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Agronomic biofortification with selenium impacts storage proteins in grains of upland rice

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ABSTRACT

Selenium (Se) is an essential element for humans and animals. Rice is one of the most consumed cereals in the world, so agronomic biofortification of cereals with Se may be a good strategy to increase the levels of daily intake of Se by the population. This study evaluated agronomic biofortification of rice genotypes with selenium (Se) and its effects on grain nutritional quality. Five rates of Se (0, 10, 25, 50 and 100 g ha^{-1}) were applied as selenate via the soil to three rice genotypes under field conditions. Selenium concentrations in the leaves and polished grains increased linearly in response to Se application rates. A highly significant correlation was observed between the Se rates and the Se concentration in the leaves and grains, indicating high translocation of Se. Application of Se also increased the concentrations of albumin, globulin, prolamin and glutelin in polished grains. Biofortifying rice genotypes using 25 g Se ha^{-1} could increase the average daily Se intake from 4.64 to 66 µg day^{-1}. Considering that the recommended daily intake of Se by adults is 55 µg day^{-1}, this agronomic strategy could contribute to alleviating widespread Se malnutrition.

Keywords: biofortification, seed proteins, micronutrients, selenium, rice (*Oryza sativa* L.)

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1. INTRODUCTION

Selenium (Se) is an essential micronutrient for humans and animals.[1-2] However, the concentration of Se in soils and foods varies greatly.[1-4] The recommended daily intake of Se is 55 μg kg^{-1} for human adults.[5-7] It is estimated that more than 1 billion people suffer from Se deficiency globally,[8,9] which can lead to numerous health issues including hypothyroidism, cardiovascular disease, viral diseases, male infertility, cognitive impairment, weakening of the immune system and increased incidence of various forms of cancer.[6,7,10,11]

Since most Se in the human diet is derived directly or indirectly from edible plants, Se deficiency in humans is attributed to agricultural production on soils with little phytoavailable Se.[6,7,12-15] Despite the consequences of Se deficiency having been recognized for decades, strategic interventions to meet the shortfall of Se in food are still limited.[16,17] Biofortification is the process of enriching edible crops with mineral nutrients based on management strategies that increase the phytoavailabilty of nutrients and/or genotypes that partition more nutrient into their edible portions.[18] In the case of Se, biofortification of crops can be achieved through the application of Se fertilisers and/or adopting cultivars that partition more Se to their edible portions.[12] Previous studies have indicated that the application of low concentrations of Se does not lead to a loss of crop productivity or harvest index.[17,20,21] In addition, fertilisation with Se at low concentrations can mitigate oxidative stress and increase photosynthetic activity thereby resulting in greater plant growth.[22] Selenium can also affect nitrogen metabolism in plants by regulating nitrate reductase activity and increasing nitrogen assimilation and protein biosynthesis.[11,23] In our former study, Reis et al[15] observed the interaction between nitrogen and Se in upland rice under field conditions showing that plants treated with nitrogen can accumulate more Se in seeds. In addition, Se can

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affect sulphur metabolism and molybdenum concentration in plants, which is co-factor of nitrate reductase enzyme showing a direct effect on nitrogen metabolism.[23,36]

The application of Se along with NPK fertiliser is an effective way to address the low concentrations of Se typically found in human and animal food, as demonstrated by the results of decades of study in Finland.[24-26] After the adoption of the Se-biofortification programme in Finland, the Se-status of the population has improved to optimal levels.[26]

Rice is a major cereal crop consumed in large quantities across the globe.[15] Therefore, it forms a suitable a target crop for biofortification programmes to improve human nutrition. This study evaluated the agronomic biofortification of upland rice with Se and its effect on grain nutritional quality. It provides important information regarding suitable Se application rates to achieve Se biofortified grains and the contribution this might have on dietary Se intakes.

2. MATERIALS AND METHODS

Experimental Conditions. The study was conducted from January 3rd to May 27th 2015 at the Research Farm of the Faculty of Engineering of Ilha Solteira (FEIS-UNESP), located in Selvíria, Mato Grosso do Sul, Brazil (20º22'S and 51º22'W, altitude of 335 m). The experimental area belongs to the Cerrado biome and has been cultivated for more than 25 years, with the last 10 years of cultivation occurring under a no-tillage system. According to the Köppen classification scheme, the climate of the region is type Aw, humid tropical, with a rainy season in the summer and dry season in the winter. The average annual rainfall is 1,232 mm, and the average temperature is 24.5 °C.[17] During the experiment, the average daily temperature varied between 27.2 ºC and 15.3 ºC, the average rainfall was 3.0 mm d^{-1}, and the average relative humidity was 86%.

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The soil of the area was classified as Typic Dystrophic Red Latosol (LVd) and very clayey, corresponding to the *Oxisol* order. Soil analysis was performed according methods described by Raij et al.[27] and the soil had the following chemical characteristics: phosphorus (resin), 29 mg dm^{-3}; organic matter, 21 g dm^{-3}; pH calcium chloride ($CaCl_2$), 5.3; potassium, 3.5 mmolc dm^{-3}; calcium, 38 mmolc dm^{-3}; magnesium, 22 mmolc dm^{-3}; H^+ + Al, 29 mmolc dm^{-3}; aluminium, 0 mmolc dm^{-3}; nickel, 0.1 mg dm^{-3}; Se, 62 µg kg^{-1}; cation exchange capacity, 92.5 mmolc dm^{-3}; and base saturation, 69%.

