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IODINE APPLICATION INCREASED ASCORBIC ACID CONTENT AND MODIFIED THE VASCULAR TISSUE IN *OPUNTIA FICUS-INDICA* L.

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Abstract

The objective of this study was to discern the effect of applying both iodide and iodate to *Opuntia ficus indica* irrigation. The effect of iodate (KIO_3, 10^{-4} M) and iodide (KI, 10^{-4} M) on plant growth, yield and morphology was studied. Experiments were carried in three samples under tunnel conditions. In the last sampling, iodine species (KIO_3, KI) caused a negative effect in biomass. The amount of ascorbic acid, however, was increased over 51% in both iodine treatments. Phosphorus (0.26%), iron (50 ppm), and magnesium (1402 ppm) increases were also observed with iodate treatment in the first sampling, and increases in potassium (46.8 ppm) were apparent in the second. Iodide treatment increased the amounts of copper (1.02 ppm) and manganese (32.80 ppm) in the first sampling. Iodate treatment modified the number of xylem vessels and increased both the mucilage area and amount of druses. In general this study shows that iodate increases the amount of ascorbic acid and the morphology of the vascular tissue.

Introduction

Nopal is a native plant of Mexico's semiarid regions. Its cropping surpasses 777, 413 t nationally and 7% is exported (SAGARPA, 2011). Interest in nopal production has increased in the last decades due to its diverse value as a vegetable (Pimienta–Barrios, 1994; Flores-Valdés *et al*., 1995; Rodríguez-Salazar & Nava-Cedillo, 1999), fodder (Rusell & Felker, 1987), substratum for exploiting cochineal (Tovar *et al.,* 2005), element of reforestation (Stintzing & Carle, 2005), biofuel (Varnero *et al.,* 1992), and as a medicinal resource (Feugang *et al*., 2006; Sher *et al.,* 2011). Moreover, it has multiple applications in agroindustry, pharmaceutics, biotechnology (Shedbalkar *et al.,* 2010), and industrial production. The consumers' demand for improving the nutritional, functional, organoleptic, and health related properties of nopal generates the requirement for better understanding of its physiology, agronomical handling, and postharvest technology (Flores-Hernández *et al*., 2004; Valdéz-Cepeda *et al.,* 2006; Robles-Ozuna *et al.,* 2007).

The functional properties of nopal refer to the amount of fiber, hydrocolloids, pigments, Ca, K, and ascorbic acid (Sáenz *et al.,* 2004). In this context, to achieve a higher nutritional and functional value, key environmental factors and necessary handling practices must be identified. Particularly, mineral nutrition provides great benefits for this crop. For *Opuntia ficus-indica*, existing work indicates the optimal concentrations, and critical and sufficient levels of essential mineral nutrients that improve biomass production (Magallanes-Quintanar *et al.,* 2005; Blanco-Macías *et al.,* 2006; Blanco-Macías *et al.,* 2011), but little is known about how other elements, such as iodine, affect its application. Iodine is a non-metallic element found in small amounts in vegetable tissues. Research conducted in the early 20th century claimed that iodine was not essential for plants (Brady & Weil, 1996); it was even found to be phytotoxic in sensitive species like corn, peas, chinese cabbage, and rice at doses higher than 10^{-3} M (Loué, 1988; Mackowiak & Grossl, 1999; Dai *et al*., 2004). The metabolic function of iodine in plants has not been elucidated (Benton-Jones, 1998), but it is known to be important in fertilization programs for biofortification (Dai *et al.,* 2004). Additionally, iodine is an inductor of pathogen and stress tolerance (Blasco *et al*., 2011).

To our knowledge, there is no literature on nopal addressing the effect of iodine application. The objective of this study was to determine the effect of iodine in the form of iodate and iodide in nopal nutrient solutions for cladode production using tunnel cultivation system. We additionally assess the chemical composition quality and amount of vitamin C.

