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Outcomes of foliar iodine application on growth, minerals and antioxidants in tomato plants under salt stress

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ABSTRACT

Plant biostimulants have been used to reduce the damage caused by different types of biotic and abiotic stresses. Iodine (I) is a non-essential element in plants. Still, it is considered beneficial and a biostimulant, since exogenous application can enhance the redox metabolism, which improves antioxidants, synergies with essential minerals and increases tolerance to adverse factors. However, little is known about the mechanism of action of iodine; so, it is advantageous to undertake research that elucidates the impact of this element on plant physiology, which is expected to encourage the productive agricultural sector to use this element with additional biofortification benefit. The objective of this research was to evaluate the effect of foliar KIO_3 applications every 15 days at 100 µM, on growth, mineral content and antioxidants in tomato plants grown under greenhouse conditions subjected to salinity stress (100 mM NaCl). The results showed that iodine did not mitigate the adverse impact of salinity on fresh or dry biomass but increased fruit production by 23%. A greater amount of N and Fe was also found in the leaves but not in the fruits; the same happened with the iodine concentration, which was high in the leaves of the treated plants but not in tomato fruits. The content of Ca and Mg in fruits was decreased in plants treated with iodine, as well as the activity of the GPX, lycopene and the antioxidant potential. None of the fruit quality variables were affected by salinity with or without application of iodine.

Keywords: biostimulants, greenhouse, nutraceutical, redox metabolism, salt tolerance, soilless

Abbreviations: ANSA, amino naphtol sulfonic acid; APX, ascorbate peroxidase; ASA, ascorbic acid; CA, antioxidant capacity; CAT, catalase; CO_2, carbon dioxide; CyTb6f, cytochrome b6f complex; DAT, days after transplantation; DPPH, 2,2-diphenyl-1-picrylhydrazyl; DTNB, 5,5 dithio-bis-2 nitro benzoic acid; Eh, redox potential; GPX, glutathione peroxidase; GSH, gluthatione; HPLC, high-performance liquid chromatography; ICP-OES, inductively coupled plasma-optical emission spectrometry; IU, international units; KI, potassium iodide; KIO_3, potassium iodate; LHC, light harvesting complex; Lic, lycopene; M, molarity; mM, millimolar; mmol, millimole; nm, nanometer; Prot, proteins; Ps, photosystems; ROS, reactive oxygen species; rpm, revolutions per minute; SOD, superoxide dismutase; T0, time zero; T1, time one; UV-VIS, ultraviolet visible spectra.

INTRODUCTION

The challenge that plants the cope to cope with adverse environmental conditions leads to the overproduction of reactive oxygen species (ROS) such as peroxide, superoxide, hydroxyl and singlet oxygen, among others.

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In large quantities, these species cause oxidative stress, which triggers a reduction in growth and production, and even cell death (Gill and Tuteja, 2010). Currently the planet is undergoing an accelerated change of environmental conditions. It can be argued that the most serious among these for vegetation is a high content of salt in soils, which leads to a hydric, osmotic and photosynthetic imbalance in plant metabolism (Acosta-Motos et al., 2017). Therefore, strategies to enhance stress tolerance are used by applying organic or inorganic molecules called biostimulants; this protection is known as molecular priming (Kerchev et al., 2020).

An inorganic biostimulant that offers a wide variety of benefits is the element iodine (Medrano-Macías et al., 2016a), which, due to its broad oxido-reducing power, can act as both an inorganic antioxidant (Venturi, 2011; Medrano-Macías et al., 2016b) and as promotor of the synthesis of a plethora of molecules with reducing power such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) (Blasco et al., 2011) and phenolic compounds (Blasco et al., 2013; Gonzali et al., 2017). Furthermore, iodine has also been associated with salicylic acid metabolism, probably by participating in the systemic induced resistance (Halka et al., 2019) and the increase in the content of essential oils (Kiferle et al., 2020). Recently, the incorporation of iodine in protein complexes, such as photosystems I and II, cytochrome b6f complex (Cytb6f) and ATPase, has been studied, for which the non-essentiality of this element has been questioned (Kiferle et al., 2021).

In addition, some research works have found a close relationship between the application of iodine and the content of the essential elements; this phenomenon is poorly understood, but it is attributed to a change in the redox state, pH and redox potential (*Eh*) of the system in which it is absorbed (Venturi et al., 2002). However, the interactions between iodine, macro- and microelements have not shown a general tendency; synergies and antagonisms have been found. An example was the reported in lettuce plants after the application of potassium iodide (KI) and potassium iodate (KIO_3) – a decreased content of N, P, K, Mg, S, Ca and some microelements such as B, Cu and Fe was found, but without physiological impact (Smoleń et al., 2011). On the other hand, in various crops, a synergy with essential microelements such as Mn and Cu has been found (Hageman et al. 1942; García Osuna et al. 2014). Furthermore, at least for Cu, it has been reported that in some bacteria, iodine is metabolised by copper-dependent enzymes, and possibly a similar process occurs in plants (Suzuki et al., 2012).

