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Rice (*Oryza sativa* L.) Biofortification with Selenium: Enrichment Index and Interactions among Nutrients †

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Abstract: Selenium is an antioxidant trace mineral important for human health and development. Therefore, the growing demand for efficient, bioeconomic and sustainable strategies to increase Se content in cereals, namely rice, is therefore justified. In this context, biofortification is a strategy that can promote nutrient enhancement in food crops and, therefore, increased nutrient uptake in the human body. In this framework, a technical itinerary was implemented using a rice genotype (OP1509), through foliar spraying with two selenium concentrations (25 and 100 g $Se.ha^{-1}$) of sodium selenate (Na_2SeO_4) and sodium selenite (Na_2SeO_3). It was found that the average of Se biofortification index was 1.8–4.7 and 5.4–6.0 fold in selenate and selenite treatments, respectively. The contents of Se, Ca, Fe, K, P, C, H and O in brown rice grains, were also quantified being found that both forms of fertilizers increased Zn contents with 25 g $Se.ha^{-1}$ but decreased with 100 g $Se.ha^{-1}$. Moreover, Ca only increased significantly with selenate pulverization. The application of both forms also increased grain weight but did not affect the colorimetric analysis. It is concluded that the applied itinerary can be implemented to minimize Se malnutrition.

Keywords: selenate; selenite; selenium biofortification

1. Introduction

Selenium is an essencial micronutrient humans and may be a benefit to higher plants [1,2]. The low presence of micronutrients (namely Se) in staple foods, namely rice, results in the evolution of deficiencies in more than half of the world population [2,3]. Selenium deficiency affects about 800 million (15%) population of the world [4], triggering hypothyroidism, male infertility, weakness in the imune system, cardiovascular and carcinogenic diseases [5–7]. To surpass this deficiency, the World Health Organization (WHO) recomends a daily intake of 30–40 μg Se for adults [8], but problems linking this nutrient deficiency is highly significant among staple food, namely in cereal crops [9]. Nevertheless, positive effects of Se on growth of plants have been reported particularly under abiotic stress [10,11].

Biofortification of food crops is reported to provide a sustainable solution for Se deficiency in human diets [12]. Indeed, it has been reported that rice is one of the most social and economic important cereal in the world yet, in spite of its nutritional properties, a global study of rice grains showed that prevails an insufficient concentration of Se for humans [13–15]. Accordingly, agronomic biofortification itineraries can be adopted as a strategy to increment Se content of rice grains [16]. Selenium contents in rice grains can increase when foliar fertilizers such as sodium selenate and sodium selenite are applied [17,18], however agronomic biofortification depends upon its uptake either through roots or foliage and translocation [19].

Considering the importance of rice for humam consumption, this study aimed at developing an agronomic itinerary for Se biofortification through foliar fertilization with sodium selenate and selenite in the new advanced rice line (*Oryza sativa* L. Poaceae).

2. Experiments

2.1. Experimental Fields

Field trials were conducted, from 30 May to 2 November of 2018, at the experimental station of the Rice Technological Center (COTARROZ), located in the middle of the lezíria ribatejana—Portugal (39°02′21.8″ N; 8°44′22.8″ W). One new advanced rice (*O. sativa* L. Poaceae) line (coded as OP1509) of the national Breeding Program carried out by the Instituto Nacional de Investigação Agrária e Veterinária (INIAV, Elvas, Portugal) was used as a test system.

OP1509 was sown in six row plots and then immediately irrigated. The experimental design was performed in randomized blocks and a factorial arrangement (3 concentrations × 2 forms selenium x 1 genotypes × 4 replicates = 24 plots). The plot size for each replication was 9.6 m^2. The agronomic management of trials, namely the application of nitrogen fertilizers, control of weeds, insect pests and diseases and the water management (irrigation) was the recommended and typically used for rice crops.

The agronomic Se biofortification comprised three distinct phases. First Se application occurred at the end of booting, the second at anthesis and the third during the milky grain stage. Biofortification was carried out by foliar spraying with solutions (at 25 and 100 g $Se.ha^{-1}$) of sodium selenate (Na_2SeO_4) and sodium selenite (Na_2SeO_3). Control plants were not sprayed at any time. Grain harvest occurred at 2 November 2018.

