UNIVERSIDADE FEDERAL DE LAVRAS

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STRATEGIES FOR AGRONOMIC BIOFORTIFICATION OF RICE WITH SELENIUM IN TROPICAL AGROECOSYSTEMS

LAVRAS – MG
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Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Ciência do Solo, área de concentração em Fertilidade do Solo e Nutrição de Plantas, para a obtenção do título de Doutor.

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Aos meus pais Francisco e Cleusa.
Ao meu filho Guilherme, presente de Deus.
A minha amada esposa Aline.
A minha querida irmã Josilene.

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RESUMO GERAL

O selênio (Se) é um elemento essencial para humanos e animais. O seu papel nos humanos e nos animais está relacionado à atividade de mais de 25 selenoproteínas, as quais são formadas a partir dos aminoácidos selenocisteína e selenometionina (*e.g.* glutationa peroxidase). A ingestão de Se pelos seres humanos depende fundamentalmente da qualidade nutricional dos alimentos quanto aos teores desse elemento. Em muitas regiões do mundo, especialmente as tropicais, os solos apresentam baixos teores de Se, sendo essa a principal razão da baixa disponibilidade de Se para as plantas. Nesse sentido, a biofortificação agronômica de alimentos com Se é uma boa estratégia que pode ser utilizada para enriquecer os alimentos básicos (como o arroz), diminuindo a incidência de doenças em populações vulneráveis. Este trabalho teve como objetivo investigar os efeitos da aplicação de Se via solo (SeVI) e via folha (SeIV e SeVI) sobre: i) produtividade de grãos; ii) teor de Se em diferentes partes da planta e do grão; iii) variáveis fisiológicas; e iv) distribuição espacial de Se e outros elementos em grãos de arroz biofortificados. Os experimentos foram realizados em dois campos de produção agrícola. Doses de Se variando de 5 a 120 g ha^{-1} foram avaliadas em quatro repetições nos municípios de Lambari-MG (safras 2015/2016 e 2016/2017) e Patos de Minas-MG (safra 2016/2017). A aplicação de SeVI via solo foi realizada em revestimento do fertilizante monoamônio fosfato (MAP) ou como solução salina. A aplicação de SeIV e SeVI via folha foi realizada utilizando solução salina nas fases de floração e enchimento de grãos de arroz. As variáveis fisiológicas em todos os tratamentos foram avaliadas nas folhas. Após a colheita, a produtividade de grãos e o teor de macro e micronutrientes foram determinados em diferentes partes da planta de arroz. Os resultados mostraram que as doses de Se fornecidas afetaram positivamente algumas variáveis fisiológicas e melhoraram a qualidade nutricional dos grãos polidos quanto aos seus teores de Se. Apenas uma baixa porcentagem de Se fornecida às plantas foi absorvida pelos grãos. Pela aplicação das doses de Se estudadas, uma quantidade adequada de Se no grão polido foi alcançada, permitindo o consumo seguro de Se para humanos. Houve variação na distribuição espacial dos elementos dentro do grão, sendo a maior concentração de Se encontrada no endosperma. Outros elementos, como Fe, Ca, Zn, K e Mn, foram localizados na casca ou no embrião do grão de arroz biofortificado. Conclui-se que a biofortificação agronômica do arroz com Se é uma estratégia promissora para solos tropicais. Entretanto, a descasca e polimento do grão de arroz pode levar a perdas significativas de Se, macronutrientes (como K e P) e micronutrientes (como Zn e Fe).

Palavras-chave: Agricultura funcional. Selenoproteínas. Selenato. Selenito. MAP enriquecido com Se. Endosperma µXRF.

GENERAL ABSTRACT

Selenium (Se) is an essential element for humans and animals. Its role in humans and animals is related to the activity of more than 25 selenoproteins, formed from the naturally occurring amino acids selenocysteine and selenomethionine (*e.g.* glutathione peroxidase). Selenium intake by humans depends fundamentally on the nutritional quality of the food with respect to this nutrient. In many regions of the world, especially in the tropics soils present a low content of Se. This is the main factor responsible for the low availability of Se for plants. In this sense, the agronomic biofortification of food with Se is a good strategy that can be used to enrich agricultural crops (as rice) decreasing the incidence of diseases in vulnerable populations. This study aimed to investigate the effects of the application of Se via soil (SeVI) and via leaf (SeIV and SeVI) on: i) grain yield; ii) contents of Se in different parts of the plant and of the grain; iii) physiological variables; and, iv) spatial distribution of Se and other elements in biofortified rice grains. Experiments were carried out in two agricultural production fields. Selenium rates ranging from 5 to 120 g ha^{-1} were evaluated in four replicates on Lambari-MG (Cropping seasons of 2015/2016 and 2016/2017) and Patos de Minas-MG (Cropping season of 2016/2017) municipalities. The application of SeVI via soil was carried out coating the fertilizer monoammonium phosphate (MAP) or as saline solutions. The application of SeIV and SeVI via leaf was carried out using saline solution at the flowering and grain-filling stages of rice. The physiological variables in all treatments were evaluated in the leaves. After harvest, grain yield, Se, macro, and micronutrient contents were determined in different parts of the rice plant. The results showed that the supplied Se rates positively affected some physiological variables and improved the nutritional quality of the polished rice grains in terms of their Se contents. Only a low percentage of the Se supplied to the plants was uptake by the grains. Adequate Se contents in the polished grain were achieved with the assessed Se rates, allowing safe consumption of Se for humans. The spatial distribution of the elements varied in the grain, with the highest concentration of Se being found in the endosperm. Other elements, as Fe, Ca, Zn, K, and Mn were located in the husk or in the embryo of the grain. We conclude that concluded that the agronomic biofortification of rice with Se is a promising strategy for tropical soils. However, peeling and polishing the rice grain can lead to significant losses of Se, macronutrients (as K and P), and micronutrients (as Zn and Fe).

Key-words: Functional agriculture. Selenoproteins. Selenate. Selenite. Se-rich MAP. Endosperm µXRF.

SUMMARY

1 GENERAL INTRODUCTION

Selenium (Se) is an essential element for humans and animals, being a constituent of selenoproteins (*e.g.* glutathione peroxidase). Selenium deficiency in the human body is related to hypothyroidism, low immunity, male infertility and increased incidence of various types of cancers (FAIRWEATHER-TAIT et al., 2011; FORDYCE, 2013; RAYMAN, 2012).

According to the World Health Organization (WHO), at least half of the children under 5 years old suffer from one or more vitamin and mineral deficiencies (WORLD HEALTH ORGANIZATION – WHO, 2018). Worldwide, nutritional deficiency affects more than 3 billion people (STOLTZFUS; MULLANY; BLACK, 2004; UNICEF, 2004). Nutritional deficiency in humans is related to the ingestion of foods that are poor in minerals and vitamins, such as cereals (MAYER; PFEIFER; BEYER, 2008) grown on soils with low fertility (FORDYCE, 2013; JOY et al., 2015; LOPES; ÁVILA; GUILHERME, 2017; NATASHA et al., 2018), poor nutritional quality due to industrial processing (*e.g.* rice polishing) (COMBS JÚNIOR; COMBS, 1986; LU et al., 2013) and genetic improvement for better productivity (MORAES et al., 2012; WHITE; BROADLEY, 2009).

Iron (Fe), zinc (Zn), iodine (I), and selenium (Se) are the main nutrients related to mineral deficiency in humans (CAKMAK, 2008; CHATTHA et al., 2017; COMBS JÚNIOR, 2001; MORENO et al., 2013; STOFFANELLER; MORSE, 2015; WHITE; BROADLEY, 2009, 2011; WHO, 2006). Combs Júnior (2001) revealed that among 48 countries around the world, in 21 of them more than 50% of the population is Se deficient (*e.g.* New Zealand, Spain and, Nigeria). Moreover, in 16 countries nearly 10-50% of the population presents moderate Se deficiency (*e.g.* Belgium and India).

The Institute of Medicine of the United States of America recommends the ingestion of 55 μg day^{-1} of Se for adult humans (BOYD, 2011). On the other hand, the German, Austrian and Swiss nutrition societies recently updated the intake values of Se to 60 μg day^{-1} and 70 μg day^{-1} for women and adult men, respectively (KIPP et al., 2015).

In order to alleviate health problems related to low Se intake, the agronomic biofortification of staple foods (*e.g.* maize, wheat and rice) has been an efficient strategy adopted worldwide (CAKMAK, 2008). Agronomic biofortification provides food that is rich in bioavailable nutrients (COMBS JÚNIOR; COMBS, 1986; GONG et al., 2018), without specific processing costs (BOUIS, 2003), as well as plants that present greater tolerance to pests (HANSON et al., 2003) and resistance to stress caused by water deficit (ANDRADE et al., 2018).

Inorganic species of Se applied to the soil can be converted into its organic forms, such as amino acids and proteins, in agricultural products (GONG et al., 2018). Organic forms are easily absorbed and used by humans (EICHE et al., 2015; GONG et al., 2018). Selenium is highly bioavailable as selenomethionine (up to 90%), whereas inorganic forms such as selenate, have lower bioavailability (< 50%) (FILIPPINI et al., 2014). According to Haba, Ding and Zhang (2005), proteins represent only 8% of the total biomass of rice. However, most Se present in rice grains is in the form of proteins (ZHANG et al., 2008).

Williams et al. (2009) found that nearly 95% of the accumulated Se species in rice grains were organic, and 59% of them were identified as methyl-selenocysteine (Se-MeSeCys). Farooq et al. (2018) suggested that the organic form of Se is at least 80% of the total Se in polished rice, regardless of the implemented method (applied either via soil or leaf). Gong et al. (2018) also found high percentages of organic Se in rice grains (56.9 to 74.1%) enriched with the application of selenite via soil, with about 50% of Se as glutelin.

In addition to the content and speciation of a particular element, its location in the grain is another important factor related to the nutritional quality of the food. Lu et al. (2013) showed that only 14% Ca, 15% K, 56.6% Zn, 34.6% Fe and 8.4% Mn accumulate in the rice grain endosperm. Using X-ray fluorescence microtomography images, Carey et al. (2012) observed Se in the ovular vascular trace, located at the entry point of nutrients in the grain of rice plants treated with selenite. On the other hand, in grains treated with selenomethionine, Se appeared distributed throughout the grain, including the endosperm. In grains treated with selenomethylcysteine, there was a significant accumulation of Se in the embryo.

Rice is the staple food for half of the world population, being an important source of minerals, besides supplying about 80% of the daily caloric intake of more than 30 countries (LUCCA; POLETTI; SAUTTER, 2006; MENG; WEI; YANG, 2005). Currently, world rice production ranks third among the major cereals, with approximately 25% (polished grain), behind only wheat (35%) and maize (40%), ranking second in terms of consumption among all cereals (40%) (AMIS MARKET DATABASE, 2018). However, the contents of Se in rice grains produced in various regions of the world are considered low. For a total of 1092 commercial rice samples analyzed from 11 countries, the average Se concentration was 67 μg kg^{-1} (WILLIAMS et al., 2009). According to White (2016), one of the main causes of Se-deficient food is related to low Se concentration in the soil.

Tan (1989) classified soils into four categories according to the possible bioaccumulation of Se in humans via food consumption: (i) deficient (< 0.125 mg kg^{-1}), (ii) marginal (0.125 and 0.175 mg kg^{-1}), (iii) moderate-high (0.175 and 3 mg kg^{-1}) and (iv) excessive (> 3 mg kg^{-1}). Low levels of Se in soils are related the parent material (GABOS; ALLEONI; ABREU, 2014; MATOS et al., 2017), or pedogenetic due to the severe weathering, resulting in the removal of the element from the environment (CARVALHO et al., 2019; CHRISTOPHERSEN et al., 2013; LOPES; ÁVILA; GUILHERME, 2017).

The United Kingdom, Australia, Siberian center, New Zealand, Thailand, Africa, Finland, Turkey, Nepal, Denmark, Brazil, India and parts of China and Bangladesh are examples of countries presenting low contents of Se in the soil (COMBS JÚNIOR, 2001; LOPES; ÁVILA; GUILHERME, 2017; RAYMAN, 2012; YASIN et al., 2015).

In Finland, Se-enriched mineral fertilizers have been used in agriculture since 1985 to ensure the content of Se recommended for humans (ALFTHAN et al., 2015). Since 2007, 15 mg kg^{-1} of Se in the form of sodium selenate is added to solid fertilizers. In particular, for fertilizers intended for agricultural areas, the content of Se can reach up to 25 mg kg^{-1}. In liquid fertilizers, the Se levels are: 10 g ha^{-1} of Se via soil and 4 g ha^{-1} to fertilizers applied via leaf (ALFTHAN et al., 2015). Other countries, such as Malawi (CHILIMBA et al., 2011), Mozambique (MANGUEZE et al., 2018), Portugal (LIDON et al., 2018), China (LU et al., 2013), Australia (PREMARATHNA et al., 2012) and Brazil (ANDRADE et al., 2018; BOLDRIN et al., 2013; RAMOS et al., 2010, 2012; REIS et al., 2014, 2018) have also developed studies aimed at adopting strategies to increase the concentration of Se in foods.

In Brazil, the legislation allows the addition of Se in fertilizers for soil or foliar application, and > 300 mg kg^{-1} in micronutrient fertilizers for leaf application (BRASIL, 2016). However, the definition of rates of Se to be added to Brazilian fertilizers still requires fine adjustments to meet the wide diversity of agricultural crops, climatic conditions, and types of soils, among others. Moreover, there is little knowledge about the specific area of Se accumulation in the rice grain, as well as its likely removal in industrial processing.

Because of the importance of Se for humans and animals and it dynamics in the soil-plant system, we propose to study some strategies of adding Se for biofortification of rice with Se in soils from two municipalities, both located in the Brazilian Cerrado region. These municipalities have different climate conditions and the soils present different chemical and physical characteristics.

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Agronomic biofortification of rice (*Oryza sativa* L.) with selenium and its effect on element distributions in biofortified grains

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Abstract

Aims: Taking into account the relevance of biofortification of crop plants with selenium (Se) - an essential element for humans and animals -, this study assessed the effect of adding Se in the soil (as selenate) on: i) rice grains yield; ii) Se contents in polished rice grains; iii) physiological traits in rice leaves; and, iv) spatial distribution of Se and other elements in biofortified grains.

Methods: A field trial was conducted using five Se rates (12, 21, 38, 68, and 120 g ha^{-1}). Physiologic and enzymatic evaluations were carried out in rice leaves. Rice grain yield and Se contents were assessed. Spatial distributions of Se and other elements were visualized mapping the biofortified rice grains with µXRF at Brazilian Synchrotron Light Source.

Results: Results showed that soil Se application was effective in producing rice grains with higher Se contents, yet no effects were verified on rice yield. Antioxidant enzyme activities and gas exchanges in rice leaves changed following the application of Se. The spatial distribution of different elements in biofortified grains varied, with Se being accumulated mainly in the rice endosperm.

Conclusion: Our results suggest that soil application of 47 and 36 g ha^{-1} of Se (as sodium selenate) may guarantee the production of rice grains with adequate Se levels for human consumption in Brazil and worldwide, respectively.

Keywords: Tropical soil; Selenate; Staple food; Antioxidant activity; Se-rich endosperm; Polished rice grain.

Introduction

Selenium (Se) is an essential element for humans and animals (Fairweather-Tait et al. 2011) and its recommended intake for adult humans is 70 μg person^{-1} day^{-1} (Kipp et al. 2015). In most countries, the traditional diet is not able to provide the daily recommended Se allowance (Saha et al. 2017; White and Broadley 2009). It is estimated that about 1 to 1.5 billion people are Se deficient worldwide, presenting disorders often associated with cardiovascular problems, hypothyroidism, male infertility, and cancers (Jones et al. 2017; White 2016).

It is known that Se is not essential for plants (Feng et al. 2013). However, beneficial effects of Se have been reported, at low concentrations, on plant metabolism (Ramos et al. 2013). These positive effects are mainly related to plant resistance to water stress (Andrade et al. 2018), cold and UV-B radiation tolerance (Yao et al. 2010), and also due to its role in plant antioxidant activity, which allows for increased plant biomass production (Ramos et al. 2013). Also, other results have shown a relevant effect of Se on the prolongation of shelf life of tomato, due to the reduction of the ethylene production rate (Zhu et al. 2017).

Plants cultivated in soils with low Se contents (< 0.5 mg kg^{-1}) are not able to accumulate this micronutrient in adequate amounts for human health (White and Broadley 2009). To overcome this issue, Se must be added to soils or incorporated into fertilizers that are commonly broadcasted in the field. Such action aims to increase Se content in the soil and, as a result, to enhance its level in plant edible parts. However, physiological and genetic factors also interfere in the plant's ability to absorb and accumulate Se into edible parts.

