Journal of Cereal Science 79 (2018) 508–515

Contents lists available at ScienceDirect

Journal of Cereal Science

journal homepage: www.elsevier.com/locate/jcs

Agronomic biofortification of upland rice with selenium and nitrogen and its relation to grain quality

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ARTICLE INFO

Article history:

Keywords:
Reserve proteins
Physiological responses
Human health
Sodium selenate

ABSTRACT

The objective of the present study was to evaluate agronomic biofortification with nitrogen (N) and selenium (Se) with the aim of increasing the daily Se intake by the population and the nutritional quality of the grain. A randomized block experimental design with a 5 × 2 factorial scheme was used; the factors were the five levels of N (0, 20, 40, 80, and 120 kg ha^{-1}), applied as topdressing fertilization, and the two levels of Se (0 and 25 g ha^{-1}). The reserve protein fractions in the seeds, glutelin, and globulin increased significantly when Se application was combined with N fertilization. Grain Se content varied from 0.03 to 0.35 mg kg^{-1}, which was within the food safety limit of 0.3 mg kg^{-1} established by the *Codex Alimentarius*. The estimated daily Se intake originating from Se-biofortified rice varied between 2.05 and 24.7 μg per day, representing an increase from 3.72% to 44.9% of the daily Se requirement. Because the recommended Se daily intake for adults is 55 μg per day, the present study presents relevant information about agronomic biofortification to increase Se concentrations in edible plant parts, with possible benefits to human health.

1. Introduction

Food is the main nutrient source, and its demand increases with the increase in population. The current world's population is 7.2 billion inhabitants and is estimated to reach 12.3 billion in 2100 (Gerland et al., 2014). Cereal production has been growing at the same rate as the population. Ray et al. (2013) estimated that food production should increase between 60 and 110% until 2050 to meet the growing global demand. In turn, malnutrition has been increasing, affecting half of the world's population, especially pregnant women, adolescents, and children (Graham et al., 2007). This includes selenium (Se) deficiency in humans, which is increasingly more frequent (Reis et al., 2017).

Combs (2011) estimated that approximately 1 billion people are likely deficient in Se. In humans, Se deficiency is associated with hypothyroidism, cardiovascular diseases, fragile immune system, male infertility, and an incidence of several types of cancer (Fordyce, 2013). The recommended Se intake is 55 μg per day for adults (White, 2016). Considering that dietary Se is basically derived from food, Se deficiency in humans is related to the consumption of foods with low Se content in the edible parts (Reis et al., 2017). This is due to the low soil Se available for plant uptake (White, 2016).

Se may increase growth and improve the nutritional status of vascular plants (Graham et al., 2007). The role of Se in grain formation is still little understood, but Seregina et al. (2001) showed an interaction effect between Se and N on increased wheat productivity. The authors also observed stronger positive effects of Se on wheat grain formation when combined with higher N levels. In addition to its nutritional role, Se also plays an important part in plant antioxidant protection. Depending on the dose, Se may activate enzymes such as superoxide dismutase (SOD) and catalase (CAT). These enzymes are activated in the presence of Se, decreasing the lipid peroxidation and hydrogen peroxide formation

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https://doi.org/10.1016/j.jcs.2018.01.004
0733-5210/

rates in plant cells and resulting in reduced senescence (Saidi et al., 2014).

According to the results of decades-long monitoring of biofortification programs in Northern European countries, such as Finland, currently, the addition of Se to the NPK fertilizers used in agricultural areas seems to be the most effective and safest way to avoid Se deficiency in humans and animals (Haug et al., 2007). The authors also state that after these programs were established, mortality indexes greatly decreased, especially those related to heart diseases, cancer, and incidence of vitamin E deficiency.

Attempts to increase the Se content in edible plant parts may change the crop metabolism and nutritional status because Se interferes with the sulfur metabolism, and such an increase probably affects the whole nitrogen (N) metabolism and grain yield (Ríos et al., 2010; White, 2016). Se addition via fertilizers is involved in the quality improvement of agricultural products. Agronomic biofortification with Se increases the selenocysteine and selenomethionine contents, which are essential for human and animal health (Rayman et al., 2012). Studies are therefore needed to evaluate the interactions between the N and Se levels and facilitate a better understanding of Se accumulation in rice grains, as rice is one of the planet's main food sources. The objective of the present study was to evaluate the potential of agronomic biofortification with Se, used in combination with N topdressing fertilization of upland rice, under field conditions, with the aim of increasing daily Se intake by the Brazilian population, which may result in improved human health.

2. Materials and methods

2.1. Description of the experimental site

The study was performed between January and May 2015 in Selvíria, Mato Grosso do Sul, Brazil (20°22′S and 51°22′W, 335 m altitude). The experimental site belongs to the Cerrado biome, has been cultivated for more than 25 years, and has been under no-tillage for the last 10 years. According to the Köppen climate classification, the region's climate is Aw, tropical humid, with a rainy season in the summer and dry season in the winter. The mean annual rainfall is 1232 mm, and the mean annual temperature is 24.5 °C. During the experiment, the mean daily temperature varied between 27.2 °C and 15.3 °C, the mean rainfall was 3.0 mm, and the mean relative humidity was 86%.

