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Research Article

Iodine application in *Vitis vinifera* L. cv. 'Cabernet Sauvignon' improve bioactive compounds and enzymatic activity in berries

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Abstract

Iodine (I) deficiency disorders represent a worldwide public health problem, with at least 1.9 million people estimated to have an unsatisfactory intake of this trace element. I content in plant foods is particularly low; however, it can be improved by biofortification. In this study, the effect of foliar fertilization with I (0, 0.25, 0.5, 0.75, 1.0, and 1.25 mg L^{-1}) on yield, bioactive compound content, and bioaccumulation in grapevine berries was evaluated. Biofortification with I has positively modified yield, bioactive compound content and bioaccumulation. Intermediate doses (0.75 mg L^{-1}) increased yield (57%), while high doses (1.25 mg L^{-1}) decreased yield (28%) and incremented the phenols, flavonoids, antioxidant capacity, vitamin C, anthocyanin (50,34,31,71,26% respectively), catalase (73%) and peroxidase activity (57%), and their bioaccumulation in berries (59%). Agronomic biofortification with I is an alternative to increase yield, enzymatic and non-enzymatic antioxidants, as well as the concentration of this trace element in grape berries.

Keywords: agronomic biofortification; bioactive compounds; *Vitis vinifera* L.; yield

Introduction

Trace element deficiency represents a health problem in the population and impacts more severely on women and children (Rami *et al*., 2022); micronutrient deficiencies are considered to be responsible for more than half of child deaths in developing countries (Barreto, 2017). Among the main causes of trace element malnutrition in the population is their low concentration in soils; consequently, their content in plant-based foods is insufficient to face the nutritional requirements of the population (Eastman and Zimmerman, 2018).

Iodine (I) deficiency disorders affect at least 1.5 million people (of which 0.25 million are schoolchildren), especially in developing countries such as Africa, Asia, Latin America and a large part of Europe (Cesar *et al*., 2020). Iodine is an element crucial for thyroid hormone biosynthesis in humans (Sorrenti

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et al., 2021). I deficiency diseases are caused by unsatisfactory dietary intake (Walle *et al*., 2020) and associated with inadequate thyroid hormone synthesis produce negative effects on the human organism, such as reproductive failure, hearing loss, impaired growth, cretinism and numerous types of brain lesions (Wang *et al*., 2021). Iodization of table salt has been used to mitigate this situation; however, it is not sufficient to cover the entire daily requirement (90-250 µg) in the population (Sularz *et al*., 2020). In addition, it is difficult to control the loss during storage, transport and cooking due to the inorganic I is volatile (Kumar *et al*., 2017). A feasible alternative to combat human malnutrition is the biofortification of crops, in order to obtain better nutritional quality and with a higher bioavailability of essential trace elements for the population that has limited access to dietary diversity (Garg *et al.,* 2018)

On the other hand, grapevine (*Vitis vinifera* L.), due to its economic and cultural importance is one of the oldest crops in the world, it is considered as a functional food due to the high content of bioactive compounds among which are phenols, flavonoids and anthocyanins, which provide antioxidant properties and are related to the prevention of diseases in humans (Franco-Bañuelos *et al*., 2019). In addition, it contains vitamins (A, C, E, B1, B3, B6 and B9), and minerals (Ca, P, Na, K, Fe, Cu, Mg, and Zn). As well as in the peel and seed, grapes contain polyphenols, vitamins (C, E), and flavonoids that provide protection against oxidative stress in humans (Huber *et al*., 2021). Therefore, in the present study, the effect of I foliar spray on yield and biosynthesis of antioxidant compounds and enzymatic activity as well as their bioaccumulation in grapevine berries of *Vitis vinifera* L. cv. 'Cabernet Sauvignon'.