Materials and Methods

This work was conducted in Municipio of General Cepeda Coahuila (25º 22' 58" North, 101º 27' 08" West and 1440 msnm), 50 km Southeast of Saltillo, Coahuila. Cladodes of *Opuntia ficus-indica* var. Copena V1 were planted in three tunnels with plastic cover caliber 600. The dimension for each tunnel was 50 m length x 3 m width. We incorporated 1300 cactus leaves per tunnel: each unit consisted of two beds with five columns and 130 rows. Iodine treatments were potassium iodate (KIO_3, 10^{-4} M), potassium iodide (KI, 10^{-4} M), and a water control.

Irrigation: Each treatment consisting of Steiner nutritional solution (Steiner, 1961) containing KIO_3 (10^{-4}M), KI (10^{-4}M)) or water control was irrigated independently.

Plant sampling: Nopal cladodes *Opuntia ficus-indica* var Copena V were used. When transplanted, all nopal plants had one stratum (1 mother cladode) and were allowed to grow to the second stratum. Data for determining productivity were obtained by harvesting and weighting the cladodes of the second stratum. We carried out chemical analysis of extracts from cladodes over 16cm long on twenty randomly chosen plants. The iodine effect on growth was calculated by counting the sprouting amount in the second stratum.

Quality of cladodes

Length, width and thickness of cladodes: Measurements were performed using a 30 cm ruler at the base of the cladode apex and middle of the cladode for length and width, respectively. Thickness was measured in millimeters from the middle section of the cladode with a vernier.

Weight: Fresh weight of each cladode was determined in grams with a mechanical balance (OHAUS). Dry weight was measure after drying samples placed in aluminum platters on an oven at 60ºC.

Color: Color and brightness parameters were analyzed with a colorimeter (Minolta CR-300) using ten cladodes per sample. Three independent measurements were obtained per cladode.

pH: The pH was measured in cladode parenchyma by inserting a potentiometer (Hanna) (Flores-Hernández, *et al.,* 2004) into a manually produced incision.

Total soluble solids (ºBrix): 30 g of sample was homogenized and placed in a refractometer (Atago) at a 0-30º range. Results were expressed as a percentage of soluble solids (Anon., 2000).

Firmness: Resistance to penetration was determined with a compact digital penetrometer Gauge (MECMESIN EUA). The measurement was taken in the central section of the cladode and registered in kilograms (kg).

Cladode chemical composition: Samplings were performed in cladodes of the second stratum on August 20, September 24, and October 23 of 2009. Five cladodes were collected per treatment in each sample. Harvested cladodes were placed in aluminum platters inside an oven at 80°C until constant weight was obtained. The dried material was finely ground in a porcelain mortar and stored in glass containers. Organic matter, raw protein (Kjeldahl), ether extract, raw fiber, and ashes were analyzed (Anon., 2000).

Ascorbic acid quantification: The amount of ascorbic acid was determined through titration. 10 g of fresh sample was weighted and grounded in a mortar with 10 ml of 2% HCl. Sample was then filtered and brought to 100 ml with deionized water. A 10 ml aliquot was labeled as 2,6-dichlorophenolindophenol (0.001 N) solution.

Mineral content of cladodes: Minerals were determined based on dry weight. Sample was subjected to a mineralization process with perchloric and nitric acids (3:1) in a heating plate at 100ºC, after which the solutions were filtered and diluted to 100 ml with deionized water. Minerals Zn, Cu, Fe, Mn, Mg, K, and Ca were analyzed through humid digestion using an atomic absorption spectrophotometer (Varian 1275). Sulfur content was determined by turbidimetry with 10% barium chloride ($BaCl_2$) and glycerin by colorimetry at an 800 nm wavelength. Phosphorus content was determined through colorimetry at 640 nm.

