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Efficiency of zinc biofortification in rice grains

Efeito da biofortificação de zinco em grãos de arroz

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ABSTRACT

The objective of this study was to evaluate the efficiency of biofortification in rice. The samples analyzed went through three treatments: A (control – no biofortification), B (in soil fertilization with $ZnSO_4.7H_2O$), and C (two foliar fertilization with $ZnSO_4.7H_2O$). The atomic absorption spectroscopy technique was used to find the Zn content in each treatment in different parts of the grain. The biofortification did not result in alteration in the mineral concentration in the endosperm, which is the portion of the grain consumed by humans. Additionally, it was observed that elevated Zn concentrations, generally, are found in the bran alongside the germ. The treatment C, which consisted of two foliar applications, presented higher mineral concentration in the grain coat. Therefore, in order for a good biofortification performance, it is indicated the consumption of the grains in their whole form.

Keywords: agronomic biofortification, *Oryza sativa*, zinc content.

RESUMO

O objetivo deste estudo foi avaliar a eficiência da biofortificação em arroz. As amostras analisadas passaram por três tratamentos: A (controle – sem biofortificação), B (adubação do solo com $ZnSO_4.7H_2O$) e C (duas adubações foliares com $ZnSO_4.7H_2O$). A técnica de espectroscopia de absorção atômica foi utilizada para encontrar o teor de Zn em cada tratamento nas diferentes partes do grão. Os resultados mostraram que a biofortificação não alterou significativamente a concentração mineral no endosperma, que é a parte do grão consumida pelo homem. Além disso, observou-se que maiores concentrações de Zn, geralmente, são encontradas no farelo junto ao germe. O tratamento C, que consistiu em duas aplicações foliares, apresentou maior concentração de minerais na casca. Assim, para um bom desempenho da biofortificação, indica-se o consumo dos grãos na sua forma integral.

Palavras-chave: biofortificação agronômica, *Oryza sativa*, teor de zinco.

INTRODUCTION

Rice (*Oryza sativa*) is a highly consumed food, predominantly cultivated in Asian countries, and contributes with up to 70% of the daily caloric intake of half the world´s population. It is a cereal with low micronutrient content, as that that is often removed during grain processing. Rice can be an alternative for biofortification and a sustainable solution for the "hidden hunger" since it can help to reduce the deficiency of essential micronutrients in the organism of the global population (Majumder et al., 2019, Senguttuvel et al., 2023, Roda et al., 2020, Prom-U-Thai et al., 2020).

The goal of biofortification is to enhance the nutrition value of food in the field during production. This can be performed in two different ways. Genetic biofortification entails the food improvement through the genetic modification of the cultures, whether transgenic or conventional. Meanwhile, the agronomic biofortification is performed through the management of the cultures (Vergütz et al., 2016) during the fertilization of leaves (foliar) or of the soil.

The issue of malnutrition due to zinc (Zn) deficiency is a growing concern. It can affect a large part of the world population, particularly in the developing countries. In these countries, the elderly, young women, children, and individuals with a restricted diet are susceptible to developing Zinc deficiency (Mello and Coelho, 2011). A deficiency of this mineral results in a weak immune system and stunted growth (Ryu and Aydemir, 2020).

It is essential to include external zinc sources in the diet, as the human body is unable to synthesize this mineral. Zn is found in significant amounts in eggs, whole wheat flour, beef, nuts, seafood, fish, beans, poultry, milk and other dairy products. Zn is essential to the functioning of multiple enzymes, so it is important to biological processes in humans, plants, and animals (Empresa de Pesquisa Agropecuária de Minas Gerais, 2016). In humans, Zn plays a pivotal role in the enzymatic activation and composition, in the mitigation of the effects of active oxygen, and in the protein synthesis (Cakmak et al., 2008).

The results of the studies of Prom-U-Thai et al. (2020), conducted in 21 field locations across five major rice-producing countries (Brazil, China, India, Pakistan, and Thailand) over two crops cycles, showed that the foliar application of a micronutrient cocktail solution, or the use of Zn in the form of $ZnSO_4.7H_2O$, was highly effective in increasing the concentration of Zn in whole rice grains. The aforementioned study shows the efficiency and importance of the biofortification.

