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SÃO PAULO STATE UNIVERSITY - UNESP
CAMPUS OF JABOTICABAL

AGRONOMIC BIOFORTIFICATION OF BROCCOLI WITH SELENIUM

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TÍTULO DA TESE: AGRONOMIC BIOFORTIFICATION OF BROCCOLI WITH SELENIUM

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AUTHOR'S CURRICULUM DATA

CAROLINA SENO NASCIMENTO – was born on September 20, 1990, in Diadema, São Paulo, Brazil to Yaeko Seno and Jorge José de Luna Nascimento. In March 2010, she started the Agronomic Engineering course at São Paulo State University (UNESP), campus of Jaboticabal. Throughout her undergraduate years, she was involved with research in different areas of Agronomy such as seed analysis, nematology, biochemistry, plant nutrition, and horticulture. She was a CNPq scholarship holder in the Department of Technology (process number 180258/2011-7), where she worked on the project entitled "Identification, extraction, characterization, and sustainability of biodiesel obtained from seeds in the Amazon". She also had a scientific initiation scholarship in the acarology and horticulture sectors. In the Horticulture sector, she developed her knowledge in hydroponics, greenhouse production, biofortification, intercropping systems, and nutrient management for vegetables. Since the beginning of her undergraduate, she has been involved in extracurricular activities, working in extension projects, event organization, tutoring, volunteer work, and faculty groups. From August 2014 to December 2015, Carolina participated in the Science without Borders program, where she attended academic classes at Western Illinois University (Macomb, USA), during this time she also did a three-month internship at the University of Wisconsin (Madison, USA). In August 2016, she started her master's degree in Agronomy (Soil Science) at São Paulo State University (UNESP), campus of Jaboticabal (Thesis Title: Agronomic biofortification of arugula with Selenium in a hydroponic system). Her project was sponsored by the Coordination for the Improvement of Higher Education Personnel (CAPES) through the granting of a scholarship. In March 2018, she began her Ph.D. in Agronomy (Crop Production) at the same university (Doctoral Research Project Title: Agronomic biofortification of broccoli with Selenium). During her graduation period, she volunteered in the 'Vegetable Program' as a coordinating assistant.

The scientist is not the man who provides the real answers; he's the one asking the real questions.

Claude Lévi-Strauss

DEDICATE

For my mother Yaeko Senô and my grandparents Ziro Senô (*In memoriam*) and Ana Akemi de Britto Senô (*In memoriam*) for being my examples of conduct, love, work, and dedication. They always encouraged and supported me at every step of my life, without their support it would be impossible to achieve this goal. To my sister Camila Seno Nascimento for her encouragement, trust, understanding, and unrestricted support. To my sister Juliana Yukiko Senô and my niece Beatriz Akemi Senô Matayoshi for their support and advice. To all my friends and family who have always been at my side in this journey.

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SUMMARY

AGRONOMIC BIOFORTIFICATION OF BROCCOLI WITH SELENIUM

ABSTRACT - The increase in the selenium (Se) content in plants via fertilization has been adopted in biofortification programs aimed at reducing important nutritional deficiencies in human food, since this micronutrient can contribute to the lower incidence of a range of diseases, including cancer, hyperthyroidism, and heart disease. Se is considered an essential micronutrient for humans and animals, however, its essentiality has not yet been considered for plants, although research shows that it plays a beneficial role in plants, especially when they are under biotic and/or abiotic stresses conditions. In this context, this study aimed to investigate the agronomic biofortification of broccoli with Se, and the effect of Se on growth, nutritional status, physiology, and production of broccoli plants, besides the effects of two cooking methods (boiled and steamed) on Se content. Five Se concentrations (0, 5, 10, 20, and 40 µM) were applied as sodium selenate in two phenological growth stages of broccoli (Experiment I - seven days after transplanting the seedlings; Experiment II – at the beginning of floret development). Broccoli plants exposed to 40 µM of Se for 84 days (Experiment I) exhibited a decrease of 20.04% in the florets' dry biomass. In experiment II, the application of 20 µM of Se led to an increase of 47.71% in the floret's fresh biomass when compared to untreated plants. Broccoli plants achieved the highest productivity at 20 µM of Se. The results revealed that regardless of the Se application time, Se content in the leaf and florets increased linearly in response to Se concentrations, showing to be an effective agricultural management to biofortify broccoli plants and reduce widespread Se malnutrition. The cooking process had a negative effect on broccoli quality since boiling and steaming promoted Se losses. Boiling caused a decrease of 39 and 40% whilst steaming reduced 13 and 17 % of florets Se content in biofortified broccoli plants treated with 20 µM of Se, in experiments I and II, respectively.