So, the use of iodine has been tested against some types of abiotic stress. It is worth mentioning that till date the works related to this topic are scarce, but the results have been quite encouraging. For example, an experiment carried out in *Glycine max* plants subjected to stress by Cd^{+2} evidenced that iodine induced an increase in antioxidant compounds and enhancement in defence response. It was also demonstrated that the exogenous iodine application in strawberry plants under salinity stress improves the yield and fruit quality, as well as the antioxidant and microelement uptake (Medrano et al., 2021). Experiments conducted with hydroponic lettuce plants (Leyva et al., 2011) indicated an association of iodine with antioxidant metabolism.

Considering the potential of using iodine as a biostimulant and as an element to biofortify crops, the objective of this work was to evaluate the effect of iodine on growth, production, essential elements content and antioxidants in tomato plants subjected to salinity stress.

MATERIALS AND METHODS

Site description

The experiment was carried out in a chapel-type greenhouse with passive temperature control, located in the Horticulture Department of the Autonomous Agrarian University, Antonio Narro, Coahuila, Mexico, located at 25°21′12.8″ north latitude and 101°01′51.9″ west longitude. The experiment was conducted at 1,711 mslm with a temperature range of 20 °C and 35 °C, a relative humidity between 50% and 60%, 70% of natural irradiance and a 430 ppm of carbon dioxide (CO_2).

Plant material

Tomato (*Solanum lycopersicum* L.) variety 'Rio Fuego' seedlings were used as plant material, which was transplanted 4 weeks after sowing, on 10 May 2019, in 10 kg capacity polystyrene containers. Peat moss + perlite was used as a substrate in a 1:1 ratio.

Plant nutrition

Fertilisation was started 1 day after transplantation. Steiner (1961) nutrient solution was applied daily through an automatised system in irrigations of 200 mL for 5 min, five times a day. The fertilisers used and their concentrations were the following: $Ca(NO_3)_2{\cdot}4H_2O$ – 4.5 mmol, KH_2PO_4 – 1 mmol, $MgSO_4{\cdot}7H_2O_4$ – 2 mmol, KNO_3 – 3 mmol, K_2SO_4 – 1.5 mmol, Fe chelated – 3 mg · L^{-1}, H_3BO_3 – 0.5 mg · L^{-1}, $MnSO_4$ – 0.7 mg · L^{-1}, $ZnSO_4$ – 0.09 mg · L^{-1} and $CuSO_4$ – 0.02 mg · L^{-1}. The nutrient solution was maintained at pH 6.3 and an electrical conductivity (EC) of 1.8 dS · m^{-1}.

Iodine treatments and salt stress

Iodine application was started 10 days after transplantation (DAT) using KIO_3. A total of three foliar treatments were carried out: (1) absolute control (SN), (2) saline control (NaCl at 100 mM) and (3) plants subjected to saline stress + iodate at 100 µM (NaCl + KIO_3). A total of 35 plants were used in each

Table 1. Representation of treatments and controls.

Salt conditions	NaCl (salt control) 35 plants	NaCl + KIO_3 35 plants
Normal conditions	Absolute control (only Steiner solution) 35 plants	

KIO_3, potassium iodate.

treatment, giving rise to a cumulative figure of 105 plants (Table 1).

The foliar applications were in both adaxial and abaxial sides of the leaves at a rate of 16 mL per plant or 400 L · ha^{-1}; every plant received 39 mg of iodine per litre, 20 dat (30 May 2019) and the stress treatment was started by adding NaCl as nutrient solution at 100 mM, through a completely randomised experimental design.

Sampling

Three randomised samplings were carried out for the quantification of growth; the first one was carried out at 41 dat, in seedling stage; the second one at 71 dat, in the flowering stage; and the third sampling at 125 dat, in full production. In each sampling, five plants were taken, and were evaluated to assess the number of leaflets, counting each leaf blade within the compound leaves; the height of the plant was measured with a flexible tape from the base of the stem to the apex; the dry weight (DW) was obtained after placing the samples in an oven for 76 h at a temperature of 70 °C.

