SÃO PAULO STATE UNIVERSITY - UNESP
CAMPUS OF JABOTICABAL

AGRONOMIC BIOFORTIFICATION OF BROCCOLI WITH SELENIUM

Carolina Seno Nascimento
Agronomist Engineer
MSc. in Agronomy (Soil Science)

2022

SÃO PAULO STATE UNIVERSITY - UNESP
CAMPUS OF JABOTICABAL

AGRONOMIC BIOFORTIFICATION OF BROCCOLI WITH SELENIUM

Carolina Seno Nascimento
Advisor: Prof. Dr. André Rodrigues dos Reis
Co-advisor: Prof. Dr. Arthur Bernardes Cecílio Filho

Thesis submitted to the College of Agricultural and Veterinary Sciences - Unesp, campus of Jaboticabal, in partial fulfillment of the requirements for the degree of Doctor of Science in Agronomy (Crop Production)

2022

N244a

Nascimento, Carolina Seno
Agronomic biofortification of broccoli with selenium / Carolina Seno Nascimento. -- Jaboticabal, 2022
55 p. : il., tabs.

Tese (doutorado) - Universidade Estadual Paulista (Unesp), Faculdade de Ciências Agrárias e Veterinárias, Jaboticabal
Orientador: André Rodrigues dos Reis
Coorientador: Arthur Bernardes Cecílio Filho

1. Brassica oleracea var. italica. 2. biofortified food. 3. cooking process. 4. mineral nutrition. 5. sodium selenate. I. Título.

Sistema de geração automática de fichas catalográficas da Unesp. Biblioteca da Faculdade de Ciências Agrárias e Veterinárias, Jaboticabal. Dados fornecidos pelo autor(a).

UNIVERSIDADE ESTADUAL PAULISTA

Câmpus de Jaboticabal

CERTIFICADO DE APROVAÇÃO

TÍTULO DA TESE: AGRONOMIC BIOFORTIFICATION OF BROCCOLI WITH SELENIUM

AUTORA: CAROLINA SENO NASCIMENTO
ORIENTADOR: ANDRÉ RODRIGUES DOS REIS
COORIENTADOR: ARTHUR BERNARDES CECILIO FILHO

Aprovada como parte das exigências para obtenção do Titulo de Doutora em AGRONOMIA (PRODUÇÃO VEGETAL), pela Comissão Examinadora:

André Rodrigues dos R
Prof. Dr. ANDRÉ RODRIGUES DOS REIS (Participaçao Virtual)
Departamento de Engenharia de Biossistemas / FEI UNESP Tupa

André Rodrigues dos R
Dr. HILÁRIO JÚNIOR DE ALMEIDA (Participaçao Virtual)
Agrônomo Autônomo / Jaboticabal/SP

André Rodrigues dos R
Pesquisador Dr. ROBERTO BOTELHO FERRAZ BRANCO (Participaçao Virtual)
Centro de Análise e Pesquisa Tecnológica do Agronegócio de Horticultura / IAC(APTA) - Ribeirão Preto/SP

André Rodrigues dos R
Prof. Dr. AURÉLIO PAES BARROS JUNIOR (Participaçao Virtual)
Universidade Federal Rural do Semi-Árido/UFERSA / Mossoró/RN

André Rodrigues dos R
Dr. VÍCTOR MANUEL VERGARA CARMONA (Participaçao Virtual)
Engenheiro Agrônomo Autônomo / Chile

Jaboticabal, 24 de janeiro de 2022

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.

ACKNOWLEDGMENT

To God for the strength and protection that guides me and made me get here.

To Prof. Dr. Arthur Bernardes Cecílio Filho for all your support. I will be eternally grateful for all your guidance, encouragement, professionalism, understanding, and teachings imparted. Thank you for all your contribution to my learning process since undergraduate. You are an example of dedication, competence, ethics, and commitment. Thank you so much for your patience and for always being willing to help me.

To the São Paulo State University (UNESP) - College of Agricultural and Veterinary Sciences, Agricultural Sciences and Biology Departments, and Graduate Program in Agronomy (Crop Production) for the opportunity to do the doctorate degree.

To the staff of the Horticulture Sector, Messrs. Inauro Santana de Lima, Reinaldo Aparecido dos Santos, and Cláudio Oian, who helped me conduct this work and for this, I have great consideration.

To Sidnéia de Aguiar Ferreira and Sônia Maria Carregari for all their help in the laboratory analyses and Rosane Aparecida Betioli Innocente for your constant collaboration and availability.

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (Capes) – Finance Code 001.

To my entire family, especially my grandparents Ziro Senô (*In memoriam*) and Ana Akemi de Britto Senô (*In memoriam*), my mother Yaeko Senô, my sisters Camila Seno Nascimento and Juliana Yukiko Senô, and my nieces Beatriz Akemi Senô Matayoshi and Izzy Ayumi Senô de Oliveira for all their understanding and support.

To my friends who have always been there encouraging me during this journey Danieli Roberta Scarpa, Ana Paula Leme de Almeida Alves, Seila Aparecida Leme de Almeida, Vítor Gavazzi de Marco, and Nathielly da Silva de Souza. Thanks for the advice, support, and trust. You all are my second family.

To all the professors of the Graduate Program in Agronomy (Crop Production) for contributing to my scientific training.

To the thesis defense examining board members, Dr. Aurélio Paes Barros Júnior, Dr. Hilário Júnior de Almeida, Dr. Roberto Botelho Ferraz Branco, and Dr. Victor

Manuel Vergara Carmona, who accepted my invitation and took the time to share their points of view on the research, contributing to the enrichment of the work.

To the brother that graduate school gave me Paulo Henrique Soares Silva, thank you for the friendship, support, encouragement, and for making the daily life at graduate school so pleasant. Your joy and charisma infect everyone around you.

To my friends on the research group Isaías Reis, Beliza Queiroz, Breno Pereira, Maria José, Júlia Mercês, and Danilo Passos for all the contributions and friendship. It was profoundly enriching to know and work with each one of you

To Prof. Dr André Rodrigues dos Reis and Profa. Dra. Priscila Lupino Gratão for all the help and guidance.

To everyone who in any way contributed to the realization of this work.

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.

.

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

LIST OF TABLES

LIST OF FIGURES

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.

2 LITERATURE REVIEW

2.1 The culture of broccoli

Broccoli (*Brassica oleracea* var*.* itálica) is an annual herbaceous plant, with an erect stem, pivoting root system, with large, simple, alternating, and spiral ordering leaves (Trevisan, 2013).

Broccoli plants are allogamous and have a sporophytic self-incompatibility system. The crop presents a less compact central inflorescence, with color that can vary from green to bluish, emitting numerous shoots in the leaf axils, which end in imperfect flower heads. The plant has floral apexes consisting of buds with yellow petals, separated into four. Stamens are distributed into six long segments. The pistil is elongated with a spherical stigma and the fruit is a silique with an elongated shape (Embrapa, 2015).

Broccoli has multiple inflorescences (branch) and single inflorescence (popularly known as ninja broccoli or head broccoli) varieties. Cultivars that fall into the branching group have lateral shoots and are characterized by having multiple inflorescences of reduced size and coarse-graining. It is possible to harvest the inflorescences several times throughout its cycle. Its commercialization is carried out through the joining of several stalks forming bundles. Single inflorescence cultivars are characterized by the formation of a central inflorescence with fine grain and large dimensions. The harvest is carried out only once and its commercialization can be carried out through the entire inflorescence in its natural form or industrially processed in the form of frozen florets (Filgueira, 2013). The cultivars that fit into the single inflorescence group have been gaining prominence in the Brazilian market (Velho and Dal Magro, 2015), especially due to the great development of the frozen food market.

Broccoli is characterized by its high nutritional value, being rich in nutrients such as calcium (Ca), vitamins A and B2, iron (Fe), Se, and fiber (Cecílio Filho et al., 2012). Furthermore, it, like other brassicas, belongs to the group of Se accumulators, that is, it can accumulate expressive Se contents when cultivated in a medium containing the element. In addition, among the Brassicaceae, broccoli is currently one of the most valued crops for having high levels of glucosinolates, a group of compounds highly beneficial to human health, being scientifically recognized for having anti-cancer properties (Sarvan et al., 2018).

2.2 Selenium plant-soil

Plants are the main sources of nutrients for humans and animals, however, due to the mineral imbalance existing in soils around the world, many of these foods do not have adequate levels of essential elements, such Se (Preciado-Rangel et al., 2021). While some regions exhibited Se deficiency, others have excessive amounts causing Se poisoning problems (El-Ramady et al., 2020).

The concentration of Se in the soil is usually between 0.01 and 2.0 mg kg^{-1}, whereas soils considered to be seleniferous usually contain more than 5 mg kg^{-1} (Saha et al., 2017), which demonstrates its great variability. This variation can be related to several factors, such as the parent materials of the soil, atmospheric deposition of volcanic plumes, anthropogenic sources (irrigation, fertilizer use, farmyard manure applications, waste incineration, among others (Liu et al., 2021; Reynolds and Pilon-Smits, 2018).

The accumulation of Se by plants depends not only on the total Se content in the soil but also on its availability (Wang et al., 2019a, 2019b). The availability can be affected by several factors, including soil physicochemical properties, such as pH, organic matter (OM) content, Fe/Al oxides content, and soil mineral composition (Xue et al., 2020). According to Xiao et al. (2020) and Ghosh and Singh (2005), as soil pH decreases, there is also a decrease in Se availability. In the case of OM, its content and composition play a decisive role in the binding speciation with Se, thus resulting in an increase or decrease in the availability of Se in the soil. Fe/Al oxides and minerals have a strong adsorption capacity and large surface area, which make them have an important piece in the adsorption and fixation of Se in the soil (Muller et al., 2012), directly affecting its availability. For all these reasons, many parts of the world, such as the United Kingdom, Finland, New Zealand, and especially China, are Se deficient (Xu et al., 2012) while other countries like Ireland, India, and the USA have seleniferous soils (Saha et al., 2017).

Plant species have different abilities to accumulate Se in their biomass. Plants can be divided into three classes: sensitive to Se (they cannot tolerate concentrations greater than 10 mg kg^{-1} of dry biomass (DW), Se indicators (can tolerate Se concentrations between 100-1000 mg kg^{-1} DW), and Se accumulators (tolerate concentrations > 1000 mg kg^{-1} DW) (Sarwar et al., 2020) such as garlic (*Allium sativum* L.), onion (*Allium cepa* L.) and broccoli (*Brassica oleracea* L.) (Hasanuzzaman et al., 2010). Only a few organisms can uptake high amounts of Se without negative biological consequences. Se hyperaccumulators can tolerate high concentrations because they are able to accumulate, immobilize, transform, and volatilize high concentrations of Se (Amos et al., 2012). Thus, establishing adequate doses to be used in agronomic biofortification programs becomes extremely important, since Se can be considered a beneficial or toxic element, depending on the concentration present in the culture medium and the plant species in question (Natasha et al., 2018).

2.3 Se transport in plants

The sources of Se exhibit considerable differences concerning absorption, translocation, and metabolism by plants, making agronomic biofortification programs aim to develop research to better understand the behavior of each source for the proper establishment of doses and times of application. The main sources studied by biofortification programs are sodium selenate and sodium selenite.

According to Pyrzynska (2009) selenite is transported by a symplastic mechanism while selenate is transported by the apoplastic mechanism. The translocation of sodium selenate from the root system to the aerial part is easily accomplished. Its absorption occurs directly by sulfate transporters and this source is translocated especially in inorganic form (Longchamp et al., 2015). Selenate is more easily absorbed by plants since it is weakly adsorbed to the solid phase by electrostatic traction. Most plants involuntarily absorb selenate because it is similar to sulfate and then it is metabolized via the sulfur (S) assimilation pathway in the chloroplast (Terry et al., 2000).

Most of selenium's harmful effects are due to its chemical similarity to S. As most enzymes have a comparable affinity for S and Se, Se is also metabolized via the S assimilation pathway. When excess Se is available, excess selenoamino acids are synthesized and then erroneously incorporated into non-selenoproteins with toxic consequences (Amos et al., 2012).

