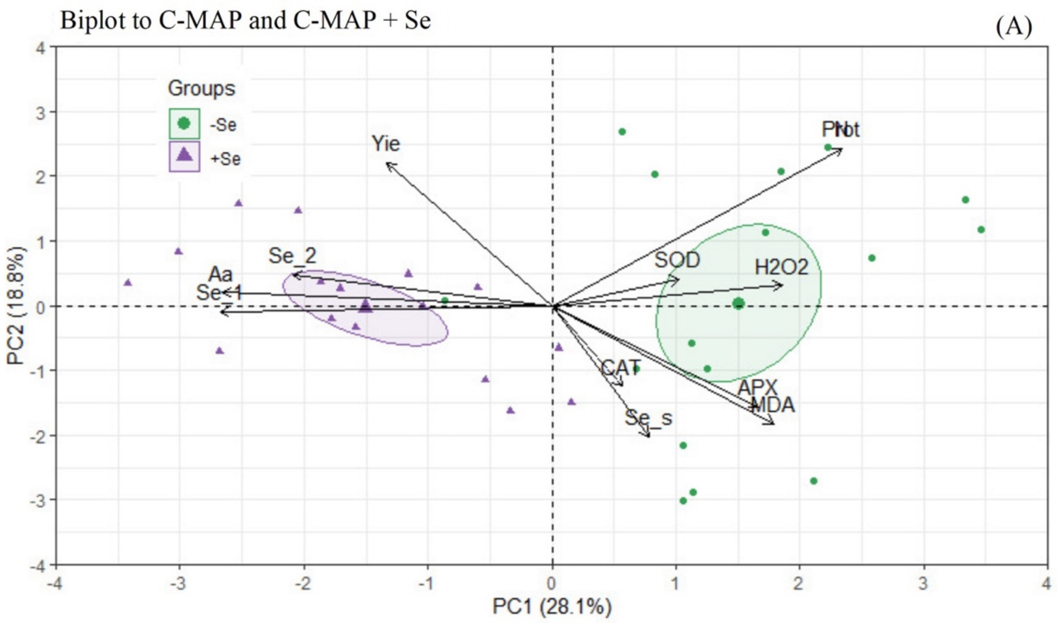
Genotype	**Fertilizer**	**SOD (U SOD min^{-1} g^{-1} FM)**	**CAT (ηmol H_2O_2 min^{-1} g^{-1} FM)**	**APX (ηmol ASA min^{-1} g^{-1} FM)**	**MDA (ηmol MDA g^{-1} FM)**	**H_2O_2 (μmol H_2O_2 g^{-1} FM)**
M5917	C-MAP	610.82 ± 18.66	2.97 ± 0.16	26.38 ± 2.01	15.94 ± 1.94 aAB	2.16 ± 0.23
TMG7061		616.14 ± 15.12	3.78 ± 0.47	29.37 ± 1.90	19.84 ± 0.58 aA	1.73 ± 0.27
N5909		647.83 ± 12.53	2.99 ± 0.30	23.55 ± 2.40	17.48 ± 1.45 aA	2.35 ± 0.09
58I60 LANÇA		658.31 ± 19.96	2.71 ± 0.29	23.36 ± 1.71	12.65 ± 1.31 aB	2.13 ± 0.24
M5917	C-MAP + Se	618.59 ± 31.55	3.36 ± 0.57	24.79 ± 2.29	13.40 ± 0.86 aA	2.00 ± 0.22
TMG7061		532.09 ± 25.74	3.37 ± 0.62	23.82 ± 2.49	13.48 ± 1.15 bA	1.35 ± 0.27
N5909		637.13 ± 13.51	2.23 ± 0.20	21.09 ± 2.50	13.08 ± 1.13 aA	1.87 ± 0.32
58I60 LANÇA		642.33 ± 24.15	1.59 ± 0.18	23.20 ± 2.87	13.94 ± 0.84 aA	1.89 ± 0.12
M5917	E-MAP	611.05 ± 24.87	2.46 ± 0.67	22.93 ± 4.04	11.77 ± 0.69 aA	2.10 ± 0.35
TMG7061		536.86 ± 21.09	3.35 ± 0.63	22.09 ± 4.12	12.45 ± 0.30 bA	1.13 ± 0.23
N5909		583.80 ± 21.11	2.58 ± 0.70	21.93 ± 2.08	15.71 ± 2.27 aA	1.88 ± 0.23
58I60 LANÇA		613.77 ± 26.17	2.69 ± 0.68	23.71 ± 3.31	14.25 ± 1.46 aA	2.11 ± 0.21
M5917	E-MAP + Se	619.08 ± 26.87	3.56 ± 0.47	28.84 ± 5.57	11.50 ± 1.42 aB	1.53 ± 0.20
TMG7061		546.71 ± 14.76	2.60 ± 0.57	21.29 ± 4.71	14.23 ± 0.59 bAB	0.97 ± 0.13
N5909		600.64 ± 18.22	2.78 ± 0.30	27.38 ± 4.84	12.86 ± 1.72 aAB	1.53 ± 0.29
58I60 LANÇA		639.01 ± 13.65	2.66 ± 0.77	26.23 ± 3.41	16.34 ± 1.93 aA	2.17 ± 0.28
M5917	General average to genotypes	614.88 ± 11.63 A	3.09 ± 0.25 AB	25.73 ± 1.77 ns	13.16 ± 0.75 ns	1.95 ± 0.13 A
TMG7061		557.95 ± 12.44 B	3.27 ± 0.28 A	24.14 ± 1.34 ns	15.00 ± 0.81 ns	1.29 ± 0.13 B
N5909		617.35 ± 10.06 A	2.65 ± 0.20 AB	23.49 ± 1.54 ns	14.78 ± 0.91 ns	1.91 ± 0.13 A
58I60 LANÇA		638.36 ± 10.47 A	1.09 ± 0.27 B	24.13 ± 1.34 ns	14.30 ± 0.73 ns	2.08 ± 0.10 A
C-MAP	General average to fertilizers	633.27 ± 16.57 ns	3.11 ± 0.30 ns	25.66 ± 2.00 ns	16.48 ± 1.32 ns	2.09 ± 0.21 a
C-MAP + Se		607.54 ± 23.74 ns	2.64 ± 0.39 ns	23.23 ± 2.54 ns	13.47 ± 0.99 ns	1.78 ± 0.23 ab
E-MAP		586.37 ± 23.31 ns	2.77 ± 0.67 ns	22.67 ± 3.39 ns	13.55 ± 1.18 ns	1.81 ± 0.26 ab
E-MAP + Se		601.36 ± 18.37 ns	2.90 ± 0.53 ns	25.93 ± 4.63 ns	13.73 ± 1.42 ns	1.55 ± 0.22 b

Lowercase letters compare among fertilizers in each genotype and capital letters compare among genotypes in each fertilizer source at the level of 5% ($p < 0.05$) by the Tukey test. No significance analysis was performed for SOD, CAT, APX, and H_2O_2 within soybean genotypes, as this was not the purpose of this study. C-MAP, conventional monoammonium phosphate; C-MAP + Se, conventional monoammonium phosphate + Se; E-MAP, enhanced-efficiency monoammonium phosphate; and E-MAP + Se: enhanced-efficiency monoammonium phosphate + Se. ns, no significant.

Malonaldehyde (MDA) levels were affected by the interaction between genotypes and fertilizers ($p < 0.05$), with genotype TMG7061 being the only one that showed a difference among fertilizers. In this genotype, MDA levels were higher with the application of C-MAP, indicating an increase in lipid peroxidation.

Principal component analysis

With the application of the conventional MAP with and without Se (C-MAP and C-MAP + Se), 46.9% of the covariances were explained by the PC1 and PC2 axes (Figure 5A). For E-MAP and E-MAP + Se, 46.2% of the covariances were explained by the PC1 and PC2, but the confidence intervals overlapped (Figure 5B). For fertilizers C-MAP and C-MAP + Se, the PCA showed that the concentration of total free amino acids correlates positively with the application of Se. In addition, the soybean grain yield from the cropping season of 2018/2019 was favored by Se application. The significance of the correlation among the studied variables was confirmed by Pearson’s linear correlation matrix ($p < 0.05$; Supplementary Figure 1).



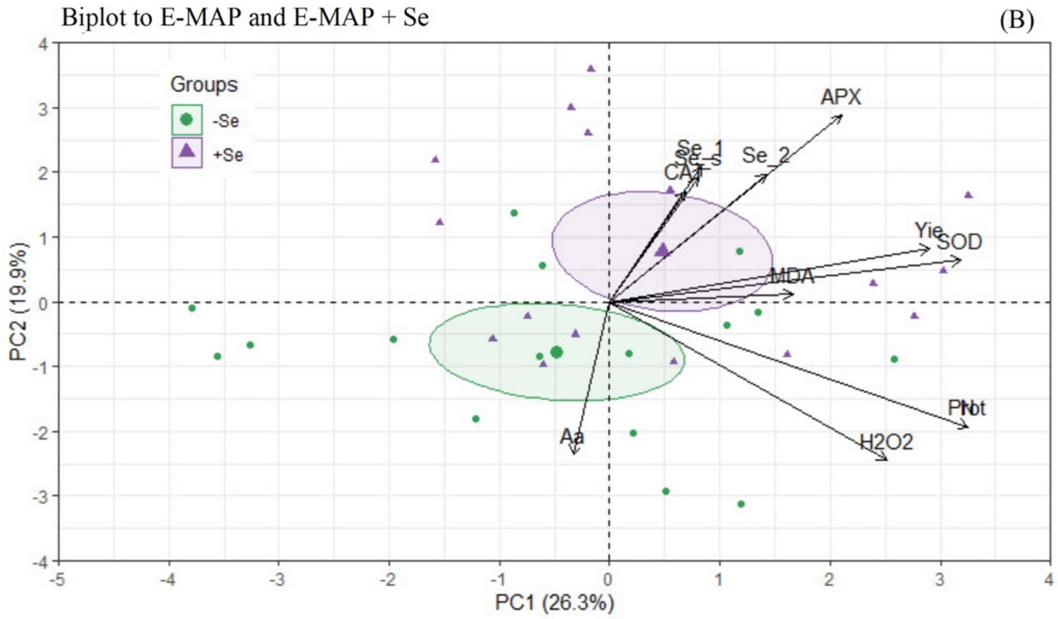


FIGURE 5. Biplot of principal component analysis (PCA) separated according to the fertilizers, (A) C-MAP and C-MAP + Se and (B) E-MAP and E-MAP + Se. Se content in grains of the cropping season of 2018/2019 (Se_1), Se content in grain of the cropping season of 2019/2020 (Se_2), Se in soil (Se_s), yield (Yie), protein in grains (Prot), amino acids in grains (Aa), lipid peroxidation (MDA), hydrogen peroxide (H_2O_2), catalase (CAT), superoxide dismutase (SOD), and ascorbate peroxidase (APX).

Discussion

Yield

The average yield found in this study (89.7 bags ha^{-1}) was above the national average (50.0 bags ha^{-1}; Conab, 2022). This high average yield is related to the management adopted by the Uva farm and to the high soil fertility, based on soil attributes and nutrient concentration (e.g., P and K). To establish homogeneity in the final stand of plants and because all field operations were performed using commercial planting machines, the number of seeds that were sown per linear meter was the same for all genotypes, even though a higher number of seeds per linear meter was recommended for genotype TMG7061. Due to the presence of a larger stand of plants for this genotype (TMG7061), lodging of the plants occurred during the grain filling stage. Under high planting density, the light capture is reduced, reducing photosynthetic activity and carbohydrate accumulation in the stem, which leads to lodging (Song et al., 2020).

In addition to the high average yield, Se application increased grain yield for the TMG7061 genotype (Figure 2). The response of Se application to plant yield may vary depending on the genotype used (Thavarajah et al., 2015; Liu et al., 2021; Sher et al., 2022). At present, there are still very few specific reports on Se application in the soybean yield. In the principal component analysis, this increase in yield, correlated better with Se in the grains of soybean, when the plant was grown in soil fertilized with C-MAP + Se fertilizer (Figure 5A). In the work carried out by Deng et al. (2021), soil Se application also increased soybean yield compared with a control treatment. Previous studies have shown that Se can improve growth and increase antioxidant capacity in plants, which can affect yield, mainly when plants are exposed to stress factors (Boldrin et al., 2013; Nawaz et al., 2015; Mateus et al., 2021; Ravello et al., 2021).

Enzymes

It has previously been established that Se can mitigate oxidative stress due to ROS regulation. This regulation can occur by stimulating the dismutation of O_2^- into H_2O_2, by the regulation of enzymatic and non-enzymatic compounds, by the direct elimination of ROS by Se species, and by regulation of photosynthetic compounds (Silva et al., 2020). With Se application via C-MAP + Se, the MDA production was negatively correlated with Se content in grains, i.e., the production of MDA by leaves was lower as the Se content in grains increased (Supplementary Figure 1A). This reduction in MDA production demonstrates a clear ability to control ROS and thus oxidative stress, maintaining the integrity of cell membranes, allowing the maintenance of photosynthetic and productive performance of the plant, in addition to increasing Se contents in grains.

The activity of SOD and CAT enzymes was not influenced by the Se application, yet the formation of hydrogen peroxide was higher with the application of C-MAP, compared with E-MAP + Se in all genotypes (Table 1). In addition, the genotype TMG7061 was more sensitive to this change than the others, resulting in higher production of MDA when C-MAP was applied. However, the higher production of hydrogen peroxide acted as a priming/beneficial stress effect, allowing the plant to adjust for grain yield, not exceeding its limit of physiological plasticity capacity, which could lead to a decrease in productivity (Agathokleous et al., 2020). According to the PCA and the Pearson's correlation matrix, ascorbate peroxidase activity in plants treated with Se application via E-MAP is positively correlated with Se content in grains. Lessa et al. (2020) showed that CAT, SOD, and APX activity had minimal interference from Se application via soil or leaf in rice, at a dose of 80 g ha^{-1}.

According to Djanaguiraman et al. (2005), Se foliar application to soybean (50 ppm) increased the activity of SOD, glutathione peroxidase (GSH-Px), and proline, causing a decrease in lipid peroxidation and the reduction in plant senescence. The activity of stress mitigation enzymes, such as SOD, is increased under conditions with high ROS production. Moreover, with adequate levels of Se, the enzyme GSH-Px acts on the spontaneous reduction of O˙2- (Hartikainen et al., 2000; Feng et al., 2013).

Nutritional quality of grains and Se content in the soil

The average Se content found in the studied soil (0.73 mg dm^{-3}) is within the range of Se contents reported for soils of the State of São Paulo (where the Uva farm is located), which varies from <0.08–1.61 mg dm^{-3}. Soil Se content is influenced by characteristics such as pH (Schiavon et al., 2020), presence of competing ions such as sulfate and phosphate (Lessa et al., 2016; Santos et al., 2022), soil texture (Araujo et al., 2018), organic matter (Li et al., 2017), and presence of microorganisms (Gregorio et al., 2006).

Selenium content in grains harvested in the first crop season was higher in all genotypes with Se application, either via C-MAP + Se or via E-MAP + Se (Figure 3A).

Considering the daily soybean intake of 50 g per person and the concentration of 2.37 mg kg^{-1} of Se in grains with the application of E-MAP + Se in the N5909 genotype, the concentration of Se ingested would be 118.5 µg day^{-1}, a value that lies above the average daily intake of Se recommended for adults (70 µg day^{-1}; Kipp et al., 2015).

The consumption of soybean by humans, for the most part, occurs indirectly as in the case of soybean sauce. The production of soybean sauce using biofortified soybean with Se is an alternative to increasing the Se intake by population utilizing supplementation of dietary change. Indeed, soybean sauce represents a strong antioxidant system, which keeps Se stable and non-toxic during storage (Gao et al., 2019, 2022). A study carried out by Gao et al. (2022) showed that soybean sauce produced from soybeans containing 259 µg kg^{-1} of Se contains 79.2 µg kg^{-1} of Se, with 24.8% being inorganic Se and 75.2% existing as organic Se form. This suggests that it is possible to produce a biofortified sauce using Se-enriched soybeans in the field.

The Se recovery observed in soybean ranged from 6.49 to 9.74% (Figure 3B). These values were higher than those reported by Lessa et al. (2020), who worked with soil Se fertilization in rice (maximum recovery = 2.7) and by Lara et al. (2019), who studied foliar application of Se in wheat (maximum recovery = 3%). This higher Se recovery by soybean grains can be attributed to its high protein concentration (about 40%). In the plant, sulfur present in selected amino acids can be replaced by Se, forming selenoaminoacids, which later form selenoproteins (White, 2016). Chan et al. (2010) found that selenospecies - including SeCys and SeMet - represent about 74% of the Se total in soybean grains, when this crop was treated with sodium selenite. Again, such results reinforce that soybean is an effective species when considering the biofortification of crops with Se.

Another factor that may have contributed to the greater Se recovery in soybean is the Se application associated with phosphate fertilizer. According to Qingyun et al. (2016), soils with nutrient deficiencies, especially P, may lead to reduced accumulation of Se in grains by crops. Phosphorus in soils occurs in anionic forms, which means that Se (as selenite-$NaSeO_4^-$) might compete with phosphate molecules for adsorption sites. However, the rates of phosphate fertilizers are much higher (nearly three orders of magnitude) than the amount of Se applied in this trial, making the retention of P more likely in these soils instead of the retention of Se.

In tropical soils, this competition between phosphate and selenite as well as between selenate and sulfate due to chemical similarities between them is acknowledged in the literature (Lessa et al., 2016; Lopes et al., 2017). The selenate adsorption process occurs mainly via formation of outer-sphere complexes, i.e., thru non-specific adsorption. However, for selenite, the formation of inner-sphere complexes occurs with the exchange of ligands, as well as phosphate, which for the most part is irreversible (McBride, 1994).

In the 2019/2020 cropping season, Se content in grains was lower (0.54 mg kg^{-1}) than the first season, and there was no difference among treatments (Supplementary Table 1). This shows that there is a low residual effect of the soil-applied Se in the 2018/2019 season, irrespectively of the fertilizer applied, mainly after the cultivation of a winter crop (wheat). The low residual effect can be confirmed by the low Se concentration found in the soil in the R4 development phase (soil sampling time) during the first crop season. Indeed, studies have reported that part of the soil-applied Se can be fixed within a few months after application, making it unavailable for plant uptake (Gissel-Nielsen and Bisbjerg, 1970; Mikkelsen et al., 1989), which might be especially relevant for the case of the oxidic soil used in this study.

When applied as selenate, Se is found to be more available in soils than selenite in the short term. However, over time, SeVI can be reduced to lower valence state species (e.g., SeIV), leading to further adsorption of the reduced species onto surfaces, including Fe/Mn/Al oxides. This effect occurs faster in acidic soils than in alkaline soils (Wang et al., 2017). Indeed, Ramkissoon et al. (2021) have reported that when selenate was applied in an Oxisol (pH = 6.8 and clay = 52%), 75% was adsorbed during the first day, which impaired the quantification of soluble Se 300 days after the application. The authors presumed that the oxides present in soil were responsible for Se sorption in this case. By contrast, soluble Se have decreased only 29% on a calcareous soil (pH = 8.2 and clay = 13%) after 300 days (Ramkissoon et al., 2021). This fact supports our findings, indicating that the low residual effect of Se at the second season is most likely related to selenium adsorption by soil.

In soils with low Se concentration (e.g., tropical regions), Se supply via fertilization is essential for biofortification strategies, especially in areas with no or low Se addition. However, the beneficial effects of fertilizer Se carried out in one season does not persist and that successive applications, associated with the application of other oxyanions that can compete with Se for oxidic sorption sites (e.g., phosphate and sulfate) as well as the addition of organic compounds via soil tend to increase the residual effect of Se in the soil (Qingyun et al., 2016). Indeed, the application of NPK fertilizer, associated or not with organic compost, has been reported to increase Se availability by 38.39 and 33.04% over 20 years (Qingyun et al., 2016).

The amount of total protein in soybeans was not increased by Se treatment. This fact supports the findings made by Yang et al. (2003) and Deng et al. (2022). However, the application of C-MAP + Se increased the free total amino acid content in genotypes N5909, Lança, and TMG7061. The results were consistent with previous studies indicating that an increase of Se in the crop could promote amino acids synthesis and thus improve amino acid content of Se-enriched soybean grains (Zhao et al., 2019).

Conclusion

This present study showed that the application of C-MAP + Se and E-MAP + Se fertilizers is a promising method for biofortifying soybean with Se in tropical soils. This fact was especially relevant in the TMG7061 genotype when, the application of these fertilizers increases crop yield. In addition, the TMG7061 genotype showed greater recovery of Se by the grains. In summary, soybean is a good crop to be used in biofortification programs due to its high protein content and high capacity of Se recovery by the grains. Lastly, it is noteworthy the positive effect of the application of C-MAP + Se in grain quality, as it not only increased Se but also the amino acids content in the grains.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

MAS: conceptualization, resources, writing-original draft, and writing-review and editing. GFS: conceptualization, resources, and writing-review and editing. APBC, JLL, and GSD: resources and writing-review and editing. CO: writing-review and editing. GL, DA, and PB: conceptualization and writing- review and editing. LRGG: conceptualization, funding acquisition, resources, and writing-review and editing. All authors contributed to the article and approved the submitted version.

