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Impacts of Selenium Foliar Biofortification on the Biochemical Composition and Grain Yield of Rice Cultivars

Danilo Pereira Ramos[a], Guillermo Arturo Herrera Chan[b], Taynar Coelho de Oliveira Tavares[c], Manoel Mota dos Santos[d], Sergio Alves de Sousa[e], Geovane Macedo Soares[f], Danilo Alves Veloso[g], Wanessa Rocha de Souza[h], Antônio Carlos Francisco de Oliveira[i], Natália Martins Veloso[j], Vitor Stefanello Fernandes[k], Nivaldo Ribeiro Mascena Júnior[l], Vanessa Silveira Jorge[m], Rodrigo Ribeiro Fidelis[n]*

[a]Doctor in Plant Production, ORCID: https://orcid.org/0000-0002-6559-6218
[b]Doctor in Plant Production, ORCID: https://orcid.org/0000-0003-1093-4125
[c]Doctor in Plant Production, ORCID: https://orcid.org/0000-0002-2202-9264
[d]Doctor in Phytotechnic, ORCID: https://orcid.org/0000-0002-6984-1399
[e]Doctor in Plant Production, ORCID: https://orcid.org/0000-0002-0481-9609
[f]Bachelor of Engineering in Agronomy, ORCID: https://orcid.org/0000-0003-2857-9539
[g]Doctor in Plant Production, ORCID: https://orcid.org/0000-0002-9668-0009
[h]Doctor in Plant Production, ORCID: https://orcid.org/0000-0002-4740-3451
[i]Bachelor of Engineering in Agronomy, ORCID: https://orcid.org/0000-0001-6306-849X
[j]Academic of Agronomic Engineering, ORCID: https://orcid.org/0000-0002-4689-6768
[k]Master in Vegetable Production, ORCID: https://orcid.org/0000-0002-4697-3313
[l]Bachelor of Engineering in Agronomy, ORCID: https://orcid.org/0000-0002-5158-8874
[m]Master in Agroenergy, ORCID: https://orcid.org/0000-0002-3573-4800
[n]Doctor in Phytotechnics, ORCID: https://orcid.org/0000-0002-7306-2662)
[a,b,c,d,e,f,g,h,i, j,h,l,m,n] Federal University of Tocantins, Street Badejós, Lot 7, Ranches 69/72, Countryside, Gurupi, 77404-970, Tocantins-Brazil
[n]Email: fidelisrr@mail.uft.edu.br

Abstract

Selenium Se is an essential micronutrient for humans and animals linked to important biological functions in the body, where its deficiency has been associated with serious diseases.

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* Corresponding author.

Rice is essential in feeding more than half of the world's population. Some studies show beneficial effects of Se in higher plants. Thus, the objective of this work was to determine the effect of leaf Se doses on biofortification, agronomic characteristics and biochemical characteristics of the grains of two irrigated rice cultivars. The factors under study were five doses of Se (0, 30, 60, 90 and 120 g ha^{-1}) using as sodium selenate source (Na_2SeO_4), and two rice cultivars (IRGA-424 and IRGA-426). The leaf application of Na_2SeO_4 allowed the biofortification of rice grains with Se, however, it affected its yield and some biochemical components. The cultivar IRGA-426 Se shows superior because it allows a higher concentration of Se in the grains, presents higher yield and better nutritional content. The cultivar IRGA-426 with the dose 60 g ha^{-1} Se was the viable combination to increase the content of Se in the grains, which may result in benefits for the health and food safety of the population.

Keywords: Oryza sativa L; grain yield; fertilization; food composition; food security.