Histological characterization: For each treatment, second stratum cladode samples were taken at four weeks of growth. Cross sections of 0.5 cm thickness were made in the middle, apical and basal sections. Tissue samples were fixed with FAA (alcohol: acetic acid: formalin; 85:5:10). Histological analysis was performed by techniques described by Sass (1958) on both cladode sides. Epidermal tissue was prepared by placing a thin, homogeneous layer of adhesive PVC plastic on the cladode surface. The adhesive film was removed with pincers, and the samples were finally placed on slides.

Observations and measurements: To generate anatomical descriptions, features of vascular tissues were observed and recorded. Idioblasts and crystals were also described. The number of xylem vessels, thickness of walls, and area of vessel lumen were determined from preparations of cross cut sections. Mucilage cells and druse distribution were also assessed. All measurements were performed with an optical image analyzer Axion Vision 4, and pictures were taken with a Pixera PVC 100 C model digital camera.

Data analysis: Groups were compared using analysis of variance, and a Tukey means comparison test was performed with a significance level of ($p \leq 0.05$) via R® version 2.8.1. (2008).

Results and Discussion

Iodide and iodate at 10^{-4}M application was studied in nopal (*O. ficus-indica* L.) to determine their effect on yield and physiological related traits.

Yield: Table 1 represents data for cladode sprout number, yield, length wide, thickness, fresh and dry weight. Sprout number was less than reported by Vázquez-Alvarado *et al.,* (2009) under hydroponic conditions. Our yield was not significant between treatments and control, but was higher than the value reported by both Ruiz-Espinoza *et al.,* (2008) in cultivating CEN-1 and by Vázquez-Alvarado *et al.,* (2009) under hydroponic and salinity conditions.

Table 1. Effect of iodate and iodide on physiological parameters of *Opuntia ficus indica* var. Copena V1.

	Sample 1			Sample 2			Sample 3		
	Control	KIO_3	KI	Control	KIO_3	KI	Control	KIO_3	KI
Cladode sprouts	3.60	3.60	3.80	4.20	4.40	4.00	4.40	4.20	3.80
Yield (kg,m^2)	7.70	7.61	9.09	7.47	7.46	8.46	7.47	7.33	8.10
Length (cm)	18.24	19.20	17.80	21.02	20.60	20.02	22.96	19.10	19.7
Width (cm)	12.70	13.20	12.02	11.56	11.48	11.27	10.70	9.54	9.74
Thickness (mm)	2.07	1.93	1.83	1.14	1.18	1.11	1.06	1.00	1.02
Fresh weight (g)	316.38	333.48	275.98	177.36	163.12	151.46	173.02 a	106.06b	115.88b
Dry weight (g)	7.04	7.16	7.46	7.52	7.78	7.16	7.88 a	5.09 b	5.38 b

Measurements with same letters are not statistically different (Tukey, 0.05);* significative

Quality of cladodes: Only the third sampling of the treatment group showed a decrease in dry sampling weight compared to control. A decrease in biomass of iodine treated plants has been documented in crops (Huang *et al.,* 2003; Ozyigit, 2012) under hydroponic growth (Hageman *et al.,* 1942; Weng *et al*., 2008a; Blasco *et al*., 2008) or soil cultivation (Hong *et al*., 2008; Weng *et al.,* 2008b). According to Muramatsu *et al*., (1989) this is due to the iodine concentration used. Meanwhile, Blasco *et al*., (2008) connected decreasing biomass in lettuce plants under hydroponic growth with a decrease in photosynthetic rate. Biomass is a parameter related to photosynthesis. *Opuntia ficus-indica* is a plant with constitutive CAM metabolism (Borland, 1996), but young cladodes upregulate a C_3 metabolism during their development, requiring more water from basal cladodes (Wang *et al*., 1997). During daytime, a stomatal opening occurs in young cladodes (Osmond, 1978). Additionally, under conditions of irrigation, stomata remain open more frequently (Hanscom & Ting, 1978), causing a higher demand for water. This causes a physiological drought, resulting in an assimilation of CO_2 in shade and a loss of chlorophyll (Pimienta-Barrios *et al*., 2007). Biomass loss in the last sample can be related to a residual effect of iodine on soil when iodine-organic material complexes forms (Yamaguchi *et al*., 2010). Dai *et al*., (2004) mentions such an effect by applying iodate on soil at a concentration of 5mg/kg. This suggests the importance of controlling application of iodine through fertilizers, given the soil diversity and the environmental conditions.

