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RESEARCH ARTICLE

Onion quality and yield after agronomic biofortification with selenium

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ABSTRACT

The hidden hunger for Se in the world population is well known. As a strategy to address this issue, this study aimed to biofortify onion (*Allium cepa* L.), since it is a condiment vegetable consumed by different economic classes and is widely used in the food industry. To this end, Se doses (0, 10, 25, 50, 100 and 200 g ha^{-1}) and application forms (soil and foliar) were evaluated on the biofortification of the bulb and crop yield. The Se source was sodium selenate. Via soil, Se was applied together with NPK fertilizer in the pre-transplantation of the seedlings. Via foliar application, Se was sprayed 77 d after seedlings were transplanted. The Se increased yield up to 99 g ha^{-1}, which was 14.7% higher than the yield obtained in the crop not fertilized with Se. Foliar application of Se was more efficient than soil application in the biofortification of onion, although both were effective. With the dose that promoted the highest yield, the Se concentrations reached 0.487 and 0.317 mg Se kg^{-1} in onion dry mass, when the supply was made via foliar and soil applications, respectively. The increase in Se supply did not influence bulb quality, as demonstrated by the macro- and micronutrients content and pungency.

Key words: *Allium cepa*, biofortified industrial condiment, nutritional deficiency, sodium selenate.

INTRODUCTION

Selenium (Se) is an essential element for humans protecting the body from free radicals and oxidative damage (Das et al., 2018). Its low content in food due to low contents in the soil has prevented adequate Se intake for the organism (Pyrzynska and Sentkowska, 2021), causing health problems, such as low immunity, growth delay, impaired bone metabolism, and abnormal thyroid function (Kieliszek et al., 2022). Agronomic biofortification can be achieved by fertilizing crops and has been widely studied (D'Amato et al., 2020; Almeida et al., 2022; Nascimento et al., 2022) to increase the Se concentration in edible crops parts, and to provide the population with organic Se, which, according to Hadrup and Ravn-Haren (2020), is the less toxic form and has less risk of reaching toxic levels compared to synthetic forms. In addition, biofortified vegetable raw material can be used to manufacture functional food products (Mayurnikova et al., 2020). Research in this field has been carried out, for example, on the use of wheat biofortified with Se for the production of bread and noodles (Wang et al., 2021), and on carrots for juice production with a high Se content (Skoczylas et al., 2020).

However, when ingested in high quantities, Se is toxic. Hadrup and Ravn-Haren (2020) reported several cases of toxicity and mortality following acute poisoning. In their review, they described the acute toxic effects of Se, including exposure, and blood and urine levels associated with mortality that may occur with the ingestion of 1-100 mg kg^{-1} body weight. According to the Institute of Medicine (2000), the tolerable daily intake of Se is 0.045 mg d^{-1} for a newborn baby up to 4 mo old, 0.090 mg d^{-1} for children aged 1 to 3 yr, 0.150 mg d^{-1} for children aged 4 to 8 yr and 0.280 for children aged 9 to 13 yr. For young people over 14 yr old to adults, as well as for pregnant and lactating women, the tolerable daily intake is 0.400 mg d^{-1}.

Therefore, to successfully establish biofortification protocols, it is very important to know the crop response to fertilization, which varies with the cultivation region, because its efficiency and effectiveness are affected by climatic and edaphic factors. Another issue is how Se is supplied to plants. In theory, foliar application should be more efficient than soil application since it can favor faster Se uptake and assimilation, and Se losses due to Se immobilization in the soil (Hasanuzzaman et al., 2020).

In addition, the species to be biofortified plays an important role because species have different Se accumulation capacities. Among the crops that benefit from the presence of Se and have the ability to accumulate it in their tissues, the onion (*Allium cepa* L.) stands out. This vegetable is classified as a secondary accumulator of Se because it has the ability to accumulate up to 1 g kg^{-1} of Se in its tissues (Pyrzynska and Sentkowska, 2021), which makes it suitable for biofortification studies. Furthermore, onion is one of the main condiment vegetables consumed in the world, mainly for its various forms of consumption, either fresh or processed as a seasoning. It is also considered a nutraceutical food because, in addition to meeting the daily needs of vitamins and minerals, it also has substances that help prevent diseases.

