

## Use of Iodine as a Tolerance Inducer in Tomato Seedlings Under Salinity Stress

### Uso del Yodo Como Inductor a la Tolerancia en Plántulas de Tomate Bajo Condiciones de Estrés por Salinidad

Pérez-Salas, S.<sup>1</sup> and Medrano-Macías, J.<sup>1\*</sup>

<sup>1</sup>Departamento de Horticultura, Universidad Autónoma Agraria Antonio Narro, Calzada Antonio Narro número. 1923, Col. Buenavista, 25315 Saltillo, México.

\*Autor para correspondencia: [jmedmac@gmail.com](mailto:jmedmac@gmail.com).

Recibido: 5 de noviembre de 2020

Aceptado: 20 de diciembre de 2020

#### Resumen

Aunque el yodo no es considerado esencial en las plantas terrestres, su aplicación exógena se ha relacionado con la potenciación del metabolismo redox y una mayor tolerancia al estrés. La salinidad es un estrés recurrente que afecta la producción de una gran variedad de especies hortícolas como el tomate; el presente experimento se realizó bajo la hipótesis de que la aplicación de  $\text{KIO}_3$  100  $\mu\text{M}$ , puede funcionar como inductor de tolerancia al estrés salino. Los resultados desde la perspectiva univariante no fueron claros ni concluyentes. Sin embargo, desde la perspectiva multivariada, se encontró un aumento en la acumulación de yodo en el tejido foliar con los tratamientos  $\text{KIO}_3$  y  $\text{KIO}_3 + \text{NaCl}$ , y correlación positiva con el potencial antioxidante. También se evidenció una correlación negativa con P, N y la altura.

En las plántulas sometidas a estrés salino ( $\text{NaCl}$  y  $\text{KIO}_3 + \text{NaCl}$ ) se encontró correlación positiva con peso seco, peso fresco, número de hojas, ancho del tallo, así como con el Ca, Na y Cu, así como una correlación negativa con el contenido de Mg, K, Fe y Mn. Por tanto, la aplicación foliar de yodo ejerce efectos benéficos incrementando la tolerancia al estrés salino.

**Palabras clave:** antioxidantes, elementos beneficiosos; cultivo; potencial redox.

#### Abstract

Although iodine is not considered essential in terrestrial plants, its exogenous application has been related to potentiation of redox metabolism and increased tolerance to stress. Salinity is recurrent stress, affecting the production of a great variety of horticultural species such as tomato plants; the present experiment was carried out under the hypothesis that application of 100  $\mu\text{M}$   $\text{KIO}_3$ , can function as an inducer of tolerance to salinity stress. The results from the univariate perspective were not clear. However, from the multivariate perspective, it was found an increase in the accumulation of iodine in foliar tissue with the  $\text{KIO}_3$  and  $\text{KIO}_3 + \text{NaCl}$ , and positive correlation with the antioxidant potential. A negative correlation with P, N, and height was also evidenced.

In the seedlings that were subjected to salinity stress ( $\text{NaCl}$  and  $\text{KIO}_3 + \text{NaCl}$ ), a positive correlation was found with dry weight, fresh weight, number of leaves, stem width, as well as with Ca, Na and Cu, and a negative correlation with the content of Mg, K, Fe and Mn. Therefore, the foliar iodine application exerts beneficial effects enhancing salinity stress tolerance.

**Keywords:** antioxidants; beneficial elements; crop; redox potential.

## INTRODUCTION

To date, iodine is considered a nonessential nutrient for terrestrial plants because it is not directly required in their nutrition (Arnon & Satout, 1939). Nevertheless, the iodine content in plants increases when is applied exogenously as iodide, iodate, organic forms ( $\text{CH}_3\text{COOI}$ , iodosalicylates, and others), either in nutrient solution or through foliar aspersions (Smoleń et al., 2019; Weng et al., 2008). Iodine uptake beneficially affects redox metabolism, probably acting as a moderate pro-oxidant, promoting non-enzymatic and enzymatic antioxidant synthesis, increasing tolerance to various adverse conditions (Gupta et al., 2015; Leyva et al., 2011; Medrano-Macías et al., 2016). Additionally, it has been argued that iodine directly acts as an electron donor (an inorganic antioxidant), at least in the case of the superoxide radical (Küpper & Carrano, 2019). Others have suggested that iodine was one of the first inorganic antioxidants used by photosynthetic organisms, that it is used in that capacity by marine algae during periods of oxidative stress, and that something similar could be occurring in terrestrial plants (La Barre et al., 2010; Venturi, 2011). However, iodine as a biostimulant, antioxidant metabolism promoter, and stress tolerance inducer is poorly documented.

On the other hand, exogenous iodine applications for biofortification purposes have demonstrated that tomato plants grown in soil are capable of withstanding high iodine concentrations (up to 5000  $\mu\text{M}$ ) without severely affecting their growth and production (Kiferle et al., 2013). Tomato is an ideal candidate for biofortification, as it is one of the most widely consumed crops and, therefore, one of the most commercially important crops both in open field and greenhouse cultivation (SAGARPA, 2017).

Salinity is the most common abiotic stress encountered during tomato production around the globe. High salt stress provokes osmotic imbalances, cell membrane disorganization, reactive oxygen species (ROS) overproduction, and photosynthesis inhibition, among other problematic effects (Parihar et al., 2015). According to the FAO, there are approximately 110 million hectares affected by high salinity, of which around 20–30 million are considered severely damaged (FAO, 2009). The use of iodine as an inducer of salt stress tolerance could be a viable option for dealing with this type of global problem. The present study was carried out to explore that possibility, focusing on determining if the foliar application of  $\text{KIO}_3$  (100  $\mu\text{M}$ ) on tomato seedlings modify their tolerance to the adverse effects generated by the presence of high salt stress during growth.