Determination of mineral nutrients

The K, Ca, Mg, Mn, Fe, Zn and Cu were mineralised by acid digestion as follows; 1 g of dry tissue was weighted in OHAUS analytical balance after 30 mL of nitric acid was added, and then they were placed on a heating plate until the clarification of the sample. Finally, it was made up to 100 mL with deionised water and filtered on Whatman #1 paper (Helrich, 1990). The sample was analysed using a Varian spectra *fs-240* Atomic Absorption Spectrophotometer (AAS).

Total nitrogen was determined using the micro Kjeldhal technique (Muller, 1961), which was carried out by weighing 50 mg of dehydrated tissue, and placing it in a flask + 3 mL of digester mixture; once it had turned to a transparent green colour, the sample was transferred to the distiller tube adding 25 mL of sodium hydroxide to 50%; after this, 30 mL of boric acid + four drops of mixed indicator + distillation were placed in a beaker, until 60 mL of solution with a blue-green colour had been obtained. Next, the titration was carried out by placing a burette with 0.025 N sulphuric acid and dropping it into the distilled sample until a pink colour was obtained. Finally, the determination of the N content was made based on the spent volume (mL) of sulphuric acid.

Total phosphorous was determined by the spectrophotometric technique of aminonaphtholsulfonic acid (ANSA), according to the method suggested by Peterson (1978). First, 1 mL of the digested sample was taken and placed in a test tube along with 5 mL of an ammonium molybdate solution + 2 mL of ANSA solution; once stirred, it was allowed to stand for 20 min; the reading was carried out in a UV-VIS Thermo Genesys 102 spectrophotometer at 650 nm.

Determination of iodine content

Iodine was extracted by alkaline ash technique (Ujowundu et al. 2009); 1 g of dry plant tissue was weighed and placed in crucibles; later 2 mL of 2 M KOH, and 1 mL of 2 M KNO_3 was placed in an oven at 100 °C for 2 h. Subsequently, the crucibles were put in a muffle at 580 °C for 3 h. Finally, the iodine was extracted with 2 mL of 2 mM KOH. The quantification was carried out using an inductively coupled plasma-optical emission spectrometry (ICP-OES) device, Agilent 725. The results were expressed in milligram of iodine per kilogram of DW.

Yield

Every fruit produced by the plants was counted and weighed in each treatment until the end of the cycle, and the total fresh weight was expressed in grams.

Biomolecules extraction

The lyophilised plant tissue was macerated and weighted at 100 mg. It was then transferred to centrifuge tubes with 10 mg of polyvinyl pyrrolidone; 2 mL of 0.1 M of phosphate buffer pH 7.2 was added and thereafter sonication was carried out for 10 min. Subsequently, the samples were subjected to microcentrifugation at 12,000 rpm for 10 min at 4 °C, and the supernatant was collected and filtered with a nylon syringe filter (Ramos et al., 2010). Finally, it was diluted 1:15 with phosphate buffer.

Antioxidant capacity test (CA)

Assessment of CA was carried out by 2,2-diphenyl-1-picrylhydrazyl (DPPH) technique, wherein 50 µL of the biomolecule extract plus 50 µL of DPPH at concentration of 0.5 mM solution were added; they reacted for 15 min, and the reading was reckoned at 530 nm in a plate reader Biotek Exl 8000 (Mareček et al., 2017).

Lycopene

Lycopene was quantified spectrophotometrically according to the method suggested by Bunghez et al. (2011); 100 mg of dried tomato tissue was weighed and placed in 2 mL centrifuge tubes. After 1.5 mL hexane was added to the above, they were homogenised in vortex for 30 s, sonicated for 5 min and centrifuged at 4 °C for 10 min at 10,000 rpm. The supernatant was extracted

and filtrated with syringe filters and read at 472 nm in ultraviolet visible spectra (UV-VIS) using Genesys 10S Thermo Scientific, Santa Clara, California, USA.

Ascorbic acid

For the extraction of ascorbic acid (AsA), 100 mg of the lyophilised leaves were weighed, and 1 mL of water : acetone solution was added in a 1:1 ratio (Yu and Dahlgren, 2000). It was vortexed for 30 s, followed by sonication for 5 min. Finally, it was centrifuged at 4 °C at 12,500 rpm for 10 min, and the supernatant was extracted and filtered. Quantification was carried out using high-performance liquid chromatography (HPLC), for which a Thermo Spectra system P4000® was used, under the following conditions: wavelength 230 nm, mobile phase NaH_2PO_4 50 mM, pH 2.8, flow 1 mL · min^{-1}. Further, aquasil C-18 column was used at a temperature of 60 °C. Units were reported in grams per kilogram.

