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Use of selenium to increase antioxidant activity and water use efficiency in arugula (*Eruca vesicaria* ssp. Sativa) exposed to drought stress

Uso de selênio para aumentar a atividade antioxidante e a eficiência do uso de água em rúcula (*Eruca vesicaria* ssp. Sativa) exposta ao estresse hídrico

Uso de selenio para aumentar la actividad antioxidante y la eficiencia del uso del agua en rúcula (*Eruca vesicaria* ssp. Sativa) expuesta al estrés por sequía

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Danielle Paula de Oliveira Mangarotti
ORCID: https://orcid.org/0000-0002-4515-8077
Universidade Estadual de Maringá, Brasil
E-mail: danipbio@yahoo.com.br
Roberto Rezende
ORCID: https://orcid.org/0000-0002-6213-1845
Universidade Estadual de Maringá, Brasil
E-mail: rrezende@uem.br
Reni Saath
ORCID: https://orcid.org/0000-0002-6610-2873
Universidade Estadual de Maringá, Brasil
E-mail: rsaath@uem.br
Tiago Luan Hachmann
ORCID: https://orcid.org/0000-0002-1298-5682
Universidade Estadual de Maringá, Brasil
E-mail: tiagohach@gmail.com
Paula Toshimi Matumoto-Pintro
ORCID: https://orcid.org/0000-0002-9182-5758
Universidade Estadual de Maringá, Brasil
E-mail: ptmpintro@uem.br
Fernando Antônio Anjo
ORCID: https://orcid.org/0000-0001-6886-0304
Universidade Estadual de Maringá, Brasil
E-mail: fernandoaanjo@hotmail.com

Abstract

Environmental stress can directly or indirectly affect the formation of reactive oxygen species. Oxidative stress damages cell constituents such as carbohydrates, lipids, nucleic acids and proteins, reducing plant growth, respiration and photosynthesis. In recent decades, evidence has shown that small doses of selenium act as an antioxidant and plant biostimulant, promoting growth and improving resistance to abiotic stress such as drought. As such, the aim of this study was to assess the effect of selenium foliar feeding (0, 150 and 300 ppm) on the antioxidant activity, water use efficiency and yield traits of arugula grown with and without drought stress (50% and 100% ETc) in a protected environment. A randomized block design was used, with a 2x3 factorial scheme and four repetitions. Antioxidant activity increased in treatments with 150 ppm of fertilizer and exposure to drought stress. Plants in these treatments obtained higher water use efficiency, yield and leaf area values than those not submitted to drought stress.

Keywords: *Eruca sativa;* Biostimulant; Reactive oxygen species; Environmental stress.

Resumo

O estresse ambiental tem efeito direto ou indireto na formação de espécies reativas de oxigênio. Esse estresse oxidativo danifica constituintes celulares, como carboidratos, lipídios, ácidos nucléicos e proteínas que reduzem o crescimento das plantas, a respiração e a fotossíntese. As evidências das últimas décadas demonstraram que em pequenas doses o selênio é considerado um antioxidante, agindo como um bioestimulante da planta, promovendo o crescimento e aumentando a resistência a estresses abióticos, tais como, déficit hídrico. Dessa forma, o objetivo deste trabalho foi avaliar o efeito da aplicação de adubação foliar de selênio (0, 150 e 300 ppm) sobre a atividade antioxidante, eficiência do uso da água e características produtivas em rúcula cultivada com e sem déficit hídrico (50% e 100% ETc) em ambiente protegido. O delineamento experimental foi em blocos casualizados, em esquema fatorial 3x2 com quatro repetições. Houve um aumento das atividades antioxidantes nos tratamentos que receberam adubação com a dose de 150 ppm e foram expostos ao estresse hídrico. Neste mesmo tratamento as plantas superaram os valores de eficiência do uso da água, produtividade e área foliar das plantas que não foram expostas ao déficit hídrico.

Palavras-chave: *Eruca sativa;* Bioestimulante; Espécies reativas de oxigênio; Estresses ambientais.