## MATERIALS AND METHODS

The experimental work was carried out in a greenhouse (7 m wide  $\times$  14 m long) with passive temperature control belonging to the Antonio Narro Agrarian Autonomous University (UAAAN) Department of Horticulture, located in the south of Saltillo, Coahuila, Mexico (25° 21' 19" N, 101° 01' 49" W) at 1777 m.a.s.l.

## Plant material and sampling

Tomato (*Solanum lycopersicum* L.) variety Rio Grande seeds were sown in 200-cavity polystyrene trays filled with peat moss-perlite mix (5:1) to a depth of 0.5 cm. The seedlings were transplanted 40 days after sowing (DAS)—when they had at least four leaves—to 10 L polyethylene pots filled with 1:1 peat moss-perlite mix. Fertilization was carried out with an automatic irrigation system dispensing nutrient solution (Steiner, 1961). The solution contained the following macroelement content:  $\text{Ca}(\text{NO}_3)_4 \cdot 4\text{H}_2\text{O}$  2.25 mEq,  $\text{KNO}_3$  3 mEq,  $\text{K}_2\text{SO}_4$  1.75 mEq,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  1 mEq, and  $\text{KH}_2\text{PO}_4$  0.25 mEq. It also contained the following microelements: Fe 0.75 ppm,  $\text{HBO}_3$  0.125 ppm,  $\text{MnSO}_4$  0.175 ppm,  $\text{ZnSO}_4$  0.002 ppm, and  $\text{CuSO}_4$  0.005 ppm and electrical conductivity (EC) 2.0  $\text{mS cm}^{-1}$ .

The experimental design was completely randomized and consisted of four treatments with 20 replicates each one: control (1),  $\text{KIO}_3$  (2), NaCl (3), and  $\text{KIO}_3$  + NaCl (4). Each experimental unit was a single plant. Two weeks after transplanting, 100  $\mu\text{M}$  iodine was sprayed on the plant foliage, ensuring that the adaxial and abaxial leaf sides were covered in solution yet not dripping. Salt stress was induced three weeks after transplanting by adding 100 mM NaCl to the irrigation solution (EC 7.5  $\text{mS cm}^{-1}$ ) (Zahedi et al., 2019).

Plants were chosen for sampling at random, four weeks after transplanting, when they presented at least 7 leaves at the main stage 1 and sub-stage 17 of maturity, according to the BBCH maturity scale (Hack et al., 1992). Five plants per treatment were collected to evaluate growth parameters (height, stem diameter, leaf number, and plant fresh weight). For the evaluation of fresh weight, the plants were weighed on a digital balance (OHAUS), after all soil residue from the roots had been removed, and the results were recorded. Afterwards, the same plants were placed in a drying oven at 70 °C for 24 hours before being weighed again to record their dry weight.

For the analysis of biochemical compounds, the leaves of five other plants per treatment were harvested. The harvested leaves were frozen and then lyophilized. The leaves from the five plants dried for the dry weight evaluation were subsequently used for the mineral analysis assays.

## Iodine content analysis

Energy dispersive micro X-ray fluorescence ( $\mu$ -EDXRF) was used to quantify the iodine in tomato's seedlings leaves. Assays were performed on an M4 Tornado micro-XRF spectrometer (Bruker). The x-ray generator was operated at 50 kV and 100  $\mu\text{A}$  with a 12.5A filter. The fluorescence radiation was detected using an XFlash<sup>TM</sup> silicon dispersion detector with a detection area of 30  $\text{mm}^2$  and an energy resolution of 142 eV. The results were expressed as mg I per kg dry weight (mg I/kg DW) (Shelor & Dasgupta, 2011).

### Plant growth parameter evaluation

Plant height was measured with a flexible tape from the plant's base stem up to the apex. The plant stem diameter was measured at the base using digital Vernier calipers. All the foliole within each compound leaf were counted, and that number was recorded.

Plant fresh weight (FW) was evaluated by removing growth media residue from the roots and then weighting the plants on a digital balance (OHAUS). Afterward, the same plants were placed in a drying oven at 70 °C for 24 hours. Once they reached a constant weight, that value was recorded as the plant dry weight (Cortés-Flores et al., 2016).

### Mineral content analysis

A portion from the samples of dehydrated leaves (1 g) was measured out then placed in a glass beaker to which 30 mL of nitric acid was added. The samples were placed on a heating plate at 150 °C until the samples were clarified. The sample volume was adjusted to 100 mL with deionized water, then filtered on Whatman #1 paper (Helrich, 1990).

The acid extraction samples were analyzed on an atomic absorption (AA) spectrophotometer (Varian Spectra FS-240). The content of K, Ca, Mg, Mn, Fe, Zn, and Cu were recorded.

Nitrogen (N) content was determined according to the micro Kjeldahl technique (Muller, 1961). A sample of dehydrated tissue (50 mg) was weighed out, then added to a flask with 3 mL of digestion mixture. After digesting for 30 minutes, the sample was transferred to a distillation tube, and 25 mL of 50% sodium hydroxide was added. Boric acid (30 mL) and four drops of indicator solution were added to a beaker and mixed with the samples, resulting in light blue-green color. The samples titration was carried out, adding 0.025 N sulfuric acid drop-by-drop from a burette until a light rose color developed. The N content was determined from the volume of sulfuric acid spent.