1. Introduction

Selenium Se is an essential micronutrient for humans and animals because it is a basic component of selenoproteins and selenoenzymes with important biological functions in the body [1]. It is estimated that there are about one billion people with a deficiency of this nutrient [2], which is considered the fourth most severe mineral deficiency in the world [3]. This deficiency has been associated with serious diseases in humans, such as viral infections, impaired immune function, hypothyroidism, reduced fertility, cardiovascular diseases, and cancer [1, 4]. Unlike the case for humans and animals, there is no definitive evidence on the essentiality of Se for higher plants [5], however, at low concentrations, this element can stimulate growth, development and increase plant yield [6,7]. At high concentrations, it can cause toxic effects and lead to lower production [8]. The accumulation of Se in plants varies according to plant species and genotype, the concentration of the element in the soil, soil properties and the chemical form of S [9]. Plants metabolize Se by sulfur assimilation route Se, producing selenocysteine, selenomethionine and other Se isologists from various metabolites of S [10]. Among the various strategies that can improve the intake of Se by the population and supply their deficiency, fertilization of plants with Se (agronomic biofortification) or selection of genotypes with greater capacity of accumulation of the element in tissues (genetic biofortification) is a useful, safe technique, and can have positive effects on long-term health [11,12]. Several recent studies such as Deng and Ramos [13,14] show the efficiency of agronomic biofortification in increasing the Se content in several crops. In biofortification programs, one must take into account the source and form of Se to be applied. In this context, sodium selenate (Na^2SeO^4) is generally more soluble and bioavailable than other inorganic sources of Se [15], and has shown greater efficiency by providing greater absorption, greater utilization, greater translocation to shoots, and greater accumulation of Se in grains [13, 8]. Like the roots, the leaves of plants can also absorb micronutrients, and the leaf application of Se has Se shown safe, with low cost, and effective in increasing the content of the element in the edible parts of agricultural crops [6,13,12]. The quality of the agricultural product is defined as the set of characteristics related to nutritional, commercial, industrial or aesthetic value [16]. The nutritional quality or biochemical composition is one of the characteristics used to evaluate the quality of rice, considering carbohydrate, protein, mineral and vitamin contents [17]. The level of Se in a population is linked to the content of this element in the food consumed [18], therefore, the choice of widely consumed crops provides greater success in biofortification programs. In this context, rice (*Oryza sativa L.*) is of great importance because it is a staple food for more than half of the world's population, being a cheap energy source, and responsible for providing 27% of carbohydrates, 20% of proteins and 3% of food lipids in developing countries [19, 20]. Considering the outstanding role of rice in human food, this agricultural crop has enormous potential for use in biofortification programs aimed at reducing the deficiency of Se in the world population. Therefore, the objective of this work was to evaluate the effect of leaf Se doses on biofortification, agronomic characteristics and biochemical characteristics of the grains of two irrigated rice cultivars.

2. Methodology

2.1 Experimental conditions

The study was conducted using plastic pots in cultivation in an unprotected environment, from April to August 2017, in Lagoa da Confusão (10º46'51.0”S, 49º37'01.5” W and 188 m altitude), Tocantins State, Brazil. The climate of the region is Aw (tropical with summer rains) or tropical savannah [21]. The average annual temperature is 27.2 °C. Total annual rainfall is 1882 mm. In the cultivation of plants, a mixture of soil, the substrate (Bioflora®) and medium sand (0.5 mm) were used in the proportion of 6:1:1. This mixture was placed in plastic pots with 8 dm3 volume, where the plants were grown. The soil used came from the 0 to 20 cm depth layer of a dystrophic Yellow-Red Latosol of clayey texture [22]. Physical and chemical analyses indicated that this soil had 58.0 dag kg^{-1} sand, 5.0 dag kg^{-1} of silte, 37.0 dag kg^{-1} clay, pH em $CaCl_2$ de 5.3, 3.4 dag kg^{-1} de M.O., P (Mehlich 1) de 59.7 mg dm^{-3}, 106.0 mg dm^{-3} de K, 4.3 cmolc dm^{-3} de Ca, 1.2 cmolc dm^{-3} de Mg, 0.0 cmolc dm^{-3} de Al, 3.1 cmolc dm^{-3} de H+Al, 48.0 mg dm^{-3} of Fe and base saturation of 65%. Sowing fertilization was performed according to the physicochemical analyses of the soil and constituted in 200 mg dm^{-3} of the fertilizer NPK 05-25-15 which supplied plants 10, 50, 30, 9.6, 3.2 and 0.4 mg dm^{-3} nitrogen (N), P_2O_5 (P), K_2O (K), calcium (Ca), sulfur (S) and zinc (Zn), respectively. Besides, micronutrient fertilization was performed with the application of 12.5 mg dm-3 of the FTE-BR12 (Peninsula) fertilizer that supplied plants 1.12, 0.22, 0.10, 0.25, 0.42 and 0.012 mg dm^{-3} zinc (Zn), boron (B), copper (Cu), manganese (Mn), Fe iron) and molybdenum (Mo), respectively.