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Keywords: *Brassica oleracea* var. *italica*, biofortified food, cooking process, mineral nutrition, sodium selenate.

SELÊNIO NA BIOFORTIFICAÇÃO AGRONÔMICA DE BRÓCOLIS

RESUMO - O aumento do teor de selênio (Se) nas plantas via fertilização tem sido adotado em programas de biofortificação que visam reduzir importantes deficiências nutricionais na alimentação humana, uma vez que, este micronutriente pode contribuir para a menor incidência de uma série de doenças, incluindo câncer, hipertireoidismo e doenças cardíacas. O Se é considerado um micronutriente essencial para humanos e animais, porém, sua essencialidade ainda não foi considerada para as plantas, embora pesquisas mostrem que ele desempenha um papel benéfico nas plantas, principalmente quando estas estão sob condições de estresse biótico e / ou abiótico. Neste contexto, este estudo teve como objetivo investigar a biofortificação agronômica do brócolis com Se, e o efeito do Se no crescimento, estado nutricional, fisiologia e produção de plantas de brócolis, além dos efeitos de dois métodos de cozimento (cozido na água e cozido no vapor) sobre o teor de Se. Cinco concentrações de Se (0, 5, 10, 20 e 40 µM) foram aplicadas por meio de selenato de sódio, em dois estádios fenológicos do brócolis (Experimento I - sete dias após o transplante das mudas; Experimento II - no início do desenvolvimento dos floretes). Plantas de brócolis expostas a 40 µM de Se por 84 dias (Experimento I) apresentaram redução de 20,04% na massa seca dos floretes. No experimento II, a aplicação de 20 µM de Se ocasionou um aumento de 47,71% na massa fresca dos floretes em relação às plantas não tratadas. Máxima produtividade de brócolis foi obtida com a aplicação de 20 µM de Se. Os resultados revelaram que independente da época de aplicação do Se, houve um aumento linear do teor de Se nas folhas e floretes em resposta às concentrações de Se na solução nutritiva, mostrando ser um manejo agrícola eficaz para biofortificar plantas de brócolis e reduzir a desnutrição generalizada desse elemento. O processo de cocção teve efeito negativo na qualidade do brócolis, uma vez que, os métodos de cozimento na água e a vapor promoveram perdas de Se. O cozimento na água ocasionou um decréscimo de 39 e 40%, enquanto o cozimento a vapor reduziu em 13 e 17% o teor de Se nos floretes de plantas de brócolis biofortificadas com 20 µM de Se, nos experimentos I e II, respectivamente.

Palavras-chave: *Brassica oleracea* var. *italica*, alimento biofortificado, processos de cocção, nutrição mineral, selenato de sódio.

LIST OF ABBREVIATIONS

Se – Selenium

WHO - World Health Organization

pH - Hydrogen potential

DW - Dry biomass

MO – Organic matter

Al – Aluminum

SeMet - Selenomethionine

SeMeSeCys - Selenomethylcysteine

ROS - Reactive oxygen species

SeO_3^{2-} Selenite

SeO_4^{2-} Selenate

N - Nitrogen

P - Phosphor

K - Potassium

Ca - Calcium

Mg - Magnesium

S - Sulfur

Cu - Copper

Fe - Iron

Mn - Manganese

Zn - Zinc

B – Boron

AEse - Absorption efficiencies

H_2O_2 - Hydrogen peroxide

SOD - Superoxide dismutase

CAT – Catalase

APX - Ascorbate peroxidase

GSH-Px - Glutathione peroxidase

DAS - Days after sowing

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1. INTRODUCTION

Food security has been the main concern on this planet for the last few decades, however, currently, countries are also turning their attention to nutrition security, which means providing nutrient-rich foods and beverages for all the population. Poor nutrition can lead to a “hidden hunger” and cause several diet-related diseases since micronutrients and vitamins are essential for human development. Therefore, developing strategies, such as biofortification of foods is an excellent strategy to improve the content of these elements in foods, promote a healthy diet, and mitigate malnutrition around the world. (Lal et al., 2020).