The positive effects of Se on the biofortification and yield of onion plants have been proven under controlled growing conditions (Sharma et al., 2007; Põldma et al., 2013; Mobini et al., 2019). However, these studies were performed under different soil and climate conditions from those of the Brazilian onion-producing regions, a fact that influences plant metabolism.

Therefore, the objective of the study was to evaluate the efficiency of soil and foliar application of Se in the biofortification of onion bulbs and to determine whether the increase in Se supply affects the yield, nutritional (macro- and micronutrients) and organoleptic (pungency) quality of the bulb.

MATERIALS AND METHODS

Location and characterization of the experimental area

The experiment was conducted from April to August 2019 in the experimental area (Oxisol with a very clayey texture) of Universidade Estadual Paulista, Jaboticabal campus (21°15'22" S, 48°15'58" W, 615 m a.s.l.), Brazil. The region's climate is classified as subtropical, with a rainy summer and a relatively dry winter, an annual average rainfall of 1423.9 mm, and an average temperature of 22.3 °C.

In the experimental period, 97.6, 24.2, 11.5, 9.3 and 10.4 mm of rainfall and monthly insolation of 228, 227, 225, 251 and 243 h were recorded in April, May, June, July, and August, respectively. The average temperature during the experimental period was 21°C, with average minimum and maximum temperatures of 9 and 32 °C, respectively.

Treatments and experimental design

The 12 treatments resulted from the combination of the following factors: Se doses (0, 10, 25, 50, 100 and 200 g ha^{-1}) and form of application (soil or foliar). The Se source used was sodium selenate (Na_2O_4Se). The randomized complete block design was used in a 6 × 2 factorial scheme with four replicates. The experimental plot consisted of eight 1.2 m long rows spaced 0.30 m apart with an area of 2.88 m^2.

For soil application, the element was dissolved in water and incorporated into the granular NPK fertilizer through a sprinkler containing a solution with the Se concentration for each dose evaluated. After drying, the fertilizer was applied and incorporated into the soil at a depth of 0.20 m in the pre-transplantation of the onion (*Allium cepa* L.) seedlings. For the foliar Se application, sprinkling was carried out 77 d after transplant (DAT) when the plants had eight leaves using a manual sprinkler with a volume of 100 mL $plot^{-1}$. An adhesive spreader (Tween) was used at a concentration of 30 mL 100 L^{-1} solution. Sprinkling was carried out between 16:00 and 18:00 h, a period with adequate weather conditions (21 °C and 53.8% RH). For foliar application, each plot was isolated with a plastic curtain to protect the plants of the other plots from possible spray drift.

Installation and conduction of the experiment

Prior to soil preparation, soil samples were collected in the 0.0-0.2 m layer for chemical analysis obtaining the following results: 30 g dm^{-3} organic matter (by spectrophotometry); 47 mg dm^{-3} P (resin by spectrophotometry); 8 mg dm^{-3} S (by turbidimetry); 4.2 mmol dm^{-3} K (by atomic absorption spectrometry); 28 mmol dm^{-3} Ca (by atomic absorption spectrometry); 10 mmol dm^{-3} Mg (by atomic absorption spectrometry); pH (in $CaCl_2$ by potentiometry) of 5.9; base saturation (V) of 69%; and non-detectable Se content of < 0.1 mg kg^{-1} (by atomic absorption spectrometry). It was not necessary to perform liming in the area because the base saturation was very close to adequate value (70%-80%). The soil was prepared by ploughing and harrowing and the plant beds were subsequently prepared. The application of 750 kg ha^{-1} NPK 4:14:8 and 750 kg ha^{-1} single superphosphate was carried out.