Experimental Design and Treatments. Soil acidity correction was not performed due to the optimal base saturation for upland rice requirements. The experimental area was desiccated with glyphosate (4.0 L ha^{-1}), carfentrazone-ethyl (200 mL ha^{-1}) and 0.5% mineral oil 20 days before sowing. Upland rice genotypes were sown with a density of 70 kg ha^{-1}. Rice seeds were treated with Standak commercial product (2 mL kg^{-1} of seeds). All plots received 250 kg ha^{-1} of NPK fertilisation using the formulation 08-28-16. The experiment was sown on January 5, 2015, and the seedlings emerged 7 days after sowing. When necessary, irrigation was performed by a central pivot with an average water depth of 14 mm and 72 h irrigation shift. Phytosanitary control was carried out during the plant development cycle with metsulfurom-methyl (3.3 g ha^{-1}), chlorantraniliprole (50 mL ha^{-1}), flubendiamide (60 mL ha^{-1}), and imidacloprid + beta-cyfluthrin (0.8 L ha^{-1}).

The study was conducted in a randomized complete block design, in a 3 × 5 factorial scheme, corresponding to three rice genotypes (ANa 5015, AN Cambará and ANa 7007) and five rates of Se (equivalent to 0, 10, 25, 50 and 100 g ha^{-1}) applied in the form of sodium selenate. A total of four replicate plots were used per genotype and Se treatment, forming a total of 60 plots. Each plot

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consisted of five rows, which were 5 m in length and with 35 cm spacing between the rows. All the Se required for each treatment across all four replicates was weighed and diluted in 2 L of water, generating a stock solution for each treatment. The stock solution was then subdivided into four portions of 500 mL each. Solutions were applied to the planting furrow at 30 days after seedlings emergence. In each plot, 100 mL of solution was applied to each line near de plants using a small malleable polyethylene bottle with a pierced cap.

~~Selenium treatments were prepared from stock solution, which were diluted in 300 mL bottles for distribution in the sowing furrow 30 days after emergence of the seedlings.~~

Leaves and seed harvest. The flag leaves from 20 plants from each plot were collected randomly 15 days after treatment application. At the end of the experiment, the panicles were collected and weighed. The husk of seeds was removed and the grain was polished. After polishing, the grains were milled in a ball mill, and the flour was sent for elemental analysis as described below and analyses of storage protein concentrations.

Determination of Albumin, Globulin, Prolamin and Glutelin Fractions. The quantification of protein fractions was performed as described by Reis et al.[15] Dried, ground samples (0.25 g) were weighed and placed in 15-mL Falcon tubes for the sequential extraction of the storage proteins.

For albumin extraction, 10 mL of deionized water was added to each tube, and the samples were shaken for 1 minute. After being shaken, the tubes were centrifuged at a temperature of 4 °C at 9.000 rpm for 20 minutes for phase separation. The supernatant was removed for albumin quantification. The precipitate was extracted with 10 mL NaCl 5% according to the procedures described above, and the supernatant was collected for globulin quantification. The precipitate was

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resuspended again with 5 mL of 60% ethanol, and the supernatant was collected for prolamin quantification. Finally, the glutelin fraction was extracted with 10 mL NaOH 0.4% and quantified.

The concentrations of albumin, globulin, prolamin and glutelin were determined according to the method of Bradford[29] with bovine serum albumin (BSA) used as the standard. For protein quantification, a 100 µL aliquot of supernatant and 5 mL of Bradford reagent were pipetted into test tubes. The absorbance reading was performed in a spectrophotometer at 595 nm, and the results were expressed in mg protein g^{-1} DM (dry mass).

~~**Seed harvest.** At the end of the experiment, the panicles were collected and weighed. The husk of seeds was removed and the grain was polished. After polishing, the grains were milled in a ball mill, and the flour was sent for elemental analysis as described below and analyses of storage protein concentrations.~~

Elemental Analysis. The leaves and seeds were dried and used for quantification of macro- and micronutrient concentrations. ~~Polished grains collected at the end of the crop cycle were also analysed.~~ Following sulphuric acid digestion of the grains, nitrogen concentrations were determined using the semi-micro-Kjeldahl method. For the quantification of other mineral elements, the material was subjected to nitric acid-hydrogen peroxide digestion according to Chilimba et al.[13] The samples were digested in a microwave oven for 45 minutes under controlled pressure (20 bar) in 3 mL of 70% nitric acid (Merck), 2 mL of hydrogen peroxide (Merck) and 3 mL of milli-Q water. Selenium and nutrient analysis was performed using Inductively Coupled Plasma Mass Spectroscopy (ICP-MS).

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Statistical Analysis

The normality and homoscedasticity of the data were assessed by the Anderson-Darling and Levene's tests. The data were subjected to analysis of variance by the F test ($p \leq 0.05$) for treatments (Se rates and rice genotypes). The results were subjected to the Tukey test at the level of $p \leq 0.05$. Correlations between the dependent variables (Se, sulphur, nitrogen, molybdenum, albumin, globulin, prolamin, and glutelin) were obtained using the Pearson correlation coefficient (CORR) procedure and illustrated as a heatmap graph. Analyses were conducted using R and Minitab softwares, and graphs were prepared using SigmaPlot 12.5.

