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**UNIVERSIDADE ESTADUAL PAULISTA (UNESP)
FACULDADE DE CIÊNCIAS AGRÁRIAS E VETERINÁRIAS
CÂMPUS DE JABOTICABAL**

**SELENIUM ABSORPTION KINETICS AND LOCALIZATION
IN COWPEA GENOTYPES USING XRF TECHNIQUE
ASSOCIATED WITH PHYSIOLOGICAL AND BIOCHEMICAL
RESPONSES**

Maria Gabriela Dantas Bereta Lanza

Biosystems Engineering

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TÍTULO DA DISSERTAÇÃO: SELENIUM ABSORPTION KINETICS AND LOCALIZATION IN COWPEA GENOTYPES USING XRF TECHNIQUE ASSOCIATED WITH PHYSIOLOGICAL AND BIOCHEMICAL RESPONSES

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“Nothing in life is to be feared, it is only to be understood. Now is the time to understand more, so that we may fear less.”

- Marie Curie,
Physicist, chemist, and winner of the 1903 and 1911 Nobel Prize in Physics

DEDICO

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ANALYSIS OF SELENIUM ABSORPTION KINETICS AND LOCALIZATION IN COWPEA GENOTYPES USING THE XRF TECHNIQUE ASSOCIATED WITH PHYSIOLOGICAL AND BIOCHEMICAL RESPONSES

RESUMO – O selênio (Se) é um elemento essencial para humanos e animais e benéfico para as plantas. Os solos são pobres em Se, tornando a dieta da população deficiente. A biofortificação agronômica é uma estratégia para aumentar os teores de Se nas plantas. Nesse trabalho, avaliou-se a eficiência da suplementação mineral dependente de fatores genotípicos e da fonte adequada de Se. Foram conduzidos 3 experimentos, sendo 2 experimentos preliminares, e 1 experimento crítico. No experimento 1 avaliou-se concentrações de Na_2SeO_4 (0, 20, 40, 80 e 120 μM) em feijão-caupi (*Vigna unguiculata* [L.] Walp cv. BRS Rouxinol), a fim de determinar a condição com melhor detecção de sinal para a avaliação *in vivo* das plantas pela técnica de fluorescência de raios x (XRF). No experimento 2, a melhor concentração resposta (40 μM) foi utilizada para avaliar a eficiência de absorção de Se de 20 genótipos comerciais de feijão-caupi, por meio da concentração de Se na parte aérea e raiz pela técnica de fluorescência de raios-X dispersiva de energia de bancada (EDXRF), afim de determinar genótipos com capacidade de absorção contrastante. No experimento 3, selecionou-se o genótipo com maior (BRS Xiquexique) e menor (BRS Urubuquara) capacidade de absorção de Se avaliando as respostas fisiológicas da aplicação de duas fontes de Se (Na_2SeO_4 e Na_2SeO_3), afim de identificar a fonte mais eficiente para a absorção e translocação de Se para a parte aérea das plantas, visando a geração de plantas com maior valor nutricional. Os resultados demonstram a presença de variação genotípica na cultura do feijão-caupi, e relaciona a capacidade de plantas eficientes em translocar Se das raízes para a parte aérea com uma alta eficiência do metabolismo fotossintético. Além disso, a fonte Na_2SeO_4 demonstrou ser uma ótima alternativa para garantir a translocação de Se raiz-parté aérea de plantas de feijão-caupi.

Palavras-chave: biofortificação agronômica, fluorescência de raios x, selenato, selenito, variação genotípica, *Vigna unguiculata*,

Selenium absorption kinetics and localization in cowpea genotypes using XRF technique associated with physiological and biochemical responses

ABSTRACT – Selenium (Se) is an essential element for humans and animals and is beneficial to plants. Soils are poor in Se, making the population's diet deficient. Agronomic biofortification is a strategy to increase Se levels in plants. In this work, we investigated the efficiency of mineral supplementation dependent on genotypic factors and the adequate source of Se. Three experiments were conducted, two preliminary experiments and one critical experiment. In experiment 1, concentrations of Na₂SeO₄ (0, 20, 40, 80, and 120 µM) in cowpea plants (*Vigna unguiculata* [L.] Walp cv. BRS Rouxinol), were evaluated to determine the best concentration-response for the in vivo evaluation of plants using the X-ray fluorescence (XRF) technique. In experiment 2, the best concentration-response (40 µM) was used to evaluate the Se absorption efficiency of 20 commercial cowpea genotypes, using Se concentration in shoots and roots by the X-ray fluorescence technique bench energy dispersive (EDXRF) to determine genotypes with contrasting absorption capacity. In experiment 3, the genotype with the highest (BRS Xiquexique) and lowest (BRS Urubuquara) Se absorption capacity was selected. Moreover, evaluating the physiological responses of the application of two Se sources (Na₂SeO₄ and Na₂SeO₃), to identify the most efficient source for the absorption and translocation of Se to the shoot of the plants, aiming at the generation of plants with greater nutritional value. Our results demonstrate the presence of genotypic variation in the cowpea crop and relate the ability of efficient plants to translocate Se from roots to shoots with the high efficiency of photosynthetic metabolism. Furthermore, the Na₂SeO₄ source proved to be an excellent alternative to ensure the root-shoot Se translocation in cowpea plants.

Keywords: Agronomic biofortification, x-ray fluorescence, selenate, selenite, genotypic variation, *Vigna unguiculata*.