Onion seedlings of the ‘Dulciana’ hybrid (Nunhems, Santo Antônio de Posse, São Paulo, Brazil) were transplanted at a spacing of 0.3 m between rows and 0.1 m between plants in the row. Sprinkler irrigation was carried out according to crop demand. After foliar Se application, irrigation was carried out with at an interval of 16 h. Pest, disease, and weed management was carried out according to the need. Topdressing with N and K was carried out at 21 and 44 DAT using urea and potassium chloride as the sources of these nutrients, respectively. Additionally, foliar application of B and Zn was carried out at 26, 44, and 62 DAT at concentrations of 1 and 2 g L^{-1}, respectively.

Harvest was carried out at 135 DAT, when 70% of the plants fell. The plants from the usable area were harvested and left for 5 d in the sun with bulbs protected by the leaves. Then, bulb curing was completed for more than 10 d in the shade. Leaves were eliminated by cutting the pseudostem 2 cm from the bulb.

Analyzed characteristics

The yield was calculated by adding the mass of the bulbs from the plants in the useful plot and estimated at t ha^{-1}. The bulbs were peeled, sliced, and placed to dry in a forced air circulation oven at 40 °C until reaching a constant mass. The DM content of the bulb was determined. Onion pungency (P) was determined by quantifying the pyruvic acid content, which was determined using the reagent 2,4-dinitrophenylhydrazine (DNPH), according to the method proposed by Schwimmer and Weston (1961). The reading was performed in a spectrophotometer at 420 nm, and the pyruvic acid concentration was determined using the standard curve of sodium pyruvate as reference. The enzymatic production of pyruvic acid was expressed in µmol pyruvic acid g^{-1} onion. Subsequently, the macro- and micronutrient content in the bulb were determined according to recommendations by Miyazawa et al. (2009). The Se content in the bulb (bulb Se) was determined. Samples of dry bulb material were digested and analyzed by inductively coupled plasma mass spectrometry (ICP-MS; Agilent 7500ce, Agilent Technologies, Tokyo, Japan). The samples were placed into digestion vessels of a perfluoroalkoxy (PFA) liner material and polyethylethylketone (PEEK) pressure jacket with 2 mL 70% trace analysis grade HNO_3, 1 mL Milli-Q water, and 1 mL H_2O_2. Digestion was performed in a microwave system comprising a Multiwave 3000 platform with a 48-vessel MF50 rotor. The settings for digestion were as follows: Power = 1400 W, temp = 140 °C, pressure = 2 MPa and time = 45 min. The Se content was expressed in mg kg^{-1} DM. The accuracy and precision of the analytical method were assured by the use of the standard reference material BCR 402 (white clover) (Sigma-Aldrich) with 99% Se recovery according to the methodology described by Silva et al. (2019).

Statistical analysis

Data were subjected to ANOVA using the F test. Soil and foliar application were compared by Tukey’s test at a 5% probability. Additionally, a regression study for Se doses was performed, and the significant equations of higher order and coefficient of determination were chosen. The AgroEstat statistical program was used (Barbosa and Maldonado Junior, 2015) to fit the statistical analysis.

RESULTS

The yield of the crop increased up to 99 g ha^{-1} Se when it reached 76.5 t ha^{-1}, which was 14.7% higher than that obtained without Se fertilization. However, the increase in Se doses caused a continued reduction in yield, reaching 66.2 t ha^{-1} with the application of 200 g ha^{-1} Se, and a decrease of 11% in relation to the Se dose 99 g ha^{-1}, resulting in a yield lower than that obtained in plants not fertilized with Se (Figure 1). The DM content of the bulb was not influenced by treatments and it have had 5.23% as mean.