RESULTS AND DISCUSSION

Concentration of sulphur (S), molybdenum (Mo), selenium (Se) and nitrogen (N). The concentrations of S, Mo and Se in leaves and polished grains of upland rice were influenced by the Se application rates and the genotype of rice evaluated, whereas N concentrations were influenced only by genotype (Figures 1 and 2). The cultivars ANa 5015 and AN Cambará did not show altered concentrations of S in leaves with increasing rates of Se application (Figure 1A). On the other hand, S concentration in leaves of the cultivar ANa 7007 decreased with increasing rates of Se application (Figure 1A). Beyond 50 g ha^{-1} applied Se, the concentration of S in the cultivar ANa 7007 was smaller than that in the other cultivars. In the polished grains, the concentration of S was different from that found in the leaves. In general, no difference was observed between cultivars for S concentration in polished grains, except at 25 g ha^{-1} applied Se in cultivar ANa 7007, which had a lower grain S concentration. When 100 g Se ha^{-1} was applied, the cultivar AN Cambará showed higher S concentrations than the other cultivars (Figure 2A).

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Leaf N concentration was lower in ANa 5015 and greater in ANa 7007 than AN Cambará (Figure 1B). On the other hand, polished N concentrations for cultivars ANa 5015 and ANa 7007 were statistically similar and greater than that of AN Cambará.

Leaf Mo concentration increased in response to Se application in cultivars ANa 5015 and ANa 7007 at the rates of 25 and 10 g Se ha^{-1}, respectively (Figure 1C). When 100 g Se ha^{-1} was applied, ANa 7007 showed a greater concentration of leaf Mo. The concentration of Mo in the polished grains of AN Cambará decreased with increasing rates of Se applied (Figure 2C). In general, the application of Se did not affect the concentrations of Mo in grains of ANa 5015 and ANa 7007, although the application of 25 g Se ha^{-1} to Ana 2015 did reduce the concentration of Mo in polished grains. Regardless of the Se application rate, ANa 7007 showed greater Mo concentration in the polished grains than other cultivars.

Selenium concentration in leaves (Figure 1D) and in polished grains (Figure 2D) increased in response to Se application rates. ~~AN Cambará had greater concentrations of Se in the leaves relative to the other genotypes.~~ Leaf Se concentration range was from 0.347 to 2.87 mg kg^{-1} in AN Cambara, 0.015 to 2.97 mg kg^{-1} in ANA 5015, and 0.014 to 2.49 mg kg^{-1} in ANA 7007 (Figure 1 D). Grain Se concentration ranged from 0.239 to 1.297 mg kg^{-1} in AN Cambara, 0.143 to 2.16 mg kg^{-1} in ANA 5015, and 0.099 to 2.062 mg kg^{-1} in ANA 7007. The linear increase in grain Se concentration indicates high Se translocation from source (leaves) to sink (grains)~~. When 100 g ha^{-1} of Se was applied, leaf Se concentration of ANa 5015 was statistically equal to the leaf Se concentration in AN Cambará, whereas grains of ANa 5015 had a greater Se concentration than other genotypes. Under the same Se application rate, ANa 7007 had a polished grain Se concentration similar to that of ANa 5015.~~

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While no evidence exists for the essentiality of Se in higher plants, a number of beneficial effects of Se on plant physiology have been recognized in several plant species.[1,4,15] Selenium can be acquired by plant roots as selenate, selenite and from organic forms such as selenocysteine (SeCys) and selenomethionine (SeMet).[2,12] When in the form of selenate, Se is taken up by root cells using sulphate transporters.[2] A reduction in leaf S concentration and an increase in the concentration of Se were observed in the cultivar ANa 7007 (Figure 1). In addition, AN Cambará showed lower concentrations of S in the grains at the highest applied rate of Se (Figure 2), which suggests an antagonism between S and Se. This is also consistent with the reduction in the concentration of S with Se fertilisation reported in other plant species.[1,2,4]

In general, the cultivar AN Cambará showed the greatest response to the application of Se in terms of leaf Se concentration but showed a lesser response in grains (Figure 2D). This observation probably reflects differences between cultivars in the activity of sulphate transporters providing different functions (uptake, translocation, intracellular compartmentation) in plants[31-33] between cultivars. Selenium concentration increased in all tested rice cultivars in response to Se application rates. Agronomic biofortification with Se via soil appears a good strategy to increase the Se concentration in leaves and grains of upland rice. In general, the effect of genotypic variation on Se concentration in rice grains is clear. This observation suggests a need for more studies with a greater number of rice cultivars and Se sources.

Dietary Se intake. Brazilian eating habits vary widely due to social, economic and geographical differences, but foods such cow milk and eggs are commonly consumed throughout the country. The mean Se concentrations in Brazilian cow milk and eggs are 0.08 and 0.21 μg g^{-1}, respectively.[43,44] The daily intake of cow milk and eggs in the southeastern region of Brazil are 37

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and 9 g, respectively.[43,44] Multiplying Se concentration by the daily consumption of each of these foods, the total Se daily intake from them is about 5 µg Se day^{-1} per capita. Subtracting this value from the recommended dietary allowance of 55 µg Se d^{-1}, it is possible to establish a guide for the daily Se intake provided by rice grains of 50 µg d^{-1}, which is represented by the red line in Figure 3. When the average intake of rice by the Brazilian population (68.5 g d^{-1}) is considered, the application of 25 g Se ha^{-1} to cultivar ANa 7007 would be sufficient to reach and exceed the recommended daily intake (Figure 3).

