UNIVERSIDADE FEDERAL DE LAVRAS

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PRIMING EFFECT WITH SELENIUM AND IODINE ON BROCCOLI SEEDLINGS: ACTIVATION OF BIOCHEMICAL MECHANISMS TO MITIGATE COLD DAMAGES

LAVRAS – MG
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Dissertação apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Ciência do Solo, área de concentração em Fertilidade do solo e nutrição de plantas, para a obtenção do título de Mestre.

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EFEITO PRIMING COM SELÊNIO E IODO EM MUDAS DE BRÓCOLIS: ATIVAÇÃO DE MECANISMOS BIOQUÍMICOS PARA MITIGAR DANOS CAUSADOS PELO FRIO

Dissertação apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Ciência do Solo, área de concentração em Fertilidade do solo e nutrição de plantas, para a obtenção do título de Mestre.

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ABSTRACT

The present research work focused on the study of the biochemical acclimatization mechanisms of broccoli cultivar Avenger when exposed to low temperatures (2ºC). The objective of this work was to investigate the *priming* effect of various doses of Se and I on broccoli seedlings exposed to low temperatures to improve physiological defense mechanisms. In this way, it was intended to explain the defense mechanisms activated in this crop at the seedling stage by the application of Se, I and the interaction of Se+I when exposed to stress. Because it is in the initial stages where the crop is more prone to suffer some kind of damage, compared to a fully developed plant. Since it has not yet fully developed and lacks the mechanisms to protect itself from stress. This work was conducted in the greenhouse at the Federal University of Lavras and then the plants were transferred to a phytotron to simulate stress conditions for three days. Selenium and I were applied to broccoli seedlings seven days before stress at different doses. The leaves were then collected in liquid nitrogen and biochemical analyses were performed at the Cultivated Plant Physiology Laboratory of the Department of Biology (DBI)-UFLA. Biochemical analyses of the osmoprotectant content and the enzymatic antioxidant system were performed to try to explain how the broccoli crop acted to reduce lipid peroxidation through the quantification of MDA. Finally, the results indicated that indeed the application of Se, I and Se+I managed to visually decrease leaf damage and this is explained by the bitplots of the Principal Component Analysis (PCA).

RESUMO

O presente trabalho de pesquisa concentrou-se no estudo dos mecanismos bioquímicos de adaptação da cultivar de brócolis Avenger quando exposta a baixas temperaturas (2ºC). O objetivo deste trabalho foi investigar o efeito *priming* de várias doses de Se e I em mudas de brócolis expostas a baixas temperaturas para melhorar os mecanismos de defesa fisiológica. Dessa forma, buscou-se explicar os mecanismos de defesa ativados nessa cultura no estágio de plântula pela aplicação de Se, I e a interação de Se+I quando exposta ao estresse. Isso se deve ao fato de que é nos estágios iniciais que a cultura está mais propensa a sofrer algum tipo de dano, em comparação com uma planta totalmente desenvolvida. Ela ainda não se desenvolveu totalmente e não possui os mecanismos para se proteger do estresse. Esse trabalho foi realizado na estufa da Universidade Federal de Lavras e, em seguida, as plantas foram transferidas para o *phytotron* para simular condições de estresse por três dias. Selênio e I foram aplicados antes do estresse em diferentes doses. As folhas foram então coletadas em nitrogênio líquido e as análises bioquímicas foram realizadas no Laboratório de Fisiologia de Plantas Cultivadas do Departamento de Biologia (DBI)-UFLA. Foram realizadas análises bioquímicas do teor de osmoprotetores e do sistema antioxidante enzimático para tentar explicar como a cultura de brócolis atuou na redução da peroxidação lipídica por meio da quantificação de MDA. Finalmente, os resultados indicaram que, de fato, a aplicação de Se, I, Se+I foi capaz de diminuir visualmente os danos às folhas, o que é explicado pelos *bitplots* da Análise de Componentes Principais (PCA).

INDICADORES DE IMPACTOS

O objetivo deste trabalho foi investigar o efeito da aplicação de selênio (Se) e iodo (I) em mudas de brócolis para melhorar a tolerância ao estresse por frio. Esse é um desafio na produção agrícola em regiões vulneráveis a baixas temperaturas. Por meio da aplicação foliar de várias concentrações de Se e I, tanto isoladamente quanto em combinação, os resultados mostraram uma redução notável dos danos foliares. Por meio da ativação dos mecanismos de defesa da planta, como o aumento da atividade das enzimas antioxidantes e o acúmulo do conteúdo de osmoprotetores, o que permitiu que as mudas tivessem maior tolerância ao frio. Essa pesquisa teve um impacto social e econômico, pois aumentou a tolerância das plantas ao frio. Ela pode contribuir para a segurança alimentar, minimizando as perdas agrícolas em condições climáticas adversas e promovendo uma agricultura mais resiliente e sustentável. Do ponto de vista tecnológico, este estudo fornece resultados interessantes e inovadores sobre o uso de Se e I como indutores de resposta de defesa da planta. E alinhado com os Objetivos de Desenvolvimento Sustentável (ODS) das Nações Unidas, especialmente o ODS 2 (Fome Zero e Agricultura Sustentável) e o ODS 13 (Ação Climática). Esse trabalho também tem caráter de extensão, envolvendo a comunidade acadêmica e também os agricultores na implementação e divulgação dos resultados, o que poderá beneficiar diretamente essas populações com inovações tecnológicas que poderão ser utilizadas para otimizar o cultivo de brócolis, mas também de outras espécies de plantas sensíveis ao frio. Essa descoberta tem implicações econômicas importantes, pois a redução dos danos causados pelo frio poderia reduzir as perdas na produção agrícola, beneficiando diretamente os produtores de regiões afetadas por baixas temperaturas. Do ponto de vista social, este trabalho se destaca por sua abordagem extensionista, envolvendo tanto a comunidade acadêmica quanto os agricultores locais no desenvolvimento e na aplicação dessas técnicas, gerando um impacto direto nas comunidades rurais dependentes da agricultura de clima frio. Além disso, os resultados obtidos estão alinhados com os Objetivos de Desenvolvimento Sustentável (ODS), especialmente o ODS 2 (Fome Zero e Agricultura Sustentável) e o ODS 13 (Ação contra a Mudança Global do Clima), contribuindo para o cumprimento da Agenda 2030 da ONU. Esse trabalho também visa à transferência de conhecimento entre a academia e a sociedade, facilitando assim o uso de tecnologias inovadoras para melhorar a resiliência das culturas às mudanças climáticas.

IMPACT INDICATORS

The aim of this work was to investigate the effect of selenium (Se) and iodine (I) application on broccoli seedlings in order to improve tolerance to cold stress. This is a challenge in agricultural production in regions vulnerable to low temperatures. Through foliar application of various concentrations of Se and I, both in isolation and in combination, the results showed a remarkable reduction of foliar damage. Through the activation of defense mechanisms in plants, such as the increase of antioxidant enzyme activity and the accumulation of osmoprotectant content, which allowed the seedlings to have a greater tolerance to cold. This research had a social and economic impact, since by increasing the tolerance of plants to cold. It can contribute to food security, minimizing agricultural losses under adverse weather conditions and promoting a more resilient and sustainable agriculture. From a technological perspective, this study provides interesting and innovative results on the use of Se and I as plant defense response inducers. And aligned with the Sustainable Development Goals (SDGs) of the United Nations, especially SDG 2 (Zero Hunger and Sustainable Agriculture) and SDG 13 (Climate Action). This work also has an extensionist character, involving the academic community as well as farmers in the implementation and dissemination of the results, being able to directly benefit these populations with technological innovations that could be used to optimize the cultivation of broccoli, but also of other cold-sensitive plant species. This advance has important economic implications, since the reduction of cold damage could reduce losses in agricultural production, directly benefiting producers in regions affected by low temperatures. On the social aspect, this work stands out for its extensionist approach, involving both the academic community and local farmers in the development and application of these techniques, generating a direct impact on rural communities dependent on cold climate agriculture. In addition, the results obtained are aligned with the Sustainable Development Goals (SDGs), especially SDG 2 (Zero Hunger and Sustainable Agriculture) and SDG 13 (Action against global climate change), which contributes to the fulfillment of the UN Agenda 2030. This work also aims to transfer knowledge between academia and society, thus facilitating the use of innovative technologies to improve crop resilience to climate change.

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FIRST SECTION

1 INTRODUCTION

Climate change is caused mainly by the increase in greenhouse gases released into the atmosphere due to human activities. This, in turn, intensifies environmental stresses such as extreme temperatures (heat, cold) water deficit, flooding and salinity, exacerbating adverse conditions that affect the growth, reproduction, quantity, quality, and yield of crops (Raza *et al.*, 2019). It is estimated that there may be a loss of up to 70% in the yield of important food crops such as rice, wheat and maize due to climate change (FAO, 2021). In addition to reduced productivity, farmers may face additional costs to mitigate the effects of abiotic stresses, such as advanced irrigation systems, specific fertilizers, and genetically modified crops (Liliane e Charles, 2020). In the United States alone, agricultural losses due to drought are estimated to reach about US $9 billion annually. Moreover, these losses are expected to increase over the years due to the increased frequency and intensity of extreme environmental events on crops (Raza *et al.*, 2019).

Fully developed plants are more resistant to adverse environmental conditions than seedlings, because they have developed more robust physical structures, such as extensive root systems that allow them to access water resources and nutrients in more significant quantities and from deeper soil layers (Suprasanna, 2020). In addition, they possess a greater amount of lignified tissues that offer physical protection against adverse factors such as wind or extreme temperatures. They also possess more sophisticated and efficient signaling systems and stress responses (Yadav e Chattopadhyay, 2023). On the other hand, fully developed plants have a more developed photosynthetic apparatus. This allows greater efficiency in light capture, regulation of energy balance, and resistance to photo oxidation, making them more resistant to abiotic stress (Muhammad *et al.*, 2021).

Therefore, exposure to abiotic stress in the early stages of cultivation can affect seed germination and subsequent development (Ashraf *et al.*, 2022; Sousa *et al.*, 2023). At the morphological level, they show reduced growth in both roots and leaves, which translates into lower biomass production. At the physiological level, one of the most common responses is the closure of stomata to reduce transpiration, in turn limiting the entry of CO_2, essential for photosynthesis (Muhammad *et al.*, 2021). From a biochemical point of view plants increase the production of protective compounds such as antioxidants and osmolytes to maintain cellular homeostasis and mitigate oxidative damage from the accumulation of free radicals such as reactive oxygen species (ROS), which causes oxidation of lipids, proteins and DNA, exacerbating stress, reducing the plant's ability to grow and develop properly (Astaneh *et al.*, 2018; El-Badri *et al.*, 2022).

In the face of this, initial treatment before the onset of stress pre-induces various physiological and biochemical changes for better adaptation to (Ashraf *et al.*, 2022). Several studies demonstrated that initial treatment or priming with appropriate levels of chemicals (organic/inorganic) improves tolerance by modulating various physiological processes such as photosynthesis and by modulating multiple stress response pathways such as reactive oxygen species (ROS) (Sousa *et al.*, 2023). It has been demonstrated in several crops that pretreatment or preparation in the early stages (seedlings/seeds) of the plant with Se can achieve abiotic stress alleviation, as was observed in coffee, rice, quinoa and maize crops (Nawaz *et al.*, 2021; Raza *et al.*, 2024; Sousa *et al.*, 2023). Therefore, this work was designed to provide information on the supplementation of selenium (Se), iodine (I), and the interaction of both elements (Se+I), which will undoubtedly help to implement strategies for other types of crops and against different types of abiotic stresses.

2 Plants of the Brassica genus

Plants of the Brassica genus include important crop plants such as cauliflower, cabbage, collards, Brussels sprouts and broccoli. Broccoli stands out for its nutritional, economic and agricultural importance. It is widely consumed due to its high nutritional value, as it contains high amounts of antioxidant, anticancer and antidiabetic compounds that are associated with bioactive secondary metabolites, including glucosinolates, sulforaphane, polyphenols and vitamin C (Garousi, 2017). They also possess a high potential to accumulate high levels of Se when grown in Se-rich environments (Bouranis *et al.*, 2023). This is because it can store high levels of sulfur compounds. And Se is chemically similar to sulfur (S). Selenium can partially substitute it in some compounds, giving rise to selenoproteins and selenoamino acids (selenocysteine and selenomethionine). This characteristic makes it a good candidate for selenium supplementation (Galić *et al.*, 2021).