Color: No significant difference was detected in the color of cladodes between treatments (Table 2). However, the values-a and b did not match those reported by Rodríguez-Félix and Villegas-Ochoa (1998) for this plant. These variables are related to the amount of chlorophyll and can be modified by degrading it (Heaton & Marangoni, 1996). For variable L, the values shown in Chart 2 are similar to those of Rodríguez-Félix *et al.,* (2007).

pH and total soluble solids: A minor difference in the cladode pH was found when the first sample was treated with potassium iodate. No differences were observed in the last two samples or in total soluble solids (Table 3). Soluble solids, pH, and titrable acidity are related to taste and consumer preferences (Bosquez, 1992; Aguilar-Sánchez *et al.,* 2007). Total soluble solid variables hereby reported are lower than those reported by Aguilar-Sánchez *et al.,* (2007). Additionally, these variables are related to polyphenoloxidase activity. Polyphenoloxidase oxidizes phenolic compounds showing little activity at pH 4.5 and this enzyme is inactive at any pH lower than 3 (Whitaker, 1994; Whitaker & Lee, 1995). Polyphenoloxidases are responsible for darkening fresh products and for deteriorating young cladodes (Whitaker &. Lee, 1995).

Firmness: No difference in cladode firmness between treatments was detected.

Chemical composition analysis: Results from bromatological variables show a significant difference in nitrogen composition in two samples. However, these results do not exhibit consistency (Table 4). No significant differences were found in remaining variables such as organic matter, lipids, fiber and ashes.

The amount of ashes is greater than the number found by Rodríguez-Félix and Catwell (1988). However, the lipids (2.7%) and nitrogen content (10.3%) is less than the reported for this specie at this development stage (Rodríguez-García *et al.,* 2007; Ramírez-Tobías *et al.,* 2010).

Table 2. Effect of iodate and iodide on color parameters of *O. ficus-indica* var. Copena V1 at harvest time.

	Sample 1			Sample 2			Sample 3		
	Control	KIO_3	KI	Control	KIO_3	KI	Control	KIO_3	KI
L	45.62	44.68	43.71	45.04	45.11	43.81	44.15	44.44	44.29
-a	15.64	15.86	15.46	15.44	15.31	15.09	14.89	15.62	15.47
B	23.06	23.62	22.45	23.65	23.08	23.29	27.78	21.87	22.02

n= 5

Table 3. Effect of iodate and iodide on postharvest parameters of *O. ficus-indica* var. Copena V1.

	Sample 1			Sample 2			Sample 3		
	Control	KIO_3	KI	Control	KIO_3	KI	Control	KIO_3	KI
pH	4.28 b	4.43 a	4.33 ab	4.35	4.46	4.38	4.49	4.47	4.35
ºBrix	1.62	1.66	1.70	1.68	1.68	1.66	1.72	1.64	1.68
Firmness(kg)	2.39	2.77	2.44	2.33	2.52	2.47	2.40	2.52	2.49

Measurements with same letters are not statistically different (Tukey, 0.05);* significative ; n= 5

Table 4. Bromatological parameters and amount of vitamin C in *Opuntia ficus-indica* var. Copena V1 cladodes under KIO_3 and KI treatment.