Selenium (Se) is an essential micronutrient for humans and animals, it is part of about twenty-five selenoproteins that participate in several physiological and biochemical processes (Schiavon et al., 2020). A diet deficient in this micronutrient can cause great harm to health. Several diseases are associated with Se deficiency, including osteochondropathy, poor immune function, cardiovascular disease, cancer, liver disease, and hyperthyroidism (Michalke, 2018; Natasha et al., 2018; Newman et al., 2019), however, the level between beneficial and toxic contents are narrow. Daily intake should not exceed 400 µg (Liu et al., 2021), since high Se intake can be toxic and causes hair and nail losses, liver injury, and damage to the central nervous system and gastrointestinal tract. (Reis et al., 2020; Loomba et al., 2020).

Populations residing in regions with low levels of Se in the soil may suffer from disturbances caused by its deficiency since Se is incorporated into human nutrition mainly via agricultural products. According to the World Health Organization (WHO), the recommended daily intake of Se is 55µg/day for healthy adults (Wesselink et al., 2019; USDA – ARS 2012; WHO 2009), however, it is observed that about 15% of the world's population are deficient in this mineral (Zhou et al., 2020), with the vast majority in underdeveloped countries. This occurs due to the food restriction resulting from scarce financial resources in these regions that make it almost impossible to have a diversified daily diet, which includes the consumption of vegetables, fruits, cereals, and animal protein that guarantee an adequate nutrient intake (Fairweather et al., 2011). Thus, for the reduction of these indices, techniques such as food biofortification have been increasingly used.

Plant biofortification is a viable and efficient means of providing micronutrients to populations that have limited access to a diversified diet. Since 2003, HarvestPlus and its partners have demonstrated that biofortification is a promising tool in improving global nutrition. Through its use, it is possible to insert and enrich agricultural products with micronutrients, vitamins, and minerals (Bouis and Saltzman, 2018).

This process can be carried out through agronomic biofortification, in which occurs soil and foliar application of fertilizers containing the desired mineral or through genetic biofortification, in which plant improvement is carried out to develop cultivars with the ability to accumulate higher levels of the desired mineral, which can be realized by conventional genetic improvement methods and/or transgenic/biotechnology methods (Lidon, 2018).

Agronomic biofortification is an efficient, sustainable, and low-cost technique used to combat nutritional deficiency in developed and underdeveloped countries to satisfactorily guarantee that a greater number of people have access to biofortified foods, reducing health problems related to nutrient deficiencies (Reis et al., 2013; Ávila et al., 2014; Alfthan et al., 2015).

Alongside the positive effect on human health, numerous studies have shown that Se is also important in plant development. When present in adequate levels, Se acts beneficially on the growth and tolerance of plants to biotic and abiotic stresses, positively influencing biochemical and physiological processes of great importance for plant development, quality, and productivity (Mengel et al., 2001; Silva et al., 2018).

Several aspects from the food production system to cooking methods can influence the Se content in the edible parts of food crops. At home, most foods are usually processed before being consumed, having as objective improve taste and palatability, however, these processes can lead to major changes in the chemical composition, thereby affecting the content and bioavailability of bioactive compounds in foods.

Due to the importance of agronomic biofortification of plants with Se and the lack of information on the effect of cooking process on Se content, this study aimed to better understand the effect of Se on the growth, physiology, nutritional status, productivity, and quality of broccoli plants biofortified with Se.

6. CONCLUSION

The results demonstrated that the greater Se concentration to enhance agronomic traits of broccoli was the supply of 20 µM Se applied at the beginning of the development of florets. The results of this study provided solid evidence that agronomic biofortification with Se is a successful strategy to increase the contents of Se in broccoli plants grown in NFT hydroponic system, contributing to alleviating human deficiency in this element. It also evidence that cooking procedures can dramatically decrease Se content in broccoli florets, since boiled decreased Se content in 39 and 40% and steamed decreased Se content in 13 and 17 % in biofortified florets treated with 20 µM of Se, in experiments I and II, respectively, providing valuable information to optimize cooking procedures to minimize Se losses in broccoli and delivery optimal concentrations of Se for human consumption.

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