Selenium availability in soils depends on several factors, with the interaction between Se species and soil solid components a major factor to be considered. Tropical soils present high amounts of iron (Fe) and aluminum (Al) oxides, which have great capacity to retain anions as selenate, diminishing its availability in the soil solution (Araujo et al. 2018; Lessa et al. 2016).

It is well-known that agronomic biofortification is a good strategy to increase the natural intake of Se by the population, as verified in Finland (Alfthan et al. 2015). In Brazil, Lopes et al. (2017) and Reis et al. (2017) emphasized that agricultural crops often accumulate low Se amounts due to its low contents in soils. According to a review conducted by Reis et al. (2017), evaluating 29 municipalities from 7 states, the median Se content value in Brazilian soils is 70 μg kg^{-1}, which is considered low and not enough to produce foods with adequate Se levels. Indeed, a recent study by Carvalho et al. (2019) found Se contents in Brazilian Cerrado soils ranging from 22 to 72 μg kg^{-1}. Because of the low Se contents in agricultural Brazilian soils, the Ministry of Agriculture, Livestock, and Supply recently published a normative Nº 46/2016 (Brasil 2016) allowing the addition of Se in fertilizers used in Brazil. Such initiative requires new research to select the best strategy to enhance Se availability in soils and, as a results, to increase Se uptake by agricultural crops. Hence, studies evaluating the application of Se in the soil to biofortify agricultural crops consumed in large amounts by the population are still required. Rice (*Oryza sativa*) is an important crop in this context, as it is the predominant staple food of billions of people worldwide (Lucca et al. 2006). This cereal is an important source of human nutrients (Meng et al. 2005) and its agronomic biofortification in tropical soils may represent a great advance in improving this crop´s nutritional quality.

Therefore, the general aim of the present study was to evaluate the agronomic biofortification of rice, a staple food consumed in large scale worldwide, including in Brazil, with Se rates applied via soil. More specifically, this study aimed to assess the effect of adding Se in the soil on: i) rice grains yield; ii) Se contents in polished rice grains; iii) physiological traits in rice leaves; and, iv) spatial distributions of Se and other elements in biofortified rice grains, via µXRF.

Material and Methods

Experimental area and treatments

This study was performed with an early upland rice cultivar (BRSMG Caravera), which presents high grain quality and is commonly grown in Brazil (Soares et al. 2008). The experiment was carried out under field conditions during the cropping season of 2015/2016 in an experimental farm of the Agricultural Research Company of Minas Gerais State (EPAMIG), located in Lambari, Minas Gerais State, Brazil (21°56'51"S; 45°18'55"W) (Fig. 1).

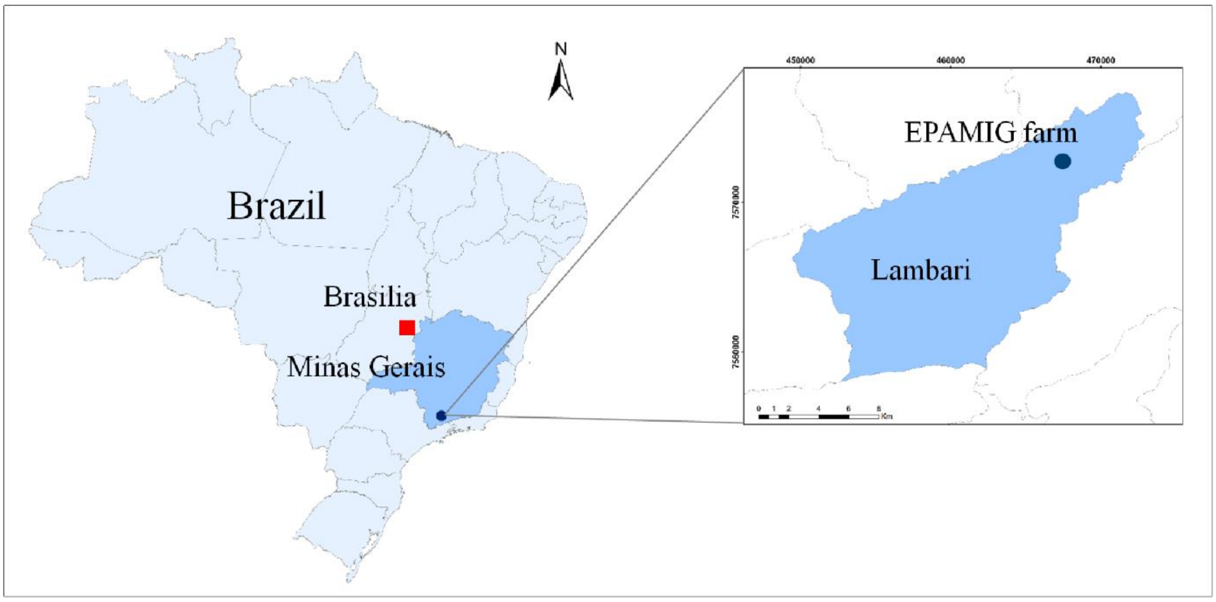

Fig. 1 Location of the experimental area.

Soil testing results of the experimental area before treatment applications are shown in Table 1. The experimental design was a randomized complete block with four replications, with field plots of 5 x 2 m, and 6 lines per plot (0.34 m between lines), totaling 10 m². Plant variables were assessed in an area comprised by 3 rice lines, each one 3 meters long, i.e., excluding 1 meter in each extremity of the plot (hereafter called useful area). Planting was made with 80 rice seeds per linear meter and fertilization was carried out applying 250 kg ha^{-1} of a commercial fertilizer with a N-P_2O_5-K_2O content of 08-28-16. Additional nitrogen (N) and potassium (K) were top-dressed at the rate of 40 kg ha^{-1} of urea and 20 kg ha^{-1} of potassium chloride, respectively.

Table 1 Soil attributes of the experimental area before the treatment application.

pH in water	K	P	S	H+Al	SB	CEC	V
	------------------ mg dm^{-3} ------------------			------------- cmol$_c$ dm^{-3} --------------			%
5.7	72.48	34.77	12.69	4.88	3.43	8.31	41.23
Fe	**Mn**	**Zn**	**Se**	**SOM**	**Clay**	**Silt**	**Sand**
----------------- mg dm^{-3} ------------------			mg kg^{-1}	---------------------- g kg^{-1} --------------------			
53.83	25.1	7.31	0.37	32.1	670	110	220

Soil properties determined according to Brazilian Company of Agricultural Research (EMBRAPA, 2017). P is the available phosphorus extracted by anion-exchange resin. SB is the sum of bases Ca^{2+}, Mg^{2+} and K; CEC is the cation exchange capacity at pH 7.0; V is the percentage of bases retained at a CEC; SOM is the soil organic matter. The “Total” contents of Se in soil sample was extracted with aqua regia, as performed by Gabos et al. (2014).

Sodium selenate (Na_2SeO_4, Sigma-Aldrich, Saint Louis, MO, USA) was applied as follows: 12, 21, 38, 68, and 120 g ha^{-1} of Se. The tested Se rates were applied as solutions. For that, the Se salt was diluted in deionized water and then applied with the aid of a pressurized pump with a carbon dioxide container, spraying the solution over the soil in the rice lines. In order to avoid spraying Se in rice leaves, the solution jet was applied close to the ground. Selenium application was done sixty days after sowing and the control treatment received only deionized water.

Paddy and polished rice grain yield

After maturation, the useful area of each experimental plot was harvested. Rice grains were dried at ambient conditions for further determination of their moisture content. The rice yield of each plot was assessed weighting the grains and extrapolating the data for the whole hectare (i.e., yield in kg ha^{-1}), after a moisture correction to 13%. Afterwards, samples were submitted to the milling process, where husk and bran were removed and then the grains were polished. Lastly, we measured the percentage (%) of polished rice grains, bran, and husk obtained from 100 g of paddy rice.

Table 2 Mean yield of paddy rice and harvest index obtained in the field experiment.

Selenium rates (g ha^{-1})	**0**	**12**	**21**	**38**	**68**	**120**	**Overall mean**
Paddy rice grain yield (t ha^{-1})	4.1a	3.4a	4.4a	3.7a	3.9a	3.9a	3.9
Harvest index							
Polished rice grains (%)	67.6a	67.8a	67.9a	67.6a	67.5a	67.9a	67.7
Bran (%)	6.7a	6.7a	6.7a	6.7a	6.7a	6.7a	6.7
Husks (%)	25.7a	25.5a	25.4a	25.7a	25.8a	25.4a	25.6

Polished rice grains percentage were assessed after removed husks and bran from a 100 g paddy rice grain sample (n = 24). Lowercase letters in the lines compare the effect of Se rates.

Selenium in rice grains

Selenium contents in rice grains were determined in extracts following an acid digestion using concentrated HNO_3 and closed vessels in a microwave oven, according to the 3051A method of the United States Environmental Protection Agency - USEPA (USEPA 2007).

Selenium contents in the digested solutions were 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). The calibration curve for Se measurement was obtained from a standard solution with 1 g kg^{-1} of Se (≥ 98% of purity, Fluka, Buchs, Switzerland).

For quality assurance and control in Se measurements, a standard reference material from the Institute for Reference Materials and Measurements (White Clover - BCR 402, IRMM, Geel, Belgium) and a blank sample were included in each batch of digestion. The mean recovery value obtained for the standard material was 96.78 ± 1.88 % (n = 13).

The fraction of applied Se that was incorporated in the polished rice grains (Se recovery by polished rice) was calculated using the Equation 1 below.

$$\mathrm{Se-recovery} = \frac{\{[\mathrm{Se}]_{\mathrm{Treated}} - [\mathrm{Se}]_{\mathrm{Control}}\} * \mathrm{Y} * \mathrm{P}}{\mathrm{Se}_{\mathrm{Applied}}} \qquad \text{Equation 1}$$

Where: Se-recovery (%) is the use efficiency of the Se rates applied in the soil by polished rice grains (Se recovery by the polished rice); $[Se]_{Treated}$ (g kg^{-1}) is the Se contents in polished rice grains from rice plants grown in treatments that received Se applications in the soil; $[Se]_{Control}$ (g kg^{-1}) is the Se contents in polished rice grains of the control treatment; $Se_{Applied}$ (g ha^{-1}) is the Se rates applied in the soil; Y (kg ha^{-1}) is the yield of paddy rice; and P (%) is the percentage of polished rice grain determined at 13% moisture, after removing the rice husk and bran, as presented in table 2.

According to the Food and Agriculture Organization of the United Nations/Agricultural Market Information System (FAO/AMIS), the mean consumption of polished rice in the world recorded for the last decade is 59.11 kg $person^{-1}$ $year^{-1}$ (162 g $person^{-1}$ day^{-1}), while this value for Brazil is 40.66 kg $person^{-1}$ $year^{-1}$ (111 g $person^{-1}$ day^{-1}) (FAO/AMIS 2019). Based on this information and taking into account the Se contents determined in polished grains of the present study, we have also calculated the possible Se intake from the consumption of these biofortified polished grains, using the Equation 2 below:

$$\mathrm{Se-Intake} = [\mathrm{Se}] * \mathrm{C} \qquad \text{Equation 2}$$

where: Se-Intake (μg $person^{-1}$ day^{-1}) is the daily Se intake estimation per person, [Se] (μg kg^{-1}) is the Se contents in polished rice grains verified for the studied treatments and C (kg $person^{-1}$ day^{-1}) is the mean consumption of polished rice grains per person (FAO/AMIS 2019).

Gas exchanges

Measurements of gas exchanges were performed at the pre-flowering period, i.e., 30 days after Se application. For that, we have used an infrared gas exchange analyzer (IRGA, model LICOR 6400, Li-COR Biosciences, Lincoln, NE, USA) and the measurements were made in the rice flag leaf, from 08:00 to 10:00 am, with an air relative humidity of 70% and ambient temperature of nearly 25°C. The density of the photosynthetically active photon flux inside the camera was fixed at 1000 μmol m^{-2} s^{-1}. The following variables were assessed: CO_2 assimilation rate (A - μmol CO_2 m^{-2} s^{-1}), stomatal conductance (g_s - mol H_2O m^{-2} s^{-1}), transpiration (E - mmol H_2O m^{-2} s^{-1}), internal CO_2 concentration (Ci - μmol CO_2 mol air^{-1}), and the ratio of internal CO_2 concentration in the leaf by the atmospheric CO_2 concentration (Ci/Ca). From these data, estimates of both instantaneous carboxylation efficiency [$EiC = A/Ci$ (mol air^{-1})] and water use efficiency [$WUE = A/E$ (μmol CO_2 $mmol^{-1}$ H_2O)] were made.

Chlorophyll relative index (SPAD)

The chlorophyll relative index (SPAD) was assessed using a portable chlorophyll meter (SPAD 502 Plus, Konica Minolta, Osaka, Japan) in the same day that gas exchanges were measured. For that, three rice flag leaves were randomly chosen within the experimental plot and the SPAD readings were performed in three regions of each leaf (basal, medium, and apical leaf regions).

Antioxidant enzyme activities

After the SPAD readings, leaves were collected and immediately frozen in liquid nitrogen and afterward stored at -80°C until analysis of antioxidant enzyme activities.

To evaluate enzyme activities, 0.2 g of fresh leaf tissue was homogenized in 1.5 mL of an extraction buffer (0.1 mol L^{-1} potassium phosphate, pH 7.8, 0.1 mmol L^{-1} EDTA, pH 7.0, 0.01 mol L^{-1} ascorbic acid and 22 mg polyvinylpolypyrrolidone - PVPP) in a pre-chilled mortar and pestle with liquid nitrogen, according to Biemelt et al. (1998). Then, the suspension was centrifuged at 13,000 g for 10 min at 4°C and the supernatant was collected for enzymatic activity analysis, evaluating the following enzyme activities: superoxide dismutase (SOD) (Giannopolitis and Reis 1977), catalase (CAT) (Havir and McHale 1987), and ascorbate peroxidase (APX) (Nakano and Asada 1981).

Spatial distribution of Se, macronutrients, and micronutrients in biofortified rice grains

Rice plants were also grown in pots containing 5 dm^3 of soil (an Oxisol with 23% clay), where 6 mg kg^{-1} of Se as Na_2SeO_4 were applied to obtain Se-rich rice grains (20.9 mg kg^{-1} of Se), allowing to evaluate Se distribution and accumulation in the grain. It has to be stated that such high Se rate was applied in order to produce rice grains with Se contents high enough to be mapped and analyzed by µXRF. After harvesting, paddy rice grains were prepared with the aid of a blade using a microscope in order to obtain images and quantify the elements by µXRF. The focus of this analysis was in the apical region of the rice grain, including the embryo, part of the endosperm, aleurone, and husk in longitudinal cuts (Fig. 2A).

XRF maps were performed to obtain the spatial distributions of Se, Ca, K, P, S, Zn, Fe, Cu, and Mn in the rice grain. To accomplish this, the X-ray microprobe available at the XRF beamline of the Brazilian Synchrotron Light Laboratory (LNLS), was used. It consists of a pair of elliptical curved mirrors arranged in the so-called Kirkpatrick-Baez configuration allowing to focalize the white beam in a 30 microns spot within the sample focal plane. This optical configuration can deliver a flux density at the focal spot of around 1011 photons/sec in a normal operation condition of the LNLS machine (1.37 GeV and 100 mA).

Rice grain samples were placed at 45° from the incoming X-ray beam direction whereas the X-ray fluorescence and scattered radiations coming from samples were detected at 45° from the sample surface. Samples were mounted on motorized XYZ stages, which allows to keep the samples on focus as well as to perform a fast scan within 0.5 μm precision.

XRF imaging was acquired in a raster-scanning mode with 30 microns step size and a dwell time of 500 ms per pixel. The XRF spectra were normalized to the intensity of the incoming beam monitor. The XRF signal was collected with a Silicon Drift Detector (SSD), model AXAS-A, with 7 mm^2 active area and an energy resolution of less than 139 eV (FHWM) at 5.9 keV. XRF spectra were fitted using the PyMca software, version 5.2.2 (Solé et al. 2007). The XRF imaging of several elements including their co-distributions and concentrations were processed and analyzed using the RGB correlator tools available in PyMca. Typical synchrotron radiation X-ray fluorescence (SR-XRF) microprobe spectrum for scanning points of rice grain is shown in Fig. 2B.