Glutathione

Glutathione was quantified following the spectrophotometric technique using 5,5 dithio-bis-2 nitro benzoic acid (DTNB) (Xue et al., 2001); 0.48 mL of the extract, 2.2 mL of dibasic sodium phosphate (Na_2HPO_4 at 0.32 M) and 0.32 mL of DTNB at 1 mM were placed in a centrifuge tube. It was mixed and read on a UV-VIS spectrophotometer at 412 nm. The results were expressed in grams per kilogram.

Total proteins

For the quantification of the total proteins, 100 µL of the biomolecule extract plus 1 mL of the Bradford reagent were added; the result was mixed and reacted for 2 min. Finally, it was read at 595 nm in the UV-VIS spectrophotometer, using bovine serum protein as a standard (Bradford, 1976).

Total phenols

Hundred milligrams of lyophilised leaves plus 1 mL of water-acetone solution was centrifuged at 10,000 rpm at 4 °C for 10 min, and the supernatant was obtained. Next, 50 µL of supernatant was taken, and 200 µL of Folin–Ciocalteu reagent plus 500 µL of 20% Na_2CO_3 and 5 mL of distilled water were added. Subsequently, it was incubated at 45 °C for 30 min. The samples were read with the spectrophotometer at 750 nm (Urias-Lugo et al., 2015).

Catalase

Testing for catalase was carried out by measuring two reaction times, time zero (T0) and reaction time 1 min (T1). For T1, it was prepared by adding 0.1 mL of biomolecule extract plus 1 mL of H_2O_2, and after 1 min of reaction, 0.4 mL of 5% H_2SO_4 was applied. The substrate concentration (H_2O_2) was read at 270 nm in a spectrophotometer (Cansev et al., 2011).

Glutathione peroxidase

Assessment for glutathione peroxidase was carried out with the method established by Xue et al. (2001) using H_2O_2 as a substrate. First, 0.2 mL of the biomolecules extract was placed in a test tube, with the addition of 0.4 mL of reduced glutathione (0.1 M) and 0.2 mL of Na_2HPO_4 (67 mM). Subsequently, 0.2 mL of 1.3 mM H_2O_2 was added to start the catalytic reaction. After 10 min, 1 mL of trichloroacetic acid (1%) was added to stop the reaction. This mixture was placed in an ice bath for 30 min. Then it was centrifuged at 3,000 rpm for 10 min. Next, 0.48 mL of supernatant was placed in a test tube, and then 2.2 mL of Na_2HPO_4 (0.32 M) and 0.32 mL DTNB were added. Readings were taken on the UV-VIS spectrophotometer at 412 nm.

Ascorbate peroxidase (APX)

The APX enzymatic activity was carried out according to the method of Nakano and Asada (1987); 100 µL of biomolecules extract, 500 µL of ascorbate (10 mg · L^{-1}) and 1 mL of H_2O_2 (100 mM) were added in a microcentrifuge tube. AsA was measured at 266 nm in a spectrophotometer (Thermo Scientific Genesys 10S UV-VIS) for T0, after 10 min (T1). The activity units (IU) were expressed in mM of ascorbate · min^{-1} / total proteins.

Superoxide dismutase

The determination of the enzymatic activity of SOD was carried out using the SOD Cayman 706002® kit. A mixture of 20 µL of extract, 200 µL of radical detector (tetrazolium salt) and 20 µL of xanthine oxidase solution was placed in a microplate, shaken for 10 s and then incubated at 26 °C for 30 min. The absorbance was measured at 450 nm using a plate reader. SOD activity was expressed as percentage inhibition rate.

Experimental design and statistical analyses

The experimental design was completely randomised with three treatments and 35 repetitions per treatment, resulting in a total of 105 plants for the experiment; each plant was considered as an experimental unit.

The data was analysed in univariate form, using a one-way analysis of variance (ANOVA) with five repetitions per treatment, followed by a least significant difference *post-hoc* test (LSD, $p \leq 0.05$) using the Infostat software package (2018 version).