3.2 Treatments and experimental design

The experimental design was randomized in a 5x2 factorial arrangement scheme, with four replications. The first factor consisted of five doses of Se (0, 30, 60, 90 and 120 g ha^{-1}) using as source sodium selenate (Na^2SeO^4, 41.8% Se, Sigma-Aldrich, Saint Louis, USA). Doses that did not compromise plant development were chosen, based on a previous study with rice [23]. The second factor under study consisted of two irrigated rice cultivars (IRGA-424 and IRGA-426). Two rice cultivars were chosen to obtain information on the general response of different genotypes about the application of Se. These cultivars were selected because they were in the group of the most cultivated in Brazil. Each experimental unit consisted of a pot with four plants spaced in 1 m between blocks and 0.35 m within the blocks, each block consisted of 10 pots (all treatments). Eight rice seeds were sown per pot. At 15 days after plant emergence, thinning was performed leaving four seedlings per pot, enough to obtain the total grain needed for the analysis, and not compromising the development of the plants by competition. As cover fertilization, 40 mg of nitrogen and 20 mg K_2O per dm^{-3} of soil were applied, divided into two applications at 25 and 45 days after sowing (DAS), using urea and potassium chloride (KCl), respectively. During the conduction of the experiment, the soil was kept moist through individual daily irrigations in each experimental unit. The application of doses of Se was performed by leaf in the morning (between 8:00 and 9:00 hours) when the plants were in the grain filling stage. Due to the difference between cultivar cycles, these applications were performed at 105 and 112 days after sowing for cultivar IRGA-426 and IRGA-424, respectively, using standard Na^2SeO^4 solution (0.25 mg mL^{-1}). For example, 1.15 mL of the solution per pot with four plants was used for the treatment of 30 g ha^{-1} Se. These doses of the standard solution were completed at 50.0 mL of water and applied evenly on the plants of each experimental unit. The doses were converted to hectare considering the population of plants generally used for irrigated rice (1,000,000.00 plants per hectare), extrapolating the doses per pot to the hectare. For the application, a manual pre-compression spray (Tramontina) with a maximum pressure of 2.5 bar (36.26 psi), a flow of 300 mL per minute, and a copper adjustable tip was used.

3.3 Evaluation of plant growth and yield

At the end of the crop cycle, at 132 DAS, the plants were harvested and the plant height (cm), number of panicles per pot, a mass of 100 grains (g) and grain yield (g pot^{-1}) were evaluated. To quantify the mass of 100 grains (g) and grain yield (g $vase^{-1}$), the samples were placed in paper bags measuring 10 x 5 x 17 cm (length x width x height) and inserted in a greenhouse with forced air circulation (Solab, model SL-102) at 70ºC. The grains remained in the greenhouse until they reached a constant weight. Grain mass was evaluated on a precision scale of 0.01 g (Gehaka BK4000).

3.4 Determination of Se and S concentrations in grains

The concentration of Se in grains (mg kg^{-1}) was determined by the Laboratory of Chemical Analysis (LACHEM) of the Federal University Santa Maria in the Rio Grande do Sul, Brazil, according to the methods described by [24, 14]. In summary, after drying in a greenhouse the grains were peeled and the brown rice samples were ground and homogenized. An aliquot (approximately 500 mg) was digested in nitric acid, hydrogen peroxide and hydrochloric acid until complete mineralization using glass tubes for sample digestion (Merck, Darmstadt, Germany) with threaded caps and a capacity of 22 mL. The volume of the digested extract was completed at 25 mL with ultrapure water. The determination of the total Se content in the samples was performed by atomic absorption spectrometry with hydride generation. An atomic absorption spectrometer (Analytik Jena, model NovAA 300) was used for this. For the calibration of the instrument, standards prepared by dilution of a standard solution of Se (Se 1000 ppm, Specsol brand) were used. Initially, a calibration curve was estimated by dilution of this pattern. After obtaining the concentration of Se in the digested extract, the concentrations of Se in the mass of the samples used were calculated. The concentration of S in grains (mg kg^{-1}) was determined according to the method described by [25], using nitric-perchloric acid digestion ($HNO_{3-}HClO_4$) and spectrophotometer readings (Shimadzu UV-1800, Vernon Hills, IL, USA). The Se/S ratio was calculated from the data obtained in the laboratory analysis.