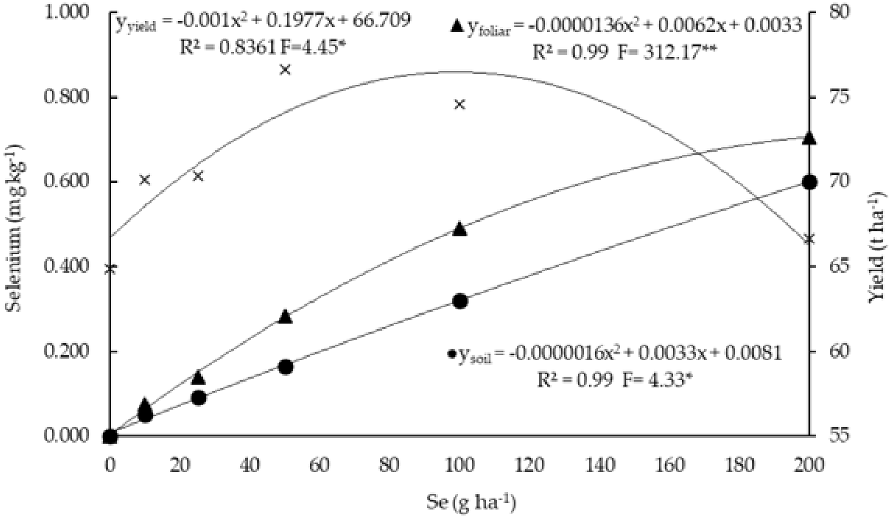


Figure 1. Yield (Yyield) and Se content in the bulb DM of the onion 'Dulciana' as a function of doses and forms (●soil and ▲ foliar) of Se application. *, **Significant at $p \leq 0.05$ and $p \leq 0.01$, respectively.

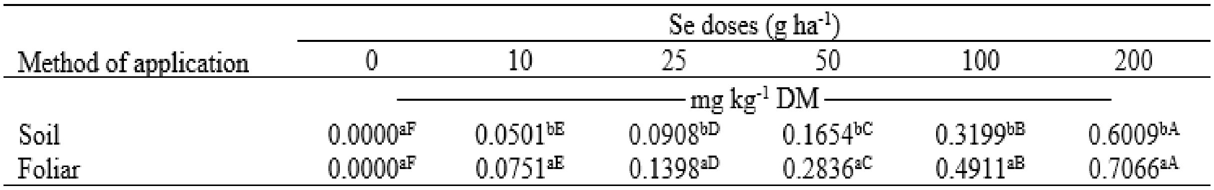
Regarding the quality of the bulb, the pungency and macro- and micronutrient contents were not influenced by the factors evaluated, and the means are showed in Table 1. For the Se content, there was interaction of the factors (Table 2).

The higher the Se dose applied, the higher the bulb Se content, regardless of the form of application. The Se concentration in the bulb increased from 0.041 to 0.600 mg kg^{-1} DM when 10 and 200 g Se ha^{-1}, respectively, were applied to the soil, and from 0.064 to 0.705 mg kg^{-1} DM, respectively, when the same doses were applied via foliar spraying (Figure 1). Table 2 shows that foliar application was more efficient in increasing Se in the bulb from the lowest concentration used.

Table 1. Pungency and macro- and micronutrients contents in the onion bulb after Se supply.

Pungency	N	P	K	Ca	Mg	S	Cu	Fe	Mn	Zn
µmol g^{-1}	g kg^{-1}						mg kg^{-1}			
0.109	24.9	4.1	19.3	2.3	1.8	6.7	5.9	115.1	30.2	27.5

Table 2. Selenium content in onion bulbs as a function of Se doses and application methods. Means followed by uppercase letters in the row and lowercase letters in the column do not differ by Tukey test ($p > 0.05$).



Method of application	Se doses (g ha^{-1})					
	0	10	25	50	100	200
	mg kg^{-1} DM					
Soil	0.0000^{aF}	0.0501^{bE}	0.0908^{bD}	0.1654^{bC}	0.3199^{bB}	0.6009^{bA}
Foliar	0.0000^{aF}	0.0751^{aE}	0.1398^{aD}	0.2836^{aC}	0.4911^{aB}	0.7066^{aA}