Phosphorus content was determined according to the aminonaphthylsulfonic acid (ANSA) technique. The acid digestion sample (1 mL) was placed in a test tube, along with 5 mL of ammonium molybdate solution and 2 mL of ANSA. The test tubes were agitated then allowed to rest for 20 minutes. The treated samples absorbance was measured in a UV/Vis spectrophotometer (Thermo Genesys 102) at 650 nm (Peterson, 1978).

### Antioxidant compound evaluation

Determination of antioxidant capacity was performed on previously lyophilized leaves. A sample of lyophilized leaves (100 mg) was macerated, then placed in 2 mL Eppendorf tubes, to which 1.5 mL of phosphate buffer solution (pH 7.2) were added. The samples were agitated by vortexing for 20 sec, then sonicated for 10 min. The sonicated samples were centrifuged at 12000 rpm, and then the supernatant was filtered through

syringe filters (13 mm diameter, pore size 0.45 µm). After filtering, 50 µL of each sample was taken, placed in a microwell plate well, and had 50 µL of DPPH solution added. The microwell plate was left to rest for 15 minutes, then the absorbance at 530 nm was measured with a plate reader Biotek Elx 808, for the calibration Curve, Trolox was used (Tang et al., 2010).

Chlorophyll content in young, east-facing leaves was measured using the method described by (Munira et al., 2015). A sample of fresh vegetable material (1 g) was homogenized using a mortar and pestle. Acetone (90%; 5 mL) was added during homogenization. Magnesium carbonate (10 mg) was also added to the acetone extract. A portion of the acetone extract (2 mL) was transferred to an Eppendorf tube and centrifuged for 5 min. at 10,000 rpm and 2 °C. The supernatant was decanted, and its absorbance was measured at both 663 and 645 nm. Acetone (90%) was used as the blanking solution. Total chlorophyll content was expressed in µg g<sup>-1</sup>, and calculated using the following formulas:

$$\text{Chlorophyll a } (\mu\text{g g}^{-1}) = 25.38 \times A_{663} + 3.64 \times A_{645}$$

$$\text{Chlorophyll b } (\mu\text{g g}^{-1}) = 30.38 \times A_{645} - 6.58 \times A_{663}$$

$$\text{Total chlorophyll } (\mu\text{g g}^{-1}) = 18.8 \times A_{663} + 34.02 \times A_{645}$$

### Statistical analysis

*Univariate Analysis.* The obtained data were first subjected to analysis of variance (ANOVA). To determine the significance between means were used Fisher's least significant difference (LSD), the level of significances was expressed for both analyses as \* $P \leq 0.05$  and \*\* $P \leq 0.01$ .

*Multivariate Analysis.* The statistical analysis was performed using principal components (PCA) obtained from the correlation matrix, thus reducing the dimensionality of the database, it was performed using the Infostat software package (2017 version).

## RESULTS

### Plant iodine content

Table 1 shows the concentrations of accumulated iodine in tomato seedling leaves. The highest concentrations were found in the leaves of seedlings treated with KIO<sub>3</sub>. Those seedlings had an iodine concentration of 19.76 mg I kg<sup>-1</sup> DW. Seedlings treated with KIO<sub>3</sub> and then subjected to salt stress had iodine concentrations of 12.76 mg I kg<sup>-1</sup> DW. The iodine concentration in control plants did not exceed the detection limit (0.1 ppm).

**Table 1.** Iodine accumulation in tomato seedling leaves.

Treatment Number	Treatment	Iodine concentration (mg kg <sup>-1</sup> DW)
1	Control	0.1c ±0.001
2	KIO <sub>3</sub>	19.76a ± 3.4
3	NaCl	0.1c ±0.001
4	KIO <sub>3</sub> + NaCl	12.76 b ±5.3

\*Means with the same letter are not statistically different according to LSD analysis ( $p \geq 0.05$ ). Standard deviation is included.

### Tomato seedling growth

The iodine application did not significantly affect the growth of tomato seedlings, including those seedlings that were also subjected to salt stress (Table 2).

**Table 2.** Response of the growth in the different treatments

Treatments	Fresh weight (g)	Dry weight (g)	Height (cm)	Stem diameter (cm)	Number of leaves
Control	25.33 a ±5.9	2.67 a ±0.5	18.80 a ±3.3	0.74 a ±0.08	44 a ±4.5
KIO <sub>3</sub>	26.76 a ±11.2	2.66 a ±1.0	19.80 a ±1.8	0.76 a ±0.05	43 a ±5
NaCl	34.90 a ±10.2	3.10 a ±0.78	17 a ±1	0.76 a ±0.08	46 a ±5.1
KIO <sub>3</sub> + NaCl	32.98 a ±6.5	3.50 a ±0.23	17.6 a ±1.5	0.78 a ±0.04	47 a ±4.5

### Antioxidant capacity of tomato seedlings

Table 3 contains the mean values of tomato seedling antioxidant capacity and chlorophyll content (a, b, and total) following each treatment. Based on the ANOVA and LSD statistical tests results, only chlorophyll b content demonstrated a significant increase in salt stress (KIO<sub>3</sub> + NaCl and NaCl).