RESULTS

Growth

As shown in Figure 1, the highest amount of biomass was found in the absolute control plants, in the three samplings. On the other hand, the saline control (NaCl)

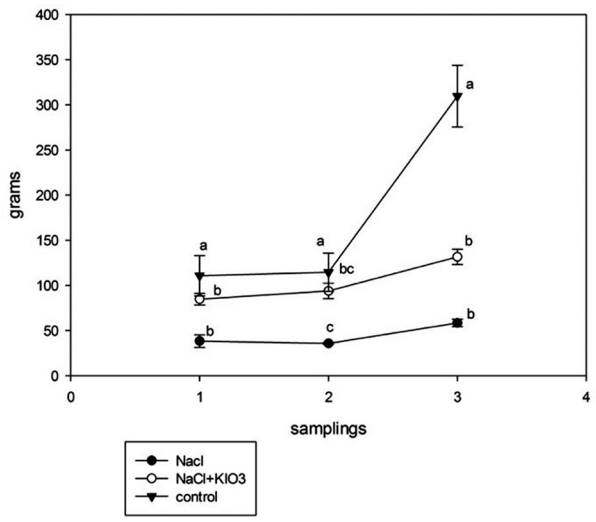

Figure 1. Effect of iodine application in vegetative biomass, in three samplings [(1) 41 dat, (2) 71 dat, (3) 125 dat]; the units are expressed in grams. Means with same letters do not show statistically significant differences, $p \leq 0.05$, vertical bars represent standard deviations. KIO_3, potassium iodate.

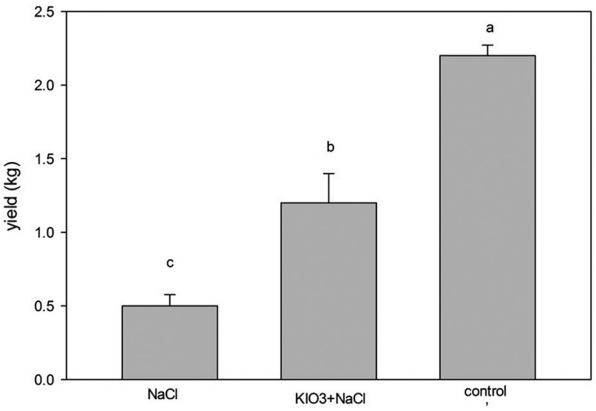

Figure 2. Effects of iodine application in total tomato yield expressed in kg F.W.; treatments control, salt control (NaCl) and KIO_3. Means with same letters do not show statistically significant differences, $p \leq 0.05$, vertical bars represent standard deviations. KIO_3, potassium iodate.

and treated-with-KIO_3 plants (NaCl + KIO_3) did not show statistical differences, indicating that iodine applications did not prevent the biomass loss.

However, although iodine did not result in avoiding the loss of plant biomass, it improved yield (raising it by 23%), and resulted in a more significant number of fruits per plant, as indicated in Figure 2.

Table 2 shows other growth variables, such as fresh weight, height, number of leaflets and stem diameter, but no differences were found between salt control (NaCl), and KIO_3-treated plants.

Mineral content

Leaves

Table 3 shows the macroelement content in leaves, expressed in units of grams per kilogram, and it was observed that the nitrogen content increased by 31% with the application of KIO_3 under salinity stress conditions. On the other hand, a reduction in calcium content was evidenced in both groups of plants under stress conditions, both in the presence and absence of KIO_3; however, the content of P, K and Mg was not affected. In addition, higher Na content was found in plants under high salinity.

Table 4 shows the content of essential microelements in the leaves; it can be seen that under stress conditions, a higher concentration of Fe and Cu was found; regarding the content of Zn and Mn, no statistical differences were found between treatments.

Fruits

Table 5 shows the results obtained from macrominerals in tomato fruits. An increase in nitrogen content in plants subjected to salinity stress (NaCl) compared to the absolute control was evident; however, the plants treated with iodine (KIO_3 + NaCl) showed a lower concentration by 22% compared to the NaCl control. On the other hand, a reduction in Ca concentration was found, while the content of P, K and Mg did not show changes between treatments or stress conditions. Finally, the concentration of Na was increased in both treatments under stress.

Table 2. Effects of iodine on fruit number, plant fresh weight, height, number of foliole and stem diameter of tomato plants.

Treatment	Fruit number	Fresh weight (g)			Height (cm)		
	Sampling	1	2	3	1	2	3
NaCl	21.6 c*	231.96 b	609.32 b	784.80 b	52.40 b	61.70 b	73.60 b
KIO_3 + NaCl	34.2 b	245.96 b	683.16 b	760.22 b	51.10 b	61.80 b	70.80 b
Control	48.8 a	394.42 a	833.06 a	1914.92 a	59.80 a	94.70 a	96.00 a
		Foliole number			Stem diameter (cm)		
		1	2	3	1	2	3
NaCl		151.00 b	294.60 b	780.80 b	9.80 b	11.80 a	15.00 b
KIO_3 + NaCl		142.40 b	307.40 b	603.00 b	10.80 b	13.20 a	15.20 b
Control		217.50 a	462.20 a	1476.00 a	14.40 a	12.80 a	18.60 a

*Means with same letters do not show statistically significant differences, $p \leq 0.05$.