3.5 Biochemical analysis of grains

To verify the effect of biofortification with Se on the biochemical composition of rice grains, the following characteristics were evaluated: oil content (%), soluble carbohydrate content (mg g^{-1}), total amino acid content (mg g^{-1}), starch content (mg g^{-1}) and protein content (mg g^{-1}). A sample of grains (750 mg each repetition) was used in the extraction with pure hexane [26]. After quantifying the oil content, the precipitate became the basis for additional quantification using ethanolic extraction [26]. From the overeating, quantifications of soluble carbohydrates and total amino acids were performed, and from the precipitate, the quantifications of starch and total proteins were performed. The quantification of soluble carbohydrates was performed by reaction with phenol-sulfuric acid [27]. The quantification of total amino acids was performed using the methodology based on the ninhydrin reaction [28]. The starch analysis was performed with a phase of the breaking of the molecule with perchloric acid, followed by quantification by the reaction of phenol-sulfuric acid [29, 27]. Protein quantification was performed according to Bradford [30] and previously described in Nascimento [26].

3.6 Statistical analyses

For the cultivar factor, the data were submitted to variance analysis (R Core Team, 2015) [31] and f test (= 0.05). For the dose factor, the data were submitted to regression analysis. The criteria used in the selection of regression curves were: the significance of the equation and its coefficients (= 0.05), higher regression coefficient (R^2) and simplicity of the equation [32].

4. Results

The rice cultivars showed a significant difference ($P<0.05$) for plant height, a mass of 100 grains, grain yield, the concentration of Se and S in grains, Se/S ratio and protein content. Significant effect ($P<0.05$) of the doses of Se was detected on grain yield, the concentration of Se and S in grains, Ratio Se/S, carbohydrate content, proteins and oils. As for the interaction between rice cultivars and doses of Se, the concentration of Se and S in the grains, the Se/S ratio, the carbohydrate, protein and oil content were significantly affected ($P<0.05$). No Se detected significant effects ($P>0.05$) between rice cultivars, doses of Se and interaction between them on panicle number per vessel, amino acid content and starches. The plants of the cultivar IRGA-424 presented higher height (Figure 1A) and a mass of 100 grains (Figure 1C). On the other hand, the plants of the cultivar IRGA-426 presented the highest grain yield (Figure 1D).

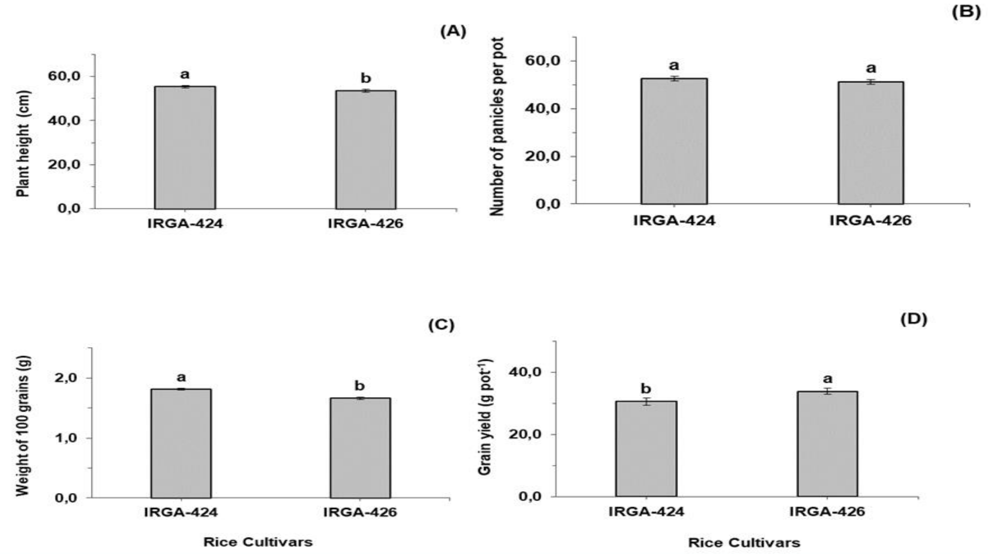

Figure 1: Plant height (A), number of panicles per pot (B), weight of 100 grains (C) and grain yield (D) of two irrigated rice cultivars, regardless of Se doses. Averages followed by the same lowercase letter do not differ according to the F test at $p<0.05$.

