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EFFECT OF ANTIOXIDANTS ON CITRUS LEAF ANATOMICAL STRUCTURE GROWN UNDER SALINE IRRIGATION WATER

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Abstract

Citrus trees have been classified as a salt sensitive crops, to increase plant tolerance, antioxidants foliar application as ascorbic acid at four concentrations (0, 400, 600 and 800 ppm) and or salicylic acidat four concentrations (0,400,600 and 800 ppm). 800 ppm was the best evaluated and chosen concentration to study the anatomical structure of both Valencia orange and Chinese tangerine budded on Volkamer lemon rootstock grown under saline conditions. The experiment was conducted at El Alamin – Wady El Natroun high way (Beer Hooker Area) in a private orchid in a sandy soil under drip irrigation system with saline water (1700 ppm). The results indicated that, under saline conditions in both varieties Valencia orange and Chinese tangerine the upper leaves epidermis, thickness of lamina, leaf blade through midrib area, were increased compared with the treated plants with either salicylic or ascorbic acids. On the other hand, the treated trees showed a decrement effect on upper epidermis, thickness of lamina as well as blade through midrib area. However ascorbic acid with 800 ppm was more effective in this respect, the cross sections showed also that, length and width of vascular bundles as well as xylem rows showed apparent increase compared with untreated trees. In this respect ascorbic acid was more effective than salicylic acid.

***Keywords*:** Citrus, Rootstock, Ascorbic acid, Salicylic acid and anatomical structure.

Introduction

Citrus grown in many areas of the world. It's a subtropical crop that's not tolerant to freezing temperature or salinity. According to the United Nations of food and agriculture (FAO, 2016) there were 140 citrus producing countries, producing 115,650,545 tons around 70% of the world's total citrus production is grown in northern hemisphere, in particular countries around the Mediterranean and United Nations. Even when Citrus irrigated with water of good quality, the use of fertilizers and other agro-chemicals raises the likelihood of salt building up in the soil causing salinity stress (Syvertsen *et al*., 1995). Relative to many crop plants, citrus trees have been classified as a salt sensitive crop (Story and Walker, 1999), because saline irrigation water reduced citrus tree growth and fruit yield relatively more than in many other crops (Grieve *et al*., 2007).

Ascorbic acid is one of non-enzymatic molecule which plays a substantial role in counteracting oxidative stress caused by stresses. It is a small, water soluble antioxidant molecule which act as a primary substrate in the cyclic pathway for enzymatic detoxification, it acts directly to neutralize superoxide radicals, signal oxygen or superoxide as a secondary antioxidant during reduction cycling of the oxidized for α tocopherol which another lipophilic antioxidant molecule (Noctor and Foyer, 1998). Ascorbic acid has been associated with several types of biological activities in plants such as enzyme Co-Factor and as a donor/acceptor in electron transport in plasma membrane or in chloroplast. Much evidence has suggested that ascorbic acid effects biosynthesis, level and signaling of many phytohormones including ethylene, gibberellic acid acid and abscisic acid (Barth *et al* ., 2006), all of which are related to oxidative stress resistance Conklin (2001), Hussein and Alva (2014) Çavuşoğlu and Bilir (2015), El-Sayed and Abdel-Rahman *et al*. (2015), Alhasnawi *et al*. (2015) and Kostopoulou *et al.* (2015).

Salicylic acid (SA) or orthohydroxy benzoic acid is distributed in whole plant kingdom. Salicylic acid is considered to be protect plant hormone (Raskin, 1992) because of its divers regulating roles in plant metabolism Popova, *et al*. (1997). Salicylic acid is an endogenous plant growth regulator of phenolic nature that possesses an aromatic ring with hydroxyl group or its functional derivate. The effect of exogenous salicylic acid on growth and bio productivity and might play a key role in regulating their growth and productivity. The main objective of the present study is to study the effect of antioxidants salicylic and / or ascorbic acid on the anatomical structure of Valencia orange and Chinese tangerine budded three citrus rootstocks under saline irrigation water.

Materials and Methods

In this work two filed experiments separately were carried out during two seasons to evaluate the effect of foliar application of salicylic and ascorbic acids on leave anatomical structure of Valencia orange and Chinese tangerine, budded on three rootstocks namely sour orange (*Citrus aurantum, mion*), Volkamer lemon (*Citrus volkameriana*, Tem) and X639 hybrid between (*Cleopatra mandarin* × *Ponicurus trifoliata*) under saline irrigation water. The experiment was conducted at El Alamin – Wady El Natroun high way (Beer Hooker Area) in a private orchid in a sandy soil under drip irrigation system with saline water (1700 ppm).

