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Iodine Effects on Phenolic Metabolism in Lettuce Plants under Salt Stress

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ABSTRACT: Iodine, applied as iodate in biofortification programs (at doses of ≤80 μM), has been confirmed to improve the foliar biomass, antioxidant response, and accumulation of phenol compounds in lettuce plants. The changes in phenolic compounds induced by the iodate application appear to have functional consequences in the response of salt-stressed plants. Thus, the aim of the present study was to determine whether the application of iodate can improve the response of severe salinity stress and whether the resistance can be attributed to the phenolic metabolism in lettuce (*Lactuca sativa* cv. Philipus), a glycophyte cultivated for food and consumed year round. In this work, the application of iodate, especially at 20 and 40 μM, in lettuce plants under salinity stress (100 mM NaCl) exerted a significantly positive effect on biomass and induced higher activity in the enzymes shikimate dehydrogenase and phenylalanine ammonia-lyase as well as the lower MW phenol-degrading enzyme polyphenol oxidase. This increased hydroxycinnamic acids and derivatives in addition to total phenols, which appear to act as protective compounds against salinity. This study reveals that in agricultural areas affected by this type of stress, the application of iodate may be an effective strategy, as it not only improves lettuce plant growth but also supplements the human diet with phenolic compounds and the trace element iodine.

KEYWORDS: *iodate, Lactuca sativa, salinity stress, phenolic metabolism*

INTRODUCTION

An estimated 6% or more of the world's land and some 30% of the world's irrigated areas suffer from salinity, a problem that is expected to spread in the context of global change, particularly in the arid and semiarid regions of the world.[1] Salinity stunts plant growth by depressing the shoot-water potential and altering several metabolic activities at the cellular level, including enzyme inhibition, solute accumulation, specific ion effects, or a combination of these factors.[2] Reduced productivity in several plant species exposed to salinity is often associated with the overproduction of reactive oxygen species (ROS), which have the potential of interacting with many cell components, significantly damaging membranes and other structures.[3] The ROS scavenging ability relies on the primary antioxidant defense system, which is composed of enzymatic as well as nonenzymatic components.[4] Nonenzymatic components of this system include various secondary metabolites, such as hydrophilic phenolics and flavonols, lipophilic α-tocopherols and carotenoids, and water-soluble ascorbate and glutathione.[4]

Secondary compounds are among a range of metabolites that accumulate in lettuce.[5] For example, wild lettuce (*Lactuca indica*) contains simple phenols, phenylpropanoids, and flavonoid derivatives, and their antioxidant properties have been demonstrated in animal-cell models and in vitro DNA strand cleavage assays.[6] Recents studies have demonstrated that phenol synthesis depends on abiotic factors. Particularly when plants are submitted to saline treatment, variation in antioxidant pools, notably in phenols, has been found.[7] For instance, mild salt treatment dramatically augmented the total phenol content in halophytic species such as *Cakile maritima*[8] as well as in the glycophyte *Raphanus sativus*.[9] Studying the genotypes of lettuce Verte (NaCl tolerant) and Romaine (NaCl sensitive), Mahmoudi et al.[10] observed that NaCl-treated Verte, as compared to similarly treated Romaine, displayed better growth and had superior antioxidative capacity due to enhanced phenolic biosynthesis. Recently, Falleh et al.[11] confirmed that phenols play a significant physiological role in the salinity tolerance of the halophyte *Mesembryanthemum edule*, particularly against salt-induced oxidative damage.

Currently, one of the strategies used to induce different salt resistance responses in plants is the nutrient supply. In this sense, numerous studies are being made with trace elements such as selenium silicon (Se)[12] and (Si),[13] the former acting against stress by intensifying the antioxidant activity that stimulates plant growth. On the other hand, Si application triggers the mechanisms that act on plant growth during salinity stress related to the ionic damage caused by the entry of Na^+ into the plant. Recently, it has been confirmed that in biofortification programs the exogenous application of iodine (at doses of ≤80 μM) in the form of iodate (IO_3^-) bolsters the foliar biomass and the antioxidant capacity of lettuce plants by stimulating the biosynthesis and accumulation of phenol compounds.[14] The changes in these compounds induced by the IO_3^- application may be functional in the salt-stress response of plants. These compounds are thought to protect the plant against salt-induced oxidative stress. Efficient antioxidants acting as radical scavengers and lipid peroxidation inhibitors,[15] phenolics are also electron donors and thus could mitigate oxidative stress by acting as excellent substrates for antioxidant enzymes such as peroxidases.[16] Furthermore,