**Table 3.** Response of tomato seedling biochemical variables in response to iodine and salt stress

Treatments	Antioxidant potential (mM Trolox)	Chlorophyll a (µg g <sup>-1</sup> )	Chlorophyll b (µg g <sup>-1</sup> )	Chlorophyll total (µg g <sup>-1</sup> )
Control	35.65 a ±8.3	61.95 a ±7.4	12.87 b ±1.8	74.82 a ±9.2
KIO <sub>3</sub>	37.44 a ±5.5	58.81 a ±17	13.01 b ±4.9	71.82 a ±22
NaCl	32.07 a ±13	72.17 a ±9	18.64 a ±4.4	90.81 a ±13.3
KIO <sub>3</sub> + NaCl	35.98 a ±10	66.79 a ± 10	14.20 a ±3.7	80.99 a ±13

\*Means with the same letter are not statistically different according to LSD analysis ( $p \geq 0.05$ ). Deviation standard is included.

### The Mineral content of tomato seedling

The results from the macronutrient and micronutrient content analyses of tomato seedlings are shown in Table 4. Following statistical analysis of the results, there were no significant differences seen in N, P, Ca, and Mg content. Zn content was reduced after saline treatment but was not reduced when KIO<sub>3</sub> and salt stress were applied. Sodium content was increased following all treatments involving salt stress.

Iron content was reduced across all treatments (KIO<sub>3</sub>, KIO<sub>3</sub>+NaCl, and NaCl), while Cu content was increased in all the seedlings subjected to salt stress (KIO<sub>3</sub> + NaCl and NaCl). There were no significant changes in Mn content across all treatments.

**Table 4.** Response of mineral content in the different treatments

Treatments	Macronutrients (g kg <sup>-1</sup> )					
	N	P	Ca	K	Mg	Na
Control	39.39 <sup>a</sup>	3.73 <sup>a</sup>	14.40 <sup>a</sup>	14.92 <sup>a</sup>	4.48 <sup>a</sup>	2.96 <sup>b</sup>
KIO <sub>3</sub>	34.30 <sup>a</sup>	2.64 <sup>a</sup>	15.20 <sup>a</sup>	14.04 <sup>ab</sup>	4.08 <sup>a</sup>	2.84 <sup>b</sup>
NaCl	33.55 <sup>a</sup>	3.02 <sup>a</sup>	15.70 <sup>a</sup>	9.10 <sup>b</sup>	4.04 <sup>a</sup>	3.66 <sup>a</sup>
KIO <sub>3</sub> +NaCl	28.63 <sup>a</sup>	3.09 <sup>a</sup>	14.88 <sup>a</sup>	13.56 <sup>ab</sup>	4.16 <sup>a</sup>	3.84 <sup>a</sup>
Treatments	Micronutrients (mg kg <sup>-1</sup> )					
	Fe	Mn	Cu	Zn		
Control	89.6 <sup>a</sup>	118 <sup>a</sup>	5.20 <sup>b</sup>	47.20 <sup>a</sup>		
KIO <sub>3</sub>	76.4 <sup>b</sup>	96 <sup>a</sup>	3.20 <sup>b</sup>	41.20 <sup>a</sup>		
NaCl	70 <sup>b</sup>	88 <sup>a</sup>	9 <sup>a</sup>	36 <sup>b</sup>		
KIO <sub>3</sub> +NaCl	69.2 <sup>b</sup>	96.40 <sup>a</sup>	10 <sup>a</sup>	40.8 <sup>a</sup>		



The univariate analysis did not indicate a strong impact in tomato seedlings by iodine or salt stress. However, to verify the hypothesis that iodine can function as an inducer of tolerance against salinity, an exploratory multivariate analysis by principal components (PC) was carried out. Through PC analysis it was possible to review whether the distribution of treatments and experimental variables on a Cartesian plane represented the data's multidimensional distribution.

After carrying out the analysis of all principal components, the dimensionality of 19 variables was reduced to two principal components (PC1 and PC2), which together explain 85.9% of the variance. PC1 alone captured 61.7% of the variance, and PC2 captured 24.3%. The magnitude and signs of the principal component coefficients are shown in Table 5. Based on those values, PC1 was interpreted as having a positive correlation with stem diameter, leaf number, plant fresh and dry weight, chlorophyll, calcium, copper, and sodium content. It also appeared to have a negatively correlated with plant height, nitrogen, potassium, magnesium, iron, zinc, and manganese content. On the other hand, PC2 had a positively correlated with iodine content and antioxidant potential and a negative relationship with phosphorous content.

A clear trend can be observed on the distribution of treatments within the biplot (Figure 1), right side is the seedling subjected to salt stress, with NaCl in the first quadrant (PC1 +, PC2 -) and KIO<sub>3</sub> + NaCl in quadrant 2 (PC1 +, PC2 +). On the left are the seedlings without salt stress. Plants treated with KIO<sub>3</sub> in quadrant 3 (PC1 -, PC2 +). The control plants can be seen in quadrant 4 (PC1 -, PC2 -). It can be seen how the controls are found towards the ends of PC1, while the seedlings treated with iodine can be found towards the center. Those plants that were treated with iodine and subjected to salt stress tend to separate from the NaCl treatment and cluster near the plants only treated with iodine. These results illustrate the mitigating impact of iodine application on salt stress.