Materials and Methods

Study site and experimental conditions

The experiment was carried out in a commercial orchard located in Monterrey, Durango, Mexico, at coordinates 25°29'20''N, 103°37'37''W. The climate of the study area is dry steppe; average temperature is 21 °C and annual precipitation is 253 mm. The soil texture is sandy loam (81% sand, 14% silt and 5% clay); bulk density 1.67 g cm^{-3}; pH 8.37; water holding capacity 25.2%; electrical conductivity 1.28 dS m^{-1}; organic matter content 1.18 mg kg^{-1}; total nitrogen 32.8 mg kg^{-1}; available phosphorus 24.4 mg kg^{-1} and removable potassium 90.43 mg kg^{-1}.

Planting material

Eight-year-old 'Cabernet Sauvignon' grapevine plants were used. The planting system was 1 m between plants and 3 m between rows, with a plant density of 3,333 plants ha^{-1}. The study lot received the same agronomic management applied to the entire vineyard by the producer, regarding irrigation, fertilization (80,20 units of N and P_2O_5 ha^{-1}), phytosanitary control and pruning.

Treatments and experimental design

Potassium iodide (KI, 99% purity, Jalmek®) was used. Five doses of iodine were prepared: 0.25, 0.5, 0.75, 1.0 and 1.25 mg L^{-1} and a control treatment (0 mg L^{-1}), using distilled water and a non-toxic commercial surfactant (INEX-A®, 0.02% v:v). The distribution of the treatments was under a completely randomized design, each treatment consisted of 10 plants, each representing one experimental unit. The treatments were applied by foliar sprays at fruit formation, in veraison and 15 days before harvest.

Variables evaluated

Harvesting was carried out on the same day for all treatments, when the total soluble solids (°Brix) of randomly harvested berries remained constant for a few days. Yield per plant and average bunch weight were

recorded for each plant using a hanging scale (Truper® BAS-10C). Cluster weight was calculated by dividing the yield per plant by the number of bunches harvested.

Oenological parameters

Total soluble solids (TSS) and titratable acidity (TA) were measured in the juice of 50 berries per treatment and replicate. TSS were measured with a 0-32% manual refractometer (Sper Scientific® 30001, Sper Scientific LTD, Scottsdale Az, USA) at 20 °C and expressed in °Brix. TA was determined following AOAC methodology (AOAC, 1990), using NaOH (0.1 N) and phenolphthalein (1%) as indicator; results were expressed as percentage of tartaric acid per 100 g. The pH was measured with an electronic pH meter (Thermo Scientific®, USA), and finally the maturity index (MI) was calculated with the TSS/TA ratio. The probable degrees of alcohol (PDA) were calculated using the formula: PDA = (0.675*°Brix)-2.0839.

Non-enzymatic antioxidants

Preparation of extracts were taken two grams of fresh pulp was mixed in 10 mL of ethanol in a screw-capped plastic tube, which was placed on a rotary shaker (ATR Inc., USA) for 6 h at 5 °C and 20 rpm. Subsequently, the tubes were centrifuged at 3,000 rpm for 5 min and the supernatant was removed for analytical testing.

Phenolic content

Total phenolic content was determined by the Folin-Ciocalteau method (García-Nava, 2009). Samples were quantified in an ultraviolet (UV)-Vis spectrophotometer at 760 nm (Master Spectrum Fisher Scientific 415). The standard was prepared with gallic acid. The results were expressed in mg GAE 100 g^{-1} fresh weight (FW).

Flavonoid content

Total flavonoids were determined by colorimetry (García-Nava, 2009). Samples were quantified in a UV-Vis spectrophotometer at 510 nm (Master Spectrum Fisher Scientific 415). The standard was prepared with quercetin dissolved in absolute ethanol ($y = 0.0122x-0.0067$; $r^2 = 0.965$). Results were expressed as mg QE 100 g^{-1} FW.

Antioxidant capacity

Total antioxidant capacity was measured by the in vitro $DPPH^+$ method (Brand-Williams *et al.*, 1995). Samples were quantified in a UV-Vis spectrophotometer at 517 nm (Master Spectrum Fisher Scientific 415). The standard was prepared with Trolox (0.1-1.0 mM, $r^2 = 0.998$). Results were expressed as μM Trolox equivalent 100 g^{-1} FW.