According to the *Codex Alimentarius*, food biofortified with Se should not exceed 0.3 mg kg^{-1} Se.[13] The application of 10 g Se ha^{-1} yielded a concentration of 0.373 mg Se kg^{-1} in polished grains, which is based on the average concentration of Se in the grains of the three cultivars. However, since the Brazilian population only consumes, on average, 25 kg of rice year^{-1} or 68.5 g day^{-1},[4] the daily Se intake of rice biofortified at a rate of 10 g ha^{-1}, would not exceed the recommended daily Se intake of 55 µg day^{-1} [42] even though the Se concentration in grain exceeds the maximum concentration permitted by *Codex Alimentarius*.

The consumption of 68.5 g d^{-1} of rice biofortified at the rate of 25 g ha^{-1} could provide an average daily Se intake of 34.52 µg day^{-1} for ANa 7007, 44.57 µg day^{-1} for AN Cambará and 66.80 µg day^{-1} for ANa 5005. When 50 g Se ha^{-1} Se was applied, calculated daily Se intakes increase to 78.44 g day^{-1} for ANa 7007, 63.91 g day^{-1} for AN Cambará and 77.04 µg day^{-1} for ANa 5005, which is greater than the recommended daily Se intake. This study is one of the first field studies informing agronomic strategies for the biofortification of upland rice with Se in the Brazilian Cerrados. However, since genotypic variation is large, further studies must be carried out using a greater number of upland rice genotypes to establish the best rate of Se application to be used for different cultivars.

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Considering the recommended daily intake of Se, the ideal rate for agronomic biofortification of upland rice appears to be 25 g Se ha^{-1}. The application of this dosage in the cultivar AN Cambará gives an average concentration of 0.92 mg Se kg^{-1} in leaves and 0.65 mg Se kg^{-1} in the grain. When the Brazilian diet is considered, the average daily intake of Se would be 44.47 g day^{-1} from AN Cambará (Table 1).

Storage Proteins in Grains. Total protein concentration and storage protein fractions differed in their responses to the rate of Se application and among the cultivars studied (Figure 4). ANa 7007 had the greatest albumin concentration in the control treatment, whereas ANa 5015 had the lowest (Figure 4A). Selenium application rates beyond 25 g ha^{-1} increased the albumin concentration in ANa 5015. In AN Cambará, an increase was observed in the albumin concentration with the application of 10 g Se ha^{-1}, followed by a reduction with increasing Se application rates. The cultivar ANa 7007 showed a general increase in albumin concentrations with the application of Se, up to a rate of 100 g ha^{-1}, at which its concentration was lower than under the control treatment.

The cultivar ANa 5015 had a lower grain albumin concentration than the other cultivars (Figure 4A). The cultivars ANa 5015 and AN Cambará showed an increase in globulin concentration at the rate of 25 g Se ha^{-1}. For AN Cambará, globulin concentration was higher with the application of 100 g Se ha^{-1}. As with the albumin fraction, ANa 7007 had a greater concentration of glutelin, and ANa 5015 had the lowest concentration of glutelin in grains (Figure 4C). A significant increase occurred in glutelin concentrations (main protein fraction in rice grains) when Se was applied to the cultivar ANa 5015. For this variety, the application of 25 g Se ha^{-1} led to the highest concentration of glutelin, with an increase from 206 mg g^{-1} DM (control) to 270 mg g^{-1} DM (25 g Se ha^{-1}). For AN Cambará, a significant increase in glutelin occurred only for at 50

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g Se ha^{-1} applied Se, ranging from 235.79 mg g^{-1} DM (control treatment) to 265.34 mg g^{-1} DM (50 g Se ha^{-1}).

In the control treatment, AN Cambará had a greater prolamin ~~glutelin~~ concentration than the other genotypes (Figure 4D). The application of Se increased the concentrations of prolamin in cultivars ANa 5015 and ANa 7007. Up to a rate of 25 g Se ha^{-1}, all three varieties had statistically equal concentrations of prolamin. AN Cambará and ANa 7007 had a greater grain prolamin concentration with the application of 50 g Se ha^{-1}.

Total protein concentration in the control treatment had a response similar to that presented by the albumin and glutelin fractions, with the cultivar ANa 7007 showing greater protein concentration and the cultivar ANa 5015 showing the smallest concentration (Figure 4E).

~~Considering the recommended daily intake of Se, the ideal rate for agronomic biofortification of upland rice appears to be 25 g Se ha^{-1}. The application of this dosage in the cultivar AN Cambará gives an average concentration of 0.92 mg Se kg^{-1} in leaves and 0.65 mg Se kg^{-1} in the grain. When the Brazilian diet is considered, the average daily intake of Se would be 44.47 g day^{-1} from AN Cambará (Table 1).~~

In the cultivar ANa 5015, the application of 25 g Se ha^{-1} yields an average concentration of 0.65 mg Se kg^{-1} in the leaves and 0.97 mg Se kg^{-1} in grains. With the application of 25 g Se ha^{-1}, the grains contain approximately 10.69% albumin, 13.78% globulin, 3.74% prolamin and 71.81% glutelin (Table 1). When considering the Brazilian diet, the average daily intake would be 66.80 µg Se d^{-1} from ANa 5015, which is above the recommended daily Se intake for the population.

For the cultivar ANa 7007, the application of 25 g Se ha^{-1} yields the lowest Se concentration in leaves among the varieties, with 0.44 mg Se kg^{-1} in leaves and 0.65 mg Se kg^{-1} in grains. The grains contain approximately 15.28% albumin, which is the highest albumin concentration among

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293 the varieties (Table 1). In addition, the grains contain 11.81% globulin, 4.63% prolamin and
294 68.26% glutelin. The mean daily intake of Se from the consumption of ANa 7007 would be 44.52
295 μg day^{-1} of Se.