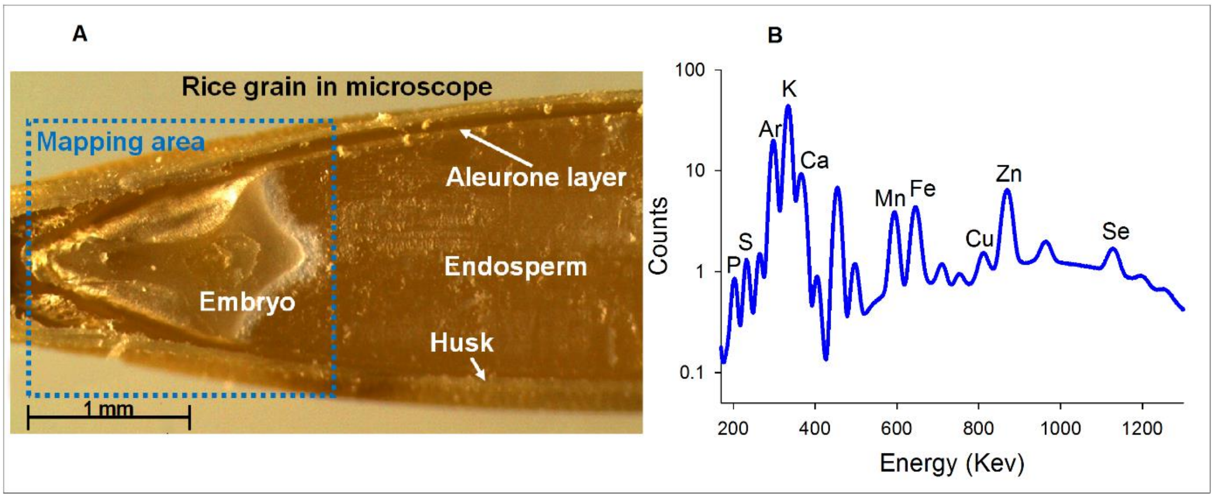

Fig. 2 Details of rice grain structure observed using microscope. Blue rectangle indicates the area for chemical elements mapping (A). Typical synchrotron radiation X-ray fluorescence (SR-XRF) microprobe spectrum for scanning points in a rice grain region (B).

Statistical analysis and data processing

Data were submitted to analysis of variance ($P < 0.05$) using the software Sisvar, version 5.6 (Ferreira 2011). Graphics and regression models were performed using the Sigma Plot software, version 12.5 (Systat Software Chicago, IL, USA).

Results

Paddy and polished rice grain yield

The yields of paddy and polished rice grains were not affected by the tested Se rates (Table 2). The mean paddy rice grain yield verified in the experiment was 3.9 t ha^{-1} (Table 2), which is lower than the Brazilian rice mean yield (4.8 t ha^{-1}) but higher when compared with the mean rice grain yield of 2.5 t ha^{-1} reported for the State of Minas Gerais, from 2007 to 2017 (SEAPA 2017).

Selenium content in polished rice grains

Selenium contents in the polished rice grains increased linearly upon increasing Se rates applied in the soil (Fig. 3A), while the fraction of the applied Se that was incorporated in the polished rice grains (Se recovery by the polished rice) followed a quadratic increase, reaching the maximum value (4.45%) close to 108.72 g ha^{-1} of Se (Fig. 3B). The greatest Se content that rice grains accumulated was 1.95 mg kg^{-1} of Se, which was possible with the application of 120 g ha^{-1} of Se (Fig. 3A), a dose where no toxicity symptoms were observed in the plants.

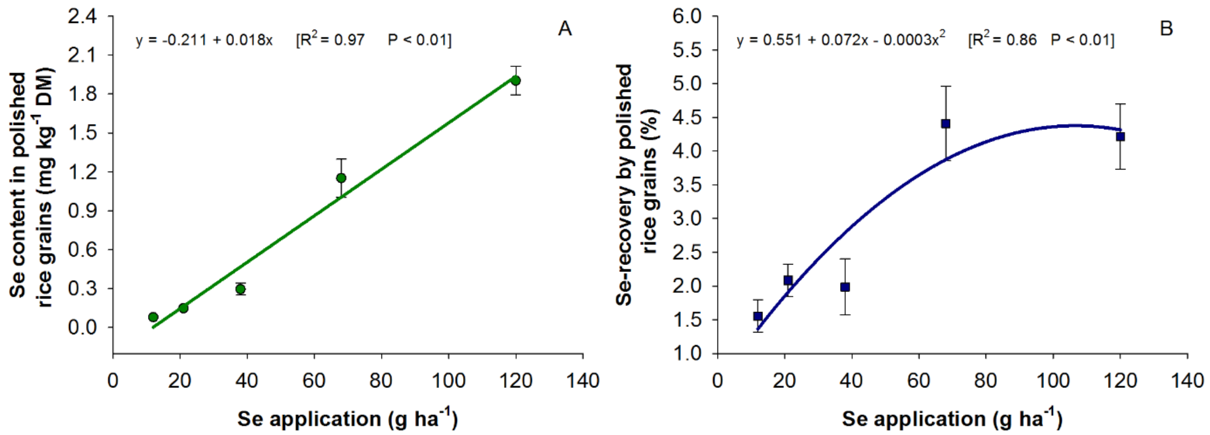

Fig. 3 Selenium contents (A) and Se-recovery percentages (B) in polished rice grains as a function of Se rates applied in the soil. The vertical bar refers to the standard error (n = 4).

The recommended daily allowance (RDA) of Se for healthy adults is 70 µg day^{-1} (Kipp et al. 2015). Considering the amount of polished rice consumed in Brazil (~111 g $person^{-1}$ day^{-1}) and in the world (~162 g $person^{-1}$ day^{-1}) (FAO/AMIS 2019), and the results of Se in the grains found in the present study, as a function of the different Se rates applied in the soil, the suggested Se soil application rate should be ~47 and ~36 g ha^{-1} in Brazil and in the world, respectively, to achieve the aforementioned RDA (70 µg day^{-1}) (Fig. 4A and 4B).

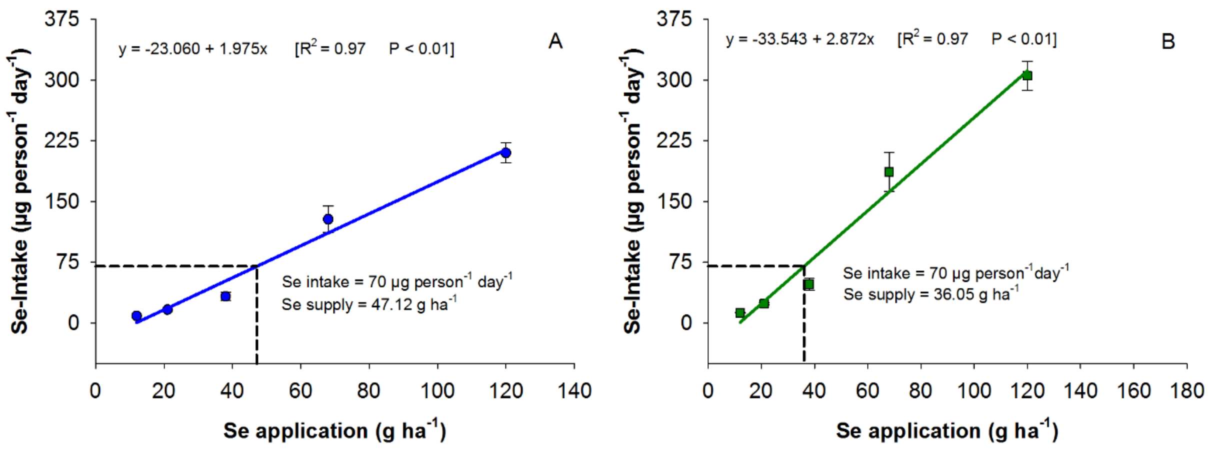

Fig. 4 Estimation of daily Se intake per person in Brazil (A) and in the world (B) considering the amounts of rice consumed reported by FAO/AMIS (2019) and the biofortified rice grains of the present study. The dotted lines mark the Se rate required for producing polished rice grains to guarantee all the Se intake recommendation (70 µg person^{-1} day^{-1}). The vertical bar refers to the standard error (n = 4).

Gas exchanges, chlorophyll relative index (SPAD), and enzyme activities in rice leaves

The variables CO_2 assimilation rates (*A*), the ratio of internal CO_2 concentration in the leaf by the atmospheric CO_2 concentration (*Ci*/*Ca*), instantaneous carboxylation efficiency (*EiC*), and water use efficiency (*WUE*) were not affected by the tested Se rates, presenting the following mean values: *A* = 22.47 µmol CO_2 m^{-2} s^{-1}, *EiC* = 0.074 mol air^{-1}, and *WUE* = 3,321 µmol CO_2 mmol^{-1} H_2O (data not shown). On the other hand, the stomatal conductance followed a quadratic regression with the increase in Se rates, reaching its higher value (0.68 mol H_2O m^{-2} s^{-1}) in the Se rate of 73.62 g ha^{-1} (Fig. 5A). Transpiration also increased upon increasing Se rates (Fig. 5B).

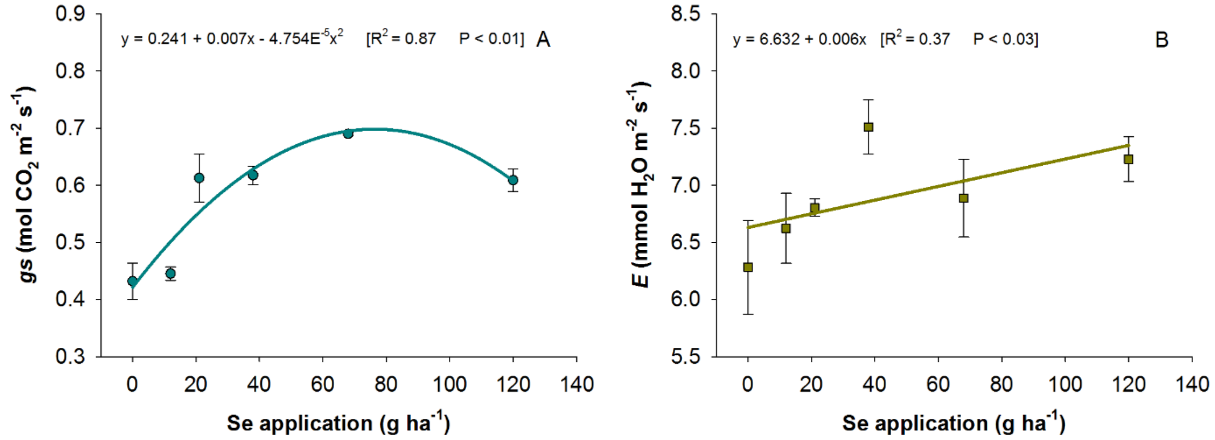

Fig. 5 Effects of Se rates applied in the soil on the stomatal conductance (gs) (A) and transpiration – (E) (B). Vertical bars indicate the standard errors (n = 4).

The chlorophyll relative index (SPAD) was not influenced by the tested Se rates and its mean value was 40.74 (data not shown). Regarding the behavior of antioxidant enzymes, SOD and APX activities followed quadratic regressions, while the CAT activity increased linearly upon increasing the Se rates applied in the soil (Fig. 6). Maximum values of SOD and APX activities were 0.253 U mg^{-1} and 8.654 µmol H_2O_2 min^{-1} mg^{-1}, which were reached with Se application at the rates of 82.58 and 86.66 g ha^{-1}, respectively (Fig. 6).

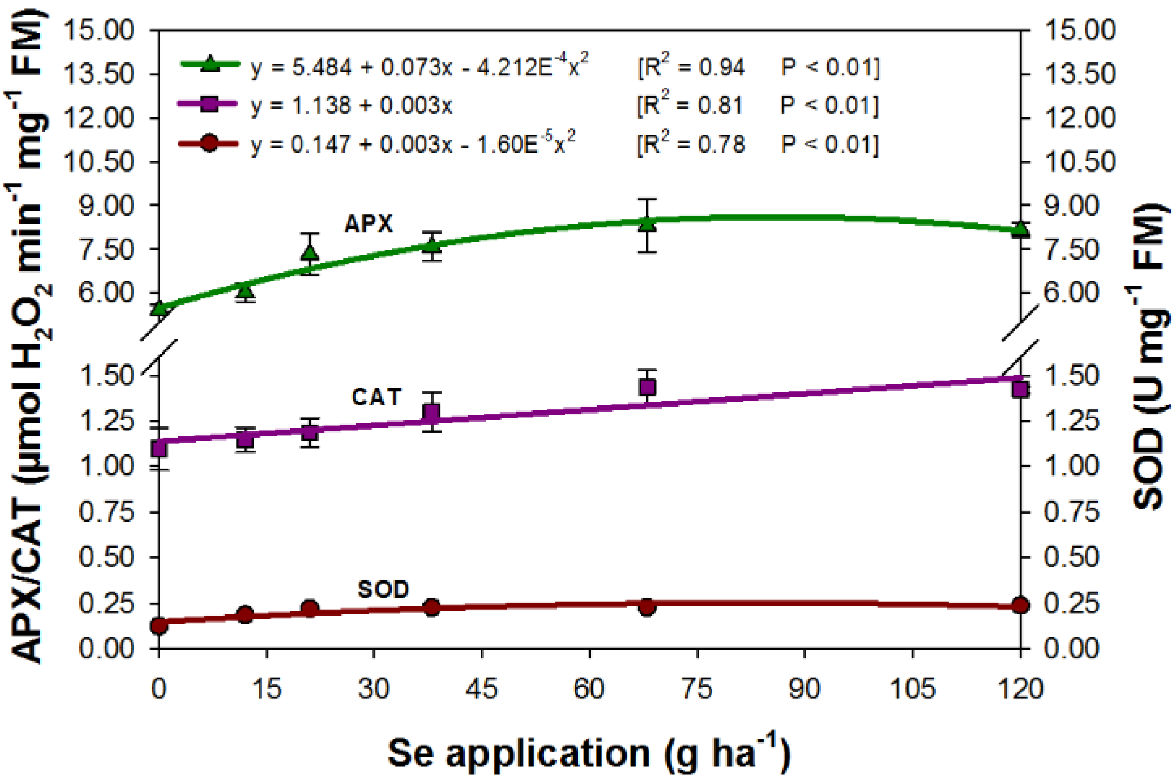

Fig. 6 Effects of Se rates applied in the soil on superoxide dismutase (SOD), ascorbate peroxidase (APX), and catalase (CAT) activities in rice leaves. Vertical bars indicate the standard errors (n = 4).

Spatial distribution of selenium and other elements in rice grains

The mapping of the biofortified rice grains carried out by µXRF showed a wide variation in terms of regions of accumulation for the different chemical elements, as in the embryo, aleurone, endosperm, and husk. Calcium (Ca) was mainly located in the rice husk and in the embryo region, whereas iron (Fe), zinc (Zn), and sulfur (S) appeared in the husk close to the region between the grain and the pedicel, and also in the embryo. Phosphorus (P), copper (Cu), and potassium (K) clearly were concentrated in the embryo. The spatial distribution of Se trough the biofortified rice grain occurred mainly in the endosperm region, being concentrated in two longitudinal bands along the grain (Fig. 7).

