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Selenium biofortification of rice grains and implications on macronutrients quality

Fernando Cebola Lidon [a, *], Karliana Oliveira [a, e], Maria Manuela Ribeiro [a], João Pelica [a], Inês Pataco [a], José Cochicho Ramalho [b], António Eduardo Leitão [b], Ana Sofia Almeida [c], Paula Scotti Campos [c], Ana I. Ribeiro-Barros [b], Isabel P. Pais [c], Maria Manuela Silva [d], Maria Fernanda Pessoa [a], Fernando Henrique Reboredo [a]

[a] GeoBioTec, Departamento de Ciências da Terra, Faculdade de Ciências e Tecnologia (FCT), Universidade Nova de Lisboa (UNL), Caparica, Portugal
[b] Plant Stress & Biodiversity Lab, Linking Landscape, Environment, Agriculture and Food (LEAF), Departamento de Recursos Naturais, Ambiente e Território (DRAT), Instituto Superior de Agronomia (ISA), Universidade de Lisboa (ULisboa), Oeiras, Portugal
[c] Unidade Estratégica de Investigação e Serviços de Biotecnologia e Recursos Genéticos, Instituto Nacional de Investigação Agrária e Veterinária, Oeiras Elvas, Portugal
[d] ESEAG, COFAC – Escola Superior de Educação Almeida Garrett, Rua de São Paulo, Lisboa, Portugal
[e] Bolsista da CAPES – Process 153113-1 – CAPES Fundação, Ministério da Educação do Brasil, Brasilia, Brazil

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ABSTRACT

Selenium is an essential element for human health but its intake is low. Accordingly, biofortified rice with this trace element can be prophylactic to consumers. In this context, this study aimed to develop an agronomic itinerary for rice biofortification with selenium, considering sodium selenite and selenate as foliar fertilizers. Since both forms of selenium fertilizers have different metabolic specificity among genotypes, the implications on sugars, fatty acids and proteins quality were also assessed. Biofortification was performed in field trials, in four target genotypes, applying both foliar fertilizers with concentrations ranging between 0 and 300 g Se ha^{-1}. It was found that biofortification with sodium selenite caused, relatively to sodium selenate, a higher accumulation of selenium in the grain. Application of high concentrations of sodium selenite and selenate increased total lipids in all the genotypes, mostly oleic acid (C18:1), linoleic (C18:2) and palmitic acid (C16:0). Sugars (with the concentration pattern being sucrose > glucose > raffinose > fructose) and proteins showed a similar trend. It is concluded that biofortification of crops with selenium is more effective with 120–300 g Se ha^{-1}, but macronutrients quality in the flour varies significantly within rice genotypes.

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1. Introduction

In past decades, breeding programs were mainly driven to increase productivity and yields. Accordingly, these programs were mostly focused, among other essential characteristics, in the selection of resistant varieties to diseases, considering plant height, biomass, and harvest index. Nevertheless, productivity increases are also essential to feed the growing world population, being the nutritional composition of food staples, especially micronutrient and protein quality also important.

In developing countries, a large percentage of the world population suffers from hidden hunger and has a daily intake largely focused in food cereals, being beneath the needs in trace elements and vitamins (Mayer et al., 2008). Moreover, agronomic biofortification of staple food, namely cereal crops, which might be attained through fertilizers application to soils and by foliar application, can understate nutrients deficiencies in human consumers. In this context, to overcome Se deficiency that affects about 15% of world population (White and Broadley, 2009), rice biofortification has a huge potential (Wang et al., 2013). Still, although this strategy can stimulate accumulation of Se in rice crops, adequate genotype characteristics are also required to enhance root uptake and optimize nutrients use efficiency (thus, to achieve maximum yields). Besides, among plant genotypes, differences in retention and concentration of metabolic compounds in

* Corresponding author.
E-mail address: fjl@fct.unl.pt (F.C. Lidon).

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biofortified crops can also inhibit or enhance nutrients bioavailability and bioassimilation to human consumers.

As a staple food, rice is an excellent source of energy, prevailing its consumption in over 30 countries, and providing about 80% of daily caloric intake to *ca.* 3 billion individuals (Lucca et al., 2006). Rice also represents about 21%, 14% and 2% of global energy, protein and fat meal, respectively (Kennedy and Burlingame, 2003). Still, the assessment by nutritionists to the degree of macronutrients accumulation in biofortified crops are also a required baseline to determine the absorption rates in the intestinal mucosa, using chemical models or by direct study in human consumers. Indeed, absorption is a prerequisite to demonstrate that biofortified crops can improve the micronutrient and macroelements status with long-term intake. Accordingly, this study aimed at developing an agronomic itinerary for selenium biofortification through foliar fertilization with sodium selenite and selenate in field trails and assesses macronutrients quality in the flour of four genotypes of rice (*Oryza sativa* L.).

2. Materials and methods

2.1. Experimental design

Field trails were carried out in Salvaterra de Magos - Ribatejo - Portugal (39.03 °N; -8.74 °W) for two consecutive years (2013 and 2014). Soil composition had 0.85–1.36% of organic matter, coarse texture, pH 5.6 and 61, 25 and 56 mg $Kg^{-1}{}_{Soil}$ of P_2O_5, K_2O and Mg, respectively. Considering these parameters, basal fertilization of field trails was performed with NPK - "20-8-10 (300 kg ha^{-1})" and sulfamide - 37% N (150 kg ha^{-1}). The conductivity of the irrigation water was 0.327–0.623 dS m^{-1}, with pH remaining between 6.53 and 6.55 and the contents of HCO_3, Na, K, Ca and Mg were 73.2–136.4, 65.7–125.4, 3.82–4.42, 16.7–43.1 and 6.40–18.12, respectively.