CHAPTER 1 – Roles of selenium in mineral plant nutrition: physiological and biochemical responses

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ABSTRACT

Agronomic biofortification of crops with selenium (Se) is an important strategy to minimize hidden hunger and increase nutrient intake in poor populations. Se is a nutrient that has several physiological and biochemical characteristics, such as the mitigation of different types of abiotic stress. Selenoproteins act as powerful antioxidants in plant metabolism through the glutathione peroxidase (GSH) pathway, and provide an increased activity for enzymatic (SOD, CAT, and APX) and non-enzymatic (ascorbic acid, flavonoids, and tocopherols) compounds which combat reactive oxygen species (ROS) and cell detoxification. Se can mitigate the damage caused by climate changes such as drought, salinity, heavy metals, and temperature. Also, it is related to the regulation of the antenna complex of photosynthesis, protecting chlorophylls by raising photosynthetic pigments. However, Se concentrations in soils vary widely in the earth's crust. Soil availability influences the concentrations in food, in addition to the capture, transport, accumulation, and speciation of Se in plants. The foliar application of Se until the concentration of 50 g ha^{-1} in the sodium selenate source increases the antioxidant, photosynthetic metabolism, and productivity of the cultures. The foliar application of Se is a strategy to minimize soil adsorption and root accumulation. However, the limit between the beneficial and toxic effects of Se requires research to establish an optimal concentration for each plant species in different edaphoclimatic conditions. In this review, we present the compilation of several studies on agronomic biofortification of plants with Se to ensure food production and food security to mitigate hidden hunger and improve the health of the population.

Keywords: abiotic stress, antioxidant metabolism, food security, biofortification, hidden hunger, selenium.

1. INTRODUCTION

According to FAO (2017), there are about 2 billion people with nutritional deficiencies in the world. The nutritional deficit is caused by the production of staple foods in soils with low mineral availability. The deficiencies caused by the lack of iron (Fe), iodine (I), selenium (Se), zinc (Zn), and vitamin A are currently the ones that cause the greatest concern regarding human health, especially in developing countries (White, 2016).

Eating vegetables rich in Se can be a safe way to combat Se deficiency in humans, however, most soils are considered poor in Se, and nutrient absorption depends on several factors that reduce their incorporation. (Yang et al., 2019). Agronomic biofortification is an agricultural practice used to enrich food production with nutrients, such as Se, to increase food intake by the population (Adebayo et al., 2020).

The application of exogenous Se can increase the concentration of Se in the edible parts of plants (Oliveira et al., 2019). Se is beneficial for crop productivity, promoting increased photosynthesis by increasing secondary metabolites and accumulating carbohydrates (Andrade et al., 2018). Also, their synergistic and antagonistic relationships with other nutrients favor plant nutrition and mitigate abiotic stresses such as drought, high temperatures, salinity, and heavy metals (Gupta and Gupta, 2017).

According to Hartikainen et al. (2000), selenium has a wide antioxidant capacity due to the promotion of selenoproteins such as glutathione peroxides, which act to combat reactive oxygen species (ROS) promoted in plant osmotic imbalance under stressful situations. It may be an osmoprotective strategy to mitigate the harmful effects of abiotic stresses such as drought (Rady et al., 2020), salinity (Habibi, 2017), heavy metals (Shekari et al., 2019), temperature (Seliem et al., 2020) and light (Jaiswal et al., 2018).

Excess Se can be toxic to plants and humans (Gouveia et al., 2020; Martens et al., 2015). Characterized by the presence of chlorosis in the leaves of vegetables, and hormonal changes in the human body (Vinceti et al., 2018; Lanza et al., 2021). The determination of ideal doses and sources of Se is extremely important to ensure the food security of the population through the consumption of legumes (Silva et al., 2018),

cereals (Lara et al., 2019), fruits (Deng et al., 2019), vegetables (Tian et al., 2018), and even medicinal plants used as herbal medicines (Stonehouse et al., 2020).

Thus, the present literature review seeks to present the most recent discoveries about Se, analyzing its reactions in different living organisms and their interactions with the abiotic environment. Demonstrating that the element is widely explored as a strategy to increase the metabolism of plants, but that it also has several opportunities and challenges to be unveiled.

5. CONCLUSION

The results show that i) the application of one μM of Na_2SeO_4 was more efficient in assimilation, translocation, and concentration of Se in shoots compared to the performance of plants treated with Na_2SeO_3 . Thus, the use of selenate proves to be a source with a better use for use in mineral supplementation of crops, and the objective of these programs is to increase the Se content in the edible parts of plants. Se sources that are trapped in plant roots can be an inefficient and cost-effective way of crop nutrition. Furthermore, it was found that ii) plants treated with selenate increase the levels of Chlorophylls, Gs, E, Ca, and K, demonstrating a strong correlation with stomatal opening, osmotic adjustment, and greater uptake of solar energy, which is one of the indispensable factors for increased productivity. On the other hand, plants supplemented with selenite tend to modulate their metabolism to increase accessory pigments (pheophytin and carotenoids), which are associated with protecting plants against intracellular damage. Finally, the use of iii) genotypes with high (BRS Xiquexique) and low (BRS Urubuquara) ability to assimilate Se demonstrated that the two Se sources have the same behavior about the evaluation of the absorption efficiency of the element. And that the union between more responsive genotypes with sources of greater assimilation of Se generates the highest concentration of the element in the shoot of the plant, and consequently, proves to be an effective combination in the nutrition of cowpea with Se.

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