KIO_3, potassium iodate.

Table 3. Effects of iodine on macroelement content in tomato leaves.

Treatments	N	P	Ca	K	Mg	Na
(g · kg^{-1} D.W.)						
NaCl	19.10 b*	3.56 a	36.83 bc	21.03 a	16.03 a	46.88 a
KIO_3 + NaCl	22.60 a	4.05 a	31.60 c	23.63 a	13.16 a	35.27 a
Control	17.23 b	2.94 a	44.52 a	29.66 a	19.91 a	7.14 b

*Means with same letters do not show statistically significant differences, $p \leq 0.05$.
KIO_3, potassium iodate.

Table 4. Effect of iodine on microelement content in tomato leaves.

Treatments	Fe	Zn	Cu	Mn
(mg · kg^{-1} D.W.)				
NaCl	192.34 a*	51.60 a	13.43 a	245.23 a
KIO_3 + NaCl	145.80 a	43.90 a	17.12 a	241.68 a
Control	100.86 b	41.50 a	5.63 b	183.29 a

*Means with same letters do not show statistically significant differences, $p \leq 0.05$.
KIO_3, potassium iodate.

Table 5. Effects of iodine on macroelement content in tomato fruit.

Treatments	N	P	Ca	K	Mg	Na
(g · kg^{-1} D.W.)						
NaCl	24.02 a	2.59 a	1.00 bc	58.31 a	2.72 a	6.12 a
KIO_3 + NaCl	18.27 b	2.51 a	0.53 c	52.32 a	2.01 a	9.04 a
Control	15.39 c	2.12 a	2.02 a	58.29 a	2.68 a	2.32 b

*Means with same letters do not show statistically significant differences, $p \leq 0.05$.
KIO_3, potassium iodate.

Table 6. Effect of iodine on microelement content in tomato fruits.

Treatments	Fe	Zn	Cu	Mn
(mg · kg^{-1} D.W.)				
NaCl	2.96 b	27.69 a	11.87 a	12.81 a
KIO_3 + NaCl	1.83 b	24.35 a	10.12 a	10.89 a
Control	28.04 a	23.64 b	8.38 a	13.29 a

*Means with same letters do not show statistically significant differences, $p \leq 0.05$.
KIO_3, potassium iodate.

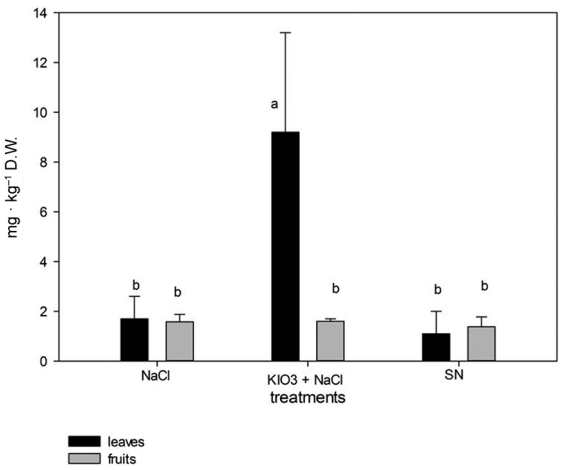

Figure 3. Iodine concentration in leaves and fruits of tomato, expressed in mg · kg^{-1} D.W. Means with same letters do not show statistically significant differences, $p \leq 0.05$, vertical bars represent standard deviations. KIO_3, potassium iodate.

The results of microelement content obtained in fruit can be observed from Table 6, together with a reduction in the Fe concentration in the plants under salt stress. In contrast, this same group of plants showed an increase in the Zn content, while the Mn and the Cu content did not show statistical differences ($p \leq 0.05$).

Iodine content

As shown in Figure 3, the highest concentration of iodine was found in the leaves of the plants treated with KIO_3 (KIO_3 + NaCl) compared to NaCl control, with an iodine average of 9.25 mg · kg^{-1}. In the fruit, the range was between 1.3 mg · kg^{-1} and 1.6 mg · kg^{-1} D.W., with no statistical differences between treatments.

Table 7. Effect of iodine on antioxidant content in tomato fruit.