Resumen

El estrés ambiental tiene un efecto directo o indirecto sobre la formación de especies reactivas de oxígeno. Este estrés oxidativo daña los componentes celulares, como carbohidratos, lípidos, ácidos nucleicos y proteínas que reducen el crecimiento, la respiración y la fotosíntesis de las plantas. La evidencia de las últimas décadas ha demostrado que en pequeñas dosis el selenio se considera un antioxidante, actuando como bioestimulante de la planta, promoviendo el crecimiento y aumentando la resistencia a los estreses abióticos, como el déficit hídrico. Así, el objetivo de este trabajo fue evaluar el efecto de la aplicación de fertilización foliar con selenio (0, 150 y 300 ppm) sobre la actividad antioxidante, la eficiencia del uso del agua y las características productivas en rúcula cultivada con y sin déficit hídrico (50% y 100% ETc) en un entorno protegido. El diseño experimental fue en bloques al azar, en un esquema factorial de 3x2 con cuatro repeticiones. Hubo un aumento en las actividades antioxidantes en los tratamientos que recibieron fertilizante a una dosis de 150 ppm y estuvieron expuestos a estrés hídrico. En este mismo tratamiento, las plantas superaron los valores de eficiencia en el uso del agua, productividad y área foliar de las plantas que no estuvieron expuestas al déficit hídrico.

Palabras clave: *Eruca sativa;* Bioestimulante; Especies reactivas al oxígeno; Estreses ambientales.

1. Introduction

Drought is one of the major constraints of agricultural productivity worldwide, particularly in arid and semiarid areas, where demand for water is high (Cabral, Maekawa, Zuffo, & Steiner, 2020; Cechin, Corniani, Fumis, & Cataneo, 2010). It is also one of the most critical environmental stresses for global food security, since water is a major factor of production.

Price & Hendry (1991) studied the physiological effect of drought on different plants and found that drought stress inhibits protein synthesis, deactivates chloroplast enzymes, compromises electron transport and increases membrane permeability and H_2O_2 elimination activity.

Additionally, environmental stress can directly or indirectly affect the formation of reactive oxygen species (ROS). Oxidative stress damages cell constituents such as carbohydrates, lipids, nucleic acids and proteins, reducing plant growth, respiration and photosynthesis (Ahmad, Waraich, Nawaz, Ashraf, & Khalid, 2016).

In recent decades, evidence has shown that small doses of selenium act as an antioxidant and plant biostimulant, promoting growth and improving resistance to abiotic stress such as high solar radiation (Pennanen, Xue, & Hartikainen, 2002), salinity (Bybordi, 2016), senescence (Hartikainen, Xue, & Piironen, 2000), heavy metals (Kumar, Bijo, Baghel, Reddy, & Jha, 2012), cold and high temperatures (Feng, Wei, & Tu, 2013), and drought (Valadabadi, Shiranirad, & Farahani, 2010).

Although selenium does not participate in free radical scavenging, it is considered an antioxidant because it acts as a cofactor for the enzyme glutathione peroxidase (GSH-Px), which plays an important role in eliminating ROS (Cartes, Gianfreda, & Mora, 2005; Freitas et al., 2020). As such, it activates protection mechanisms against oxidative stress and can contribute to reducing the negative effects of drought stress on plants, thereby improving water use efficiency (WUE) (Hajiboland, Sadeghzadeh, & Sadeghzadeh, 2014).

Water use efficiency is the ability of crops to produce biomass per unit of water applied or evapotranspired by the plants. Vegetable growth is generally heavily influenced by soil moisture conditions.

Arugula (*Eruca sativa* L.) is a fast-growing annual herbaceous vegetable with significant crop treatment and water needs (Freitas et al., 2017; Reghin, Otto, Olinik, & Jacoby, 2005). It belongs to the family Brassicacea and, in addition to its use as a food source containing vitamin C, potassium, sulfur and iron, is also considered a medicinal plant, with digestive, diuretic, stimulant, laxative and anti-inflammatory properties (Reghin et al., 2005).

Considering the effect of selenium in reducing abiotic stress, especially drought stress, few field studies have been conducted to assess the response of plants grown under these conditions. Thus, the aim of this study was to assess the effect of selenium foliar feeding on the antioxidant activity, water use efficiency and yield traits of arugula grown with and without drought stress in a protected environment.

