

Iodine increases the concentration of phenolic compounds and photosynthetic pigments in three cultivars of *Ficus carica* L. subjected to salt stress

Pablo Alan Rodríguez-Salinas¹
Fernando de Jesús Carballo-Méndez^{1§}
Juan Carlos Rodríguez-Ortiz²
Guillermo Niño-Medina¹
Emilio Olivares-Saénz¹
Carlos Alberto Garza-Alonso¹

¹Autonomous University of Nuevo León-Faculty of Agronomy. Av. Francisco Villa s/n, col. Former Hacienda El Canada, General Escobedo, Nuevo Leon, Mexico. ZC. 66050. (palanvf@hotmail.com; emolivares@gmail.com; nino.medina.g@gmail.com; crls-alonso@hotmail.com). ²Autonomous University of San Luis Potosí-Faculty of Agronomy and Veterinary Medicine. San Luis-Matehuala highway km 14.5, ejido Palma de la Cruz, Soledad de Graciano Sánchez, San Luis Potosí, Mexico. CP. 78321. (jcrodor@hotmail.com).

[§]Corresponding author: ing.fercarballo@gmail.com.

Abstract

Iodine (I) is a non-essential element for plants; however, the application of the element has shown positive effects on plants grown in optimal conditions or under stress. The objective of this experiment was to evaluate the impact of iodine on the concentration of phenolic compounds, antioxidant capacity and photosynthetic pigments in leaves of three fig cultivars subjected to salt stress. Eight-month-old fig plants were established under a completely randomized experimental design with a 3x2x2 factorial arrangement: three fig cultivars (*Ficus carica* L.): Brown Turkey, Kadota and Black Mission; two levels of NaCl (0 and 100 mmol L⁻¹) and two levels of iodine (0 and 10 mg L⁻¹). The analyses of variance showed the impact of the main factors (cultivars, NaCl and I) and the interaction between them. The application of iodine on fig seedlings increased the concentration of chlorophyll a, regardless of the cultivar and the concentration of NaCl. The number of new leaves and their dry weight were affected by the interaction between NaCl and I, these variables increased with the presence of I in saline condition. The relative content of total phenols, total flavonoids, antioxidant capacity by DPPH and photosynthetic pigments (chlorophylls and carotenoids) showed interaction between the cultivars, the levels of NaCl and the concentration of I, where the values of these variables were increased by the presence of I under salinity conditions. Due to the above, iodine could be considered as an alternative to mitigate the stress caused by NaCl in *Ficus carica* L. plants.

Keywords: chlorophylls, fig, salinity, total flavonoids, total phenols.

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Introduction

Iodine (I) is a non-essential element for plants; however, it has been shown that the application of this element generates positive effects on the growth of various plants (Medrano-Macías *et al.*, 2016). Small amounts of this element, in the range of microelement concentrations required by plants, have improved plant growth, development and productivity (Cakmak *et al.*, 2017; Lyons, 2018; Duborská *et al.*, 2020). This positive impact has been observed from modulation in gene expression to being a structural part of several proteins (Kiferle *et al.*, 2021), as well as the increase in the concentration of essential minerals in the leaves (Cortés-Flores *et al.*, 2016; Sularz *et al.*, 2020).

Similarly, applications of I have shown diverse effects on antioxidant capacity in several species; this antioxidant capacity varies depending on sources, concentration and type of application (Halka *et al.*, 2020; Sabatino *et al.*, 2021). Contrary to the above, in some species, high levels of I in the nutrient solution have caused a marked reduction in leaf expansion and photosynthetic activity, which was attributed to a large accumulation of this element in leaf tissues (Incrocci *et al.*, 2020).

On the other hand, low concentrations of I have improved the response to salinity stress, particularly caused by sodium chloride (NaCl). The effect of the application of I decreased the concentration of toxic ions such as Na^+ and Cl^- ; in addition to increasing the concentration of soluble sugars (sucrose, glucose and fructose) that participate as osmoprotectants during osmotic adjustment, as well as causing the activation of antioxidant enzymes such as superoxide dismutase (SOD) and ascorbate peroxidase (APX), and the non-enzymatic antioxidant system in which phenolic compounds are found, in order to maintain reactive oxygen species (ROS), such as O_2 and H_2O_2 , at minimum levels (Leyva *et al.*, 2011; Gonzali *et al.*, 2017; Kiferle *et al.*, 2019).