The application of Se negatively influenced the grain yield of the rice cultivar's understudy, so that, with an increase in the applied doses, there was a decrease in grain yield regardless of the cultivars used (Figure 2A). The highest dose of Se (120 g ha^{-1}) reduced approximately 20% of grain yield when compared to the control treatment.

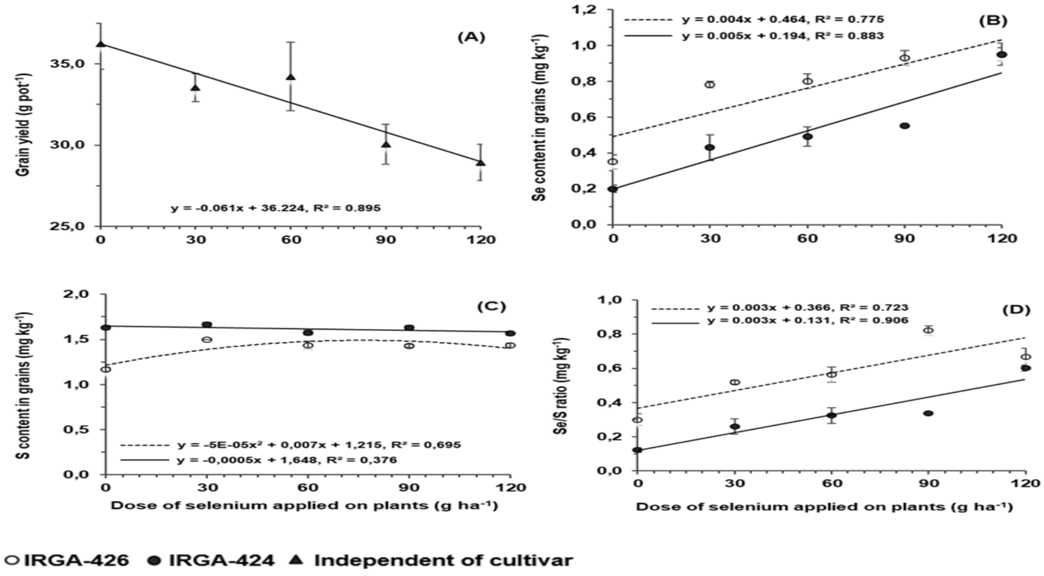

Figure 2: Grain yield (A), Se content in grains (B), S content in grains (C) and Se/S ratio (D) of two irrigated rice cultivars depending on the application of Se doses. The vertical line segments represent the average ± SE of the data.

The increase in the doses of Se applied resulted in a linear increase in the concentration of the element in the grains of the crop (Figure 2B). The rice cultivars showed different behavior for this characteristic, however, although the cultivar IRGA-426 presented a higher concentration of Se in the grains at the lower doses (0, 30, 60 and 90 g ha^{-1}), at the maximum dose (120 g ha^{-1}) the concentration of the element was the same for both cultivars. There was an increase in the concentration of Se in grains of approximately 4.75 and 2.72 times for cultivar IRGA-424 and IRGA-426, respectively, when Se compared the highest dose of Se with the control treatment. Although the cultivar IRGA-424 presented a lower concentration of Se in the grains at all doses, except for the maximum dose, this cultivar provided a greater increase in the concentration of the element compared to the cultivar IRGA-426.

Rice cultivars present a different pattern of S accumulation in grains (Figure 2C). It was observed that the doses of Se did not influence the S content in the grains of the cultivar IRGA-424, while for IRGA-426 it provided an increase up to the dose 72 g ha^{-1}, and from this dose presented some stabilization. Although the doses of Se did not influence the S content in the grains of the cultivar IRGA-424, it presented a higher concentration of the element than IRGA-426. Regarding the proportion Se and S in the grains, we have a Se/S ratio that presents the same pattern observed for the content of Se (Figure 2D).