The transport of selenite is carried out by phosphate transporters, considered to be less efficient and, thus, tends to present a greater accumulation in the roots, due to the difficulty of translocation within the plant (Ríos et al., 2008; Malagoli et al., 2015).

Selenite is a source easily converted into selenoamino acids in the roots (Souza et al., 1998; Zayed et al., 1998) and translocated to the aerial part in organic form, this form is quickly incorporated into proteins in substitution for S, being able of causing a toxic effect on the plant, even when applied in low concentrations (Hopper and Parker, 1999; Galinha et al., 2015). Selenite has a high affinity with Fe and Al oxides/hydroxides, clay minerals, and MO, so that, in the presence of any of these factors, its bioavailability is reduced (Yang et al., 2021).

According to Terry et al. (2000), Se is first transported to leaves before accumulating in seeds or fruits in accumulating plants, whereas, in non-accumulating plants, the accumulation is the same in roots, seeds, or grains.

2.4 Selenium and its toxicity to plants

High levels of Se can lead to symptoms of toxicity, influencing negatively in biochemical and physiological processes, causing reductions in the development, quality, and productivity of the plant (Hasanuzzaman et al., 2020). Se toxicity is the result of excess accumulation in plant cells and competition between Se and S due to their similarity in chemical structure, which can result in the addition of Se to structural components or participation in biochemical reactions, which can negatively affect plant development. The Se uptake/transport pathways are like those adopted by sulfate, as a result, S may eventually be replaced by Se at the cellular level to synthesize meaningful macromolecules, which include, specific structural and functional proteins, potentially toxic non-specific proteins, and other seleno-compounds. Toxic compounds and non-specific proteins can cause severe cell toxicity, through the alteration of the function and structure of important biomolecules (proteins and enzymes) and/or through the production of ROS (Sarwar et al., 2020). High concentrations of Se can result in growth alterations, leaf chlorosis, branch dwarfism, necrosis, wilt, and dryness. In some plant species, Se toxicity is also related to the increased accumulation of anthocyanins in leaves. Se can result in plant toxicity due to the substitution of the amino acids cysteine and methionine by selenomethionine (SeMet) and selenomethylcysteine (SeMeSeCys) in proteins, which can destroy protein functions and lead to Se poisoning (Reynolds et al., 2020). Another mechanism of Se toxicity to plants is oxidative stress caused by inorganic selenate and selenite (Van Hoewyk, 2013), also it is believed that Se can cause a dysfunction in photosynthesis which results in increased reactive oxygen species (ROS) accumulation and oxidative stress (Hasanuzzaman et al., 2020).

Research has shown that Se can interact with minerals, affecting plant metabolism. The presence of high levels of Se can result in a lack of macronutrients and micronutrient uptake, which are essential for the maintenance of vital processes for the plant (Hawrylak-Nowak, 2008). This occurs, because Se ions interact with nutrients in plant tissues, resulting in the alteration of important physiological processes regulated by these nutrients. These interactions will depend on the concentrations of Se in the cultivation medium, which may cause antagonistic and/or synergistic effects (Hawrylak-Nowak, 2008). According to Nawaz et al. (2015), the permeability coefficient of some ions in biomembranes can be affected by the presence of Se, thus affecting its transport.

Researches demonstrate that the toxicity of Se depends on the age of the plant and the Se chemical form. For example, seedlings are more sensitive than mature plants and selenite (SeO_3^{2-}) is much more toxic than selenate (SeO_4^{2-}) (Hasanuzzaman et al., 2020).

2.5 Selenium beneficial effects in plants

When present in adequate levels in the plant, Se acts beneficially on the growth, development, and tolerance to biotic and abiotic stresses (Li et al., 2020, Alves et al., 2020). Studies have shown that the application of Se increases plant protection against oxidative damage since it raises the activity of antioxidant defense systems (SOD, APX, CAT, GSH-Px, among others.), which helps in the elimination of ROS and lipid peroxides, improving the resistance of plants to stress and aging (Andrade et al., 2018; Sarwar et al., 2020; Yang et al., 2021). Besides that, Se improves crop quality and productivity, since Porphyrin biosynthesis can be regulated by its presence. Porphyrin is related to the formation of chlorophyll, so the addition of Se can promote the formation of this photosynthetic pigment (Lidon et al., 2018), increasing the photosynthetic activity of plants which reflects in an increase in the growth and production of biomass (Sharma et al., 2017).

According to Gargouri et al. (2013), Se can increase the activity of nitrate reductase, increasing protein synthesis (Sager, 2006). Possibly Se is directly involved in protein synthesis through Se-amino acids such as Se_2Cys and Se_2Met, increasing the plasma content in plants (Yang et al., 2021).

3. MATERIAL AND METHODS

3.1 Experimental site

Two experiments were carried out in a greenhouse at São Paulo State University (UNESP), campus of Jaboticabal, São Paulo, Brazil, (21°15'22” S, 48°18'58” W and 575 m above sea level) using the hydroponic system NFT (Nutrient Film Technique). The mean minimum, mean maximum, and average temperature during the experimental period were 20 °C, 42.4 °C, and 34.8 °C, respectively.

3.2 Experimental design and treatments

The experiments differed in terms of Se application timing. In experiment I, Se was applied seven days after transferring the seedlings to the definitive channels while in experiment II it was applied at the beginning of the florets development.

The experiments were designed in a complete randomized block with four replications. The treatments consisted of five concentrations of Se (0, 5, 10, 20, and 40 µmol L^{-1}) applied as sodium selenate via nutritive solution. The experimental unit in both experiments consisted of five broccoli plants grown in 2.0 m long and 0.20 m diameter PVC channel, a tank of 150 L, and a submerged pump (model Power Head CX-300) with a flow rate of 1000 L h^{-1}, used for circulating the nutrient solution. The useful area was represented by the three central broccoli plants; a plant at each end was not considered to obtain data.

3.3 Experimental setup

The pump activation was controlled through a timer. The circulation started at 6:30 am and ended at 7:00 pm uninterrupted. The complete nutrient solution recommended by Hoagland and Arnon (1950) was used, but with changes in the concentrations of N, K, and Ca to 170, 211, and 150 mg L^{-1}, respectively.

The Hybrid broccoli 'Avenger' (Sakata®) was seeded in a 5 × 5 × 3 cm phenolic foam block. The phenolic foams were irrigated with water and kept in a greenhouse during the germination period. When the seedlings presented expanded cotyledons, they were transferred to the initial growth PVC channels (5 cm in diameter) in a NFT hydroponic system, receiving during this period a complete nutrient solution recommended by Hoagland and Arnon (1950).

As soon as the plants had three leaves, they were transferred to the final growth channel. The spacing of 1.0 × 0.4 m was used in this phase. The nutrient solution was monitored daily using a potentiometer and a digital conductivity meter. Nitric acid and sodium hydroxide were used to maintain the nutrient solution between pH 5.5 and 6.5. Water replacement was performed daily to restore the initial volume of the tank. The nutrient solution was renewed when there was a 40% reduction in the initial electrical conductivity (EC).

The broccoli florets in both experiments were harvested at 104 days after sowing (DAS) when the heads were fully formed and exhibited market harvest maturity (before flowers started to open).

3.4 Characteristics evaluated

3.4.1 Plant growth and production:

The parameters were evaluated at the end of both experiments were as follows.

3.4.2 Leaf area (cm2 per plant)

At the end of the experiment 15 leaves were collected and leaf area was measured using a Licor 3100 electronic meter.

3.4.3 Fresh biomass of florets (g per plant)

At the end of the experiment broccoli florets present in the useful area were collected and immediately weighed.

3.4.4 Dry biomass of florets (g per plant)

At the end of the experiment broccoli florets were harvested, washed, and dried in an oven with forced air circulation at 40ºC until the samples had constant masses, then weighing was carried out to obtain the dry biomass.

3.4.5 Nutritional analysis

The first newly developed leaf was collected, as recommended by Trani and Raij (1997), washed, dried, ground, and prepared for the determination of macronutrients and micronutrients contents, according to the methodology proposed by Miyazawa et al. (2009). The same procedure was performed for the florets.

3.4.6 Absorption efficiencies (AEse)

It was obtained using the equation proposed by Hammond et al. (2009).

$$AE_{Se} = \frac{(Setrat)\ x\ (\mathrm{MS}trat)-(Secon\ x\ \mathrm{MS}con)}{\Delta QSe}$$

AESe = absorption efficiency of Se (µg g^{-1} of Se applied);

Setrat = Se content in the florets from the treatments with Se application (µg g^{-1} DW);

MStrat = dry biomass of florets from the treatment with Se application (g $plant^{-1}$);

TSecon = Se content in the florets from the control treatment (µg g^{-1} DW);

MScon = dry biomass of florets from the control treatment (g $plant^{-1}$);

ΔQSe = difference between the amount of Se applied in the Se treatment to the control treatment.

3.4.7 Carotenoids and chlorophyll contents

At the end of the experiment, leaf discs with defined areas were collected in the central region of the newly developed leaf. The material was inserted into Eppendorf tubes wrapped in aluminum foil with 80% acetone solution. Contents of carotenoids (470 nm), chlorophyll a (662 nm), and chlorophyll b (645 nm) were determined in a spectrophotometer at the indicated wavelengths. Calculations were performed using the method proposed by Lichtenthaler (1987).

3.4.8 Lipid peroxidation

At the end of the experiment, newly developed leaves were collected from each plot. The material was snap-frozen in liquid nitrogen and stored at -80 °C. The lipid peroxidation was determined based on the content of thiobarbituric acid reactive substances, according to the method described by Heath and Packer (1968).

The malondialdehyde content was obtained by spectrophotometry in wavelength between 535 and 600nm.

3.4.9 Hydrogen peroxide (H_2O_2)

Obtained through the methodology proposed by Alexieva et al. (2001). Fresh plant tissue was homogenized with thiobarbituric acid (0.1%) and centrifuged at 10,000 rpm for 10 min. The supernatant obtained was inserted into a medium containing 100 mM potassium phosphate buffer (pH 7.5) and 1 M potassium iodide, then remained in a container with ice for 1 h. The reading was carried out at 390 nm. The concentration of H_2O_2 was determined by a standard curve of H_2O_2.

3.4.10 Protein concentration (mg g $^{-1}$ FW)

Protein was determined according to Bradford (1976), a bovine serum albumin calibration curve was used.

3.4.11 Enzymes activity analysis

At the end of the experiment, for enzymatic analysis, newly developed leaves were collected from each plot. The material was snap-frozen in liquid nitrogen and stored at -80 °C. Extraction for enzyme determination was performed using 0.1 M potassium phosphate buffer (pH 7.5), 3 mM dithiothreitol, 1 mM ethylenediaminetetraacetic acid and 5% polyvinylpolypyrrolidone. The mixture was centrifuged at 10,000 rpm for 30 min at 4 °C, the supernatant was collected and stored at -80 °C.

3.4.12 Superoxide dismutase (SOD, E.C. 1.15.1.1)

SOD activity (U mg^{-1} protein) was determined according to Giannopolitis and Ries (1977).

3.4.13 Catalase (CAT, E.C. 1.11.1.6)

CAT activity (μmol min^{-1} mg^{-1} of protein) was determined according to Azevedo et al. (1998) by monitoring the decomposition of H_2O_2 at 240 nm for 1 min.

3.4.14 Ascorbate peroxidase (APX, E.C. 1.11.1.11)

APX determination (nmol/min/mg of protein) was obtained through a reaction containing plant extract, 80 mM potassium phosphate buffer (pH 7.0), 5 mM ascorbate, 1 mM EDTA and 1 mM L^{-1} H_2O_2 (Gratão et al., 2012). The APX activity was obtained by monitoring the oxidation rate of ascorbate at 290 nm, 30 ^{o}C for 1 min.

3.4.15 Gas exchange parameters

Determination of gas exchange parameters was carried out at 100 DAS with the aid of a portable infrared gas analyzer (LC-PRO+, ADC Bioscientific Ltda Herts England), with artificial radiation of 1200 μmol m^{-2} s^{-1}. The following parameters have been determined: Internal concentration of CO_2 (Ci) (µmol m^{-2} s^{-1} CO_2), stomatal conductance (gs) (mmol m^{-2} s^{-1} H_2O), transpiration rate (E) (mmol m^{-2} s^{-1} H_2O), and photosynthesis rate (A) (µmol m^{-2} s^{-1} CO_2). The analysis was carried out between 8:00 am and 12:00 pm on the first newly developed leaf of two plants from each experimental unit.