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Table 1. Effects of Iodate Supplementation on Foliar Biomass and Concentrations of Sodium, Chloride, and Iodine in Leaves of NaCl-Stressed Lettuce Plants[a]

treatment	foliar biomass (g dw)	sodium (mg g^{-1} dw)	chloride (mg g^{-1} dw)	iodine (mg kg^{-1} dw)
control	4.16 ± 0.11 a	8.44 ± 0.28 cd	1.90 ± 0.02 d	nd
100 mM NaCl	2.81 ± 0.08 c	11.80 ± 0.38 a	6.01 ± 0.12 a	nd
100 mM NaCl + 20 μM IO_3^-	3.62 ± 0.14 b	10.24 ± 0.39 b	4.98 ± 0.10 c	196.66 ± 1.77 c
100 mM NaCl + 40 μM IO_3^-	3.48 ± 0.12 b	8.20 ± 0.35 d	5.47 ± 0.10 b	279.99 ± 3.03 b
100 mM NaCl + 80 μM IO_3^-	3.18 ± 0.07 bc	9.32 ± 0.25 bc	4.83 ± 0.09 c	360.71 ± 2.87 a
$LSD_{0.05}$	0.45	0.95	0.15	60.1
p value	***	***	***	***

[a]Values are the mean ± SE (n = 9). Means followed by the same letter do not differ significantly. Levels of significance: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, not significant. dw, dry weight; nd, not detectable.

phenolics may help shield the photosynthetic apparatus from photodamage in salt-stress conditions.[17]

In this context, the aim of the present study was to determine whether the application of IO_3^- can improve the response to severe salinity stress and whether the resistance can be attributed to the phenolic metabolism in lettuce (*Lactuca sativa* cv. Philipus), a glycophyte cultivated for food and consumed year round.

MATERIALS AND METHODS

Plant Material and Treatments. Seeds of *L. sativa* L. var. Phillipus were germinated and grown for 35 days in cell flats (cell size = 3 × 3 × 10 cm) filled with perlite mixture, and the flats were placed on benches in an experimental greenhouse in southern Spain (Saliplant S.L., Motril, Granada, Spain). The 35-day-old seedlings were transferred to a growth chamber under controlled environmental conditions with 250 μmol m^{-2} s^{-1} photosynthetically active radiation (measured at the top of plants with a 190 SB quantum sensor; LI-COR Inc., Lincoln, NE, USA), generated by white fluorescent lamps (HPI-T 250 W; Phillips, Eindhoven, The Netherlands), with a 12/12 h (25/15 °C) light/dark photoperiod and relative humidity of 60−80%. The plants were grown in individual 8 L pots (25 cm upper diameter, 17 cm lower diameter, and 25 cm in height) filled with vermiculite. Throughout the experiment, the plants received a growth solution, which was composed of 4 mM $Ca(NO_3)_2$, 6 mM KNO_3, 2 mM $MgSO_4{\cdot}7H_2O$, 1 mM $NaH_2PO_4{\cdot}2H_2O$, 5 μM H_3BO_3, 2 μM $MnCl_2{\cdot}4H_2O$, 1 μM $ZnSO_4{\cdot}7H_2O$, 0.1 μM $Na_2MoO_4{\cdot}2H_2O$, 0.25 μM $CuSO_4{\cdot}5H_2O$, and 10 μM iron ethylenediamine di(*O*-hydroxyphenyl acetic) (EDDHA). The nutrient solution (pH 5.5−6.0) was renewed every 3 days and the vermiculite partly rinsed with Millipore-filtered water to avoid nutrient over accumulation.