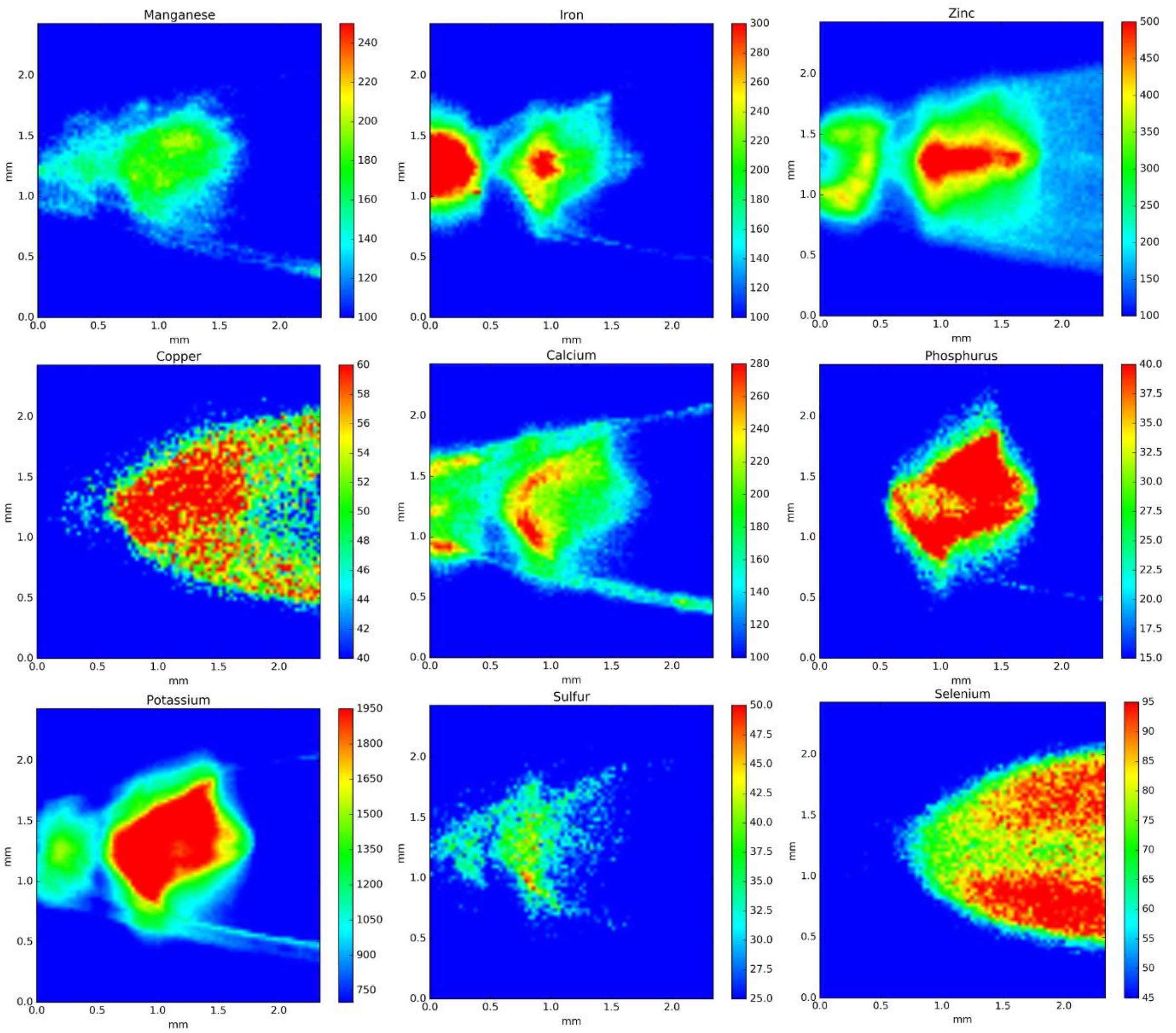

Fig. 7 Images of mapping region and spatial distribution of different elements assessed by µXRF in rice grains biofortified with Se (20.90 mg kg^{-1} of Se).

Discussion

Rice yield and selenium content in polished grains

The rice variety used in the experiment (BRSMG Caravera) was developed for the State of Minas Gerais and has an expected grain yield of 4.7 t ha^{-1}, with an yield of whole grain milled polished rice of 70%. The polished rice grains percentage in the present study was similar to that found by Soares et al. (2008).

Studies evaluating the effect of applying Se from 0 to 25 g ha^{-1} reported an improvement in the nutritional quality of rice grains (Reis et al. 2018). Moreover, in a study carried out under greenhouse conditions, Andrade et al. (2018) verified that rice plants receiving 2 mg kg^{-1} of Se via soil (as selenate) had 20.78 mg kg^{-1} of Se in the polished grains, without exhibiting toxicity symptoms. Thus, considering the Se contents in rice grains showed in Fig. 3, the Se rates tested in the present study are not of concern in terms of causing toxicity, being considered safe for rice plants.

In general, the Se-recovery percentages by polished rice grains were low within the assessed Se rates (from 0 to 4.87%) (Fig. 3B). Haug et al. (2007) reported that only a small fraction of Se applied in the soil via mineral fertilizers (from < 10 to 18%) is used by plants. This fact is explained by the different processes that Se may be involved, such as volatilization, leaching, adsorption on soil particles, and its accumulation in other plant organs as roots, leaves, grain husk, and others.

According to Ajwa et al. (1998), soil microorganisms (mainly anaerobic) play an important role in Se volatilization processes. Selenium stimulates microorganism activities, enhancing the reduction of selenate or selenite to elemental Se and selenide, which are later lost to the atmosphere as dimethylselenide (Losi and Frankenberger, 1997). Plants also have the ability to convert selenate to elemental Se or selenide, creating organic compounds that are volatiles such as dimethylselenide and dimethyldiselenide. These compounds are discharged to the atmosphere during the respiration process and release a characteristic smell of garlic, as reported for Se hyperaccumulator plants (Mayland 1994).

The fact that the Se-recovery percentages increased upon enhancing the Se rates applied via soil (Fig. 3B) may be attributed to the higher Se availability in the soil solution when greater Se rates were applied. In lower Se rates, most of the applied Se can be adsorbed onto soil particles, which compromises its uptake by plants. Tropical soils, such as the Brazilian ones, present as major mineralogical phases 1:1 clays (kaolinite) and Fe and Al oxides, which have all high affinity to retain anions such as phosphate, silicate, and selenate (Araujo et al. 2018; Carvalho et al. 2001; Lessa et al. 2016. Because of that, anionic concentrations in soil solution are reduced, especially, for those anions that are applied in low rates in the soil, such as SeO_4^{2-}.

The Se amount absorbed and used by the human body is highly dependent on the dietary behavior of the population. Ideally, nutritional needs of humans might be satisfied by different types of food (i.e., the concept of “food basket”). Excessive Se intake by humans, usually higher than 400 µg day^{-1}, may lead to toxicity effects, causing health problems, such as hair and nail loss, skin lesions, nervous system dysfunction, and even death (Fairweather-Tait et al. 2011).

On the other hand, our findings show that lower Se rates can satisfy smaller requirements, as estimated by the regression equations showed in Fig. 4A and 4B. Yet, it has to be pointed out that these estimates are considering that the rice grain will reach humans as polished grain and do no consider possible Se losses during other industrial processing and/or cooking. Also, according to Williams et al. (2009) only a small fraction of the Se accumulated in rice grains is present as inorganic Se species, i.e., 94.5% of the detected species were organic Se, and among them, 59% were identified as methyl-selenocysteine (Se-MeSeCys). Notwithstanding, it is known in the literature that Se bioaccessibility from organic Se compounds present in foods is high, within the range 70 to 95% (Combs Jr. and Combs 1986).

Influence of selenium on gas exchanges, chlorophyll relative index (SPAD) and enzyme activities in rice leaves

The effect of Se upon the traits g_s and E has been previously reported in rice (Andrade et al., 2018), wheat (Nawaz et al. 2015), and olive plants (Proietti et al. 2013). According to these studies, Se acts in several plant organs and its role in dismutation reactions of reactive oxygen species (ROS), such as H_2O_2 and O_2^- in cells is commonly emphasized. These reactions decrease ROS accumulation, allowing plants to better adapt in the environment and, in some cases, even to reach higher yields (White 2016). Our data also showed variations in g_s and E, yet they were not enough to change rice yield, as showed in Table 2.

Although E and g_s increased in the presence of Se, thus increasing the possibility of CO_2 assimilation, an instantaneous increased in carboxylation efficiency as well as on the assimilation of CO_2, which could result in improved yield, did not occur. This is because E and g_s are only a few of the factors that affect the photosynthetic rate, while other factors that affect CO_2 assimilation have not been positively affected by Se, resulting in no increased yield.

Selenium plays a fundamental role for plant protection against several biotic and abiotic stresses, resulting in many benefits, e.g., improved photosynthetic apparatus, delayed leaf and fruit senescence, increased plant yield, reduction in oxidative stress induced by heavy metals, cold, or water stress, such as drought (Moulick et al. 2018; Natasha et al. 2018).

Although SOD is not a selenoenzyme, the presence of Se increased its activity in the present study (Fig. 6). Jiang et al. (2017) emphasized that the genes involved in SOD activation are significantly regulated in corn roots after few hours of Se application. Increases in the activity of SOD in corn plants following the application of Se have been also reported elsewhere (Nawaz et al. 2016).

Based on the above statements and as verified in the present study (Fig. 6), the increases in the activities of CAT, APX, and SOD achieved due to soil Se application indicate an important role for protecting the rice plant against critical stresses, as reported by Yao et al. (2010).

Spatial distribution of selenium and other elements in rice grains

The distribution of the evaluated elements in rice grains of the present study was shown in Fig 7. Similar spatial distributions of Ca, Fe, K, Zn, and Mn were reported for rice grains by Lu et al. (2013). Since milling processes might take over part of nutrients, these authors also observed that 43% of the total Zn, 65% of the total Fe, and from 85% to 92% of the total K, Ca, and Mn were removed with the elimination of the husk and bran tissues (embryo + aleurone layer) during the milling process (Lu et al. 2013). It is well known that a considerable fraction of Ca is mainly accumulated in the rice husk (Marschner 1986).

Losses of nutrients during rice processing to obtain the polished rice, as reported for Fe and Zn by Lu et al. (2013) are a matter of concern, taking into account that these losses may decrease the nutritional value of biofortified rice with respect to these micronutrients, after the industrial processing. This is indeed very relevant, as Fe and Zn are the micronutrients with higher nutritional deficiency index among humans worldwide (Ciccolini et al. 2017).

The fact that Se accumulated mainly in the endosperm region of the biofortified rice grains (Fig. 7) is positive and lead to a success of employing agronomic biofortification to produce Se-rich rice grains. Note that Se is also accumulated in the rice seed endosperm (Fig. 7), which reduces the possibility of its losses during industrial processing.

Carey et al. (2012) verified that the region where Se is accumulated in rice grains produced by agronomic biofortification techniques depends on the Se source applied and where Se is applied (soil or rice plant, i.e., foliar sprays). The authors showed that, when Se was applied as organic Se (selenometionine and selenocysteine), higher Se contents were found in the internal parts of the grain, such as in the embryo and in the endosperm.

Finally, based on our findings, we may emphasize that: i) agronomic biofortification - i.e., applying Se rates (as sodium selenate) in the soil as in the present study - can be effective in improving the nutritional quality of rice grains in terms of their Se contents; ii) the evaluated Se rates influenced gas exchanges and the activities of antioxidant enzyme (SOD, CAT, and APX), yet they did not affect the chlorophyll relative index and rice grain yield;

and, iii) spatial distribution of different elements in the biofortified rice grains varied, with some elements accumulating preferentially in the endosperm while others were concentrated mainly in the husk (e.g., Ca). More specifically, Se accumulated primarily in the endosperm, a region that is rich in carbohydrates and which is the major edible part after the industrial processing. Such Se accumulation is positive and highlights the success of employing the agronomic biofortification as an strategy to produce Se-rich rice grains.

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Strategies for agronomic biofortification of rice with selenium in tropical soils

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Abstract

This study aimed to investigate the effects of the selenium (Se) application via soil (SeVI) and via leaf (SeIV and SeVI) on agronomic attributes of rice and its spatial distribution of Se and other elements in biofortified rice grains. Experiments were carried out in two agricultural areas applying 0, 5, 10, 20, 40, and 80 g ha^{-1} of Se. The results showed that the supply of Se to the plants was effective for producing Se-rich rice grains. The spatial distribution of the different elements found in the biofortified rice grains varied substantially, with Se accumulations mainly in the endosperm, whereas P, K, Fe, and Zn accumulate in the embryo. This study showed that the agronomic biofortification of rice with Se, via soil or via leaf, is a promising strategy to be adopted in tropical soils in order to produce rice grains with adequate Se contents for human consumption.

1. Introduction

Selenium (Se) has been regarded as an essential micronutrient for animals and humans (Schwarz, Bieri, Briggs & Scott, 1957). Its role is related to the activity of more than 25 selenoproteins, formed from the naturally occurring amino acids selenocysteine and selenomethionine, which are the major organic forms of Se (Kryukov et al., 2003).

In humans, Se deficiency in the body is often associated with health problems such as Keshan's disease, Kashin-Beck, HIV/AIDS, type-2 diabetes, hypothyroidism, low immunity, male infertility, and the occurrence of cancers (Lopes, Ávila & Guilherme, 2017; Saha, Fayiga & Sonon, 2017). Worldwide, estimates indicate that nearly 3 billion people suffer from Se deficiency, especially children (Saha, Fayiga & Sonon, 2017).

Currently, the global scientific community has been devoting resources to address health problems related to the low intake of Se by the population (Global Panel, 2014). Many of the actions to combat selenium-related malnutrition around the world are based on daily intake reference values (Kipp et al., 2015). Thus, it is possible to infer about food nutritional quality and its suitability for consumption regarding the intake of Se by humans in many regions of the world where low levels of Se are found in soils, including Brazil (Carvalho, Oliveira, Curi, Schulze & Marques, 2019; Gabos, Alleoni & Abreu, 2014; Lidon et al., 2018).

Considering a classification proposed by Tan (1989), most Brazilian soils can be classified as Se deficient (< 0.125 mg kg^{-1}). In Brazil, the legislation allows the addition of Se in fertilizers for soil or foliar application, and > 300 mg kg^{-1} in micronutrient fertilizers for leaf application (Brasil, 2016).

In this context, the adoption of food biofortification programs (agronomic and/or genetic) has been a promising strategy to improve the nutritional quality of foods (Cakmak, 2008). The biofortification of plants allows the production of food rich in nutrients, such as Se, which becomes bioavailable by the conversion of inorganic species into their organic forms present in amino acids and proteins (Gong, Zhang & Cheng, 2018; Sun, Liu, Williams & Zhu, 2010).

In grain crops such as rice, wheat, and maize, in addition to the content and speciation of Se, its location in the grain is an important factor related to the nutritional quality of the food. Industrial processing of grain peeling and polishing might reduce its nutritional quality due to the loss of considerable amounts of nutrients (Lu et al., 2013).

Rice is a staple food for more than half of the world population, considered an important source of minerals. It provides nearly 80% of the daily caloric intake of the population of more than 30 countries (Lucca, Poletti & Sautter, 2006; Meng, Wei & Yang 2005). Currently, the world's rice production ranks third among the major cereals, with approximately 25% (polished grain) (FAO/AMIS, 2019). Thus, considering that rice is a staple food of great importance, its biofortification is highly recommended, and studies are need specially involving agronomic biofortification of Se in tropical soils.

In this sense, this study aimed to evaluate the effects of the Se application via soil (SeVI) and via leaf (SeIV and SeVI) in rice grown at two experimental fields on: i) grain yield; ii) contents of Se and other elements in different fractions of the plant and grain; iii) antioxidant enzymatic activity in the leaf; and, iv) spatial distribution of Se and other elements in biofortified rice grains by synchrotron-based X-ray microfluorescence (μXRF) imaging.

2. Materials and Methods

2.1 Experimental areas and treatments

An early upland rice cultivar (BRSMG Caravera), which is commonly grown in Brazil and presents high grain quality (Soares et al., 2010), was used in this study. The experiment was carried out during the cropping season of 2016/2017 under field conditions at two experimental areas belonging to the Agricultural Research Company of Minas Gerais State

(EPAMIG), located in Lambari (21°56'51"S, 45°18'55"W) and Patos de Minas (18°31'04.24" S, 46°26'22.12" W), state of Minas Gerais, Brazil (Supplementary Fig.1).

The physical and chemical parameters of the soils prior to the experiment are shown in Supplementary table 1. The experiment was conducted using a randomized complete block design, composed of five Se rates, one control treatment without Se application, and four replicates. The experimental plots had 5 meters long by 2 meters wide (rice row spacing at 0.5 m), totaling 10 m^2. For measurements of the different variables that were assessed, the useful area considered was two rice lines and four meters long. A total of 80 rice seeds were sown per linear meter. For both experimental areas, fertilization was carried out by applying 163 kg ha^{-1} of monoammonium phosphate (MAP = 10% N and 46% P_2O_5) in the rice lines. Forty days after sowing, fertilizations with ammonium sulfate and potassium chloride was performed at both experimental areas.

The agronomic biofortification of rice was tested based on three strategies: i) Se-enriched MAP supplied via soil during sowing of rice (MAP-SeVI); ii) selenate applied via leaf during the reproductive phase of the plant (Foliar-SeVI); and iii) selenite applied via leaf during the reproductive phase (Foliar-SeIV). The following Se rates were used for each treatment: 5, 10, 20, 40, and 80 g ha^{-1} of Se, which was applied as sodium selenate and sodium selenite (Na_2SeO_4 or Na_2SeO_3, Sigma-Aldrich, Saint Louis, MO, USA). As for the foliar Se application, Se salts were diluted in deionized water and applied using a carbon dioxide-pressurized pump, spraying the solutions in the rice leaves. Leaf Se rates were equally divided at two applications, during the rice flowering (70 days after sowing) and in the rice grain-filling stage (90 five days after sowing). The control treatment received only deionized water and surfactant. Selenium-enriched MAP was prepared by adhering Se to the fertilizer granule by the addition of diethanolamine and dye. The dye was used to aid in the visualization of the homogeneity of the compound. Given the rates of Se applied in the experiment, the amount of Se in the Se-enriched MAP treatment was: 30; 60; 120; 240, and 480 mg kg^{-1}. The control treatment received only MAP, diethanolamine and dye.