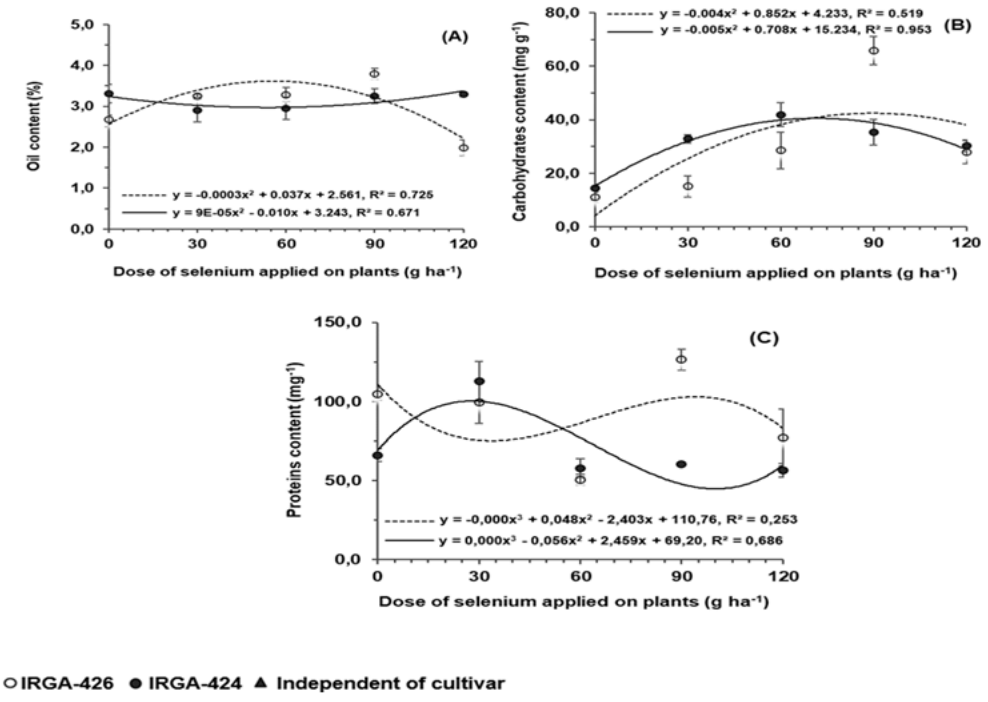

Figure 3: Oil content (A), carbohydrates content (B) and proteins content (C) in grains of two irrigated rice cultivars depending on the application of Se doses. The vertical line segments represent the average ± SE of the data.

The cultivar IRGA-424 showed similarity between the doses for the characteristic of oil percentage in the grains (Figure 3A). For the cultivar IRGA-426, there was an increase up to the dose of 63 g ha^{-1} (Se), when it presented maximum oil content (3.6%), while the minimum percentage (2.2%) was found at the dose 120 g ha^{-1}. Although there was no significant difference between rice cultivars for this characteristic, the cultivar IRGA-426 showed mild superiority, since it increased the oil concentration in the grains as a function of the doses of Se up to the dose of 63 g ha^{-1}, when compared with IRGA-424, which showed no significant difference between the doses of Se for this characteristic.

The grains of cultivar IRGA-426 showed an increase in soluble carbohydrate content up to the dose of 89 g ha^{-1}, with an average of 43.0 mg g^{-1} and later decrease (Figure 3B). The minimum concentration (4.0 mg g^{-1}) is observed at the control dose. For the cultivar, IRGA-424 similar response was presented up to the dose of 71 g ha^{-1}, where it obtained an average of 41.0 mg g^{-1}. There was a difference between the cultivars at doses 30, 60 and 90 g ha^{-1} (Se), where the first two doses were favorable to cultivar IRGA-424 and the latter favored the cultivar IRGA-426. The total mean soluble carbohydrate content was 30.35 mg g^{-1} (3.03%).

The cultivar IRGA-426 presented higher protein content in the grains (Figure 3C), and the control dose was the most beneficial for this. For the cultivar IRGA-424, the dose of 26 g ha^{-1} provided higher protein content in the grains and the application of the highest doses caused its decrease.