2.2. Analysis of Macro and Micronutrients Contents

Quantification of Se, Zn, Ca, Fe, K, P, C, H and O in tissues was determined in harvested grains from control and sprayed plots, with selenate and selenite, at 0 and 100 g $Se.ha^{-1}$, using a *μ-EDXRF system* (*M4 Tornado™, Bruker, Germany*) [20]. The X-ray generator was operated at 50 kV and 100 μA without the use of filters, to enhance the ionization of low-Z elements. To a better quantification of Se, a set of filters between the X-ray tube and the sample, composed of three foils of Al/Ti/Cu (with a thickness of 100/50/25 μm, respectively) was used. All the measurements were perform with 600 μA current. The values of the content of the elements were obtained through the average of four readings.

Measurements were carried out under 20 mbar vacuum conditions. These point spectra were acquired during 200 s.

2.3. Thousand Grains Weight and Colorimetry Analysis

For each treatment, 1000 grains were picked randomly and weighed in triplicate. Subsequently, grains were hulled and whitened, as described in [18]. Determination of the colorimetric parameters of grain samples, using a fixed wavelength, followed [21]. The color parameters, using fixed wavelength, adopted the methodology described by Ramalho et al. [22]. Brightness/brightness (L) and chromaticity parameters (a* and b* coordinates) were obtained with a Minolta CR 300 colorimeter (Minolta Corp., Ramsey, NJ, USA) coupled to a sample vessel (CR-A504). Using the illuminant D_{65}, the system of the Commission Internationale d'Éclaire (CIE) was applied. The parameter L represents the brightness of the sample, translating the variation of the tonality between dark and light, with a range between black (0) and white (100). Parameters a* and b*, indicate color variations. The value of a*characterizes coloring in the region from green (−60) to red (+60) and the value b* indicates coloring in the range of between blue (−60) to yellow (+60). The approximation of these coordinates to the null value translates neutral colors like white, gray and black. Chroma is the relationship between the values of a* and b*, where the real color of the analyzed object is obtained. Hue is the angle formed between a* and b*, indicating the saturation of the object's color. To calculate Chroma (C), equation (1) was used, and to calculate Hue-Angle (H*) equation (2). Measurements were carried out in quadruplicate in the grains of rice at harvest.

$$C *= \sqrt{a^{*2} + b^{*2}} \quad (1)$$

$$H^* = arctg\,\frac{b^*}{a^*} \quad (2)$$

2.4. Statistical Analysis

Data were statistically analyzed using a One-Way or Two-Way ANOVA ($p \leq 0.05$), to assess differences among treatments. Based on the results, a Tukey's for mean comparison was performed, considering a 95% confidence level. Statistical analysis was performed with a IBM SPSS Statistics 20 program.

3. Results

3.1. Accumulation of Chemical Elements in Rice Grains

Foliar spraying with sodium selenate and sodium selenite promoted the accumulation of Se in the brown grains (Figure 1). Relatively to the control, the index of Se biofortification ranged between 1.8–4.7 fold and 5.4–6.0 fold after pulverization at 100 g $Se.ha^{-1}$ with selenate and selenite, respectively. Accordingly, the selenite form revealed a higher Se accumulation in rice crops.

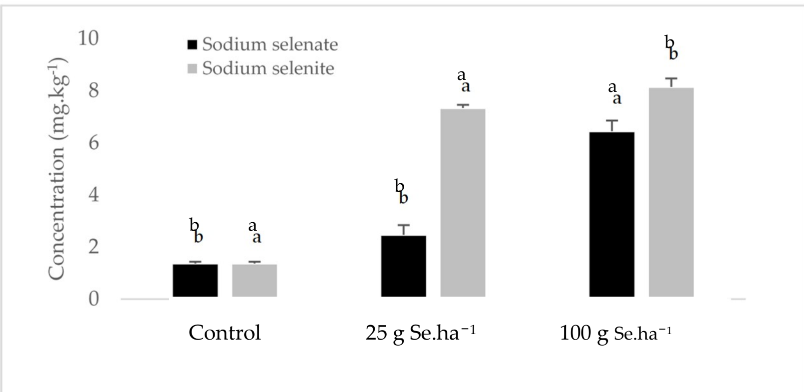

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Figure 1. Accumulation of Se in brown grains of O. sativa L. Poaceae, line OP1509 in the control and after foliar fertilization with selenate and selenite, at 25 and 100 g $Se.ha^{-1}$. Letters a and b indicate significant differences among treatments ($p \leq 0.05$).