	Sample 1			Sample 2			Sample 3		
	Control	KIO_3	KI	Control	KIO_3	KI	Control	KIO_3	KI
% Organic material	63.16	68.93	66.87	71.60	70.30	69.10	68.20	81.00	77.70
% Ether extract	2.40	2.38	1.94	1.61	1.40	1.50	2.10	2.87	2.04
% Raw fiber	11.88	11.56	11.36	11.59	10.62	10.69	12.87	15.40	11.61
% Nitrogen	1.79 b	2.15 a	1.94 ab	2.14 a	1.72 b	2.03 ab	1.88	1.97	2.21
% Ashes	28.58	25.00	27.20	25.20	22.61	20.91	23.9	20.68	24.12
Vitamin C (mg/100g)	19.70b	35.90a	35.90a	17.50b	37.60a	37.60a	20.80b	37.40a	39.10a

Measurements with same letters are not statistically different (Tukey, 0.05);* significative; n= 5

Table 5. Mineral content in *Opuntia ficus-indica* var. Copena V1 samples after iodine applications through fertilizing irrigation.

Minerals	Sample 1			Sample 2			Sample 3		
	Control	KIO_3	KI	Control	KIO_3	KI	Control	KIO_3	KI
S (%)	0.30	0.19	0.16	0.14	0.17	0.30	0.16	0.16	0.12
P (%)	0.16 b	0.26 a	0.20 b	0.25	0.15	0.19	0.26	0.27	0.12
Zn (ppm)	47.00	39.40	38.20	28.80	34.20	38.60	37.80	41.80	34.00
Cu (ppm)	0.86 b	0.84 b	1.02 a	1.10	1.10	1.20	1.00	1.54	0.96
Fe (ppm)	36.20 b	50.00 a	45.20 ab	48.40	54.60	44.00	38.80	37.20	29.80
Mn (ppm)	22.60 b	30.60 b	32.80 a	17.8	14.6	13.8	17.60	13.60	13.80
Mg (ppm)	890 b	1402 a	760 b	862 b	1760 a	630 b	728	592	468
K (ppm)	22.80	24.20	19.60	23.20 b	46.8 a	17.00 b	27.80 a	25.80 a	19.60 b
Ca (ppm)	1456	1208	908	1616	1142	816	2270	1784	1370

Measurements with same letters are not statistically different (Tukey, 0.05);* significative; n= 5

Vitamin C: The amount of vitamin C increased more than 45% with I^- and IO_3^- treatments at 10^{-4}M. Weng *et al.,* (2008a) mentioned a 22-40% increase in vitamin C concentration in lettuce when iodine (I^-) was applied at 0.05-0.1 mg L^{-1}, whereas uptake of IO_3^- and CH_2ICOO^-, however, vitamin C concentrations were below the control level. Blasco *et al.,* (2011) mentioned iodate capacity to induce plant antioxidant accumulation. Ascorbic acid, participates in H_2O_2 detoxification, the growth process regulation and xylem lignification (Antonova *et al.,* 2005). Ascorbic acid concentration is affected by environmental conditions (Antonova *et al*., 2005; Zhang *et al.,* 2007). In CAM plants, carboxylates function as energetic reservoirs. Some cyclic CAM plants tend to accumulate more ascorbic acid than malate due to lower energetic costs (Borland *et al.,* 1994).

Mineral amount in the cladodes: Mineral amount results obtained (Table 5) showed a significant difference in certain elements such as P, Fe, Mg, and K for iodate, and Cu and Mn for iodide. Fraps and Fudge (1939) mention a positive correlation between phosphorus and iodine found in Texas soil. Hageman *et al.,* (1942) studied an increase in magnesium concentration (until 1.36%) in tomato leaves and stems when they are treated with an iodide solution at 100 mg/kg (7.8 x 10^{-4} Molar). Iodide also stimulates absorption of Cu and Mn (Hageman *et al.,* 1942). Higher amount of Cu is related to oxidative stress (Azmat & Khan, 2011) and this could be correlated with a higher amount of ascorbic acid found in this study.