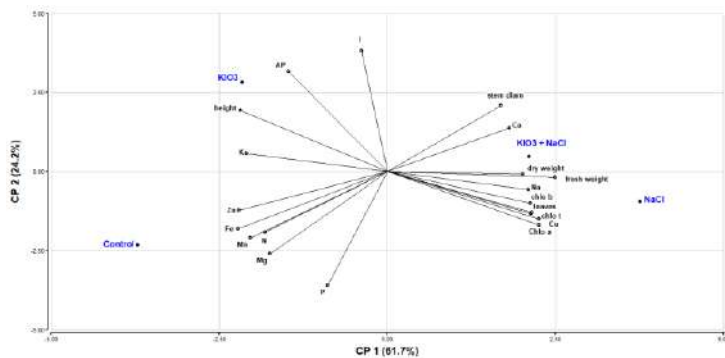
The coefficients and signs, shown in Table 5, were considered the positions in the biplot (Figure 1) when evaluating the relationships between the variables and experimental treatments. A positive correlation was found between the accumulation of iodine in leaves and antioxidant potential with the KIO<sub>3</sub> and KIO<sub>3</sub> + NaCl treatments. There was a negative correlation between seedling height, phosphorus content, and nitrogen content with those same treatments.

For treatments involving exposure to salinity (NaCl and KIO<sub>3</sub> + NaCl), a positive correlation was seen with stem diameter, leaf number, dry weight, fresh weight, chlorophyll content (a, b, and total), copper, calcium, and sodium content. Conversely, magnesium, potassium, zinc, iron, and manganese content a negatively correlated with those treatments.

Thus, in this preliminary study, iodine's application was generally observed to improve plant tolerance to the presence of salt stress. This was concluded from the treatment's distribution tendencies following a multivariate analysis of all the experimental variables.

**Table 5.** Correlation coefficients between evaluated variables and PC

Variable	PC 1	PC 2
<b>Height</b>	-0.87	0.48
<b>Stem diameter</b>	0.68	0.52
<b>Number of leaves</b>	0.86	-0.33
<b>Fresh weight</b>	0.95	-0.05
<b>Dry weight</b>	0.81	-0.02
<b>Antioxidant Potential</b>	-0.59	0.79
<b>Chlorophyll a</b>	0.90	-0.42
<b>Chlorophyll b</b>	0.85	-0.25
<b>Chlorophyll total</b>	0.90	-0.38
<b>N</b>	-0.73	-0.48
<b>P</b>	-0.35	-0.90
<b>K</b>	-0.83	0.14
<b>Ca</b>	0.72	0.34
<b>Mg</b>	-0.70	-0.65
<b>Fe</b>	-0.89	-0.45
<b>Zn</b>	-0.88	-0.31
<b>Mn</b>	-0.81	-0.53
<b>Cu</b>	0.85	-0.33
<b>Na</b>	0.84	-0.14
<b>I</b>	-0.15	0.95



**Figure 1.** Biplot of principal components for growth, mineral elements, iodine, and biochemical variables of tomato seedlings treated with iodine in the presence and absence of salt stress.

## DISCUSSION

The present study found an increase in foliar concentrations of iodine in tomato seedlings treated with the said element ( $KIO_3$  and  $KIO_3 + NaCl$ ) compared to the controls in both statistical analyses. This result is consistent with those previously reported. Foliar application of KI (22000  $\mu M$ ) resulted in alfalfa plants accumulating up to 2 mg iodine per kilogram of fresh weight (Altinok et al., 2003). This was also seen in other plant species, where the foliar application of 1.5 M  $KIO_3$  led to the accumulation of that element (0.5–1.5 mg I  $kg^{-1}$  FW) in leaves (Lawson et al., 2016). Application of a micronutrient cocktail that included 2330  $\mu M$  iodine resulted in variable iodine accumulation in wheat grains (0.36–0.633 mg I  $kg^{-1}$  grain), depending on location (Zou et al., 2019).

In this study, the concentrations of iodine in tomato seedlings following foliar application of 100  $\mu M$  iodine were 19.7 mg I  $kg^{-1}$  DW in the absence of salt stress and 12.7 mg I  $kg^{-1}$  DW with salt stress. The reported values varied greatly with those previously reported, though those differences could be due to the plant species. It has been demonstrated that the activity of iodine transfer factors is higher in leaf species than in grasses (Medrano-Macías et al., 2016). However, other factors such as the season during which the element is applied, the presence of surfactant, and the plant developmental stage, can also influence iodine transfer factors.

The specific mechanisms through which iodine is absorbed via leaves are not fully elucidated, although it has been reported that plants can accumulate and metabolize this element in either its KI or  $KIO_3$  form (Golubkina et al., 2018), its absorption causes changes in the plant metabolism (Halka et al., 2019). An observation confirmed by the results obtained in the present work, where  $KIO_3$  application was positively correlated with improved antioxidant capacity, under both conditions. That change in antioxidant capacity indicates enhancement in the synthesis of low molecular weight, reducing molecules, mainly non-enzymatic antioxidants. Based on the chemical extraction method employed during analysis, those would be polar molecules such as reduced glutathione, ascorbic acid, and

phenolic compounds (Kasote et al., 2015). The biosynthesis of such antioxidant molecules is generally increased when there is an overproduction of free radicals (Foyer & Noctor, 2013).

Unfortunately, more detailed information of iodine's effect on redox metabolism and stress tolerance is scarce within the literature, even more so for its effects following foliar application (Medrano-Macías et al., 2016). Increases in the antioxidant potential, ascorbic acid content, and total phenolic compounds in *Brassica juncea L.* plants were observed following foliar application of KI (6 mM) (Golubkina et al., 2018). Similarly, applying 0.2% KI to radish plants increased their ascorbic acid content (Strzetelski et al., 2010). However, the authors of that study note that more research into the effects of iodine on antioxidant metabolism and its action mechanisms is required.