DISCUSSION

Soil in many regions of the world has a low Se content, which conditions the production of foods with low levels of the element that are insufficient to meet the daily requirement of humans, causing several pathologies (D'Amato et al., 2020; Hasanuzzaman et al., 2020). Agronomic biofortification of crops has been used as a tool for nutritional enrichment of food to end or at least mitigate malnutrition in the population. The present study confirmed the success of the technique, because the increase in the supply of sodium selenate via soil or foliar application was effective in biofortifying onion bulbs. As the onion is a Se-accumulating plant (Pyrzynska and Sentkowska, 2021), high concentrations of the element were still observed at low doses, and the higher the dose of sodium selenate, the higher the Se concentration in the bulb (Figure 1, Table 2). The results corroborate those obtained by Sharma et al. (2007) and Põldma et al. (2013), who verified the high capacity of the plant to concentrate Se in the bulb in response to fertilization with the element.

Among the Se supply methods, foliar application was more efficient and promoted higher Se contents in the bulb than that with soil application (Table 2), which can be attributed to faster processes of Se uptake and assimilation by the leaves, since there is no need for the element to be translocated from the root to the leaves and bulb. Furthermore, Se losses due to Se immobilization in the soil are avoided when Se is supplied by foliar spraying (Hasanuzzaman et al., 2020). In the present study, the biofortification of onion bulb by foliar spraying was up to 71.5% more efficient than when Se was supplied through the soil and this efficiency was achieved at a dose of 50 g ha^{-1} Se (Table 2).

The recommendation for an Se dose to obtain biofortified onion needs to consider two points: Se content in the bulb and the acceptable daily limit of Se intake, which according to the Institute of Medicine (2000) is up to 0.400 mg d^{-1} for healthy adult men and women (including pregnant and lactating women), 0.045 mg d^{-1} for a newborn baby up to 4 mo old and 0.090 mg d^{-1} for children 1 to 3 yr old. As observed in Figure 1, the maximum yield was obtained with 99 g ha^{-1} Se, and this dose obtained 0.487 and 0.317 mg kg^{-1} Se in the onion DM, when Se was supplied by foliar and soil application, respectively. Therefore, considering the average of 5.23% for DM content in the onion, the Se concentrations in the fresh bulb were 0.025 and 0.017 mg kg^{-1} by foliar and soil application, respectively. These Se concentrations do not pose a risk to the population. If a 10% loss in yield is acceptable, a Se dose of 185 g ha^{-1} can be recommended, which would make it possible to obtain concentrations of 0.69 and 0.56 mg kg^{-1} in dehydrated bulb, corresponding to increases of 42% and 77% in the intensity of biofortification using the foliar and soil methods, respectively.

As an alternative to the ingestion of Se via the consumption of fresh food, biofortified plant raw material can be used to manufacture functional food products (Mayurnikova et al., 2020). Research in this field has been carried out, for instance with the use of Se-biofortified wheat for the production of bread and pasta (Wang et al., 2021), as well as carrots for the production of juice with a high Se content (Skoczylas et al., 2020). As onions are widely used in processed foods, the use of this biofortified vegetable in the food industry is an appropriate strategy to increase Se intake in the population. Considering the results obtained in this study, biofortification through onion fertilization can promote a high Se contents in dehydrated onion through soil and foliar application. Therefore, a small amount of this dehydrated onion would be successful in increasing the diversity of commercially available Se products, and increasing Se reach across multiple segments of the population.

Another relevant aspect of biofortification programs is its relationship with yield. Biofortification should not be achieved through a reduction in food size, as an effect of the concentration of the applied element. In the present study, there was a positive effect of Se on yield of the ‘Dulciana’ onion up to 99 g ha^{-1}, respectively (Figure 1). The result corroborates those obtained by Sharma et al. (2007), who verified a 16% increase in bulb yield when applying 25 g Se ha^{-1} (as sodium selenate) by foliar spraying, and Põldma et al. (2013), who obtained a 52% increase in yield when applying 1 kg Se ha^{-1} in the form of sodium selenate.