296 The positive relationship between Se concentration in rice leaves and grains and glutelin
297 and prolamin concentrations in grains is shown in Figure 5. Similar results were observed by Fang
298 et al.[38] and Tao et al.[39] who reported that an increase in Se concentrations in rice plants increased
299 the glutelin concentration in grains. In the endosperm, glutelin is the main protein fraction,
300 corresponding to approximately 80% of the protein, with lower concentrations of albumin and
301 globulin (15%) and prolamin (5-8%). To obtain polished white rice, buffing is used to remove the
302 bran (pericarp, tegument, aleurone layer and germ), which represents 8.5-14.8% of brown rice.[40-
303 41] Thus, polishing is not recommended for rice and the whole grain should be consumed to avoid
304 losses of proteins and Se. The effect of Se on nitrogen metabolism and an increase in the
305 concentration of albumin, glutelin and prolamin in rice grains is consistent with previous
306 observations by Reis et al.[15].

307

308 **CONCLUSIONS**

309 Selenium concentrations in leaves and grains, and the concentrations of storage proteins in
310 rice grains increased in response to the application of Se as selenate via soil. The application of 10
311 g Se ha^{-1} provided increases in the grain Se concentration above 0.3 mg kg^{-1}, the upper limit
312 allowed by the *Codex Alimentarius*. However, if adults consumed 68.5 g d^{-1} rice the application
313 of 10 g Se ha^{-1} would supply less Se through rice than their recommended dietary Se intake (55 μg
314 day^{-1}). The application of 25 g Se ha^{-1} to rice could increase the average daily intake of Se from

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4.64 to 66 μg day^{-1}. Thus, agronomic biofortification of rice with Se could prove a suitable strategy to increase human dietary Se intakes to reduce widespread Se malnutrition.

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Author Contributions

ARR designed the experiment, HPGR, JPQB; VMS performed the experiment, EFS, SDY performed the chemical analysis, RFRT and FFT performed the statistical analysis, HPGR, JPQB, VMS and EFS wrote the manuscript, which was revised by SDY, MRB, PJW and ARR.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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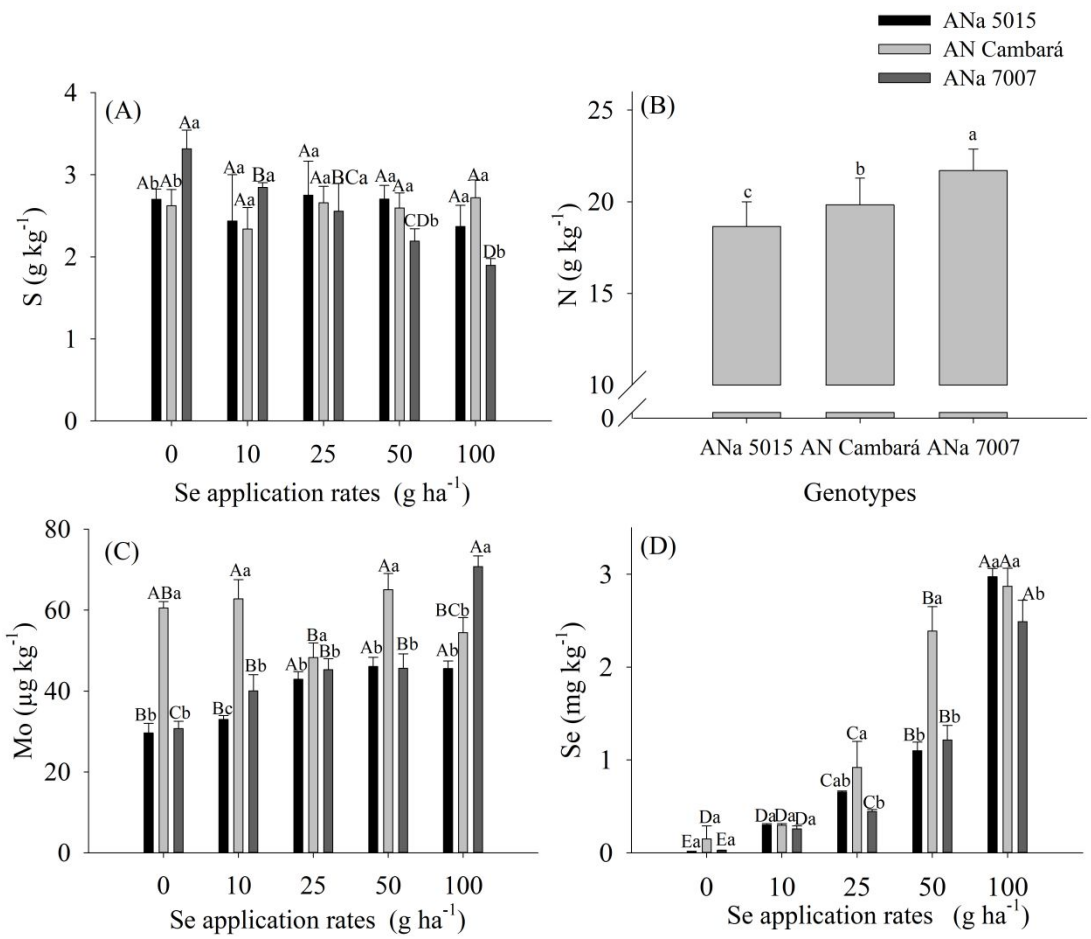

Figure 1. Foliar concentrations of sulphur (A), nitrogen (B), molybdenum (C) and selenium (D) in response to Se application in three rice cultivars (ANa 5015, AN Cambará and ANa 7007). Means followed by the same lowercase letters are not significantly different among the compared cultivars. Means followed by the same uppercase letters compare the cultivars as a function of the Se doses according to the t-test ($p \leq 0.05$). The error bars represent the standard error of the mean ($n = 4$).