The soil in the area was classified as Oxisol. The soil analysis revealed the following chemical characteristics: phosphorus (resin) 29 mg dm^{-3}; organic mater 21 g dm^{-3}; pH calcium chloride ($CaCl_2$) 5.3; potassium 3.5 $mmol_c$ dm^{-3}; calcium 38 $mmol_c$ dm^{-3}; magnesium 22 $mmol_c$ dm^{-3}; $H^+ + Al$ $mmol_c$ dm^{-3}; aluminum 0 $mmol_c$ dm^{-3}; Se 62 μg kg^{-1}; cation exchange capacity 92.5 $mmol_c$ dm^{-3}; and base saturation (V%) - 69%.

A randomized block experimental design was used, with a 5 × 2 factorial scheme, with four replicates, totaling 40 plots. The factors were five levels of N (0, 20, 40, 80, and 120 kg ha^{-1}), applied as urea, and there were two levels of Se (0 and 25 g ha^{-1}), applied as sodium selenate. A Se stock solution was prepared, diluted in 500-mL bottles, and distributed over the planting furrow. The Se dose applied (25 g ha^{-1}) was selected based on preliminary results (Reis, 2015).

2.2. Experimental setup

The experiment was performed under a no-tillage system. Soil acidity was not corrected because the base saturation was adequate for upland rice. Twenty days before sowing, the area was desiccated with glyphosate (4.0 L ha^{-1}), carfentrazone-ethyl (200 mL ha^{-1}), and 0.5% mineral oil.

The upland rice cultivar used was ANa 5015, sown at a density of approximately 70 kg ha^{-1}. The experimental plots consisted of four 6-m-long rows, spaced 0.35 m apart. The useful area consisted of the two central rows, excluding 0.5 m of the row ends. Concurrently, seeds were treated with fipronil at 2 mL c.p. (commercial product) kg^{-1} seeds. Fertilization was performed at sowing, with the application of 250 kg ha^{-1} 08-28-16 NPK. Irrigation was performed as needed, using a center-pivot sprinkler irrigation system, with a mean water depth of 14 mm, and 72-h irrigation frequency. Plant health management during the crop cycle was performed with the application of metsulfuron-methyl (3.3 g ha^{-1}), chlorantraniliprole (50 mL ha^{-1}), flubendiamide (60 mL ha^{-1}), and imidacloprid + beta-cyfluthrin (0.8 L ha^{-1}).

2.3. Chlorophyll index

Fourteen days after the treatment application, indirect chlorophyll measurements were performed in five plants from each plot, on three different points of the flag leaf, using a portable chlorophyll meter (SPAD-502, MINOLTA).

2.4. Antioxidant system

Twenty days after the treatment application, the flag leaves from 15 plants from each plot were collected, placed in liquid nitrogen, and used for quantification of the hydrogen peroxide content, lipid peroxidation, soluble protein content, and SOD and CAT activity. The plant material was ground in liquid N and stored at −80 °C until analyzed.

2.4.1. Hydrogen peroxide content

Hydrogen peroxide (H_2O_2) content was determined after reaction of the plant material with potassium iodide (KI), according to Alexieva et al. (2001). Leaf hydrogen peroxide contents were calculated based on a standard curve, and the results were expressed in nmol g^{-1} FW (fresh weight).

2.4.2. Lipid peroxidation

Lipid peroxidation was determined by the production of 2-thiobarbituric acid (TBA)-reactive substances, especially malondialdehyde, according to Heath and Packer (1968).

2.4.3. Protein and antioxidant enzyme extraction

The plant material stored at −80 °C was ground in liquid nitrogen with a mortar and pestle. For protein extraction, approximately 0.3 g processed plant material was placed into 15-mL Falcon tubes and extracted with 5 mL of 100 mM potassium phosphate buffer, pH 6.8, with 1 mM ethylenediaminetetraacetic acid (EDTA), 3 mM dithiothreitol (DTT), and 4% polyvinylpolypyrrolidone (PVPP) (w/v). The supernatant was transferred to Eppendorf tubes and used for protein and antioxidant enzyme activity quantification.

2.4.4. Reserve protein determination

For extraction of the reserve proteins, 0.25 g of dry and ground grain was weighed and subjected to sequential extraction with 5 mL of deionized water (for albumin), 5 mL of 5% NaCl (for globulin), 2.5 mL of 60% ethanol (for prolamin), and 5 mL of 0.4% NaOH (for glutelin). Protein concentration was determined according to Bradford (1976), using BSA as the standard.

2.4.5. Superoxide dismutase (SOD, EC 1.15.1.1)

SOD activity was determined according to Giannopolitis and Ries (1977).