Vitamin C content

Vitamin C was determined according to Hernández-Hernández *et al.* (2019). Ten g of fresh fruit were ground with 10 mL of 2% hydrochloric acid, then with the help of a funnel and filter paper, the sample was filtered and the extract obtained was made up to 100 mL with deionized water. Then, 2,6 dichlorophenolindophenol (1×10^{-3} N) was used to titrate 10 mL of the dilute. To determine the titration, the reddish color should persist for a few seconds. If it disappears when the sample is shaken, it means that there is still vitamin C without oxidation. Once we obtain the reddish color, we stop adding dye and calculate with the volume spent. The result is reported as mg 100 g^{-1} FW.

Anthocyanins

The total anthocyanin content was determined according to the methodology indicated by Lira *et al*. (2017), one g of grape sample was weighed in a 25 mL capacity Erlenmeyer flask and a 10 mL anthocyanin extraction solution consisting of HCl: methanol: water: (0.02:8:1.8; v/v/v/v) was added. The flask was placed in a sonicator (Branson, 1,800: 1.8 L/0.5 GAL) at 50 °C for 1 h. The extract obtained was centrifuged. The extract obtained was centrifuged at 3,000 rpm for 10 min at 4 °C and the supernatant was collected and stored in an amber vial at 4 °C until use. Finally, absorbance was measured at a wavelength of 525 nm in spectrophotometer and the concentration of total anthocyanins in the grape sample was reported as μg cyanidin 3-glucoside equivalent g^{-1} grape sample.

Enzymatic antioxidant

For the determination of catalase activity (CAT) (EC 1.11.1.6) (the mM equivalent of H_2O_2 consumed per milliliter per minute), it was carried out according to David *et al.* (2008). An enzyme extract was prepared, and 0.5 g of grape pulp was weighed, to which 5 mL of a potassium and sodium phosphate solution with the following characteristics was added: 100 mM, pH 7.0 at 4 °C and 50 mg of polyvinylpyrrolidone (PVP) was added. After this procedure, it was mixed in a mortar. The mixture was centrifuged at 11,000 g for 11 min at 4 °C and the supernatant was taken to determine the catalase activity, then mixed with 3 mL of sodium phosphate buffer at 300 μM, pH 6.8, 1 mL of H_2O_2 at 100 μM, and 1 mL of the enzyme (from the enzyme extract) as recommended diluted at a ratio of 1:20. The measured reaction time was 1 min at 240 nm.

Peroxidase activity (PXA) (EC 1.11.1.7) (the mM equivalent of H_2O_2 consumed per milliliter per minute) was determined using a modification of the method of Nickel and Cunnnigham (1969), where absorbance was measured at 420 nm, the reaction mixture contained 20 mL H_2O, 2 mL enzyme extract plus 1 mL guaiacol and 1 mL H_2O_2. Then, the reaction time was taken as 10 min.

Iodine content analysis

Iodine was determined with the alkaline digestion technique (Medrano *et al*., 2021). The preparation of the reagents, as well as the washing of the material was carried out with deionized water. 0.5 g of sample was weighed in a porcelain crucible, 1 mL of 0.5 M sodium hydroxide and 0.1M potassium nitrate were added. The samples were covered with aluminum and placed in a brand muffle (Thermo Scientific Thermolyne®, USA) at a temperature of 580 °C and counted 3 hours from temperature. Samples were removed and placed in a glass desiccator with lid at room temperature. Then, 2 mL of sodium hydroxide at 1.0 mM was added. It was centrifuged at 2500 rpm for 20 minutes and the supernatant was obtained for iodine determination. 1 mL of the supernatant was decanted and made up to 10 mL with 2 M potassium hydroxide (Cortés-Flores *et al*., 2016). The readings were performed by Coupled Plasma Atomic Emission Spectroscopy, ICP-OES (Perkin Elmer®, USA) Model: Optima 7300 DV.