2. Methodology

2.1 Description of the experimental area

The study was carried out in the municipality of Maringá, Paraná state (PR), Brazil (23º25'S, 51º57'W, altitude of 542 m). The climate is classified as humid subtropical (Cfa), according to Köppen's classification. The experiment was conducted from February to March 2017, in a greenhouse with an arched roof, covered in 150 µm-thick polyethylene film and white shade cloth on the sides.

The soil in the experimental area is classified as Distroferric Red Nitisol. Fertilization was based on chemical analysis of the soil. Soil chemical analysis provided the following results: pH= 6.7; OM= 23.72 g dm^{-3}; CEC= 22.03 $mmol_c$ dm^{-3}; K= 0.63 $cmol_c$ dm^{-3}; Ca= 17.84 $cmol_c$ dm^{-3}; Mg=16.47 $cmol_c$ dm^{-3}; Z= 28.6 mg dm^{-3}; Fe= 127.68 mg dm^{-3}; Bo= 4.1 mg dm^{-3}; Base saturation = 89%.

2.2 Treatments and experimental design

A randomized block design was used, with a 3x2 factorial scheme and four repetitions, totaling 24 treatments with 26 plants in double rows. The first factor consisted of three selenium doses (0, 150 and 300 ppm) and the second irrigation depths related to crop evapotranspiration (ETc), namely 50% ETc (drought stress) and 100% ETc (without drought stress). The Astro arugula variety was used and seedlings were grown in 200- cell expanded polyethylene (EPE) trays, containing a commercial coconut fiber substrate.

Drip irrigation was performed via 16 mm high density polyethylene tubing (lateral line), equipped with 12 pressure compensating drippers (4 L h^{-1} flow rate) spaced 0.25 m apart.

The crop water need was determined based on crop evapotranspiration (ETc), measured using three constant water table lysimeters installed in the greenhouse. Seedlings were transplanted into the lysimeters using the same spacing previously applied, so that the water extracted by the plants was automatically replenished by the system. Readings and water replacement in the tank were performed at 8 a.m. every day, followed by the treatments using different irrigation depths.

Foliar spraying of selenium was performed by diluting each of the doses in 1 L of water. The selenium source used was sodium selenite (Na_2SeO_4), in the form of a wettable powder containing 42% Se and 24% Na.

The solution was applied with a backpack sprayer in two growth stages, the first seven days after transplanting (DAT) and the second at 14 DAT. A 12 mL spray solution was used, enough to completely wet the leaves. It is important to underscore that the doses used here were determined based on a literature review to identify minimum and maximum selenium doses. Harvesting was performed at 23 DAT, when the leaves were at the recommended size.

2.3 Evaluation of antioxidant activity and production variables

The following production variables were evaluated, the yield value, obtained by dividing the fresh weight per plant by the area of the bed (gm^{-2}); leaf area (LA): calculated using a LI-COR® LI 3100 area meter (cm^2/plant); water use efficiency (WUE): ratio between yield and the amount of water applied ($gm^{-2}mm^{-1}$).

For the tests of total polyphenols and antioxidant activities, arugula leaves were crushed in a food processor to obtain an aqueous extract. The extraction of bioactive compounds was carried out by mixing 1 mL of aqueous arugula extract and 9 mL of methanol. The methanol extract was homogenized in a vortex for 10 seconds, agitated for 10 minutes, and then centrifuged for 10 minutes at 3000 rpm for complete phase separation and the supernatant was used in the assays.

The ferric reducing antioxidant power (FRAP) was determined with an aliquot (250 µL) of methanol extract was mixed with 50 mM sodium phosphate buffer pH 7.0 (1250 µL), 1% potassium ferricyanide (1250 µL) and incubated at 50 °C for 20 min. After trichloroacetic acid (10%) (1250 µL) was addition and centrifuged at 3000 rpm for 10 min. The supernatant (2500 µL) was mixed with 0.1% ferric chloride (500 µL) and the absorbance was measured at 700 nm. The results obtained were expressed by standard curve of gallic acid ranging from 0 to 300 mgL^{-1} (Zhu, Hackman, Ensunsa, Holt, & Keen, 2002).