Funding

This work was financially supported by funds from the National Council for Scientific and Technological Development (CNPq), the Coordination for the Improvement of Higher

Education Personnel (CAPES), the Foundation for Research of the State of Minas Gerais (FAPEMIG), and ICL South American Group.

Acknowledgments

We are grateful to CNPq, CAPES, and FAPEMIG from Brazil for their financial support to the research project and for scholarships. In addition, we thank Uva Farm (Elizana Baldissera) and ICL for their help with logistics and for supplying equipment and technical support for field experiments.

Conflict of interest

GSD was employed by ICL South American.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

GSD declares that this study received support from ICL South America. The funder had the following involvement in the study: providing support for travel expenses to/from the field experiments and helping with sample collection.

Publisher's note

References

Agathokleous, E., Kitao, M., and Calabrese, E. J. (2020). Hormesis: highly generalizable and beyond laboratory. Trends Plant Sci. 25, 1076–1086. doi: 10.1016/j. tplants.2020.05.006

Alvares, C. A., Stape, J. L., Sentelhas, P. C., de Gonçalves, J. L. M., and Sparovek, G. (2013). Köppen's climate classification map for Brazil. Meteorol. Z. 22, 711–728. doi: 10.1127/0941-2948/2013/0507

Andrade, F. R., Da Silva, G. N., Guimarães, K. C., Barreto, H. B. F., De Souza, K. R. D., Guilherme, L. R. G., et al. (2018). Selenium protects rice plants from water deficit stress. Ecotoxicol. Environ. Saf. 164, 562–570. doi: 10.1016/j.ecoenv.2018.08.022

Araujo, A. M., Lessa, J. H. D. L., Ferreira, L. A., Guilherme, L. R. G., and Lopes, G. (2018). Soil management and ionic strength on selenate retention in oxidic soils. Cienc. Agrotecnol. 42, 395–407. doi: 10.1590/1413-70542018424007318

Avery, J. C., and Hoffmann, P. R. (2018). Selenium, selenoproteins, and immunity. Nutrients 10:1203. doi: 10.3390/nu10091203

Boldrin, P. F., Faquin, V., Ramos, S. J., Boldrin, K. V. F., Ávila, F. W., and Guilherme, L. R. G. (2013). Soil and foliar application of selenium in rice biofortification. J. Food Compos. Anal. 31, 238–244. doi: 10.1016/j.jfca.2013.06.002

Boldrin, P. F., Faquin, V., Ramos, S. J., Guilherme, L. R. G., Bastos, C. E. A., Carvalho, G. S., et al. (2012). Selenato e selenito na produção e biofortificação agronômica com selênio em arroz. Pesqui. Agropecu. Bras. 47, 831–837. doi: 10.1590/S0100-204X2012000600014

Brazil (2016). Normative instruction n° 46. Minist. Agric. Livestock. Supply, 1–44.

Brazilian Agricultural Research Company (EMBRAPA) (1997). Manual de Métodos de Análise de Solo 2nd Edn. Rio de Janeiro: Centro Nacional de Pesquisa de Solos, 212p

Bremner, J. M. (1996). "Nitrogen total". In Methods of Soil Analysis: Part 3 Chemical Methods. ed. J. M. Bartels (Wisconsin, U.S.A: SSSA), 1085–1121.

Brown, P. H., Zhao, F. J., and Dobermann, A. (2021). What is a plant nutrient? Changing definitions to advance science and innovation in plant nutrition. Plant Soil 476, 11–23. doi: 10.1007/s11104-021-05171-w

Buege, J. A., and Aust, S. D. (1978). Biomembranes - Part C: Biological Oxidations. Methods Enzymol. 52, 302–310. doi: 10.1016/S0076-6879(78)52032-6

Cakmak, I. (2008). Enrichment of cereal grains with zinc: agronomic or genetic biofortification? Plant Soil 302, 1–17. doi: 10.1007/s11104-007-9466-3

Chan, Q., Afton, S. E., and Caruso, J. A. (2010). Selenium speciation profiles in selenite-enriched soybean (Glycine max) by HPLC-ICPMS and ESI-ITMS. Metallomics 2, 147–153. doi: 10.1039/b916194e

Conab (2022). Acompanhamento da Safra Brasileira. Bol. da Safra 2021. Brasília: Conab 9, 60. Available at: https://www.conab.gov.br/info-agro/safras/

Deng, X., Liao, J., Zhao, Z., Qin, Y., and Liu, X. (2022). Distribution and speciation of selenium in soybean proteins and its effect on protein structure and functionality. Food Chem. 370:130982. doi: 10.1016/j.foodchem.2021.130982

Deng, X., Zhao, Z., Lv, C., Zhang, Z. Z., Yuan, L. X., and Liu, X. (2021). Effects of sulfur application on selenium uptake and seed selenium speciation in soybean (*Glycine max* L.) grown in different soil types. Ecotoxicol. Environ. Saf. 209:111790. doi: 10.1016/j.ecoenv.2020.111790

Djanaguiraman, M., Devi, D. D., Shanker, A. K., Sheeba, J. A., and Bangarusamy, U. (2005). Selenium-An antioxidative protectant in soybean during senescence. Plant Soil 272, 77–86. doi: 10.1007/s11104-004-4039-1

Feng, R., Wei, C., and Tu, S. (2013). The roles of selenium in protecting plants against abiotic stresses. Environ. Exp. Bot. 87, 58–68. doi: 10.1016/j.envexpbot. 2012.09.002

Gao, X., Liu, E., Zhang, J., Yang, M., Chen, S., Liu, Z., et al. (2019). Effects of sonication during moromi fermentation on antioxidant activities of compounds in raw soy sauce. LWT 116:108605. doi: 10.1016/j.lwt.2019.108605

Gao, X., Shan, P., Feng, T., Zhang, L., He, P., Ran, J., et al. (2022). Enhancing selenium and key flavor compounds contents in soy sauce using selenium-enriched soybean. J. Food Compos. Anal. 106:104299. doi: 10.1016/j.jfca.2021.104299

Giannopolitis, C. N., and Ries, S. (1977). Superoxide dismutases. Plant Physiol. 44, 316–317. doi: 10.1080/09553008314551231

Gissel-Nielsen, G., and Bisbjerg, B. (1970). The uptake of applied selenium by agricultural plants-2. The utilization of various selenium compounds. Plant Soil 32, 382–396. doi: 10.1007/BF01372878

Gregorio, S., Lampis Di, S., Malorgio, F., Petruzzelli, G., Pezzarossa, B., and Vallini, G. (2006). Brassica juncea can improve selenite and selenate abatement in selenium contaminated soils through the aid of its rhizospheric bacterial population. Plant Soil 285, 233–244. doi: 10.1007/s11104-006-9010-x

Hartikainen, H., Xue, T., and Piironen, V. (2000). Selenium as an anti-oxidant and pro-oxidant in ryegrass. Plant Soil 225, 193–200. doi: 10.1023/A:1026512921026

Havir, E. A., and McHale, N. A. (1987). Biochemical and developmental characterization of multiple forms of catalase in tobacco leaves. Plant Physiol. 84, 450–455. doi: 10.1104/pp.84.2.450

Kipp, A. P., Strohm, D., Brigelius-Flohé, R., Schomburg, L., Bechthold, A., Leschik-Bonnet, E., et al. (2015). Revised reference values for selenium intake. J. Trace Elem. Med. Biol. 32, 195–199. doi: 10.1016/j.jtemb.2015.07.005

Lara, T. S., Lessa, J. H. D. L., De Souza, K. R. D., Corguinha, A. P. B., Martins, F. A. D., Lopes, G., et al. (2019). Selenium biofortification of wheat grain via foliar application and its effect on plant metabolism. J. Food Compos. Anal. 81, 10–18. doi: 10.1016/j.jfca.2019.05.002

Lessa, J. H. D. L., Araujo, A. M., Silva, G. N. T., Guilherme, L. R. G., and Lopes, G. (2016). Adsorption-desorption reactions of selenium (VI) in tropical cultivated and uncultivated soils under Cerrado biome. Chemosphere 164, 271–277. doi: 10.1016/j. chemosphere.2016.08.106

Lessa, J. H. D. L., Raymundo, J. F., Branco Corguinha, A. P., Dias Martins, F. A., Araujo, A. M., Melo Santiago, F. E., et al. (2020). Strategies for applying selenium for biofortification of rice in tropical soils and their effect on element accumulation and distribution in grains. J. Cereal Sci. 96:103125. doi: 10.1016/j.jcs.2020.103125

Li, Z., Liang, D., Peng, Q., Cui, Z., Huang, J., and Lin, Z. (2017). Interaction between selenium and soil organic matter and its impact on soil selenium bioavailability: a review. Geoderma 295, 69–79. doi: 10.1016/j.geoderma.2017.02.019

Liu, Y., Huang, S., Jiang, Z., Wang, Y., and Zhang, Z. (2021). Selenium biofortification modulates plant growth, microelement and heavy metal concentrations, selenium uptake, and accumulation in black-grained wheat. Front. Plant Sci. 12, 1–15. doi: 10.3389/fpls.2021.748523

Lopes, G., Ávila, F. W., and Guilherme, L. R. G. (2017). Selenium behavior in the soil environment and its implication for human health. Ciênc. Agrotecnol. 41, 605–615. doi: 10.1590/1413-70542017416000517

Lyons, G. H., Genc, Y., Soole, K., Stangoulis, J. C. R., Liu, F., and Graham, R. D. (2009). Selenium increases seed production in brassica. Plant Soil 318, 73–80. doi: 10.1007/s11104-008-9818-7

Mateus, M. P. D. B., Tavanti, R. F. R., Tavanti, T. R., Santos, E. F., Jalal, A., and Reis, A. R. D. (2021). Selenium biofortification enhances ROS scavenge system increasing yield of coffee plants. Ecotoxicol. Environ. Saf. 209, 111772. doi: 10.1016/j. ecoenv.2020.111772

McBride, M. B. (1994). Environmental Chemistry of Soils. New York: Oxford University Press.

Mikkelsen, R. L., Page, A. L., and Bingham, F. T. (1989). “Factors affecting selenium accumulation by agricultural crops” in Selenium in Agriculture and the Environment. ed. L. W. Jacobs (USA: American Society of Agronomy). 65–94.

Mora, M., Durán, P., Acuña, J., Cartes, P., Demanet, R., and Gianfreda, L. (2015). Improving selenium status in plant nutrition and quality. J. Soil Sci. Plant Nutr. 15:41. doi: 10.4067/S0718-95162015005000041

Nakano, Y., and Asada, K. (1981). Hydrogen peroxide is scavenged by ascorbatespecific peroxidase in spinach chloroplasts. Plant Cell Physiol. 22, 867–880. doi: 10.1093/oxfordjournals.pcp.a076232

Nawaz, F., Ahmad, R., Ashraf, M. Y. Y., Waraich, E. A. A., and Khan, S. Z. Z. (2015). Effect of selenium foliar spray on physiological and biochemical processes and chemical constituents of wheat under drought stress. Ecotoxicol. Environ. Saf. 113, 191–200. doi: 10.1016/j.ecoenv.2014.12.003

Oliver, M. A., and Gregory, P. J. (2015). Soil, food security and human health: A review. Eur. J. Soil Sci. 66, 257–276. doi: 10.1111/ejss.12216

Qingyun, W., Jiabao, Z., Bingzi, Z., Xiuli, X. I. N., Xihai, D., and Zhang, H. (2016). Influence of long-term fertilization on selenium accumulation. Pedosph. An Int. J. 26, 120–129. doi: 10.1016/S1002-0160(15)60028-5

R Core Team (2020). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna.

Ramkissoon, C., Degryse, F., Young, S., Bailey, E. H., and McLaughlin, M. J. (2021). Effect of soil properties on time-dependent fixation (ageing) of selenate. Geoderma 383:114741. doi: 10.1016/j.geoderma.2020.114741

Ramos, S. J., Faquin, V., De Almeida, H. J., Avila, F. W., Guilherme, L. R. G., Alves Bastos, C. E., et al. (2011). Selenate and selenite on yield, mineral nutrition and biofortification with selenium in lettuce cultivars. Rev. Bras. Cienc. Do Solo 35, 1347–1355. doi: 10.1590/S0100-204X2012000600014

Ravello, R. A. V., de Oliveira, C., Lessa, J., Boas, L. V. V., de Castro, E. M., Guilherme, L. R. G., et al. (2021). Selenium application influenced selenium biofortification and physiological traits in water-deficit common bean plants. Crop Past. Sci. 73, 44–55. doi: 10.1071/CP20519

Rayman, M. P. (2012). Selenium and human health. Lancet 379, 1256–1268. doi: 10.1016/S0140-6736(11)61452-9

Santos, M. J. V. D., Lessa, J. H. D. L., De Assis, M. B., Raymundo, J. F., Ribeiro, B. T., Guilherme, L. R. G., et al. (2022). Selenium desorption in tropical soils by sulfate and phosphate, and selenium biofortification of Mombaça grass under increasing rates of phosphate fertilisation. Crop Past. Sci. 73, 56–66. doi: 10.1071/CP21059

Schiavon, M., Nardi, S., Dalla Vecchia, F., and Ertani, A. (2020). Selenium biofortification in the 21st century: status and challenges for healthy human nutrition. Plant Soil 453, 245–270. doi: 10.1007/s11104-020-04635-9

Sher, A., Ul-Allah, S., Sattar, A., Ijaz, M., Ahmad, W., Bibi, Y., et al. (2022). The effect of selenium concentration on the quantitative and qualitative yield of four safflower (*Carthamus tinctorius* L.) genotypes. J. Soil Sci. Plant Nutr. 22, 2663–2669. doi: 10.1007/s42729-022-00834-8

Silva, V. M., Rimoldi Tavanti, R. F., Gratão, P. L., Alcock, T. D., and dos Reis, A. R. (2020). Selenate and selenite affect photosynthetic pigments and ROS scavenging through distinct mechanisms in cowpea (*Vigna unguiculata* (L.) walp) plants. Ecotoxicol. Environ. Saf. 201:110777. doi: 10.1016/j.ecoenv.2020.110777

Soil Survey Staff, United States Department of Agriculture Natural Resources Conservation Service (2014). Keys to soil taxonomy, 360 p. Available at: http://www. nrcs.usda.gov/Internet/FSE_DOCUMENTS/nrcs142p2_051546.pdf

Song, D., Tariq, A., Pan, K., Khan, S. U., Saleh, T. A., Gong, S., et al. (2020). Influence of planting distance and density on the yield and photosynthetic traits of sweet potato (*Ipomoea balatas* L.) under an intercropping system with walnut (*Juglans regia*) saplings. Soil Tillage Res. 196, 104484. doi: 10.1016/j. still.2019.104484

Thavarajah, D., Thavarajah, P., Vial, E., Gebhardt, M., Lacher, C., Kumar, S., et al. (2015). Will selenium increase lentil (*Lens culinaris* Medik) yield and seed quality? Front. Plant Sci. 6, 1–9. doi: 10.3389/fpls.2015.00356

Usepa, U. S. E. P. A. (2007). Test methods for evaluation solid waste physical and chemical methods: microwave assisted acid digest of sediments, sludges, soils and oils. SW-846. Washington, U.S., Gov. Print. Off., 1–30.

Velikova, V., Yordanov, I., and Edreva, A. (2000). Oxidative stress and some antioxidant systems in acid rain-treated bean plants. Plant Sci. 151, 59–66. doi: 10.1016/s0168-9452(99)00197-1

Wang, D., Zhou, F., Yang, W., Peng, Q., Man, N., and Liang, D. (2017). Selenate redistribution during aging in different Chinese soils and the dominant influential factors. Chemosphere 182, 284–292. doi: 10.1016/j.chemosphere.2017.05.014 White, P. J. (2016).

Selenium accumulation by plants. Ann. Bot. 117, mcv180– mcv235. doi: 10.1093/aob/mcv180 White, P. J., and Broadley, M. R. (2009). Biofortification of crops with seven mineral elements often lacking in human diets-iron, zinc, copper, calcium, magnesium, selenium and iodine. New Phytol. 182, 49–84. doi: 10.1111/j.1469-8137.2008.02738.x

Yang, F., Chen, L., Hu, Q., Pan, G., Science, E., Yang, F., et al. (2003). Effect of the application of selenium on selenium content of soybean and its products. Biol. Trace Elem. Res. 93, 249–256. doi: 10.1385/BTER:93:1-3:249

Yao, Y., Pei, F., and Kang, P. (2011). Selenium, iodine, and the relation with Kashin-Beck disease. Nutrition 27, 1095–1100. doi: 10.1016/j.nut.2011.03.002

Yemm, E. W., Cocking, E. C., and Ricketts, R. E. (1954). Estimation of amino acids by ninhydrin. Biochem. J. 58, 7–8.

Zhao, X., Zhao, Q., Chen, H., and Xiong, H. (2019). Distribution and effects of natural selenium in soybean proteins and its protective role in soybean β -conglycinin (7S globulins) under AAPH-induced oxidative stress. Food Chem. 272, 201–209. doi: 10.1016/j.foodchem.2018.08.039

Supplementary material

Supplementary Table 1. Analysis of variance (ANOVA).

Variable	Fertilizer	Genotype	Fertilizer x Genotype
Yield 2018/2019	*	***	***
Yield 2019/2020	ns	ns	ns
Selenium 2018/2019	***	ns	**
Selenium 2019/2020	ns	ns	ns
Selenium recovery	ns	*	ns
Selenium in soil	ns	ns	ns
Amino acids	***	***	***
Nitrogen	***	***	***
Protein	***	***	***
SOD	ns	***	ns
CAT	ns	*	ns
APX	ns	ns	ns
MDA	ns	ns	**
H_2O_2	**	***	ns

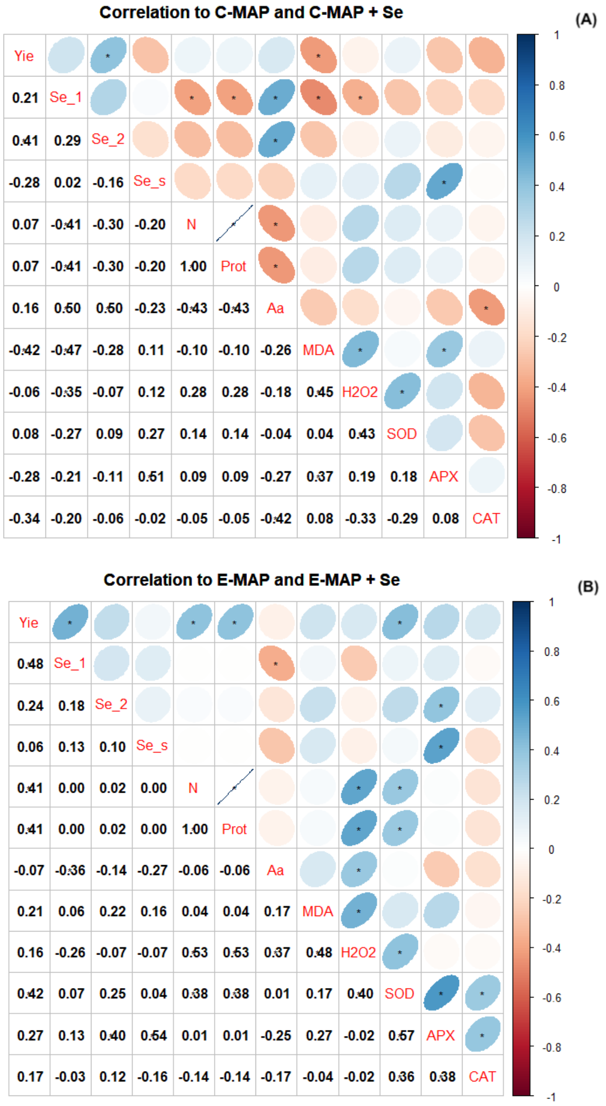
* Significance 0.05; ** Significance 0.01; *** Significance 0.001; ns = no significance by F test.

Supplementary Table 2. Selenium in soil, Se in grain (2019/2020 season), N content, and S content in grains.