For these reasons, I can be considered as a beneficial element to help counteract the harmful effects of salinity stress, but it is necessary to investigate in a specific manner the impact on the various species and stages of development (Leyva *et al.*, 2011; Pérez-Salas and Medrano-Macías, 2021). Considering the above, the objective of this experiment was to evaluate the impact of the application of iodine on the concentration of phenolic compounds, antioxidant capacity and photosynthetic pigments in leaves of three fig cultivars subjected to salt stress.

Materials and methods

Location of the experiment and plant material

The research was carried out at the Center for Protected Agriculture of the Faculty of Agronomy of the Autonomous University of Nuevo León, Mexico, inside a Spanish-type greenhouse. The average environmental conditions inside the greenhouse were 25 °C temperature and 70% relative humidity. Eight-month-old fig plants, with six leaves each, were transplanted into black polyethylene bags of a capacity of 10 L filled with perlite substrate. The experiment was established under a completely randomized experimental design with a 3x2x2 factorial

arrangement: three fig cultivars (*Ficus carica* L.): Brown Turkey (BT), Kadota (K) and Black Mission (BM); two levels of NaCl (0 and 100 mmol L⁻¹) and two levels of I (0 and 10 mg L⁻¹) using potassium iodate (KI) as a source of iodine. Both factors, NaCl and I were applied by gravity irrigation.

The base irrigation water had the following characteristics: Ca: 3.5 Mg, 1.8, Na: 0.9, HCO₃: 2.5, Cl: 1.35, SO₄: 2.35; HCO₃: 2.5 mEq L⁻¹; pH: 7.5 and electrical conductivity (EC): 0.6 dS m⁻¹. From the combination of the aforementioned factors, 12 treatments emerged, with six repetitions each. Each plant was considered as an experimental unit. The relative chlorophyll content, expressed in SPAD units, was measured using the portable SPAD-502 of Minolta (Konica Minolta, Osaka, Japan). SPAD units were measured in the middle lobe of leaves number 4, 5 and 6 from the base to the apex.

The values of this variable are the average of three measurements made 5, 10 and 15 days after the beginning of treatments. Also, after the last measurement, samples of four plants per treatment were collected for the determination of dry weight of new leaves, antioxidant compounds and pigments. The dry weight was obtained by means of a drying oven (Yamato model DX602C, Yamato Scientific America, Santa Clara, CA, USA) where the samples were subjected to a temperature of 60 °C for three days. The resulting material was weighed with an analytical balance with precision of 0.001 g.

Extraction of phenolic compounds

The extraction of phenolic compounds was carried out based on what was reported by Carballo-Méndez *et al.* (2019) with slight modifications. Four grams of fresh leaf tissue were suspended in 40 mL of water:methanol (20:80 v/v) (80%) and homogenized with an Oster blender (M4655-813/465-42, Sunbeam, Mexico City, Mexico) in a crystal glass for porridge for 30 s. It was then filtered with an organza fabric, placed in 50 ml Corning tubes and centrifuged at 4 500 rpm for 5 min at 25 °C. The supernatant was recovered and stored protected from light at -20 °C, until its further analysis.

Total phenols and flavonoids

The content of total phenols and total flavonoids was evaluated based on what was reported by Rodríguez-Salinas *et al.* (2020), with the following modifications: the total phenols were evaluated based on the colorimetric method of the Follin-Ciocalteu colorimetric reaction, with 200 µl of the methanolic extract in 2 600 µl of water, followed by the addition of 200 µl and alkalization by adding 2 000 µl of Na₂CO₃, then it was left to react for 90 min and the absorbance was measured in an SP-830 Plus spectrophotometer (Barnstead, Turner, USA) at a wavelength of 750 nm.

The standard curve was prepared with gallic acid in concentrations from 0 to 200 mg L⁻¹, 80% methanol was used as a blank. The results were expressed in milligrams of gallic acid equivalents per gram of fresh tissue (mgGAE g⁻¹). The total flavonoids were measured by the method of aluminum chloride (AlCl₃) and sodium hydroxide (NaOH), briefly, 200 µl of the methanolic extract was added to 3 500 µl of water, followed by 150 µl of a 5% solution of NaNO₂ and it was left to stand for 5 min, then 150 µl of a 10% solution of AlCl₃ was added and finally 5 min later, 1 000 µl of NaOH at 1 N was added.