2.2 Agronomic attributes of rice

After maturation, the useful area of each experimental plot was harvested. Rice grains were dried at room conditions and their humidity were determined. The paddy rice yield (kg ha^{-1}) of each plot was assessed by weighting the grains and it was extrapolated for the whole hectare, having the moisture corrected to 13%. For assessing the polished rice grains, rice bran and husks from 100 g of paddy rice samples were peeled and weighed.

2.3 Selenium, macronutrient, and micronutrient contents in biofortified rice

The contents of Se were quantified in polished rice grains for all treatments, whereas, for the treatment in which the rice plants received the highest Se dose (80 g ha^{-1}), the contents of the macronutrients calcium (Ca), magnesium (Mg), phosphorus (P), sulfur (S), and the micronutrients zinc (Zn), manganese (Mn), iron (Fe), and copper (Cu) in the stem, leaf, husk, bran, whole rice and polished rice were also measured. Thus, samples of rice grains were processed for peel removal and polishing of the grains (Supplementary Fig. 2). Leaves and stems were also collected at the time of rice harvest for analysis. Thereafter, the material was finely milled for further acid digestion.

The contents of Se, Ca, Mg, P, S Zn, Mn, Fe, and Cu in rice grains were determined in extracts following acid digestion using concentrated HNO_3 in microwave oven, according to the 3051A method of the United States Environmental Protection Agency (USEPA) (USEPA, 2007).

The fraction of the applied Se that was incorporated in the polished rice grains (Se recovery percentage) was calculated using the Equation 1 below as performed by Lessa et al. (2019).

$$\mathrm{Se-recovery} = \frac{\{[\mathrm{Se}]_{\mathrm{Treated}} - [\mathrm{Se}]_{\mathrm{Control}}\} * \mathrm{Y} * \mathrm{P}}{\mathrm{Se}_{\mathrm{Supply}}} \qquad \text{Equation 1}$$

Where: Se-recovery (%) is the use efficiency of the Se rates applied in the soil by polished rice grains (Se utilization percentage); $[Se]_{Treated}$ (g kg^{-1}) is the Se contents in polished rice grains from rice plants grown in treatments that received Se applications in the soil; $[Se]_{Control}$ (g kg^{-1}) is the Se contents in polished rice grains of the control treatment; Se_{Supply} (g ha^{-1}) is the Se rates applied in the soil; Y (kg ha^{-1}) is the yield of paddy rice; and P (%) is the fraction of polished rice grain determined at 13% of moisture after removing the rice husk and bran, as showed in table 2.

According to the Food and Agriculture Organization of the United Nations/Agricultural Market Information System (FAO/AMIS), the average consumption of polished rice in the world recorded for the last decade is 59.11 kg $person^{-1}$ $year^{-1}$ (162 g $person^{-1}$ day^{-1}) (FAO/AMIS 2019). Based on this information and taking into account the Se contents determined in polished grains of the present study, the possible Se intake from the consumption of these biofortified polished grains were estimated using Equation 2 below:

$$\mathrm{Se-Intake} = [\mathrm{Se}] * \mathrm{C} \qquad \text{Equation 2}$$

where: Se-Intake (μg $person^{-1}$ day^{-1}) is the daily Se intake estimation per person, [Se] (μg kg^{-1}) is the Se contents in polished rice grains verified for the studied treatments and C (kg person^{-1} day^{-1}) is the mean consumption of polished rice grains per person (FAO/AMIS 2019).

Selenium contents in the extracts were 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). The calibration curve for Se measurements was obtained from a standard solution with 1 g kg^{-1} of Se (≥ 98% of purity, Fluka, Buchs, Switzerland). The macro and micronutrient contents in the extracts were measured using inductive coupled plasma emission spectrometry (ICP-OES) (Spectro, Blue model, Germany), with background correction. The operating parameters and the sample introduction system were as per manufacturer's specification: 1400 W plasma power, 12 L min^{-1} cooling gas flow, 0.8 L min^{-1} auxiliary gas flow and gas flow rate in the nebulizer 0.85 L min^{-1}. The gas used was argon with purity greater than 99.99%.

For quality assurance and control of Se measurements in rice plant, a standard reference material from the Institute for Reference Materials and Measurements (White Clover - BCR 402, IRMM, Geel, Belgium) and a blank sample were included in each batch of analysis. The mean recovery value obtained for the standard material was 96.78 % ± 1.88 % (n = 13).

2.4 Antioxidant enzyme activities

Due to the effect of Se in the antioxidant system of plants, some antioxidant enzymes were measured in rice leaves as performed by Lessa et al. (2019). For that, 0.2 g of fresh leaf tissue was homogenized in 1.5 mL of extraction buffer (0.1 mol L^{-1} potassium phosphate, pH 7.8, 0.1 mmol L^{-1} EDTA, pH 7.0, 0.01 mol L^{-1} ascorbic acid and 22 mg polyvinylpolypyrrolidone - PVPP) in a pre-chilled mortar and pestle with liquid nitrogen. Then, the suspension was centrifuged at 4°C, and the supernatant was analyzed for enzymatic activity analysis of the following enzymes: superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX).

2.5 Spatial distribution of Se and macro and micronutrient contents in biofortified rice grains

Rice plants were also grown in pots containing 5 dm^3 of soil (Oxisol with 23% of clay), where SeVI (Na_2SeO_4) was applied via soil or via leaf while SeIV (Na_2SeO_3) was applied only via leaf to obtain Se-rich rice grains. The amount of Se supplied was 6 mg pot^{-1} for all treatments. A total of 10 rice seeds were sown per pot. After harvesting, rice grains were prepared with the aid of a blade, using microscope to obtain images and quantify the elements by synchrotron-based X-ray microfluorescence (μXRF). The analysis was performed in the ventral region of the rice grain, including embryo, parts of the endosperm, aleurone, and husk in longitudinal cuts.

XRF maps were performed to obtain the spatial distributions of Se, Ca, K, P, S, Zn, Fe, Cu, and Mn in the rice grain. To accomplish this, the X-ray microprobe available at the XRF beamline of the Brazilian Synchrotron Light Laboratory (LNLS) was used. It consists of a pair of elliptical curved mirrors arranged in the so-called Kirkpatrick-Baez configuration that focalizes the white beam in a 30 microns spot within the sample focal plane. This optical configuration can deliver a flux density at the focal spot of around 1011 photons/s in a normal operation condition of the LNLS machine (1.37 GeV and 100 mA).

Rice grain samples were placed at 45° from the incoming X-ray beam direction, whereas the X-ray fluorescence and scattered radiations coming from samples were detected at 45° from the sample surface. Samples were mounted on motorized XYZ stages, which allows to keep the samples on focus as well as to perform a fast scan within 0.5 μm precision.

XRF imaging was acquired in a raster-scanning mode with 30 microns step size and a dwell time of 500 ms per pixel. The XRF spectra were normalized to the intensity of the incoming beam monitor. The XRF signal was collected with a silicon drift detector (SSD), model AXAS-A, with 7 mm^2 active area and an energy resolution of less than 139 eV (FHWM) at 5.9 keV. XRF spectra were fitted using the PyMca software, version 5.2.2 (Solé, Papillon, Cotte, Walter & Susini, 2007). The XRF imaging of several elements including their co-distributions was processed and analyzed using the RGB correlate tools available in PyMca.

2.6 Statistical analysis and data processing

Data were submitted to analysis of variance using the Sisvar software, version 5.6 (Ferreira, 2011). Graphics and regression models were performed using the Sigma Plot software, version 12.5 (Systat Software Chicago, IL, USA).

3. Results and Discussion

3.1 Agronomic attributes of rice

The biofortification methods and the applied Se rates did not affect the agronomic characteristics (i.e., yield) of rice ($p > 0.05$) at both cultivation areas (Lambari and Patos de Minas) (Table 1).

The highest grain yield and percentage of polished grains were obtained in the experimental field Patos de Minas (Table 1). The high levels of phosphorus and potassium available in the soil, associated with the pH close to neutrality of the area (Supplementary table 1) might have favored the development of plants and the grain filling (Table 1).

Table 1. Average yield of paddy rice grains and percentage (mass of fraction/mass of paddy rice grain) of different fractions of the rice grains.

Experimental field	Paddy rice grain	Polished rice grains	Husk	Bran
	kg ha^{-1}	----------------------------	%	-----------------------------
Lambari	1,936 ± 39	58.88 ± 2.77	34.67 ± 2.31	6.45 ± 0.17
Patos de Minas	4,635 ± 86	72.41 ± 0.94	20.41 ± 1.45	7.19 ± 0.99

Polished rice grains percentage were assessed after remove husk and bran from a 100 g paddy rice grain sample. The number after the average indicates the standard error (n = 72).

3.2 Selenium contents in polished rice grains

The content of Se in polished grains increased linearly upon increasing the Se rates supplied via soil and leaf in Lambari and Patos de Minas (Fig. 1). Rice plants treated with SeIV and SeVI via leaf presented the highest levels of Se (Fig. 1). The polished rice from Patos de Minas presented the lowest contents of Se when compared with polished rice from Lambari (Fig. 1).

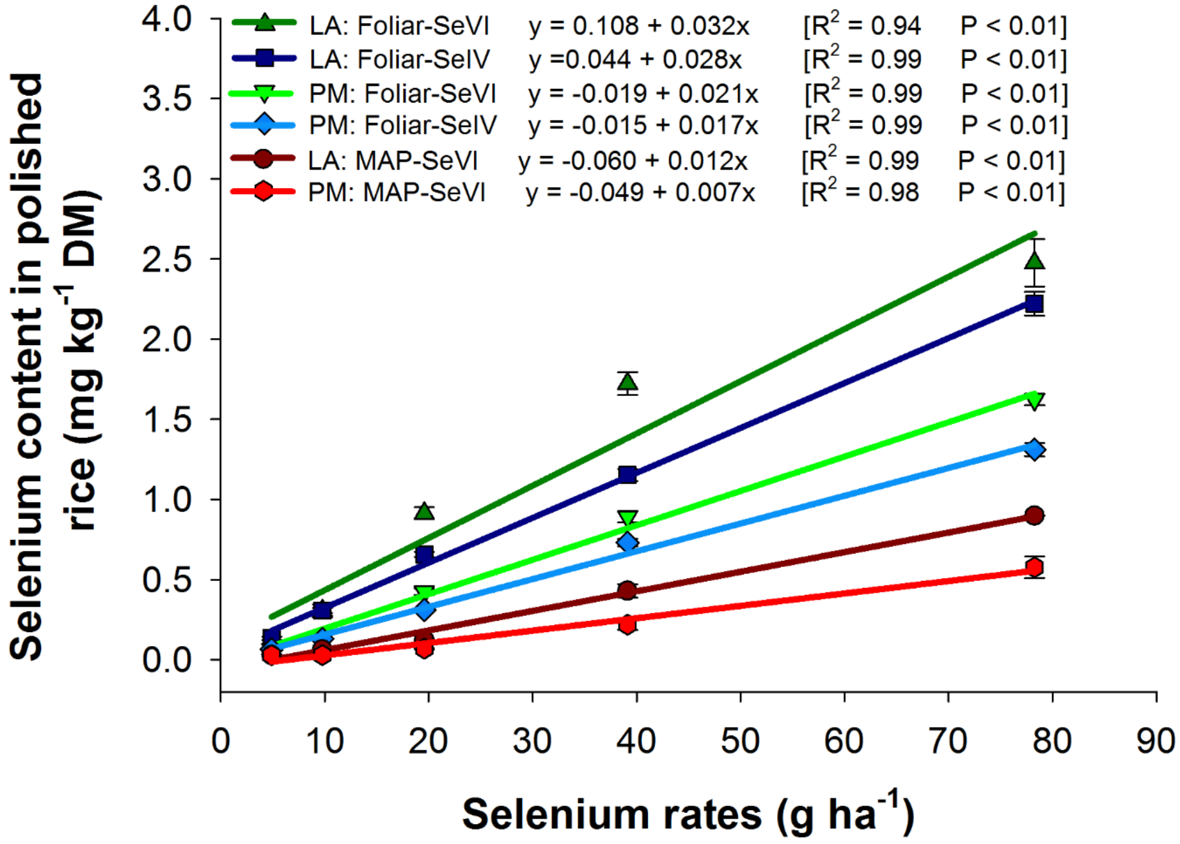

Figure 1. Selenium contents in polished rice grains as a function of Se rates applied via soil and via leaf. DM = Dry matter; LA = Lambari, and PM = Patos de Minas. The vertical bars refer to the standard error (n = 4).

Foliar fertilization can cause lower losses of nutrients due to direct nutrient and vegetal contact (Marschner, 2012). Studies have shown that foliar nutrient application is several times more efficient than soil application (Boldrin, Faquin, Ramos, Boldrin, Ávila & Guilherme, 2013; Curtin, Hanson, Lindley & Butler, 2006).

The lower content of Se found in polished rice from Patos de Minas when compared with Lambari was likely due to the dilution effect, which occurs when the element concentration is diluted due to higher plant growth and grain yield (Jarrell & Beverly, 1981), which happened as it can be seen in Table 1.

3.3 Selenium utilization by polished rice grains

The fraction of the applied Se that was incorporated in the polished rice grains (Se-recovery percentages) from Patos de Minas increased with the increase of the dose of Se. The rice treated with MAP-SeVI showed the lowest percentages of Se (Supplementary table 2).

In general, the highest percentage of Se use by polished rice in treatments that received soil application (MAP-SeVI) in Patos de Minas compared to Lambari (Supplementary table 2) is due to the higher availability of Se in the soil solution as well as P and S contents of the soil (Supplementary table 1). On the other hand, the percentage of Se utilization for rice treated with SeVI and SeVI via leaf might be related to the greater development of the plants. The higher leaf density may have reduced Se loss, favoring foliar absorption.

In Lambari and Patos de Minas, the low use efficiency of Se applied via soil (MAP-SeVI) (Supplementary table 2) might be related to a series of chemical (i.e. sorption phenomena) and physical (i.e. leaching) soil obstacles that could have reduced the Se availability and its root absorption. Tropical soils, such as those from the Brazilian Cerrado biome, present high levels of iron and aluminum oxides, which increases the adsorption capacity of SeVI (Araujo, Lessa, Ferreira, Guilherme & Lopes, 2018; Lessa, Araujo, Silva, Guilherme & Lopes, 2016; Lopes et al., 2017). Thus, the content of iron and aluminum oxides of the soil could be the main factor responsible for the low transfer of Se from the soil to the rice grains.

According to Haug, Graham, Christophersen and Lyons (2007), Se applied via soil is rapidly converted into unavailable forms, resulting in the absorption of a small fraction (up to 10%) by the plants. Nevertheless, the percentage of Se use can reach up to 18% for grain crops (Lyons et al., 2004). The application of Se via soil in the initial growth phase of the plant can increase its use efficiency (Curtin et al., 2006).

The relationship between Se rates and human daily Se intake based on the average consumption of rice (0.162 kg person^{-1} day^{-1}) (FAO/AMIS, 2019) and the contents of Se in polished rice (µg kg^{-1}) (Fig. 1) can be seen in the supplementary fig. 3. Thus, the rate of Se that should be supplied to the plant to obtain Se contents in polished rice that are suitable for human consumption would be (reaching 70 µg of Se day^{-1}): 11 g ha^{-1} (Foliar-SeVI), 14 g ha^{-1} (Foliar-SeIV), and 40 g ha^{-1} (MAP-SeVI) for Lambari (Supplementary fig. 3A) and 21 g ha^{-1} (Foliar-SeVI), 26 g ha^{-1} (Foliar-SeIV), and 63 g ha^{-1} (MAP-SeVI) for Patos de Minas (Supplementary fig. 3B). These rates of Se are equivalent to ~ 0.42 mg kg^{-1} of Se in polished rice.

Comparatively, the supply of Se via soil (MAP-SeVI) should be, on average, 3.3 and 2.5 times higher in relation to the application of Foliar-SeVI and Foliar-SeIV for Lambari (Supplementary fig. 3A) and Patos de Minas (Supplementary fig. 3B), respectively, in order to meet the recommended daily intake of Se (70 µg person^{-1} day^{-1}).

The bioavailability/bioaccessibility is a key factor in the assimilation of Se in the human and animal organism, presenting a strong relation with the organic Se content present in the food. According to some studies with rice grains, the content of organic Se varies from 50 to 95% (Farooq et al., 2018; Gong et al., 2018; Williams et al., 2009) and its bioaccessibility ranges from 52 to 76% (Gong et al., 2018; Sun et al., 2010).