Mechanisms that facilitate iodine absorption by plants have not yet been elucidated. In algae, iodine is oxidized and enters the cell through facilitated diffusion (Küpper *et al*., 2008), and in land plants, authors suggest iodate is reduced to I^- for internal plant localization (Umaly & Poel, 1971; Whitehead, 1975; Blasco *et al.,* 2008). Differential absorption of minerals under iodate and iodide treatment suggests a transport mechanism mediated by transporters, as it occurs with selenium, that are moved as selenite ions or selenium in organic compounds (Zayed *et al*., 1998; Broadley *et al*., 2006; Zhao *et al*., 2010), sulfates (Terry *et al*., 2000) and silicon (Zhao *et al*., 2010).

Histological characterization: The analysis of variance showed significant differences for the number of xylem vessels, the increase in idioblasts, and density of druses in both iodine treatments. This was not the case for all other variables (Table 6). Our results differ from those at the morphological level performed by Pimienta-Barrios, *et al.,* (2003). Arnold and Mauseth (1999) mention stability in the morphogenetic mechanisms present in the xylem development (Fig. 1). Grouping and increasing the number of xylem vessels improves conductivity and security in transporting water so that when a vessel is obstructed, conduction through an adjacent vessel is established (Clarquist, 1984; Gibson, 1996; Hacke *et al*., 2006), Also the presence of more narrow diameter vessels constitutes a response to a water deficit (Laskowski, 2000). Increase in idioblast area (Fig. 2) relates to the increase in mucilage, and the amount and composition of mucilage varies with age and species (Trachtenberg & Fahn, 1981; Terrazas & Mauseth, 2002). It is interesting to point out that, in xylem development, the substrates for ascorbic acid formation are galacturonic and uronic acids, two compounds that form the mucilage. The density of druses we report surpasses the value reported by Tovar-Puente *et al.,* (2007). The function of calcium oxalate crystals has not yet been elucidated, but these crystals have been proposed to function in sequestration and maintenance of calcium levels within plants and as deterrents against herbivory (Jáuregui-Zuñiga & Moreno, 2004). Our data suggests that applying iodine highlights the adaptive capacity of plants when facing oxidative metabolic stimuli.

Table 6. Histological variables at four weeks post iodine treatment on *Opuntia ficus-indica* var. Copena V1.

Morphological character	Control	KIO_3	KI
Density of stomata (mm^2)	10	10	10
Length of stomata (µm)	29.18	37.34	36.4
# Druses.mm-^{2}	40.60 b	68.00a	72.60a
# vessels/vascular bundle	10.00 b	17.60 a	11.40 b
Area of vessels (μm^2)	306.92	466.45	230.60
Vessel wall thickness (µm)	2.40	2.81	2.54
Idioblasts area (μm^2)	946.35 b	1222.32 a	1512.76 a

Measurements with same letters are not statistically different(Tukey, 0.05);* significative;n=5