It was left to react for 15 min and the absorbance of the samples was measured in an SP-830 Plus spectrophotometer at the length of 510 nm. Concentrations were calculated based on a catechin curve made at concentrations from 0 to 200 mg L⁻¹. The results were expressed in milligrams of catechin equivalents per gram of fresh tissue (mg CE g⁻¹).

DPPH antioxidant capacity

The determination of antioxidant capacity by the method of 2,2-diphenyl-1-picrylhydrazyl (DPPH) of the methanolic extracts of the fresh fig leaf tissue was carried out based on what was described by Rodríguez-Salinas *et al.* (2020), with slight modifications described below: DPPH, it was evaluated using the working stock solution prepared at 60 µM dissolved in 80% methanol, adjusted to an absorbance at 1 nm at a wavelength of 517 nm. The reaction was performed by adding 0.5 µl of the methanolic extract to 1.5 ml of the stock solution, it was left to stand for 30 min and the absorbance was measured on an SP-830 Plus spectrophotometer at a wavelength of 517 nm. As a standard curve, Trolox was used in concentrations from 0 to 500 µmol L⁻¹. Results were expressed in Trolox equivalents per each gram of fresh tissue (µmol TE g⁻¹).

The IC₅₀ of DPPH was calculated for the amount of antioxidant needed to inhibit 50% of the oxidation of the radical, the absorbance adjusted to 1 in the working solutions was considered as 100% of oxidation and methanol was used as a control. The results were expressed in milligrams per gram of fresh leaf tissue (mg g⁻¹).

Photosynthetic pigments

The photosynthetic pigments chlorophylls (a, b and total) and total carotenoids were extracted and calculated based on what was reported by Rajput and Patil (2017), with slight modifications. Briefly, on average 4 g of fresh leaf tissue was homogenized in an Oster blender with a crystal glass for porridge for 30 sec. in the presence of 40 ml of water:acetone (20:80 v/v) (80%) that contained 200 mg of Na₂CO₃. The homogenized was then passed through an organza fabric, placed in 50 ml capacity corning tubes and centrifuged at 6 000 rpm for 5 min at room temperature. The supernatant was recovered and stored protected from light at -20 °C, until its subsequent analysis by spectrophotometry.

Chlorophylls and total carotenoids were quantified in an SP-830 Plus spectrophotometer at wavelengths of 663-645 and 480-510 nm, respectively. The results were expressed in milligrams per gram of fresh tissue (mg g⁻¹). Calculation: chlorophyll a= $12.7(A_{663}) - 2.69(A_{645}) \times V / (1000) \times SW$; Chlorophyll b= $22.9(A_{645}) - 4.68(A_{663}) \times V / (1000) \times SW$; total carotenoids= $7.6(A_{480}) - 1.49(A_{510}) \times V / (1000) \times SW$. Where: A= absorbance obtained at the specified wavelength; V= volume of extraction; and SW= sample weight.

Statistical analysis

The values obtained were analyzed with the Levene test for homogeneity of variances and with the Kolmogorov-Smirnov test for the verification of normality. For the analysis of variance of the variables, the relative chlorophyll content covariate was considered, which was evaluated one day before the start of the treatments. In the variables where a significant difference was found, a comparison of means was made using the Tukey test ($p < 0.05$). For these analyses, the statistical package SPSS version 22.0, IBM, was used.

Results and discussion

The analysis of variance showed that the main factors (cultivars, NaCl levels, I levels) and the interaction between them was different in the variables evaluated (Table 1).

Table 1. F values and significance levels (P) observed in analyses of variance.

Variables		Cultivar (C)	NaCl (S)	Iodine (I)	C*S	C*I	S*I	C*S*I
Relative chlorophyll content	F	14.271	0.022	17.485	2.021	1.992	3.896	7.734
	<i>p</i>	<0.001	ns	<0.001	ns	ns	ns	0.003
Number of new leaves	F	4.878	9.936	7.288	0.798	1.153	4.851	0.165
	<i>p</i>	0.017	0.004	0.013	ns	ns	0.038	ns
Dry weight of new leaves	F	0.425	16.245	15.845	0.37	0.496	4.567	0.099
	<i>p</i>	ns	0.001	0.001	ns	ns	0.043	ns
Total phenols	F	9.275	0.241	4.588	0.163	1.197	21.886	8.205
	<i>p</i>	0.001	ns	0.043	ns	ns	<0.001	0.002
Total flavonoids	F	65.69	0.891	0.611	0.573	1.556	2.435	1.821
	<i>p</i>	<0.001	ns	ns	ns	ns	ns	ns
DPPH	F	4.497	3.566	3.501	0.846	1.027	2.312	4.028
	<i>p</i>	0.022	ns	ns	ns	ns	ns	0.032
IC50	F	5.544	3.068	4.905	0.401	6.231	2.146	0.677
	<i>p</i>	0.011	ns	0.037	ns	0.007	ns	ns
Chlorophyll a	F	20.8	23.283	7.974	0.657	2.656	0.198	2.377
	<i>p</i>	<0.001	<0.001	0.01	ns	ns	ns	ns
Chlorophyll b	F	47.641	68.021	11.578	19.475	2.202	0.157	3.927
	<i>p</i>	<0.001	<0.001	0.002	<0.001	ns	ns	0.034
Carotenoids	F	14.557	23.262	1.079	7.702	0.695	0.005	1.275
	<i>p</i>	<0.001	<0.001	ns	0.003	ns	ns	ns