Statistical analysis

Data were analysed by one-way analysis of variance (ANOVA) using STATISTICA software (version 10.0; StatSoft, Tulsa, OK, USA). Post hoc least significant difference Fisher test ($p \leq 0.05$) was used to compare means.

Results and Discussion

Yield and its components

I is not considered essential for terrestrial plants, which is why it is not included in normal fertilization programs; however, it has been shown that I can act as a biostimulant and cause positive effects on crop growth, development and productivity (Riyazuddin *et al*., 2022; Nascimiento *et al*., 2022). In the current investigation, it was found that foliar spraying of I modified yield and its components (Table 1). The application of 0.75 mg L^{-1}, showed the highest yield until 57% compared with the control plants. Our results confirm previous studies showing that I in low concentrations improves plant yield (Duborská *et al*., 2020). Such positive impact has been observed from modulation in gene expression to being a structural part of several proteins (Kiferle *et al*., 2021), as well as contributing in improving chlorophyll concentration, detoxification and maintenance of homeostasis of reactive oxygen species under normal or stressful environmental conditions (Riyazuddin *et al*., 2022); however I in high concentrations decreases yield since at high concentrations it can become toxic to the plant (Incrocci, 2019), because it reduces CO_2 assimilation by reducing leaf size, stomatal conductance and photosynthetic pigment content (Kiferle *et al*., 2019). Therefore, the beneficial effects of I depend on the concentration, source used, duration of exposure, type of application and the plant species used (Gonzali *et al*., 2017).

Table 1. Yield and its components in grapevine cultivation

Iodine mg L^{-1}	Yield per plant (kg)	Bunches per plant	Bunches weight (g)
0	1.45b*	25.33ab	65.66e
0.25	2.02ab	22.67b	90.34a
0.50	2.02ab	32.67ab	60.33d
0.75	3.41a	48.67a	70.33b
1.00	2.38ab	34.67ab	69.01bc
1.25	1.13b	19.00b	60.00d

*Different letters in each column indicate significative difference ($p \leq 0.05$).

Oenological parameters

The parameters such as pH, TSS and TA are used as maturity indices to define the optimal time of harvest. The pH indicates the strength of acids in the must (Blouin and Guimberteau, 2003) and it is estimated that at harvest time it should have on average a pH of 3.66, TSS for wine grape varieties should be between 12-24 °Brix (Cabeller, 2017); TA is a component of flavour, composed mainly of organic acids such as malic and tartaric (Saxton *et al*., 2009), which are used as metabolites during respiration during fruit growth and ripening (Dorey *et al*., 2016) and the MI for this variety is between 3 and 3.5 indicating commercial maturity (Walteros *et al*., 2012). The results show that foliar spraying with I affected these oenological parameters (Table 2). I at low concentrations has been shown to increase sugar content (12%) and at high concentrations to reduce (9%) TSS according to the reported (Li *et al*., 2017; Budke *et al*., 2021). I decreased TA in grapevine berries (20%). Similar results were reported by Budke *et al*. (2020) with the application of iodine doses from 0.2 to 1.4 mg L^{-1} with the lowest doses an increase and with higher doses a decrease of tartaric acid was found. High TSS and low TA cause a low TSS/TA ratio attributable to the fact that organic acids are used as a substrate for sugar synthesis and respiration during berry ripening (Sariñana-Navarrete *et al*., 2021). Then, the decrease in TA and the increase in TSS is due to the fact that during ripening there is an increment in membrane permeability that allows the respiration of organic acids stored in cell vacuoles and their transformation into sugars (Sabir *et al*., 2010).

Table 2. Effect of foliar spraying of I on oenological parameters in grapevine berries

Iodine (mg L^{-1})	pH	TSS (°Brix)	Probable degrees of alcohol (%)	Maturation index	Titratable acidity (%)
0	3.5c*	18.0a	10.06c	2.29c	7.89a
0.25	3.9a	20.6a	12.54a	3.00ab	7.14ab
0.50	3.8ab	20.6a	12.09a	3.34a	6.29b
0.75	3.6abc	18.6a	10.51bc	2.91ab	6.40b
1.00	3.5bc	19.6a	11.41ab	3.12ab	6.40b
1.25	3.7abc	18.6a	10.51bc	2.68bc	7.04ab

*Different letters in each column indicate significative difference ($p \leq 0.05$).