The objective of this study was to evaluate the efficiency of agronomic biofortification in rice grains and to verify the Zinc concentration in the different parts of the grain, in particular the peel, bran and germ, and endosperm of whole grains.

MATERIAL AND METHODS

Description of the samples

This work used biofortified samples of rice, variety BRSMG Caravera, in the third phase of the HarvestPlus programs cultivated by EPAMIG (Empresa de Pesquisa Agropecuária de Minas Gerais), in the city of Lambari, Minas Gerais, Brazil.

The city of Lambari is located at an altitude of 887 meters, and has the following geographic coordinates: Latitude 21° 58' South and Longitude 45° 20' West. Throughout the year, in general, the temperature varies from 10 °C to 28 °C. The climate is humid subtropical.

The samples studied include a control treatment, which was not subjected to biofortification, and two distinct biofortification treatments.

The three treatments are as follows:

A. Control (NPK) – The application of fertilizer doses in the soil consisted nitrogen (N), phosphorus (P), and potassium (K).

B. NPK + application in the soil of $ZnSO_4.7H_2O$ 50 kg of $ZnSO_4$.7H2O per hectare. Soil fertilization was done at planting.

C. NPK + two foliar applications of $ZnSO_4$: 0,5% of $ZnSO_4.7H_2O$ in 800 liters per hectare. Foliar fertilization was done 50 days after germination.

Experimental design

The cultivation of the plants was conducted using a Random Block Design, which included the analysis of two treatments (Treatments B and C, as previously described) and control (Treatment A), with four repetitions each. In each sample, the portions (peel, bran and germ, and endosperm) were analyzed individually.

The separation of the grain parts was carried out on a Suzuki testing device. 400g of paddy rice was processed from each plot. Samples were husked using the equipment's husking rollers, the procedure of passing the rice samples through the rollers was repeated three times. Then, husked rice grains were manually separated from rice with husk (rice grains that remained unhusked, even after passing through the husking rollers three times). Next, husked rice grains were burnished for one minute, in this way, the bran together with the germ was separated from rice grains.

Zn analysis

The analysis of Zn was conducted on the samples through atomic absorption spectroscopy, using the methodology described by Malavolta et al. (1997).

Statistical analysis

The analysis of variance through F-Test was performed. The means obtained from the treatments were compared and analyzed using the Scott-Knott test ($p<0,05$). The software Sisvar version 5.6 was used (Ferreira, 2014).

RESULTS AND DISCUSSION

Table 1 presents the zinc (Zn) values (mg kg^{-1}) for each treatment and for each part of the rice grain.

The concentration of minerals present varies depending on the specific portion of the grain. In general, the mineral content is influenced by the cultivation conditions (soil and fertilization conditions) as well as by the processing. Tipically, the minerals are present in greater quantities in the external layers of the grain, with values close to 72% in the bran and 28% in the polished grain (Juliano and Bechtel, 1985 cited by Walter et al., 2008). Moreover, grain processing causes changes in the chemical compounds. The process of rice polishing has been demonstrated ro result in a reduction in Zinc content (Hensawang et al., 2020).

It was observed no significant effect of the F-Test on the Zn content present in the endosperm for each treatment studied. Every sample obtained values in close proximity, with an average value of Zn content of 25.27 mg kg^{-1}. The results demonstrate that the biofortification process did not change the Zn content in the endosperm of the raw polished rice grains.

According to the TACO table (Universidade de Campinas, 2011), the Zn content in samples of raw polished rice type 2 is 13 mg kg^{-1}.This valeu is considerable higher than that observed in the present study. According to Juliano; Bechtel (1985) cited by Walter et al. (2008), there is a notable range in Zn concetrations among the genotypes of polished white rice, with values ranging from 6 mg kg^{-1} and 23 mg kg^{-1}. Thus, it is possible to verify that the results found are close to this value range once this variation might be caused by a higher concentration of Zn in the soil.

In the study conducted by Prom-U-Thai et al. (2020), the mean Zn concentration in whole rice with foliar biofortification was 28,1 mg kg^{-1}. This value is higher than that observed in the present study due to the inclusion of a non-polished rice grain with a presumed elevated Zn concentration.