Four genotypes (Ariete, Albatros, OP1105 and OP1109) were sown in paddy/wetland fields, being the life cycle completed after about 4 months. Fields trails were flooded 15 days after plant emergence, being the water kept (20 cm depth) until the physiological maturity. Biofortification was carried out by foliar application with solutions of sodium selenite (Na_2O_3Se – 45% Se) and sodium selenate (Na_2O_4SE – 41%). In the first year of the experimental design, randomized blocks and a factorial arrangement (5 concentrations x 2 forms selenium x 4 varieties x 4 replicates = 160 plots) with a surface area of 2.9 m^2 (2.5 m × 1.2 m) was used. Foliar application with selenite and sodium selenate was performed 45–48, 85–88 and 102–105 days after germination (at booting, heading and during grain filling), with 0, 4, 20, 30–60 g Se ha^{-1}. In the second year, the agronomical itinerary was identical to that adopted in the first-year testing, occurred in the same location and a completely randomized experimental design and a factorial arrangement (4 concentrations x 2 forms Se x 4 varieties x 4 replicates = 128 plots; each plot having about 4 m^2 ≅2.68 m × 1.5 m) was also implemented. Nevertheless, considering that in the first year of the experimental design visual toxicity symptoms did not occur, to further increase the levels of Se in the grain, without reaching the threshold of toxicity, foliar biofortification considered the application of 0, 120, 180, 300 g Se ha^{-1}, also using sodium selenite and sodium selenate solutions.

2.2. Analytical proceduces

Following Galinha et al. (2013), the concentration of selenium in the grains was carried out by cyclical analysis through neutron activation, being samples irradiated with a neutron flux of 1.7×10^{12} cm^2 s^{-1}, in a fast pneumatic system. In powdered grains, X-ray analysis was also performed with a Niton Thermal Scientific, XLT, according to Pataco et al. (2017).

Lipids extraction was performed according to the method of Bligh and Dyer (1959), with minor modifications, being the methylated fatty acids analyzed by GC-FID (Varian CP-3380), with a DB-Wax capillary column (J&W Scientific, 30 m, 0.25 mm internal diameter, 0.25 μm film thickness), in a programmed column temperature rise (Scotti-Campos et al., 2014). Injector and detector were maintained at 200 °C and 250 °C, respectively. The carrier gas was hydrogen (flow rate of 1 mL min^{-1}, 1:50 flow partition). Fatty acids were identified using standards mixtures (Sigma, Supelco and Restek). The unsaturation degree was expressed through the Double Bond Index (DBI), calculated according to the formula: DBI = [(% monoenes + sing 2 × % dienes + 3 × % trienes)/% saturated fatty acids], as described by Medicott and Thompson (1985).

Soluble sugars were cold extracted according to Medicott and Thompson (1985) and analyzed on HPLC - High-Performance Liquid Chromatography (Waters, USA), coupled to a refractometric detector (Waters 2414), equipped with a SugarPak 1 column (Waters 6.5 × 300 mm). For identification and quantification, standard curves were constructed for rafinose, sucrose, glucose and fructose.

Protein was determined through quantitation of total nitrogen by the Kjeldahl method (NP, 1996. 2000), considering that all nitrogen integrates the aminoacids structure of proteins.

Data were statistically analyzed using a Two-Way ANOVA ($p \leq 0,05$), to assess differences between treatments and experimental periods and, based on the results, a Tukey's for mean comparison was performed, considering a 95% confidence level.

3. Results

Using sodium selenite and selenate, until fertilization with 300 g Se ha^{-1}, all genotypes (except OP1109 with 180 g Se ha^{-1} and using selenite as fertilizer), showed a progressive increase of Se contents in biofortified grains (Fig. 1A and B). Applying sodium selenite as fertilizer, from the control until 300 g Se ha^{-1}, 427, 884, 703 and 617-fold increases were found in Ariete, Albatros, OP1105 and OP1109, respectively. Moreover, fertilization with sodium selenate caused 205, 347, 277 and 128-fold increases in Ariete, Albatros, OP1105 and OP1109, respectively. Accordingly, independently of the chemical form of the fertilizer, between the control and 300 g Se ha^{-1}, Albatros and OP1105 revealed the highest increase of Se.

In Albatros, foliar application of sodium selenite and selenate increased total lipids contents (Fig. 2A and B). In Ariete, after application of both fertilizers, relatively to the control, from 120 g Se ha^{-1} onwards, total lipids also increased significantly. In OP1105 total lipids only increased after application of sodium selenite (60 g Se ha^{-1}).

In all genotypes, within each treatment (Table 1), after fertilization with sodium selenite the lowest values of stearic acid (C18:0) occurred with 30 g Se ha^{-1} (except Albatros). Relatively to oleic acid (C18:1), the control (except OP1105 and OP1109 with foliar application of sodium selenate and Ariete after application of sodium selenite) revealed the lowest contents among genotypes (Table 1). Concerning to linoleic acid (C18:2), only Ariete (through application of sodium selenite), OP1105 (using selenate as fertilizer) and OP1109 (applying both selenium fertilizers), did not reveal the lowest values in the control (Table 1). Minimum concentrations of linolenic acid (C18:3) occurred in the control (sodium selenite block) excepting in OP1109, but in all the remaining treatments was detected in the 60 g Se ha^{-1} (except OP1105, applying sodium selenate).

After foliar fertilization with sodium selenite, the lowest values of the Double Bound Index - DBI (Table 1) occurred after application

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