Non-enzymatic antioxidants

The exogenous application of a biostimulant to the plant induces biochemical and physiological changes and, in response, activates a series of mechanisms similar to those caused by a phytopathological attack, or another kind of stress, which modifies plant metabolism and influences the synthesis of enzymatic and non-enzymatic antioxidants. The results show that the application of I increased the content of non-enzymatic antioxidants: phenols, flavonoids, antioxidant capacity, vitamin C and anthocyanins (50,34,31,71,26% respectively) in relation to untreated berries (Table 3).

These results are in agreement with previous studies reporting increases in non-enzymatic antioxidant content by the use of I (Consentino *et al*., 2022; Garcia-Fuentes *et al*., 2022; Maglione *et al*., 2022).The response of plants to I depends on the concentration and chemical species used and can range from an increase in development and productivity to a stressful condition or toxicity (Riyazuddin *et al*., 2022), in our case we believe it was a stressful condition due to a decrease in yield (Table 1) and an increase in non-enzymatic antioxidants, with the aim of preventing oxidative damage to the plant (Gonzali *et al*., 2017; Kiferle *et al*., 2019).

Table 3. Effect of foliar spraying of I on non-enzymatic antioxidants in grapevine berries

Iodine mg L^{-1}	Phenolic content (mg GAE 100 g^{-1} FW)	Flavonoids (mg QE 100 g^{-1} FW)	Antioxidant capacity (µM Trolox equivalent 100 g^{-1} FW)	Vitamin C (mg 100 g^{-1} FW)	Anthocyanin (µg of Cyanidin 3-glucoside g^{-1} FW)
0	161.78e*	148.00e	121.71d	8.06c	117.86b
0.25	188.24d	160.23d	124.64d	19.06b	156.04a
0.50	246.26c	185.96c	128.03cd	19.06b	128.94ab
0.75	286.32b	205.56b	134.31c	17.60b	140.28ab
1.00	293.64b	211.31b	153.57b	24.93a	159.29a
1.25	324.17a	226.64a	177.20a	27.86a	160.17a

*Different letters in each column indicate significative difference ($p \leq 0.05$).

Enzymatic activity

CAT and PXA belong to the enzymatic antioxidants of plants. The typical process catalysed by CAT is to use H_2O_2 as a substrate to decompose it to H_2O and O_2. CAT and PXA activities in grapevine berries were increased both enzymatic activities with the application of I (Table 4). The CAT activities were all higher than that of the control. Similar results have been reported increasing the doses of potassium iodine decreased CAT activity (Halka, *et al*., 2020). As well as, the reduction of CAT activity with the application of organic I compounds 5-iodosalicylic acid and 3,5-diiodosalicylic I in tomato plants has been reported in previous studies (Halka *et al*., 2019). This reduction with increasing dose could be due to the fact that it is a specific response of grapevine plants to the exogenous action of I application. In the other hand, PXA is affected by the inorganic

forms of I (I^- or IO_3^-) application on tomato seedlings; the treatments with I is probably connected with the activation of other protection mechanisms against ROS (Halka *et al.*, 2019). Furthermore, it was an increased according to the I concentration; it is possible that when given higher doses, grapevine plants produce more PXA as a way to reduce a probable damage (Li *et al.*, 2017). However, limited research has been carried out to examine the impact of organic I on the antioxidant capacity of vegetables (Sularz *et al.*, 2020).

Table 4. Effect of foliar spraying of I on enzymatic antioxidants in grapevine berries

Iodine (mg L^{-1})	CAT (mM min^{-1})	PXA (mM min^{-1})
0	0.0166d*	0.362c
0.25	0.0616a	0.414c
0.50	0.0566ab	0.519bc
0.75	0.0433bc	0.578b
1.00	0.0416c	0.617b
1.25	0.0300cd	0.857a

*Different letters in each column indicate significative difference ($p \leq 0.05$).