DPPH assay was performed with methanolic extract 150 µL which was mixed with 2850 µL of DPPH solution in methanol (60 µM). Samples were incubated for 30 min in the dark and were measured at 515 nm and the percentage of antioxidant activity was calculated (Brand-Williams, Cuvelier, & Berset, 1995).

Total polyphenols (TP) compounds were determined using an aliquot of 125 µL of the extract was added with 125 µL of Folin-Ciocalteu reagent (50%) and 2250 µL of sodium carbonate (28 gL^{-1}). The samples were incubated in the dark at 25 °C for 30 min and thereafter the absorbance was determined at 725 nm. The results were expressed by standard curve of gallic acid ranging from 0 to 300 mgL^{-1} (Singleton, Rossi Jr., & Rossi J A Jr., 1965).

The determination of selenium content was performed according to the methodology proposed by Malavolta, Vitti, & Oliveira, (1997). The leaves (0.5g) were crushed and digested in 6 mL of digesting solution (nitric acid and perchloric acid in a 2:1 ratio) at 200 °C until complete digestion. A sample of certified reference material (White Clover - BCR 402, Institute for Reference Materials and Measurements (IRMM), Geel, Belgium) was inserted in each analysis battery, with a known Se content (6.70 $mgkg^{-1}$), and a blank sample for the purpose of quality control and calculation of the limits of detection and quantification. To read the selenium content in the samples, an atomic absorption spectrophotometer equipped with a lamp for reading the selenium content was used. A standard selenium solution containing 1000 $mgSekg^{-1}$ was used to prepare the calibration curve. Selenium levels were presented based on dry mass and expressed in $mgkg^{-1}$.

2.4 Statistical analysis

The variance analysis procedure (ANOVA) was applied by the F test using a minimum probability of 5% to verify the significance of the factors tested and their interactions on the evaluated characteristics. As this is a factorial experiment, the developments were carried out, regardless of whether the interaction between the factors was significant or not. All the mentioned analyzes were made using the SISVAR software (Ferreira, 2014).

3. Results and Discussion

Interaction occurred between the treatments with and without drought stress and selenium doses for the variables FRAP, DPPH, TP, yield, LA and WUE (Table 1). There was an increase in antioxidant activity as a function of selenium application when plants were exposed to drought stress. Arugula plants under drought stress fertilized with 150 ppm of selenium showed higher antioxidant activity (FRAP and DPPH) and total polyphenols (Table 1).

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Table 1. Antioxidant activities, total polyphenols and arugula production variables cultivated with and without drought stress.

	FRAP (mgGAE/mL)		**DPPH (%)**		**PT (mgGAE/mL)**	
ppm	DS	WDS	DS	WDS	DS	WDS
0	29.47^{aB}	28.32^{aB}	26.58^{bB}	31.23^{aA}	150.06^{aA}	139.46^{aB}
150	35.45^{aA}	26.82^{bB}	34.53^{aA}	30.52^{aA}	169.63^{aA}	145.39^{bA}
300	27.50^{aB}	36.73^{aA}	33.86^{aA}	32.76^{aA}	149.47^{aA}	151.72^{aB}
	Yield (g/m^2)		**LA (cm^2/in)**		**WUE (g/m^{2}mm)**	
	DS	WDS	DS	WDS	DS	WDS
0	806,88^{bB}	1239,08^{aA}	850,51^{bB}	1413,32^{aA}	15,07^{aB}	11,57^{aA}
150	1272,44^{aA}	1022,40^{bB}	1561,38^{aA}	1091,26^{bB}	22,03^{aA}	11,35^{bA}
300	966,24^{aB}	1030,85^{aB}	1006,89^{bB}	1190,72^{aB}	15,37^{aB}	9,31^{bA}

The same lowercase letters do not differ for irrigation depth and the same uppercase letters do not differ for selenium doses, according to Tukey's test ($p>0.05$). FRAP: ferric reducing antioxidant power; DPPH: DPPH radical scavenging capacity; PT: Total polyphenols; LA: leaf area; WUE: water use efficiency. DS: with drought stress; WDS: without drought stress. Source: Authors.