5. Discussion

The agronomic characteristics of plant height and mass of 100 grains were significantly influenced only by rice cultivars, while panicle number per pot was not influenced by any of the sources of variation. Already Se expected that there would be no significant effect of the doses of Se on such characteristics, since the application of the element was performed in the grain filling phase, where the plants had already completed their vegetative development and were finishing and reproductive stage, where probably, in this phase, it was no longer possible to act Se on the plants. The differences in these characteristics between rice cultivars Se are due to the intrinsic genetic properties of these genotypes. Despite the significant effect between rice cultivars for plant height, the difference was only 1.88 cm (Figure 1A). According to Pinheiro [33] , the length, diameter and thickness of the nodes in rice plants determine the resistance to bed, where taller plants tend to be more susceptible. As for the mass of 100 grains (Figure 1C), [34] reported that this characteristic in rice is dependent on grain density, therefore, a cultivar with a lower number of viable spikelets per area may have higher grain mass and lower yield, since the lower number of viable spikelets per area negatively influences grain yield in rice, which probably happened with IRGA-424 in this study.

Grain yield was significantly influenced by rice cultivars and Se doses applied to plants. The difference between rice cultivars for this characteristic (Figure 1D) Se is due to plant characteristics. According to [35], rice crop yield is defined by its components: number of panicles per unit area, the number of viable spikelets per panicle and grain weight. In this work, the most productive cultivar (IRGA-426) presented a lower mass of 100 grains, however, it must have obtained a higher number of viable spikelet per panicle, resulting in a higher yield, since there was no significant difference between cultivars for the number of panicles per pot (Figure 1B), which in turn is one of the most important components of rice yield.

The increase in the doses of Se caused a decrease in grain yield, regardless of the cultivar used (Figure 2A). The reduction in grain yield in response to treatment with Se should be related to the substitution of S by Se in proteins, especially in the amino acids cysteine and methionine, forming the analogs selenocysteine and selenomethionine. This substitution occurs because Se is easily absorbed by plants through sulfate transporters due to their chemical similarities and can alter the synthesis and function of amino acids and proteins in plant tissues [36]. Besides, Sors [37] reported that isologist compounds in plants compete in biochemical processes that interfere in absorption, translocation and assimilation throughout plant development. Therefore, the decrease in grain yield as a function of the increase in the doses of Se applied, should be related to this substitution of S by Se in these amino acids and the competition of these elements in biochemical processes.

According to Rani [38], the maximum limit of Se in the plant tissues of the rice plant before the appearance of phytolith or reduction of productivity is 41.5 mg kg^{-1} of dry matter, however, in this study, the maximum accumulation of Se did not reach 1 mg kg^{-1} of dry matter. Besides, phytotoxicity was not observed caused by leaf application of this element, and it was possible to rule out the hypothesis of toxicity.

Figure 2B shows an increase in the concentration of Se in rice grains with the increase in the doses of the element applied in the plants. The low-affinity sulfate transporters expressed in the leaves of the plants are responsible for the transport of the Se applied on the surface of the cells to the inside of the cells [37], therefore, these transporters must have played a fundamental role in the movement of the Se applied to the leaves to the inside of the rice grains. The fact that cultivar IRGA-424 presented a higher increase in the concentration of Se in the grains when compared to the cultivar IRGA-426, even with a lower concentration of the element in all doses, except at the maximum dose, can be explained by the fact that cultivar IRGA-426 presented higher concentration of the element in the grains at the control dose and concentration similar to the cultivar IRGA-424 at the maximum dose, resulting in this difference. These results show the superiority of cultivar IRGA-426 concerning the concentration capacity of Se in grains at doses lower than 120 g ha^{-1}.