Iodine content in berries

The I content in soils depends on their geographical location with respect to the sea, which is why most cultivated plants are deficient in I (Mohiuddin *et al.*, 2019). In this context, biofortification can improve the content of this trace element in plant foods and decrease the health problems inherent to the deficiency of I. In this study, compared to the control, it was obtained the higher concentration of I with the application of 1.25 mg L^{-1} (59%). The results show that foliar spraying improved the bioaccumulation of I with respect to the control treatment berries (Figure 1).

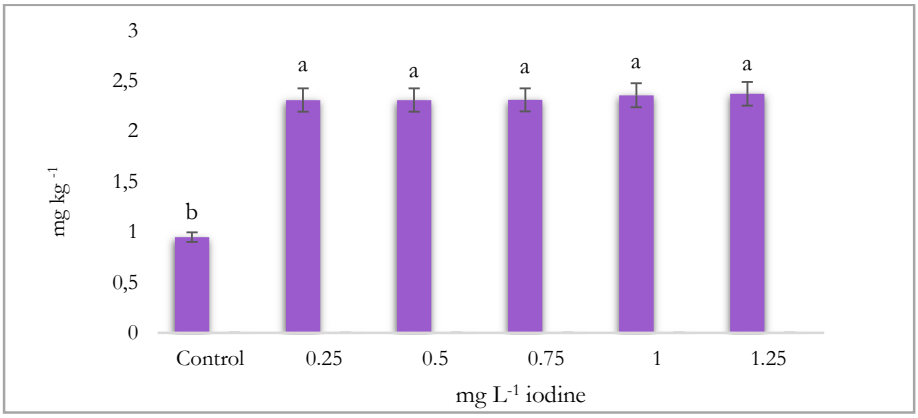

Figure 1. Effect of foliar iodine spraying on iodine bioaccumulation in grapevine berries. Different letters indicate significant difference ($p \leq 0.05$).

Several studies have shown that biofortification with I is a practical way to increase the content of this element in the edible part of the plant (Budke *et al.*, 2021; Sabatino *et al.*, 2021). Recent authors have reported that all the applied iodine compounds contributed to a significant increase in I content in leaves, stems and roots of tomato seedlings (Halka *et al.*, 2019). Inorganic form as KI have demonstrated a proportional increment according to the doses applied, it usually got accumulated in parts of the plant (Halka *et al.*, 2019). Moreover, the dose should be considerate and chemical species because leaf necrosis can be caused by high doses of I (Cakmak *et al.*, 2017; Incrocci *et al.*, 2019; Budke *et al.*, 2020; Buke *et al.*, 2021). I usually causes stronger phytotoxic effects than IO_3^-, the above could be attributed that I inhibits superoxide dismutase activity, whereas

IO_3^- can promote its activity (Halka *et al.*, 2020). A predominant accumulation of iodine applied as iodobenzoates in the roots may be related to its more complex structure compared to iodides (Halka *et al.*, 2020). Hence, the hypothesis reported by Halka *et al.* (2020) the ionic forms could be transported through the chloride's channels while iodine from aromatic compounds is characterized by their weaker translocation.

Conclusions

Foliar application of I in the vineyard promotes yield, bioactive compounds and enzymatic activity in grape berries. Intermediate doses increased yield in grapevine plants, while high concentrations decreased it, but increased the enzymatic and non-enzymatic antioxidants. Foliar spraying with I is a simple and accessible practice that induces the synthesis of bioactive compounds, as well as their bioaccumulation in grape berries.

Authors' Contributions

Conceptualization: VBRG, PPR; Methodology: MFH, RGA; Validation: VBRG; Formal analysis: MFH, Investigation: VBRG; Data curation: MFH; Funding acquisition: PPR; Project administration: RGA. Writing: VBRG, PPR; Review and editing PPR.

All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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