Foliar fertilization with Se interfered with the accumulation of other chemical entities (Table 1). In brown grains, through the application of both fertilizers, the Zn contents increased with 25 g $Se.ha^{-1}$ but decreased with 100 g $Se.ha^{-1}$. This was also verified in the concentration of all the elements when selenite was applied (except Fe and also H and O that did not varied significantly). When selenate was applied only the contents of Ca increased significantly, whereas the other chemical elements did not varied significantly.

Table 1. Average values + S.D. (n = 4) of Zinc, Ca, Fe, K, P C, H and O contents in brown grains of O. sativa, line OP1509. Letters a, b, c indicate significant differences among treatments ($p \leq 0.05$).

Treatments ($g\ Se.ha^{-1}$)		Zn	Ca	Fe	K	P	C	H	O
		mg.kg-1							
Na_2SeO_4	0	28.6 ± 1.43a	116.1 ± 5.80a	22.4 ± 1.12a	0.54 ± 0.03a	0.57 ± 0.03a	43.7 ± 2.18a	6.11 ± 0.31a	48.6 ± 2.43a
	25	30.1 ± 1.50a	147.5 ± 7.38a	17.6 ± 0.88a	0.55 ± 0.03a	0.58 ± 0.03a	43.8 ± 2.19a	6.12 ± 0.31a	48.6 ± 2.43a
	100	26.1 ± 1.31a	175.1 ± 8.76b	18.1 ± 0.90a	0.64 ± 0.03a	0.66 ± 0.66a	43.7 ± 2.19a	6.12 ± 0.31a	48.6 ± 2.43a
Na_2SeO_3	0	28.6 ± 1.43a	116.1 ± 5.80c	22.4 ± 1.12b	0.54 ± 0.03b	0.57 ± 0.03b	43.7 ± 2.18a	6.11 ± 0.31a	48.6 ± 2.43a
	25	42.4 ± 2.12b	260.4 ± 13.02b	3.8 ± 1.74a	0.86 ± 0.04a	0.90 ± 0.04a	43.5 ± 2.17a	6.08 ± 0.30a	48.2 ± 2.41a
	100	23.3 ± 1.17a	150.5 ± 7.52a	13.3 ± 0.67c	0.50 ± 0.03b	0.53 ± 0.03b	43.8 ± 2.19a	6.13 ± 0.31a	48.7 ± 2.43a

3.2. Grain Weight and Colorimetry Analysis

The application of Se, in both forms, did not significantly affected grain weight in OP1509 (Table 2), however, in the paddy grains was found to be slightly higher with the application of sodium selenate.

Table 2. Average ± S. D. (n = 4) of 1000-grain weight of *O.* sativa, line OP1509, submitted to foliar application with sodium selenate and sodium selenite. Letter a indicates absence of significant differences between treatments ($p \leq 0.05$).

Treatments ($g\ Se.ha^{-1}$)		Paddy	Brown rice	White rice
			$g.1000\ g^{-1}$	
	Control	31.56±0.67a	26.98±0.36a	23.63±0.19a
Na_2SeO_4	25	31.53±0.61a	27.25±0.57a	23.44±0.40a
	100	32.18±1.71a	27.62±0.69a	23.86±0.44a
	Control	29.26±0.52a	25.86±0.63a	24.00±0.81a
Na_2SeO_3	25	30.21±0.51a	26.97±0.27a	24.39±0.14a
	100	30.66±1.38a	25.99±0.38a	23.66±0.17a

The colorimetric analysis of the paddy, brown and white grains, regarding to the control, did not show significant variations between treatments (Table 3). Parameters L* and H*, showed high values in white rice while parameter C* presented higher values in paddy rice.

Table 3. Colorimeter parameters of the paddy, brown and white flour of *O.* sativa, line OP1509. Letter a indicates the absence of significant differences between treatments ($p \leq 0.05$). Average values are expressed ± S.D. (n = 4).