At 45 days after germination, the different treatments were applied together with the nutrient solution described above. Our experiment consisted of four treatments of 100 mM NaCl, three of which were supplemented with increasing doses of IO_3^- (20, 40, and 80 μM as KIO_3) and also had a control treatment that was applied to the nutrient solution in the absence of IO_3^- and NaCl, which were added to the growth solution and maintained for 21 days. In addition to these treatments, we carried out a control treatment that consisted of applying the complete growth solution without an iodine supplement. The experimental design was a randomized complete block with five treatments arranged in individual pots with six plants per treatment and three replications each. The experiment was repeated three times under the same conditions.

Ion Determinations. To determine the total concentration of Na^+, 0.15 g of dry plant material underwent digestion with sulfuric acid in the presence of H_2O_2 and was subsequently diluted with distilled water. The total concentration of Na^+ leaf was measured directly by flame spectrophotometry.[18] The Cl^- was analyzed by an aqueous extraction of 0.10 g of dry plant material in 10 mL of distilled water. Cl^- content was measured according to the method of Diatloff and Rengel.[19] The results were expressed as milligrams per gram dry weight (dw).

For the determination of the I^- concentration, 25 mg of dry plant tissue was subjected to a mineralization process with 2.5 mL of concentrated HNO_3 and 1 mL of H_2O_2.[14] The resulting solution was diluted in distilled water, and the concentration of the element was determined using an Agilent 7500 ICP-MS system.

Analysis of Phenolic Compounds by HPLC-UV. For the identificaiton and characterization of phenolics, 0.1 g of lyophilized leaves was extracted with 1 mL of water/methanol (1:1) by sonication for 1 h, followed by overnight maceration and another sonication period (1 h). The resulting extract was centrifuged and filtered through a 0.45 μm PVDF membrane. Chromatografic analyses were carried out on a Phenomenex reverse-phase column (250 × 4.6 mm, Luna 5 μm C18 (2), 100 Å). The mobile phase consisted of two solvents: water/acetic acid (1%) (A) and acetonitrile (B), starting with 5% B and using a gradient to obtain 50% at 30 min and 80% at 37 min. The flow rate was 1 mL/min and the injection volume, 20 μL. Spectral data from all peaks were accumulated in the range of 200−400 nm, and chromatograms were recorded at 280, 320, and 360 nm. The HPLC-UV analyses were carried out with an Agilent HPLC 1100 series (Agilent Technologies, Waldbronn, Germany). Quantification of the analytes was performed by HPLC detection, using the external standard method with calibration graphs, as a function of concentration based on peak area, detected at the wavelength corresponding to their maximum absorbance.[20]

Preparation of Enzyme Extract for Assay. For determination of shikimate dehydrogenase (SKDH, EC 1.1.1.25) and polyphenol oxidase (PPO, EC 1.10.3.2) activities, whole fresh leaf was homogenized in 50 mM potassium phosphate buffer (pH 7.0). Homogenates were centrifuged at 15000*g* for 15 min at 4 °C.

For determination of phenylalanine ammonia-lyase (PAL, EC 4.3.1.5) activity, whole fresh leaf was homogenized in 100 mM potassium phosphate buffer (pH 8.0) containing 1.4 mM 2-mercaptoethanol. The homogenate was centrifugated at 15000*g* for 15 min at 4 °C. The supernatant was desalted through a Sephadex G-25 column (24 × 100 mm) previously equilibrated with the same buffer.

For determination of 4-coumarate coenzyme A ligase (4CL, EC 6.2.1.12) the extract buffer was 0.05 M Tris-HCl (pH 8.8) containing 14 mM mercaptoethanol and 30% glycerol. Homogenates were centrifuged at 10000*g* for 15 min at 4 °C.