Genotype	Fertilizer	Selenium in soil (mg dm^{-3})	Selenium in grain - 2019/2020 (mg kg^{-1})	N (g kg^{-1})
M5917	C_MAP	0.558 ± 0.28	0.445 ± 0.01	66.38 ± 0.70
TMG7061		0.924 ± 0.10	0.473 ± 0.08	46.11 ± 0.37
N5909		0.782 ± 0.24	0.624 ± 0.06	46.90 ± 0.34
58I60 LANÇA		0.623 ± 0.11	0.563 ± 0.09	56.14 ± 0.74
M5917	C-MAP + Se	0.950 ± 0.30	0.614 ± 0.06	49.23 ± 0.53
TMG7061		0.705 ± 0.18	0.602 ± 0.06	45.88 ± 0.54
N5909		0.724 ± 0.33	0.598 ± 0.07	46.83 ± 0.31
58I60 LANÇA		0.488 ± 0.16	0.699 ± 0.04	48.70 ± 0.21
M5917	E-MAP	0.911 ± 0.23	0.488 ± 0.09	49.61 ± 0.47
TMG7061		0.765 ± 0.17	0.361 ± 0.04	47.08 ± 0.09
N5909		0.614 ± 0.12	0.634 ± 0.13	46.97 ± 0.53

58I60 LANÇA		0.656 ± 0.07	0.469 ± 0.07	49.54 ± 0.59
M5917	E-MAP + Se	0.694 ± 0.19	0.634 ± 0.01	49.95 ± 0.47
TMG7061		0.740 ± 0.23	0.556 ± 0.10	47.45 ± 0.49
N5909		0.976 ± 0.27	0.556 ± 0.06	47.15 ± 0.39
58I60 LANÇA		0.703 ± 0.13	0.477 ± 0.04	49.40 ± 0.24

3.1 Supplementary Figures



Supplementary Figure 1. Pearson's linear correlation matrix to C-MAP and C-MAP + Se (A) and to E-MAP and E-MAP + Se (A). * significant relationship of soil and plant attributes at $p < 0.05$; blue ellipse with right sloping top: positive relationship; red ellipse with left sloping top: negative correlation. Se content in the grain 2018/2019 (Se_1), Se content in the grain 2019/2020 (Se_2), Se in soil (Se_s), yield (Yie) protein in grains (Prot), amino acids in grains (aa), lipid peroxidation (MDA), hydrogen peroxide (H_2O_2), catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX).

ARTICLE 2: Foliar application of selenium associated with a multi-nutrient fertilizer in soybean: yield, grain quality, and critical Se threshold

Published in Plants
(DOI - 10.3390/plants12102028)

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Abstract: Selenium uptake and its content in soybean grains are affected by Se application methods. This study evaluated the impact of Se foliar application combined with a multi-nutrient fertilizer (MNF) on soybean, establishing a Se threshold to better understand the relationship between Se content in grains and yield of two genotypes (58I60 Lança and M5917). Two trials were conducted in a 4 × 2 factorial design: four Se rates (0, 10, 40, 80 g Se ha^{-1}) and two methods of foliar Se application (Se combined or not with MNF). Foliar fertilizers were applied twice, at phenological stages of beginning of pod development and grain filling. Grain yield increased with the application of MNF, yet Se rates increased Se contents linearly up to 80 g Se ha^{-1}, regardless of the use of MNF. Lança and M5917 genotypes had grain Se critical thresholds of 1.0 and 3.0 mg kg^{-1}, respectively. The application of Se favored higher contents of K, P, and S in grains of genotype Lança and higher contents of Mn and Fe in grains of genotype M5917. Our findings highlight the importance of addressing different Se fertilization strategies as well as genotypic variations when assessing the effects of Se on soybean yield and grain quality.

Keywords: Selenate; biofortification; selenium reference values; cereal

1. Introduction

Selenium (Se) is a trace element required by both humans and animals [1]. It acts as a co-factor for antioxidating enzymes (e.g., glutathione peroxidase) and has functions in immune system maintenance, cardiovascular disease reduction, thyroid regulation, detoxification capacity, and anti-cancer and anti-viral action [1,2]. The recommended daily dose of Se for adults is 60–70 μg day^{-1}, and it is estimated that approximately one billion people worldwide are Se deficient [3,4]. Low Se intake in humans has been closely linked to Keshan and Kashin–Beck disease [5,6].

Food biofortification has been an alternative for reducing Se deficiency in the population. Biofortification is a process that increases the content of minerals and vitamins (such as Se, zinc–Zn, iodine–I, and iron–Fe) in edible parts of plants to improve nutritional quality for humans and animals [7–9]. Approximately 25 proteins of the human body have Se as a component [10]. It also aids in the regulation of thyroid hormone, DNA synthesis, and fighting free radicals [10,11]. Selenium deficiency in the body has been linked to several diseases, including neurological disorders, cancer, and heart disease [5,12,13].

Selenium is not considered a nutrient for plants, but it has beneficial effects, such as reducing the stress caused by low temperatures in coffee [14], increasing the yield of wheat [15], increasing protein and amino acid content in soybean [16], and reducing drought stress in common bean plants [17]. Its effects on plants have been studied for approximately 70 years, and several advantages have already been reported [14,18–21]. Due to its beneficial effects on plants, recently a change of the definition of “plant nutrient” has been proposed to start a debate on the inclusion of Se, and other beneficial elements, as a plant nutrient [22].

Selenium bioavailability and transfer into the food chain are influenced by soil biophysical chemistry, presence of competing ions, plant species/genotype, method of application, and Se rates applied [9,21,23,24]. Tropical soils have a high capacity for retaining Se–due to the presence of Fe/Al oxyhydroxides–[25,26], as a result, foliar spray application has been considered as one strategy to overcome Se sorption in soils and to increase the efficiency of biofortification practices [24,27,28].

In contrast to soil application, foliar spray improves Se uptake and recovery efficiency by reducing Se immobilization in the soil and shortening the transport distance of Se from plant roots to shoots [29]. In addition, Se spray associated with a multi-nutrient fertilizer may be a promising strategy for increasing Se uptake/redistribution by plants. However, it is critical to understand the Se content in plant tissue to ensure that it does not reduce yield by causing toxicity. High Se concentrations in plants can cause phytotoxicity by directly affecting metabolism, resulting in chlorosis, cell membrane degradation, senescence, and reduced growth and grain yield [30]. Additionally, in the current Brazilian recommendation systems, there are no reference values (critical threshold) of Se for the soybean crop. Furthermore, it is unclear if there are critical threshold variations among soybean genotypes. These gaps can be filled by Bayesian modeling techniques combined with well-documented databases [31,32].

Soybean (*Glycine max* [L. Merril]) is the most widely grown crop in the world, and its grains are high in protein (about 40 percent) [33]. Soybean grains are used to produce feed for animals, in order to produce meat for human consumption. Furthermore, soybean plays a variety of roles in food processing due to its distinct protein-related food texture, high water-holding capacities, and foaming properties [34,35]. As a result, the current study aimed to assess the impact of Se foliar application associated with the use of a multi-nutrient fertilizer on soybean yield and grain quality. In addition, we want to establish a grain-Se critical threshold to understand the relationship between Se content in soybean grains and the yield of different soybean genotypes.

2. Results

2.1. Variance Components

The results obtained showed a great influence of the genotypes for most of the analyses performed (Figure 1). They were supported by principal component analyses, in which the results were also separated by genotype (Figure S1). On the other hand, Se content in grains was affected mainly by Se rates.

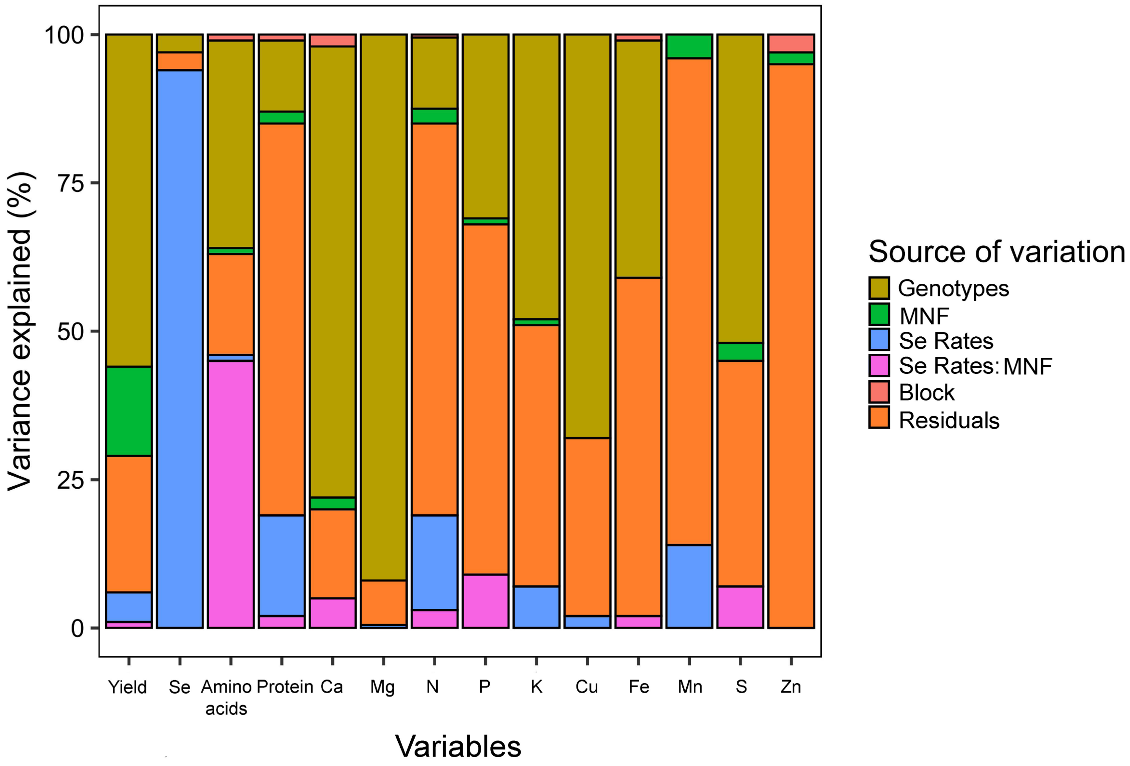

Figure 1. Visual representation of variance components. The colors represent the source of variation (genotypes, MNF, Se rates, block, and residuals). The variation proportion explained by each source of variation for each response variable is observed on the Y-axis (percentage).

2.2. Grain Yield

The spraying of Se rates did not affect grain yield ($p > 0.05$). On the other hand, MNF application influenced the yield of both genotypes ($p < 0.05$) (Figure 2A,B). The application of Se and MNF increased ~0.32 and 0.38 t ha^{-1} of grains (i.e., ~5.37 and 6.26 60-kg bags ha^{-1}) for 58I60 Lança and M5917 genotypes, respectively, compared with the application of Se without MNF. In general, the genotype M5917 showed the highest grain yield – 5.0 t ha^{-1} on average – while the genotype 58I60 Lança produced 4.2 t ha^{-1}. Average yields with the MNF application varied from 4.3 to 4.7 t ha^{-1} for genotype 58I60 Lança, and from 5.0 to 5.4 t ha^{-1} for genotype M5917.

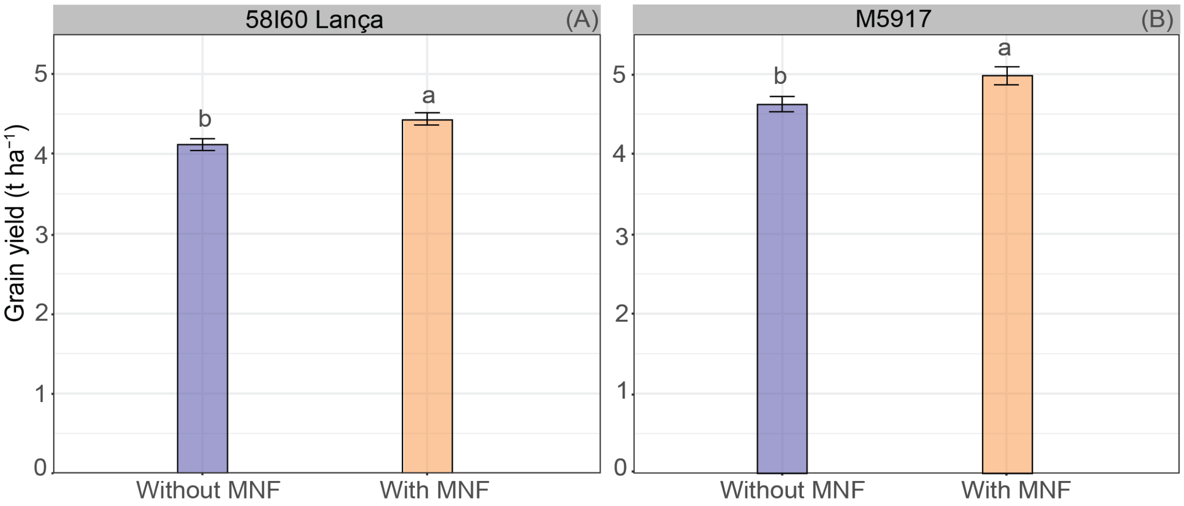

Figure 2. Grain yield (t ha^{-1}) of soybean plants of genotypes 58I60 Lança (**A**) and M5917 (**B**). Lowercase letters compare the application of Se associated or not with MNF, at the 5% significance level, according to Tukey's test. Vertical bars refer to the standard error ($n = 16$).

2.3. Selenium Content and Se Recovery in Grains

Selenium content in grains was affected by the interaction between Se rates and MNF application (Figure 3A,B). In both genotypes, increasing Se rates increased the Se content linearly up to the highest rate (80 g Se ha^{-1}), regardless of the use of MNF. In 58I60 Lança, the increase in grain Se content for each gram of Se applied via foliar was 0.063 mg kg^{-1} with MNF and 0.055 mg kg^{-1} without MNF. In genotype M5917, the Se content in grain increased by 0.087 mg kg^{-1} with MNF and 0.065 mg kg^{-1} without MNF for each gram of Se applied through foliar supply. According to the confidence interval (95%), the application of MNF did not affect the Se content in the soybean grains up to the rate of 43.8 g Se ha^{-1} for genotype 58I60 Lança and 29.4 g Se ha^{-1} for genotype M5917. However, after these Se rates, the association of Se with MNF promoted higher Se content.

The Se recovery rate (%) by soybean grains was also affected by Se foliar rates ($p < 0.05$) (Figure 3C,D). The Se recovery in genotype 58I60 Lança at the rate of 10 g Se ha^{-1} was 18.4%, whereas the application of 80 g Se ha^{-1} promoted 24.1% of Se recovery. The foliar rates of 10 and 80 g Se ha^{-1} showed different Se incorporation by the grains, but both were statistically equal to the foliar rate of 40 g Se ha^{-1}. For genotype M5917, the Se foliar rates of 40 g Se ha^{-1} and 80 g Se ha^{-1} promoted equal Se rate recovery ($p > 0.05$), 31.1% and 37.7%, respectively. The Se recovery obtained with the application of Se foliar at 10 g Se ha^{-1} was 21.5%.

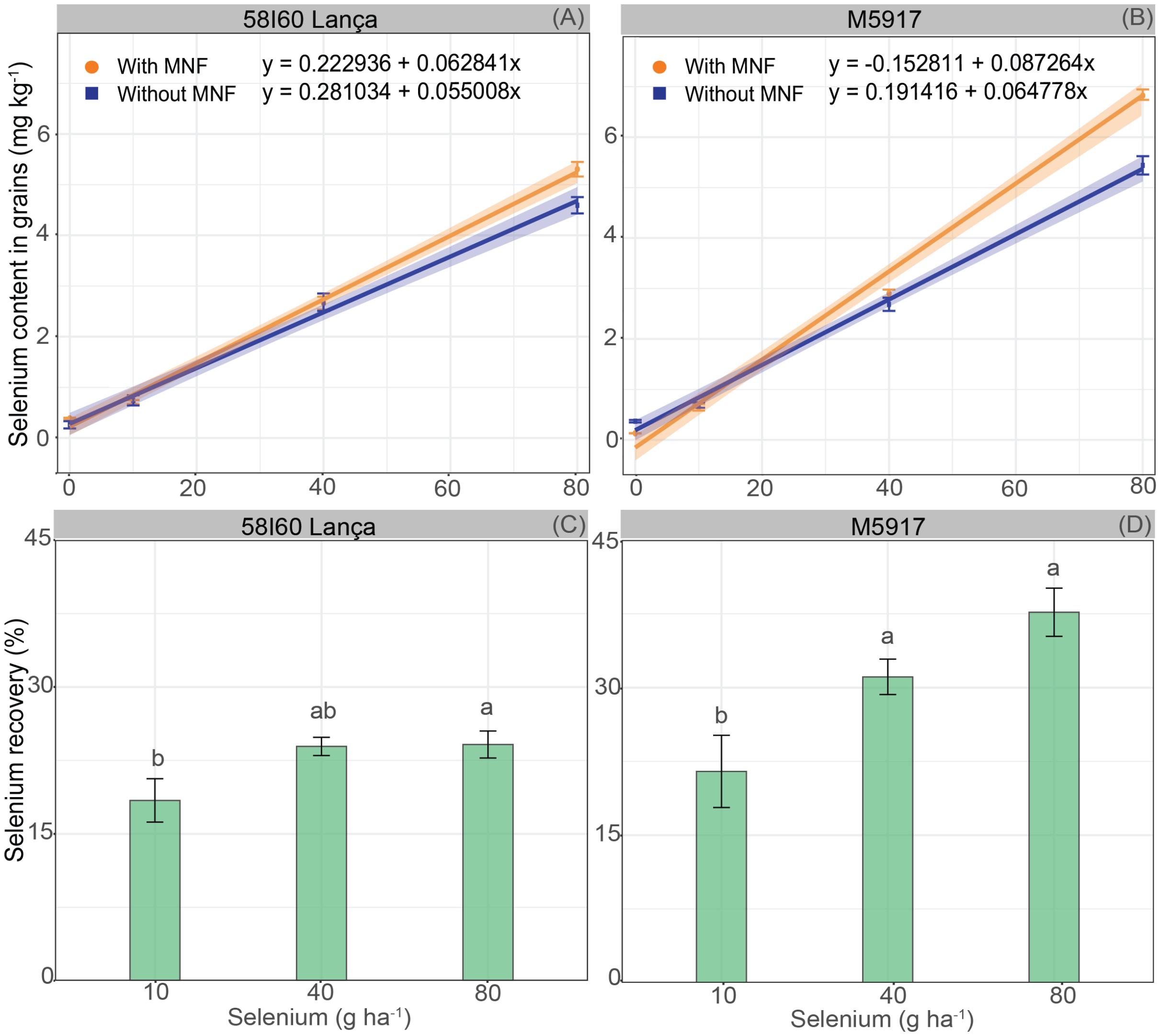

Figure 3. Selenium content in grains (mg kg^{-1}) (**A**,**B**) and Se recovery by soybean grains (%) (**C**,**D**) of soybean plants of genotypes 58I60 Lança and M5917. Lowercase letters compare Se rate, at the 5% significance level, according to Tukey's test. Vertical bars refer to the standard error (n = 4 for selenium content in grains and *n* = 8 for selenium recovery).

2.4. Macronutrients, Micronutrients, Proteins, and Amino Acids

The content of K, P, and S in the grains was affected by the interaction among the studied factors (Se foliar rates and MNF) in the 58I60 Lança genotype ($p < 0.05$) (Table S1). Despite the significant difference in the K and P content in the soybean grains, the data did not fit either the linear or the quadratic regression model. In addition, the content of K and P was higher in the grains that had received Se foliar at 80 g Se ha^{-1} combined with the MNF.

For genotype M5917, the F test for Cu and Zn showed significant differences among Se foliar rates and MNF (Table S1). On the other hand, the content of K, Mn, P, S, and N were affected by the Se rates ($p < 0.05$). The content of Mg, Ca, and Fe was not affected by the factors studied ($p > 0.05$). Following in the genotype M5917, the K content in the grains fitted quadratic regression in the levels of Se supplied via foliar application (R^2 = 77%). The highest K content in grains was 18.57 g Se ha^{-1} and it was obtained by the application of 45.6 g Se ha^{-1}.

Concerning genotype 58I60 Lança, the S content increased linearly upon increasing the Se rate, regardless of the MNF application, with a correlation coefficient of 89% being observed with MNF and 68% without MNF. The MNF promoted a higher accumulation of S in grains, regardless of the Se rate in genotype M5917. The S data were fitted to a quadratic model ($R^2 = 63\%$), and the highest S content was obtained by the rate of 37 g Se ha^{-1}.

In M5917, the interaction between Se rates and MNF affected the Zn and Cu content in grains. Additionally, N and Mn contents were affected by the Se rates, with Se rates providing a quadratic regression fit for N content in grains. Se foliar and MNF application did not influence the N, Cu, and Zn in the 58I60 Lança. Protein contents in soybean grains increased quadratically upon increasing Se rates in the M5917 ($p > 0.05$) (Table S1). The total free amino acids content was affected by the interactions among Se rates and MNF, yet there was a fitted model regression only in genotype 58I60 Lança.

2.5. Selenium Critical Threshold and Selenium Intake (µg person^{-1} day^{-1})

The critical Se threshold in soybean grains was estimated at 1.0 mg kg^{-1} and 3.0 mg kg^{-1} for genotypes Lança and M5917, respectively (Figure 4A,B). This means that above 1.0 mg kg^{-1} of Se in the grain the yield in the genotype 58I60 Lança is reduced, whereas the same effect occurs for genotype M5917 above 3.0 mg kg^{-1}. Therefore, genotype M5917 is more tolerant to Se accumulation in grains than 58I60 Lança.

Figure 4C,D shows the relationship between Se rate and human daily Se intake based on an average recommended intake of soybean protein (25 g $person^{-1}$ day^{-1}). As per the estimated daily Se intakes shown in Figure 4C,D, the rate of Se that should be supplied to the plant in order to obtain a Se content in soybean grains suitable for human consumption (considering a recommended daily intake of 70 µ of Se day^{-1}) would be: 15.1 g Se ha^{-1} (with MNF – genotype 58I60 Lança), 16.2 g Se ha^{-1} (without MNF – genotype 58I60 Lança), 15.3 g Se ha^{-1} (with MNF – genotype M5917), and 15.4 g Se ha^{-1} (without MNF – genotype M5917).