The increments in yield due to the application of Se can be justified by the beneficial action of the element in increasing chlorophyll (Ashraf et al., 2018; Lara et al., 2019; Alves et al., 2020) and, consequently, the photosynthetic rate of the plant, which leads to yield gains. In addition, Se can control the production and reduction of reactive oxygen species in plants (Feng et al., 2013; Chauhan et al., 2019), which consequently reduces oxidative stress and yield loss. However, a negative effect of high doses of Se on the onion plant was also observed in the present study. Reduction of 13% in yield was observed (Figure1). Therefore, the range between the beneficial and phytotoxic doses of Se to plants is very narrow, as reported by Zhou et al. (2020). This was also observed by Sharma et al. (2007), who obtained an increase in onion yield when a dose of 25 g Se ha^{-1} was applied, while the application of 50 g Se ha^{-1} reduced crop yield, which was pointed out by the authors as the first signs of toxicity. High concentrations increase the production of reactive oxygen species (Feng et al., 2013). This occurs because glutathione, an enzyme that also has a function in combating reactive oxygen species, is used for Se assimilation (Feng et al., 2013; Hoewyk, 2013). At high Se concentration, the enzyme becomes unable to perform both functions, allowing the plant to accumulate reactive oxygen species, causing toxicity, which is characterized by leaf chlorosis, decreased protein synthesis, growth reduction (Corbo et al., 2018; Chauhan et al., 2019) and, consequently, yield reduction (Feng et al., 2013).

Regarding bulb quality, for the two Se supply methods, the macro- and micronutrient content and pungency were not influenced by Se doses. The absence of the effect of Se on pungency agrees with the results obtained by Sharma et al. (2007). Onion pungency is a combination of flavor and aroma released when tissues are ruptured and exposed to oxygen. The main precursors of onion aroma are S compounds, which have cysteine in their composition. As a result of the close relationship between Se and S inside plants, Se application was expected to reduce pungency in onion plants, since sulphate is necessary for cysteine biosynthesis, which is an important amino acid for the biosynthesis of flavor compounds in onion (Bybordi et al., 2018). Therefore, a reduction in pungency could occur by partially replacing S with Se, which would promote the production of selenocysteine instead of cysteine (Hoewyk, 2013). However, the expected effects of increased Se supply on S content in the bulb and pungency were not confirmed, in contrast to the results observed by Kopsell et al. (1999) and Bybordi et al. (2018). The absence of an effect of Se on pungency can be attributed to the genetic characteristic of the hybrid ‘Dulciana’, which has low pungency.

In summary, the dose of 99 g Se ha^{-1}, which promoted a higher yield, also promoted a high Se content in the bulb without bringing risks to human health. As a condiment vegetable widely used in the world’s cuisine and even by low-income classes, onions are an interesting strategy for biofortification, aiming to increase Se intake in the population and to combat malnutrition and hidden hunger.

CONCLUSIONS

Selenium is a beneficial element for onions and should be incorporated into crop management because it provides yield increases up to 99 g Se ha^{-1}.

Soil and foliar applications of Se were effective in biofortifying onion bulbs, and foliar application was the most efficient method. The Se dose that provided the highest yield allowed for the biofortification of the bulb, reaching concentrations of 0.487 and 0.317 mg Se kg^{-1} in the DM of the onion, when the supply was made via foliar and soil application, respectively. Accepting a yield loss of 10%, the biofortification of the bulb reached concentrations of 42% and 77% higher than those obtained with the maximum yield.

Due to its versatility of use in the food industry and its high capacity to accumulate Se in the bulb, biofortified dehydrated onion can be used as a raw material for the biofortification of processed foods.

Author contribution

Conceptualization: A.B.C.F. Methodology: A.B.C.F. Formal analysis: B.Q.V.M. Investigation: B.Q.V.M., B.J.P., G.F.R. Resources: A.B.C.F. Writing-original draft: B.Q.V.M, B.J.P, G.F.R. Writing-review & editing: A.B.C.F. Supervision: A.B.C.F. Project administration: A.B.C.F. Funding acquisition: A.B.C.F. All co-authors reviewed the final version and approved the manuscript before submission.

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