Enzyme Assay. SKDH activity was assayed in 0.1 M Tris-HCl buffer (pH 9). The reaction mixture contained 2 mM shikimic acid, 0.5 mM NADP, and 0.1 mL of supernatant. Increase of absorbance due to reduction of NADP was read over 1 min at 340 nm.[21]

PAL activity was measured by using a modified method.[22] The reaction mixture was 100 mM Tris-HCl buffer (pH 8.8), 40 mM phenylalanine, and 0.2 mL of enzyme extract. The reaction mixture was incubated for 30 min at 37 °C, and the reaction was terminated by adding 25% trichloroacetic acid. In the control PAL assay, the same amount of phenylalanine was added after termination. To remove precipitated protein, the assay mixture was centrifuged at 10000*g* for 15 min at 4 °C, and the absorbance of the supernatant was measured at 280 nm relative to the control.

Table 2. Effects of Iodate Supplementation on Phenolic Compounds in Leaves of NaCl-Stressed Lettuce Plants[a]

treatment	hydroxycinnamic acids and derivatives (mg g^{-1} dw)	flavonoids and glycosides (mg g^{-1} dw)	others (mg g^{-1} dw)	total (mg kg^{-1} dw)
control	1.68 ± 0.03 a	0.16 ± 0.008 a	2.24 ± 0.04 a	4.01 ± 0.11 a
100 mM NaCl	0.58 ± 0.01 d	0.13 ± 0.009 a	1.58 ± 0.08 c	2.29 ± 0.09 d
100 mM NaCl + 20 μM IO_3^-	1.35 ± 0.02 b	0.15 ± 0.01 a	2.11 ± 0.07 ab	3.61 ± 0.10 b
100 mM NaCl + 40 μM IO_3^-	1.21 ± 0.03 b	0.14 ± 0.008 a	1.87 ± 0.05 b	3.22 ± 0.13 c
100 mM NaCl + 80 μM IO_3^-	0.92 ± 0.04 c	0.14 ± 0.01 a	1.85 ± 0.06 b	2.91 ± 0.07 c
$LSD_{0.05}$	0.22	0.05	0.31	0.36
p value	***	ns	***	***

[a]Values are the mean ± SE (n = 9). Means followed by the same letter do not differ significantly. Levels of significance: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, not significant. dw, dry weight.

Table 3. Effects of Iodate Supplementation on Flavonoid and Phenylpropanoid Synthesis and Degradation Related Enzymes in Leaves of NaCl-Stressed Lettuce Plants[a]

treatment	SKDH activity	PAL activity	4CL activity	PPO activity
control	0.067 ± 0.007 a	0.10 ± 0.01 a	3.34 ± 0.12 a	1.17 ± 0.08 c
100 mM NaCl	0.034 ± 0.005 c	0.05 ± 0.006 c	2.83 ± 0.11 b	1.99 ± 0.13 a
100 mM NaCl + 20 μM IO_3^-	0.052 ± 0.009 b	0.08 ± 0.009 b	3.18 ± 0.15 a	1.34 ± 0.11 bc
100 mM NaCl + 40 μM IO_3^-	0.055 ± 0.009 b	0.07 ± 0.007 b	3.15 ± 0.13 a	1.42 ± 0.12 b
100 mM NaCl + 80 μM IO_3^-	0.046 ± 0.007 b	0.07 ± 0.007 b	3.01 ± 0.11 ab	1.51 ± 0.10 b
$LSD_{0.05}$	0.009	0.006	0.23	0.19
p value	***	***	*	***

[a]Values are the mean ± SE (n = 9). Means followed by the same letter do not differ significantly. Levels of significance: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, not significant. SKDH activity expressed as ΔA_{340} h^{-1} mg^{-1} protein; PAL activity expressed as A_{240} h^{-1} mg^{-1} protein; 4CL activity expressed as A_{333} h^{-1} mg^{-1} protein, PPO activity expressed as ΔA_{390} h^{-1} mg^{-1} protein.

The 4CL activity was determined with the spectrophotometric method, using caffeic acid as the preferred phenolic substrate.[23] The reaction mixture was 5 μM p-coumaric acid, 50 μM ATP, 1 mM CoA-SH, and 15 mM Mg_2SO_4. The reaction mixture was incubated at 40 °C for 10 min, and then the absorbance was measured at 333 nm.