Considering the daily Se consumption by person, the adequate Se rates to produce enriched soybean grains are below the Se content that reduces crop yield (below the Se critical threshold). Indeed, using the model fitted for Se content in grains (Figure 2A,B), Se rates to promote 1 mg kg^{-1} in grains were 19.5 g Se ha^{-1} (with MNF) and 23.3 g Se ha^{-1}

(without MNF) for the Lança. In the genotype M5917, the rates to produce soybean grains with 3 mg kg^{-1} of Se are 36.1 g Se ha^{-1} (with MNF) and 49.3 g Se ha^{-1} (without MNF).

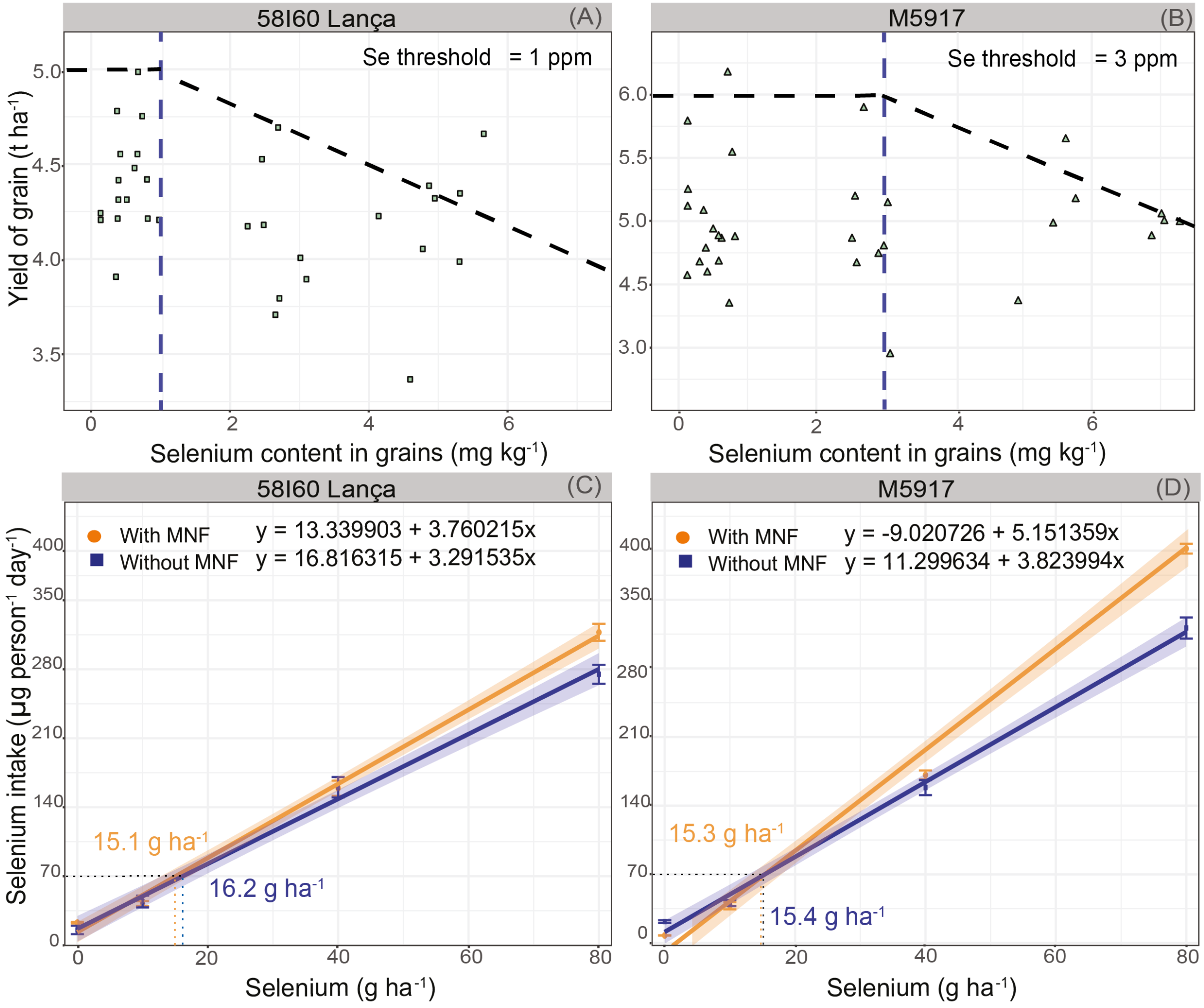

Figure 4. Selenium critical threshold in soybean grains (**A**,**B**) and selenium intake by a person by day (**C**,**D**) in the genotypes 58I60 Lança and M5917. Vertical bars refer to the standard error (n = 4). Triangles and boxes refer to grain yield values. The Blue dotted line refers to the Se critical threshold in the grain.

2.6. Pearson's Correlation Matrix

The correlation between the variables assessed is presented in Figure 5. In genotype Lança, Se content correlated positively with S (R^2 = 65%), P (R^2 = 57%), and K (R^2 = 57%) content in grains. For genotype M5917, the increase of Se in the grains increased Mn content (R^2 = 44%), and Fe (R^2 = 51%). Selenium content in grains negatively affected yield and total free amino acid content in the genotype Lança (R^2 = 29% and 73%, respectively). This effect was not observed for cultivar M5917.

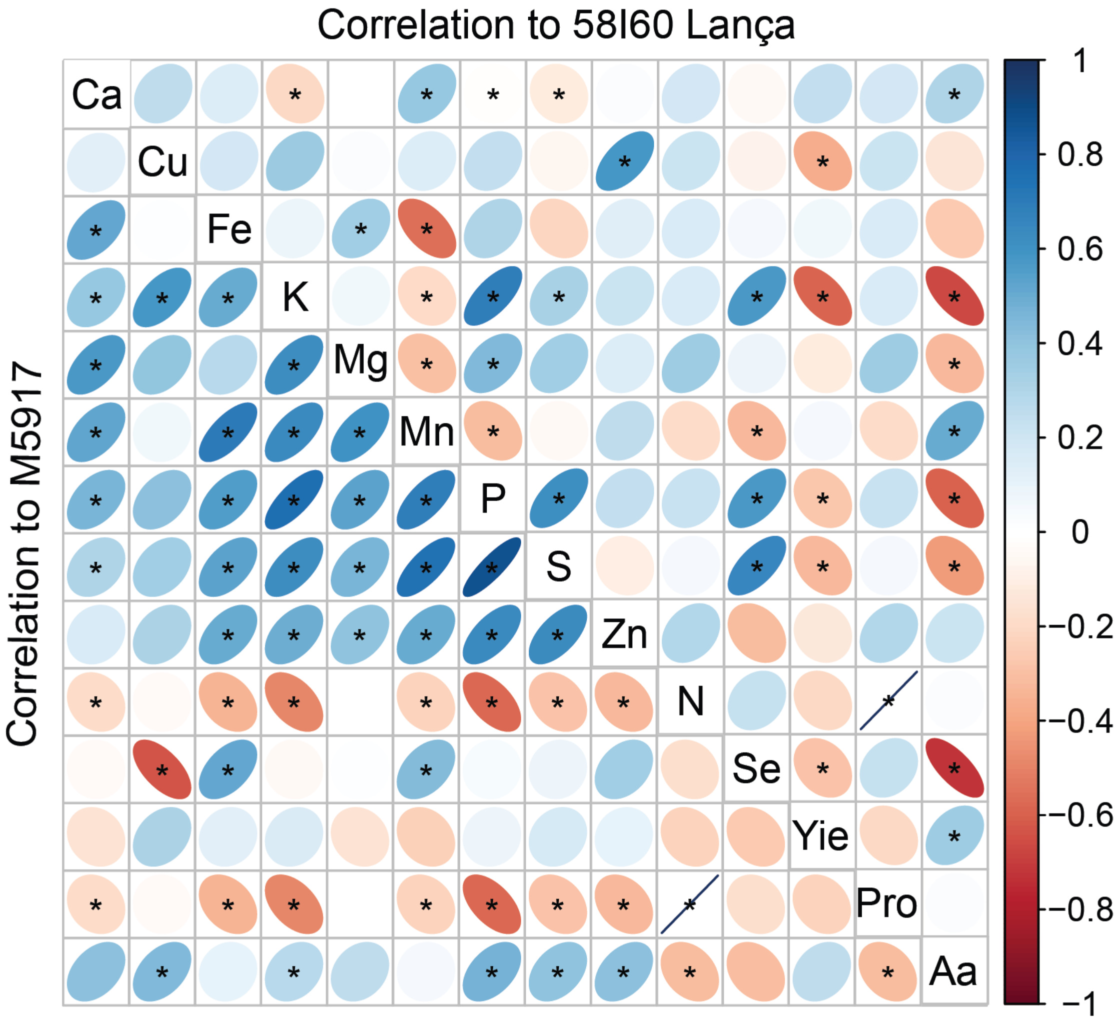

Figure 5. Pearson's correlation matrix among the variables at 58I60 Lança and M5917 genotypes. Yie = yield; Pro = protein; Aa = amino acids. * means a positive Pearson's correlation.

3. Discussion

Selenium is not considered a nutrient for plants, yet its beneficial effects are already well established [22,36]. The increase of the content of a target element (e.g., Se) in the edible part of the plant without reducing yield is one of the key assumptions for the implementation of biofortification programs. Our results showed significant differences in yield only due to the application of MNF (Figure 2A,B). Although there was no difference with Se foliar spray in soybean, some studies showed increased yields of wheat [15,37], rice [19], and coffee [38]. Such increases are mostly observed in plants under biotic and abiotic stress conditions [17,39,40]; discrepancies in results may be attributed to the various growth stages and methods of Se application in plants.

Foliar spraying with Se resulted in a linear increase of the Se content in soybean grains (Figure 3A,B). Similar results were observed in wheat [15] and rice [24]. The lowest rate at which it is possible to see a difference in Se content among the treatments (with MNF and without MNF) is 43.8 kg Se ha^{-1} for Lança and 29.4 kg Se ha^{-1} for genotype M5917, which is about 33% less, indicating that the application is more efficient for the last genotype. This also demonstrates that the interaction of Se foliar and other nutrients can enhance the uptake/redistribution of Se in plants.

Nitrogen is one of the components of MNF and it is known that it can affect Se uptake by plants [41]. The fact that Se and S use the same metabolic pathway in plants can be used to explain this interaction between N and Se. Applications of N enhance O-acetyl serine, an important regulator of S metabolism in cysteine synthesis in plants, which then increases the synthesis of cysteine and protein. Along with N, the MNF also contains Mg, K, and B, which are important nutrients for the transport of photoassimilates within plants [42–44]. Magnesium has a direct impact on the yield and quality of grains. A lack of Mg both affects the transport of assimilates from the leaves to the grains and reduces the transport of amino acids in plants [45].

Previous studies have produced conflicting findings regarding the response of various essential elements to Se treatment in several crops. In rice, Se application combined with N fertilization resulted in higher grain concentrations of N, P, K, Ca, Se, B, Cu, Zn, and Mo [41]. Wheat cultivated under drought stress showed increased Fe content and decreased Zn content on shoot with Se foliar spray [46]. The presence of Se in plants can alter the ionic permeability coefficient in the plasma membrane, thereby altering the transport and accumulation of micronutrients in plant cells [47]. Nonetheless, the mechanisms by which Se interferes with other elements require further investigation.

Several factors influence selenium recovery, including the Se source and the plant species. In a previous study carried out by our research group, the recovery of Se applied via soil was 6.5% for genotype M5917 and 8.1% for genotype Lança [21]. In the current study, the average recovery rate of Se applied via foliar was 24.1% for Lança and 37.7% for M5917 (3–5 times higher), which was remarkably higher than the wheat's maximum recovery of Se by grains (3%) [15].

Changes in the content of the nutrients in grains are shown in Table S1, suggesting potential interactions between Se and these nutrients. In fact, providing Se to plants may affect the foliar absorption of selected nutrients or their redistribution within plants, which in turn affects the concentration of those nutrients in soybean grains. Djanaguiraman et al. (2010) [48] reported that the availability of Se to plants can have an impact on the uptake and accumulation of the nutrients necessary for plant metabolism. The interaction between Se and

S is the most studied, due to the similarity between these elements; they share the same metabolic pathway in plants [49,50]. In general, Se foliar supply affected the content of P and K in grains of the genotype Lança, as well as N, P, K, S, Cu, Mn, and Zn in grains of the genotype M5917. Except for Cu, Mn, and Zn, the other nutrients affected by Se application are those found in MNF.

The critical Se threshold demonstrated that above 1.0 mg kg^{-1} of Se in grain, the yield in the genotype 58I60 Lança is reduced, while genotype M5917 showed this effect only above 3.0 mg Se kg^{-1}, which is a clear indication that genotype M5917 is more tolerant to Se accumulation in grains than 58I60 Lança. Selenium toxicity in plants has not been fully investigated, but it has been demonstrated that tolerance varies depending on plant species and genotypes [30]. Indeed, a previous experiment with two soybean genotypes demonstrated such variation in Se response [51], with the Sonja genotype being more sensitive to Se and displaying a stronger physiological response when compared with the Lucija genotype.

In another study assessing Se toxicity to various crops, brown mustard has shown a greater tolerance to Se when compared with maize, rice, and wheat [52]. From the established critical level of Se by the authors, there was a reduction of 21% in the dry matter of brown mustard, 24% in maize, 11% in rice, and 27% in wheat. These levels where related to the following Se content in the shoot dry matter of the studied crops: 18.9 mg kg^{-1} for wheat, 41.5 mg kg^{-1} for rice, 76.9 mg kg^{-1} for corn, and 104.8 mg kg^{-1} for brown mustard [52].

As seen in our study, Se intake values for biofortifying soybean, considering the selected data for adequate daily intake and average consumption of soy protein, are lower than the level of Se in grains that reduced yields (Figure 4C,D). This fact is relevant since it is possible to biofortify soybeans without decreasing the grain yield of the crop. The dietary habits of the population have a significant impact on Se intake by the population. Excessive human Se intake, typically greater than 400 µg day^{-1}, may lead to toxicity, resulting in health problems. Some of the Se toxicity symptoms are hair and nail loss, skin lesions, nervous system dysfunction, and even death [53,54]. As estimated by the regression equations shown in Figure 4C,D, our findings indicate that lower Se rates can satisfy smaller demands. However, it must be noted that these estimates assume that soybean will be consumed by humans as soybean protein and do not account for possible Se losses during other industrial processes.

4. Materials and Methods

4.1. Growth Conditions and Experimental Design

Two similar trials were conducted on a soybean field during the 2018/2019 cropping season, at Farm Uva, municipality of Capão Bonito, São Paulo state, Brazil (Lat: −24.040934, Lon: −48.262421), with an average annual rainfall of 1628 mm and an average annual temperature of 18.8 ºC, under a humid subtropical climate (Cfa) [55]. The soil is classified as Oxisol (Typic Hapludox) [56] and its chemical and physical characteristics are as follows [57]: pH (H_2O) = 6.0; H + Al = 2.96; Al = 0.06; P (Mehlich-1) = 34.8 mg dm^{-3}; K = 148 mg dm^{-3}; S = 4.11 mg L^{-1}; CEC = 9.83 $cmol_c$ dm^{-3}; Ca = 5.05 $cmol_c$ dm^{-3}; Mg = 1.44 $cmol_c$ dm^{-3}; P-rem = 28.10 mg L^{-1}; organic matter = 2.69 dag dm^{-3}; clay = 510 g kg^{-1}; silt =110 g kg^{-1}; and sand =380 g kg^{-1}.

Each experiment was carried out with one soybean genotype, 58I60 Lança, and M5917. The experiment was arranged in a randomized block with a full factorial design of 4 × 2, being four Se rates (0, 10, 40, and 80 g Se ha^{-1}) and two methods of Se application: i) Se with multi-nutrient fertilizer (hereafter called MNF) and ii) Se without MNF. The experiments were composed of four blocks, totalizing 32 experimental plots for each genotype. The treatments with MNF received 2 kg ha^{-1} of the product; its composition was as follows: nitrogen (N – 5%), phosphorus pentoxide (P_2O_5 – 10%), potassium oxide (K_2O – 20%), magnesium (Mg – 29%), sulfur (S – 12%), and boron (B – 0.5%). Sodium selenate was used to prepare the Se solution (Na_2SeO_4 Sigma-Aldrich, Saint Louis, MO, USA). Foliar spray fertilizers were applied twice, at phenological stage R3 (beginning of pod development) and R5 (grain filling), with each application containing half of the total dose. Sodium selenate and MNF (if used) were diluted in deionized water, mineral oil was added, and a pressured carbon dioxide pump was used to apply it.

The plots were composed of 4 sowing lines with a spacing of 0.5 m between lines and 7 m in length. The collection of material for analysis was carried out in the useful area of the plot. For the composition of the useful area of the plot, 0.5 m from each end of the plot and two lateral lines were disregarded. Fourteen seeds were sown per meter and fertilization was carried out with 16 kg ha^{-1} N, 80 kg ha^{-1} P_2O_5, and 28 kg ha^{-1} K_2O.

4.2. Yield

The grains from the useful area of each experimental plot were harvested after maturity and soybean yield was calculated by weighing the grains and extrapolating to a hectare (kg ha^{-1}), considering 13% moisture. After that, the grains were dried until they reached a constant weight and were ground with an electric hand mill.

4.3. Selenium, Macronutrient, and Micronutrient in Soybean Grains

To determine Se, macronutrients, and micronutrients, 0.5 g of samples were placed in Teflon vessels and 5 mL of HNO_3 (65%) was added. The extract was allowed to stand at room temperature overnight before being digested the next morning (16 h). The vessels were then hermetically sealed and heated in a Mars-5 microwave digestion oven (CEM Corp, Matthews, NC) following the 3051A methodology, proposed by the United States Environmental Protection Agency [58]. The content of macronutrients and micronutrients was obtained using inductively coupled plasma – optical emission spectroscopy (ICP-OES).

Selenium content in the solutions was measured using a graphite furnace atomic absorption spectrometer (Atomic Absorption Spectrometry with Zeeman background correction and EDL lamp for Se; AAnalyst™ 800 AAS, Perkin Elmer, Waltham, United States). The calibration curve for Se measurement was obtained from a standard solution containing 1 g kg^{-1} of pure Se (Fluka, Buchs, Switzerland). To maintain digestion quality, each batch of digestion included standard reference material from the Institute for Reference Materials and Measurements (White Clover–BCR 402, IRMM, Geel, Belgium) and a blank sample. The main recovery value for standard material was 95% (n = 5). The detection limit (LOD) was calculated by taking the standard deviation and mean of 7 blank extracts.

The fraction of the applied Se incorporated in soybean grains (Se recovery) was calculated using Equation (1) described below:

$$\text{Se recovery (\%)} = \frac{(\text{Se treatment - Se control})}{\text{Se rate}} \times 100 \tag{1}$$

where: Se recovery (%) = use efficiency of the Se rates applied in the leaves by soybean grains (Se utilization percentage);

Se treatment (g Se ha^{-1}) = Se content in soybean grains from soybean plants grown in treatments that had received Se applications, considering the yield obtained in each treatment;

Se control (g Se ha^{-1}) = Se content in soybean grains from soybean plants grown in treatments without Se applications, considering the yield obtained in each treatment;

$$\text{Se intake} = (100 \times 25/\ \text{Prot}) \times \text{Se} \tag{2}$$

where: Se intake (µg person^{-1} day^{-1}) = estimated daily Se intake per person;

Se (µg kg^{-1}) = the amount of selenium in soybean grains;

Prot (g) = the average amount of protein in soybean grains (42.4 to Lança and 41.8 to M5917).

4.4. Free Total Amino Acids and Protein

The ninhydrin method was used to determine total free amino acids [59]. The protein content of the grains was calculated by multiplying the N content by 6.25.

4.5. Statistical Analysis

The obtained data were analyzed for normality (Shapiro–Wilk test) and variance homogeneity (Bartlett's test). They were then analyzed using analysis of variance (ANOVA), and when significant, linear, or quadratic regression models were fitted. The linear models were compared to Se content in grains, using a confidence interval, created at 95% of probability. The treatments were compared using the Tukey test ($p < 0.05$) for grain yield and Se recovery analysis. Pearson's linear correlation matrix ($p < 0.05$) was also used to validate clusters and potential relationships of Se rates applied and plant attributes. The R software was used to carry out the analyses [37]. Subsequently, the obtained data were submitted and an analysis of variance components was performed to quantify the percentage contribution of each source of variation (genotypes, MNF, Se rates, block, interactions, and residues) on the total variance of each response variable (yield, protein, amino acids, and nutrients concentration). This statistical procedure was performed using the VCA package of the R statistical environment [60].