The sample of treatment B showed higher Zn concentration, with 159.93 mg kg^{-1} (Bran + germ), followed by treatment C with a concentration of 138.86 mg kg^{-1}(Bran + germ).

Lacerda et al. (2010) analyzed the quality of raw rice and identified an average Zn content of 61.8 mg kg^{-1} in their samples. Therefore, the values obtained in this study, even in the control sample (82,5 mg kg^{-1}), are above the values reported in the literature.

According to Kong et al. (2022), Zn accumulates predominantly in the aleurone layer of rice. Nevertheless, few factors or critical genes controlling the translocation of Zn from the aleurone layer to the endosperm were identified. The identification of these factors or genes may

Table 1. Zinc (Zn) values (mg kg^{-1}) for each treatment and for each part of the rice grain.

Treatments	A = Control	B = soil biofortification	C= foliar biofortification
	Zinc content (mg kg^{-1})		
Endosperm	25.31 a	24.71 a	25.79 a
Bran + germ	82.50 c	159.93 a	138.86 b
Peel	45.45 b	50.07 b	204.63 a

Means followed by the same letter (row) belong to the same group. A = Control (NPK) – The application of fertilizer doses in the soil consisted nitrogen (N), phosphorus (P), and potassium (K). B = NPK + application in the soil of $ZnSO_4.7H_2O$ 50 kg of $ZnSO_4.7H_2O$ per hectare. C = NPK + two foliar applications of $ZnSO_4$: 0,5% of $ZnSO_4.7H_2O$ in 800 liters per hectare.

greatly facilitate the process of rice biofortification, which would result in a reduction of Zn content in the germ and bran, which were found to be higher in this study.

The study of Prom-U-Thai et al. (2020) revealed a positive impact resulting from the foliar application of Zn in the grain. The results of the previous study confirm that whole rice (only peeled) is the optimal food source for zinc intake, as the grain´s bran and in the germ contain the highest concentrations of this mineral, particularly when the application occurs in the soil.

Only treatment C stood out from the control sample with a high Zn concentration of 204.63 mg kg^{-1}. The control sample and the treatment B sample exhibited Zn concentrations of 45.45 mg kg^{-1} and 50.07 mg kg^{-1}, respectively. These concentrations are considerably lower that those observed in the sample subjected to foliar biofortification.

According to Dors et al. (2009) the ash content, which represents the total amount of minerals in a sample, is higher for the peel plus bran fraction (14.5%) when compared to the endosperm fraction (0.7%) of the rice grain. It can be stated, according to the data from the present work, that the zinc content is higher in the external fractions of the rice grain (germ, bran and peel).

For Mangueze et al. (2018) zinc accumulates mainly in the outer layers of the grain and not in the endosperm, as reported in the present study. The increase in Zn concentration of whole wheat grains and their fractions is pronounced when Zn is sprayed at the final growth stage (Cakmak et al., 2010). Future studies should evaluate whether the moment of plant development, when the mineral is applied, affects the concentration of Zn in different parts of the rice.

The results of Zn content in the peel of the control and biofortified treatments suggest that foliar biofortification may result in concentration of Zn content in the peel. However, the same did not happen to the sample with soil biofortification.

It is recommended the consumption of biofortified whole rice, that is, without polishing. This is particularly relevant in the case of treatment B, which obtained high Zn values in the bran + germ samples.

Furthermore, the consumption of biofortified and parboiled rice is also interesting since parboiling consists in the immersion of rice (with peel) in water heated in an autoclave. This process facilitates the absorption, in the endosperm, of vitamins and minerals, such as Zn, retained in the peel, bran, and germ. It is recommended that parboiling be employed, in particular, for Treatment C (foliar biofortification) due to the high Zn content in the bran + germ and peel samples.

CONCLUSION

The agronomic biofortification did not result in a change in the Zn content of the endosperm of rice grains. The bran + germ samples with Zn application in the soil and in the leaves (foliar) were different from the control sample. The soil biofortification resulted in a higher concentration of Zn. In the peels, only the treatment with two foliar applications of $ZnSO_4.7H_2O$ showed a high concentration.

Thus, for better use of the Zn content through biofortification, it is recommended that the grains be consumed in the whole form (endosperm + bran + germ).

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