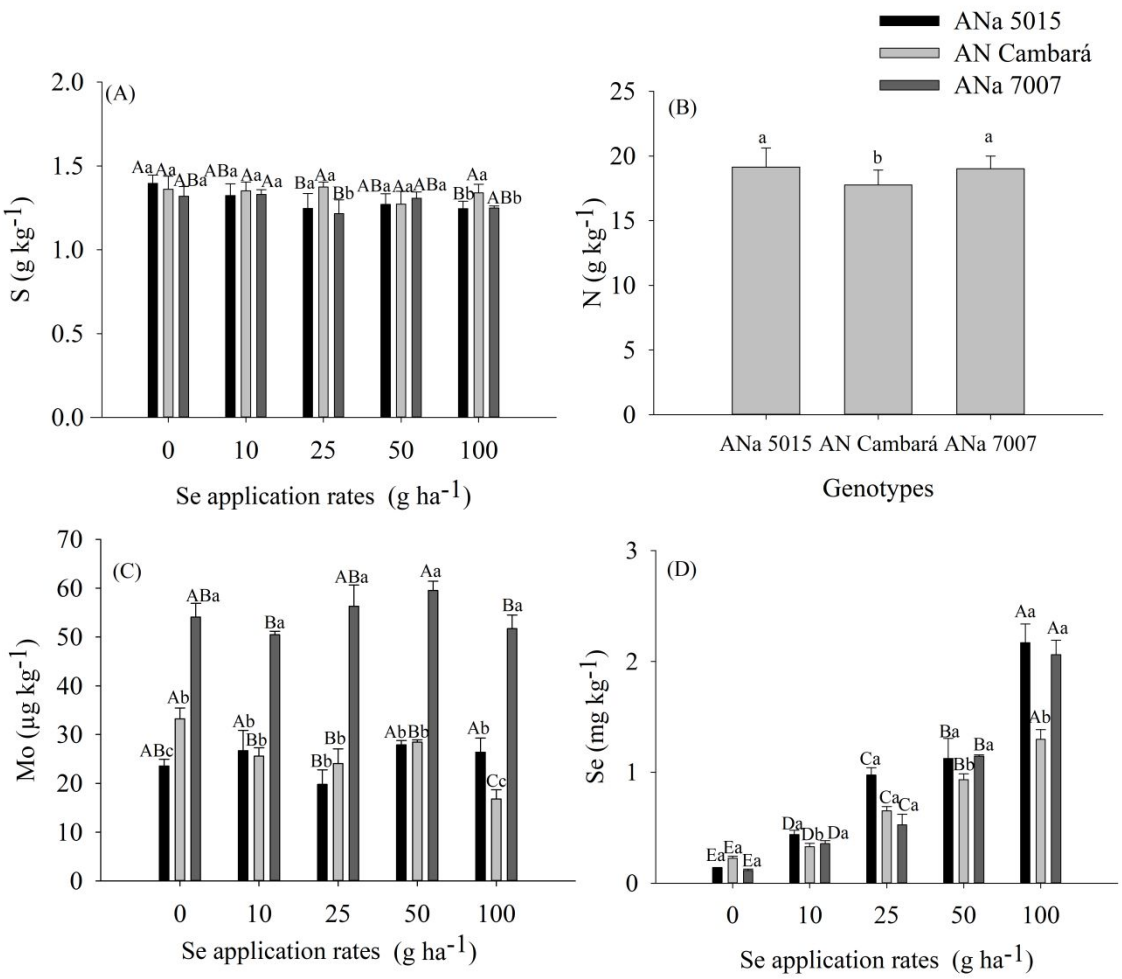

Figure 2. Concentration of sulphur (A), nitrogen (B), molybdenum (C) and selenium (D) in the grain in response to Se application in three rice cultivars (ANa 5015, AN Cambará and ANa 7007). Means followed by the same lowercase letters are not significantly different among the compared cultivars. Means followed by the same uppercase letters compare the cultivars as a function of the Se doses according to the t-test ($p \leq 0.05$). The error bars represent the standard error of the mean ($n = 4$).