Broccoli from the Avenger cultivar is a hybrid with very uniform, compact, fine-grained blue-green florets. This crop produces a single medium to large sized head, with no side shoots. In addition to having greater post-harvest durability, maintaining the color and quality of the product for longer; and versatility in marketing, which can be both fresh and processed. Other notable aspects of this cultivar are the average production cycle of around 105 days and the vigorous and rustic root system of the plant, particularities that ensure great productivity and performance in the field, being the most produced and consumed broccoli variety in the entire country. South America. 'Avenger' broccoli is a cultivar noted for both its yield and its adaptability to different growing conditions. Regarding its physiological characteristics, this cultivar has shown good tolerance to low temperatures during growth, which reduces losses due to environmental stress (EMBRAPA, 2015).

2.2 Impact of abiotic stress on crops

Plants, being sensible, are constantly challenged by environmental stresses such as drought, flooding, and extreme temperatures, fostered by climate change, without the possibility of avoiding them during their growth and development. Abiotic stress can generally affect germination, growth, and ultimately, crop quality and yield (Devireddy *et al.*, 2021; Satyakam *et al.*, 2022).

It has been shown that early growth stages (germination and seedlings) are more sensitive to environmental stresses, which can inhibit seed germination and disrupt growth and post-growth processes, compared to mature plants (Nie *et al.*, 2023). This may be because mature plants have a fully developed photosynthetic apparatus and accumulate higher levels of pigments (Ljubej *et al.*, 2021). LJUBEJ et al., (2021), found higher carotenoid contents in fully mature plants than in seedlings (Šamec *et al.*, 2022). Carotenoids are essential pigments located in photosynthetic organs together with chlorophylls. They act as photoprotectants, antioxidants and stabilizers in cell structures and also participate in signaling and regulation of stress response (Swapnil *et al.*, 2021).

Seed germination is one of the leading early stages of crop growth, which determines crop plants' future growth, position, and yield. It has been shown that seed germination can be severely affected by abiotic stress (Khan *et al.*, 2020). Under salt stress, it has been found that plant cells are unable to absorb sufficient water from the external medium, due to the reduction of water potential, which disrupts normal metabolic activities, blocks cell division and differentiation, and thus inhibits their germination (Astaneh *et al.*, 2018). This also affects morphological parameters that are sensitive indicators of stress levels, as it can often lead to a decrease in growth (Ashraf *et al.*, 2022). Under salt stress, grain sorghum variety Jinza 22 showed a lower percentage of seed germination (28.1%) and seedling height (40%) compared to normal conditions. Also, radicle length was affected, decreasing to 9.8 cm under stress compared with the control group (14.6 cm) (Nie *et al.*, 2023). This is important since roots are the most sensitive organs in crops to detect stress and initiate the response (Raza *et al.*, 2024).

Among the processes affected in plants by abiotic stress, photosynthesis is often the first process to be inhibited by stress. The intensity of photosynthesis can be affected by a decrease in photosynthetic pigments such as chlorophyll (a and b) and carotenoids, which in turn influences crop yield (Chen e Cheng, 2009). In a study on broccoli seedlings, it was found that low temperatures decreased the content of chlorophyll a, chlorophyll b and total chlorophyll by 3, 15 and 8%, respectively compared with control plants (Šola *et al.*, 2024). Similar results were found in flat-leaved kale, also exposed to cold. It significantly decreased the content of chlorophyll a, b, total chlorophylls, and carotenoids, and consequently slowed shoot growth. The consequence was a decrease in shoot mass, as well as root and shoot length by 13%, 35% and 11%, respectively (Šamec *et al.*, 2022). Similar results have been reported for other crops, such as sorghum (Nie *et al.*, 2023).

Under stress conditions both chlorophyll a and chlorophyll b tend to decrease. In contrast, the chlorophyll a/chlorophyll b ratio tends to increase, due to a more significant reduction of chlorophyll b than chlorophyll a (Muhammad *et al.*, 2021). Following stress, chlorophyll b is more affected than chlorophyll a, indicating that the aldehyde group in chlorophyll a might contribute to heat stress resistance. This may be because chlorophyll b undergoes faster degradation and eventually transforms into chlorophyll a as part of the decomposition process (Soengas *et al.*, 2018).

ROS have a dual function. At low concentrations they act as intracellular signalers during stress acclimation (Devireddy *et al.*, 2021). However, exposure of plants to any abiotic stress leads to overproduction of free radicals such as ROS in various cellular compartments, such as mitochondria, peroxisomes and chloroplasts (Mansoor *et al.*, 2022). However, as a consequence of stress, their overaccumulation occurs, among them are singlet oxygen ($^1O_2$), superoxide ion (O^{2-}), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH^-) (Mansoor *et al.*, 2022). The increase in their levels causes oxidative stress in biomolecules leading to alteration of cell membranes, inactivation of enzymes, degradation of proteins, ionic imbalance in plants and damage to biological membranes as a result of lipid peroxidation measured by the accumulation of MDA (Mittler *et al.*, 2004).

2.3 Beneficial elements

2.3.1 Selenium as a beneficial element

Plants innately activate multiple defense mechanisms. However, recent investigations have found that these processes are potentiated after the exogenous application of selenium (Liu *et al.*, 2023). Exogenous applications of this element are made because naturally this element is found in the soil in low concentrations (Bañuelos *et al.*, 2016). As the Brazilian soils showed Se contents ranging from < 0.08 to 1.61 mg kg $^{-1}$, with an average value of 0.19 mg kg $^{-1}$ (Gabos, Alleoni e Abreu, 2014). Therefore, the crop of interest can be enriched with this element by foliar, root or seed imbibition (Astaneh *et al.*, 2018; El-Badri *et al.*, 2022).

Selenium and S are analogs, i.e. both elements are part of the VIA group (chalcogens). They have similar chemical and physical characteristics. However, the two differ in at least two respects in biological systems. First, S compounds metabolize to more oxidized states, while Se compounds metabolize to more reduced states. Second, the chemical behavior of these two elements is different, especially in the acidic strengths of their hydrides H_2S and H_2Se (Tian *et al.*, 2017).

Once selenium is absorbed from the soil solution, it can be transported by high-affinity sulfate transporters. Sultr 1;1 is a transporter crucial in S and Se uptake in broccoli seedling roots (Tian *et al.*, 2017). The primary forms of Se absorbed by plants are selenate (Se VI) and selenite (Se IV). Several studies have found Se VI to be more effective than Se IV in promoting Se accumulation in different crops. In broccoli seedlings grown in nutrient solutions with Se VI and Se IV, higher concentrations of Se were found in both leaves and roots in plants supplied with Se VI (Tian *et al.*, 2017). This may be due to a more efficient translocation of Se VI to shoots compared with Se IV and also because Se IV is rapidly converted to organic forms in the root, which hinders its transport to the areal part of the plant (Schiavon e Pilon-Smits, 2017).

2.3.2 Iodine as a beneficial element

The oceans are the largest reservoirs of bioavailable iodine (I), from where iodine is distributed to the atmosphere and land areas. The second most important iodine reservoir is the soil. Its mobility will depend on the characteristics of the soil (composition, pH, texture and redox conditions). Plants absorb iodine through their leaves and roots (Izydorczyk *et al.*, 2021).

Iodine is not considered essential for plants. However, in humans, iodine is vital for thyroid metabolism and for the development of cognitive abilities, and it is associated with a lower risk of developing certain types of cancer. For this reason, great efforts are made to ensure adequate iodine intake for the population. Through iodine fortification of food products such as, for example, iodization of table salt (Duborská *et al.*, 2022). However, people following low-salt diets reduce their salt intake, which consequently leads to lower iodine intake, due to concerns about cardiovascular diseases. Thus, the agricultural application of iodine to improve growth, environmental adaptation and stress tolerance of plants has not been well explored (Mittal *et al.*, 2023).

2.4 Priming effect on stress mitigation

Plants, being sensible organisms, have developed specific mechanisms that allow them to detect environmental changes and respond to complex stress conditions, minimizing damage. Often this is not enough and the plant may yield to the intensity and duration of the stress (Mittler *et al.*, 2004; Suprasanna, 2020). As mentioned above, the critical stage in which plants are more prone to suffer damage from environmental factors are the germination and seedling stages. In recent years, pretreatment in seeds and seedlings with Se has emerged as an attractive technology in stress management in economically important crops, a strategy known as "priming effect" (Abdul Rehman, 2019).

The "priming effect" refers to the prior application of an agent (chemical preparation, such as a treatment with selenium), by which the plant is prepared to face abiotic stresses later in its development. The exogenous application of Se potentiates the plant's defense mechanisms, improving the tolerance capacity of the plants. This is an attractive proposal because compared to genetic improvement, which is more expensive, complex, and requires more time, pretreatment is a more straightforward, less costly, and easier to use than its counterpart (Abdul Rehman, 2019).

2.4.1 Priming effect with Se and I to improve plant tolerance to stress

Seed priming is a viable technique to improve the germination rate, and promote better seedling growth and seedling vigor index (Ishtiaq *et al.*, 2023). This results in higher-quality seedlings and tolerance to unfavorable environmental conditions (Raza *et al.*, 2024). This is important for farmers since economic losses are reduced (García-Locascio, Valenzuela e Cervantes-Avilés, 2024). In addition, by improving germination quality, seedlings can absorb nutrients and water more effectively, improving stress resistance (los Ángeles Sariñana-Navarrete, de *et al.*, 2024). Next, will be presented some works related to pretreatment with selenium in various crops, under different abiotic stresses and the action triggered by selenium and iodine in the potentiation of defense mechanisms.

Seed germination directly affects crop yield, as it is the first step in their growth. In a study on sorghum, seeds were primed with Na_2SeO_3 (25 µmol L^{-1}) for 12 h and then exposed to NaCl (salt stress, 120 mmol L^{-1}). The results indicated that under stress and primed with Se, seed germination, fresh weight of radicles and plumules, and seedling height increased by 23.8, 35.2, 21.7, and 52.4%, respectively, compared with plants stressed without Se (Nie *et al.*, 2023). Similarly, in *Brassica rapa* L. the final germination percentage reached 100% after Se application compared to the stressed group, which achieved only 53% germination (Hussain *et al.*, 2023). Similar results were found in rice seeds, embedded in Na_2SeO_3 solution at different concentrations of 0, 30 and 60 µmol L^{-1}. Selenium priming accelerated seed germination, increased emergence rate and final emergence percentage, and promoted seedling growth (Hu *et al.*, 2022). Finally, it can be indicated that pretreatment with Se has been shown to improve germination and promote seed growth (Liu *et al.*, 2023).

Photosynthesis is a fundamental process in plants that converts solar energy into chemical energy, stored in the form of carbohydrates. This process relies heavily on photosynthetic pigments (Swapnil *et al.*, 2021), (SWAPNIL et al., 2021), which are affected by selenium application. Quinoa (*Chenopodium quinoa Willd.*) seeds were prepared with Se (0, 3, 6 and 9 mg Se L^{-1}) for 6 hours. Selenium was found to increase leaf chlorophyll content by 35.97% under drought stress (Raza *et al.*, 2024). Besides, the preparation of turnip seeds with 100 µmol L^{-1} of Se showed beneficial effects under stress on the biosynthesis of photosynthetic contents, with increases in Chl *a*, Chl *b*, carotenoids and total chlorophyll compared with seedlings grown in NaCl (Hussain *et al.*, 2023). Also, tomato plants subjected to a water deficit and exposed to a concentration of 100 µM KI showed an increase of ~224% in net photosynthesis, *i.e.*, 1.07 µmol CO_2 m^{-2} s^{-1} for treated plants compared with those without KI under the same irrigation conditions (0.33 µmol CO_2 m^{-2} s^{-1}). Selenium and I succeeded in improving the photosynthetic properties of plants by alleviating the degradation of chlorophyll (a, b), carotenoids and total chlorophyll (Liu *et al.*, 2023).