Although biofortification is primarily important for improving the nutritional quality of food, adopting this strategy increases agricultural production costs. The dose of Se supplied via soil (MAP-SeVI) is approximately three times greater than the dose supplied via leaf (Foliar-SeVI or Foliar-SeIV), considering Se contents appropriate to consumption (Supplementary fig. 3). Thus, biofortification of rice with the application of Se via soil is even more economically disadvantageous. Therefore, low-cost Se sources can be an efficient alternative for agricultural application. According to Haug et al. (2007), there are high amounts of wastes that contains Se due to mining and industrial processing of nickel, copper, and coal, which could be better used. Moreover, the residual effect of successive applications of Se via soil associated with the application of anions, such as phosphate and sulfate, and the incorporation of straw into the soil can reduce the rates of application of Se in crops over time (Alfthan et al. 2015).

The contents of Se in leaves, stem, husk, bran, whole rice, and polished rice varied according to each method of Se application (Fig. 2A). The highest levels of Se were found in leaves (5.2 mg kg^{-1} and 6.7 mg kg^{-1}) and husks (3.5 mg kg^{-1} and 4.3 mg kg^{-1}) supplied with SeVI and SeIV via leaf, respectively. As for Se applied via soil (MAP-SeVI), the highest levels of Se were observed in leaves and bran (1.5 mg kg^{-1}). Regardless of the biofortification method, the stem was the part of the rice plant that presented the lowest Se contents (0.8 mg kg^{-1}).

When the Se distribution was evaluated only in the parts of the rice grain (husk, bran and polished rice grain), it was observed that the highest percentage of Se was found in the polished rice grain for the following biofortification methods: MAP-SeVI = 55 %, Foliar-SeVI = 51%, and Foliar-SeIV = 43% (Fig. 2B). The Se content in the husk was also quite representative, reaching 53% for foliar application (Foliar-SeIV). In addition, the treatment that received SeVI via soil produced the highest accumulation of Se in the bran (MAP-SeVI = 12%, Foliar-SeVI = 7% and Foliar-SeIV = 4%) (Fig. 2B).

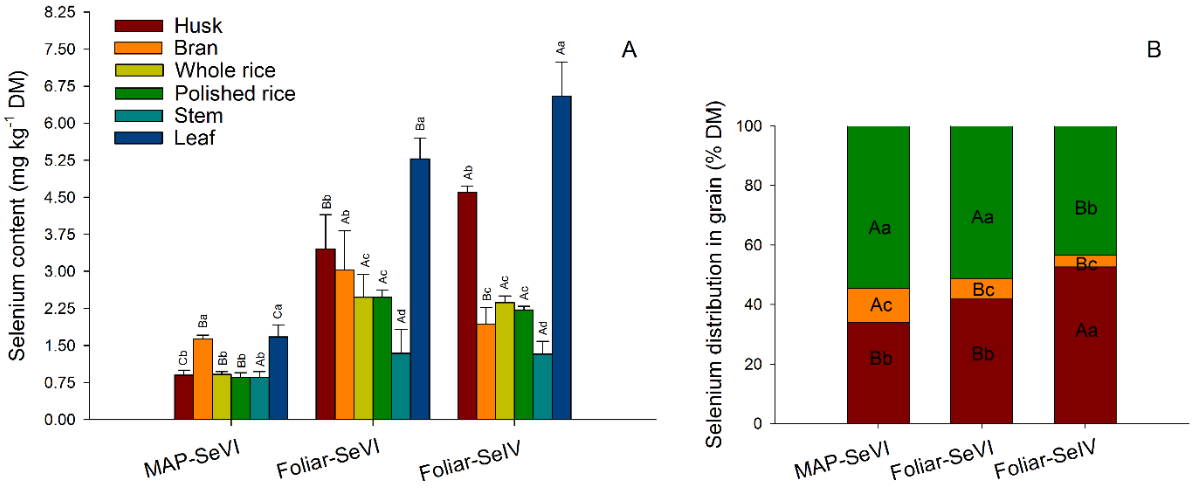

Figure 2. Selenium content in the parts of the rice plant (A) and distribution of Se in rice grains (B) for the treatment that received the dose of 80 g ha^{-1} from Lambari experimental field. DM = Dry matter. Uppercase letters in the bars compare the contents of Se between the different structures of the rice plant within each biofortification method, while lowercase letters compare the content of Se in the plant structures between the methods in the level of 5% ($p < 0.05$) by the Scott-Knott test. The vertical bars refer to the standard error (n = 4).

The results for Se applied via soil are similar to those of Sun et al. (2010), who verified that the concentration of Se in the rice bran was 1.94 times higher than in polished rice cultivated in Chinese soils. Because of rice processing (peeling and polishing), Se losses can reach up to 53% due to peeling, or up to 57% due to peeling and polishing (Fig. 2B).

3.4 Macro and micronutrient contents in polished rice

In general, the contents of macro and micronutrients in polished rice are high. The contents of Ca, Mg, Mn, Fe, and K are higher in the stem, leaf, and bran. As for S, Zn, and Cu, higher contents were found only in the bran, while the highest concentration of P was found in the bran and in the whole grain (supplementary Fig. 4).

The accumulation of macro and micronutrients was little affected by the methods of Se application to rice plants. Sulfur presented the highest accumulation in polished grain (65%), followed by K (50%). In the bran, P (45%) and Mg (50%) were present in greater amounts. Calcium was the only element that was not detected in the whole or polished rice grains (Supplementary Fig. 5).

3.5 Enzyme activities in rice leaves

In general, the activity of the enzymes superoxide dismutase (SOD), ascorbate peroxidase (APX), and catalase (CAT) increased upon increasing Se rates supplied to plants, except for SOD (SeVI via MAP), APX (SeIV e SeVI via foliar) in Lambari and APX and CAT (SeVI via MAP), SOD (SeVI via foliar) and CAT (SeIV via foliar) in Patos de Minas (Supplementary table 3).

The interference of Se in the enzymatic activities of SOD, APX, and CAT was minimal (Supplementary table 3), suggesting that the rates of Se used in the present study were not high enough to cause any effect on the agronomic characteristics of the rice (Table 1). Reis et al. (2018) showed that the application of 25 g ha^{-1} of Se via soil promoted the increase of SOD and CAT, however, grain yield was not altered either. Andrade et al. (2018), in greenhouse cultivation using high rates of Se (0.5 to 2 mg kg^{-1}), showed that water deficit strongly decreased the plant growth and yield. However, rice plants treated with Se showed higher net photosynthesis, water use efficiency, and antioxidant system.

According to Asada (2006) the effect of Se in the plant activates SOD and CAT, causing reduction of lipid peroxidation and formation of hydrogen peroxide in plant cells, resulting in reduction of senescence. SOD has the ability to eliminate the superoxide radical by disrupting two radicals and converting them to hydrogen peroxide (H_2O_2). CAT and APX convert H_2O_2 to O_2 and H_2O.

3.6 Spatial distribution of selenium and other elements in rice grains

It is noteworthy that the application of high rates of Se to plants was necessary to produce Se-rich rice grains to enable mapping analysis µXRF (Supplementary table 4).

Figure 3A shows the typical synchrotron-based X-ray fluorescence (XRF) spectrum. The map of µXRF was made in the basal region of the rice grain, close to the rachilla, surrounding the embryo (Fig. 3B). Figures 3C, D, and E show the spatial distribution of Se in the biofortified rice grain according to each treatment. The results of Figure 3C, D, and E showed that Se is localized in all fractions of rice grains such as husk, aleurone, and endosperm.

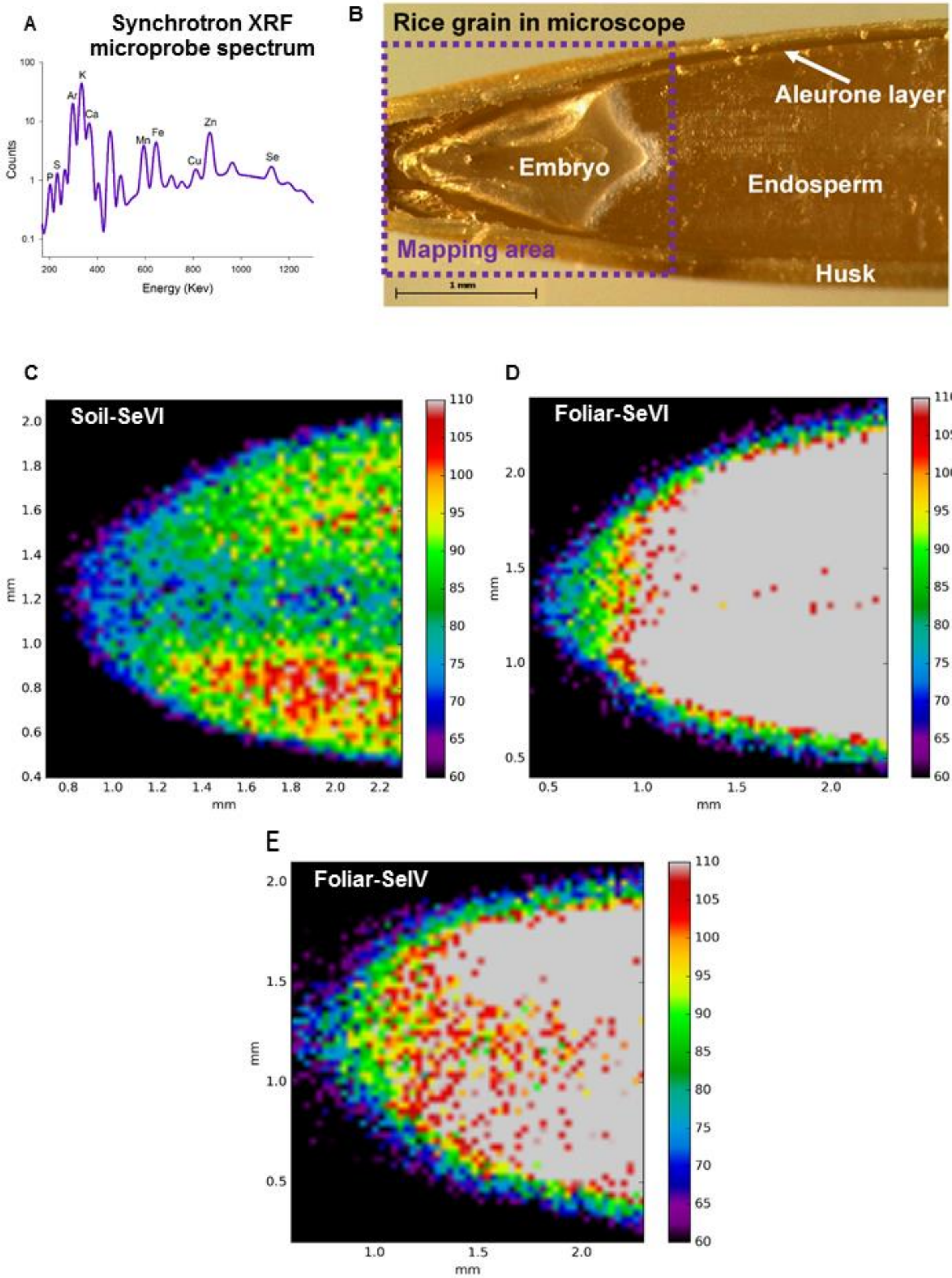

Figure 3. Typical synchrotron radiation X-ray fluorescence microprobe spectrum (A) in a basal region of the rice grain (B). Images of mapping region and spatial distribution of Se assessed by µXRF in biofortified rice grains (C-E). The color bar next to each image indicates the variation of the Se content in the sample.

In the samples treated with SeVI via soil (Fig. 3C) or leaf (Fig. 3D), Se is located predominantly in longitudinal bands in the endosperm. Figure 4A (region II) shows a higher intensity of yellow, red, and gray, indicating a higher content of Se in the endosperm, near the endosperm edge of polished grain. The central region of the grain, as well as the embryo, presented the lowest contents of Se.

On the other hand, grains treated with SeIV via leaf showed an evenly distribution in the basal endosperm (Fig. 3E). However, in the equatorial region of the grain (Fig. 4B, region I), following to the apex (Fig. 4B, region II), there is a decreasing gradient of Se.

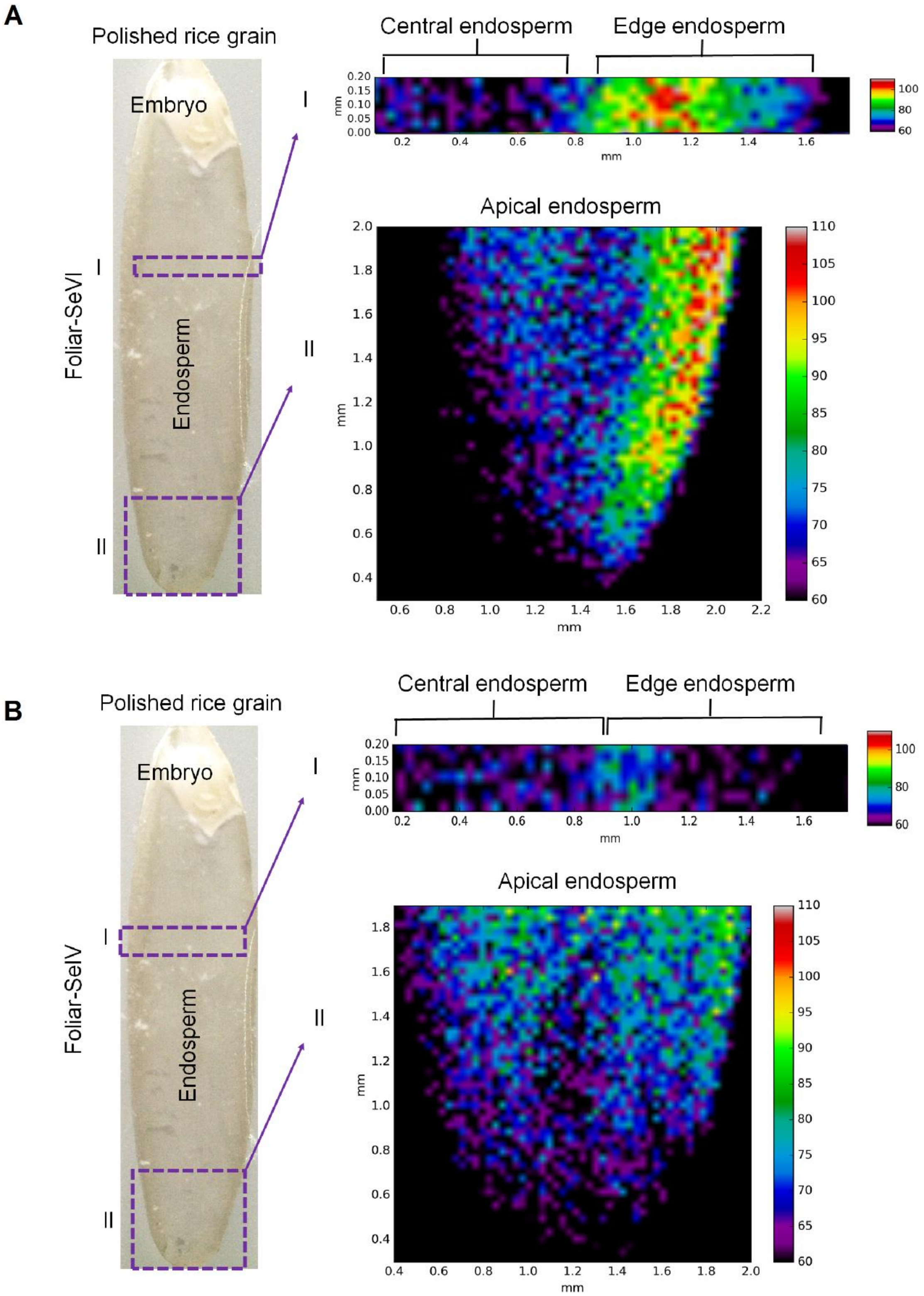

Figure 4. Spatial distribution of Se in polished rice grains treated with SeVI (A) and SeIV (B). The regions I (equatorial) and II (apical) delimited in purple represent the mapping sites of Se in the grain.

The results indicated that there is a lower content of Se in the husk than in the endosperm of the grains (Fig. 3D and E). For the treatment that received Se via soil, no difference in Se contents was observed between the grain fractions (Fig. 3C). As for the endosperm, the distribution of Se in bands also occurs in the husk (Fig. 5). Due to the thin thickness of the aleurone layer, it was not possible to map it separately.