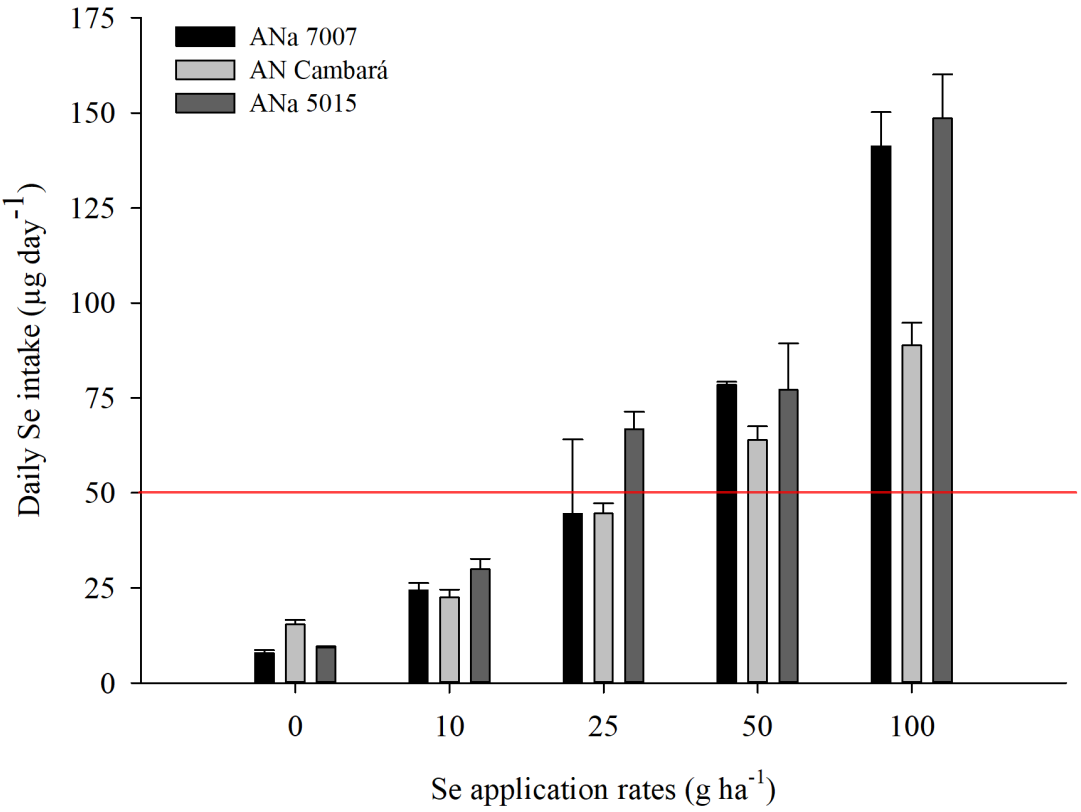

Figure 3. Estimated daily Se intake by humans from rice cultivars (ANa 5015, AN Cambará and ANa 7007) biofortified through Se application via the soil, calculated based on their grain Se concentrations and a consumption of 68.5 µg rice d^{-1}. The error bars represent the standard error of the mean (n = 4). The red line indicates the maximal Se intake by biofortified rice grains taking account the Se delivered from other food sources based on Brazilian eating habits.