Treatments	CA (mM trolox)	AsA* ($g \cdot kg^{-1}$)	Lic ($mg \cdot kg^{-1}$)	GSH ($g \cdot kg^{-1}$)	Prot ($g \cdot kg^{-1}$)	CAT ($U \cdot min^{-1}$)	GPX ($U \cdot min^{-1}$)	APX ($U \cdot min^{-1}$)	SOD ($U \cdot min^{-1}$)
NaCl	51.8 a**	37 b	19 a	0.68 a	16.1 b	0.12 a	1.6 a	0.01 a	5.68 a
NaCl+KIO_3	21.2 b	59.5 a	4.4 b	0.87 a	16.2 a	0.11 a	1.6 a	0.01 a	5.40 a
Control	29.6 b	70.8 a	22.5 a	0.99 a	12.5 c	0.14 a	3.1 b	0.01a	6.52 a

*CA, Antioxidant capacity; AsA, ascorbic acid; APX, ascorbate peroxidase; Lic, lycopene; GSH, gluthatione; Prot, total proteins; CAT, catalase; GPX, glutathione peroxidase; SOD, superoxide dismutase; KIO_3, potassium iodate.

**Means with same letters do not show statistically significant differences, $p \leq 0.05$.

Antioxidants in fruit

Table 7 shows the results obtained for enzymatic and non-enzymatic antioxidants, CA and total proteins found in tomato fruits treated with iodine and subjected to salinity stress. The most notable result was an increase in the content of AsA and total proteins in the plants treated with iodine and subjected to salinity stress; in contrast, it was found that the same treatments were characterised by a reduction in CA compared to NaCl control. In addition, an increase in glutathione peroxidase (GPX) activity was also observed in plants subjected to NaCl and KIO_3 + NaCl application. Finally, a decrease in lycopene was found with the iodine + NaCl treatment compared to controls.

DISCUSSION

There is strong evidence that plants under stress are associated with less biomass and a lower yield index (Moreno, 2009). In the present study, it was found that plants subjected to salinity conditions and treated with iodine presented a reduction in dry biomass of 34.41% in the first sampling, 35.63% in the second and 64.26% in the third; so, it is concluded that iodine applications cannot result in avoiding the loss of vegetative biomass. However, it does prevent the loss of reproductive tissue by 23%.

Scientific literature indicates no general tendency between the concentration, form and chemical species of iodine and response in terms of growth among different plant species (Weng et al., 2008). However, its exogenous application has been related to redox metabolism. Furthermore, recent findings point out its incorporation into photosynthetic proteins, such as PS 11, PS1, LHC11, plastocyanins and ferredoxin-oxidoreductase (Lo Piccolo et al., 2021), which give it a narrow range between beneficial effect and toxicity. Under salt stress, there is an osmotic imbalance caused by excess Na^+ and Cl^-; the involvement of iodine in photosynthesis could have interrupted the vegetative biomass production, and probably the adjustment of some metabolic pathway leads energy into fruit production.

Regarding the content of essential elements in leaves, an increase in nitrogen concentration was found, which could be linked to the effect of the decrease in plant size due to stress; the above would translate into N absorption not modified, and may be accompanied with a modification in the destination of assimilation. Likewise, an increase in Fe concentration was observed in the leaves of plants subjected to stress. A similar result was reported by Askary et al. (2017), who argued that due to the excess of ions and the consequent overproduction of species reactive oxygen, peroxidation of the cell membrane is promoted, affecting its integrity. So, there is a change in the modulation pattern, for both release and uptake of ions; coupled with this, a higher sodium content was evidenced in this same organ.

On the other hand, a lower calcium concentration was found in the plants under stress, compared to the

controls. However, these concentrations remained within the normal ranges (0.1–5% DW) (White and Broadley, 2003). It is known that besides the structural functions of calcium, it acts as a signaling agent under stress conditions; the effect found could be related to the absorption that occurs via the root, since it has been reported that the metabolic activities in channels are carried out through voltage-dependence and by electrophysiological properties, which could be affected by the excess of Na^+ and Cl^- ions in the surrounding medium (Thor, 2019).

A lower concentration of nitrogen in the fruits was found in iodine-treated plants compared with both controls. However, this was not below normal values (1.5–6%) (Kabata-Pendias and Pendias, 2011); a similar finding was reported in curly endive plants, where a reduction in nitrogen content was found in the aerial parts, but with an increase in uptake via root, after the application of an inorganic biostimulant. The above phenomenon was attributed to an inhibition in translocation from root to shoot, even attributed benefits to it due that photosynthesis or biomass were not compromised; also a lower nitrites production was achieved, which have adverse effects on the final consumer (Sabatino et al., 2019).

The calcium and iron content were decreased in plants under stress. Similar results have been found in plants such as beans (Ullah et al., 1993) and corn (Turan et al., 2010), suggesting an imbalance in ionic product uptake due to increased sodium in the surrounding medium. The presence of iodine applied via foliar did not prevent such imbalance.