PPO assay was performed in a mixture containing 50 mM potassium phosphate buffer (pH 7.0), 60 mM catechol, and 0.1 mL of supernatant. Increase in absorbance was read over 2 min at 390 nm.[24]

Statistical Analysis. Data compiled were submitted to an analysis of variance (ANOVA), using the Statgraphics 6.1 program, and differences between the means were compared by Duncan's multiple-range test ($P > 0.05$).

RESULTS

The minimum value of foliar biomass was found in the 100 mM treatment of NaCl with a decline of 32% with respect to the maximum value found in control plants (Table 1). The IO_3^- treatments together with NaCl also declined in foliar biomass in comparison to control, but less severely (13, 16, and 24% for IO_3^- application rates of 20, 40, and 80 μM, respectively) than in the 100 mM treatment of NaCl (Table 1).

In terms of the ion concentration, the Na^+ and Cl^- contents proved lower in control plants than in the treatment with 100 mM NaCl (Table 1), where Cl^- tripled its concentration. The IO_3^- treatment lowered the Na^+ and Cl^- concentrations in relation to the NaCl treatment (Table 1). With respect to the total concentration of I^-, as expected, the control treatments and 100 mM NaCl presented no detectable concentrations of this trace element (Table 1). On the contrary, the application of the treatments with the different IO_3^- rates resulted in foliar I^- concentrations, reaching the maximum in the 80 μM treatment of IO_3^- (Table 1).

The minimum concentrations of both hydroxycinnamic acid and derivatives as well as other types of phenols, not including the flavonoids or glyosides, fell 64 and 29% in the 100 mM NaCl treatment, respectively, in comparison to the maximum values registered in the control (Table 2). Notably, for both types of phenols, the different application rates of IO_3^- together with NaCl raised the concentration of these phenols with respect to plants treated with 100 mM NaCl, reaching maximum increases at the rate of 20 μM IO_3^- (133 and 34%, respectively; Table 2). With regard to flavonoids and glycoside, the levels were minimum in all of the treatments, without presenting significant differences (Table 2). Finally, the total phenol concentration followed a response similar to that described previously for hydroxycinnamic acids and derivatives (Table 2).

The metabolism of phenolic compounds was also affected by the different treatments applied (Table 3). Thus, the synthesis enzymes SKDH and PAL presented the same response, registering minimum activities in the treatment with 100 mM NaCl (Table 3), with reductions of 49 and 50%, respectively, with respect to the maximum activity found in control plants. The application of different rates of IO_3^- with 100 mM NaCl resulted in a significant rise in these enzymatic activities in comparison to the treatment with 100 mM NaCl, in both cases exceeding 35% (Table 3). On the contrary, the different treatments applied showed only a slightly significant variation in the response of the 4CL activity (Table 3). It bears noting that for this enzyme the minimum activity was again found in the 100 mM NaCl treatment with a decrease (15%) only with respect to control (Table 3). Finally, the enzyme PPO, responsible for phenol oxidation, responded in a way contrary to that of the synthesis enzymes, given that the maximum activity was found in the 100 mM NaCl treatment and the minimum in control (Table 3). In addition, the treatment consisting of IO_3^- and 100 mM NaCl presented lower PPO activities than in the treatment with 100 mM NaCl, in all cases registering declines of >24% (Table 3).

DISCUSSION

One of the main consequences of the exposure to salinity stress in plants is growth reduction,[25] with considerable loss in yield registered in most plant species. Therefore, one of the most widely used agricultural indices to define salt-stress tolerance is biomass production. [26] Whereas this growth reduction with respect to control was clear in the present experiment, the addition of IO_3^- to the growth medium translated as an increase with respect to the plants treated with 100 mM NaCl, especially at the rates of 20 and 40 μM IO_3^- (Table 1). These results a priori suggest that the application of this form of I may induce a mechanism that partially counteracts salinity toxicity in lettuce plants.