3.4.16 Se content in the leaf and florets (µg kg^{-1} DW)

The dry biomass (DW) of leaf and florets was used to determine Se. The extract to perform this analysis was obtained through acid digestion, using nitric acid. The Se was analyzed by atomic absorption spectrophotometry with atomization by graphite furnace (GFAAS), following method 3051A of the United States Environmental Protection Agency (USEPA, 1998).

3.4.17 Post-harvest analysis: Cooking process

At the end of the experiment, the effect of two cooking processes of broccoli (boiling and steaming) on the Se content was evaluated. 100 grams of homogeneous florets were used for each method. In the boiling method, the florets were immersed in 400 mL of deionized boiling water for 4 minutes while in the steaming method the florets remained 15 cm above 600 mL of deionized boiling water for 6 min in a vaporizer with a lid. Shortly after finishing each broccoli cooking process, the florets were immediately immersed in 500 mL of deionized water and ice for 5 minutes to stop the cooking process. Afterward, the samples were dried in an oven with forced air circulation at 40 ºC until they reached constant masses and prepared for the determination of Se.

3.5 Statistical analysis

The statistical analysis of the dataset was performed using the Agroestat software (Barbosa and Maldonado Júnior, 2015). Individual analyses of variance were conducted for Se concentration, and a joint analysis was conducted for Se application times. Multiple comparisons between treatment means were conducted using the Tukey test at 5%.

4. RESULTS

4.1 Plant growth and production

The leaf area, fresh and dry biomass of florets were significantly affected by the interaction between Se doses and Se application time (Table 1).

Table 1 Mean values of the number of leaves per plant, fresh biomass, and dry biomass of broccoli as a function of doses of Selenium applied seven days after transferring the seedlings to the definitive channels and at the beginning of the development of florets

Selenium time application	Doses of Selenium (μM)				
	0	5	10	20	40
	Leaf area cm^2				
Seven days after transferring the seedlings to the definitive channels	2511.61 Aa	2312.85 Aa	1968.73 Aa	2569.71 Aa	2008.23 Aa
At the beginning of the development of the florets	1804,73 Bb	2624.82 Aa	1891.59 Ab	1993.84 Bab	2294.61 Aab
	Fresh biomass g $plant^{-1}$				
Seven days after transferring the seedlings to the definitive channels	333.00 Ab	341.65 Aab	317.90 Ab	388.90 Ba	347.40 Aab
At the beginning of the development of the florets	302.50 Ac	361.36 Ab	351.60 Abc	446.82 Aa	384. 39 Ab
	Dry biomass g $plant^{-1}$				
Seven days after transferring the seedlings to the definitive channels	28.06 Aab	24.76 Bbc	23.16 Bbc	30.60 Aa	22.48 Bc
At the beginning of the development of the florets	24.62 Ab	29.06 Aab	27.81 Ab	34.44 Aa	29.86 Aab

* Different letters indicate the difference between means according to a Tukey test ($p \leq 0.05$). Uppercase letters correspond to Selenium time application, and lowercase letters correspond to Se application doses.

In both experiments, fresh and dry biomass of florets were influenced by Se concentrations, whereas leaf area differed only in experiment II (Figure 1).

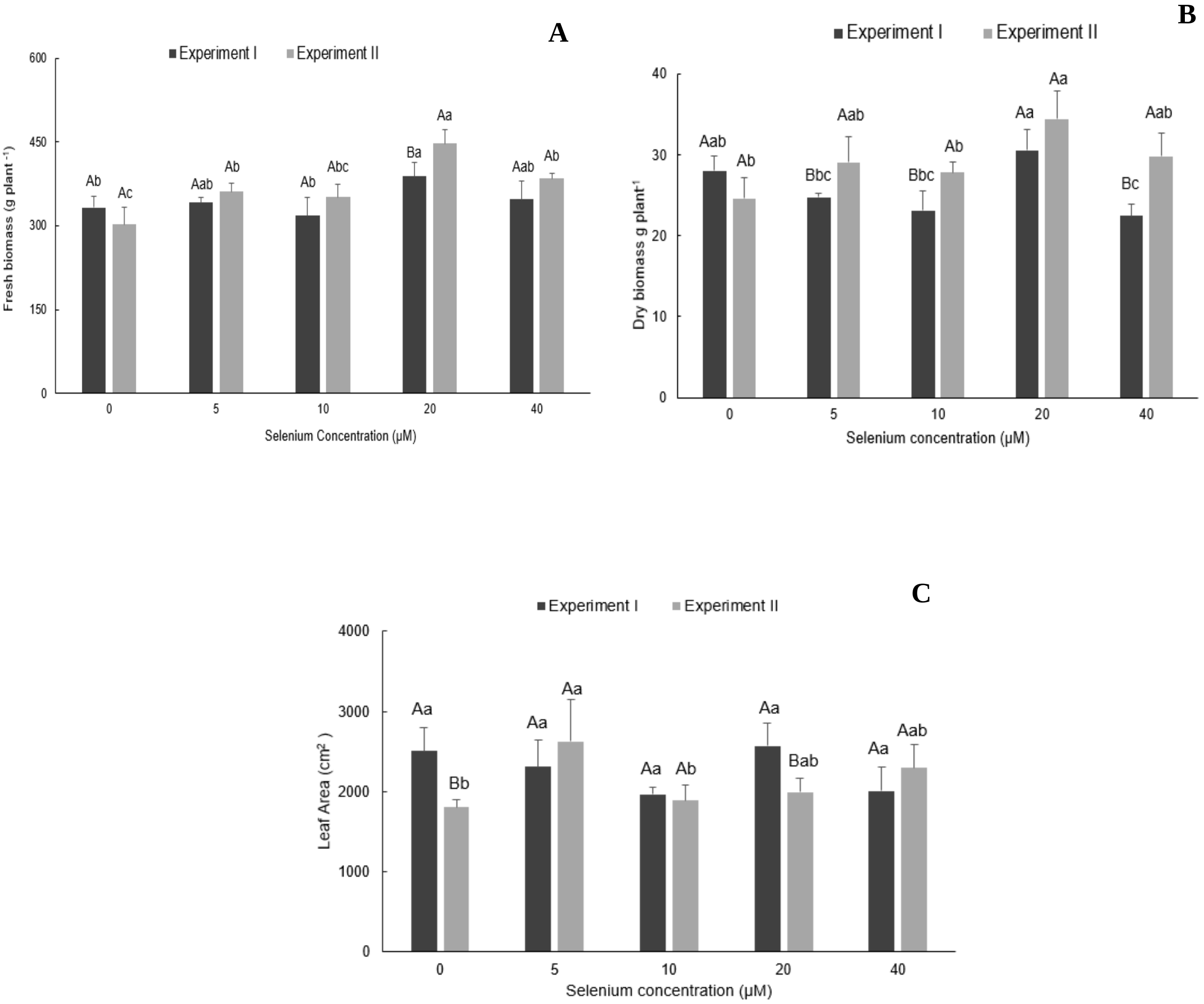


Figure 1. Florets fresh biomass (A), florets dry biomass (B), and leaf area (C) of broccoli plants in response to Se application in experiments I and II. The error bar indicates the standard error of the mean (n = 4 replications). Different letters indicate the difference between means according to a Tukey test ($p \leq 0.05$).

In experiment I, broccoli plants exhibited a decrease of 20.04% in the florets dry biomass with the supply of 40 µM Se when compared with control plants (without Se). In experiment II, plants at 20 µM Se showed an increase of 47.71% and 39.89% in the florets fresh and dry biomass, respectively when compared to untreated plants.

Broccoli plants at 20 µM Se had fresh biomass of florets 14.9% higher in experiment II than experiment I (Table 1).

For leaf area, plants with 5 µM Se showed an increase of 45.44% when compared to the control treatment.

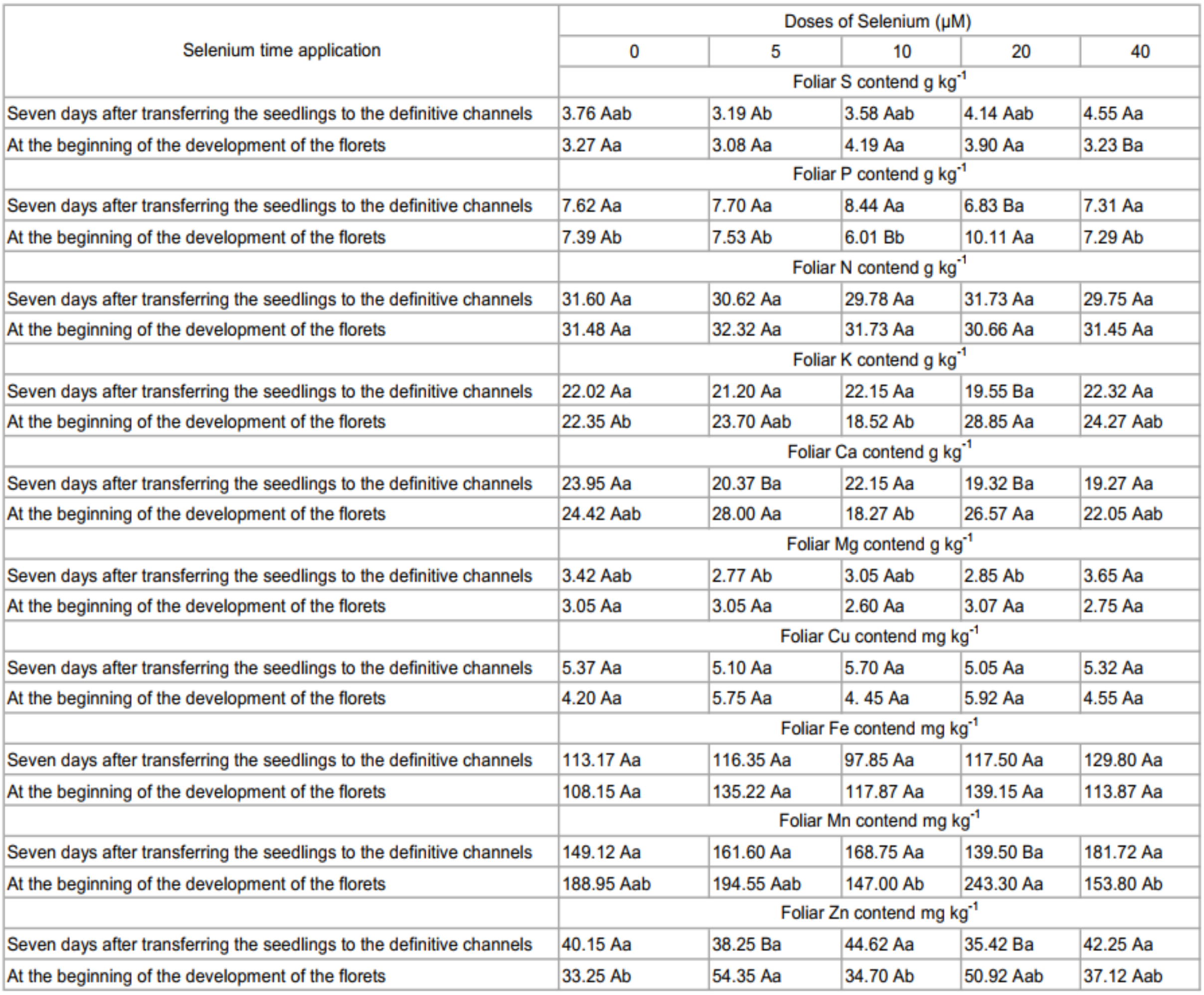
4.2 Foliar nutrition analysis

In experiment I, N, P, K, Ca, Cu, Fe, Mn, and Zn foliar contents were not affected by Se supply, regardless of Se concentration (Table 2). Foliar Mg content was 31.77% higher in plants that received 40 µM Se than those which received only 5 µM Se.