In addition, Se has also been found to stimulate increased root proliferation. This helps to increase water and nutrient uptake, especially when water availability is limited (Raza *et al.*, 2024). As was found in tomato seeds after priming with Se, root fresh weight was increased by

13.9% under water deficit stress (Ishtiaq *et al.*, 2023). Besides, in sorghum, the root activity of seedlings in the NaCl+Se group increased by 19.1% compared with the NaCl group (Nie *et al.*, 2023). In turn, improved root development can improve water relations such as water use efficiency (WUE), relative water content, and water potential in plants. RAZA et al. (2024) found that quinoa crops under Se pretreatment increased relative water content, osmotic potential, water potential, and turgor potential in leaves by 14.55, 10.32, 38.55, and 31.37%, respectively, under stress. Also, after the application of 10 and 20 µM KI in soybean, water use efficiency increased by 49% compared with the other treatments (Lima, Andrade, Santos, *et al.*, 2023). These results are relevant since plants with better developed root systems maintain cell turgor, which improves the growth and development of the crop.

Starch is the main form of carbohydrate storage in plants. Under stressful conditions, starch represents an energy reserve that can induce metabolic responses and help plants overcome harmful circumstances. SOUSA et al. (2023) observed that starch degradation was enhanced after applying selenium and under osmotic stress in coffee seedlings. Starch degradation is probably attributed to the increase in α-amylase content since this molecule can hydrolyze starch into soluble sugars that provide energy for growth under stress conditions (Thalmann e Santelia, 2017). Just as it was found in rice and turnip, the α-amylase content of Se-treated plants increased by 19.52 and 33% compared with the treatment without Se under stress; meanwhile, the MDA content was reduced, and starch hydrolysis was accelerated (Hu *et al.*, 2022).

Preparation with Se can alleviate osmotic stress by increasing the content of osmoregulatory substances (Hawrylak-Nowak, Hasanuzzaman e Matraszek-Gawron, 2018) found increased leaf sugar and soluble protein content under Se application in sorghum by 10 and 4.3%, respectively compared with the group of plants stressed without Se. Besides priming tomato seeds with selenium (75 ppm SeNPs) increased proline contents by 29% under water stress (Ishtiaq *et al.*, 2023). Similar behavior was observed (*i.e.*, increased proline content by 13%) in turnip crops after pretreatment with Se under salt stress (Hussain *et al.*, 2023). Moreover, in tomatoes, it was also observed that after the application of 50 µM KI, soluble sugar and sucrose levels increased by 96 and 46%, respectively, under stress. In contrast, the doses of 50 and 100 µM KI increased the levels of total free amino acids and proline by 146 and 132%, respectively (Lima, Andrade, Morais, *et al.*, 2023). Finally, applying selenium and iodine further enhanced the accumulation of osmolytes in stress-treated seedlings. Therefore, Se can promote the content of osmoprotectants, such as proline and soluble sugar, thus maintaining cellular osmotic pressure (Liu *et al.*, 2023).

Selenium can significantly enhance the activities of antioxidant enzymes, such as superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT), and accelerate the scavenging of excess ROS in plants (Liu et al., 2023). As reported by ISHTIAQ et al. (2023), seed preparation with 75 ppm SeNP increased SOD and CAT functioning by 35.9% and 25.4%, respectively, while reduced H_2O_2 and MDA contents by 39.3 and 28.9%, respectively under water stress. In sorghum, SOD, POD and, CAT activities increased significantly by 10.8, 16.6 and 17.8% in Se-treated plants compared with the control group, whereas a 9.6% reduction was observed in MDA levels (Nie *et al.*, 2023). LIMA et al. (2023b), also found a ~56% reduction in MDA in plants treated with 100 µM KI (14.31 nmol MDA g^{-1} FW) compared with those without KI (31.80 nmol MDA g^{-1} FW) under water stress. In contrast, SOD and CAT activity increased by 127 and 124%, respectively, influenced by the 100 µM KI treatment during water deficit. For soybeans, an average 18% reduction in MDA was observed when plants were exposed to KI compared with plants grown without KI application under stress, whereas an increase was observed for SOD (87%), APX (68%), CAT (94%) and POD (42%) (Lima, Andrade, Santos, *et al.*, 2023).

Exposure to environmental stress can negatively affect fruit quality, and applying exogenous elements can alleviate this effect. As reported by RAZA et al. (2024), Se significantly improved grain quality parameters, *i.e.*, phosphorus, potassium, and protein contents, by 21.28, 18.92, and 15.04%, respectively. As also reported by LIMA et al. (2023b) in tomatoes, the application of KI (100 µmol L^{-1}) significantly increased titratable acidity (65%) and total phenolics (81%) and decreased the pH (~3%), as well as the ripening index (35.18), suggesting its stress mitigating effect and its potential to increase the postharvest shelf life of tomatoes

As well as priming seeds, work was also carried out on seedlings as reported in a study on *Coffea arabica* cv. Catuaí. It was observed that, 5 days after exposure to osmotic stress, the leaves began to wilt and become flaccid. However, prior application of Na_2SeO_4 8 days before stress promoted an increase of 356, 771.1 and 266.5% of APX, CAT and SOD, respectively, compared with the group of stressed plants without Se. It was also observed that proline and protein levels increased to some extent after applying Se. On the contrary, starch levels were reduced in all treatments subjected to stress compared to basal levels (Sousa *et al.*, 2023).

A similar deleterious effect was found at the highest Se dose (60 µmol L^{-1}) in rice crops. In this case, Se reduced seedling biomass by 27.41% (Hu *et al.*, 2022). Just as selenium decreases stress, it can also accentuate it, fulfilling a dual role in plants, acting both as an antioxidant and as a prooxidant (Subramanyam, Laing e Damme, Van, 2019). As it was also reported in other crops such as *Brassica rapa L.* (125 µmo L^{-1}) (Hussain *et al.*, 2016), tomato (100 ppm) (Ishtiaq *et al.*, 2023), rice (> 6mg L^{-1}) (Subramanyam, Laing e Damme, Van, 2019) they showed a decrease in growth, and biomass and finally affected yield under the highest concentration of selenium.

In a rice study, both seeds and seedlings were pretreated with Se. For mode (1), in seeds were exposed to 6 mg L^{-1} Na_2SeO_4. For mode (2), two-week-old plants were foliar sprayed with the same concentration and source as mode 1. For mode (3), there was a combination of seed priming and foliar spraying under the conditions already mentioned. Next, control and Na_2SeO_4-treated plants were subjected to salt stress for two weeks. The combination of seed priming and foliar spray was more effective than the individual treatments and ultimately resulted in higher biomass (82.1%), antioxidant enzyme activities (SOD, APX and CAT were higher than in control plants by 40.7, 92.7 and 82.9 %, respectively) and proline content (191.1 %). These data suggest that Na_2SeO_4 supplied by foliar spray to plants developed from Se-prepared seeds acted as a booster dose to enhance antioxidant enzyme activities and proline levels after NaCl stress (Subramanyam, Laing e Damme, Van, 2019). A summary table of the responses of different crops to selenium under stress exposure is presented below.

Table 1. Crop responses to Se pretreatment in seeds/seedlings

Seed/Seedlings Application	**Source and applied dose**	**Crop**	**Type of abiotic stress**	**Changes at the physiological, biochemical and biochemical levels**	**Reference**
Seedlings, 8 days prior to stress	Na_2SeO_4, 80 mg L^{-1} Se	Coffee (*Coffea arabica cv. Catuaí.*), with 5–6 fully expanded leaves	Osmotic stress	CAT, APX and SOD increased by 771.1%, 356.3% and 266.5%, respectively.	(Sousa et al., 2023)

Seed	Na_2SeO_4, (0, 3, 6 and 9 mg L^{-1} Se)	Quinoa (*Chenopodium quinoa Willd.*)	Water stress	Improved growth, yield, optimized water relations and quality of quinoa grain	(Raza et al., 2024)
Seed	Na_2SeO_3, (75, 100 and 125 µmo L^{-1})	Turnip (*Brassica rapa L.*)	Salt stress	Decrease of oxidative damage and improvement of the antioxidant system and of the expression levels of genes involved in the antioxidant system of plants.	(Hussain et al., 2023)
Seed	Se nanoparticles (SeNP), 25, 50, 75 andg 100 ppm	Tomatoes (*Solanum lycopersicum L.)*	Water stress	Increased growth, as well as the enzymatic and non-enzymatic antioxidant system, decreased MDA content	(Ishtiaq et al., 2023)
Seed	Se, (0, 30 and 60 µmol L^{-1})	Rice (*Oryza sativa L.)*	Flood stress	Increased α-amylase and soluble sugar content by 19.52 and 6.69%, respectively. It accelerated seed germination, increased the emergence rate, the percentage of final emergence and promoted seedling growth.	(Hu et al., 2022)

Seed	Na_2SeO_3, 25 µmol L^{-1}	Sorghum (*Sorghum bicolor L.)*	Salt stress (NaCl, 120 mmol L-1)	Seed germination, fresh weight of radicles and plumules, as well as seedling height increased by 23.8, 35.2, 21.7 and 52.4%, respectively. In addition, leaf sugar and soluble protein content increased under Se application by 10 and 4.3%, respectively.	(M. Nie et al., 2023)
Seed/seedlings	Na_2SeO_4, foliar (2, 4, 6, 8, 10 and 12 mg L^{-1})	Rice (*O. sativa*, L. spp., Japonica, cv. Nipponbare; GSOR-100), two-week-old plants (foliar spray)	Salt stress	Enhancement of the antioxidant defense system SOD, CAT, APX	(Subramanyam et al., 2019)

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SECOND SECTION – ARTICLE

Priming effect with selenium and iodine on broccoli seedlings: activation of biochemical mechanisms to mitigate cold damages

Abstract: Optimizing crop production in cold-susceptible regions by improving stress tolerance at critical plant development stages is key to reducing food losses. This study aimed to improve cold stress tolerance in broccoli seedlings by priming them with selenium (Se) and iodine (I). Different doses of selenium (0, 25, 50, and 75 mg L^{-1}) and iodine (0, 50, 100, 250, and 500 mg L^{-1}) were applied individually and in combination, totaling 21 treatments. After foliar spray, seedlings were subjected to 20/2 °C (day/night) for three days, and next, antioxidant enzyme activity and osmoprotectant levels were analyzed. Treatments Se75, Se75+I50, and I100 significantly reduced leaf damage (2.64%, 3.11%, and 9.05%, respectively). Moreover, the results showed that Se, I, and their combination (Se+I) activate different defense mechanisms in seedlings, enhancing antioxidant enzyme activity and osmoprotectant accumulation. These findings affirm the potential of these treatments to activate diverse defense mechanisms, ultimately enhancing cold tolerance in broccoli crops.

Keywords: Selenium and iodine combination, enzymatic antioxidant system, osmoprotectants, oxidative stress, beneficial elements.

Highlights:

Se and I promote cold tolerance in broccoli seedlings.

Se and I decrease leaf damage in broccoli leaves.

Se enhances SOD activity and sucrose content.

I increase APX, CAT activities, TFAA, and TSP content.

Se and I combination increases the activity of SOD, APX, TFAA, proline, and RS content.

1. INTRODUCTION

Extreme weather events due to climate change have caused abiotic stresses, such as extreme temperatures and dehydration, which have increased in recent decades (Mohammadi et al., 2023). Low temperatures are among the most critical environmental factors affecting several aspects of the plant. They include germination, growth, development, and reproduction (Jiang et al., 2023). Consequently, it can significantly impact its productivity and quality (Janmohammadi et al., 2015), reducing crop yields by up to 70% (Liliane and Charles, 2020). This scenario substantially challenges agricultural production and rural livelihoods, affecting people partially or dependent on agriculture (Ali et al., 2017). It would also directly impact the economy, reaching global monetary losses of around 2.5 trillion annually (Lobell and Field, 2007).

Among the crops found based on the human diet, broccoli belonging to the genus Brassica L. is naturally efficient in accumulating selenium (Se) (Galić et al., 2021). This condition is due to its high level of sulfur compounds, and Se can partly substitute sulfur in some of them, as well as the formation of seleno-amino acids and seleno-proteins (Garousi, 2017). Likewise, broccoli is a crop of great nutritional and economic importance grown and consumed in several countries (Gudiño et al., 2022). It has experienced a significant increase in world production in recent years, reaching 26 million tons (FAO, 2021). The interest in this crop is due to its diverse beneficial health properties, its high content of vitamins A, C, E, and K, minerals, and essential secondary metabolites such as glucosinolates, phenolic compounds, and fatty acids (Gudiño et al., 2022).