In this study, we used Bayesian models, which allow us to explore all the possible regression lines (combinations of intercepts, slopes, and breakpoints). The yield of soybean present in the database was converted into relative yield (%) by taking into consideration each genotype and crop season to develop critical threshold estimation models. Models were developed on the boundary line [61,62] using Bayesian segmented quantile regression [31] to measure the association between dependent variables (yield) and Se concentration in soybean grains. Bayesian analysis was used to adjust the parameters of the regression models [63]. In this adjustment, the Monte Carlo simulation with Markov chains (MCMC) [64] was used based on the Gibbs sampling algorithm, with 20,000 random drawings after a warm-up period of 10,000 iterations. The sampling stage was performed through normal distribution, based on the distribution a posteriori of Se concentration. Modeling was implemented by using the 'rjags' package [65] in the R software (R version 4.2.0) [60]. Critical levels were assumed as the point at which the adjusted line reached the plateau and did not show a further increase in crop yield, with the increase in nutrient concentration. Finally, density frequency was analyzed, at a 90% confidence interval, to determine Se borderline concentrations and highest density.

5. Conclusions

Selenium supply through foliar application in soybean plants is a notably good strategy to improve intake of Se by the population, and an association with other nutrients can improve the efficiency of biofortification strategies. Despite this, Se foliar applications should be used cautiously to avoid Se toxicity and yield loss in plants. The grain yield was higher with MNF application. In both genotypes, the Se rates increased the Se content linearly up to the highest rate (80 g Se ha^{-1}), regardless of the use of MNF. The Lança and M5917 genotypes of soybean grains had Se critical thresholds up to 1.0 mg kg^{-1} and 3.0 mg kg^{-1}, respectively. The application of Se increased the content of K, P, and S in the grains of the genotype 58I60 Lança as well as the content of Mn and Fe in the grains of the genotype M5917. Finally, we recommended applying 15.1 g Se ha^{-1} to genotype 58I60 Lança and 15.3 g Se ha^{-1} to genotype M5917, combined with MNF to improve the Se content in the grains.

Author Contributions: Conceptualization, M.A.S., G.F.d.S., L.R.G.G., J.M.L., and G.L.; field research, M.A.S., G.F.d.S., A.P.B.C., and L.R.G.G.; software, M.A.S., J.M.M.B., and G.B.; formal analysis, M.A.S., G.F.d.S., G.A.Z.V.O., and G.G.A.Z.V.O.; writing—original draft preparation, M.A.S. and G.F.d.S.; writing—review and editing, L.R.G.G., G.B., J.M.M.B., G.L., and A.A.d.S.; and funding acquisition, L.R.G.G. All authors have read and agreed to the published version of the manuscript.

Funding: This work was financially supported by funds from the National Council for Scientific and Technological Development (CNPq) (number 406577/2022-6), the Coordination for the Improvement of Higher Education Personnel (CAPES), the Foundation for Research of the State of Minas Gerais (FAPEMIG), and ICL South American Group.

Data Availability Statement: The data presented in this study are available from the corresponding author upon reasonable request.

Acknowledgments: We are grateful to CNPq, CAPES, and FAPEMIG from Brazil for their financial support for the research project and for scholarships.

Conflicts of Interest: The funders had no role in the design of the study; analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results. JML was employed by ICL South American. The funder had the following involvement in the study: providing support for travel expenses to/from the field experiments and helping with sample collection.

References

1. Rayman, M.P. Selenium and Human Health. *Lancet* **2012**, *379*, 1256–1268.

2. Hoffmann, F.W.; Hashimoto, A.C.; Shafer, L.A.; Dow, S.; Berry, M.J.; Hoffmann, P.R. Dietary Selenium Modulates Activation and Differentiation of CD4+ T Cells in Mice through a Mechanism Involving Cellular Free Thiols. *J. Nutr.* **2010**, *140*, 1155–1161.

3. Kipp, A.P.; Strohm, D.; Brigelius-Flohé, R.; Schomburg, L.; Bechthold, A.; Leschik-Bonnet, E.; Heseker, H. Revised Reference Values for Selenium Intake. *J. Trace Elem. Med. Biol.* **2015**, *32*, 195–199.

4. Mora, M.L.; Durán, P.; Acuña, J.; Cartes, P.; Demanet, R.; Gianfreda, L. Improving Selenium Status in Plant Nutrition and Quality. *J. Soil Sci. Plant Nutr.* **2015**, *15*, 486–503.

5. Combs, G.F. Selenium in Global Food Systems. *Br. J. Nutr.* **2001**, *85*, 517–547.

6. Fairweather-Tait, S.J.; Bao, Y.; Broadley, M.R.; Collings, R.; Ford, D.; Hesketh, J.E.; Hurst, R. Selenium in Human Health and Disease. *Antioxid. Redox Signal.* **2011**, *14*, 1337–1383.

7. Graham, R.D.; Welch, R.M.; Saunders, D.A.; Ortiz-Monasterio, I.; Bouis, H.E.; Bonierbale, M.; de Haan, S.; Burgos, G.; Thiele, G.; Liria, R.; et al. Nutritious Subsistence Food Systems. *Adv. Agron.* **2007**, *92*, 1–74.

8. Cakmak, I. Enrichment of Cereal Grains with Zinc: Agronomic or Genetic Biofortification? *Plant Soil* **2008**, *302*, 1–17.

9. Lopes, G.; Ávila, F.W.; Guilherme, L.R.G. Selenium Behavior in the Soil Environment and Its Implication for Human Health. *Ciênc. Agrotec.* **2017**, *41*, 605–615.

10. Reeves, M.A.; Hoffmann, P.R. The Human Selenoproteome: Recent Insights into Functions and Regulation. *Cell. Mol. Life Sci.* **2009**, *66*, 2457–2478.

11. Pedrero, Z.; Madrid, Y. Novel Approaches for Selenium Speciation in Foodstuffs and Biological Specimens: A Review. *Analytica Chimica Acta* **2009**, *634*, 135–152.

12. Cardoso, B.R.; Roberts, B.R.; Bush, A.I.; Hare, D.J. Selenium, Selenoproteins and Neurodegenerative Diseases. *Metallomics* **2015**, *7*, 1213–1228.

13. Vinceti, M.; Filippini, T.; Del Giovane, C.; Dennert, G.; Zwahlen, M.; Brinkman, M.; Zeegers, M.P.; Horneber, M.; D'Amico, R.; Crespi, C.M. Selenium for Preventing Cancer. *Cochrane Database Syst. Rev.* **2018**.

14. de Sousa, G.F.; Silva, M.A.; de Morais, E.G.; Van Opbergen, G.A.Z.; Van Opbergen, G.G.A.Z.; de Oliveira, R.R.; Amaral, D.; Brown, P.; Chalfun-Junior, A.; Guilherme, L.R.G. Selenium Enhances Chilling Stress Tolerance in Coffee Species by Modulating Nutrient, Carbohydrates, and Amino Acids Content. *Front. Plant Sci.* **2022**, *13*, 1000430.

15. Lara, T.S.; Lessa, J.H.; de Lima Lessa, J.H.; de Souza, K.R.D.; Corguinha, A.P.B.; Martins, F.A.D.; Lopes, G.; Guilherme, L.R.G. Selenium Biofortification of Wheat Grain via Foliar Application and Its Effect on Plant Metabolism. *J. Food Compos. Anal.* **2019**, *81*, 10–18.

16. Zhao, X.; Zhao, Q.; Chen, H.; Xiong, H. Distribution and Effects of Natural Selenium in Soybean Proteins and Its Protective Role in Soybean β-Conglycinin (7S Globulins) under AAPH-Induced Oxidative Stress. *Food Chem.* **2019**, *272*, 201–209.

17. Ravello, R.A.V.; de Oliveira, C.; Lessa, J.; Boas, L.V.V.; de Castro, E.M.; Guilherme, L.R.G.; Lopes, G. Selenium Application Influenced Selenium Biofortification and Physiological Traits in Water-Deficit Common Bean Plants. *Crop Pasture Sci.* **2021**, *73*, 44–55.

18. Ramos, S.J.; Rutzke, M.A.; Hayes, R.J.; Faquin, V.; Guilherme, L.R.G.; Li, L. Selenium Accumulation in Lettuce Germplasm. *Planta* **2011**, *233*, 649–660.

19. Boldrin, P.F.; Faquin, V.; Ramos, S.J.; Boldrin, K.V.F.; Ávila, F.W.; Guilherme, L.R.G. Soil and Foliar Application of Selenium in Rice Biofortification. *J. Food Compos. Anal.* **2013**, *31*, 238–244.

20. de Oliveira, V.C.; Faquin, V.; Guimarães, K.C.; Andrade, F.R.; Pereira, J.; Guilherme, L.R.G. Agronomic Biofortification of Carrot with Selenium. *Ciênc. Agrotec.* **2018**, *42*, 138–147.

21. Silva, M.A.; de Sousa, G.F.; Corguinha, A.P.B.; de Lima Lessa, J.H.; Dinali, G.S.; Oliveira, C.; Lopes, G.; Amaral, D.; Brown, P.; Guilherme, L.R.G. Selenium Biofortification of Soybean Genotypes in a Tropical Soil via Se-Enriched Phosphate Fertilizers. *Front. Plant Sci.* **2022**, *13*, 988140.

22. Brown, P.H.; Zhao, F.-J.; Dobermann, A. What Is a Plant Nutrient? Changing Definitions to Advance Science and Innovation in Plant Nutrition. *Plant Soil* **2022**, *476*, 11–23.

23. Fordyce, F. Selenium Geochemistry and Health. *AMBIO A J. Hum. Environ.* **2007**, *36*, 94–97.

24. Lessa, J.; Raymundo, J.F.; Branco Corguinha, A.P.; Dias Martins, F.A.; Araujo, A.M.; Melo Santiago, F.E.; Pereira de Carvalho, H.W.; Guimarães Guilherme, L.R.; Lopes, G. Strategies for Applying Selenium for Biofortification of Rice in Tropical Soils and Their Effect on Element Accumulation and Distribution in Grains. *J. Cereal Sci.* **2020**, *96*, 103125.

25. Lessa, J.H.L.; Araujo, A.M.; Silva, G.N.T.; Guilherme, L.R.G.; Lopes, G. Adsorption-Desorption Reactions of Selenium (VI) in Tropical Cultivated and Uncultivated Soils under Cerrado Biome. *Chemosphere* **2016**, *164*, 271–277.

26. Araujo, A.M.; Lessa, J.H.; de Lima Lessa, J.H.; Ferreira, L.A.; Guilherme, L.R.G.; Lopes, G. Soil Management and Ionic Strength on Selenite Retention in Oxidic Soils. *Ciênc. Agrotec.* **2018**, *42*, 395–407.

27. Wang, J.; Wang, Z.; Mao, H.; Zhao, H.; Huang, D. Increasing Se Concentration in Maize Grain with Soil- or Foliar-Applied Selenite on the Loess Plateau in China. *Field Crops Res.* **2013**, *150*, 83–90.

28. Zhu, S.; Liang, Y.; Gao, D.; An, X.; Kong, F. Spraying Foliar Selenium Fertilizer on Quality of Table Grape (*Vitis vinifera* L.) from Different Source Varieties. *Sci. Hortic.* **2017**, *218*, 87–94.

29. Deng, X.; Liao, J.; Zhao, Z.; Qin, Y.; Liu, X. Distribution and Speciation of Selenium in Soybean Proteins and Its Effect on Protein Structure and Functionality. *Food Chem.* **2022**, *370*, 130982.

30. Gouveia, G.C.C.; Galindo, F.S.; Dantas Bereta Lanza, M.G.; Caroline da Rocha Silva, A.; Pereira de Brito Mateus, M.; Souza da Silva, M.; Rimoldi Tavanti, R.F.; Tavanti, T.R.; Lavres, J.; dos Reis, A.R. Selenium Toxicity Stress-Induced Phenotypical, Biochemical and Physiological Responses in Rice Plants: Characterization of Symptoms and Plant Metabolic Adjustment. *Ecotoxicol. Environ. Saf.* **2020**, *202*, 110916.

31. Liang, Z.; Qian, S.S.; Wu, S.; Chen, H.; Liu, Y.; Yu, Y.; Yi, X. Using Bayesian Change Point Model to Enhance Understanding of the Shifting Nutrients-Phytoplankton Relationship. *Ecol. Model.* **2019**, *393*, 120–126.

32. Stefanello, L.; Schwalbert, R.; Schwalbert, R.; Tassinari, A.; Garlet, L.; De Conti, L.; Ciotta, M.; Ceretta, C.; Ciampitti, I.; Brunetto, G. Phosphorus Critical Levels in Soil and Grapevine Leaves for South Brazil Vineyards: A Bayesian Approach. *Eur. J. Agron.* **2023**, *144*, 126752.

33. Hu, J.; Zhao, Q.; Cheng, X.; Selomulya, C.; Bai, C.; Zhu, X.; Li, X.; Xiong, H. Antioxidant Activities of Se-SPI Produced from Soybean as Accumulation and Biotransformation Reactor of Natural Selenium. *Food Chem.* **2014**, *146*, 531–537.

34. Makeri, M.U.; Mohamed, S.A.; Karim, R.; Ramakrishnan, Y.; Muhammad, K. Fractionation, Physicochemical, and Structural Characterization of Winged Bean Seed Protein Fractions with Reference to Soybean. *Int. J. Food Prop.* **2017**, *20*, 2220–2236.

35. Huang, Y.; Fan, B.; Lei, N.; Xiong, Y.; Liu, Y.; Tong, L.; Wang, F.; Maesen, P.; Blecker, C. Selenium Biofortification of Soybean Sprouts: Effects of Selenium Enrichment on Proteins, Protein Structure, and Functional Properties. *Front. Nutr.* **2022**, *9*, 849928.

36. Lyons, G.H.; Genc, Y.; Soole, K.; Stangoulis, J.C.R.; Liu, F.; Graham, R.D. Selenium Increases Seed Production in Brassica. *Plant Soil* **2009**, *318*, 73–80.

37. Xia, Q.; Yang, Z.; Shui, Y.; Liu, X.; Chen, J.; Khan, S.; Wang, J.; Gao, Z. Methods of Selenium Application Differentially Modulate Plant Growth, Selenium Accumulation and

Speciation, Protein, Anthocyanins and Concentrations of Mineral Elements in Purple-Grained Wheat. *Front. Plant Sci.* **2020**, *11*, 1114.

38. de Brito Mateus, M.P.; Tavanti, R.F.R.; Tavanti, T.R.; Santos, E.F.; Jalal, A.; dos Reis, A.R. Selenium Biofortification Enhances ROS Scavenge System Increasing Yield of Coffee Plants. *Ecotoxicol. Environ. Saf.* **2021**, *209*, 111772.

39. Jóźwiak, W.; Politycka, B. Effect of Selenium on Alleviating Oxidative Stress Caused by a Water Deficit in Cucumber Roots. *Plants* **2019**, *8*, 217.

40. Shekari, L.; Aroiee, H.; Mirshekari, A.; Nemati, H. Protective Role of Selenium on Cucumber (*Cucumis sativus* L.) Exposed to Cadmium and Lead Stress during Reproductive Stage Role of Selenium on Heavy Metals Stress. *J. Plant Nutr.* **2019**, *42*, 529–542.

41. Reis, H.P.G.; de Queiroz Barcelos, J.P.; Junior, E.F.; Santos, E.F.; Silva, V.M.; Moraes, M.F.; Putti, F.F.; dos Reis, A.R. Agronomic Biofortification of Upland Rice with Selenium and Nitrogen and Its Relation to Grain Quality. *J. Cereal Sci.* **2018**, *79*, 508–515.

42. Ahmed, N.; Abid, M.; Ahmad, F.; Ullah, M.A.; Javaid, Q.; Ali, M.A. Impact of Boron Fertilizantion on Dry Matter Production and Mineral Constitution of Irrigated Cotton. *Pak. J. Bot.* **2011**, *43*, 2903–2910.

43. Tränkner, M.; Tavakol, E.; Jákli, B. Functioning of Potassium and Magnesium in Photosynthesis, Photosynthate Translocation and Photoprotection. *Physiol Plant.* **2018**, *163*, 414–431.

44. Geng, G.; Cakmak, I.; Ren, T.; Lu, Z.; Lu, J. Effect of Magnesium Fertilization on Seed Yield, Seed Quality, Carbon Assimilation and Nutrient Uptake of Rapeseed Plants. *Field Crops Res.* **2021**, *264*, 108082.

45. Cakmak, I.; Kirkby, E.A. Role of Magnesium in Carbon Partitioning and Alleviating Photooxidative Damage. *Physiol. Plant.* **2008**, *133*, 692–704.

46. Nawaz, F.; Ahmad, R.; Ashraf, M.Y.; Waraich, E.A.; Khan, S.Z. Effect of Selenium Foliar Spray on Physiological and Biochemical Processes and Chemical Constituents of Wheat under Drought Stress. *Ecotoxicol. Environ. Saf.* **2015**, *113*, 191–200.

47. Pazurkiewicz-Kocot, K.; Kita, A.; Pietruszka, M. Effect of Selenium on Magnesium, Iron, Manganese, Copper, and Zinc Accumulation in Corn Treated by Indole-3-acetic Acid. *Commun. Soil Sci. Plant Anal.* **2008**, *39*, 2303–2318.

48. Djanaguiraman, M.; Prasad, P.V.V.; Seppanen, M. Selenium Protects Sorghum Leaves from Oxidative Damage under High Temperature Stress by Enhancing Antioxidant Defense System. *Plant Physiol. Biochem.* **2010**, *48*, 999–1007.

49. Sors, T.G.; Ellis, D.R.; Salt, D.E. Selenium Uptake, Translocation, Assimilation and Metabolic Fate in Plants. *Photosynth. Res.* **2005**, *86*, 373–389.

50. Li, Y.; Hu, W.; Zhao, J.; Chen, Q.; Wang, W.; Li, B.; Li, Y.-F. Selenium Decreases Methylmercury and Increases Nutritional Elements in Rice Growing in Mercury-Contaminated Farmland. *Ecotoxicol. Environ. Saf.* **2019**, *182*, 109447.

51. Galić, L.; Špoljarević, M.; Jakovac, E.; Ravnjak, B.; Teklić, T.; Lisjak, M.; Perić, K.; Nemet, F.; Lončarić, Z. Selenium Biofortification of Soybean Seeds Influences Physiological Responses of Seedlings to Osmotic Stress. *Plants* **2021**, *10*, 1498.

52. Rani, N.; Dhillon, K.S.; Dhillon, S.K. Critical Levels of Selenium in Different Crops Grown in an Alkaline Silty Loam Soil Treated with Selenite-Se. *Plant Soil* **2005**, *277*, 367–374.

53. Fan, A.M.; Kizer, K.W. Nutritional, Toxicologic, and Clinical Aspects. *West. J. Med.* **1990**, *153*, 160–167.

54. MacFarquhar, J.K. Acute Selenium Toxicity Associated With a Dietary Supplement. *Arch. Intern Med.* **2010**, *170*, 256.

55. Alvares, C.A.; Stape, J.L.; Sentelhas, P.C. Köppen's Climate Classification Map for Brazil. *Meteorol. Z.* **2013**, *22*, 711–728.

56. Soil Survey Staff. *Keys to Soil Taxonomy*, 13th ed.; USDA-Natural Resources Conservation Service: Washington, DC, USA, 2022.

57. *Embrapa Manual de Metodos de Análise de Solo*, 2nd ed.; r ev. atual.; Embrapa: Rio de Janeiro, Brazil, 1997; ISBN 978-85-85864-03-3.

58. Usepa Test Methods for Evaluation Solid Waste Physical and Chemical Methods: Microwave Assisted Acid Digest of Sediments, Sludges, Soils and Oils, feb. 2007. Available online: **https://www.epa.gov/sites/default/files/2015-12/documents/3051a.pdf** (accessed on 1 February 2023).

59. Yemm, E.W.; Cocking, E.C.; Ricketts, R.E. The Determination of Amino-Acids with Ninhydrin. *Analyst* **1955**, *80*, 209.

60. R Core Team. *R: A Language and Environment for Statistical Computing*; R Foundation: Vienna, Austria, 2022.

61. Webb, R.A. Use of the Boundary Line in the Analysis of Biological Data. *J. Hortic. Sci.* **1972**, *47*, 309–319.

62. Makowski, D.; Doré, T.; Monod, H. A New Method to Analyse Relationships between Yield Components with Boundary Lines. *Agron. Sustain. Dev.* **2007**, *27*, 119–128.

63. Kruschke, J.K.; Liddell, T.M. Bayesian Data Analysis for Newcomers. *Psychon. Bull. Rev.* **2018**, *25*, 155–177.

64. Gelman, A.; Hill, J. *Data Analysis Using Regression and Multilevel/Hierarchical Models*; Cambridge University Press: New York, NY, USA, 2007; ISBN 10: 0521867061.

65. Plummer, Martyn Bayesian Graphical Models Using MCMC 2022. Version 4-14, 2022. Available online: Available online: **https://mcmc-jags.sourceforge.io** (accessed on 1 February 2023).