In experiment II, the application of Se concentrations did not generate a significant alteration in S, N, Mg, Cu, and Fe in the foliar contents, when compared with control plants (Table 2). The highest foliar P and K contents were observed in plants that received 20 µM Se, representing an increase of 36.81% and 29.08%, respectively, when compared to the control treatment. The application of 5 µM Se caused an increase of 63.46% in the Zn content when compared to the control treatment.

S, P, K, Ca, Mn, and Zn were influenced by Se application time (Table 2).

Table 2. Mean values of foliar sulfur (S), phosphorus (P), nitrogen (N), potassium (K), calcium (Ca), magnesium (Mg), copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn) foliar contents as a function of doses of Selenium applied seven days after transferring the seedlings to the definitive channels and at the beginning of the development of florets.

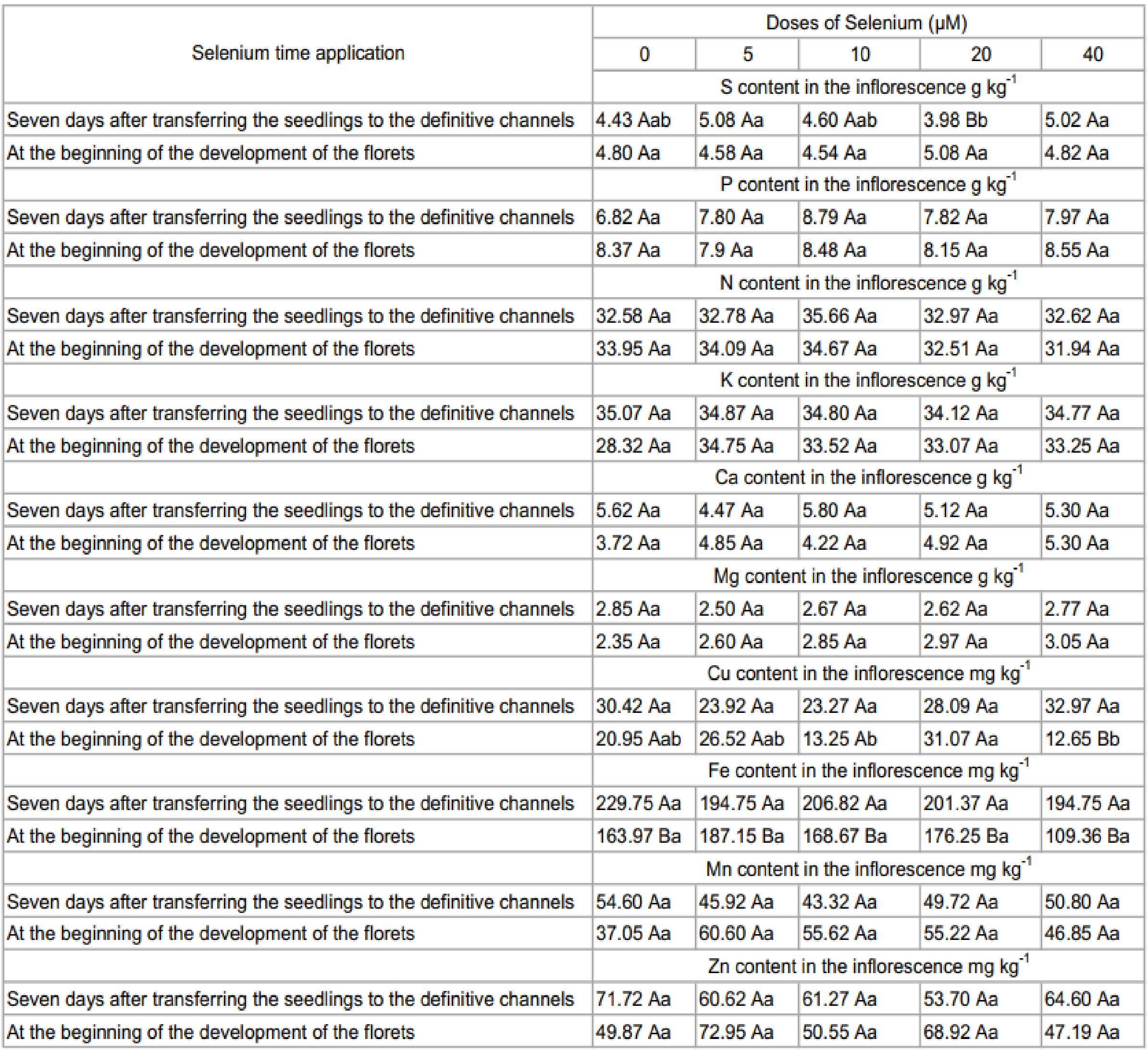


Selenium time application	Doses of Selenium (µM)				
	0	5	10	20	40
	Foliar S contend g kg^{-1}				
Seven days after transferring the seedlings to the definitive channels	3.76 Aab	3.19 Ab	3.58 Aab	4.14 Aab	4.55 Aa
At the beginning of the development of the florets	3.27 Aa	3.08 Aa	4.19 Aa	3.90 Aa	3.23 Ba
	Foliar P contend g kg^{-1}				
Seven days after transferring the seedlings to the definitive channels	7.62 Aa	7.70 Aa	8.44 Aa	6.83 Ba	7.31 Aa
At the beginning of the development of the florets	7.39 Ab	7.53 Ab	6.01 Bb	10.11 Aa	7.29 Ab
	Foliar N contend g kg^{-1}				
Seven days after transferring the seedlings to the definitive channels	31.60 Aa	30.62 Aa	29.78 Aa	31.73 Aa	29.75 Aa
At the beginning of the development of the florets	31.48 Aa	32.32 Aa	31.73 Aa	30.66 Aa	31.45 Aa
	Foliar K contend g kg^{-1}				
Seven days after transferring the seedlings to the definitive channels	22.02 Aa	21.20 Aa	22.15 Aa	19.55 Ba	22.32 Aa
At the beginning of the development of the florets	22.35 Ab	23.70 Aab	18.52 Ab	28.85 Aa	24.27 Aab
	Foliar Ca contend g kg^{-1}				
Seven days after transferring the seedlings to the definitive channels	23.95 Aa	20.37 Ba	22.15 Aa	19.32 Ba	19.27 Aa
At the beginning of the development of the florets	24.42 Aab	28.00 Aa	18.27 Ab	26.57 Aa	22.05 Aab
	Foliar Mg contend g kg^{-1}				
Seven days after transferring the seedlings to the definitive channels	3.42 Aab	2.77 Ab	3.05 Aab	2.85 Ab	3.65 Aa
At the beginning of the development of the florets	3.05 Aa	3.05 Aa	2.60 Aa	3.07 Aa	2.75 Aa
	Foliar Cu contend mg kg^{-1}				
Seven days after transferring the seedlings to the definitive channels	5.37 Aa	5.10 Aa	5.70 Aa	5.05 Aa	5.32 Aa
At the beginning of the development of the florets	4.20 Aa	5.75 Aa	4. 45 Aa	5.92 Aa	4.55 Aa
	Foliar Fe contend mg kg^{-1}				
Seven days after transferring the seedlings to the definitive channels	113.17 Aa	116.35 Aa	97.85 Aa	117.50 Aa	129.80 Aa
At the beginning of the development of the florets	108.15 Aa	135.22 Aa	117.87 Aa	139.15 Aa	113.87 Aa
	Foliar Mn contend mg kg^{-1}				
Seven days after transferring the seedlings to the definitive channels	149.12 Aa	161.60 Aa	168.75 Aa	139.50 Ba	181.72 Aa
At the beginning of the development of the florets	188.95 Aab	194.55 Aab	147.00 Ab	243.30 Aa	153.80 Ab
	Foliar Zn contend mg kg^{-1}				
Seven days after transferring the seedlings to the definitive channels	40.15 Aa	38.25 Ba	44.62 Aa	35.42 Ba	42.25 Aa
At the beginning of the development of the florets	33.25 Ab	54.35 Aa	34.70 Ab	50.92 Aab	37.12 Aab

* Different letters indicate the difference between means according to a Tukey test ($p \leq 0.05$). Uppercase letters correspond to Selenium time application, and lowercase letters correspond to Se application doses.

With exception of Cu, the application of different Se concentrations had no effect on macronutrient and micronutrient contents in broccoli florets when compared with control plants in either experiment. The S and Fe contents were influenced by the interaction between Se application time and Se doses (Table 3).

Table 3. Mean values of sulfur (S), phosphorus (P), nitrogen (N), potassium (K), calcium (Ca), magnesium (Mg) and copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn) contents in the inflorescences a function of doses of Selenium applied seven days after transferring the seedlings to the definitive channels and at the beginning of the development of the florets



Selenium time application	Doses of Selenium (μM)				
	0	5	10	20	40
	S content in the inflorescence g kg^{-1}				
Seven days after transferring the seedlings to the definitive channels	4.43 Aab	5.08 Aa	4.60 Aab	3.98 Bb	5.02 Aa
At the beginning of the development of the florets	4.80 Aa	4.58 Aa	4.54 Aa	5.08 Aa	4.82 Aa
	P content in the inflorescence g kg^{-1}				
Seven days after transferring the seedlings to the definitive channels	6.82 Aa	7.80 Aa	8.79 Aa	7.82 Aa	7.97 Aa
At the beginning of the development of the florets	8.37 Aa	7.9 Aa	8.48 Aa	8.15 Aa	8.55 Aa
	N content in the inflorescence g kg^{-1}				
Seven days after transferring the seedlings to the definitive channels	32.58 Aa	32.78 Aa	35.66 Aa	32.97 Aa	32.62 Aa
At the beginning of the development of the florets	33.95 Aa	34.09 Aa	34.67 Aa	32.51 Aa	31.94 Aa
	K content in the inflorescence g kg^{-1}				
Seven days after transferring the seedlings to the definitive channels	35.07 Aa	34.87 Aa	34.80 Aa	34.12 Aa	34.77 Aa
At the beginning of the development of the florets	28.32 Aa	34.75 Aa	33.52 Aa	33.07 Aa	33.25 Aa
	Ca content in the inflorescence g kg^{-1}				
Seven days after transferring the seedlings to the definitive channels	5.62 Aa	4.47 Aa	5.80 Aa	5.12 Aa	5.30 Aa
At the beginning of the development of the florets	3.72 Aa	4.85 Aa	4.22 Aa	4.92 Aa	5.30 Aa
	Mg content in the inflorescence g kg^{-1}				
Seven days after transferring the seedlings to the definitive channels	2.85 Aa	2.50 Aa	2.67 Aa	2.62 Aa	2.77 Aa
At the beginning of the development of the florets	2.35 Aa	2.60 Aa	2.85 Aa	2.97 Aa	3.05 Aa
	Cu content in the inflorescence mg kg^{-1}				
Seven days after transferring the seedlings to the definitive channels	30.42 Aa	23.92 Aa	23.27 Aa	28.09 Aa	32.97 Aa
At the beginning of the development of the florets	20.95 Aab	26.52 Aab	13.25 Ab	31.07 Aa	12.65 Bb
	Fe content in the inflorescence mg kg^{-1}				
Seven days after transferring the seedlings to the definitive channels	229.75 Aa	194.75 Aa	206.82 Aa	201.37 Aa	194.75 Aa
At the beginning of the development of the florets	163.97 Ba	187.15 Ba	168.67 Ba	176.25 Ba	109.36 Ba
	Mn content in the inflorescence mg kg^{-1}				
Seven days after transferring the seedlings to the definitive channels	54.60 Aa	45.92 Aa	43.32 Aa	49.72 Aa	50.80 Aa
At the beginning of the development of the florets	37.05 Aa	60.60 Aa	55.62 Aa	55.22 Aa	46.85 Aa
	Zn content in the inflorescence mg kg^{-1}				
Seven days after transferring the seedlings to the definitive channels	71.72 Aa	60.62 Aa	61.27 Aa	53.70 Aa	64.60 Aa
At the beginning of the development of the florets	49.87 Aa	72.95 Aa	50.55 Aa	68.92 Aa	47.19 Aa

* Different letters indicate the difference between means according to a Tukey test ($p \leq 0.05$). Uppercase letters correspond to Selenium time application, and lowercase letters correspond to Se application doses.