Although broccoli is a low-temperature tolerant crop (Ljubej et al., 2021), it has lower resistance during the seedling stage. The low temperature affects membrane rigidity in plant cells and alters photosynthesis, leading to increased intercellular CO_2 accumulation (Jiang et al., 2023), which contributes to oxidative stress caused by reactive oxygen species (ROS) (Huang et al., 2018). ROS such as singlet oxygen ($^1O_2$), superoxide ion (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH^-) are produced under normal conditions as an intracellular signaling mechanism that regulates plant growth and development (Sies and Jones, 2020). However, during stress, their production rate increases dramatically, causing oxidative damage to biomolecules such as lipid membranes, proteins, RNA, and DNA, which eventually causes cell damage and can eventually lead to plant death (Mateus et al., 2021; Tavanti et al., 2021).

In the face of this, it is possible to improve the defense potential of the plant through exogenously applied treatments (Izydorczyk et al., 2021). Plant priming, at different stages of plant development, as seed or seedling, acts as a preconditioner. It acts as a preconditioning or pre-treatment to achieve more remarkable survival and efficient action of defense mechanisms under subsequent stress conditions. It creates a metabolic memory effect known as the "priming effect" (Wiszniewska, 2021; Zulfiqar et al., 2022). Selenium and iodine (I) have been demonstrated in different crops such as coffee (de Sousa et al., 2022), strawberry (Huang et al., 2018), tomato (Saeed et al., 2023), soybean (Lima et al., 2023b), tea (Liu et al., 2021), and tobacco (Han et al., 2022) to mitigate stress under various abiotic stresses.

This research brings together the primary investigations carried out in recent years on the different mechanisms associated with Se and I in mitigating cold stress. Innovatively, the study provides information on the priming effect, both separately and through the combination of Se+I in broccoli seedlings, and the responses of biochemical mechanisms in abiotic stress reduction. Here, we hypothesized that applying Se and I separately and in combination can mitigate cold stress. Therefore, this study aimed to investigate the impact of the priming effect of different doses of Se and I on broccoli seedlings exposed to low temperatures (2°C) to potentiate biochemical defense mechanisms, evaluating the effects on antioxidant enzyme activity of superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), peroxidase (POD), and some osmoprotectants such as proline, total soluble proteins (TSP), total free amino acids (TFAA), carbohydrates like sucrose, and, reducing sugars (RS), in mitigating cold stress.

2. Materials and methods

2.1. Cultivation System, experimental design, and treatments

The experiment was carried out in the greenhouse of the Soil Science Department of the Federal University of Lavras (UFLA), located in Lavras, State of Minas Gerais (21° 13' 35.573'' S 44° 58' 43.219'' W), with an altitude of 913.31 m. The local climate is classified as Cwb according to Köppen (Köppen, 1936). It is characterized by a humid temperate climate with a dry winter and moderately hot summer, with the following conditions: an average daytime temperature of 25 °C and nighttime temperature of 15°C, with 57% humidity.

Seeds of the cultivar Avenger were used and put to germinate and grow in polystyrene trays using a commercial substrate and a nutrient solution (Hoagland and Arnorn, 1950) until they reached the ideal transplant stage (45 days after sowing - seedlings with 4-6 fully developed leaves). These conditions are similar to those used in commercial field crops.

The soil used for the experiment (≤ 2 mm soil fraction, collected in the 0-0.20 m layer) was classified as Latossolo Vermelho distrófico, according to the Brazilian soil classification system (Santos and Embrapa Solos, 2018), which corresponds to Ferralsols (Food and Agriculture Organization of the United Nations, 2014) or Oxisols, in the Soil Taxonomy (Survey Staff, 2022), being the last one adopted as the official classification in the present study. The chemical and physical attributes of the soil were determined (Table 1), and an incubation curve (with pure calcium carbonate) was used to assess the amount of lime needed to raise the soil pH from 4.5 to 5.5.

Table 1. Chemical and physical attributes of the Oxisol used in the experiment.

pH	**SOM**	**Clay**	**Silt**	**Sand**	**H+Al**	**Al^{3+}**	**Ca^{2+}**	**Mg^{2+}**
-	------------g kg^{-1}------------				------------cmol$_c$ kg^{-1}------------			
4.5	24.9	670	130	200	3.6	0.60	0.4	0.2
Total N	**P**	**K**	**Zn**	**Fe**	**Mn**	**Cu**	**B**	**S-SO_4**
g kg^{-1}	----------------------------------mg kg^{-1}----------------------------------							
2.3	0.4	24.8	0.2	38.0	3.4	1.2	0.01	2.9

pH in water at a ratio of 1:2.5 (w/v). Soil organic matter (SOM) was determined by the Walkley-Black method, and clay, silt, and sand were assessed by the Boyoucos method. The available contents of potassium (K), phosphorus (P), zinc (Zn), iron (Fe), manganese (Mn), and copper (Cu) were determined by the Mehlich-1 soil test. Calcium (Ca^{2+}) and magnesium (Mg^{2+}) exchangeable contents were extracted by a 1 mol L^{-1} KCl solution-soil test. The available contents of boron (B) were determined by the hot-water extraction method, and the available contents of sulfur (S) were determined by the monocalcium phosphate diluted in acetic acid method.

To determine the optimal timing for Se and I application during cold stress, a foliar spray of Se as Na_2SeO_4 was applied at a concentration of 50 mg Se L^{-1} (reagent grade, Sigma-Aldrich), using 0.7 mL of this solution per plant with 0.2% mineral adjuvant. The concentration of Se applied was based on previous work done on coffee (de Sousa et al., 2022). Foliar application was performed when the plants had 4-6 fully developed leaves. The plants were transferred to the spray chamber to avoid contamination during application. After Se application, broccoli leaf samples were collected on different days (1, 3, 5, 7, 10, 14, and 21 days). The experiment was conducted in a 2 (Se application) x 7 (days of analysis) split plot scheme with three replicates. A single dose of selenium (50 mg Se L^{-1}) was applied to establish how many days after foliar application the activity of the enzymatic antioxidant system in broccoli increased (SOD, CAT, and APX) since various concentrations could interfere with the results of Se application on different days.

Once the time of application was defined, a new experiment was developed to evaluate how the "priming effect" with Se and I could reduce the damage due to cold stress in broccoli seedlings. The experiment was completely randomized, with a 4 x 5 +1 factorial scheme (Se dose x I dose + control treatment). A total of 21 treatments were evaluated, with three replicates per treatment. The selenium doses were 0, 25, 50, and 75 mg L^{-1}; the I doses were 0, 50, 100, 250, and 500 mg L^{-1}, with 0.20% v/v mineral oil. These doses for each element studied were evaluated separately and in combination. The Se source was Na_2SeO_4, sodium selenate (Reagent grade, Sigma Aldrich), and the I source was KIO_3, potassium iodate (Reagent grade, Synth). Foliar application was made when the plants had 4-6 fully developed leaves. Plants were transferred to the spray chamber to avoid contamination during application, receiving 0.7 mL of Se, I, and Se+I foliar solutions. A positive control (PC) and negative control (NC) were applied as additional treatments using distilled water with 0.20% v/v mineral oil, as follows: the PC was not subjected to cold stress, whereas the NC was. The sprayed treatments were transplanted into cups with 100 g of the soil that was previously limed.

Table 2. Description of the treatments (Ti) used for the broccoli seedlings greenhouse experiment.

Treatment (Ti)	Condition	Se	I	Treatment (Ti)	Condition	Se	I
		mg L^{-1}				mg L^{-1}	

T1	-	0	0	T12	+	50	100
T2	+	0	0	T13	+	75	100
T3	+	25	0	T14	+	0	250
T4	+	50	0	T15	+	25	250
T5	+	75	0	T16	+	50	250
T6	+	0	50	T17	+	75	250
T7	+	25	50	T18	+	0	500
T8	+	50	50	T19	+	25	500
T9	+	75	50	T20	+	50	500
T10	+	0	100	T21	+	75	500
T11	+	25	100				

(-) = without cold stress, (+) = with cold stress

One week after foliar application, plants were taken to the growth chambers (Modelo, Conviron, Canada), where they were exposed to the following circumstances for three days: an average daytime temperature of 20 °C (12 h photoperiod, 60% relative humidity and light intensity of 260 µmol m^{-2} s^{-1}) and an average nighttime temperature of 2 °C (12 h photoperiod, 50% relative humidity).

2.2. Sample collection and preparation

Seedlings from the experiment that defined the application time were collected destructively, so each analysis time consisted of one set of plants (4 plants). Next, the samples were placed in liquid nitrogen and stored in an ultra freezer (-80 °C) to evaluate the enzymatic antioxidant system of the plants (SOD, APX, and CAT).

In the study related to the role of Se and I in mitigating cold stress, after the stress, the plants were placed again in the greenhouse, and 24 hours following the stress, the plants were analyzed. All leaves from each treatment were digitized using a flatbed scanner to measure the area affected by cold damage. The scanned leaf images were processed with GIMP 2.10.34 software to determine the stress-damaged leaf area. They were immediately frozen in liquid nitrogen, individually macerated in liquid nitrogen, and stored at -80°C. These samples were used to quantify osmoprotectant parameters sucrose, proline, total free amino acids (TFAA), reducing sugars (RS) and total soluble proteins (TSP), antioxidant enzyme activity (SOD, APX, CAT, and POD), hydrogen peroxide (H_2O_2) and lipid peroxidation (MDA).

2.3. Compatible Osmolytes

Carbohydrates (sucrose and RS), TFAA, and TSP were extracted according to Zanandrea et al. (2009), using 0.05 g of plant material and homogenate in 5 mL of 100 mmol L^{-1} potassium phosphate buffer (pH 7.0). The mixture was then placed in a water bath for 30 min at 40 °C. The homogenate was centrifuged at 5000 × g for 10 min, and the supernatant was collected.

The anthrone method determined total soluble sugars (TSS) and sucrose contents (Dische, 1962). Reducing sugars (RS) were determined by the DNS method (Gail Lorenz Miller, 1959) and TFAA by the ninhydrin method (Yemm, 1955). Total soluble proteins (TSP) in leaves were also determined (Bradford, 1976).

Proline content was determined according to the methodology of Bates. (1973), using 0.05 g of plant material macerated in 3% sulfosalicylic acid. The material was then shaken for 60 min at room temperature, filtered, added to tubes, and placed in a water bath at 100 °C for 60 min. The samples were then transferred to ice for approximately 10 min to stop the reaction. The supernatant was read in a spectrophotometer at 520 nm and quantification was performed using a proline standard curve.

2.4. Antioxidant enzymatic activity

To determine APX, CAT, SOD, and POD enzymes activity, 0.05 g of freshly ground material was weighed in liquid nitrogen and mixed with 1.5 mL of potassium phosphate buffer (0.1 mol L^{-1}, pH 7.8 + 0.1 mol L^{-1} EDTA, pH 7.0, 0.01 mol L^{-1} ascorbic acid). The supernatant was collected for analysis on an Epoch® microplate spectrophotometer (BioTek Instruments, Winooski, VT, USA). Two blanks were used on each readout plate, and samples were kept from 0 to 4 °C to ensure the quality of enzymatic analysis.

Superoxide dismutase (SOD) activity was determined by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium at 560 nm. The sample was composed of 50 mmol L^{-1} potassium phosphate buffer, pH 7.8, 14 mmol L^{-1} methionine, 0.1 µmol L^{-1} EDTA, 75 µmol L^{-1} NBT, 2 µL enzyme extract, and 2 µmol L^{-1} riboflavin (Beauchamp and Fridovich, 1971).

Ascorbate peroxidase (APX) activity was determined using the methodology of Nakano and Asada. (1981). To quantify APX, a solution containing 100 µL ascorbate solution (10 mmol L^{-1}), 100 µL H_2O_2 (30 %), and 100 µL enzyme extract (supernatant) in 2.7 mL sodium phosphate buffer was used. After gentle stirring, the absorbance was read at 290 nm with a time sweep (from 0 to 60 s) using a spectrophotometer.

The reaction solution for POD contained 100 µL of 30 mmol L^{-1} H_2O_2, 100 µL of guaiacol, and 100 µL of enzyme extract (supernatant) in 2.7 mL of sodium phosphate buffer. The same reaction solution used for POD (except guaiacol) was used for CAT activity estimation. The absorbance of POD and CAT samples was observed in a time sweep (from 0 to 60 s) at 470 and 240 nm, respectively, using a spectrophotometer (Maehly and Uflith, 1954).

The enzymatic extraction method determined the total soluble protein content to calculate the specific activity of the antioxidant enzymes. Microplates first received 294 µL of Bradford's solution (Bradford, 1976) in a 1:5 reagent dilution. Readings were taken using an absorbance microplate reader (Epoch-BioTek) at a wavelength of 595 nm, and the results were obtained from a calibration curve with BSA.