Taking into account that the average consumption per capture of rice in Brazil is 153g day^{-1} [39], that the minimum intake recommendation of Se in adults is 55 μg day^{-1} and the maximum tolerable level is 400 μg day^{-1} [40], it was found that the concentration of Se found in the grains, when Se used the cultivar IRGA-426 at the dose 60 g ha^{-1} of Se, which provided a lower reduction of grain yield (5.6%), may contribute to a daily intake of Se in approximately 122 μg day^{-1}, an intermediate value within the recommendation of ingestion of the element. Considering the contribution of the application of this dose of Se in rice crops to supply the lack of this element in the population, which may result in a reduction in the incidence of serious diseases, and the low reduction in grain yield, where the added value in biofortified rice can overcome this loss, the practice of biofortification with Se for this crop can Se make it feasible. However, further studies are still needed with agronomic biofortification with Se in rice, to adapt the doses to be applied leaf, to meet the demand of this element in human food, without reducing productivity.

Leaf application of Se increased the S content in the grains of cultivar IRGA-426. However, there was no significant effect on the cultivar IRGA-424 (Figure 2C). [8], when evaluating the effect of selenium and selenate on several lettuce germplasms, they observed a relationship of synergism between selenate and S, a case that occurred with the cultivar IRGA-426 in this study. These authors also observed that the accumulation of S varied between lettuce accesses in response to the application of (Se), ratifying the situation that occurred with the rice cultivars studied here. According to [41], treatment with Se in certain species and/or plant genotypes mimics S deficiency and activates the specific expression of the sulfate transporter, stimulating S absorption, resulting in s-induced accumulation of s, an event that probably occurred with the cultivar IRGA-426 understudy. The fact that cultivar IRGA-424 presented higher S concentration in grains, even if it did not present a significant difference between the doses applied, should be related to the situation of this cultivar having more affinity for S to the detriment of Se, since it also presented lower concentration of Se in grains and lower Se/S ratio when compared to cultivar IRGA-426.

Regarding the nutritional and biochemical characteristics, although rice cultivars presented different behavior for the oil content, there was no significant difference between them, however, the cultivar IRGA-426 presented mild superiority (Figure 3A). This difference in the behavior of cultivars should be related to their genetic properties, where this characteristic may vary depending on the cultivar used, contributing differently to the content of lipids essential to the diet [20]. Interferences in the processes of absorption, translocation and assimilation in the plant resulting from the competition between Se and S may have caused a decrease in the oil content in the cultivar IRGA-426 when Se the highest dose of Se was used. Another fact that evidence genetic variability among cultivars for this characteristic is that in this same dose the cultivar IRGA-424 had a higher oil content.

Rice cultivars showed an increase in carbohydrate content in response to the addition of Se up to a certain dose (Figure 3B). Reference [42], studying the effect of Se on wheat, they also verified a beneficial effect of Se on carbohydrate metabolism and concluded that adequate doses of Se provided greater growth and productivity of that crop. The same is evidenced in the present study, where the increase in doses of Se provided an increase in soluble carbohydrate content up to the dose of 71 g ha^{-1} for the cultivar IRGA-424 and up to 89 g ha^{-1} for IRGA-426, however, did not benefit grain yield.

Although the protein content does not present a possible behavior of adopting an efficient mathematical model through regression (Figure 3C), the average content of 81 mg kg^{-1} (8.1%) proteins are within the means obtained in the literature, ranging from 6.5 to 9.0 % [43], showing that, even with a certain decrease, rice continues as a potential source of protein, since it is responsible for about 20% of the protein consumed in developing countries, and has high quality due to the lysine and tryptophan content [16].

The biosynthesis of most compounds Se may depend on the enzymes involved in the s assimilation pathways, thus the incorporation of Se in the analogs of selenoamino acids, selenocysteine and selenomethionine, in proteins, instead of sulfurous amino acids, can interfere in biological processes in plants [44, 37], thus, this substitution of S by Se, can also result in a reduction in protein synthesis [45].

The results obtained in this study indicate that leaf biofortification is an efficient strategy, as it can provide the recommended daily amount of Se to the population, without harming the biochemical quality of the grains.

6. Conclusion

The foliar application of sodium selenate allows the biofortification of rice grains with Se, however, it can affect its yield and some biochemical components. There is genetic variability among rice cultivars as to the accumulation capacity of Se in the grains. The cultivar IRGA-426 Se shows superior because it allows a higher concentration of Se in the grains, presents higher yield and better nutritional content. The cultivar IRGA-426 with the dose 60 g ha^{-1} Se is the viable combination to increase the content of Se in the grains, which may result in benefits for the health and food safety of the population.

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