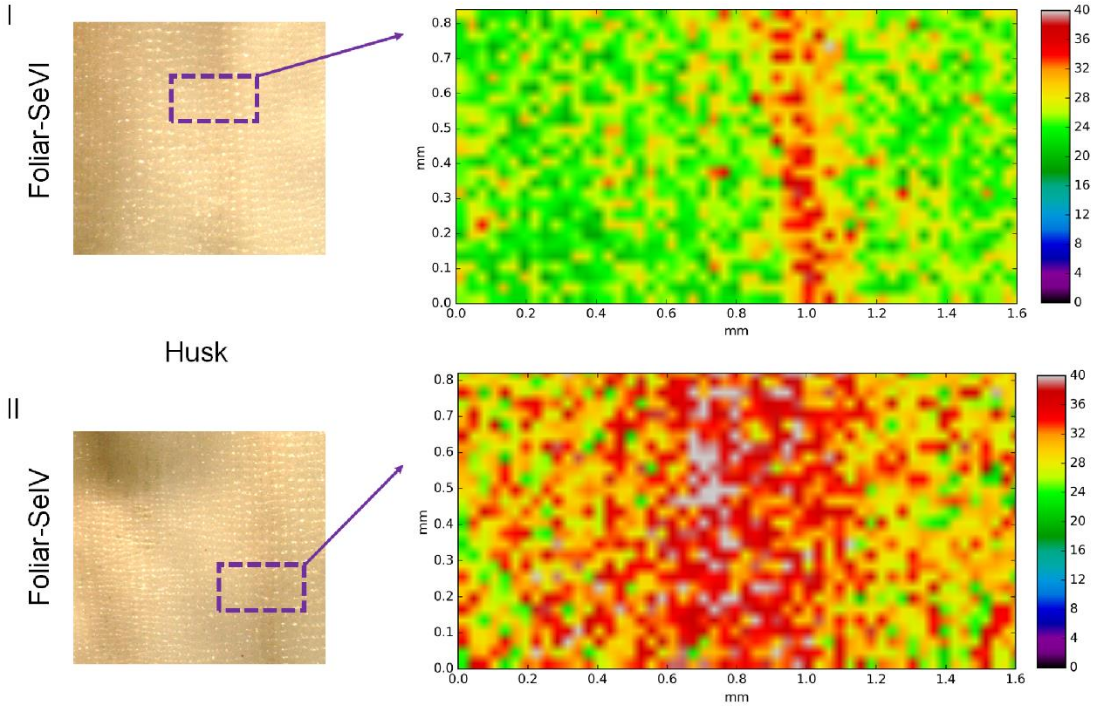

Figure 5. Spatial distribution of Se in rice husk treated with SeVI (I) and SeIV (II). The regions delimited in purple in the sample represent the mapping sites of Se in the husk.

During rice grain processing (peeling), grain structures such as sterile lemma, awn, rachilla, palea, and lemma are not separated. Thus, the highest concentration of Se in the husk (Fig. 2) may have been due to the presence of structures such as sterile lemma and rachilla in the analyzed sample. These structures have more leaf-like characteristics. However, lemma and palea present in the mapped grain consist mainly of cellulose (30%), hemicellulose (20%), lignin (27%) and silica (16%) (Soares, 2012).

The locations of Ca, P, K, Zn, Mn, and Fe in the rice grains followed the same pattern regardless of the applied treatment. A good correlation was observed between the results of the elements analyzed in the extract of the digested fractions (Supplementary Fig. 5) and their location in the grain by μXRF (Supplementary Fig. 6).

Calcium is predominantly located in the husk (Supplementary Fig. 6A), while Mn (Supplementary Fig. 6B) appears in the husk and embryo. The concentration of Ca in whole grain and polished extracts was lower than the limit of detection (Fig. 2). In the plant, Ca exerts structural function, being component of calcium pectate of the middle lamella of the cell wall, presenting low redistribution in the plant. Manganese is an activator of numerous enzymes in plant cells, and similar to Ca, it presents low redistribution in the plant. Therefore, its absence in the endosperm is expected, since the endosperm is basically composed of carbohydrates such as starch, with low metabolic activity (Marschner, 2012). In other study, Mn was also found located in the outermost part of the grain (Kyriacou et al., 2014).

The elements P, K, and Zn are located exclusively in the embryo (Supplementary Fig. 6B, C and D). Iron appears preferentially in the embryo, with slight traces in the husk, especially close to the rachilla (Supplementary Fig. 6F).

Using synchrotron X-ray fluorescence microscopy, Kyriacou et al. (2014) verified that Fe and Zn were clearly located in the scutellum and other regions of the embryo, with oxymugineic acid being the possible responsible for the mechanism of accumulation of these elements in this structure.

In rice, as in any other cereal, micronutrients are stored almost exclusively in husk, aleurone layer and embryo (Lucca et al., 2006). Thus, it is possible to infer that, in part, the losses of nutrients during grain processing can be reduced by the ingestion of whole rice.

In the present study, based on the results of supplementary material 2, it is estimated that the maintenance of the aleurone layer (bran) may contribute to the maintenance of ~ 50%

Mg, ~ 45% P ~ 35% Zn and ~ 30 % Fe in whole rice. According to Lu et al. (2013), nutrient losses due to rice processing can reach up to 43% Zn, 65% Fe and 85% to 92% K, Ca, and Mn. Lidon et al. (2018) also showed a reduction in the nutritional quality of the rice due to the polishing process of the grains with the removal of the aleurone and the embryo.

A second alternative that might contribute to the reduction of nutrient losses due to the industrial processing of rice grains is parboiling. Parboiling is the industrial process used to obtain rice grains with a substantial increase in the physical quality and also the content of minerals and vitamins. For this, grains with husk are placed in warm water for a certain time, promoting the gelatinization of the starch with the transfer of the nutrients into the grains (Soares, 2012).

On the other hand, rice by-products (straw, husk, and bran) can be harnessed, reducing nutrient losses in the agricultural system. Rice straw can be used to make hay for animal consumption or to cover the soil, avoiding erosion and providing nutrients after it has decomposed. Rice husk is commonly used as poultry bed material, which is later used as manure in agricultural crops. Bran can be used in animal feed, constituting up to 30% of animal food (Soares, 2012).

4. Conclusions

Agronomic biofortification by applying rates of Se (MAP-SeVI, Foliar-SeVI, and Foliar-SeIV) was effective in improving the nutritional quality of rice grains in relation to the content of Se.

Adding Se influenced positively the activity of the antioxidant enzymes (SOD, CAT, and APX), with some exceptions yet, it did not affect the yield of rice grains.

There was a variation in the spatial distribution of different elements in the biofortified rice grains, with elements that were preferentially accumulated in the embryo (such as Zn and

K) and others that accumulated mainly in the husk (such as Ca). Selenium accumulated mainly in the endosperm, a region rich in carbohydrates and the main edible part after the industrial processing of the rice grain. Such accumulation of Se is positive and leads to the successful use of agronomic biofortification to produce Se-rich rice grains.

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Declaration of competing interest:

The authors declare that they do not have any conflict of interest

Ethical review:

“This study does not involve any human or animal testing”.

Informed consent:

Written informed consent was obtained from all study participants.

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Supplementary materials

Table 1. Soil attributes of the experimental area before the experiment.

Experimental field	**pH in water**	**K**	**P**	**S**	**H+Al**	**SB**	**CEC**	**V**
Lambari		--------- mg dm^{-3} ---------			----- cmol$_c$ dm^{-3} -----			%
	5.90	94.2	34.8	14.5	4.50	3.77	8.27	45.6
	Fe	**Mn**	**Zn**	**Se**	**SOM**	**Clay**	**Silt**	**Sand**
	------------------ mg dm^{-3} -------------------				---------------- g kg^{-1} --------------			
	63.4	34.3	5.49	0.37	26.7	650	100	250
Experimental field	**pH in water**	**K**	**P**	**S**	**H+Al**	**SB**	**CEC**	**V**
Patos de Minas		--------- mg dm^{-3} ---------			---- cmol$_c$ dm^{-3} -----			%
	6.10	176.5	63.1	35.6	6.10	4.02	10.1	39.8
	Fe	**Mn**	**Zn**	**Se**	**SOM**	**Clay**	**Silt**	**Sand**
	------------------ mg dm^{-3} -----------------				---------------- g kg^{-1} --------------			
	45.8	206	10.4	0.39	33.8	450	100	450

Soil properties determined according to the Brazilian methods of soil (Teixeira, Donagemma, Fontana & Teixeira, 2017). P is the available phosphorus extracted by anion-exchange resin. SB is the sum of bases Ca^{2+}, Mg^{2+} and K; CEC is the cation exchange capacity at pH 7.0; V is the percentage of bases retained at a CEC; SOM is the soil organic matter. The “Total” contents of Se in soil sample was extracted with aqua regia, as performed by Gabos et al. (2014).

Table 2. Se-recovery percentages by polished rice grains as a function of Se rates applied in the soil for each experimental field.

Experimental fields	**Treatments**	**Selenium rates (g ha^{-1})**				
		5	10	20	40	80
		---------------------------- % ----------------------------				
Lambari	SeVI-MAP	0.63 ± 0.11 aB	0.69 ± 0.07 aB	0.66 ± 0.06 aB	1.18 ± 0.07 aB	1.14 ± 0.06 aB
	SeVI-Foliar	3.15 ± 0.08 aA	3.58 ± 0.20 aA	4.10 ± 0.14 aA	3.67 ± 0.28 aA	3.32 ± 0.10 aA
	SeIV-Foliar	3.23 ± 0.10 aA	3.42 ± 0.19 aA	3.38 ± 0.12 aA	3.65 ± 0.19 aA	3.66 ± 0.19 aA
Patos de Minas	SeVI-MAP	1.50 ± 0.00 cB	0.98 ± 0.17 cB	1.23 ± 0.06 cC	1.92 ± 0.27 bB	2.73 ± 0.42 aC
	SeVI-Foliar	3.93 ± 0.54 bA	4.39 ± 0.80 bA	6.26 ± 0.49 aA	5.58 ± 0.49 aA	6.45 ± 0.28 aA
	SeIV-Foliar	3.59 ± 0.20 bA	3.98 ± 0.20 bA	5.24 ± 0.33 aB	6.10 ± 0.21 aA	5.58 ± 0.20 aB

LA = Lambari; PM = Patos de Minas; Lowercase letters in the lines compare the effect of rates of Se for each biofortification method, while uppercase letters in the columns compare the effect of the biofortification methods for each dose of Se applied in each experimental field, Scott Knott test 5%, n = 4.

Table 3. Effects of Se rates applied via soil and via leaf on superoxide dismutase – SOD (U mg^{-1}), ascorbate peroxidase – APX (μmol H_2O_2 min^{-1} mg^{-1}), and catalase – CAT (μmol H_2O_2 min^{-1} mg^{-1}) activities in fresh rice leaves matter.

Experimental field	Treatments	Enzymes	Selenium rates (g ha^{-1})					
			0	5	10	20	40	80
Lambari	SeVI-MAP	SOD	0.30 ± 0.01a	0.28 ± 0.01a	0.29 ± 0.02a	0.28 ± 0.01a	0.27 ± 0.01a	0.26 ± 0.02a
		APX	2.73 ± 0.27b	3.24 ± 0.03b	3.24 ± 0.02b	5.76 ± 0.29a	4.88 ± 0.04a	4.67 ± 0.13a
		CAT	1.14 ± 0.08c	0.99 ± 0.04c	1.48 ± 0.03b	1.50 ± 0.07b	2.07 ± 0.09a	2.11 ± 0.21a
	SeVI-Foliar	SOD	0.17 ± 0.01b	0.22 ± 0.01a	0.22 ± 0.02a	0.22 ± 0.01a	0.22 ± 0.01a	0.22 ± 0.02a
		APX	7.01 ± 0.18a	6.78 ± 0.36a	6.61 ± 0.81a	6.45 ± 0.02a	7.56 ± 0.19a	8.04 ± 0.31a
		CAT	1.16 ± 0.07b	1.12 ± 0.03b	1.80 ± 0.02a	2.21 ± 0.03a	1.89 ± 0.29a	1.15 ± 0.01b
	SeIV-Foliar	SOD	0.15 ± 0.01b	0.19 ± 0.02b	0.19 ± 0.02b	0.25 ± 0.00a	0.22 ± 0.01a	0.27 ± 0.02a
		APX	6.65 ± 0.62a	7.26 ± 0.81a	6.82 ± 0.52a	7.19 ± 0.79a	6.94 ± 0.46a	8.18 ± 1.05a
		CAT	1.99 ± 0.05b	2.44 ± 0.05b	2.17 ± 0.02b	2.36 ± 0.28b	3.83 ± 0.24a	3.50 ± 0.17a
Patos de Minas	SeVI-MAP	SOD	0.29 ± 0.04b	0.36 ± 0.03b	0.31 ± 0.03b	0.35 ± 0.00b	0.43 ± 0.01a	0.42 ± 0.01a
		APX	8.12 ± 0.08a	8.44 ± 0.71a	8.55 ± 0.10a	8.64 ± 0.06a	7.18 ± 1.04a	9.26 ± 0.49a
		CAT	2.27 ± 0.08a	2.30 ± 0.27a	2.40 ± 0.08a	2.61 ± 0.10a	2.46 ± 0.15a	2.86 ± 0.34a
	SeVI-Foliar	SOD	0.30 ± 0.01a	0.32 ± 0.02a	0.34 ± 0.01a	0.28 ± 0.01b	0.34 ± 0.01a	0.26 ± 0.01b
		APX	4.37 ± 0.02d	4.54 ± 0.16d	7.23 ± 0.97c	9.85 ± 0.98b	16.97 ± 0.72a	18.25 ± 0.77a
		CAT	1.37 ± 0.17c	2.35 ± 0.08b	2.35 ± 0.23b	3.83 ± 0.45a	4.57 ± 0.21a	4.45 ± 0.30a
	SeIV-Foliar	SOD	0.17 ± 0.02c	0.22 ± 0.01b	0.26 ± 0.02b	0.25 ± 0.02b	0.24 ± 0.01b	0.33 ± 0.03a
		APX	14.45 ± 0.18b	14.43 ± 1.13b	12.25 ± 0.28b	14.64 ± 1.30b	13.15 ± 0.71b	19.24 ± 2.27a
		CAT	2.28 ± 0.19a	2.65 ± 0.50a	2.89 ± 0.23a	3.01 ± 0.28a	2.53 ± 0.19a	2.44 ± 0.29a

Lowercase letters compare the enzymatic activity in the leaves between the biofortification methods at the level of 5% ($p < 0.05$) by the Scott-Knott test. The symbol ± indicates the standard errors (n = 4).

Table 4. Selenium contents (mg kg^{-1}) in the biofortified rice to enable mapping analysis μXRF.

	Soil-SeVI	Foliar-SeVI	Foliar-SeIV
Husk	14.31	13.18	14.97
Bran	28.22	14.62	12.54
Whole rice grain	25.24	24.12	13.05
Polished rice grain	20.90	19.06	10.94

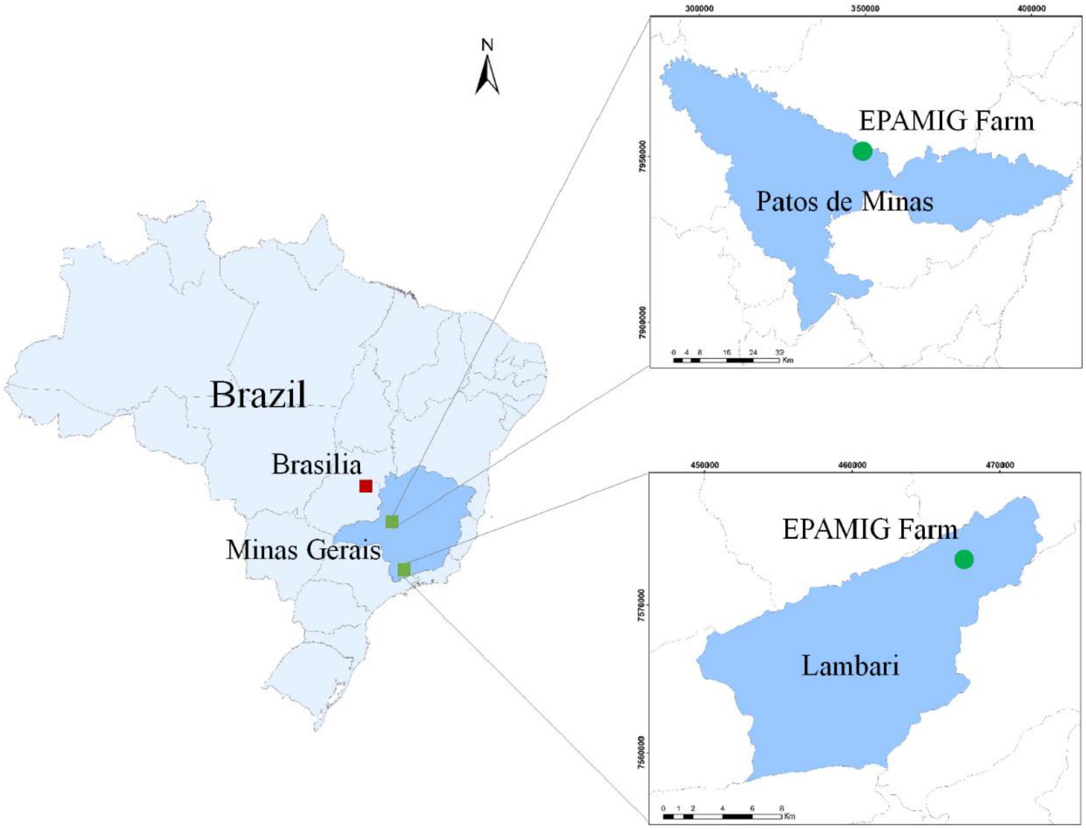

Figure 1. Location of the experimental areas.