2.5. H_2O_2 and MDA content

A mass of 0.05 g of fresh material was collected in triplicate from each treatment and macerated in a mortar with liquid nitrogen, then homogenized in 1500 µL trichloroacetic acid (TCA) and centrifuged at 12,000 × g for 15 min at 4 °C. The H_2O_2 content was determined by potassium phosphate (pH 7.0), and its absorbance was read on a spectrophotometer at 390 nm (Velikova et al., 2000). The quantification of MDA was performed according to Buege. (1978). An aliquot of 125 µL of the extraction supernatant was collected and pipetted into a 1500 µL microtube containing 250 µL of the following reaction medium: 0.5% thiobarbituric acid (TBA) and 10% TCA. Next, 350 µL of the reaction medium was collected, pipetted into microplates, and read on a spectrometer at 535 and 600 nm.

2.6. Statistical analysis

Statistical analysis was performed using the R software (Lê et al., 2008a) with the package's stats, agricolae, corrplot, factoextra, FactoMineR, and Metrics R (Kassambara and Mundt, 2020; Lê et al., 2008a, 2008b; Wei and Simko, 2021). First, an exploratory data analysis was performed to corroborate the existence of outliers. Next, the assumptions test (homogeneity, homoscedasticity) and analysis of variance (ANOVA) were performed. Means were compared using the Scott-Knott test ($p < 0.05$). In addition, a principal component analysis (PCA) was conducted to determine the correlation of the variables measured for Se, I, separately and combined with both elements under cold stress conditions. A Pearson correlational analysis ($p < 0.05$) was also performed to validate the correlations observed in the PCAs.

3. Results

3.1. Time of application

The definition of the time elapsed between Se application and cold stress simulation was based on the data in figure 1, where it can be observed that at 7 days after Se application, catalase (CAT) levels and ascorbate peroxidase (APX) activity were higher with Se application compared with no Se application. As for SOD activity, at 7 days, there was no significant difference between Se application and no Se application, an effect that was only observed at 1 and 5 days after Se application.

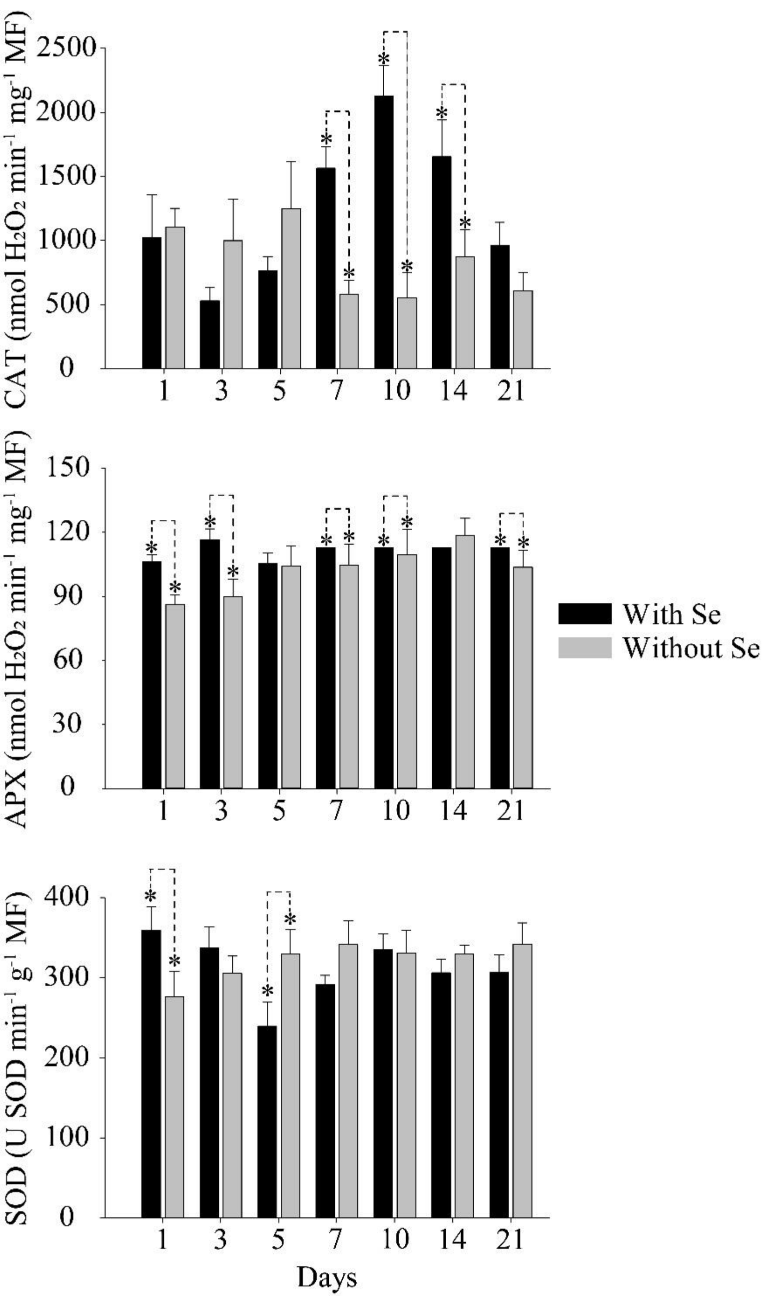

Figure 1. Evaluation of (A) catalase (CAT), (B) ascorbate peroxidase (APX), and (C) superoxide dismutase (SOD) activity across days as a function of foliar application of selenium (Se) (with and without Se). *Means differ on each day evaluated by t-test ($p < 0.05$). BoxPlot chart represents the means (n = 3).

3.2. Leaf and oxidative damage in cold stress

Once the time between Se application and exposure to cold stress (7 days) was defined (Figure 1), it was possible to observe that Se and I applications were able to attenuate the damage caused by cold stress (Figure 2). Among all treatments exposed to cold stress and sprayed with both elements, Se75 (75 mg Se L^{-1}), Se75+I50 (75 mg Se L^{-1} + 100 mg I L^{-}

^{1}), and I100 (100 mg I L^{-1}) were found to have the lowest percentages of leaf damage at 2.64, 3.11, and 9.05%, respectively compared with the treatment without application of Se and I exposed to stress (NC), which presented 31.72% leaf damage. The highest values were found in treatments Se25, Se50, and I250, similar to NC (Figure 2 A).

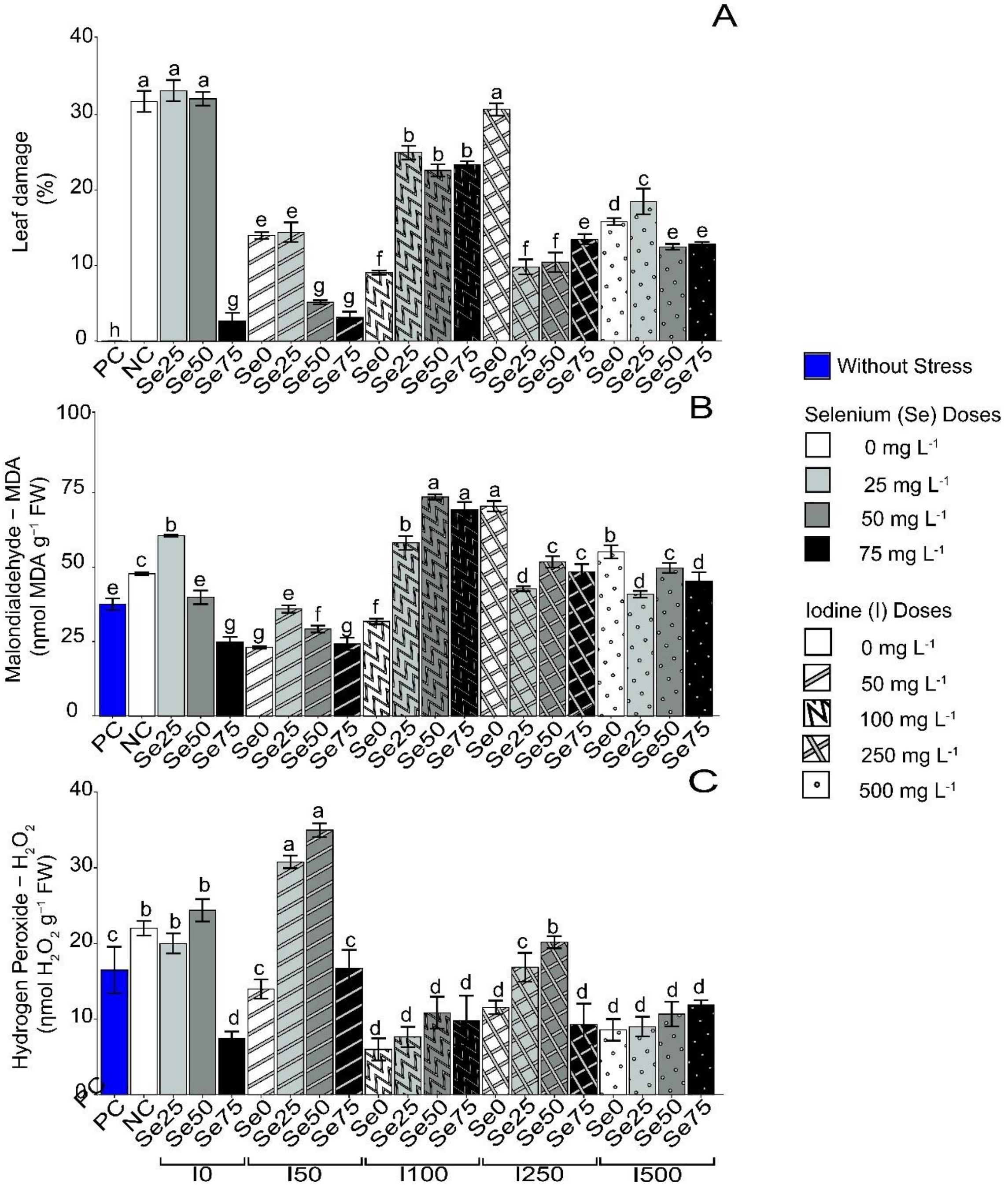

Figure 2. Effect of selenium (Se) and iodine (I) application under cold stress on broccoli seedling leaves on oxidative damage, collected one day after stress. (A) Leaf damage, (B) Malondialdehyde - MDA, and (C) Hydrogen peroxide - H_2O_2. PC: positive control, and NC: negative control. Equal letters indicate no significant differences calculated using Scott Knott's test ($p > 0.05$). The error bars represent the standard error of the means (n = 3).

Concerning MDA, its content increased by 27% in NC compared with the group that was neither exposed to stress nor sprayed (PC). Treatments Se50, Se75, I50, Se25+I50, Se50+I50, Se75+I50, I100 presented lower values than NC (47.8 nmol MDA g^{-1} FW). Among them, treatments Se75, I50, and Se75+I50 decreased (~49.7%) compared with NC. The remaining treatments presented values similar to or higher than NC (Figure 2B). Regarding H_2O_2, Se25+I50 and Se50+I50 presented the highest levels (30.8 and 35 µmol H_2O_2 mg^{-1}, respectively). Similarly, Se25, Se50, and Se50+I250 presented values similar to NC (22 µmol H_2O_2 mg^{-1}). The remaining treatments presented values comparable to or lower than PC (16.5 µmol H_2O_2 mg^{-1}) (Figure 2C).

3.3. Antioxidant Enzymatic Activity (SOD, CAT, POD, APX)

Regarding the enzymatic antioxidant system, POD, CAT, and APX presented higher values in NC in contrast to PC, except for SOD, which presented lower values (Figure 3).