ns= not significant.

Relative chlorophyll content and growth

In the SPAD units, there was a triple interaction between cultivars, NaCl level and presence of I. In the BT cultivar, I reduced the SPAD units by 3% under conditions of low salinity, while in the condition of salinity, it increased them by 6%. For the K cultivar, the behavior was similar in both NaCl conditions, increasing these units by 11 and 3% under conditions of low and high salinity, respectively. In the BM cultivar, the effect of I was only present at the high level of salinity, with an increase of 13% (Figure 1A). These results found in fig leaves are similar to those reported by Cortes *et al.* (2016), who recorded that the presence of I increased by up to 5% the concentration of nitrogen related to the SPAD units, in leaves of bell pepper (*Capsicum annum* L.).

The number of new leaves showed a difference between the cultivars, with BT being the one with the highest quantity. For this same variable, there was an interaction between the level of NaCl and I, where the presence of I increased the number of leaves in the solution with NaCl by 204% (Figure 1B). The dry leaf weight only showed interaction between NaCl and I level, where I increased the dry leaf weight by 273% in the presence of NaCl (Figure 1C). Like our results, Salimpour *et al.* (2019) reported that different cultivars of fig show differences in leaf growth, as a consequence of genetic diversity. Kiferle *et al.* (2019) reported that, in non-saline conditions, the presence of I did not increase the dry matter in basil (*Ocimum basilicum* L.), while Leyva *et al.* (2011); Blasco *et al.* (2013) reported that, under salinity conditions, the presence of I increased the aerial biomass of lettuce (*Lactuca sativa* L.), which was attributed to the reduction of Na and Cl and the increase in the K/Na ratio.

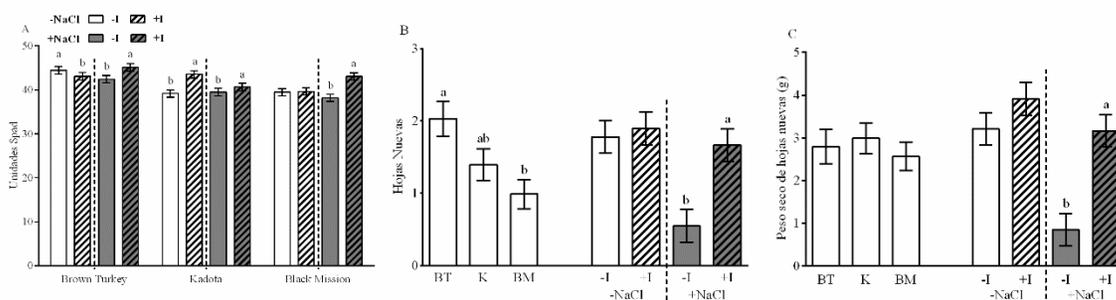


Figure 1. Effect of cultivars, NaCl and I, the relative chlorophyll content (SPAD) (A); on the number of new leaves (B); and dry weight of new leaves (C). The lines on the bars represent the standard error. The different letters on each bar mean that the treatments were statistically different (Tukey, $p < 0.05$).