4.3 Se absorption efficiency

The Se absorption efficiency in both experiments was influenced by Se concentrations (Figure 2). In experiments I and II, the highest Se absorption efficiency was obtained with the application of 20 µM Se, representing an increase of 47.95% and 57.39% respectively when compared to plants that received 5 µM Se. Broccoli plants fertilized with 20 µM Se had higher Se absorption efficiency at experiment II than experiment I.

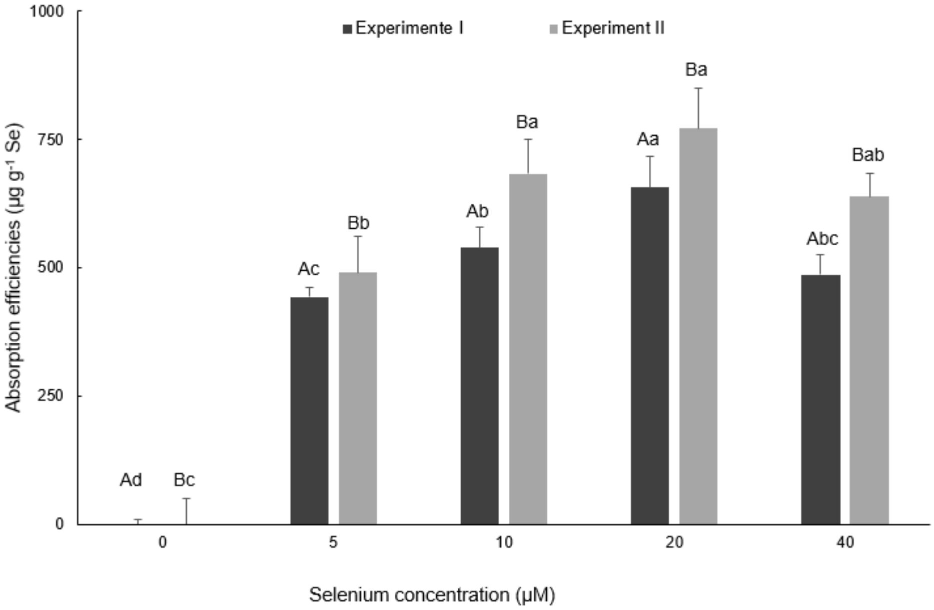


Figure 2. Se absorption efficiency of broccoli plants in response to Se application in experiments I and II. The error bar indicates the standard error of the mean (n = 4 replications). * Different letters indicate the difference between means according to a Tukey test ($p \leq 0.05$). Uppercase letters correspond to Selenium time application, and lowercase letters correspond to Se application doses.

4.4 Gas exchange parameters

With exception of gs, in experiment I, Se application had no effect in the Ci, E, and A in either experiment (Figure 3). The application of 40 µM Se, caused a decrease of 11.76% in the gs when compared to the control treatment.

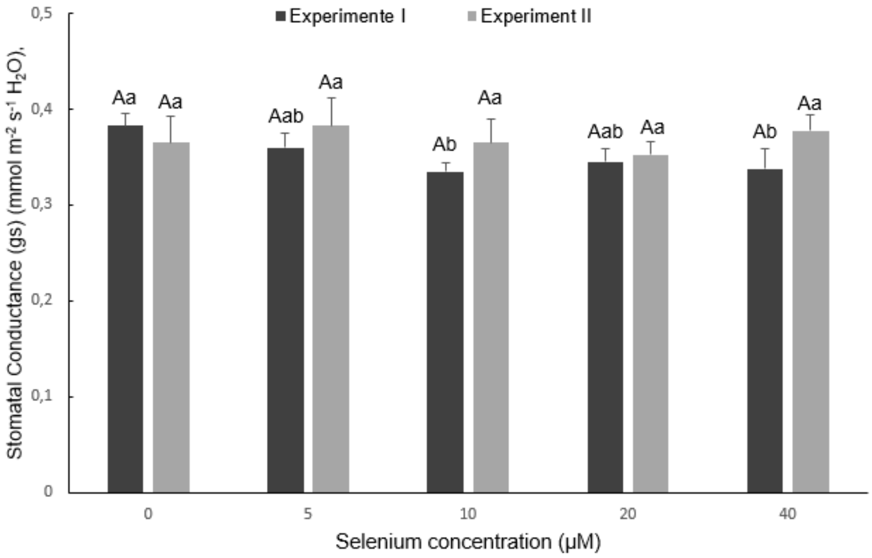


Figure 3. Stomatal conductance (gs) (mmol m^{-2} H_2O) in response to Se application in experiments I and II. The error bar indicates the standard error of the mean (n = 4 replications). * Different letters indicate the difference between means according to a Tukey test ($p \leq 0.05$). Uppercase letters correspond to Selenium time application, and lowercase letters correspond to Se application doses.

4.5 Carotenoids and chlorophyll contents

In experiment I, chlorophyll *a, b*, total chlorophyll, and total carotenoids were not affected by Se supply, regardless of Se concentration applied. In experiment II, the application of 20 µM Se decreased 28.43%, 25.93%, 27.64%, and 29.64% of chlorophyll *a*, chlorophyll *b*, total chlorophyll, and total carotenoids respectively when compared to untreated plants. (Figure 4 A - D). Photosynthetic pigments were not affected by Se application timing.

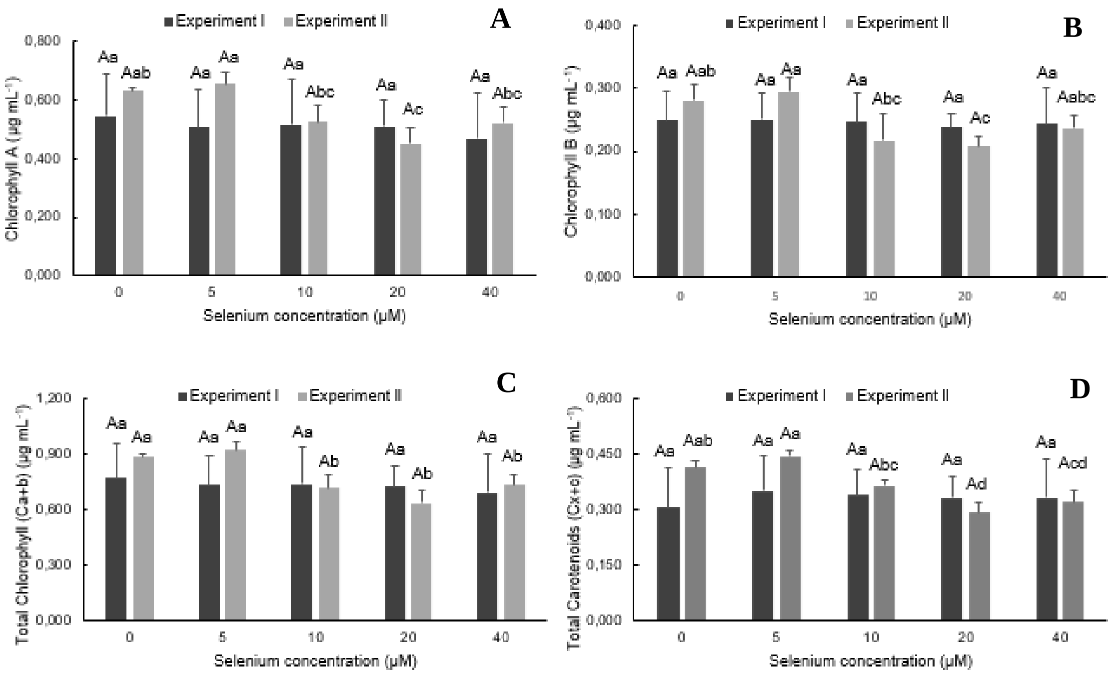


Figure 4. Effects of Se on, chlorophyll *a* (A), chlorophyll *b* (B), total chlorophyll (C), and total carotenoids concentration (D) in response to Se application in experiments I and II. The error bar indicates the standard error of the mean (n = 4 replications).
* Different letters indicate the difference between means according to a Tukey test ($p \leq 0.05$). Uppercase letters correspond to Selenium time application, and lowercase letters correspond to Se application doses.

4.6 Enzymes activity

In experiment I, MDA, SOD, and CAT were affected by Se supply (Figure 5). The application of 20 µM Se, caused an increase of 21.55% in MDA when compared to the control treatment. The SOD and CAT activity at 40 µM Se concentration were 19.45% and 31.35% lower than untreated plants, respectively. In experiment II, APX activity was 31.54% higher in the 40 µM Se concentration when compared to the control treatment (Figure 5E). The CAT activity at 5 µM Se was 37.51% lower when compared to untreated plants (Figure 5D).

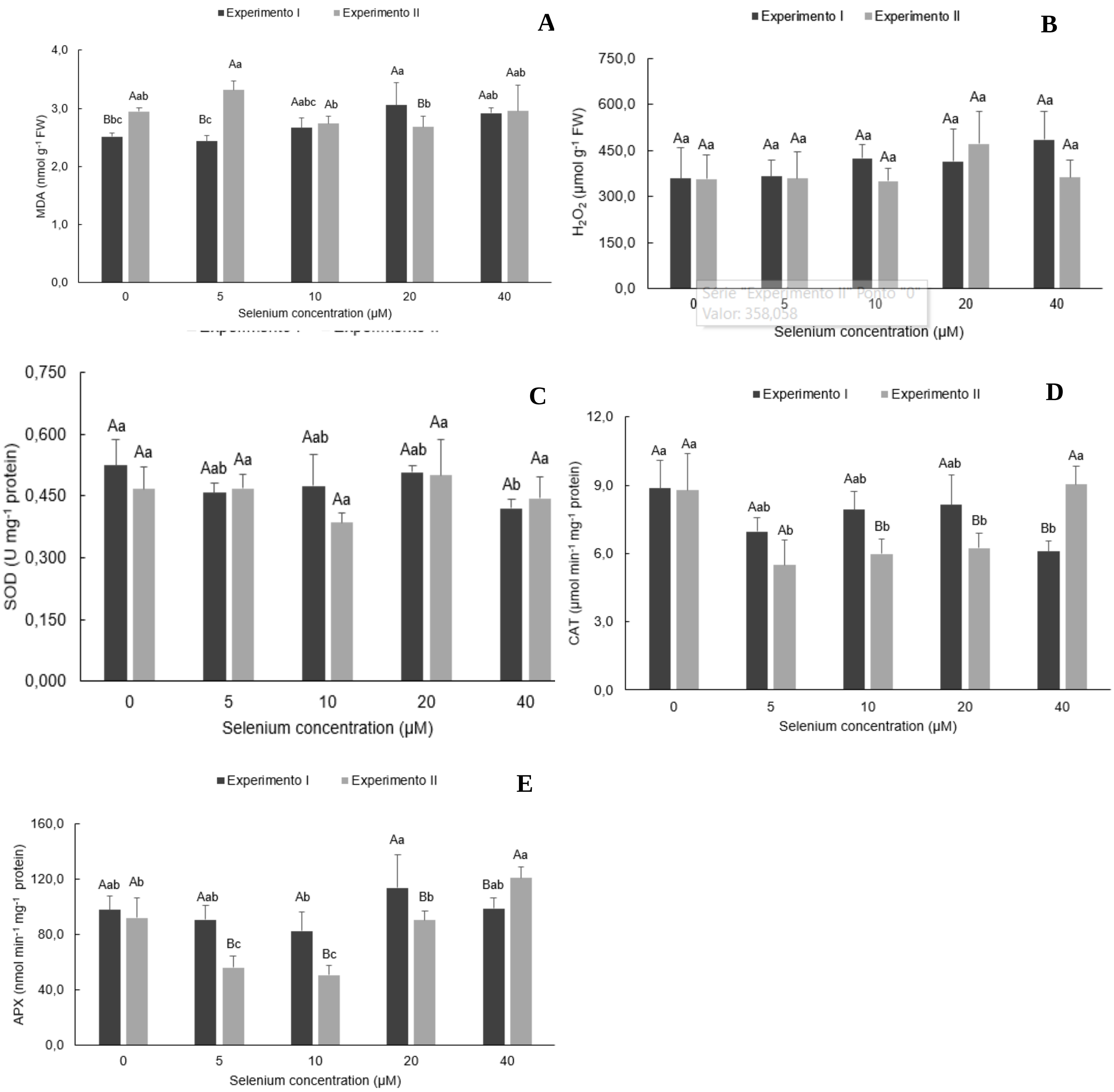


Figure 5. Effects of Se on lipid peroxidation essayed by MDA concentration (A), hydrogen peroxide (B), superoxide dismutase (C), catalase (D), and ascorbate peroxidase (E) in response to Se application in experiments I and II. The error bar indicates the standard error of the mean (n = 4 replications). Different letters indicate the difference between means according to a Tukey test ($p \leq 0.05$).