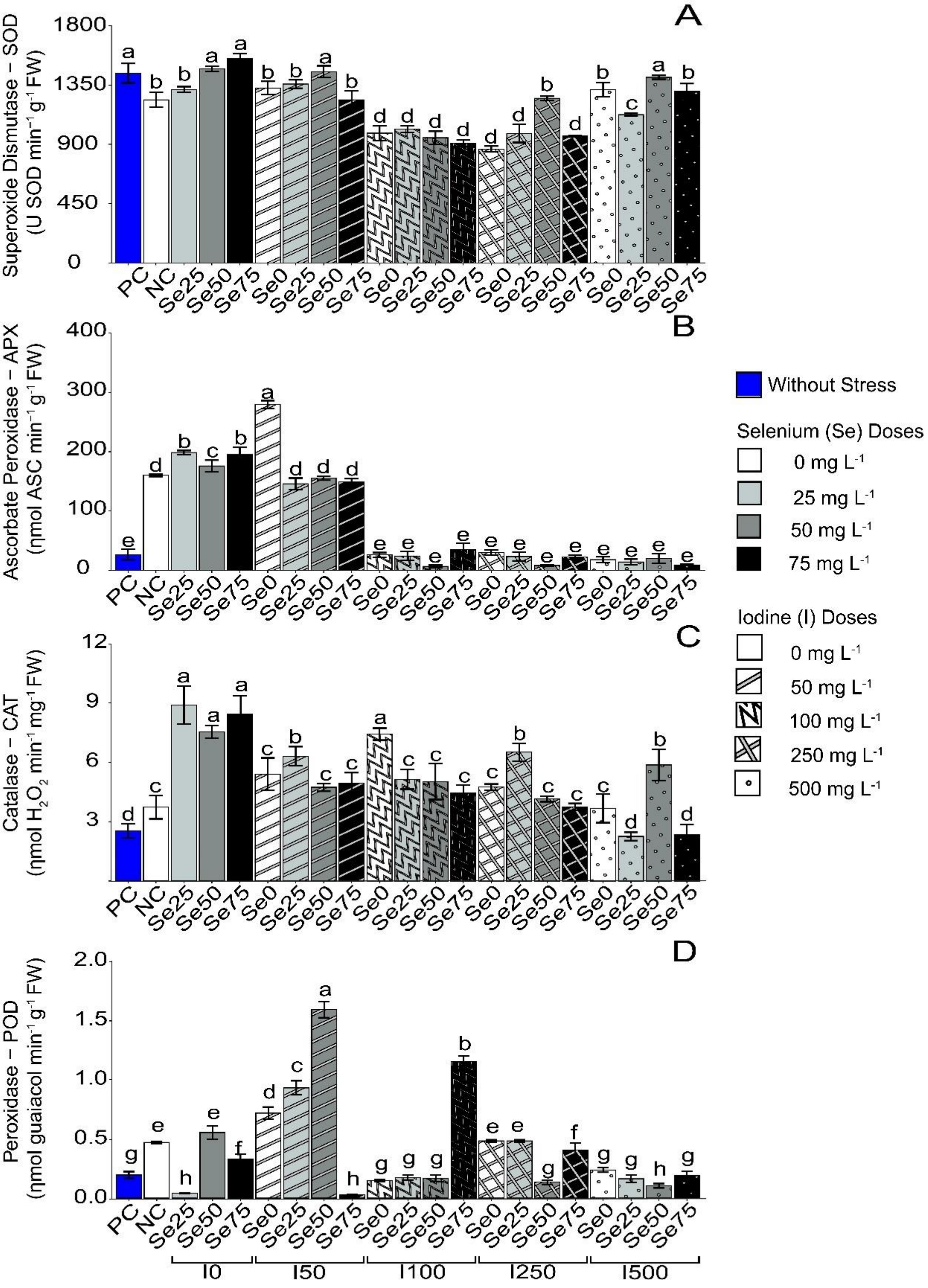

Figure 3. Effect of selenium (Se) and iodine (I) application on the antioxidant enzyme activity of broccoli seedlings collected one day after stress. (A) superoxide dismutase - SOD, (B) ascorbate peroxidase - APX, (C) catalase - CAT, and (D) peroxidase - POD. PC: positive control, and NC: negative control. Equal letters indicate no significant differences calculated using the Scott Knott test (*p* > 0.05). The error bars represent the standard error of the means (n = 3).

In SOD, activity was statistically similar in Se50, Se75, Se50+I50, and Se50+I500 compared with PC (1440.2 U SOD min^{-1} g^{-1} FW). Similarly, Se25, I50, Se25+I50, Se75+I50, Se50+I250, I500, and Se75+I500 showed results comparable to NC (1238.9 U SOD min^{-1} g^{-1} FW). The remaining treatments showed lower values than PC and NC (Figure 3A). For APX activity, statistically superior results ($p < 0.05$) were obtained in Se25, Se50, Se75, and I50, among which I50 showed the highest value (279.3 µmol ASA min^{-1} g^{-1} FW), for PC and NC (26.6 and 160.2 µmol ASA min^{-1} g^{-1} FW, respectively).

Treatments Se25+I50, Se50+I50, and Se75+I50 obtained results similar to those of NC. The remaining treatments were statistically identical to PC (Figure 3B). In addition, the highest CAT values were found in Se25, Se50, Se75, and I100, increasing by 85.5 and 180.2 % concerning NC and PC, respectively. On the other hand, Se25+I50, Se25+I250, and Se50+I500 presented similar values (~6.1 µmol H_2O_2 min^{-1} mg^{-1} FW). The remaining treatments presented values comparable to or lower than PC and NC (Figure 3C). In turn, POD activity was higher in I50, Se25+I50, Se50+I50, and Se75+I100, among which Se50+I50 presented the highest value (1.6 nmol guaiacol min^{-1} g^{-1} FW) compared with PC and NC (0.2 and 0.5 nmol guaiacol min^{-1} g^{-1} FW, respectively). The remaining treatments presented values similar to or lower than NC. Se75 decreased by 30.5% compared with NC and increased by 67.7% compared with PC (Figure 3D).

3.4. Osmolytes in cold stress

Concerning osmolytes, the level of sucrose in Se75 was the highest value observed among the treatments, with Se and I application being similar to NC (3.7 µmol of glucose mg^{-1} FW) and higher than PC (1.6 µmol of glucose mg^{-1} FW). The remaining treatments showed lower concentrations than NC (Figure 4A). Concerning RS, NC (4.6 mmol glucose g^{-1} FW) presented the highest value, followed by Se25+I50 and Se50+I500 (3.7 and 3.8 mmol glucose g^{-1} FW, respectively), among all treatments subjected to cold stress. Treatments Se25, Se75, Se75+I50, and Se25+I250 presented similar values and were significantly higher than those found in PC (1.9 mmol glucose g^{-1}) (Figure 4B).

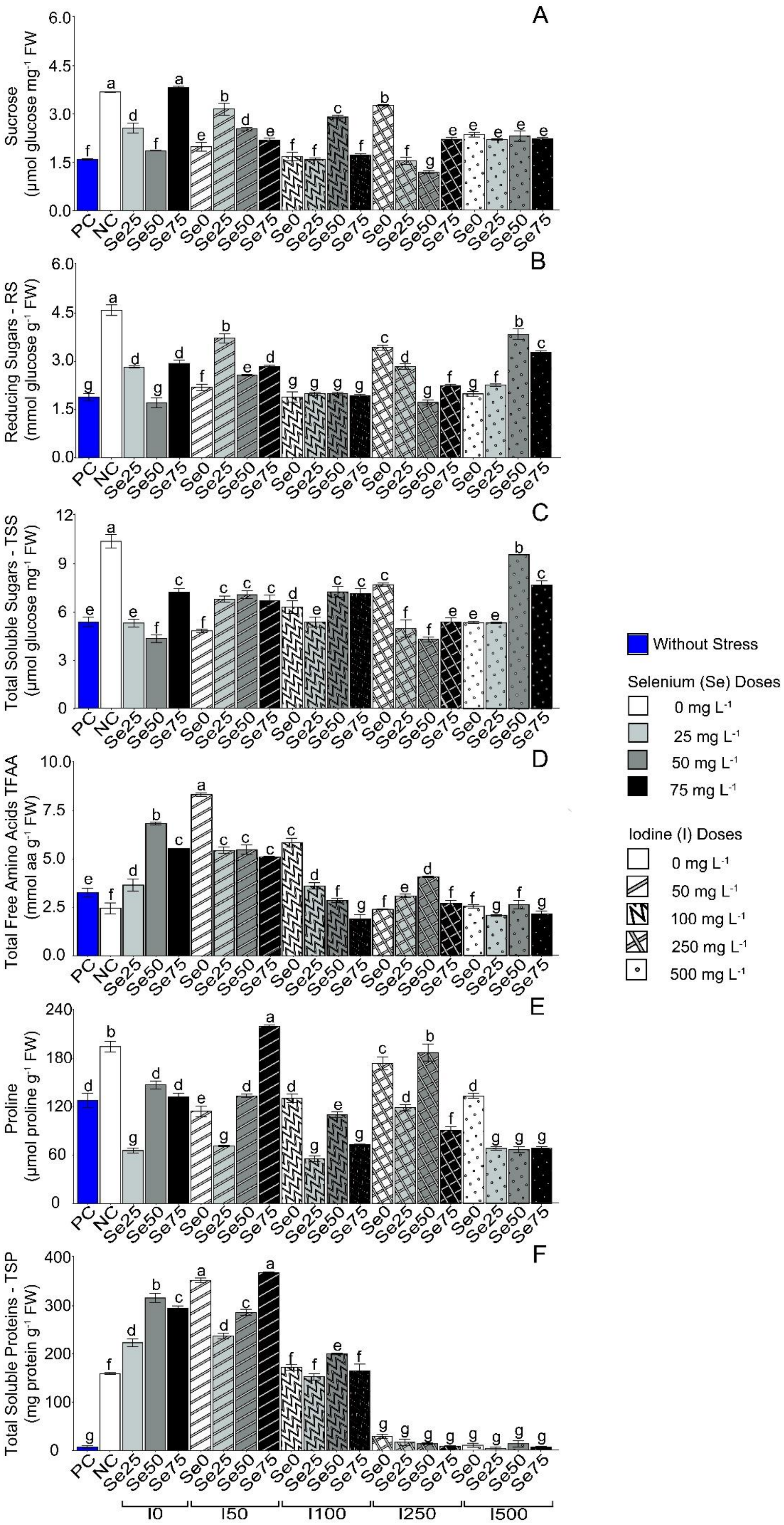
A
Sucrose (µmol glucose mg⁻¹ FW)
6.0
4.5
3.0
1.5
0.0
f a d f a e b d e f f c f b f g e e e e e
B
Reducing Sugars - RS (mmol glucose g⁻¹ FW)
6.0
4.5
3.0
1.5
0.0
g a d g d f b e d g g g g c d g f g f b c
C
Total Soluble Sugars - TSS (µmol glucose mg⁻¹ FW)
12
9
6
3
0
e a e f c f c c c d e c c c f f e e e b c
D
Total Free Amino Acids TFAA (mmol aa g⁻¹ FW)
10.0
7.5
5.0
2.5
0.0
e f d b c a c c c c d f g f e d f f g f g
E
Proline (µmol proline g⁻¹ FW)
240
180
120
60
0
d b g d d e g d a d g e g c d b f d g g g
F
Total Soluble Proteins - TSP (mg protein g⁻¹ FW)
400
300
200
100
0
g f d b c a d c a f f e f g g g g g g g g
PC NC Se25 Se50 Se75 Se0 Se25 Se50 Se75 Se0 Se25 Se50 Se75 Se0 Se25 Se50 Se75 Se0 Se25 Se50 Se75
I0
I50
I100
I250
I500
Without Stress
Selenium (Se) Doses
0 mg L⁻¹
25 mg L⁻¹
50 mg L⁻¹
75 mg L⁻¹
Iodine (I) Doses
0 mg L⁻¹
50 mg L⁻¹
100 mg L⁻¹
250 mg L⁻¹
500 mg L⁻¹

Figure 4. Effect of the application of selenium (Se) and iodine (I) on (A) Sucrose, (B) Reducing sugars - RS, (C) Total soluble sugars - TSS, (D) Total free amino acids - TFAA, (E) Proline, (F) Total soluble proteins - TSP. PC: positive control, and NC: negative control. Equal letters indicate no significant differences calculated using the Scott Knott test ($p > 0.05$). The error bars represent the standard error of the means (n = 3).

Regarding TSS, NC (10.4 µmol glucose mg^{-1} FW) presented the highest value concerning this variable. Treatments Se50, I50, Se25+I250, and Se50+I250 presented lower values than PC and NC (5.4 and 10.4 µmol glucose mg^{-1} FW, respectively). The other treatments presented concentrations equal to or higher than PC (Figure 4C).

In the TFAA variable, I50 (8.3 mmol aa g^{-1} FW) presented the highest concentration, followed by Se50 (6.8 mmol aa g^{-1} FW). Se75, Se25+I50, Se50+I50, Se75+I50, and I100 presented statistically similar results (~8.3 mmol aa g^{-1} FW) and higher by 67.7 and 122.3 % in contrast to PC and NC, respectively (Figure 4D).