On the other hand, experiments on the enrichment of the legume *Pisum sativum L.* managed to increase the concentrations of iodine in various plant tissues following foliar application of KI and  $KIO_3$  (1000 mg  $L^{-1}$ ). However there were no observed changes in the concentrations of anthocyanins or glutathione antioxidant (Jerše et al., 2018). The observed phenomena could be related to iodine's oxidoreductive capacity, since it can behave either as an electron acceptor or donor (Küpper et al., 2008; Venturi et al., 2002). In this case, iodine is probably acting as a moderate pro-oxidant in that it did not have adverse effects on plant growth but did lead to an increase in the antioxidant potential of plants treated with  $KIO_3$ .

Plants subjected to salt stress, either with or without iodine treatment ( $NaCl$  and  $KIO_3 + NaCl$ ), demonstrated a growth increase compared to control plants. That improvement could be linked to the increase in chlorophyll content (a, b, and total). The preferential accumulation of iodine and chlorine in chloroplasts when those elements reach foliar tissue has been reported (Eichert & Fernández, 2011; H.-X. Weng et al., 2008). The effects on growth could also be coupled to the positive correlations with calcium and copper content. The mechanisms through which iodine absorption antagonizes or synergizes with other essential elements are not fully understood. The existing research has focused on the radicular uptake process, and what has been seen are significant changes in the redox metabolism of the surrounding environment, affecting the bioavailability of some essential elements, such as manganese, copper, and iron (Venturi, 2011). When iodine is present as  $KIO_3$ , it is reduced by nitrate reductase, thus competing directly with the process of nitrate uptake (Kato et al., 2013).

The uptake of minerals via aerial plant tissues occurs mainly through cuticle pores rather than stomata. Translocation of minerals from the aerial tissues down to the roots has been observed when the mineral root concentrations in question are lower than the aerial tissue concentrations (Clarkson & Scattergood, 1982). A similar translocation may be occurring from the tomato leaves to the roots, and that is where the aforementioned oxidoreductive changes are potentiating the absorption of calcium and copper.

As for the negative correlation between salt stress and the content of Mg, K, Zn, Fe and Mn, that could be linked to the

osmotic and ionic imbalances caused by excess  $\text{Cl}^-$  and  $\text{Na}^+$  (Acosta-Motos et al., 2017). However, it is worth mentioning that the negative relationship was not reflected in the physiological parameters evaluated. A similar finding was found by Smolen and Sady 2012 (Smoleń & Sady, 2012) in lettuce plants, where they argue that this phenomenon is likely due to a series of additional factors as the added salt dose, chemical composition, growth medium, and environmental factors.

## CONCLUSIONS

It is concluded that the foliar iodine application provides the potential to achieve an increase in tolerance to salinity stress. Which gives the guideline to extrapolate the experiment to other horticultural species in different stages of maturity.

## ACKNOWLEDGEMENTS

This research was funded by el Consejo Nacional de Ciencia y Tecnología (CONACYT), through a postdoctoral funding grant (CVU 304747).