Zinc was increased under salt stress conditions, this phenomenon has been previously reported in *Brassica juncea* and this has been at least partially explained as being due to an increase in Zn mobility due to Cl^- presence, which leads an enhancement in the Zn transport to the roots due to an extra bioavailability in the rhizosphere (Novo et al., 2014). However, these same authors present the opinion that more research is necessary to elucidate this phenomenon better.

The highest accumulation of iodine was found in the leaves, probably because the mobility of this element is mainly carried out via the xylem, while the phloem pathway is limited (Humphrey et al., 2018).

Although various studies have shown the effectiveness of iodine biofortification in horticultural plants (Dai et al., 2004; Landini et al., 2011; Smoleń and Sady, 2012; Lawson et al., 2015), little information is available about the mechanism of absorption and transportation via foliar of iodine. It has been evidenced that iodate is reduced to iodide in roots, probably due to existence of iodate reductase enzyme (Kato et al., 2013), and transported via symplastic and apoplastic to finally reach the xylem. The transport via phloem was believed to be scarce at first but in recent studies it was concluded that it also has an important participation (Humphrey et al., 2019); for this reason, the bioaccumulation of iodine in fruits is possible, even in species such as cereals (Cakmak et al., 2017). However, the phenomenon of reduction from iodate to iodide via foliar has not been evidenced, but it could occur in a similar way to that in root, and thus a competition between transport of chloride and iodide takes place due to the fact that the transporters used are the same (Blasco et al., 2013); the above could explain the observation that iodine accumulation in leaf tissue occurred but it was not transported to fruit. Only two similar studies were found in the current literature; one in lettuce (Leyva et al., 2011) and the other in strawberries (Medrano et al., 2021); in the second research work, the phenomenon was similar, i.e. accumulation in the aerial part but not in fruit, leading to the conclusion that more studies are needed to elucidate the iodine transport mechanism under salt conditions.

Regarding the content of molecules with reducing power, such as AsA, a higher concentration was found in the plants treated with iodine compared to controls. AsA is a hydrophilic antioxidant, the most abundant in the cytoplasm, and is largely responsible for the redox buffer. It acts in different ways as a direct electron donor and cofactor for enzymes such as APX participating in the Halliwell-Asada cycle (Gill and Tuteja, 2010). Similar results have been found in soilless crops such as lettuce (Blasco et al., 2008), but besides the increase in AsA concentration, a biomass reduction has been observed; so, it was concluded that iodine could act as a pro-oxidant promoting the increase of ROS, synthesis of antioxidants and total CA.

An increase in total proteins was also observed. A similar result was found in tomato seedling leaves treated with KI and KIO_3 applied by foliar application, which is attributed to possible effects of signalling and gene expression (Medrano-Macías et al., 2016b). Kiferle et al. (2021) monitored the impact of iodine application in plants on genomics and proteomics, and found important data that is in conformity with the results obtained in this experiment; they established the assimilation of iodine binding to some specific proteins, linked to photosynthetic processes, suggesting a functional involvement of iodine in plant nutrition.

On the other hand, a reduction in the concentration of lycopene, the primary carotenoid in tomato fruit, was observed. Martínez-Damián et al. (2018) found similar results after iodine application, observing a reduction in the content of this pigment, and suggested a possible modification in the biosynthetic pathway of some secondary metabolites. There are two biosynthetic pathways for lycopene; one dependent on ethylene (Cazzonelli and Pogson, 2010) and the other through jasmonate signalling (Liu et al., 2012); so, more specific research is required on these to achieve a more specific elucidation on the impact of iodine on lycopene.

CONCLUSION

In the present research, it was found that the foliar application of iodine did not prevent the loss of

vegetative biomass in plants under salt stress conditions, but induced a 23% improvement in fruit production. The accumulation of iodine was reached in leaves but not in fruits, probably due to competition with chlorine.

Regarding effect on minerals was found synergy with nitrogen in leaves but antagonism with calcium, in fruits decreased nitrogen content.

Some modifications in the concentrations of antioxidant molecules were found with foliar iodine application; the content of AsA and total proteins was increased, but there was decreased lycopene and capacity antioxidant in fruit.

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J.E.G. and B.H.C. carried out the experimental part of the study. E.R.M. and W.N.O. reviewed the manuscript, A.B.M. designed the experiments and J.M.M. conceived the principal idea and wrote the paper.

CONFLICT OF INTEREST

Authors declare that no conflict of interest exist.

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