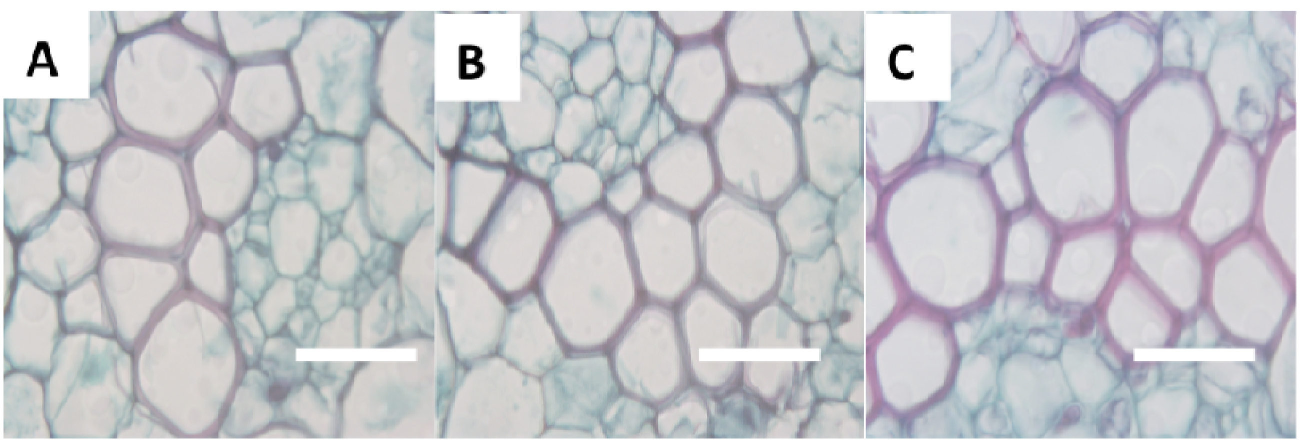

Fig. 1. Xylem vessels in cladodes of *Opuntia ficus- indica* under treatments A= Control, B= Iodate, C=Iodide with objective of 40X. Bar is equal to 100µm.

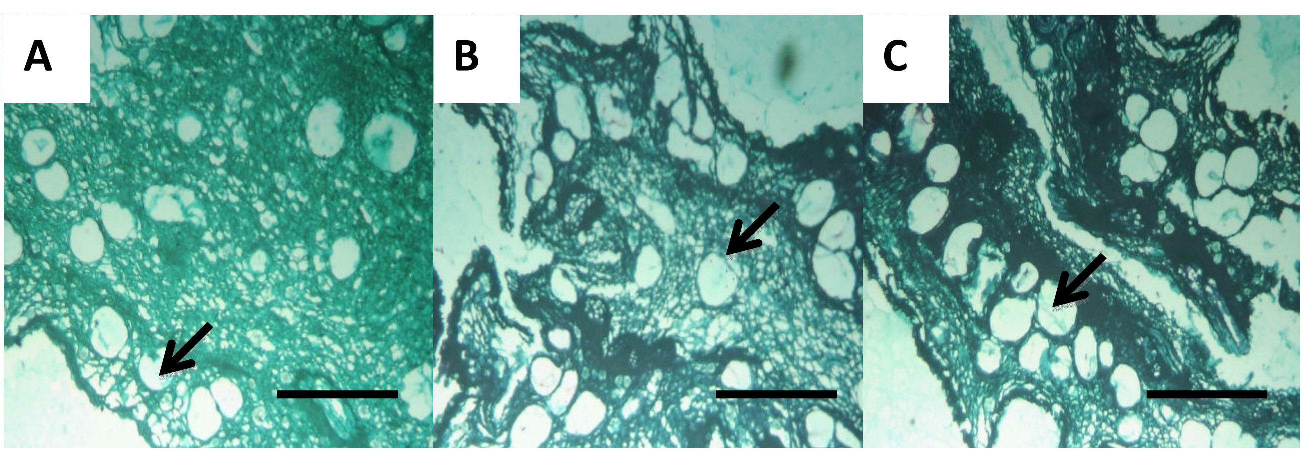

Fig. 2. Cross cuts in *Opuntia ficus-indica* taken with a 10x objective, showing the mucilaginous matrix (arrow) in the different treatments: A= Control, B= Iodate, C=Iodide. Bar represents 1000 µm.

Conclusions

Both iodate and iodide application at 10^{-4} M through irrigation resulted in an increase in the amount of P, Mg, Fe, K, Cu, and Mn (62.5%, 57.5%, 38.12,101.7, 18.6, 45.13%, respectively) as well as increasing ascorbic acid above 5%. Both fresh weight and dry weight were negatively affected by treatment in the last sample. Lastly, the xylem vessel number increased with iodide treatment and an increase in mucilage cell area and druse amount in both iodate and iodide applications were observed.

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