4.7 Se content

In both experiments, Se content in broccoli leaf and florets increased with increasing concentrations of Se applications. The highest Se content in both experiments was obtained with 40 µM Se, which was 3472.26 and 3158.96 times higher in the leaf and 3542.76 and 3805.30 times higher in the raw florets than that obtained in the untreated plants, in experiment I and II, respectively (Figure 6).

Cooking methods significantly influence Se content in broccoli florets (Figure 6). All cooking procedures lead to a considerable loss of Se. The Se loss increased in the following order: Fresh raw florets> Steamed Florets > Boiled florets. At an application rate of 40 µM Se, broccoli florets showed 41% and 43% times less Se after boiling and 13% and 15% less Se after steaming when compared with fresh raw florets, in experiment I and II, respectively.

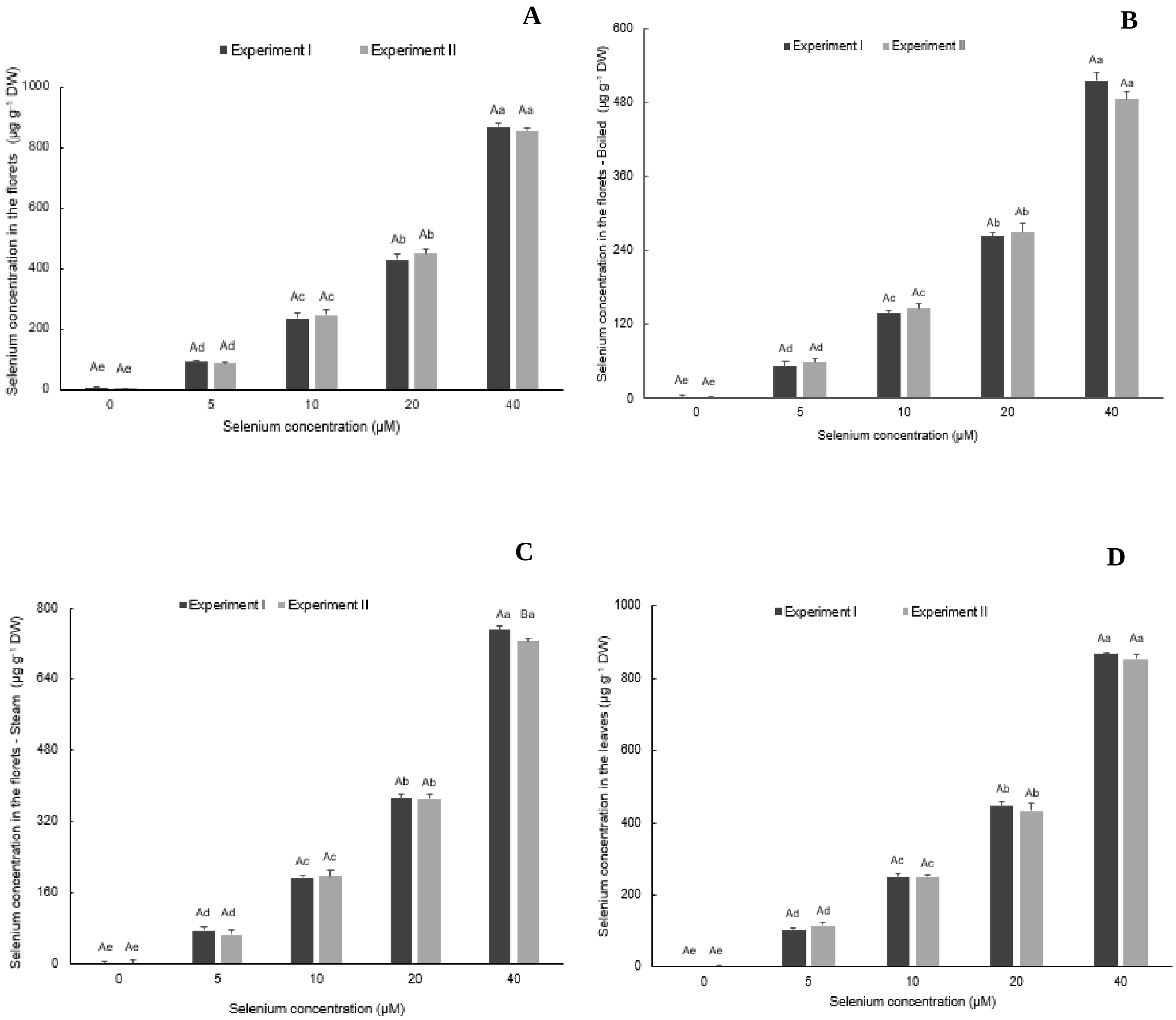


Figure 6. Se concentration in the fresh raw florets (A), boiled florets (B), steamed florets (C) and leaf (D) of broccoli in response to Se application in the T1 - experiment I (A) and T2 – experiment II (B). The error bar indicates the standard error of the mean (n = 4 replications). For each condition, different letters indicate difference between means according to Tukey test ($p \leq 0.05$). * Different letters indicate the difference between means according to a Tukey test ($p \leq 0.05$). Uppercase letters correspond to Selenium time application, and lowercase letters correspond to Se application doses.

4.8 Productivity

The application rate of 20 µM Se in experiment II provided the highest productivity (Figure 7). It was observed an increase of 47.89% when compared to the control treatment.

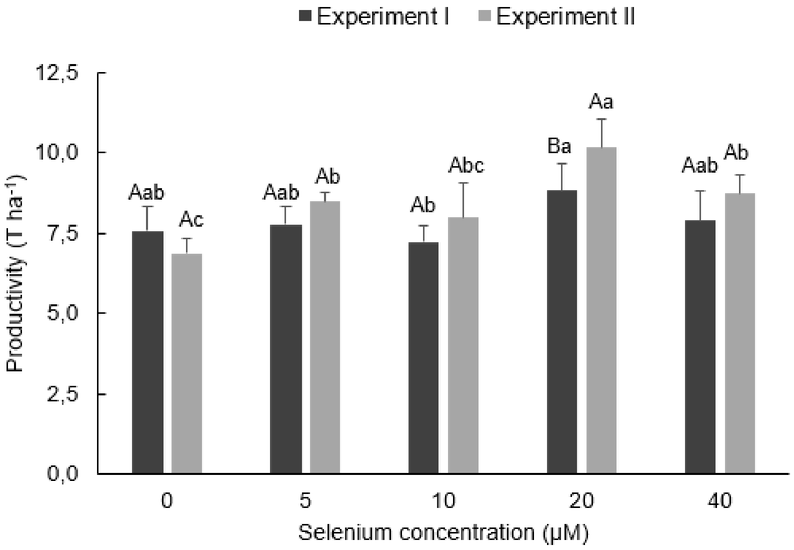


Figure 7. Productivity in response to Se application in the T1 - experiment I (A) and T2 – experiment II (B). The error bar indicates the standard error of the mean (n = 4 replications). For each condition, different letters indicate difference between means according to Tukey test ($p \leq 0.05$). * Different letters indicate the difference between means according to a Tukey test ($p \leq 0.05$). Uppercase letters correspond to Selenium time application, and lowercase letters correspond to Se application doses.

5. DISCUSSION

Se is an important trace element that has been used in agriculture as a promising strategy to reduce Se-malnutrition and improve plant development (Rios et al., 2013; Reis et al., 2020). The beneficial effect of Se to plants is dependent on the concentration applied, being higher concentrations harmful to plant development. So, defining the appropriate dose for each crop is fundamental (Alves et al., 2020).

In this study, broccoli plants exposed to 40 µM Se for 84 days (Experiment I) exhibited a decrease of 20.04% in the florets' dry biomass when compared with the control treatment. The toxic effect of Se at this concentration may be due to the overproduction of ROS, causing oxidative stress and by the excessive presence of malformed selenoproteins caused by the misincorporation of cysteine and methionine in the proteins chain by selenomethylcysteine and selenomethionine, negatively affecting the growth and development of plants (Gupta and Gupta, 2017; Mitller, 2017).

Plants exposed to Se for 24 days (Experiment II) showed the highest fresh and dry biomass of florets at 20 µM Se. The beneficial effect observed with low Se concentration may be due to the short supply period of Se and the mitigation of the ROS effects. Studies show that Se at low concentrations is beneficial for the plant since increases the activity of antioxidant enzymes and the synthesis of antioxidant metabolites, decreasing the electrolytic leakage, and promoting the recovery of cell integrity when plants are grown under stress conditions (Schiavon et al., 2020).

In experiment I, broccoli plants when exposed to 20 µM Se had an increase in the lipid peroxidation concentration, indicating stress in the plants. A factor that may have contributed to this result was the long period of exposure to Se. In experiment II, at 40 µM Se was observed higher activity of APX enzyme. APX is an enzyme that uses ascorbate as an electron donor to reduce H_2O_2 to H_2O (Caverzan et al., 2012), the increase in the enzymatic activity may have contributed to maintaining the concentration of MDA and H_2O_2 at the same levels of the control treatment.

The enzymes SOD, CAT, and APX have activity variations at different Se concentrations, indicating that this element can stimulate or inhibit different enzymes, depending on their concentration.

The content of ROS, like hydroxyl radicals (OH), superoxide (O_2^-), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2) and lipid peroxide radicals, created by plants when not under stress conditions are low, however when the plant is under biotic and/or abiotic stress these levels increase rapidly. It is observed that the presence of Se at low concentrations acts as a stress regulator and inhibits the accumulation of ROS during stress, operating as an inhibitor and antioxidant of ROS (Feng et al., 2013).

In experiment II, plants with 20 µM Se showed an increase of 47.71% in the florets fresh biomass, while florets dry biomass increased 39.89% when compared to untreated plants. For leaf area, plants with 5 µM Se showed an increase of 45.44% when compared to the control treatment. As observed in this experiment, plants subjected to low concentrations and short period of exposure to Se had beneficial results. This factor can be explained because Se can regulate the production and accumulation of ROS in stressed plants through methods such as (1) boost the spontaneous dismutation of O_2 into H_2O_2; (2) regulate the antioxidants enzymes and non-enzymatic systems; (3) directly eliminating ROS through Se species. Another way is related to the positive effect of Se on the integrity of the photosynthetic complex and on the photosynthesis mechanism, which in turn will lead to a reduction in ROS production (Feng et al., 2013; Chauhan et al., 2019).

In experiment II, the Se applied at 20 µM Se provided a decrease in pigment concentrations, as observed in chlorophyll *a* and *b*, total chlorophyll, and carotenoids contents. The concentration of photosynthetic pigment is very sensitive to variation in the concentration of ROS, thus it can be used as an indicator of the cellular metabolic state (Chutipaijit et al., 2011) and indicative of stress.

For biofortification, the Se supply should not cause an imbalance in nutrients uptake by plants or cause a productivity reduction (Longchamp et al., 2016). The results in both experiments demonstrate that the Se application did not influence the florets' nutrient content (Table 2 and 3), except for the micronutrient Cu. On the other hand, the Se supply affects the foliar nutrient content. The changes observed in the foliar mineral content in this study may be due to a redox phenomenon in the cell membrane that causes an alteration in cell transport and metabolism processes. The presence of Se can alter the permeability of the cell membrane to certain cations, thus affecting transport in plant cells.