Phenolic compounds and antioxidant capacity

The concentration of phenolic compounds was affected by the interaction of the different factors. The BR and K cultivars reduced the concentration of total phenols by 26 and 45% in the absence of NaCl, while with the presence of NaCl, these compounds increased by 25 and 17%, respectively. Contrary to the above, in the BM cultivar, the presence of I in saline situation reduced total phenols by 9% (Figure 2A). The concentration of total flavonoids was only affected by cultivars, where the BT cultivar exceeded K and BM; in general, 150% (Figure 2B). The antioxidant activity by DPPH showed interaction between the three factors. In the BT cultivar, the presence of I in the solution without NaCl decreased this activity by 11%, while in the presence of NaCl, I increased it by 455%. In the BM cultivar without the presence of NaCl, the presence of I increased this activity by 22% (Figure 2C).

The IC₅₀ was only affected by the interaction of cultivars and I, particularly in the BM cultivar where the presence of this element reduced this concentration by 21% (Figure 2D). The increase in antioxidant capacity by the DPPH assay of plants reveals an increase in the synthesis of low molecular weight reducing molecules, mainly non-enzymatic antioxidants (Pérez-Salas and Medrano-Macías, 2021), such as phenolics (Leyva *et al.*, 2011). Because DPPH bases its foundation on the ability to measure compounds with the ability to donate H atoms (Schaich *et al.*, 2015).

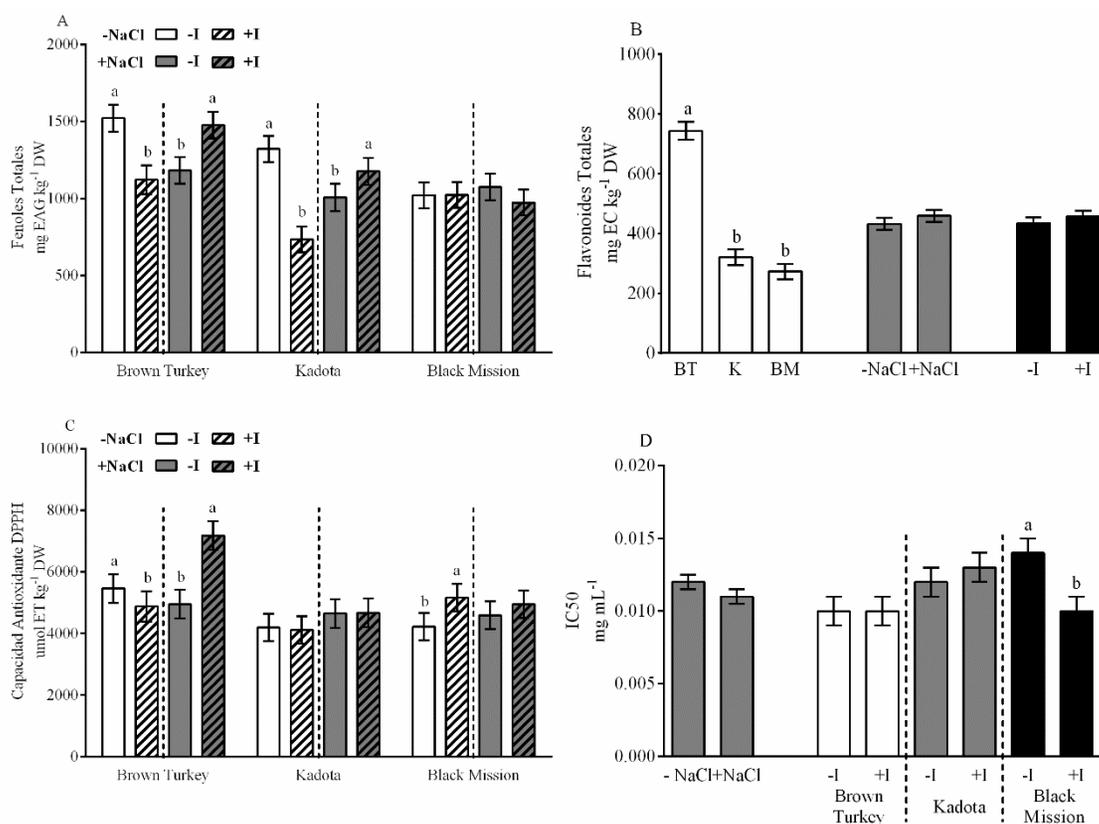


Figure 2. Effect of cultivars, NaCl and I on the concentration of total phenols (A); total flavonoids (B); DPPH antioxidant activity (C); and mean inhibitory concentration (IC₅₀). The lines on the bars represent the standard error. The different letters on each bar mean that the treatments were statistically different (Tukey, $p < 0.05$).