One of the toxic effects of salinity stress that directly affects plant productivity is the accumulation of Na^+ and Cl^- in the leaves.[27] The accumulation of these ions can upset the ionic balance, triggering a nutritional imbalance due to the blockage of other cations such as K^+, Ca^{2+}, and Mg^{2+} or anions such as NO_3^- and thereby provoking symptoms of nutritional deficiency. In our work, the Na^+ and Cl^- contents proved lower in control plants than in the treatment with 100 mM NaCl (Table 1). The accumulation of the ions Na^+ and Cl^- in plants treated with 100 mM NaCl could explain the lower foliar biomass found in this treatment (Table 1), given that under salinity conditions it is one of the causes for ROS formation.[27] In addition, salt treatments with IO_3^- decreased in the Na^+ and Cl^- concentration in relation to the NaCl treatment (Table 1), presumably from the antagonism between the salts KIO_3 and NaCl. In the specific case of IO_3^-, this must first be reduced to I^- prior to its uptake by the plant[14] and, therefore, could inhibit Cl^- uptake. In fact, Table 1 reflects that the greater application of IO_3^- in the nutrient solution implies higher foliar concentrations of this trace element. However, the fall in foliar concentrations of Na^+ and Cl^- prompted by the different IO_3^- treatments in our work were minimal with respect to the concentrations of the 100 mM NaCl treatment (Table 1). According to our data, this fact would not explain the improvement caused by the different IO_3^- treatments in terms of foliar biomass in lettuce plants subjected to salinity. In this sense, Leyva et al.[28] indicated that the main benefit of IO_3^- regarding the salinity responses of plants is the improvement in the antioxidant capacity induced by this trace element. This may involve, as confirmed below, the metabolism of phenolic compounds.

Phenolic compounds are C-rich metabolites that represent the largest group of secondary plant metabolites. For example, they are important antioxidants and, in situations of abiotic stress, may scavenge free radicals and other oxidative species.[29] Few studies are available on the response of phenolics to salinity in lettuce plants. Kim et al.[30] observed that the phenolic content of Romaine lettuce declined with short-term (2 days) saline irrigation ($\geq$50 mM NaCl), whereas no significant differences were found among salt treatments (5−200 mM NaCl) exposed to long-term (15 days) saline irrigation. Contrary to these findings, our results indicate that long-term exposure to 100 mM NaCl significantly lowered the concentrations of all the phenolic compounds analyzed (Table 2). This discrepancy in findings may be due either to the greater exposure time to salinity in our experiments (21 days) or else to the degree of sensitivity to salinity of the genotype chosen. Results similar to ours have been reported by López-Berenguer et al.[31] in broccoli, which under long-term treatments of 40 and 80 mM NaCl registered lower values for phenolic compounds both in the young and in the old leaves. Also, previous results have shown that phenolic compounds in *Bruguiera parviflora* and in the shoots of two clones of Saccharum sugar canes decreased when the plants were subjected to long-term NaCl treatments.[32,33]

On the contrary, the application of the IO_3^- treatments under saline conditions, especially at rates of 20 and 40 μM, in our work raised the phenolic concentrations with respect to the plants submitted to 100 mM NaCl (Table 2). The increases in phenolic compounds induced by IO_3^- application appear to play functional roles in the of response salt-stressed plants, this being confirmed by the greater foliar biomass registered in these treatments compared to the treatment with 10 mM NaCl (Table 1). These compounds are thought to protect the plant against salt-induced oxidative stress. Phenolics are efficient antioxidants acting as radical scavengers and lipid peroxidation inhibitors.[15] Furthermore, phenolics are electron donors and thus could mitigate the effect of oxidative stress as excellent substrates for antioxidant enzymes such as peroxidases.[16] Phenolics also may protect the photosynthetic apparatus from photodamage in salt-stress conditions.[17] In this sense, the application of IO_3^- under saline conditions very significantly induces hydroxycinnamic acids and derivatives (Table 2), which have been defined under different types of abiotic stress as exerting high radical-scavenging activity, stimulating antioxidant activity in these plants.[34] In short, the beneficial effect of IO_3^- application, that is, fortifying salinity resistance by inducing the accumulation of phenolic compounds, is corroborated by the results of Mahmoudi et al.[10] These authors, studying the genotypes of lettuce Verte (NaCl tolerant) and Romaine (NaCl sensitive), observed that, compared with the latter, NaCl-treated Verte displayed better growth and possessed superior antoxidative capacity due to enhanced phenolic biosynthesis.