In experiment I, the 31.53% increase in Mg content, observed at a concentration of 40 µM Se when compared to plants fertilized with 5 µM Se, agrees with the results obtained by Nawaz et al. (2015), who reported that the application of Se in the 40 mg content L^{-1} in leaves can increase the transpiration rate and stomatal conductance of wheat. These physiological processes are related to the absorption and translocation of elements moved by mass flow, which may partially explain the positive correlation between the Se content and the Mg content. (Narváez-Ortiz et al., 2018).

The effect of Se, observed in experiment II, at concentration 20 µM Se, which resulted in an increase in K, may have contributed to better control of water loss by this vegetable, favoring metabolism even under high-temperature conditions, observed during the conduct of this experiment. Table 1 shows the appropriate foliar nutrient contents (Raij et al., 1997) and the observed contents.

The biofortification of the florets was observed in both experiments. This research allowed us to observe that the application of Selenium can occur later and still provide satisfactory results as we can see in experiment II. The results obtained in these experiments provide important information about the ideal time for the beginning of the application of Se. In biofortification programs, the later application of Se can result in numerous advantages, such as the reduction of costs and mitigation of toxicity due to prolonged contact with the nutrient.

The increasing Se concentration in broccoli florets with the increase in Se concentrations applied showed the high efficiency of using the agronomic biofortification method to increase Se concentration in edible parts of broccoli. Brassicas are known for their high capacity to accumulate Se, this ability is because these plants have a high concentration of sulfur (S) compounds (Puccinelli et al., 2017). S and Se show chemical and physical similarities, which allows Se as an analog of S to utilize S uptake and assimilation routes in plants, enabling plants to accumulate high levels of Se when cultivated in Se-rich environments (Schiavon; Pilon-Smits, 2017).

The feature of broccoli accumulates high rates of Se associated with its health benefit phytonutrients making this vegetable an exceptional functional food (D’Amato et al., 2020; Duan et al., 2021). However, studies about the influence of the cooking process on total Se content are scarce. Broccoli usually is cooked before consumption to improve its palatability, affecting the bioavailability of nutrients, chemical composition, and physical characteristics.

This study showed that steaming, but mainly boiling led to a considerable loss of Se concentration when compared with raw florets. This loss can be due to thermal degradation, absorption of Se into the utensil wall, volatilization, but mainly by the leaching of Se into the cooking water (Lu et al., 2018). The cooking process results in the softening and breakdown of cellular components, consequently in the release of molecules in the boiling water (Miglio et.al., 2008).

Zhang et al. (2021) observed similar results in an experiment with potatoes, the authors have a Se loss of 28.64% and 42.44%, during steaming and boiling methods, respectively. According to the authors, a probable reason for obtaining these results is that the water when in contact with the vegetable dissolves the nutrients, including selenium, the dissolved nutrients are eventually retained in the decoction. A study by Thavarajah et al. (2008) with lentils showed a loss of approximately 50% of Se after the boiling method.

The application rate of 20 μM Se in experiment II provided the highest productivity (Figure 3). Considering this concentration for both experiments, it was observed for raw, steamed, and boiled florets 429.2, 372.6, and 263.7 μg g^{-1} DW (dry weight) of Se in experiment I, and 448.1, 369.8, and 270.0 μg g^{-1} of Se in experiment II, respectively. The broccoli florets contain 92% of water. Assuming that the average consumption of broccoli for an adult is 7.7 g per day, this Se-biofortified broccoli provides 264, 229, and 162 μg of Se per day in experiment I and 276, 228, and 166 μg of Se per day in experiment II, respectively. In all the cases we can assume that the recommended daily intake of Se, which ranges within 55 to 400 μg will be attended (Tóth; Csapó, 2018).

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.

7. REFERENCES

Alfthan G, Eurola M, Ekholm P, Venäläinen ER, Root T, Korkalainen K, Hartikainen H, Salminen P, Hietaniemi V, Aspila P, Aro A (2015) Effects of nationwide addition of selenium to fertilizers on foods, and animal and human health in Finland: From deficiency to optimal selenium status of the population. Journal of Trace Elements in Medicine and Biology 31:142-147.

Alexieva V, Sergiev I, Mapelli S and Karanov E (2001) The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. **Plant, Cell & Environment** 24:1337-1344.

Alves LR, Rossatto DR, Rossi ML, Martinelli AP, Gratão PL (2020) Selenium improves photosynthesis and induces ultrastructural changes but does not alleviate cadmium-stress damages in tomato plants. **Protoplasma** 257: 597–605.

Amos W, Webb S, Liu Y, Andrews JC, LeDue DL (2012) Anal. **Analytical and Bioanalytical Chemistry** 404: 1277-1285.

Ávila FW, Yang Y, Faquin V, Ramos SJ, Guilherme LRG, Thannhauser TW, LI L (2014) Impact of selenium supply on Se-methylselenocysteine and glucosinolate accumulation in seleniumbiofortified Brassica sprouts. **Food Chemistry** 165: 578-586.

Azevedo RA, Alas RM, Smith RJ, Lea PJ (1998) Response of antioxidant enzymes to transfer from elevated carbon dioxide to air and ozone fumigation, in the leaves and roots of wild-type and a catalase-deficient mutant of barley. **Physiologia Plantarum** 104: 280-292.

Barbosa JC, Maldonato Júnior W (2015) **Experimentação Agronômica e Agroestat** – Sistema para análises estatísticas de ensaios agronômicos. 1. ed. Jaboticabal: Gráfica Multipress Ltda.

Bouis HE, Saltzman A (2018) Improving nutrition through biofortification: A review of evidence from HarvestPlus, 2003 through 2016. **Global Food Security** 12: 49-58.

Bradford MM, (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of proteinedye binding, **Analytical Biochemistry** 72:248e259.

Caverzan A, Passaia G, Rosa SB, Ribeiro CW, Lazzarotto F, Margis-Pinheiro M (2012) Plant responses to stresses: role of ascorbate peroxidase in the antioxidant protection. **Genettics and Molecular Biology** 35:1011–1019.

Cecilio Filho AB, Schiavon Junior AA, Cortez JWM (2012) Produtividade e classificação de brócolos para indústria em função da adubação nitrogenada e potássica e dos espaçamentos entre plantas. **Horticultura Brasileira** 30:12-17.

Chauhan R, Awasthi S, Srivastava S, Dwivedi S, Pilon-Smits EA, Dhankher OP,Tripathi RD (2019) Understanding selenium metabolism in plants and its role as a beneficial element. Critical Rev. **Environmental Science and Technology** 49:1937–1958.

Chutipaijit S, Cha-Um S, Sompornpailin K (2011). High contents of proline and anthocyanin increase protective response to salinity in Oryza sativa L. spp. Indica. **Australian Journal of Crop Science** 5: 1191–1198.

D'Amato R, Regni L, Falcinelli B, Mattioli S, Benincasa P, Dal Bosco A, Pacheco P, Proietti P, Troni E, Santi C, Businelli D (2020) Current knowledge on selenium biofortification to improve the nutraceutical profile of food: a comprehensive review. **Journal of Agricultural and Food Chemistry** 68: 4075–4097.

Duan Y, Santiago FEM, Reis AR, Figueiredo MA, Zhou S, Thannhauser TW, Li L (2021) Genotypic variation of flavonols and antioxidant capacity in broccoli. **Food Chemistry** 338:127997.

El-Ramady H, Faizy SED, Abdalla N, Taha H, Domokos-Szabolcsy É, Fari M, et al. (2020). Selenium and nano-selenium biofortification for human health: opportunities and challenges. **Soil Systems** 4: 57.

EMBRAPA. Empresa Brasileira de Pesquisa Agropecuária: Centro Nacional e Pesquisa em Hortaliças. **A cultura dos Brócolis** 2015. Brasília, p.1-7.

Fairweather-Tait SJ, Bao Y, Broadley MR, Collings R, Ford D, Hesketh JE, Hurst R (2011) Selenium in human health and disease. **Antioxid Redox Signal** 14: 1337–1383.

Feng R, Wei C, and Tu S (2013). The roles of selenium in protecting plants against abiotic stresses. **Environmental and Experimental Botany** 87: 58–68.

Filgueira FAR. (2013) Agrotecnologia moderna na produção e comercialização de hortaliças. **Novo manual de Olericultura**, Viçosa: UFV, 421p, 2013.

Galinha C, Martinez MS, Pacheco AMG, Freitas MC, Coutinho J, Maçãs B, Almeida AS, Corona MTP, Madrid Y, Wolterbeek HT (2015) Characterization of selenium-enriched wheat by agronomic biofortification. **Journal of Food Science Technology** 52:4236-4245.

Gargouri M, Magne C, Dauvergne X, Ksouri R, Feki AE, Metges MAG, Talarmin H (2013) Cytoprotective and antioxidant effects of the edible halophyte Sarcocornia perennis L. (swampfire) against lead-induced toxicity in renal cells. **Ecotoxicology and Environmental Safety** 95: 44–51.

Giannopolitis CN and Ries SK (1977) Superoxide dismutases: occurrence in higher plants. **Plant Physiology** 59:309-314.

Ghosh M, Singh S (2005) A review on phytoremediation of heavy metals and utilization of it's by products. **Asian J Energy Environ**. 3:1–18.

Gratão PL, Monteiro CC, Carvalho RF, Tezotto T, Piotto FA, Peres LEP, Azevedo RA (2012) Biochemical dissection of diageotropica and Never ripe tomato mutants to Cd-stressful conditions. **Plant Physiology and Biochemistry**, 56: 79–96.

Gupta M, Gupta S (2017) An overview of selenium uptake, metabolism, and toxicity in plants. **Frontiers in Plant Science.** 7:2074.

Hammond JP, Broadley MR, White PJ, King GJ, Bowen WC, Hayden R, Meacham MC, Mead A, Overs T, Spracklen WP, Greenwood DJ (2009) Shoot yield drives phosphorus use efficiency in Brassica oleracea and correlates with root architecture traits. **Journal of Experimental Botany** 60:1953–1968.

Hasanuzzaman M, Bhuyan MHMB, Raza A, Hawrylak-Nowak B, Matraszek-Gawron R, Mahmud JA, Nahar K, Fujita M (2020) Selenium in plants: Boon or bane? **Environmental and Experimental Botany** 178:104170.

Hasanuzzaman M, Hossain MA, Fujita M (2010) Selenium in higher plants: physiological role, antioxidant metabolism and abiotic stress tolerance. **Journal of Plant Sciences** 5:354–375

Hawrylak-Nowak B (2008) Effect of selenium on selected macronutrients in maize plants. **Journal of Elementology** 13:513–519.

Heath RL and Packer L (1968) Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. **Archives of Biochemistry and Biophysics** 125:189-198.

Hoagland DR, Arnon DL (1950) The water culture methods for growing plants without soil. Berkeley: University of California, 32p (Circular 347).

Hopper J & Parker D (1999) Plant availability of selenite and selenate as influenced by the competing ions phosphate and sulfate. **Plant and Soil** 210:199-207.

Lal MK, Kumar A, Kardile HB, Raigond P, Changan SS, Thakur N, Dutt S, Tiwari RK, Chourasia KN, Kumar D, Singh B (2020) Biofortification of Vegetables. In: Sharma

TR, Deshmukh R, Sonah H (eds) **Advances in agri-food biotechnology** 105-129.

Li H, Liu X, Wassie M, Chen L (2020) Selenium supplementation alleviates cadmiuminduced damages in tall fescue through modulating antioxidant system, photosynthesis efficiency, and gene expression. **Environ. Sci. Poll. Res.** 1–13

Liu N, Wang M, Zhou F, Zhai H, Qi M, Liu Y, Li Y, Zhang N, Ma Y, Huang J, Ren R, Liang D (2021) Selenium bioavailability in soil-wheat system and its dominant influential factors: A field study in Shaanxi province, China. **Science of the Total Environment** 770: 144664.

Lichtenthaler HK (1987) Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. **Methods in Enzymology** 148:350-382.

Lidon FC, Oliveira K, Ribeiro MM, Pelica J, Pataco I, Ramalho JC, Leitão AE, Almeida AS, Campos PS, Ribeiro-Barros AI, Pais IP, Silva MM, Pessoa MF, Reboredo FH (2018) Selenium biofortification of rice grains and implications on macronutrients quality. **Journal of Cereal Science** 81:22-29.