	Treatments (g $Se.ha^{-1}$)		L*	C*	H*
Paddy	**Na_2SeO_4**	**0**	57.16 ± 0.90a	31.43 ± 0.59a	77.36 ± 0.66a
		25	57.91 ± 0.93a	31.07 ± 0.78a	78.15 ± 0.33a
		100	57.02 ± 0.99a	31.11 ± 0.48a	77.63 ± 0.38a
	Na_2SeO_3	**0**	57.71 ± 1.24a	30.49 ± 0.44a	77.54 ± 0.44a
		25	56.94 ± 0.89a	30.01 ± 0.28a	77.96 ± 0.32a
		100	57.78 ± 1.12a	31.30 ± 0.71a	77.09 ± 0.13a
Brown rice	**Na_2SeO_4**	**0**	70.31 ± 3.98a	20.66 ± 2.23a	84.16 ± 2.04a
		25	69.53 ± 3.97a	20.18 ± 2.39a	84.14 ± 1.68a
		100	69.70 ± 4.56a	20.46 ± 2.58a	84.19 ± 1.26a
	Na_2SeO_3	**0**	69.07 ± 3.57a	20.80 ± 1.69a	84.50 ± 1.29a
		25	68.13 ± 4.97a	20.90 ± 2.61a	83.65 ± 1.51a
		100	69.71 ± 4.14a	20.85 ± 1.94a	84.21 ± 1.39a
White rice	**Na_2SeO_4**	**0**	76.24 ± 0.10a	10.87 ± 0.68a	94.56 ± 1.11a
		25	75.86 ± 0.45a	9.65 ± 0.68a	95.24 ± 0.98a
		100	75.93 ± 0.63a	9.58 ± 0.64a	94.74 ± 0.97a
	Na_2SeO_3	**0**	74.38 ± 1.57a	10.40 ± 0.85a	94.46 ± 1.24a
		25	76.16 ± 0.38a	10.46 ± 0.95a	94.30 ± 1.29a
		100	76.06 ± 0.93a	10.48 ± 0.68a	93.65 ± 0.58a

4. Discussion

The response of rice plants to Se application has been reported previously [24]. Several cultivars exposed to similar doses showed differences in Se content [25], since its accumulation depends on the characteristics of the genotype, concentration and form of Se applied [15]. This study showed that for this genotype, both forms of the Se applied promoted biofortification (Figure 1). The average of Se contents in brown grains ranged between 1.8–4.7 and 5.4–6.0 fold with selenate and selenite, respectively. According to our findings, other studies also reported that, in rice foliar, application of sodium selenite is more effective than sodium selenate [26], because selenite is very mobile and easily absorbed by the plants [27].

Our data showed that 25g $Se.ha^{-1}$ promoted the accumulation of Zn in the grain, however, with a higher dose, the value decreased (Table 1). This trend also agrees with other studies reporting that there is no effect on Zn when 15 g $Se.ha^{-1}$ was applied [28], which suggests that these elements are metabolized and assimilated in different pathways [29]. As previously found [30], the application of selenate also promoted the accumulation of Ca in rice grains, whereas Fe and Zn did not varied with higher concentrations of this fertilizer. The highest grain weight was verified in paddy grains (Table 2) with selenate fertilization. Besides, the applied itinerary of biofortification produced higher brown grains, as verified in studies with application of selenite [15].

As our research team previously confirmed [18], after industrial processing, such as dehusking, whitening and milling, luminosity (L) and saturation (H) parameters increased (Table 3). Additionally, the highest values of real color (C*) were observed in the paddy rice, although there were no significant changes since the threshold of toxicity was not reached. Nevertheless, besides the genotype characteristics it is necessary to consider external factors that can influence the color of the grain [31].

5. Conclusions

Foliar spraying with selenate or selenite concentrations (25 and 100 g $Se.ha^{-1}$) did not surpass the threshold of toxicity in O. *sativa* L. Poaceae. The average of Se biofortification index was 1.8–4.7 times in selenate treatment and 5.4–6.0 times in selenite treatment. The contents of Ca, Fe, K and P varied according to the form and concentration applied however the C, H and O contents did not vary significantly. The application of both forms increased grain weight in the genotype and did not affect the colorimetric analysis. Accordingly, it is concluded that the applied itinerary applied for biofortification in rice (variety OP1509) can be implemented to minimize Se malnutrition.

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