Se75+I50 presented the highest proline content among the treatments (220 µmol proline g^{-1} FW), followed by Se50+I250, which presented similar values to NC (195 µmol proline g^{-1} FW). In addition, Se50, Se75, Se50+I50, I100, Se25+I250, and I500 presented statistically similar values ($p < 0.05$) to PC (128.2 µmol proline g^{-1} FW), the rest of the treatments presented lower levels than PC (Figure 4E).

Concerning total soluble proteins (TSP), treatments I50 and Se75+I50 presented the highest values at 352.6 and 368.5 mg protein g^{-1} FW, respectively, followed by Se50, Se75, and Se50+I50 (316.2, 295.2, and 286.2 mg protein g^{-1} FW, respectively). In addition, it was observed that I100, Se25+I100, and Se75+I100 were statistically similar ($p < 0.05$) to NC (159.37 mg protein g^{-1} FW). Likewise, Se25 and Se25+I50 were statistically similar ($p < 0.05$) to each other (230.2 mg protein g^{-1} FW). The remaining treatments obtained values comparable to those in PC (7 mg protein g^{-1} FW) (Figure 4F).

4.4. Multivariate Analysis

Principal component analysis (PCA) of the application of Se and I were performed separately and in combination for the variables of osmolytes, enzyme activity, oxidative damage, and percentage of leaf damage with and without stress treated with different concentrations of Se and I.

For the PCA of the treatments in which only Se was applied (Figure 5), the principal components PC1 and PC2 accounted for 71.9% of the total variance of the data. The biplot showed a strong positive relationship between H_2O_2, MDA, and percentage leaf damage. In turn, H_2O_2 and MDA were negatively correlated with sucrose and SOD, respectively. This result is interesting because SOD is the first enzymatic antioxidant barrier, which decomposes superoxide anion into hydrogen peroxide, a less harmful ROS, and thus can reduce cell damage caused by ROS. Furthermore, the negative correlation with MDA demonstrates reduced cell damage through decreased lipid peroxidation. The negative correlation between SOD and leaf damage demonstrates this. Among the treatments applied, the dose of 75 mg Se L^{-1} (Se75) possibly favored the SOD content. Concerning TFAA, a high positive correlation was observed with total soluble protein content.

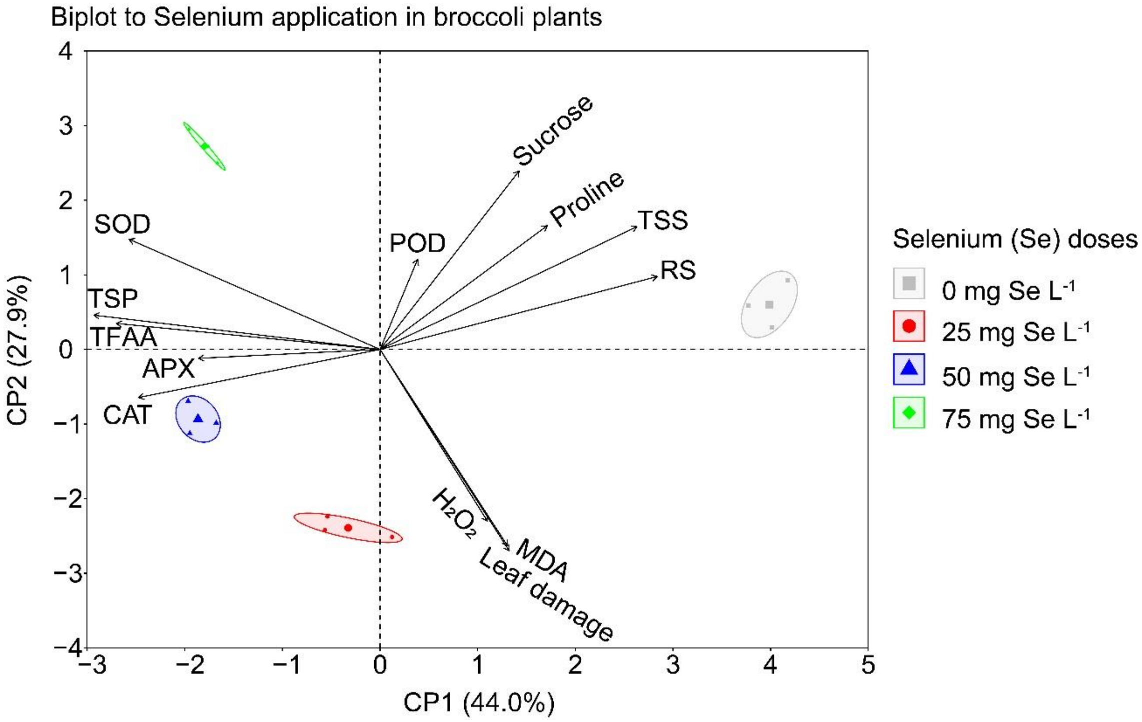

Figure 5. Principal component analysis (PCA) using data on antioxidant activity, percent leaf damage, and oxidative damage under cold stress conditions in separate selenium (Se) applications. Reducing sugars (RS); Total soluble sugars (TSS); Total free amino acids (TFAA); Total soluble proteins (TSP); Superoxide dismutase (SOD); Ascorbate Peroxidase (APX); Catalase (CAT); Peroxidase (POD); Lipid peroxidation (MDA); Hydrogen peroxide (H_2O_2).

For the PCA of the treatments that only applied I (Figure 6), the principal components PC1 and PC2 accounted for 81% of the total variance of the data. The MDA and H_2O_2 variables were positively correlated with the percentage of leaf damage. In the same way, the following negative correlations were observed for H_2O_2 with CAT, as well as MDA with TFAA, APX, and total soluble protein content. The treatment with 250 mg L^{-1} I may have influenced the MDA content. In addition, the higher CAT activity is related to applying 100 mg L^{-1} I. The positive correlation between H_2O_2, MDA, and leaf damage shows that as H_2O_2 increases, lipid peroxidation increases, which is demonstrated by the increase in MDA, and consequently, there is more significant leaf damage. On the other hand, there is a negative correlation between these variables (H_2O_2, MDA, and leaf damage) and CAT. However, these results are interesting because CAT is an enzyme whose activity increases under high concentrations of H_2O_2, which could indicate that higher activity reduces ROS and decreases the damage caused on broccoli seedling leaves.

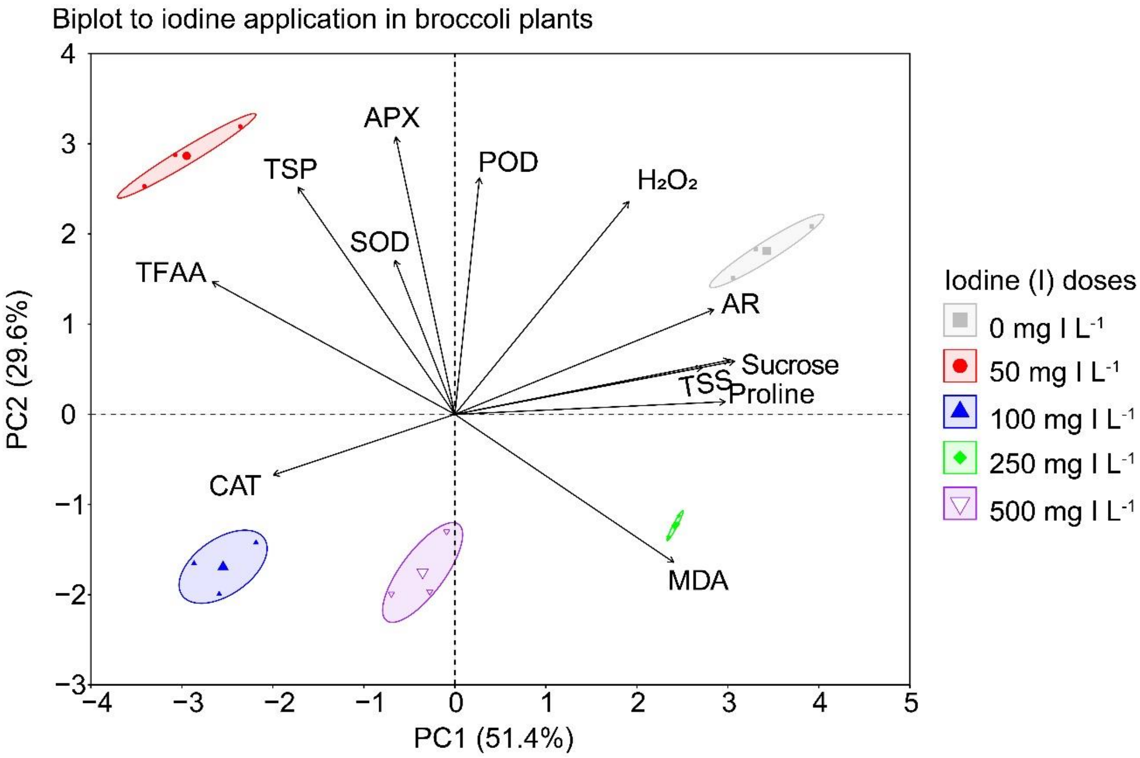

Figure 6. Principal component analysis (PCA) using data on antioxidant activity, percent leaf damage, and oxidative damage under cold stress conditions and iodine application separately. Reducing sugars (RS); Total soluble sugars (TSS); Total free amino acids (TFAA); Total soluble proteins (TSP); Superoxide dismutase (SOD); Ascorbate Peroxidase (APX); Catalase (CAT); Peroxidase (POD); Lipid peroxidation (MDA); Hydrogen peroxide (H_2O_2).

For the PCA of the Se and I treatments (Figure 7), the principal components PC1 and PC2 accounted for 58.4% of the total variance of the data. MDA presented a positive correlation with the percentage of leaf damage. In addition, for MDA, a negative correlation was observed with the variables TFAA, APX, proline, SOD, and RS. Moreover, for the percentage of damage, a negative correlation was observed with SOD and TFAA. Moreover, the dose of 25 mg Se L^{-1} + 50 mg I L^{-1} tends to influence the content of APX, total soluble proteins, and SOD, and the concentration of 75 mg Se L^{-1} + 50 mg I L^{-1} tends to influence the content of TFAA.

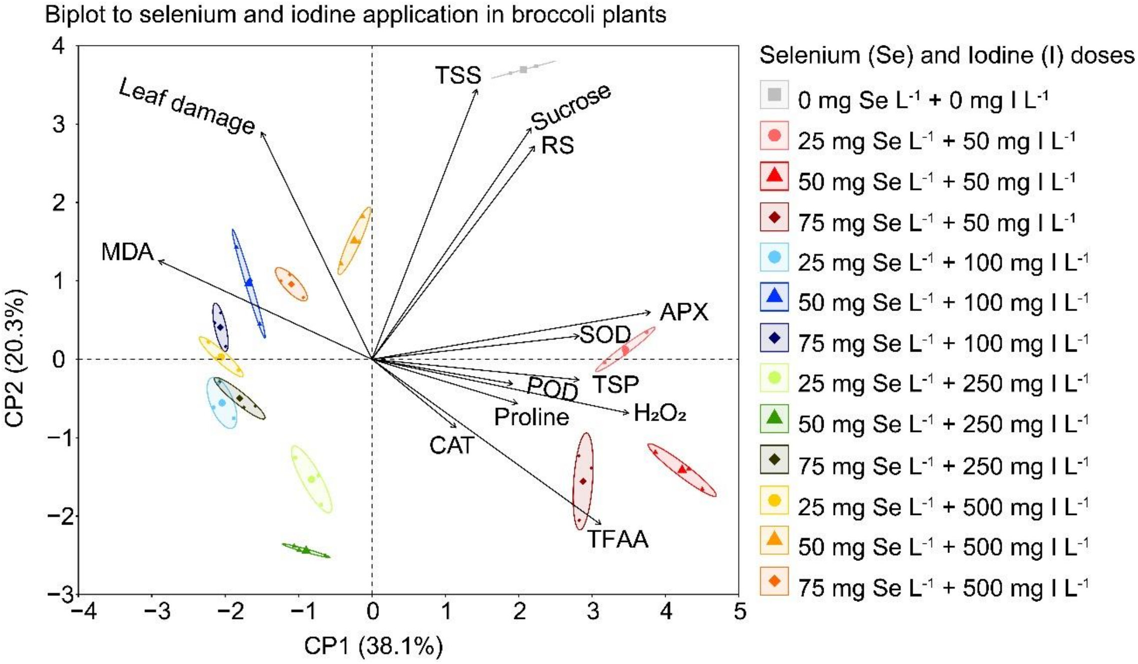

Figure 7. Principal component analysis (PCA) using data on antioxidant activity, percent leaf damage, and oxidative damage under cold stress conditions and combined application of selenium (Se) and iodine (I). Reducing sugars (RS); Total soluble sugars (TSS); Total free amino acids (TFAA); Total soluble proteins (TSP); Superoxide dismutase (SOD); Ascorbate Peroxidase (APX); Catalase (CAT); Peroxidase (POD); Lipid peroxidation (MDA); Hydrogen peroxide (H_2O_2).