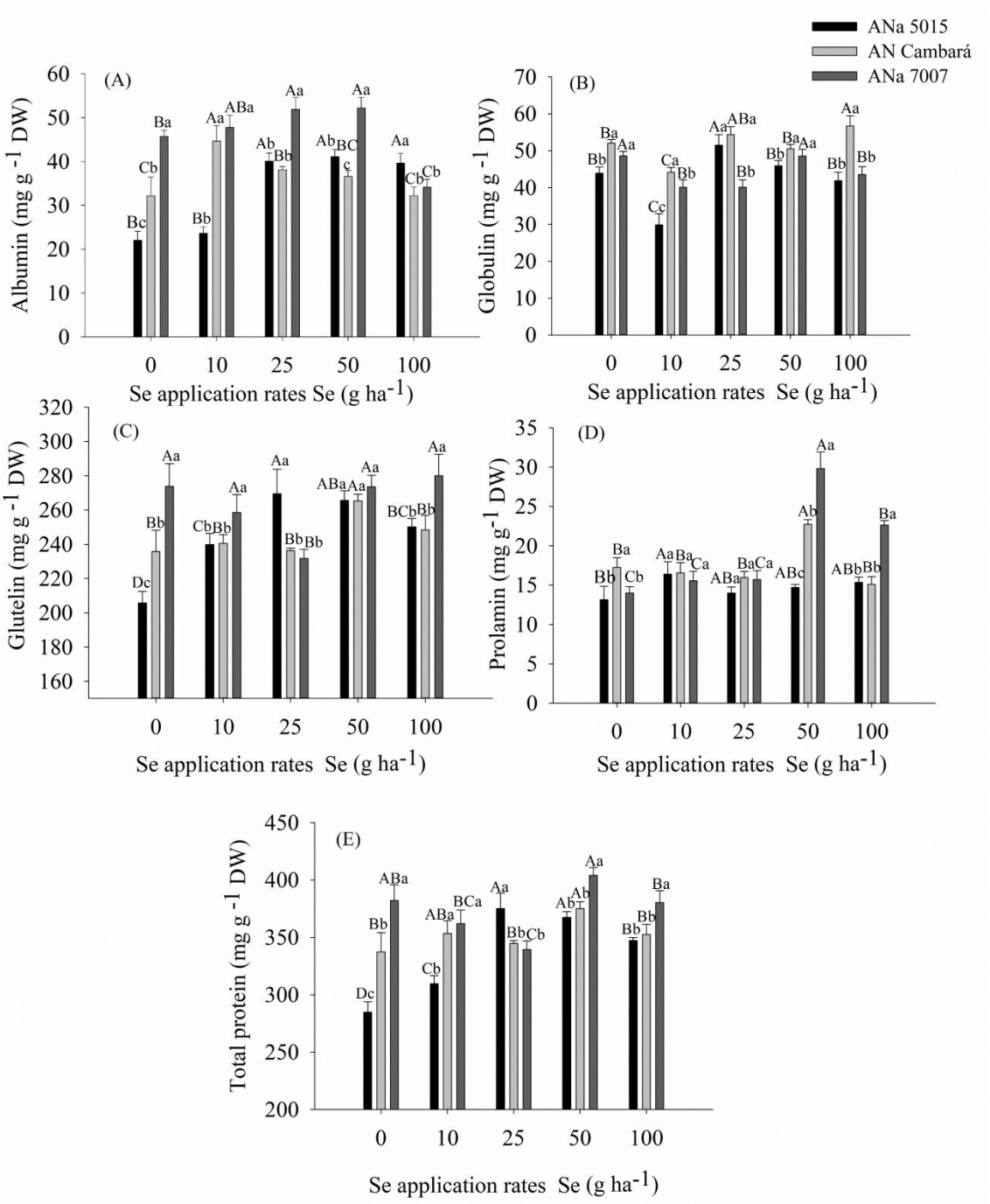

Figure 4. Concentration of albumin (A), globulin (B), prolamin (C), glutelin (D) and total protein (E) in response to Se application in three rice cultivars (Ana 7007, AN Cambará and ANa 5015). Means followed by the same lowercase letters are not significantly different among the compared cultivars. Means followed by the same uppercase letters compare the cultivars as a function of the Se doses according to the t-test ($p \leq 0.05$). The error bars represent the standard error of the mean ($n = 4$).

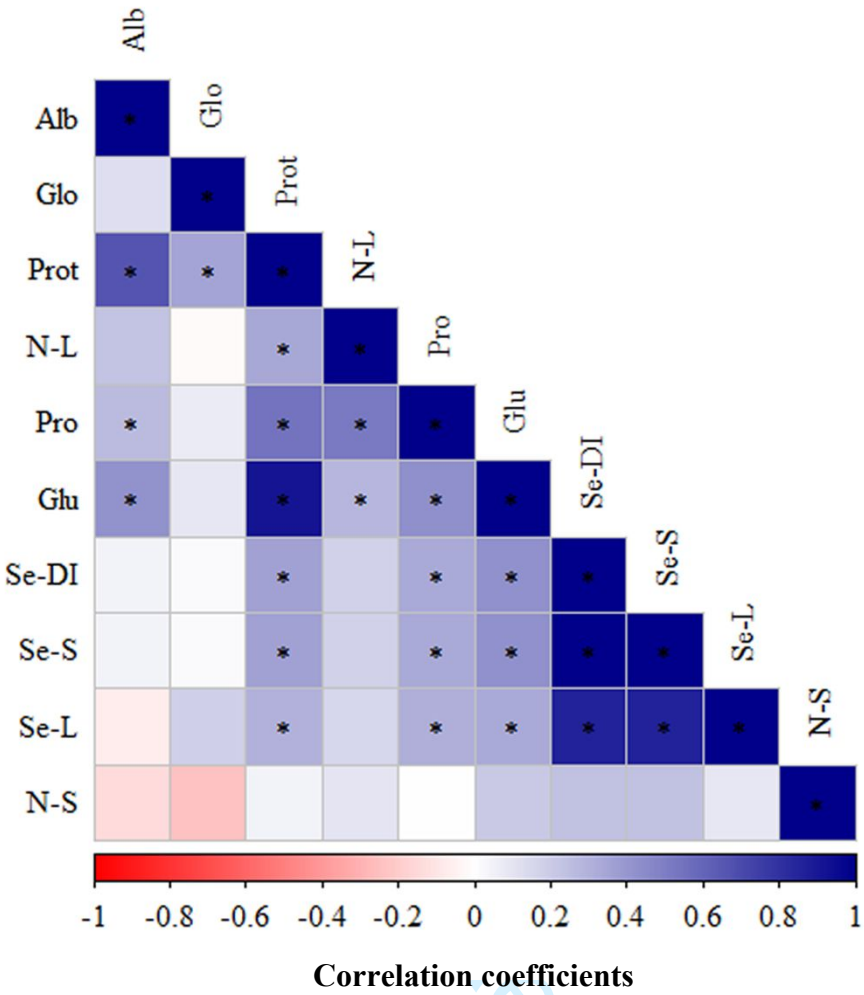

Figure 5. Heatmap of the Pearson correlation coefficients obtained from variables analysed in rice genotypes (ANa 5015, AN Cambará and ANa 7007) in response to Se application. * indicates significant correlation ($p < 0.05$). Abbreviations: Alb - albumin, Glo - globulin, Prot - total protein, N-L - N in leaves, Pro - prolamin, Glu - glutelin, Se-DI - Daily intake of Se, Se-S - Se in seeds, Se-L - Se in leaves, N-S - N in seeds.

Table 1. Genotypic variation in the concentration of Se in the leaves, grains, daily intake of Se and storage proteins (albumin, globulin, prolamin and glutelin) in cultivars biofortified using 25 g Se ha^{-1} applied via soil. The ± represent the standard deviation (n = 4).

	AN Cambara	**ANa 5015**	**ANa 7007**
Se in leaves (mg kg^{-1})	0.92 ± 0.14	0.65 ± 0.003	0.44 ± 0.01
Se in seeds (mg kg^{-1})	0.65 ± 0.01	0.97 ± 0.03	0.65 ± 0.14
Se daily intake (µg day^{-1})	44.47 ± 1.35	66.80 ± 2.25	44.52 ± 9.76
Albumin (%)	11.06 ± 0.20	10.69 ± 0.47	15.28 ± 0.84
Globulin (%)	15.76 ± 0.54	13.78 ± 0.99	11.81 ± 0.41
Prolamin (%)	4.63 ± 0.24	3.74 ± 0.34	4.63 ± 0.32
Glutelin (%)	68.53 ± 0.40	71.81 ± 1.57	68.26 ± 0.33

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