The results show that selenium positively affected the antioxidant system of arugula plants. A similar effect was observed in lettuce (Ríos et al., 2008) and broccoli (Bachiega et al., 2016) fertilized with selenium. Moreover, phenolic compound and flavonoid accumulation was observed in tomatoes as a function of selenium application (Schiavon et al., 2013). Djanaguiraman et al. (2005) studied the effect of selenium (selenite) on senescence in lettuce and soybean and reported a less marked decline in antioxidant activity in selenium-treated plants.

The results obtained in DPPH analysis showed the same basic behavior as the FRAP assay and total polyphenols (Table 1). At a selenium dose of 0 ppm the DPPH percentage was higher in treatments without drought stress, whereas 150 ppm had a positive effect on plants under drought conditions, whose antioxidant activity was greater in relation to the other treatments (Table 1).

The results of this study corroborate those of Xu et al. (2003) and Yu et al., (2007), who conducted DPPH analyses and found that antioxidant activity increased in green tea plants treated with selenium. Hartikainen et al. (2000) and Xu & Hu (2004) observed a dose-dependent rise in antioxidant activity when selenium was applied to ryegrass and rice, respectively.

Plants not submitted to drought stress exhibited higher yield and leaf area values at a selenium dose of 0 ppm (Table 1). The results confirm the sensitivity of arugula plants to water shortages, since a decline in growth was observed under drought conditions, likely due to reduced photosynthesis from stomatal closure, a plant defense mechanism. Similar findings were reported by Vasco et al. (2011), who obtained the highest arugula yields for irrigation depths between 75% and 100%, while 50% ETc was deemed insufficient for leaf vegetable production.

In the present study, selenium caused an increase in yield and leaf area in plants exposed to drought stress (Table 1). In treatments with a selenium dose of 150 ppm, plants under a limited water supply showed higher yields and leaf areas than those without these restrictions, indicating that selenium had a beneficial effect on drought tolerance. Nawaz et al. (2015) observed a significant increase in photosynthesis and stomatal conductance in selenium-treated wheat, suggesting that the mineral had a positive effect on maintaining turgor pressure and activating antioxidant metabolism.

The superior yield of arugula in the present study may be related to the fact that plants under drought stress were protected from the effects of oxidative stress by enzymes activated by the presence of selenium, such as superoxide dismutase, ascorbate peroxidase, glutathione reductase and catalase (Habibi & Hajiboland, 2011).

A selenium dose of 300 ppm did not obtain the same positive yield and leaf area results as 150 ppm (Table 1). Similar findings were reported by Hartikainen et al., (2000), who found that selenium had a dual effect on ryegrass growth and metabolism, with low concentrations increasing antioxidant activity and high doses exerting a prooxidant effect. Ramos et al. (2010) recorded the lowest biomass yield in lettuce treated with high selenium doses.

These findings reinforce the hypothesis that high selenium concentrations can be toxic to cell metabolism. Some of the main toxic effects occur because selenium is chemically very similar to sulfur and therefore cannot be differentiated by cells during protein synthesis, resulting in poorly formed non-functional proteins (Voet & Voet, 2013), altering plant cell metabolism.

The highest WUE values were recorded in the drought stress treatment with 150 ppm of selenium (Table 1). These results are consistent with those obtained by Seciu et al. (2016), who found that selenium mitigated the damage caused by drought, improving WUE and increasing cauliflower head production to a level similar to that observed under an optimal water supply. As such, selenium may be involved in delaying plant senescence and increasing drought resistance (Djanaguiraman et al., 2005).