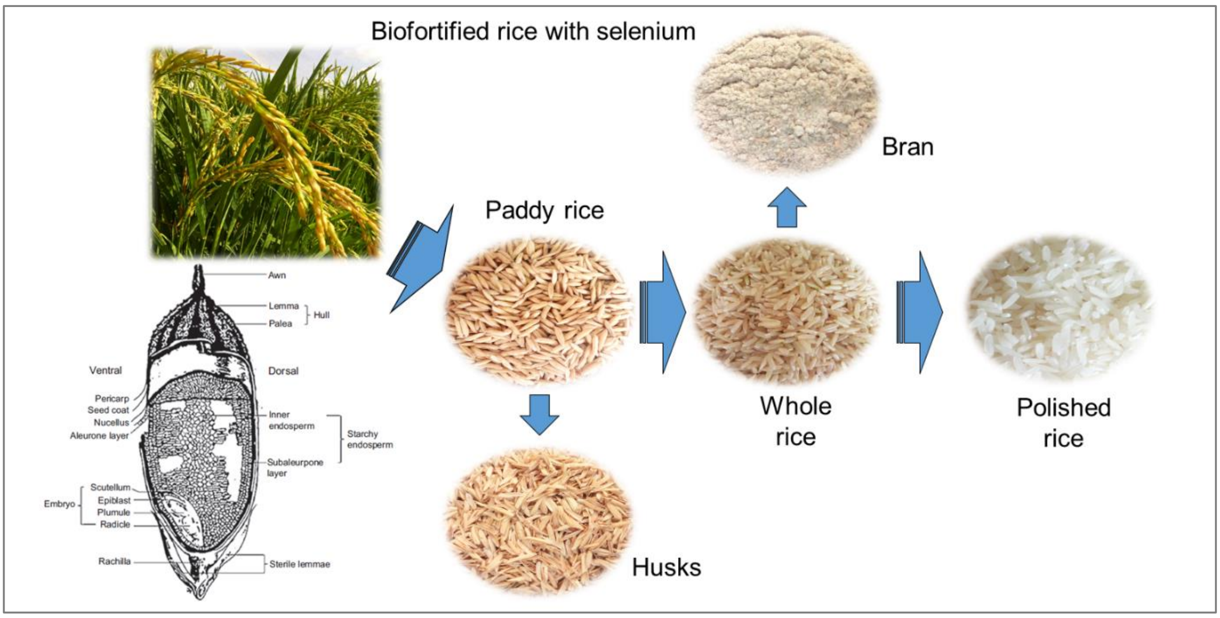

Figure 2. Scheme evidencing rice grain structure and the processing of the rice to obtain polished grains. The whole and polished rice were obtained after the peeling and polishing of 100 g of paddy rice grains, respectively.

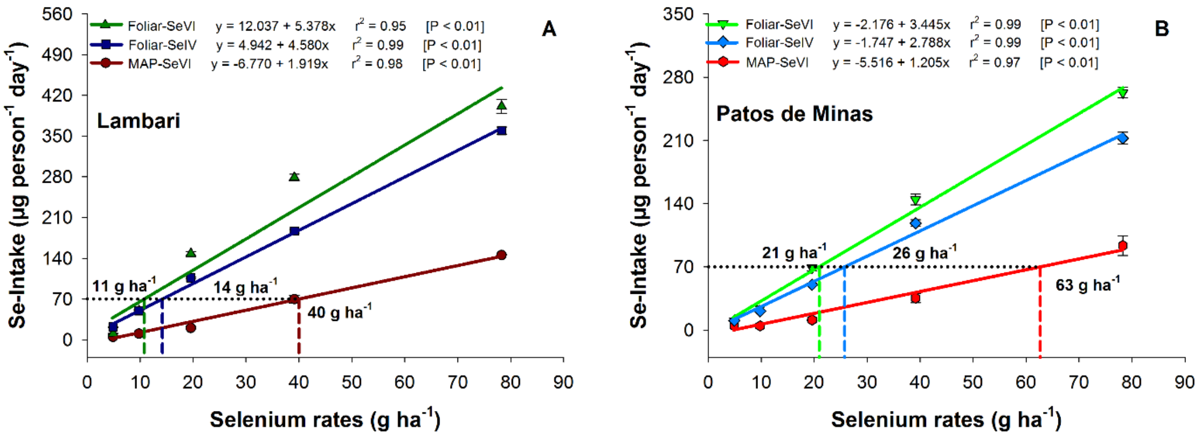

Figure 3. Estimation of daily Se intake per person considering the amounts of rice consumed reported by FAO/AMIS (2019) for Lambari (A) and Patos de Minas (B) experimental fields. The dotted lines mark the Se rates required for producing polished rice grains to guarantee all recommended Se intake (70 µg person^{-1} day^{-1}). The vertical bar refers to the standard error (n = 4).

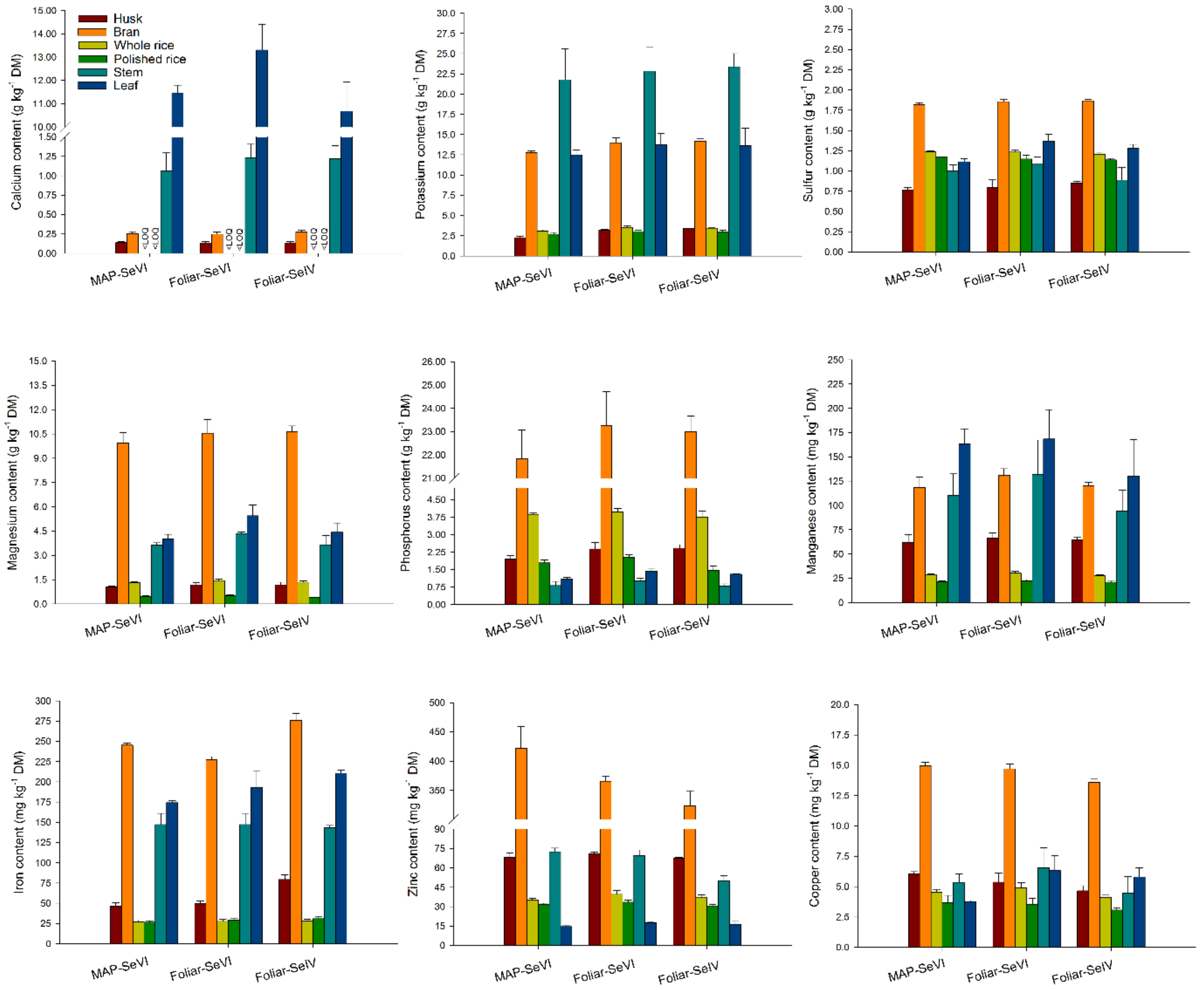

Figure 4 The contents of macro and micronutrients in different parts of the rice plant. The vertical bars refers to the standard error (n = 4).

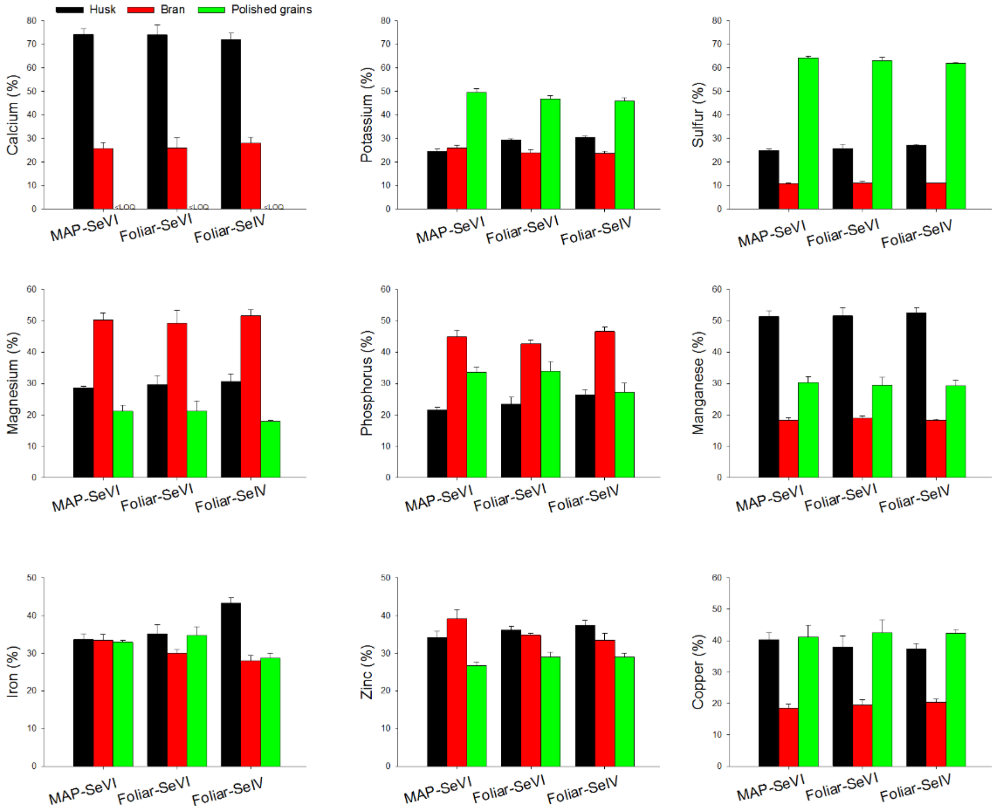

Figure 5 The contents of macro and micronutrients in different parts of the rice grains. The vertical bars refers to the standard error (n = 4).

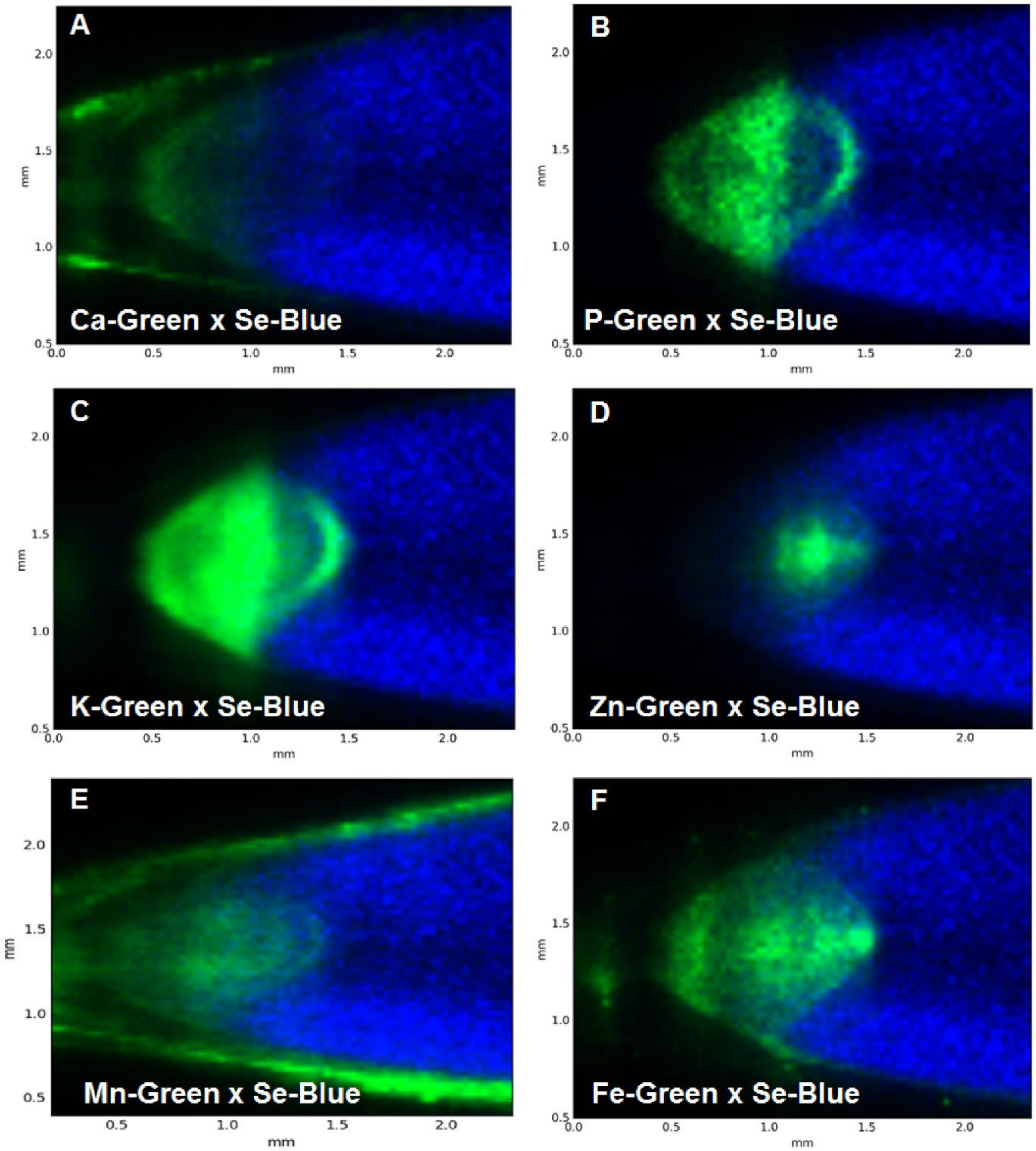

Figure 6. Correlation maps between Se and Ca, P, K, Zn, Mn, and Fe on rice grains biofortified with Se.

Concluding remarks

The rice biofortication studies with Se carried out by this present work provided innovative information, being useful for improving the nutritional quality of this cereal for human consumption.

The agronomic biofortification of rice in the field conditions by applying Se via leaf provides greater increases in Se content in rice grains than the application of Se via soil. However, in the long-term, soil application of Se may provide the residual effect of Se on soil and its use by subsequent agricultural crops, which should be evaluated in other/future studies.

The fact that Se accumulates preferentially in the endosperm, as shown in this present thesis, increases the success chances in adopting rice biofortification strategies. Associated with this, the high consumption of this cereal worldwide (staple food) also contributes to the increase of Se intake by humans and may lead to the reduction of health problems related to Se deficiency.