## REFERENCES

- Acosta-Motos, J. R., Ortuño, M. F., Bernal-Vicente, A., Diaz-Vivancos, P., Sanchez-Blanco, M. J., & Hernandez, J. A. (2017). Plant responses to salt stress: Adaptive mechanisms. *Agronomy*, 7(1), 1–38. <https://doi.org/10.3390/agronomy7010018>
- Altinok, S., Sozudogru-Ok, S., & Halilova, H. (2003). Effect of iodine treatments on forage yields of alfalfa. *Communications in Soil Science and Plant Analysis*, 34(1–2), 55–64. <https://doi.org/10.1081/CSS-120017415>
- Arnon, D. I., & Satout, P. R. (1939). The essentiality of certain elements in minute quantity for plants with special reference to cooper. *Plant Physiology*, 14(2), 371–375. <https://doi.org/https://dx.doi.org/10.1104%2Fpp.14.2.371>
- Clarkson, D. T., & Scattergood, C. B. (1982). Growth and phosphate transport in barley and tomato plants during the development of, and recovery from, phosphate-stress. *Journal of Experimental Botany*, 33(5), 865–875. <https://doi.org/10.1093/jxb/33.5.865>
- Cortés-Flores, C., Rodríguez-Mendoza, M. de las N., Benavides-Mendoza, A., García-Cué, J., Tornero-Campante, M., & Sánchez-García, P. (2016). El yodo aumenta el crecimiento y la concentración de minerales en plántulas de pimiento morrón. *Agrociencia*, 50(6), 747–758. <https://doi.org/30247467007>
- Eichert, T., & Fernández, V. (2011). Uptake and Release of Elements by Leaves and Other Aerial Plant Parts. *Marschner's Mineral Nutrition of Higher Plants: Third Edition, December*, 71–84. <https://doi.org/10.1016/B978-0-12-384905-2.00004-2>
- FAO. (2009). The State of Food Insecurity in the World. In *Notes*. Food and Agriculture Organization of the United Nations.
- Foyer, C. H., & Noctor, G. (2013). Redox Signaling in Plants. *Antioxidants & Redox Signaling*, 18(16), 2087–2090. <https://doi.org/10.1089/ars.2013.5278>
- Golubkina, N., Kekina, H., & Caruso, G. (2018). Yield, Quality and Antioxidant Properties of Indian Mustard (*Brassica juncea L.*) in Response to Foliar Biofortification with Selenium and Iodine. *Plants*, 7(4), 80. <https://doi.org/10.3390/plants7040080>
- Gupta, N., Bajpai, M., Majumdar, R., & Mishra, P. (2015). Response of iodine on antioxidant levels of Glycine max L. Grown under Cd<sup>2+</sup> stress. *Advances in Biological Research*, 9(1), 40–48. <https://doi.org/10.5829/idosi.abr.2015.9.1.9183>
- Hack, H., Bleiholder, H., Buhr, L., Meier, U., Schnock-Fricke, U., Weber, E., & Witzemberger, A. (1992). Einheitliche Codierung der phänologischen Entwicklungsstadien mono- und dikotyler Pflanzen. -Erweiterte BBCH-Skala, Allgemein-. In *Nachrichtenbl. Deut. Pflanzenschutzkd.* (Vol. 44, Issue 12, pp. 265–270).
- Halka, M., Smoleń, S., Czernicka, M., Klimek-Chodacka, M., Pitala, J., & Tutaj, K. (2019). Iodine biofortification through expression of HMT, SAMT and S3H genes in *Solanum lycopersicum L.* *Plant Physiology and Biochemistry*, 144(June), 35–48. <https://doi.org/10.1016/j.plaphy.2019.09.028>
- Helrich, K. (1990). *AOAC. Official methods of analysis. 15. Arlington, VA, USA: Association of official Analytical Chemists.* [https://scholar.google.com.mx/scholar?q=AOAC+%281990%29.+In+K.+Helrich+%28Ed.%29%2C+Official+Methods+of+Analysis+%2815th+ed.%29.+Arlington%2C+VA%2C+USA%3A+Association+of+Official+Analytical+Chemists%2C+Inc&btnG=&hl=es&as\\_sdt=0%2C5](https://scholar.google.com.mx/scholar?q=AOAC+%281990%29.+In+K.+Helrich+%28Ed.%29%2C+Official+Methods+of+Analysis+%2815th+ed.%29.+Arlington%2C+VA%2C+USA%3A+Association+of+Official+Analytical+Chemists%2C+Inc&btnG=&hl=es&as_sdt=0%2C5)
- Jerše, A., Kacjan Maršič, N., Kroflič, A., Germ, M., Šircelj, H., & Stibilj, V. (2018). Is foliar enrichment of pea plants with iodine and selenium appropriate for production of functional food? *Food Chemistry*, 267, 368–375. <https://doi.org/10.1016/j.foodchem.2018.02.112>
- Kasote, D. M., Katyare, S. S., Hegde, M. V., & Bae, H. (2015). Significance of antioxidant potential of plants and its relevance to therapeutic applications. *International Journal of Biological Sciences*, 11(8), 982–991. <https://doi.org/10.7150/ijbs.12096>
- Kato, S., Wachi, T., Yoshihira, K., Nakagawa, T., Ishikawa, A., Takagi, D., Tezuka, A., Yoshida, H., Yoshida, S., Sekimoto, H., & Takahashi, M. (2013). Rice (*Oryza sativa L.*) roots have iodate reduction activity in response to iodine. *Frontiers in Plant Science*, 4(3), 227. <https://doi.org/10.3389/fpls.2013.00227>
- Kiferle, C., Gonzali, S., Holwerda, H. T., Ibaceta, R. R., & Perata, P. (2013). Tomato fruits: a good target for iodine biofortification. *Frontiers in Plant Science*, 4(June), 205. <https://doi.org/10.3389/fpls.2013.00205>
- Küpper, F. C., Carpenter, L. J., McFiggans, G. B., Palmer, C. J.,