To confirm the different responses to phenolic compounds after the application of different treatments in our work, we analyzed the activity of the main enzymes involved in phenolic metabolism. From the metabolism of carbohydrates and glycolysis, shikimate is biosynthesized by SKDH, which is one of the enzymes that controls the carbon flow toward phenolic metabolism. The pathway continues, producing phenylalanine, the aromatic amino acid, which is afterward deaminated by PAL, the key enzyme in phenolic biosynthesis. PAL catalyzes the nonoxidative deamination of L-phenylalanine to form cinnamic *trans*-acid. Finally, the action of the enzyme 4CL results in the formation of the compound *p*-coumaroyl CoA, producing a great number of secondary products derived from phenylpropanoids in plants, such as flavonoids and isoflavonoids, coumarins, lignins, hydroxycinnamic acids, esters, and phenolic compounds.[35] In our work, the minimum activities of the enzymes SKDH, PAL, and 4CL appeared in the plants treated with 100 mM NaCl (Table 3), corresponding to the lowest phenol concentration in these plants (Table 2). On the contrary, the IO_3^- application, together with the salinity, increased primarily the activity of SKDH and PAL with respect to the 100 mM NaCl treatment (Table 3). These results would explain the greatest phenol concentrations (Table 2), and the protective role of these secondary compounds against stress would also explain the greater foliar biomass presented by the treated plants, especially with 20 and 40 μM IO_3^- (Table 1). For example, stronger activities of enzymes related to phenolics and the accumulation of phenolic compounds have been correlated with the resistance of cereals

to abiotic stress.[29] Supporting our results, Oh et al.[15] and Sánchez-Rodríguez et al.[36] have associated intensified PAL activity with better adaptation to stress in lettuce and tomato plants submitted to water deficit. The function that IO_3^- may have in prompting the activity of the enzymes SKDH and PAL could be related to the effect of this trace element on the increase of photosynthesis and carbohydrate synthesis,[37] which, under stress conditions, could supply the extra carbohydrates needed to augment the synthesis of phenolic compounds.

Phenolic compounds are oxidatively degraded primarily by PPO. The enzyme PPO catalyzes the oxidation of *o*-diphenols to *o*-diquinones, as well as the hydroxylation of monophenols. The activity of PPO involves the production of quinines and ROS, and thus it has been demonstrated that a rise in its activity exacerbates oxidative stress.[36] Our results show that the plants that underwent the most severe saline stress with the lowest foliar biomass (treatment with 100 mM NaCl; Table 1) were those presenting the greatest PPO activity (Table 3), which is correlated in these plants with a greater oxidative stress and ROS formation.[28] On the contrary, the application IO_3^- together with 100 mM NaCl depressed PPO activity (Table 3) and, therefore, the possible formation of ROS in these plants. These data coincide with findings by Thipyapong et al.,[38] who proposed that in tomato plants diminished PPO activity lowers the H_2O_2 concentration, thereby improving resistance against an abiotic stress.

Our data reveal that the application of IO_3^- under salinity stress, especially at the rates of 20 and 40 μM, induced higher activity in the synthesis pathway of phenolic compounds together with lower oxidation. This resulted in an increase in hydroxycinnamic acids and derivatives and total phenols, which could act as protective compounds against salinity, improving the antioxidant activity in these plants. Finally, the present study reveals that in agricultural areas affected by salinity, the application of IO_3^- could be an effective strategy, given that, in addition to improving lettuce plant growth, it would provide this crop added nutritional value for the human diet, consisting of intake of phenolic compounds and the trace element iodine.

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Notes

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ABBREVIATIONS USED

4CL, 4-coumarate coenzyme A ligase; I, iodine; IO_3^-, iodate; PAL, phenylalanine ammonia-lyase; PPO, polyphenol oxidase; ROS, reactive oxygen species; SKDH, shikimate dehydrogenase

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