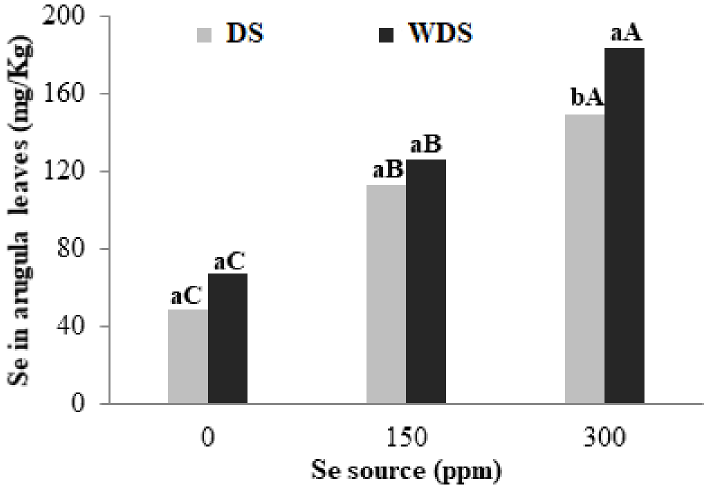
A selenium dose of 150 ppm had the same positive effect on WUE and production variables (Yield, LA) (Table 1). This may be due to the rise in antioxidant activity since selenium is beneficial to plants, and correct concentrations contribute to growth and mitigate the effects of biotic and abiotic stress (Pilon-Smits & Quinn, 2010).

Wheat plants treated with selenium and exposed to drought stress showed significantly higher stomatal conductance and photosystem II efficiency (Tadina, Germ, Kreft, Breznik, & Gaberščik, 2007). Also, in wheat, Yao et al. (2009) reported that selenium favored seedling growth under drought stress; however, growth and physiological responses were dose dependent.

Selenium fertilization raised its content in arugula leaves, with the highest concentrations recorded in treatments without drought stress and a dose of 300 ppm (Figure 1). Xu & Hu (2004) reported that selenium foliar feeding increased concentrations of the mineral in all the parts of rice plants. In tea plants, leaf application of selenite significantly increased leaf selenium content (Hu, Xu, & Pang, 2003).

This indicates that arugula accumulated selenium in accordance with the dose applied and that the selenite added was efficiently adsorbed. This element is used in several countries for biofortification (Mckenzie, Lill, Trovole, & Brummell, 2015) and adds value to selenium fertilization of arugula because, in addition to helping plants withstand environmental stress and providing metabolic benefits to their antioxidant system, accumulated selenium can contribute to human nutrition.

Figure 1. Selenium content in arugula leaves. DS: with drought stress; WDS: without drought stress. The same lowercase letters do not differ for irrigation depth and the same uppercase letters do not differ for selenium doses, according to Tukey’s test (p>0.05).



Fonte: Autores.

Low levels of selenium uptake and accumulation are beneficial to plants and determined by their ability to absorb and metabolize the element. In most plants, selenium concentrations remain low, except those belonging to the families Brassicacea and Alliacea, which can tolerate concentrations up to 103 times higher than other plants.

Recent studies show that low selenium concentrations can protect plants from different types of abiotic stress (Feng et al., 2013). However, selenium doses for crop application have yet to be standardized, which leads to varying results that are difficult to compare, even in crops from the same family. As such, it is important to determine the ideal doses that provide the best physiological and production results.

It is important to underscore that the narrow range between phytotoxicity and deficiency, particularly for micronutrients, means it is vital to establish the correct doses. Given that selenium can interfere in plant physiology and human health, it is important to identify the best dose to ensure its accumulation in compatible edible plant tissue and a beneficial effect for plants and human health.

The analyses and discussions presented here for the conditions studied allow the following conclusions to be drawn: selenium had a beneficial effect on the yield and leaf area of arugula plants under drought stress. A 150 ppm dose of selenium affected the plant antioxidant system, improving drought resistance and increasing antioxidant activity, water use efficiency and leaf area in plants exposed to drought stress.

4. Conclusion

For the conditions studied and equipment used, the analyzes and discussions presented allowed to conclude that, selenium had beneficial effects for arugula plants under drought stress, provided positive changes in the plant's metabolism, increasing productivity, even if the plant is in drought stress. Selenate at a dose of 150 ppm induced the antioxidant system of plants, improving resistance to stress and promoting greater efficiency in the use of water in plants exposed to drought stress.

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Porcentagem de contribuição de cada autor no manuscrito

Danielle Paula de Oliveira Mangarotti – 35%

Roberto Rezende – 20%

Reni Saath – 15%

Tiago Luan Hachmann – 10%

Paula Toshimi Matumoto-Pintro – 10%

Fernando Antônio Anjo – 10%