Supplementary material

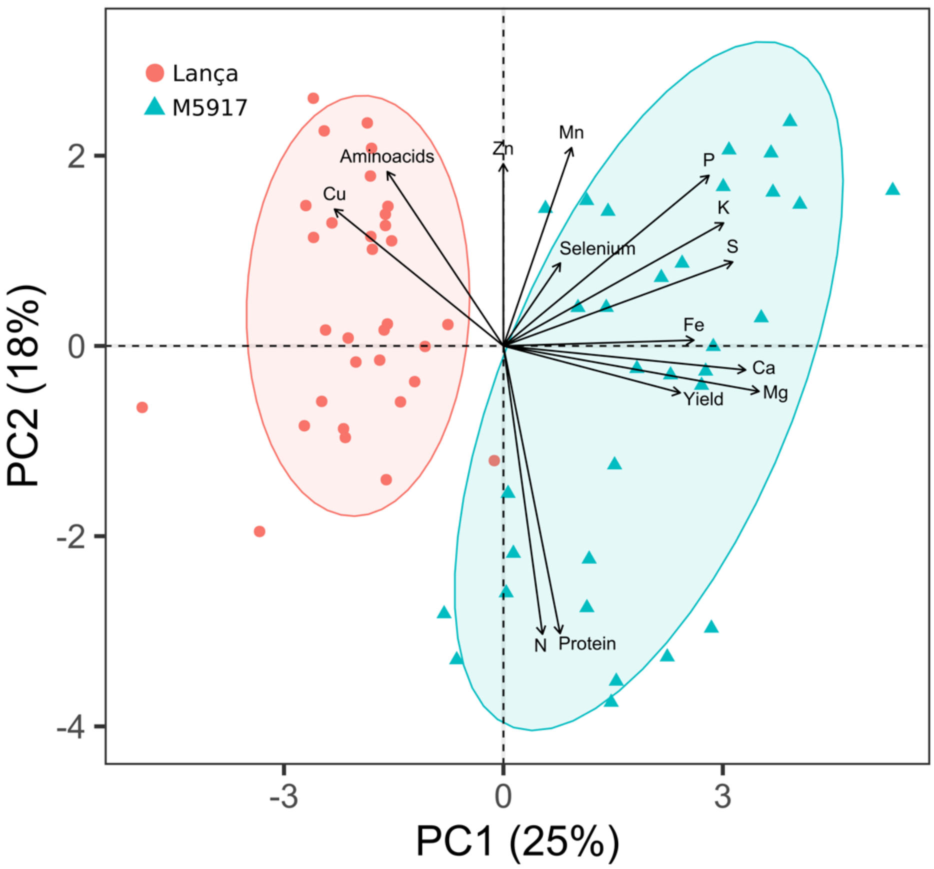

Figure S1: Biplot of principal component analysis (PCA)

Table S1: Summary of F Test (ANOVA) and regression MNF for macronutrient, micronutrient, protein, and amino acid analyses.

Variable	Significance			Adjusted Equation/Mean	R^2 (%)	CV (%)
	Rate	MNF	Rate*MNF			
				58I60 LANÇA		
N	ns	ns	ns	ns	-	2.2
P	*	ns	*	With MNF: no adjusted Without MNF: $y = 5.4726 - 0.0029x$	- 44	2.2
K	ns	ns	*	no adjusted	-	2.4
Ca	ns	ns	ns	ns	-	5.8
Mg	ns	ns	ns	ns	-	3.4
S	ns	ns	***	With MNF: $y = 2.4497 + 0.0012x$ Without MNF: $y = 2.5319 - 0018x$	89 68	1.9
Cu	ns	ns	ns	ns	-	7.9
Fe	ns	ns	ns	ns	-	4.2
Mn	ns	ns	ns	ns	-	3.6
Zn	ns	*	ns	ns	-	5.9
Protein	ns	ns	ns	ns	-	2.2
Amino acids	*	ns	*	With MNF: $y = 139.1267 – 0.3811x$ Without MNF = no adjusted	60 -	7.6
				M5917		
N	*	ns	ns	General equation: $y = 69.1895 - 0.1130x + 0.0011x^2$	-	2.2
P	*	*	*	With MNF: $y = 5.4893 + 0.0193x - 0.0002x^2$ Without MNF: no adjusted	50 -	3.0
K	*	ns	ns	General equation: $y = 17.5397 + 0.0459x - 0.0005x^2$	77	2.9
Ca	ns	ns	ns	ns	-	6.7
Mg	ns	ns	ns	ns	-	4.6
S	*	*	ns	General equation: $y = 2.5731 + 0.0074x - 0.0001x^2$	63	4.2
Cu	ns	*	*	With MNF: $y = 10.1170 + 0.0387x - 0.0007x^2$ Without MNF: no adjusted	80 -	
Fe	ns	ns	ns	ns	-	6.1

Mn	*	ns	ns	General equation: $y = 28.0131 + 0.0190x$	67	3.4
Zn	*	ns	*	General equation: $y = 39.7419 + 0.1295x - 0.0013x^2$	55	3.1
Protein	*	ns	ns	General equation: $y = 43.3434 – 0.0707x + 0.0007x^2$	94	2.2
Amino acids	*	ns	*	no adjusted	-	6.8

* Significance 0.05; ns = no significance by F test; CV = Coefficient of variation; R^2 = regression coefficient

ARTICLE 3: Selenium speciation in Se-enriched soybean grains from biofortified plants grown under different methods of selenium application

Published in Foods
(DOI - 10.3390/foods12061214)

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Abstract: Since soybean is widely cultivated around the world and has a high protein content, it is a great nutritional vehicle for increasing the dietary uptake of selenium (Se). Several studies have evaluated biofortification with Se through fertilizer application in several crops. However, it is not clear how each method and source affect the total Se content or Se species in soybean grains. This work aimed to assess the total Se content and Se speciation in Se-enriched soybean grains produced under different Se application methods in the field. The treatments consisted of Se application (soil or foliar), using organic or inorganic Se sources at 10 g ha^{-1} or 80 g ha^{-1}, in two genotypes. The results showed that all treatments with inorganic Se (soil and foliar) increased the Se content in grains compared with the control. More than 80% of the total Se in grains was present as selenomethionine (SeMet), and the speciation was affected by the Se source and the method of application. The treatments using inorganic Se, applied via soil or foliar, produced the highest content of Se as SeMet in soybean grains. Finally, we propose that the preservation of the Se species in products derived from soybean grains be evaluated as the following step.

Keywords: selenium amino acids; biofortification; selenium fertilizers; food composition; selenomethionine; food security

1. Introduction

Selenium (Se) is a micronutrient for animals and humans and is needed for hormone regulation, antioxidant defenses, and immune and muscle systems [1]. The average intake recommendation for adults is 55 μg day^{-1}, yet it can reach 60 and 70 μg day^{-1} in pregnant and lactating women, respectively [2]. Up to one in seven people worldwide are estimated to have deficient dietary Se intake [3]. The reduced Se intake occurs mainly because agricultural technologies in many parts of the world have focused on promoting grain yield, rather than on increasing the nutrient content [4].

Soil Se is the primary natural source of Se in staple crops, but its availability is largely affected by parent rocks, climate, and soil chemistry (e.g., soil pH and redox potential) [5,6]. Moreover, the Se soil content is not always enough to supply the daily-recommended intake of 55 μg day^{-1} for the population. Disorders related to Se deficiency are more common in regions with severely low Se content in soils [2,7,8], including soils in the tropics [6].

Selenium can be added to the diet of humans by supplementing foods directly or by biofortifying plants, i.e., by increasing Se levels in crops during plant growth/development. Biofortification can be performed through conventional plant breeding or modern biotechnology (genetic biofortification) or by increasing Se uptake via Se supplementation to plants (agronomic biofortification) [4,9,10,11]. Agronomic biofortification has been effective in increasing the content of Se in several grain crops, such as wheat [12,13], rice [14], common bean [15,16], and sorghum [17].

The effectiveness of Se fertilization in grain crops is highly affected by genotype, chemical source applied, and method of Se application [18,19]. The main strategies for agronomic biofortification are through soil and/or foliar applications, though some authors have suggested that foliar supply is more efficient for increasing the Se content than soil application supply due to the direct contact of the element with the crop and prevention of Se sorption in the soil [20,21].

Both the total content and the bioavailability of Se in food exert a great role in Se assimilation by humans and animals. Selenium species are frequently found in plants in both organic Se compounds (i.e., selenomethionine (SeMet), methyl selenocysteine (MeSeCys), selenocysteine (SeCys)) and inorganic compounds (i.e., selenite (SeO_3^{2-}), selenate (SeO_4^{2-})) [22]. Selenium bioavailability differs based on the chemical form of the element present in the food and is considerably greater for organic forms [23].

The variation in characteristics of different genotypes has a significant influence on Se content and on its chemical forms in edible parts [24,25]. Indeed, Se biofortification efforts may profit from this natural variation among plant genotypes, choosing those that can naturally accumulate higher Se levels in edible parts. In this context, soybean has been suggested to be a promising alternative to producing biofortified cereal grains given its higher protein content and, hence, greater propensity to produce organic Se forms such as SeMet, MeSeCys, and SeCys [26]. Soybean is one of the most produced grains in the world, which makes this crop a good Se vehicle for increasing Se in the population. This study aims to evaluate the Se content and speciation in Se-enriched soybean grains produced under different Se application methods in the field.

2. Materials and Methods

2.1. Area Characterization

Soybean target grain samples were selected from three different trials previously grown under the same field conditions [27], during the season 2018/2019 in Capão Bonito (SP), Brazil (Lat: -95 24.040934, Lon: -48.262421). The target samples were selected for the study based on earlier analyses of the total Se content in Se-enriched grains under different Se application conditions. The average annual precipitation in the cultivated field was 1628 mm, and the average annual temperature was 18.8 °C. The soil of the experimental region is an Oxisol, classified as Typic Hapludox [28]. Physical and chemical characteristics were determined according to the methodology suggested by the Brazilian Agricultural Research Company [29] as follows: pH (H_2O) = 6.0; H + Al = 2.96; Al = 0.06; P (Mehlich-1) = 34.8 mg dm^{-3} ; P-rem = 28.10 mg L^{-1}; K = 148 mg dm^{-3}; S = 4.11 mg L^{-1}; CEC = 9.83 $cmol_c$ dm^{-3}; Ca = 5.05 $cmol_c$ dm^{-3}; Mg = 1.44 $cmol_c$ dm^{-3}; organic matter = 2.69 dag dm^{-3}; Se = 0.92 mg dm^{-3}; clay = 510 g kg^{-1}; silt = 110 g kg^{-1}; and sand = 380 g kg^{-1}.

2.2. Selenium Application Methods

The treatments comprised both soil and foliar application of Se (Table 1). When Se was applied to soil, two different phosphate fertilizers coated with Se were used at planting time (conventional monoammonium phosphate and enhanced efficiency monoammonium phosphate). The fertilizers were coated with 500 mg kg^{-1} of Se after granulation (using a solution of sodium selenate-Na_2SeO_4, Sigma-Aldrich, Saint Louis, MO, USA). Given that 80 kg ha^{-1} of P_2O_5 was applied as MAP (~50% P_2O_5), the addition of Se-rich fertilizers (500 mg Se kg^{-1}) resulted in a Se dose of 80 g ha^{-1}.

Table 1. Treatments description applied in soybean plants.

Abbreviation	Genotype	Method of Application	Se Source/Vehicle	Dose (g ha^{-1})
L-Cnt	Lança	-	-	0
LF-In10	Lança	Foliar	Inorganic [1]	10
LF-In80	Lança	Foliar	Inorganic	80
LF-Or10	Lança	Foliar	Organic [2]	10
LS-C80	Lança	Soil	C-MAP [3] + Se	80
LS-E80	Lança	Soil	E-MAP [4] + Se	80
M-Cnt	M5817	-	-	0
MF-In10	M5817	Foliar	Inorganic	10
MF-In80	M5817	Foliar	Inorganic	80
MF-Or10	M5817	Foliar	Organic	10
MS-C80	M5817	Soil	C-MAP + Se	80
MS-E80	M5817	Soil	E-MAP + Se	80

[1] Inorganic Se source = sodium selenate; [2] organic Se source = acetylselenide; [3] phosphate fertilizer applied via soil = C-MAP: conventional monoammonium phosphate; [4] phosphate fertilizer applied via soil = E-MAP: enhanced efficiency monoammonium phosphate (coated with humic and fulvic substances).

Foliar spray with an inorganic Se source was applied at two doses, 10 and 80 g ha^{-1}, as sodium selenate (Na_2SeO_4-Sigma Aldrich 98.9%, Saint Louis, MO, USA). Another treatment with an organic Se source (acetylselenide-25% of Se) was applied at 10 g ha^{-1} via foliar spray. Fertilizers were applied at phenological stages R3 (beginning of pod development) and R5 (grain filling), with each application containing half of the total rate. Sodium selenate was diluted in deionized water (500 mL of water plot^{-1}) before being applied with a pressurized carbon dioxide pump, and no Se application was used as a control treatment for both genotypes. Mineral oil was used with each application solution (0.5% v/v of mineral oil). All treatments were applied to two different genotypes (50I60 Lança and M5917) (Table 1).

2.3. Selenium Extraction: Soluble and Protease

The samples from each treatment were dried at 40 °C (to avoid protein denaturation) in a drying oven with air circulation after being harvested. In addition, they were ground in an electric hand mill (ka-A11 basic BS32, IKA, Staufen, Germany). Analyses of total Se and Se speciation in the Se-enriched soybean grain followed the methodology described by Bañuelos et al. (2012) and Bañuelos et al. (2012) [22,30]. Soluble Se compounds (non-protein bound) and insoluble Se compounds (protein-bound) were separated and identified/quantified using methanol-chloroform-water solvent extraction and methanolchloroform-water enzymatic digest (with protease), respectively.

The methanol-chloroform-water extraction was performed using 1 g of the ground sample placed in 40 mL glass vials equipped with Teflon caps and divided into two groups (soluble and protease). Protease (*Streptomyces griseus* Type XIV-Sigma-Aldrich) was added (50 mg) to the protease set [31]. Following that, 10 mL of ultrapure water at room temperature was added to the protease-containing vials. The set of soluble samples received 17 mL of methanol. The matched samples were mixed by vortex, and the protease sample set was maintained at 37 °C for 20 h, while the methanol sample set was kept at 4 °C overnight. Protease-digested samples received 17 mL of methanol, and soluble-digested methanol extractions received 10 mL of water (to inhibit enzymatic action and denature the protease enzyme).

Each tube was stirred continuously and left in a refrigerator at 4 °C overnight. Then, chloroform (8.5 mL) was joined to all vials, which were sealed, shaken quickly, and refrigerated at 4 °C overnight until the material was totally extracted. The top aqueous phase (methanol-water) was completely separated from the chloroform phase. This phase (methanol-water) containing the extracted Se compounds was removed and put into a centrifuge tube. One-quarter of the aqueous phase (methanol-water) was then pipetted into 50 mL ICP digestion tubes for drying (using a heating block at 50 °C), which was followed by acid digestion and analysis of total aqueous Se by Agilent 7500 CX ICP-MS (Agilent Technologies, Santa Clara, CA, USA). Additionally, the nonaqueous chloroform phase remaining in the original 40 mL glass vial was also evaporated, then acid-digested and analyzed with ICP-MS. After extraction, the portion of Se in the aqueous phase was calculated as {(total Se in methanol-water phase)/[(total Se in methanol-water phase) + (total Se in chloroform phase)] × 100}.

The final part of the aqueous (methanol-water) phase was dried using a refrigerated centrifugal speed vacuum (Labconco CentriVap Concentrator), resuspended in ultrapure water to 2.5 mL, and then saved in a -80 °C freezer. Waters Se-Pak Classic C18 cartridges (360 mg 55+ 05 um) were utilized for the full cleanup of the aqueous concentrates. Each cartridge was cleaned by flushing it with 10 mL of methanol and 5 mL of ultrapure water in that order. The 2.5 mL concentrates were thawed and vortexed, and then received 11 µL of formic acid (88%-ACS grade, Fisher Chemical) before being transferred to the Sep-Pak. The sample was eluted using a syringe into a new 50 mL conical tube. Methanol (3 mL) was added to remove residuals in the conical tube. Afterward, the aliquot was dried, dissolved in

1.5 mL of water, placed in Agilent screw-top glass HPLC vials, and frozen (-80 °C) until SAX-HPLC/ICP-MS analysis.

The National Institute of Standards and Technology (NIST) wheat flour standard (SRM 1567a) was used as the standardized quality control for wet-acid digestion (total Se concentration) and Se speciation extraction (SeMet, SeCys2) content in plant material. The SRM 1567a was utilized as an internal control in the MCW extraction to account for any changes in the protease XIV efficacy and other factors during the extraction process. The total Se recovery rates were over 94% for the wheat flour standard, which has a Se concentration of 1.1 ± 0.2 µg Se g^{-1} DW, with a method detection limit of 50 ng Se g^{-1} DW. The selenoamino acid content in SRM 1567a consisted of 92% SeMet and 6% SeCys2. The NIST wheat flour standard and soybean samples were extracted in triplicate. The extraction and quality control measures are documented in detail [30].

2.4. Total Selenium, Selenium Recovery, Selenium Speciation, and Total Free Amino Acids

Total Se concentrations were analyzed by Agilent 7500 CX ICP-MS (Agilent Technologies Santa Clara, USA) [22,30]. The fraction of applied Se incorporated in soybean grains (Se recovery) was calculated using Se total data, as follows:

$$\text{Se recovery (\%)} = (\text{Se}_{\text{treatment}} - \text{Se}_{\text{control}})/\text{Se}_{\text{dose}} \times 100 \qquad (1)$$

where: $\text{Se}_{\text{treatment}}$ (g ha^{-1}) = Se contents in soybean grains from soybean plants grown in treatments that received Se applications; $\text{Se}_{\text{control}}$ (g ha^{-1}) = Se contents in soybean grains from soybean plants grown in treatments without Se applications; Se_{dose} (g ha^{-1}) = Se doses applied in the treatment.

Selenium speciation was performed on an Agilent 1200 HPLC connected to a Hamilton PRP-X100 strong anion exchange column (10 µm particle size, 250 mm length, and 4.1 mm internal diameter) coupled to the Agilent 7500 CX ICP-MS (SAX-HPLC-ICP-MS). The Agilent Chemstation software was used to combine the two instruments (Agilent 1200 HPLC and Agilent 7500 CX ICP-MS) with chromatographic data analysis. To account for any matrix-induced changes to the chromatographic analysis, the retention time of Se78 containing peaks was monitored using the ICP-MS and directly compared with the authentic standard [30]. The ICP-MS was operated in collision/reaction cell mode with hydrogen at 5 mL/min to minimize isobaric interferences. The limits of detection (LOD = 3 σ/m) for the primary 5 selenium species speciated ranged from 0.16 to 0.32 ng mL^{-1}. The limits of quantification (LOQ = 10 σ/m) for all 5 respective Se species ranged from 0.56 to 1.02 ng mL^{-1}. The correlation coefficients ranged from 0.9997 to 0.9999 for all calibration curves. Standards of sodium selenate, sodium selenite, SeMet, and SeCys from Sigma-Aldrich, St. Louis, MO, and methyl-selenocysteine (MeSeCys) from Pharma Se were used for analyses [22,30]. The ninhydrin method was used to assess total free amino acids [32].

2.5. Statistical Analyses

Analysis of variance (ANOVA) was performed to verify the effects of treatments on the attributes analyzed. Selenite, Selenate, and SeCys data were log-transformed and fitted to a linear model to address the statistical assumptions before ANOVA (normality and homoscedasticity). Due to homoscedasticity, the SeMet and MeSeCys data were also evaluated using linear models estimated by generalized least square regression. A Tukey test (5% of probability) was performed to compare the treatments. A principal component analysis (PCA) was carried out to report the interaction between Se species. The simple linear relationship between the Se species was performed using Pearson's correlation. All statistical analyses were carried out using R [33].

3. Results

3.1. Selenium Accumulation, Recovery, and Speciation in Soybean Grains

Total Se concentrations in soybean grains ranged from 0.08 to 7.71 mg kg^{-1} for treatments L-Cnt and MF-In80, respectively (Figure 1A). All treatments with inorganic Se application (soil and foliar) increased the Se content in grains compared with the control (Tukey 0.05 probability). The MF-In80 treatment produced grains with higher Se content than the LF-Ino80 treatment, indicating that total Se in the genotype M5917 was greater than in the genotype Lança when sodium selenate was applied at a dose of 80 g Se ha^{-1}.