4. DISCUSSION

Isolated and combined applications of Se and I visually attenuated cold damage in broccoli seedlings. Currently, there is plenty of information on the use of Se to fight plant stress (de Sousa et al., 2022; Han et al., 2022; Sousa et al., 2023), and recently, work is being done with I since its role in plant metabolism for stress attenuation has yet to be understood (Lima et al., 2023a, 2023b). However, information on the interaction of both elements is scarce, and whether these two elements have a synergistic or antagonistic interaction is unknown. Through this work, we wanted to select the best treatments of Se, I, and the interaction between both elements.

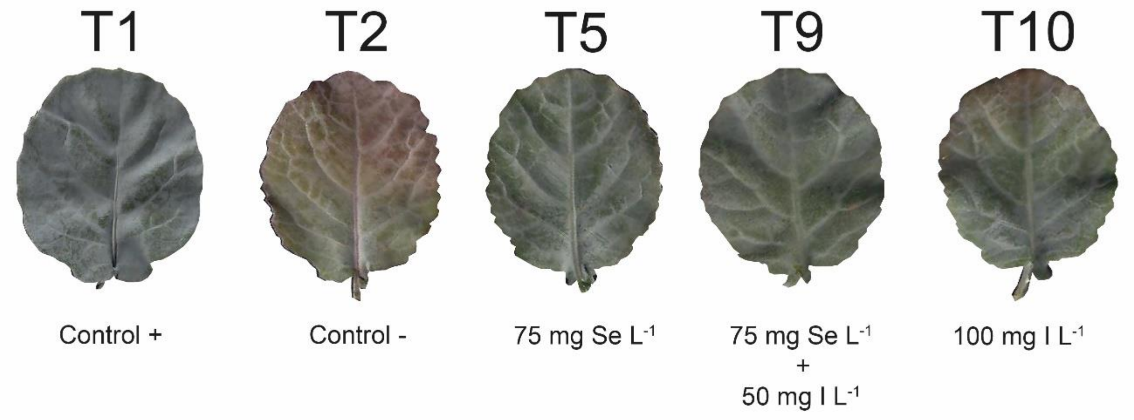

Figure 8. Visual damage to leaves of broccoli seedlings.

Several studies have found that pre-applying beneficial elements to seeds/seedlings before the stress can stimulate increased growth or yield, a response known as the "priming effect" (Sousa et al., 2023; Wiszniewska, 2021; Zulfiqar et al., 2022). The present work found that on the seventh day after foliar application of Se (50 mg L^{-1}), the highest values of CAT and APX were obtained. However, there was no difference in SOD activity (Figure 1) when the plants were subjected to 2 ºC. Similar results were found in coffee plants subjected to osmotic stress when Se was foliar-applied 8 days before the stress. They exhibited high values of CAT, APX, and SOD in 771.1, 356.3, and 266.5%, respectively, compared with plants subjected to stress and without Se (Sousa et al., 2023). This result may indicate that the pre-application of Se and I can be a strategy to counteract the detrimental effects of low temperatures on broccoli seedlings.

The brown coloration of leaves is related to H_2O_2 accumulation (Hawrylak-Nowak et al., 2018) since MDA and H_2O_2 content tends to increase in plants when exposed to low temperatures (Liu et al., 2021; Wang et al., 2023). In this study, leaf coloration changed from yellow to brown in broccoli seedlings exposed to cold (Figure 8), which is visual evidence of damage caused by low temperatures. Our results indicated that, among the treatments evaluated, Se75 (75 mg Se L^{-1}), Se75+I50 (75 mg Se L^{-1} + 100 mg I L^{-1}), and I100 (100 mg I L^{-1}) showed the lowest percentages of leaf damage, 2.64, 3.11, and 9.05%, respectively. In a study on coffee sprayed with Se (80 mg Se L^{-1}) and exposed to 4 °C, the scale of the damage was lower when Se was applied, and the opposite occurred when Se was not used (de Sousa et al., 2022). This behavior was also observed in lettuce *(Lactuca sativa)* leaves exposed to high temperatures (35/22 °C; day/night), with leaf damage lower with Se application (Hawrylak-Nowak et al., 2018). Likewise, leaves of Nicotiana tabacum L. presented less damage with the application of Se under drought stress (Han et al., 2022). This result demonstrates that the application of Se and I can visually decrease the damage in broccoli seedling leaves induced by low temperatures by reducing the levels of MDA and H_2O_2 (Figure 2). This fact is interesting because the appearance of produce is as important as its taste and is the first barrier to purchasing and consuming the product.

To alleviate oxidative stress induced by abiotic stress, plants have developed mechanisms to scavenge free radicals such as ROS (Han et al., 2022) by using antioxidant enzymes such as SOD, CAT, APX, POD, and osmolytes (Jiang et al., 2023; Lima et al., 2023b). The PCAs of this study show that treatments with Se, I, and Se+I combination act differently on low-temperature stress response mechanisms. Our results indicated that Se75 (75 mg Se L^{-1}) influenced SOD activity and sucrose content. The treatment I100 (100 mg I L^{-1}) influenced the production of enzymes such as CAT and APX, as well as osmoprotectants such as TFAA and total soluble proteins. In comparison, Se75+I50 (75 mg Se L^{-1} + 50 mg I L^{-1}) influenced the levels of SOD, APX, TFAA, proline, and RS. This result suggests that the adverse effects of abiotic stress can be mitigated by foliar application of Se and I to reduce ROS, thus increasing tolerance to low temperatures in broccoli seedlings.

With the application of Se and I, the MDA and H_2O_2 content decreased significantly in Se75, Se75+I50, and I100, by 48, 49 and 33% for MDA and 66, 23 and 72% for H_2O_2 respectively, compared with the stressed group and without foliar application (NC) (Figure 2). Similar results were found in tea plants exposed to low temperatures, where MDA and H_2O_2 content were reduced by 31.59 and 23.94 %, respectively, with Se (2 mg L^{-1}) compared with the group exposed to stress without Se. The authors attributed this to improved photosynthesis in the plant. In addition, Se application increased the activity of antioxidant enzymes such as SOD, POD, and APX by 20, 30 and 15%, respectively (Liu et al., 2021). Also, in lettuce, applying Se improved the plants' antioxidant defense by reducing the bioaccumulation of H_2O_2 when exposed to high temperatures (Hawrylak-Nowak et al., 2018). Huang et al. (2018) found that strawberry plants exposed to Se at low concentrations (5 mg L^{-1}) increased SOD, CAT, and POD activity, which alleviated the oxidative stress caused by cold (Medrano Macías et al., 2021). This happens because SOD is the first line of defense used by the plant against stress, as it catalyzes the conversion of O_2^-, which is a highly reactive element- into a less reactive one such as H_2O_2 (Tavanti et al., 2021). This molecule - H_2O_2 - is then broken down by APX and CAT to be converted into H_2O and O_2, which decreases free radicals (Sies and Jones, 2020), thus increasing plant resistance against stress.

In addition to antioxidant enzymes, plants can react to stress by accumulating osmolytes, such as carbohydrates (TSS, sucrose, RS), proteins, and amino acids (proline), which contribute to stress tolerance (Majumder et al., 2010; Suprasanna et al., 2015). Several studies have found that exogenous application of beneficial elements can increase osmolyte content (Bai et al., 2024). In our research, the foliar application of Se and I was found to influence carbohydrates (sucrose, RS), TFAA, proline, and TSP content (Figures 5, 6, and 7). Similar results were found in coffee exposed to 4 °C, where Se reduced visual damage by ~ 24% compared with the non-Se stressed group. This result is probably attributed to the fact that foliar application of Se reduced coffee leaf injury primarily through increased carbohydrate (TSS, starch, and sucrose) and amino acid contents (de Sousa et al., 2022).

Since osmoprotectants can reduce water's freezing point by interfering with water's ability to form ice crystals (Majumder et al., 2010), thus they also maintain cell turgor or osmotic balance, stabilizing the membranes and preventing electrolyte leakage. This condition causes ROS levels to be in normal ranges, avoiding the triggering of oxidative stress (Suprasanna et al., 2015). It was also found that, in tomato plants, Se induced the accumulation of soluble sugars, reducing sugar and sucrose under cold stress (Bai et al., 2024). Likewise, research has shown that after Se application in maize and tobacco under water stress, the content of proline, protein, free amino acids, and soluble sugars increased under water stress (Bocchini et al., 2018; Han et al., 2022). This result indicates that exogenous application of beneficial elements is an efficient method for maintaining osmotic pressure to cope with different crop stresses.

Like Se, I is recently being used to combat abiotic stress, as reported in tomato (Lima et al., 2023a) and soybean (Lima et al., 2023b) crops where MDA content was reduced by 56 and 18%, respectively, compared with plants without KI application, both under water stress. Stress tolerance in tomatoes may be because applying 100 μmol L^{-1} increased the photosynthetic and electron transport efficiency rates by 224 and 51%, respectively. It also increased SOD and CAT content by 127 and 124%, respectively. Also, some osmolytes increased their content, such as TFAA (146%) and proline (132%), compared with the stressed group without KI. As in tomato plants, in soybean the application of low concentrations of KI (10 µmol L^{-1}) promoted an increase in antioxidant enzyme activity (APX, CAT, and POD), promoting better protection of the photosynthetic apparatus, which resulted in the rise of biomass (Lima et al., 2023a). These contrasting results mentioned above for Se and I may be due to the different responses of each crop to various abiotic stresses and their varying abilities to mitigate these stresses through the exogenous application of Se and I.

5. CONCLUSIONS

The present work has shown that isolated foliar application of Se, I, and the combination Se+I effectively alleviated low-temperature stress in broccoli seedlings in a dose-dependent manner. Seven days after foliar application of Se, spraying showed the highest content of enzymes such as CAT and APX. Treatments Se75 (75 mg Se L^{-1}), Se75+I50 (75 mg Se L^{-1} + 100 mg I L^{-1}), and I100 (100 mg I L^{-1}) showed the lowest percentages of leaf damage at 2.64, 3.11, and 9.05%, respectively. Furthermore, it was found that the exposure of broccoli seedlings to Se, I, and the Se+I combination acted differently to decrease MDA, H_2O_2, and, consequently, the percentage of leaf damage. Selenium influenced SOD activity and sucrose content. Iodine influenced the production of enzymes such as CAT and APX and osmoprotectants such as TFAA and total soluble proteins. The combination of Se+I influenced the activity of SOD, APX, as well as the content of TFAA, proline, and RS. This condition indicates that these elements, separately and combined, act on different response mechanisms in broccoli seedlings. Further studies with different cultivars are recommended, as well as studies to identify the genes activated by selenium and iodine exposure involved in cold tolerance.

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FINAL CONSIDERATIONS

Abiotic stress is one of the main constraints to agriculture and global food security is abiotic stress. Drought, salinity, temperature extremes, and flooding negatively impact crops. In response to these various abiotic stressors, plants deploy a series of adaptive physiological mechanisms to maintain homeostasis and minimize cellular damage to them. The ability of plants to cope and adapt is crucial to sustain agricultural productivity. In this sense using of selenium and iodine as abiotic stress mitigators emerges as a promising strategy. Based on their role in antioxidant protection and regulation of physiological and biochemical processes in plants. This research is crucial for scientific development as it will allow us to clarify the biochemical mechanisms that broccoli plants use to adapt to low temperatures when exposed to Se, I and Se+I. As for the technological dimension, it inspires the development of innovative strategies to potentiate the plant's response under stress. Moreover, this allows for expanding knowledge and providing practical solutions to face the challenges of climate change, thus contributing to food security.