- Waite, T. J., Boneberg, E.-M., Woitsch, S., Weiller, M., Abela, R., Grolimund, D., Potin, P., Butler, A., Luther, G. W., Kroneck, P. M. H., Meyer-Klaucke, W., & Feiters, M. C. (2008). Iodide accumulation provides kelp with an inorganic antioxidant impacting atmospheric chemistry. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(19), 6954–6958. <https://doi.org/10.1073/pnas.0709959105>
- Küpper, F. C., & Carrano, C. J. (2019). Key aspects of the iodine metabolism in brown algae: A brief critical review. *Metallomics*, *11*(4), 756–764. <https://doi.org/10.1039/c8mt00327k>
- La Barre, S., Potin, P., Leblanc, C., & Delage, L. (2010). The halogenated metabolism of brown algae (phaeophyta), its biological importance and its environmental significance. *Marine Drugs*, *8*(4), 988–1010. <https://doi.org/10.3390/md8040988>
- Lawson, P. G., Daum, D., Czuderna, R., & Vorsatz, C. (2016). Factors influencing the efficacy of iodine foliar sprays used for biofortifying butterhead lettuce (*Lactuca sativa*). *Journal of Plant Nutrition and Soil Science*, *179*(5), 661–669. <https://doi.org/10.1002/jpln.201600213>
- Leyva, R., Sánchez-Rodríguez, E., Ríos, J. J., Rubio-Wilhelmi, M. M., Romero, L., Ruiz, J. M., & Blasco, B. (2011). Beneficial effects of exogenous iodine in lettuce plants subjected to salinity stress. *Plant Science*, *181*(2), 195–202. <https://doi.org/10.1016/j.plantsci.2011.05.007>
- Medrano-Macías, J., Leija-Martínez, P., González-Morales, S., Juárez-Maldonado, A., & Benavides-Mendoza, A. (2016). Use of Iodine to Biofortify and Promote Growth and Stress Tolerance in Crops. *Frontiers in Plant Science*, *7*, 1146. <https://doi.org/10.3389/fpls.2016.01146>
- Medrano-Macías, J., Leija-Martínez, P., Juárez-Maldonado, A., Rocha-Estrada, A., & Benavides-Mendoza, A. (2016). Efecto de la aplicación de yodo sobre antioxidantes en plántulas de jitomate. *Revista Chapingo Serie Horticultura*, *XXII*(2), 133–143. <https://doi.org/10.5154/r.rchsh.2015.12.025>
- Muller, L. (1961). Device for varying the frequency of a vibration exciter. *US Patent* 3,004,389. <https://www.google.com/patents/US3004389>
- Munira, S., Hossain, M., Zakaria, M., Ahmed, J., & Islam, M. (2015). Evaluation of Potato Varieties against Salinity Stress in Bangladesh. *International Journal of Plant & Soil Science*, *6*(2), 73–81. <https://doi.org/10.9734/ijpss/2015/15879>
- Parihar, P., Singh, S., Singh, R., Singh, V. P., & Prasad, S. M. (2015). Effect of salinity stress on plants and its tolerance strategies: a review. *Environmental Science and Pollution Research*, *22*(6), 4056–4075. <https://doi.org/10.1007/s11356-014-3739-1>
- Peterson, G. L. (1978). A simplified method for analysis of inorganic phosphate in the presence of interfering substances. *Analytical Biochemistry*, *84*(1), 164–172. [https://doi.org/10.1016/0003-2697\(78\)90495-5](https://doi.org/10.1016/0003-2697(78)90495-5)
- SAGARPA. (2017). *Agrícola Nacional JITOMATE. Planeación Agrícola Naional 2017-2030*, 2–20.
- Shelor, C. P., & Dasgupta, P. K. (2011). Review of analytical methods for the quantification of iodine in complex matrices. *Analytica Chimica Acta*, *702*(1), 16–36. <https://doi.org/10.1016/j.aca.2011.05.039>
- Smoleń, S., Kowalska, I., Kováčik, P., Halka, M., & Sady, W. (2019). Biofortification of six varieties of lettuce (*Lactuca sativa* L.) with iodine and selenium in combination with the application of salicylic acid. *Frontiers in Plant Science*, *10*(February), 1–13. <https://doi.org/10.3389/fpls.2019.00143>
- Smoleń, S., & Sady, W. (2012). Influence of iodine form and application method on the effectiveness of iodine biofortification, nitrogen metabolism as well as the content of mineral nutrients and heavy metals in spinach plants (*Spinacia oleracea* L.). *Scientia Horticulturae*, *143*(2012), 176–183. <https://doi.org/10.1016/j.scienta.2012.06.006>
- Steiner, A. A. (1961). A universal method for preparing nutrient solutions of a certain desired composition. *Plant and Soil*, *15*(2), 134–154. <https://doi.org/10.1007/BF01347224>
- Strzetelski, P., Smoleń, S., Rozek, S., & Sady, W. (2010). The effect of diverse iodine fertilization on nitrate accumulation and content of selected compounds in radish plants (*raphanus sativus* L.). *Acta Scientiarum Polonorum, Hortorum Cultus*, *9*(2), 65–73.
- Tang, X. Z., Dong, Y. X., Wei, S. Q., Zhang, X. S., & Yin, Y. P. (2010). Antioxidant activity of pigment extracted from green-wheat-bran. *Agricultural Sciences in China*, *9*(6), 825–832. [https://doi.org/10.1016/S1671-2927\(09\)60160-7](https://doi.org/10.1016/S1671-2927(09)60160-7)
- Venturi, S. (2011). Evolutionary Significance of Iodine. *Current Chemical Biology*, *5*(3), 155–162. <https://doi.org/10.2174/187231311796765012>
- Venturi, S., Donati, F. M., Venturi, A., & Venturi, M. (2002). Environmental Iodine Deficiency: A Challenge to the Evolution of Terrestrial Life? *Thyroid*, *10*(8), 727–729. <https://doi.org/10.1089/10507250050137851>
- Weng, H.-X., Hong, C.-L., Yan, A.-L., Pan, L.-H., Qin, Y.-C., Bao, L.-T., & Xie, L.-L. (2008). Mechanism of iodine uptake by cabbage: effects of iodine species and where it is stored. *Biological Trace Element Research*, *125*(1), 59–71. <https://doi.org/10.1007/s12011-008-8155-2>
- Weng, H. X., Weng, J. K., Yan, A. L., Hong, C. L., Yong, W. Bin, & Qin, Y. C. (2008). Increment of iodine content in vegetable plants by applying iodized fertilizer and the residual characteristics of iodine in soil. *Biological Trace Element Research*, *123*(1–3), 218–228. <https://doi.org/10.1007/s12011-008-8094-y>
- Zahedi, S. M., Abdelrahman, M., Hosseini, M. S., Hoveizeh, N. F., & Tran, L. S. P. (2019). Alleviation of the effect of salinity on growth and yield of strawberry by foliar spray of selenium-nanoparticles. *Environmental Pollution*, *253*, 246–258. <https://doi.org/10.1016/j.envpol.2019.04.078>
- Zou, C., Du, Y., Rashid, A., Ram, H., Savasli, E., Pieterse, P. J., Ortiz-Monasterio, I., Yazici, A., Kaur, C., Mahmood, K.,



Singh, S., Le Roux, M. R., Kuang, W., Onder, O., Kalayci, M., & Cakmak, I. (2019). Simultaneous Biofortification of Wheat with Zinc, Iodine, Selenium, and Iron through Foliar Treatment of a Micronutrient Cocktail in Six Countries. *Journal of Agricultural and Food Chemistry*, 67(29), 8096–8106.  
<https://doi.org/10.1021/acs.jafc.9b01829>