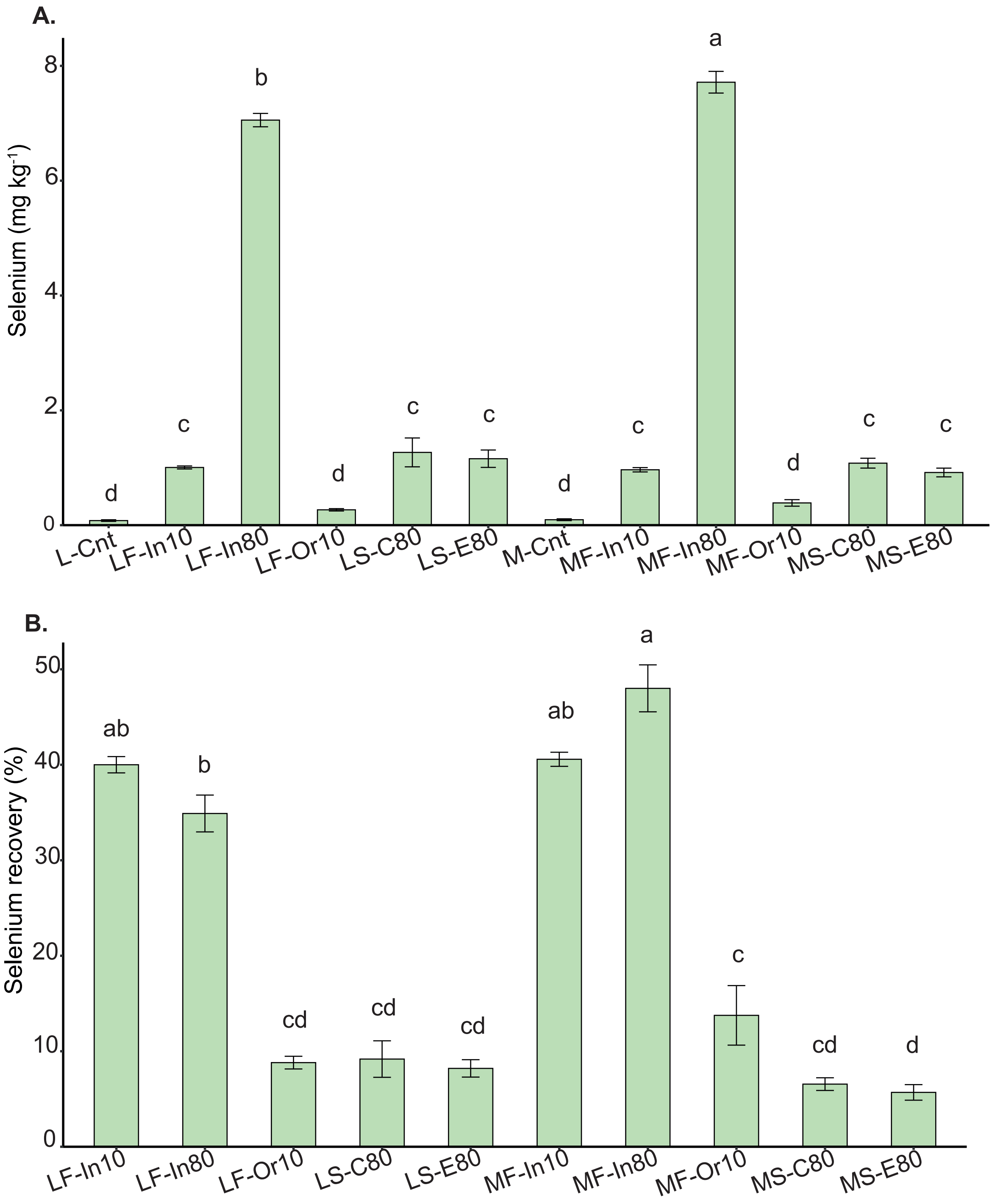

Figure 1. Selenium content (mg kg^{-1}) (**A**) and selenium recovery (%) (**B**) in soybean grains from biofortified plants growing under different methods of selenium application. Lowercase letters compare Se contents and Se recovery among the treatments at the level of 5% by the Tukey test. The vertical bars represent the standard error (*n* = 4). Genotype: L = Lança and M = M5917; Se application: F = foliar and S = soil; Se source: In = Se inorganic, Or = Se organic, C = C-MAP + Se, and E = E-MAP + Se; Rate: 10 = 10 g ha^{-1} and 80 = 80 g ha^{-1}; Cnt = control.

Treatments LF-Or10 and MF-Or10, in which Se was applied via an organic source at a dose of 10 g ha^{-1}, did not differ from L-Cnt and L-Cnt for total Se analysis. Treatments with

Se foliar application using sodium selenate at a dose of 10 g ha^{-1} (LF-In10 and MF-In10) were similar to treatments with Se soil application via phosphate fertilizer at a dose of 80 g ha^{-1} (LS-C80, LS-E80, MS-C80, and MS-E80). The total Se concentrations were 1.27, 1.16, 1.08, and 0.92 for LS-C80, LS-E80, MS-C80, and MS-E80, respectively. There was no significant difference in total Se content between conventional and enhanced efficiency phosphate fertilizer applications.

The treatments MF-In80, MF-In10, LF-In10, and LF-In80 showed higher percentages of Se recovery by grains (48.0%, 40.6%, 40.0%, and 34.9%, respectively) (Figure 1B). The percentage of Se recovery by soybean grains ranged from 5.7% to 48%. The treatment LF-In80 (foliar application) provided a recovery that was 3.8 and 4.3 times greater than LS-C80 and LS-E80 (soil application), respectively. For the genotype M5917, this recovery was 7.3 and 8.4 times greater than the treatments MS-C80 and MS-E80, respectively.

The percentages of Se species found in soybeans are shown in Figure 2A. Selenium species in soybeans were influenced by the treatments applied ($p \leq 0.05$) (Table S1). For SeCys, the percentages ranged from 0.97% (MS-E80) to 17.0% (L-Cnt). The overall percentage of MeSeCys did not differ statistically among the treatments. In general, the Se species with the lowest percentages were MeSeCys and selenite. The highest average percentages for selenite species were found in the LF-In80 treatment (2.3%), yet this treatment differed only from the M-Cnt.

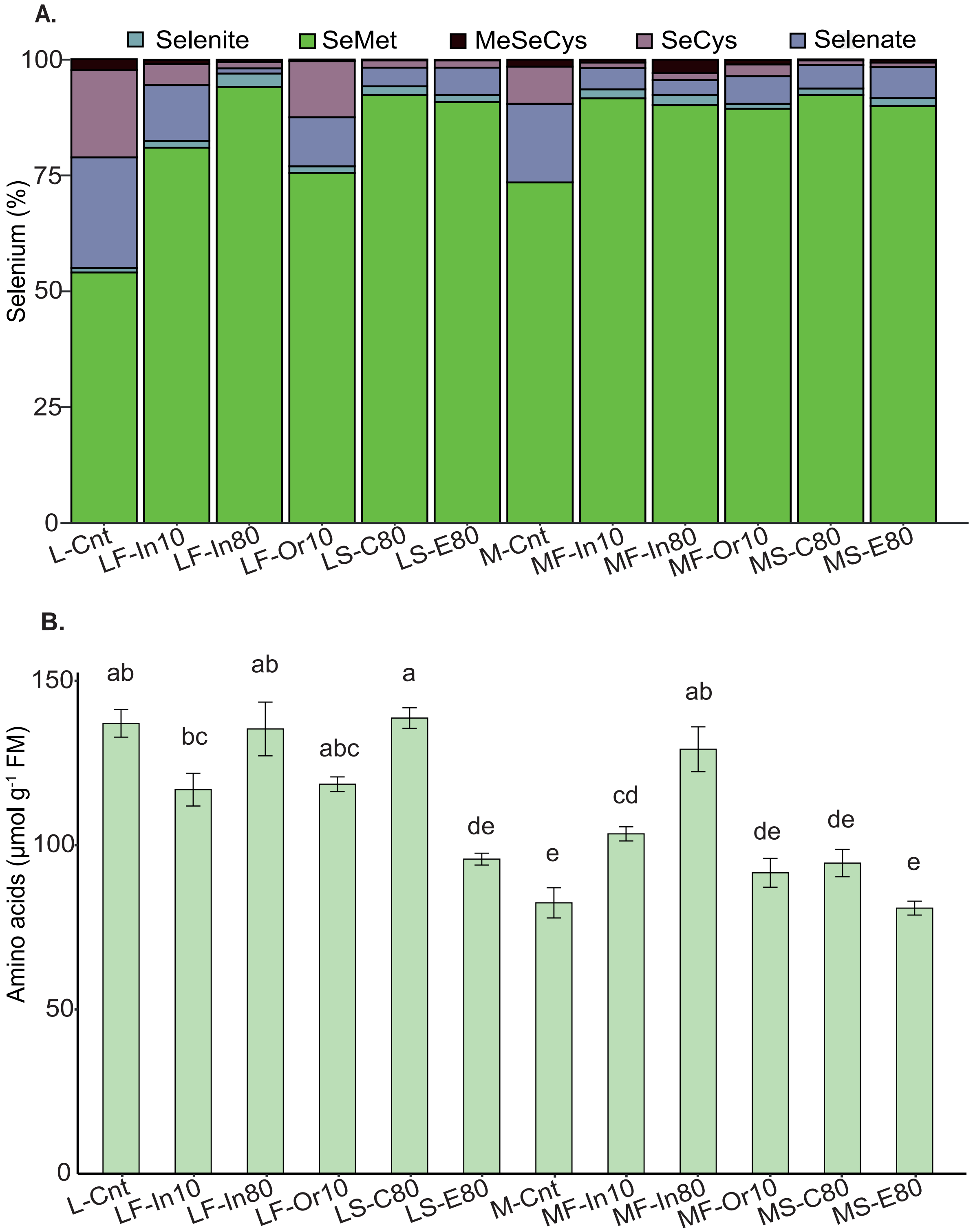

Figure 2. Selenium species (%) (**A**) and amino acids (µmol g^{-1} FM) (**B**) in soybean grains from biofortified plants growing under different methods of selenium application. Lowercase letters compare Se contents and Se recovery among the treatments at the level of 5% by the Tukey test. The vertical bars represent the standard error (*n* = 4). Genotype: L = Lança and M = M5917; Se application: F = foliar and S = soil; Se source: In = Se inorganic, Or = Se organic, C = C-MAP + Se, and E = E-MAP + Se; Rate: 10 = 10 g ha^{-1} and 80 = 80 g ha^{-1}; Cnt = control.

The Se species found in the highest percentage in the grains was SeMet, which ranged from 54.0% (L-Cnt) to 94.1% (LF-In80). The LF-In80 treatment had the highest SeMet mean (94.1%); however, it differed statistically only from the LF-Or10 and M-Cnt. Organic Se (including SeMet, SeMeCys, and SeCys) accounted for more than 84% of the total Se content in the grains following Se applications. In summary, the percentages of Se species present in the grains were higher for SeMet, followed by selenate, SeCys, selenite, and MeSeCys.

3.2. Total Free Amino Acids

The total free amino acid concentrations ranged from 80.8 to 138.7 µmol g^{-1} FM, with the LS-C80 treatment showing the highest mean (Figure 2B). The LS-C80 treatment had an average similar to the L-Cnt, LF-In80, LF-Or10, and MF-In80 treatments. The treatments M-Cnt and MS-E80 showed the lowest averages for total free amino acids, according to the results. Comparing the treatments of genotype M5917, the MF-In80 treatment showed the highest concentration of total free amino acids (129.2 µmol g^{-1} FM). In general, treatments of the genotype Lança had a higher content of total free amino acids than the others.

3.3. Principal Component Analysis

The principal component analysis (Figure 3) demonstrated that two main components accounted for 70.6% of the variation in Se species. The variable responses influenced by treatments with low Se content in grains (L-Cnt, M-Cnt, and LF-Or10) are shown on the right, indicating an increase in the content of selenate and SeCys in grains. In addition, SeMet content in these treatments was lower and it decreases as selenate content increases. This information was also confirmed by Pearson’s correlation analysis (Table S2). The other group shown on left (up to the horizontal line) is influenced by the treatments with high foliar-applied Se doses, for both genotypes (LF-In80 and MF-In80). This group indicates an increase in the Se total and selenite in grains.

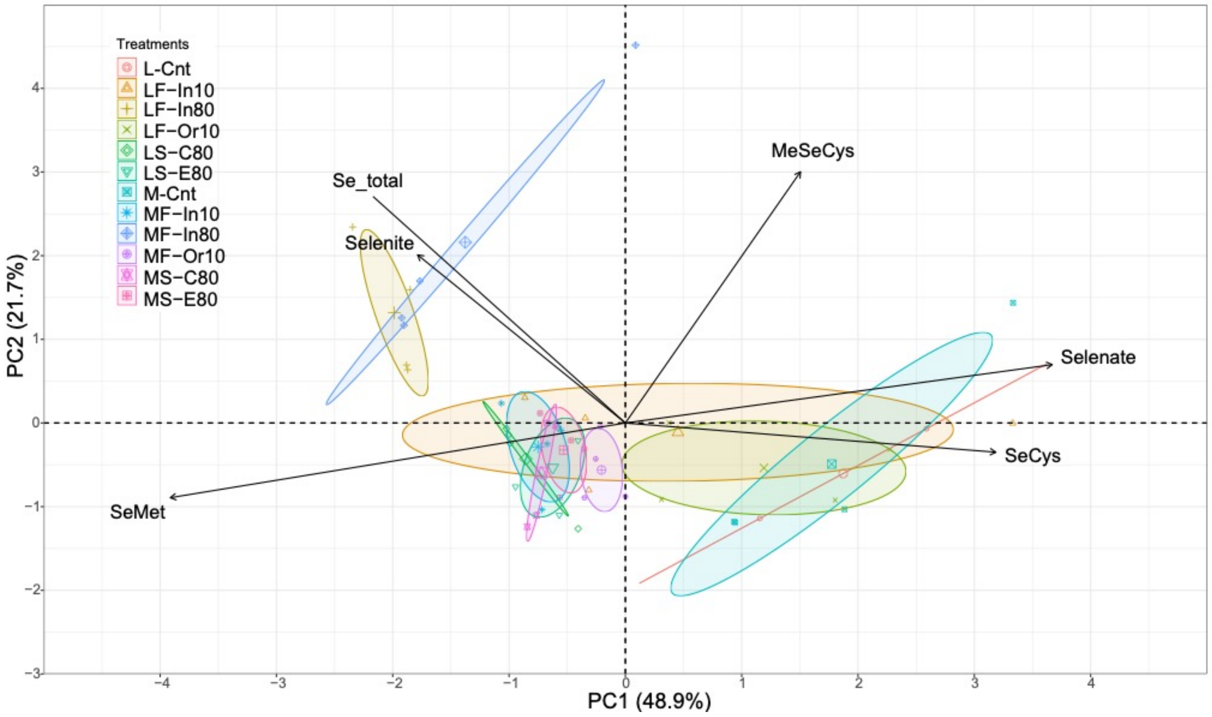

Figure 3. Principal component analysis. Abbreviations: SeMet = selenomethionine; MeSeCys = methyl selenocysteine; SeCys = selenocysteine. Genotype: L = Lança and M = M5917; Se application: F = foliar and S = soil; Se source: In = Se inorganic, Or = Se organic, C = C-MAP + Se, and E = E-MAP + Se; Rate: 10 = 10 g ha^{-1} and 80 = 80 g ha^{-1}; Cnt = control.

4. Discussion

The results demonstrate that soybean is responsive to both soil and foliar biofortification with Se fertilizers, yet the Se concentration in grains depends on how Se is applied. Even though Se is not established as a nutrient for plants, it is clearly a beneficial element [34] as it can enhance plant development and increase antioxidant capacity, especially when plants are exposed to stressing conditions [16,35,36].

All treatments that used inorganic Se-in both foliar and soil application-increased the total Se content in soybean grains. Deng et al. (2021) [37], using pot experiments with soil application of Se, observed that Se content was easily increased in soybean seeds resulting in a 21–27% greater Se content than in the control treatment. Even at a lower rate, treatments LF-In10 and MF-In10 achieved an average total Se content comparable to LS-C80, LS-E80, MS-C80, and MS-E80. In rice crops, Lessa et al. (2020) [14] found that foliar application was more effective in enhancing the quality of Se content in grains than soil application of Se associated with phosphate fertilizers.

In the present study, the Se concentration achieved 7.71 mg kg^{-1} when Se was foliar sprayed in treatment MF-In80. The high recovery of Se in this treatment is due to the higher uptake efficiency of Se associated with a high distribution efficiency of Se from leaves to grains. Similarly, Se foliar application at the highest dose of Se improved the recovery efficiency of Se in the genotype M5917 (~1.3-fold) when compared with the genotype Lança in the present study. These findings suggest that different genotypes have varying capacities for Se uptake following foliar application with Se inorganic sources, particularly at high doses of Se application.

Foliar spraying transfers Se directly from the leaves to the grains, making it more bioavailable than when Se is applied to the soil [38]. Indeed, when Se is soil applied, it is subjected to various reactions that affect its mobility and solubility [20,39,40]. As a result of the changes in Se mobility and solubility, the efficiency of Se absorption and distribution by plants is also impacted. Soil redox potential, soil texture, sorption/desorption reactions, pH, presence of competing ions, and dissolution processes in soils are the factors that affect Se availability in the soil, affecting the absorption of Se by plants [39–42].

The bioavailability of Se for animals and humans is based on the Se species consumed and the total Se content [43]. Consequently, the speciation of Se in plant tissue is important for understanding the efficiency of Se absorption. When Se is taken up by the plant, it is transformed into different species in plant tissues. For vegetables, such as garlic, onion, and broccoli, MeSeCys is the main Se species of Se, while in most grains, the main Se species is SeMet [36,44]. Soybean can accumulate Se both as organic and inorganic forms of Se. Here, we found that SeMet was the main Se species produced. These results corroborate the results found by Lu et al. (2018) [45] and Deng et al. (2021) [37]. According to Bañuelos et al. (2020) [46], most non-Se-accumulating plant species are expected to accumulate Se as SeMet.

Plants utilize sulfate transporters to uptake Se when it is provided as selenate [47,48]. The physical and chemical similarity of Se-as selenate- and S-as sulfate-indicates that both elements shared similar metabolic pathways in plants [49]. The transformation of Se into selenite is the first step of assimilation. Later, the cysteine-synthase enzyme helps change selenite into selenide, which is then changed into SeCys. Based on the species and surrounding circumstances, SeCys can then be transformed into elemental Se, MeSeCys, or SeMet. The SeMet species can be either transformed into methyl-SeMet (Me-SeMet) or used to produce selenoproteins [50]. The percentages of Se species found in grains are influenced by how Se is applied. The treatments L-Cnt and M-Cnt accumulate less SeMet and more SeCys than those that received inorganic Se supply via soil application. In other words, under low Se availability, soybeans did not form as much SeMet as when this plant is under high Se availability.

Selenoamino acids, such as SeMet and SeCys, are key for the formation of selenoproteins in plants. The production of SeMet and SeCys in selenoproteins offers even more benefits because they are known to have promising biological properties in vitro and in vivo against free radicals such as $HO^•$ and O_2^- [51]. Selenoproteins, such as glutathione peroxidase, play important roles in Se transport and cellular redox balance regulation [52]. Organic Se forms (e.g., Se amino acids) are more bioavailable for humans than inorganic forms such as selenite and selenate. In fact, the human body takes more than 90% of SeMet but only about 50% of Se from selenite [10,53].

Total free amino acids varied according to the treatments applied. The treatment MSE80 had an amino acids content that was higher than MS-C80, MS-E80, MF-In80, MF-In10, M-Cnt, and MF-Or80. This observation indicates that foliar application of Se at a dose of 80 g ha^{-1} increased the total free amino acids for genotype M5917. However, this result was not observed for treatments applied in genotype Lança, which showed a variable response among the treatments. In lettuce accessions, Ramos et al. (2011) [54] reported a large discrepancy in total free amino acids using selenate and selenite as Se sources. In contrast, Huang et al. (2022) [55] found an increase in total amino acids in soybean sprouts that received Se at concentrations smaller than 40 mg L^{-1} and a decrease in sprouts treated with Se greater than 60 mg L^{-1}. According to these authors, low-level Se doses promote amino acid synthesis, whereas high-level Se doses have an inhibitory effect. In general, the treatments with high total free amino acids were L-Cnt, LF-In80, LS-C80, and MF-In80. According to Sanmartín et al. (2014) [56], adequate Se accumulation in plants can enhance their nutritional values by increasing the total amino acid content.

5. Conclusions

Our results show that different methods of Se application in soybean plants affect total Se and Se species in soybean grains. Foliar application using inorganic Se at 80 g ha^{-1} is more efficient for improving total Se content in soybean grains, for both genotypes. The foliar spray at the dose of 10 g ha^{-1} provides Se content in the grains similar to that found as a result of the application of 80 g ha^{-1} via soil. More than 80% of total Se in soybean grains was present in the organic form, with SeMet as the main Se species accumulated by soybean grains. The highest levels of SeMet in soybean grains were obtained in the treatments with the application of inorganic Se, either via soil or via foliar application, in both genotypes evaluated. In order to assess the mechanism by which Se sources and Se carriers (i.e., the different soil fertilizers) affect Se species in soybean grains, further research is required. Lastly, we propose future trials to evaluate the preservation of the Se content and Se species in products originating from soybean grains after industrial processing.

Supplementary Materials: The following supporting information can be downloaded at: https: //www.mdpi.com/article/10.3390/foods12061214/s1, Table S1: Means (% of Se species) and Tukey test for Se species. Table S2: Correlation coefficients among Se species in soybean grains.

Author Contributions: Conceptualization, L.R.G.G., M.A.S. and G.F.d.S.; methodology, M.A.S., G.F.d.S. and G.B.; formal analysis, M.A.S., G.F.d.S. and G.B.; writing-original draft preparation, M.A.S. and G.F.d.S.; writing-review and editing, L.R.G.G., G.B., P.H.B. and D.A.; supervision, L.R.G.G. and P.H.B.; project administration, L.R.G.G.; funding acquisition, L.R.G.G., P.H.B. and G.B. All authors have read and agreed to the published version of the manuscript.

Funding: This work was financially supported by funds from the National Council for Scientific and Technological Development (CNPq), the Coordination for the Improvement of Higher Education Personnel (CAPES), the Foundation for Research of the State of Minas Gerais (FAPEMIG), and funds provided by California State University Agricultural Research Initiative (ARI).