Loomba R, Filippini T, Chawla R, Chaudhary R, Cilloni S, Datt C, Singh S, Dhillon KS, Vinceti M (2020) Exposure to a high selenium environment in Punjab, India: Effects on blood chemistry. **Science of theTotal Environment** 716:135347.

Longchamp M, Angeli N, Castrec-Rouelle M (2016) Effects on the accumulation of calcium, magnesium, iron, manganese, copper and zinc of adding the two inorganic forms of selenium to solution cultures of Zea mays. **Plant Physiology and Biochemistry** 98:128–137.

Longchamp M, Castrec-Rouelle M, Biron P, Bariac T (2015). Variations in the accumulation, localization and rate of metabolization of selenium in mature Zea mays plants supplied with selenite or selenate. **Food Chemistry** 182:128-135.

Lu X, He Z, Lin Z, Zhu Y, Yuan L, Ying L, Yin X (2018) Effects of Chinese Cooking Methods on the Content and Speciation of Selenium in Selenium Bio-Fortified Cereals and Soybeans. **Nutrients** 10:317.

Malagoli M, Schiavon M, Dall'acqua S, Pilon-Smits EA (2015) Effects of selenium biofortification on crop nutritional quality. **Frontier Plant Science** 6:280.

Mengel K, Kirkby EA, Kosegarten H, Appel T (2001) Elements with More Toxic Effects. In.: Mengel K, Kirkby EA Principles of plant nutrition. Bern: **International Potash Institute**, v.5, cap 20, p. 657-673.

Michalke B (2018) Selenium in Human Health and Disease: An Overview. **Molecular and Integrative Toxicology** 1: 3–26.

Miglio C, Chiavaro E, Visconti A, Fogliano V, Pellegrini N (2008) Effects of different cooking methods on nutritional and physicochemical characteristics of selected vegetables. **Journal of Agricultural and Food Chemistry** 56: 139–147.

Mittler R (2017) ROS Are Good. **Trends in Plant Science** 22: 11-17.

Miyazawa M, Pavan MA, Muraoka T, Carmo CAFS, Melo WJ. Análises químicas de tecido vegetal. In: SILVA, F. C. (Org.). Manual de análises químicas de solos, plantas e fertilizantes. Brasília: EMBRAPA, 2009. v.2, cap 1, parte 2. 107-184 p.

Muller J, Abdelouas A, Ribet S, Grambow B (2012) Sorption of selenite in a multicomponent system using the “dialysis membrane” method. **Appl. Geochem**. 27: 2524–2532.

Narváez-Ortiz WA, Becvort-Azcurra AA, Fuentes-Lara LO, Benavides-Mendoza A, Valenzuela-García JR, González-Fuentes JA (2018) Mineral Composition and Antioxidant Status of Tomato with Application of Selenium. **Agronomy** 8:185.

Natasha SM, Niazi NK, Khalid S, Murtaza B, Bibi I (2018) A critical review of selenium biogeochemical behavior in soil-plant system with an inference to human health. **Environmental Pollution** 234:915–934.

Nawaz F, Ahmad R, Ashraf M, Waraich E, Khan S (2015) Effect of selenium foliar spray on physiological and biochemical processes and chemical constituents of wheat under drought stress. **Ecotoxicology and Environmental Safety** 113: 191–200.

Newman R, Waterland N, Moon Y, Tou JC (2019) Selenium biofortification of agricultural crops and effects on plant nutrients and bioactive compounds important for human health and disease prevention-a review. **Plant Food for Human Nutrition**. 74: 449–460.

Preciado-Rangel P, Hernández-Montiel LG, Valdez-Cepeda RD, de la Cruz-Lázaro E, Lara-Capistrán L, Morales-Morales B, Gaucin-Delgado JM (2021) Biofortification with selenium increases bioactive compounds and antioxidant capacity in tomato fruits. **Terra Latinoamericana** 39: 1-10

Puccinelli M, Malorgio F, Pezzarossa B (2017) Selenium enrichment of horticultural crops. **Molecules** 22: 933.

Pyrzynska K (2009) Selenium speciation in enriched vegetables. Food Chemistry 114: 1183-1191.

Raij B van, Cantarella H, Quaggio Já, Furlani AMC (1997) Recomendações de Adubação e Calagem para o Estado de São Paulo, 2 ed. rev. ampl. Campinas, Instituto Agronômico & Fundação IAC, 285p. (Boletim Técnico, 100).

Reis HPG, de Queiroz Barcelos JP, Silva VM, Santos EF, Tavanti RFR, Putti FF, Young SD, Broadley MR, White PJ, Dos Reis AR (2020) Agronomic biofortification with selenium impacts storage proteins in grains of upland rice. **Journal of the Science Food and Agriculture** 100:1990–1997.

Reis AR, Moraes M, Ramos SJ, Guilherme LRG (2013) Agronomic biofortification of upland rice with selenium to improve human health. **Journal of Trace Elements in Medicine and Biology**, 271: 42-42.

Reynolds RJB, Jones RR, Heiner J, Crane KM, Pilon-Smits EAH (2020) Effects of selenium hyperaccumulators on soil selenium distribution and vegetation properties. **American Journal of Botany** 107: 970-982.

Reynolds RJB, Pilon-Smits EAH (2018) Plant selenium hyperaccumulation—ecological effects and potential implications for selenium cycling and community structure. **Biochim Biophys Acta Gen Subj** 1862:2372–2382

Ríos JJ, Blasco B, Leyva R, Sanchez-Rodrigues E, Rubio-Wilhelmi MM, Romero L, Ruiz JM (2013) Nutritional balance changes in lettuce plant grown under different doses and forms of selenium. **Journal of Plant Nutrition** 36:1344-1354.

RÍos JJ, Blasco B, Cervilla LM, Rosales MA, Sanches-Rodriguez E, Romero L, Ruiz JM (2008) Production and detoxification of H_2O_2 in lettuce plants exposed to selenium. **Annals of Applied Biology** 154: 107-116.

Sager M (2006) Selenium in agriculture, food, and nutrition. **Pure and Applied Chemistry** 78:111–133.

Saha U, Fayiga A, Sonon L (2017) Selenium in the soil-plant environment: a review **International Journal of Applied Agricultural Sciences** 3:1-18

Sarwar N, Akhtar M, Kamran MA, Imran M, Riaz MA, Kamran K, Hussain S (2020) Selenium biofortification in food crops: Key mechanisms and future perspectives. **Journal of Food Composition Analysis** 93:103615.

Sarvan I, Der Klauw M, Oliviero T, Dekker M, Verkerk R (2018) The effect of chewing on oral glucoraphanin hydrolysis in raw and steamed broccoli. **Journal of Functional Foods** 45:306-312.

Sharma S, Kaur N, Kaur S, Nayyar H, (2017) Selenium as a nutrient in biostimulation and biofortification of cereals. **Ind. J. Plant Physiol.** 22:1–15.

Schiavon M, Nardi S, dalla Vecchia F, Ertani A (2020) Selenium biofortification in the 21st century: Status and challenges for healthy human nutrition. **Plant and Soil** 453:245-270.

Schiavon M and Pilon-Smits, EAH. (2017) The fascinating facets of plant selenium accumulation - biochemistry, physiology, evolution, and ecology. **New Phytologist** 213:1582–1596.

Silva VM, Boleta EHM, Lanza MGDB, Lavres J, Martins JT, Santos EF, Santos FLM dos, Putti FF, Furlani Junior E, White PJ, Broadley MR, Carvalho HWP de, Reis AR dos (2018) Physiological, biochemical, and ultrastructural characterization of selenium toxicity in cowpea plants. **Environmental and Experimental Botany** 150: 172-182.

Souza MP, Pilon-Smits EAH, Lytle CM, Hwang S, Tai J, Honma TSU, Yeh L, Terry N (1998) Rate-limiting steps in selenium assimilation and volatilization by Indian mustard. **Plant Physiology** 117: 1487-1494.

Terry N, Zayed AM, Souza MP, Tarun AS (2000) Selenium in higher plants. **Annual Review of Plant Physiology and Plant Molecular Biology** 51: 401-432.

Thavarajah D, Ruszkowski J, Vandenberg A (2008) High potential for selenium biofortification of lentils (Lens culinaris L.). **Journal of Agricultural Food Chemistry** 56:10747–10753.

Toth RJ, Csapo J (2018) The role of selenium in nutrition: a review. **Acta Universitatis Sapientiae Alimentaria** 11:128-144

TRANI PE, RAIJ B van. Hortaliças. In: RAIJ, B. van. et al. (Eds.). Recomendação de adubação e calagem para o estado de São Paulo. 2. ed. Campinas: IAC, 1997, 285 p. (Boletim Técnico, 100).

Trevisan JN (2013) Crescimento, desenvolvimento e produção de brócolis de cabeça única. 105 f. Dissertação (Mestrado em Agronomia) **- Universidade Federal de Santa Maria**, Centro de Ciências Rurais, Rio Grande do Sul.

USDA–ARS (2012) USDA national nutrient database for standard reference, Release 25**. USDA–ARS**, Washington, DC. https://www.nal.usda.gov/fnic/selenium

USEPA. Method 3051A. 1998. Disponível em https://www.epa.gov/sites/production/files/2015-12/documents/3051a.pdf. Acesso em jan. 2018.

Van- Hoewyk D (2013) A tale of two toxicities: Malformed selenoproteins and oxidative stress both contribute to selenium stress in plants. Annals of Botany 112: 965-972

Velho LPS, Dal Magro T (2015) Seletividade do herbicida oxyfluorfen em pré-transplante na cultura do brócolis. **Horticultura Brasileira** 33:373-376.

Wang MK, Cui ZW, Xue MY, Peng Q, Zhou F, Wang D, Dinh QT, Liu YX, Liang DL, (2019ª) Assessing the uptake of selenium from naturally enriched soils by maize (Zea mays L.) using diffusive gradients in thin-films technique (DGT) and traditional extractions. **Sci. Total Environ** 689:1–9.

Wang D, Xue MY, Wang YK, Zhou DZ, Tang L, Cao SY, Wei YH, Yang C, Liang DL, (2019b) Effects of straw amendment on selenium aging in soils: mechanism and influential factors. **Sci. Total Environ**. 657: 871–881.

Wesselink E, Koekkoek WAC, Grefte S, Witkamp RF, van Zanten ARH (2019) Feeding mitochondria: potential role of nutritional components to improve critical illness convalescence**. Clinical Nutrition** 38:982–995.

WHO (2009) Global health risks: Mortality and burden of disease attributable to selected major risks. http://www.who.int/healthinfo/global_burden_disease/GlobalHealthRisks_report_annex.pdf.

Xiao KC, Lu LF, Tang JJ, Chen H, Li DJ, Liu YX (2020) Parent material modulates land use effects on soil selenium bioavailability in a selenium-enriched region of Southwest China. **Geoderma** 376:114554.

Xu YF, Hao Z, Li YH, Li HR, Wang L, Zang ZF, Liao XY, Zhang R, (2020). Distribution of selenium and zinc in soil-crop system and their relationship with environmental factors. **Chemosphere.** 242: 125289.

Xu ZC, Shao HF, Li S, Zheng C (2012) Relationships between the selenium content in flue-cured tobacco leaves and the selenium content in soil in Enshi, China tobaccogrowing area. **Pak. J. Bot**. 44: 1563–1568.

Yang H, Yang X, Ning Z, Kwon SY, Li ML, Tack FMG, Kwon EE, Rinklebe J, Yin R (2021) The beneficial and hazardous effects of selenium on the health of the soilplant-human system: An overview. **Journal of Hazardous Materials** 422: 126876.

Zayed AM, Lytle CM, Terry N (1998) Accumulation and volatilization of different chemical species of selenium by plants. **Planta** 206: 284-292.

Zhang H, Zhao Z, Nie B, Lyu C, Liu X (2021) Selenium loss and changes in product quality during cooking of selenium enriched potato tubers. **Journal of Food Composition and Analysis** 96: 103728.

Zhou X, Yang J, Kronzucker H J, and Shi W. (2020). Selenium biofortification and interaction with other elements in plants: a review**. Frontiers in Plant Science** 11:586421.