Several authors have reported that applications of I increase the antioxidant capacity of plant tissues. Kiferle *et al.* (2019) mention that I increased the total phenols in basil leaves, as well as Blasco *et al.* (2013), who reported that, in lettuce subjected to salt stress, I increased the concentration of total phenols, without finding differences in the concentration of flavonoids. These results are consistent with what was observed in this experiment. Contrary to the above, Golubkina *et al.* (2018) reported that, in mustard (*Brassica juncea* L.) leaves, the application of I increased the concentration of flavonoids.

Similarly, Cortés-Flores *et al.* (2016) reported that foliar application of KI increased DPPH antioxidant activity in pepper seedlings under optimal development conditions, while Kiferle *et al.* (2019) reported the decrease in this activity in basil plants. In the present experiment, this effect was different between cultivars and salinity level. In non-saline conditions, the presence of iodine decreased the DPPH antioxidant activity in the BT cultivar, while it increased it in the BM cultivar. In saline situation, the presence of I only benefited the BT cultivar. Particularly for IC₅₀, Incrocci *et al.* (2020) reported that, in basil, the presence of I reduced the IC₅₀, although the impact was different between cultivars, a situation very similar to that shown in this experiment.

Photosynthetic pigments

The concentration of chlorophyll a showed main effects of the three factors. The BT and K cultivars exceeded BM by 35%. The presence of NaCl reduced these compounds by 18%, while the presence of I increased them by 12% (Figure 3A). The concentration of chlorophyll b was affected by the interaction of the three factors. In the BT cultivar, the presence of I increased them, the concentration of chlorophyll b by 17% without the presence of NaCl and by 83% in the presence of said salt. In the K cultivar, the effect of I was different since, in the absence of NaCl, chlorophyll b increased 36%, while, in the presence of salt, these compounds decreased by 17%.

For the BM cultivar, the presence of I in salinity condition increased these pigments by 65% (Figure 3B). The carotenoid concentration was only affected by the interaction between the cultivar and NaCl. In BT and K cultivars, the presence of NaCl reduced carotenoid concentration by 36% and 20%, respectively (Figure 3C). The results shown above differ partially from those reported by Pérez-Salas and Medrano-Macías (2021), who point out that, in tomato (*Solanum lycopersicum* L.) plants subjected to salt stress, the presence of I did not modify the concentration of chlorophyll a, but it did increase the concentration of chlorophyll b.

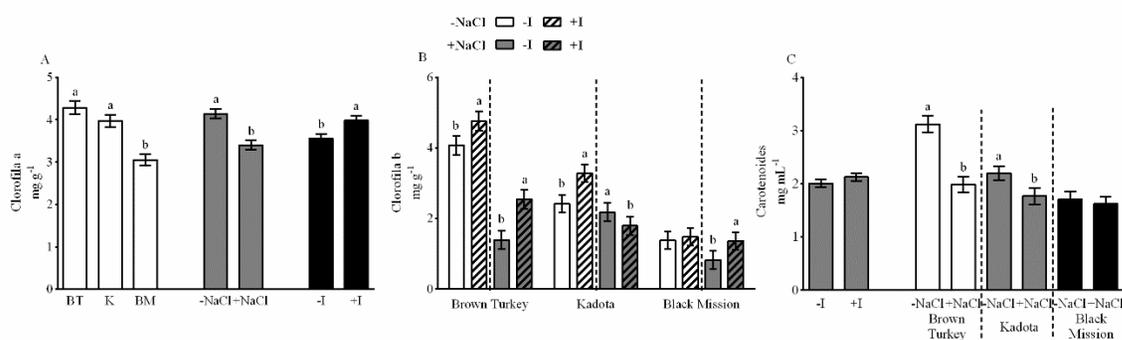


Figure 3. Effect of cultivars, NaCl and I on the concentration of chlorophyll a (A); chlorophyll b (B); and total carotenoids (C). The lines on the bars represent the standard error. The different letters on each bar mean that the treatments were statistically different (Tukey, $p < 0.05$).

Conclusions

The application of 10 mg L^{-1} of iodine in fig seedlings increased, in general terms, the relative content of chlorophyll, number and dry weight of new leaves, as well as the antioxidant activity and the concentration of chlorophylls, under salt stress condition. Therefore, iodine can be considered as an alternative to mitigate the stress caused by NaCl in *Ficus carica* L. However, in particular it is necessary to consider that there are differences in the impact of iodine between cultivars, so it is necessary to know the effect of the application of this element in species and varieties of specific interest.

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