Data Availability Statement: The data presented in this study are available from the corresponding author upon reasonable request.

Acknowledgments: We are grateful to CNPq, CAPES, and FAPEMIG from Brazil for their financial support for the research project and for scholarships, especially to CAPES PrInt and UC Davis for the financial support for scholarships during the Sandwich PhD exchange program. Furthermore, we are grateful to USDA for its partnership and to The Brazilian National Council for Scientific and Technological Development (CNPq grant #406577/2022-6-National Institute of Science and Technology on Soil and Food Security). We also appreciate the help of Teotônio Soares de Carvalho in helping us with statistics of Se speciation data.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

References

1. Rayman, M.P. Food-Chain Selenium and Human Health: Emphasis on Intake. Br. J. Nutr. **2008**, 100, 254–268.

2. Combs, G.F. Selenium in Global Food Systems. Br. J. Nutr. **2001**, 85, 517–547.

3. Jones, G.D.; Droz, B.; Greve, P.; Gottschalk, P.; Poffet, D.; McGrath, S.P.; Seneviratne, S.I.; Smith, P.; Winkel, L.H.E. Selenium Deficiency Risk Predicted to Increase under Future Climate Change. Proc. Natl. Acad. Sci. USA **2017**, 114, 2848–2853.

4. Garg, M.; Sharma, N.; Sharma, S.; Kapoor, P.; Kumar, A.; Chunduri, V.; Arora, P. Biofortified Crops Generated by Breeding, Agronomy, and Transgenic Approaches Are Improving Lives of Millions of People around the World. Front. Nutr. **2018**, 5, 12.

5. Natasha; Shahid, M.; Niazi, N.K.; Khalid, S.; Murtaza, B.; Bibi, I.; Rashid, M.I. A Critical Review of Selenium Biogeochemical Behavior in Soil-Plant System with an Inference to Human Health. Environ. Pollut. **2018**, 234, 915–934.

6. Lopes, G.; Ávila, F.W.; Guilherme, L.R.G. Selenium Behavior in the Soil Environment and Its Implication for Human Health. Ciênc. Agrotec. **2017**, 41, 605–615.

7. Garousi, F. The Essentiality of Selenium for Humans, Animals, and Plants, and the Role of Selenium in Plant Metabolism and Physiology. Acta Univ. Sapientiae Aliment. **2017**, 10, 75–90.

8. Schomburg, L. Selenium Deficiency Due to Diet, Pregnancy, Severe Illness, or COVID-19-A Preventable Trigger for Autoimmune Disease. Int. J. Mol. Sci. **2021**, 22, 8532.

9. Cakmak, I. Enrichment of Cereal Grains with Zinc: Agronomic or Genetic Biofortification? Plant Soil **2008**, 302, 1–17.

10. D’Amato, R.; Regni, L.; Falcinelli, B.; Mattioli, S.; Benincasa, P.; Dal Bosco, A.; Pacheco, P.; Proietti, P.; Troni, E.; Santi, C.; et al. Current Knowledge on Selenium Biofortification to Improve the Nutraceutical Profile of Food: A Comprehensive Review. J. Agric. Food Chem. **2020**, 68, 4075–4097.

11. Broadley, M.R.; White, P.J.; Bryson, R.J.; Meacham, M.C.; Bowen, H.C.; Johnson, S.E.; Hawkesford, M.J.; McGrath, S.P.; Zhao, F.-J.; Breward, N.; et al. Biofortification of UK Food Crops with Selenium. Proc. Nutr. Soc. **2006**, 65, 169–181.

12. Lara, T.S.; de Lessa, J.H.L.; de Souza, K.R.D.; Corguinha, A.P.B.; Martins, F.A.D.; Lopes, G.; Guilherme, L.R.G. Selenium Biofortification of Wheat Grain via Foliar Application and Its Effect on Plant Metabolism. J. Food Compos. Anal. **2019**, 81, 10–18.

13. Wang, M.; Ali, F.; Wang, M.; Dinh, Q.T.; Zhou, F.; Bañuelos, G.S.; Liang, D. Understanding Boosting Selenium Accumulation in Wheat (*Triticum aestivum* L.) Following Foliar Selenium Application at Different Stages, Forms, and Doses. Environ. Sci. Pollut. Res. **2020**, 27, 717–728.

14. Lessa, J.; Raymundo, J.F.; Branco Corguinha, A.P.; Dias Martins, F.A.; Araujo, A.M.; Melo Santiago, F.E.; Pereira de Carvalho, H.W.; Guimarães Guilherme, L.R.; Lopes, G. Strategies for Applying Selenium for Biofortification of Rice in Tropical Soils and Their Effect on Element Accumulation and Distribution in Grains. J. Cereal Sci. **2020**, 96, 103125.

15. De Araújo, S.N.; Raymundo, J.F.; Costa, F.F.R.; de Lima Lessa, J.H.; Guilherme, L.R.G.; Lopes, G. Selenium Application Methods and Rates for Biofortification of Common Bean and Their Residual Effects on Mombaça Grass. Crop Pasture Sci. **2022**, 73, 792–803.

16. Ravello, R.A.V.; de Oliveira, C.; Lessa, J.; Boas, L.V.V.; de Castro, E.M.; Guilherme, L.R.G.; Lopes, G. Selenium Application Influenced Selenium Biofortification and Physiological Traits in Water-Deficit Common Bean Plants. Crop Pasture Sci. **2021**, 73, 44–55.

17. Cipriano, P.E.; da Silva, R.F.; de Lima, F.R.D.; de Oliveira, C.; de Lima, A.B.; Celante, G.; Dos Santos, A.A.; Archilha, M.V.L.R.; Pinatto-Botelho, M.F.; Faquin, V.; et al. Selenium Biofortification via Soil and Its Effect on Plant Metabolism and Mineral Content of Sorghum Plants. J. Food Compos. Anal. **2022**, 109, 104505.

18. Fang, Y.; Wang, L.; Xin, Z.; Zhao, L.; An, X.; Hu, Q. Effect of Foliar Application of Zinc, Selenium, and Iron Fertilizers on Nutrients Concentration and Yield of Rice Grain in China. J. Agric. Food Chem. **2008**, 56, 2079–2084.

19. Li, J.; Yang, W.; Guo, A.; Qi, Z.; Chen, J.; Huang, T.; Yang, Z.; Gao, Z.; Sun, M.; Wang, J. Combined Foliar and Soil Selenium Fertilizer Increased the Grain Yield, Quality, Total Se, and Organic Se Content in Naked Oats. J. Cereal Sci. **2021**, 100, 103265.

20. Araujo, A.M.; de Lima Lessa, J.H.; de Lima, F.R.D.; Raymundo, J.F.; Curi, N.; Guilherme, L.R.G.; Lopes, G. Adsorption of Selenite in Tropical Soils as Affected by Soil Management, Ionic Strength, and Soil Properties. J. Soil Sci. Plant Nutr. **2020**, 20, 139–148.

21. Curtin, D.; Hanson, R.; Lindley, T.N.; Butler, R.C. Selenium Concentration in Wheat (*Triticum aestivum*) Grain as Influenced by Method, Rate, and Timing of Sodium Selenate Application. N. Z. J. Crop Hortic. Sci. **2006**, 34, 329–339.

22. Bañuelos, G.S.; Freeman, J.L.; Arroyo, I.S. Selenium Content and Speciation Differences in Selenium Enriched Soups Made from Selenium Biofortified Plants. J. Food Compos. Anal. **2022**, 105, 104255.

23. Khanam, A.; Platel, K. Bioaccessibility of Selenium, Selenomethionine and Selenocysteine from Foods and Influence of Heat Processing on the Same. Food Chem. **2016**, 194, 1293–1299.

24. Šindelářová, K.; Száková, J.; Tremlová, J.; Mestek, O.; Praus, L.; Kaňa, A.; Najmanová, J.; Tlustoš, P. The Response of Broccoli (*Brassica oleracea* Convar. Italica) Varieties on Foliar Application of Selenium: Uptake, Translocation, and Speciation. Food Addit. Contam. Part A **2015**, 32, 2027–2038.

25. Zhang, M.; Xing, G.; Tang, S.; Pang, Y.; Yi, Q.; Huang, Q.; Huang, X.; Huang, J.; Li, P.; Fu, H. Improving Soil Selenium Availability as a Strategy to Promote Selenium Uptake by High-Se Rice Cultivar. Environ. Exp. Bot. **2019**, 163, 45–54.

26. Hu, J.; Zhao, Q.; Cheng, X.; Selomulya, C.; Bai, C.; Zhu, X.; Li, X.; Xiong, H. Antioxidant Activities of Se-SPI Produced from Soybean as Accumulation and Biotransformation Reactor of Natural Selenium. Food Chem. **2014**, 146, 531–537.

27. Silva, M.A.; de Sousa, G.F.; Corguinha, A.P.B.; de Lima Lessa, J.H.; Dinali, G.S.; Oliveira, C.; Lopes, G.; Amaral, D.; Brown, P.; Guilherme, L.R.G. Selenium Biofortification of Soybean Genotypes in a Tropical Soil via Se-Enriched Phosphate Fertilizers. Front. Plant Sci. **2022**, 13, 988140.

28. Soil Survey Staff. Keys to Soil Taxonomy, 13th ed.; USDA-Natural Resources Conservation Service: Washington, DC, USA, **2022**.

29. Brazilian Agricultural Research Company (EMBRAPA). Manual de Métodos de Análise de Solo, 3rd ed.; Embrapa: Brasília, Brazil, **2017**; ISBN 978-85-7035-771-7.

30. Bañuelos, G.S.; Walse, S.S.; Yang, S.I.; Pickering, I.J.; Fakra, S.C.; Marcus, M.A.; Freeman, J.L. Quantification, Localization, and Speciation of Selenium in Seeds of Canola and Two Mustard Species Compared to Seed-Meals Produced by Hydraulic Press. Anal. Chem. **2012**, 84, 6024–6030.

31. Montes-Bayón, M.; Yanes, E.G.; Ponce de León, C.; Jayasimhulu, K.; Stalcup, A.; Shann, J.; Caruso, J.A. Initial Studies of Selenium Speciation in *Brassica juncea* by LC with ICPMS and ES-MS Detection: An Approach for Phytoremediation Studies. Anal. Chem. **2002**, 74, 107–113.

32. Yemm, E.W.; Cocking, E.C.; Ricketts, R.E. Ricketts Estimation of Amino Acids with Ninhydrin. Analyst **1955**, 80, 209–214.

33. R Core Team. R: A Language and Environment for Statistical Computing; R Core Team: Vienna, Austria, **2022**.

34. Brown, P.H.; Zhao, F.-J.; Dobermann, A. What Is a Plant Nutrient? Changing Definitions to Advance Science and Innovation in Plant Nutrition. Plant Soil **2022**, 476, 11–23.

35. Boldrin, P.F.; Faquin, V.; Ramos, S.J.; Boldrin, K.V.F.; Ávila, F.W.; Guilherme, L.R.G. Soil and Foliar Application of Selenium in Rice Biofortification. J. Food Compos. Anal. **2013**, 31, 238–244.

36. Zhu, Y.-G.; Pilon-Smits, E.A.H.; Zhao, F.-J.; Williams, P.N.; Meharg, A.A. Selenium in Higher Plants: Understanding Mechanisms for Biofortification and Phytoremediation. Trends Plant Sci. **2009**, 14, 436–442.

37. Deng, X.; Zhao, Z.; Lv, C.; Zhang, Z.; Yuan, L.; Liu, X. Effects of Sulfur Application on Selenium Uptake and Seed Selenium Speciation in Soybean (*Glycine max* L.) Grown in Different Soil Types. Ecotoxicol. Environ. Saf. **2021**, 209, 111790.

38. Yuan, Z.; Long, W.; Liang, T.; Zhu, M.; Zhu, A.; Luo, X.; Fu, L.; Hu, Z.; Zhu, R.; Wu, X. Effect of Foliar Spraying of Organic and Inorganic Selenium Fertilizers during Different

Growth Stages on Selenium Accumulation and Speciation in Rice. Plant Soil **2022**, volume number, 1–15.

39. Lessa, J.H.L.; Araujo, A.M.; Silva, G.N.T.; Guilherme, L.R.G.; Lopes, G. Adsorption-Desorption Reactions of Selenium (VI) in Tropical Cultivated and Uncultivated Soils under Cerrado Biome. Chemosphere **2016**, 164, 271–277.

40. Araujo, A.M.; de Lima Lessa, J.H.; Ferreira, L.A.; Guilherme, L.R.G.; Lopes, G. Soil Management and Ionic Strength on Selenite Retention in Oxidic Soils. Ciênc. Agrotec. **2018**, 42, 395–407.

41. Li, Z.; Liang, D.; Peng, Q.; Cui, Z.; Huang, J.; Lin, Z. Interaction between Selenium and Soil Organic Matter and Its Impact on Soil Selenium Bioavailability: A Review. Geoderma **2017**, 295, 69–79.

42. Schiavon, M.; Nardi, S.; dalla Vecchia, F.; Ertani, A. Selenium Biofortification in the 21st Century: Status and Challenges for Healthy Human Nutrition. Plant Soil **2020**, 453, 245–270.

43. Premarathna, L.; McLaughlin, M.J.; Kirby, J.K.; Hettiarachchi, G.M.; Stacey, S.; Chittleborough, D.J. Selenate-Enriched Urea Granules Are a Highly Effective Fertilizer for Selenium Biofortification of Paddy Rice Grain. J. Agric. Food Chem. **2012**, 60, 6037–6044.

44. Winkel, L.; Vriens, B.; Jones, G.; Schneider, L.; Pilon-Smits, E.; Bañuelos, G. Selenium Cycling Across Soil-Plant-Atmosphere Interfaces: A Critical Review. Nutrients **2015**, 7, 4199–4239.

45. Lu, X.; He, Z.; Lin, Z.; Zhu, Y.; Yuan, L.; Liu, Y.; Yin, X. Effects of Chinese Cooking Methods on the Content and Speciation of Selenium in Selenium Bio-Fortified Cereals and Soybeans. Nutrients **2018**, 10, 317.

46. Bañuelos, G.S.; Freeman, J.; Arroyo, I. Accumulation and Speciation of Selenium in Biofortified Vegetables Grown under High Boron and Saline Field Conditions. Food Chem. X **2020**, 5, 100073.

47. Schiavon, M.; Pilon-Smits, E.A.H. The Fascinating Facets of Plant Selenium Accumulation–Biochemistry, Physiology, Evolution and Ecology. New Phytol. **2016**, 213, 1582–1596.

48. White, P.J. Selenium Metabolism in Plants. Biochim. Biophys. Acta (BBA)-Gen. Subj. **2018**, 1862, 2333–2342.

49. Sors, T.G.; Ellis, D.R.; Salt, D.E. Selenium Uptake, Translocation, Assimilation and Metabolic Fate in Plants. Photosynth. Res. **2005**, 86, 373–389.

50. Gupta, M.; Gupta, S. An Overview of Selenium Uptake, Metabolism, and Toxicity in Plants. Front. Plant Sci. **2017**, 7, 2074.

51. Tapiero, H.; Townsend, D.M.; Tew, K.D. The Antioxidant Role of Selenium and Seleno-Compounds. Biomed. Pharmacother. **2003**, 57, 134–144.

52. Schiavon, M.; Lima, L.W.; Jiang, Y.; Hawkesford, M.J. Effects of Selenium on Plant Metabolism and Implications for Crops and Consumers. In Selenium in Plants; Elizabeth,

A.H., Pilon-Smits, E.A.H., Winkel, Z.Q.L., Eds.; Springer: Berlin/Heidelberg, Germany, **2017**; pp. 257–275.

53. Institute of Medicine (US) Panel on Dietary Antioxidants and Related Compounds. Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids; National Academies Press (US): Washington, DC, USA, **2000**.

54. Ramos, S.J.; Rutzke, M.A.; Hayes, R.J.; Faquin, V.; Guilherme, L.R.G.; Li, L. Selenium Accumulation in Lettuce Germplasm. Planta **2011**, 233, 649–660.

55. Huang, Y.; Fan, B.; Lei, N.; Xiong, Y.; Liu, Y.; Tong, L.; Wang, F.; Maesen, P.; Blecker, C. Selenium Biofortification of Soybean Sprouts: Effects of Selenium Enrichment on Proteins, Protein Structure, and Functional Properties. Front. Nutr. **2022**, 9, 849928.

56. Sanmartín, C.; Garmendia, I.; Romano, B.; Díaz, M.; Palop, J.A.; Goicoechea, N. Mycorrhizal Inoculation Affected Growth, Mineral Composition, Proteins and Sugars in Lettuces Biofortified with Organic or Inorganic Selenocompounds. Sci. Hortic. **2014**, 180, 40–51.

Supplementary material

Table S1: Means (% of Se species) and Tukey test for Se species.

Abbreviation	SeMet	SeCys	MetSeCys	Selenite	Selenate
L-Cnt	54.00 abc	17.00 a	2.38 a	0.87 a	23.88 ab
LF-In10	81.00 abc	4.09 bc	0.90 a	1.23 a	12.00 ab
LF-In80	94.10 a	1.29 d	0.53 a	2.34 a	1.10 b
LF-Or10	75.50 bc	11.25 ab	0.35 a	0.89 a	10.57 a
LS-C80	92.40 a	1.49 cd	0.18 a	1.24 a	4.00 b
LS-E80	90.80 a	1.51 cd	0.10 a	1.10 a	5.95 ab
M-Cnt	73.50 c	7.86 ab	1.50 a	0.00 b	16.98 ab
MF-In10	91.60 a	1.24 d	0.63 a	1.52 a	4.55 ab
MF-In80	90.20 a	1.20 d	2.93 a	2.18 a	3.15 b
MF-Or10	89.40 ab	2.38 cd	0.95 a	1.00 a	5.95 ab
MS-C80	92.4 a	1.02 d	0.20 a	0.65 ab	5.05 ab
MS-E80	90.0 a	0.97 d	0.63 a	1.37 a	6.67 ab

Letters compare Se species among the treatments

Table S2: Correlation coefficients among Se species in soybean grains.

Variables	SeCys	MeSeCys	Selenite	SeMet	Selenate
MeSeCys	0.07^{ns}	-	-	-	-
Selenite	-0.22^{ns}	-0.05^{ns}	-	-	-
SeMet	-0.78^{*}	-0.41^{*}	0.20^{ns}	-	-
Selenate	0.52^{*}	0.37^{*}	-0.28^{ns}	-0.92^{*}	-
Se-total	-0.34^{*}	0.16^{ns}	0.39^{*}	0.34^{*}	-0.36^{*}

$^{/1}$ non-significant correlation; $^{/2}$ Pearson's correlations significant.

CONCLUDING REMARKS

This research made significant contributions to the understanding of the beneficial impact of Se on soybean yield in field conditions, while shedding light on the different forms of Se found in the grains. Moreover, we successfully established a comprehensive recommendation for the biofortification of soybean grains with Se. This study stands as a pioneering effort, introducing a novel "4R management" protocol specifically tailored for Se fertilization in soybean cultivated under tropical soil environments. This protocol emphasizes the importance of selecting the appropriate source, applying the correct dose, timing the application correctly, and ensuring precise placement, thereby maximizing the effectiveness of Se utilization in soybean crops.

Our findings demonstrate the remarkable responsiveness of soybean to Se biofortification, whether applied through soil or foliar methods. However, the concentration of Se in soybean grains is significantly influenced by the mode of application. Specifically, our study highlights the promising potential of soil application of Se-carrying fertilizers, namely C-MAP + Se and E-MAP + Se, as an effective approach for biofortifying soybeans with Se, particularly in tropical soil conditions. This significance was particularly evident in the TMG7061 genotype, where the application of these fertilizers resulted in notable crop yield increases. Conversely, when employing foliar application, we observed a linear increase in Se content in soybean grains as Se doses increased.

The genotypes 58I60 Lança and M5917 exhibited critical Se thresholds of 1.0 mg kg^{-1} and 3.0 mg kg^{-1}, respectively. It is important to highlight that when considering the required daily intake and average consumption of soybean protein, the Se intake values necessary for biofortification in soybean are lower than the levels that led to reduced yields in both genotypes. Regarding Se content enhancement in soybean, foliar application of inorganic Se at a dose of 80 g ha^{-1} proved to be more efficient for both genotypes. Foliar application at a dose of 10 g ha^{-1} achieved Se levels in grains comparable to those obtained with the soil application of 80 g ha^{-1} indicating the superior efficiency of foliar application.

More than 80% of the total Se in soybeans is present in organic form, with SeMet being the primary Se species accumulated in soybean grains. The highest SeMet levels in soybean grains were achieved through the application of inorganic Se, whether via soil or foliar methods. Based on our comprehensive findings, we recommend the foliar application of 15.0 g ha^{-1} , in conjunction with MNF, for effective biofortification of soybeans with Se.

The findings of our research significantly enhance the precision and knowledge regarding the application and effects of Se on soybean cultivation. They provide information for decision-making regarding the utilization of Se, not only in soybean production but also in other crops, thereby addressing the challenges arising from Se deficiency in human nutrition.

As a recommendation for future studies, we highlight the importance of evaluating the dynamics of Se within the agricultural production system. In addition, we suggest investigating the transformation of Se in processed products derived from biofortified grains.