Soil and Water physical and chemical analysis were carried out according to the standered procedure described by Jackson (1958);

Table 1: Physical Properties of the tested soil:

Organic matter %	Soil particle size distribution				
	Sand (%)	Silt (%)	Clay (%)	Soil Texture	Field capacity (%)
0.18	94.4	2.0	3.6	Sandy	20.2

Table 2 : Chemical Properties of the tested soil:

pH	E.C dsm^{-1}	Saturation soluble extract 1:5							
		Soluble anions (mg/L.)				Soluble cations (mg/L.)			
		CO^-_3	HCO^-_3	SO^-_4	Cl^-	Ca^{++}	Mg^{++}	Na^+	K^+
7.20	1.50	0.0	2.4	1.2	4.4	2.0	1.4	4.1	0.5

Table 3 : Macro and micro elements contents of the tested soil:

Depth	Available macronutrients (mg /100g)			Available micronutrients (ppm)			
	N	P	K	Fe	Mn	Zn	Cu
0-30	30	`5.12	18.90	5.38	0.59	0.43	0.74

Table 4 : Chemical Properties of irrigation water**:**

pH	E.C dsm^{-1}	Saturation soluble extract							
		Soluble aions (meq/L.)				Soluble cations (meq/L.)			
		CO^-_3	HCO^-_3	SO^-_4	Cl^-	Ca^{++}	Mg^{++}	Na^+	K^+
7.2	2.80	-	2.8	4.6	7.5	5.0	3.2	6.5	0.2

The selected antioxidants materials namely ascorbic and salicylic acid were used in the experiments as foliar spray twice in both seasons (2015 and 2016). The first foliar application at 15^{th} of June and the second foliar application was applied 15 days later. The foliar application was conducted at the early morning using back sprayer (25 Liter). The volume of the spraying solution was maintained just to cover completely the seedling until drop the selected antioxidant materials were used as Follows**: -**

Tap water (control)

Salicylic acid (SA)

It is a poorly soluble in water, thus it was soluble in ethyl alcohol and then in distilled water at four concentrations (0,400,600 and 800 ppm).

Ascorbic Acid (ASA)

It was diluted in distilled water and used at four concentrations (0,400,600 and 800 ppm). The leaf samples at 800 ppm was selected anatomical tests. Samples were taken after 39 months from leaves to evaluate the effect of antioxidants under saline water irrigation on the anatomical structure of leaves of both varieties.

For anatomical study, samples were taken from the middle of sixth leaf from apex. Samples were killed and fixed in FAA solution (90 ml 95% ethyl alcohol + 5 ml formalin + 5 ml glacial acetic acid) for 48 hours. Thereafter, samples were washed in 50% ethyl alcohol, dehydrated and cleared in tertiary butyl alcohol series, embedded in paraffin wax of 54-56 °C m.p. Cross sections, 20µ thick, were cut by a rotary microtome, adhesive by Haupt's adhesive and stained with the crystal violet-erythrosin combination, cleared in carbolxylene and mounted in Canada balsam. The sections observed and documented using an upright light microscope (AxioPlan, Zeiss, Jena, Germany). Measurements were carried out, using a micrometer eyepiece and average of 5 readings were calculated.

Results

Leaf anatomical structure of Valencia orange young tress

Table (5) Showed leaf anatomical structure of Valenciaorangeyoung tress budded on Volkamer lemon rootstock grown under saline water irrigation and sprayed with Salicylic acid and Ascorbic acidat 800 ppm. had been selected for evaluation. Thickness of upper epidermis, thickness of lamina, thickness of midrib, length of vascular bundle, width of vascular bundle and number of xylem were studied. The cross section of Valencia orange showed that, both salicylic and ascorbic acids showed different change in upper epidermis thickness compared with the control where the decreased in upper epidermis thickness by Salicylic acid was 11.16 % compared with control while in case of ascorbic acid the decrease reached 11.15%. As for thickness of lamina the highest decrement compared with the control achieved by ascorbic acid reached 26.865 %, in addition the thickness of leaf blade through mid-rib area, antioxidants were more effective in increasing mid rib thickness, the increment over the control young tress reached 85.94% under saline conditions .

Concerning length and width of vascular bundles showed apparent decrease in control young tress, with Salicylic acid application the increment reached 47.94% over the control, as for length of vascular bundle over the control young trees and 58.95% for width of vascular bundle over the control young tress. Xylem row number increased in volkamer lemon rootstock affected by salicylic acid at 800 ppm by 75, while the same rootstock recorded 72 by ascorbic acid at 800 ppm compared with control young trees which recorded 35.

Ascorbic acid was more effective in this concern. Our results showed increment in number of xylem rows in respect to antioxidants treatments, the highest increments reached 32.85% over the control in response to Salicylic acid treatment.

Those results agree with reported by these results agreed with those reported by Sakhabutdinova *et al.* (2003) indicated that Treatment of wheat plants with 0.05mMSalicylic acidSA increased the level of cell division within the apical meristem of seedling roots which caused an increase in plant growth. Abou-Leila *et al.* (2012) on *Jatropha curcus* plant declared that foliar application of ascorbic acid interacted with salinity caused increment in number of xylem row and number of vessels/bundle but had no effect on the number of bundles of the stem. Rania (2013) found that foliar application with 450 ppm ascorbic acidinduced prominent increase in thickness of both mid vein and lamina of leaflet blades of mung bean cv. Kawny1. It was found that the thicker lamina induced by ascorbic acid was mainly due to increase induced in thickness of both palisade and spongy tissues. Also, the main vascular bundle of the mid vein was increased in size as a result of spraying ascorbic acid. Agami (2014) showed that ascorbic acidASC presoaking seeds at 1mM for barley plants caused a positive change in leaf anatomical structure, the thickness of midvein, blade, mesophyll, adaxial and abaxial epidermis, and average diameter of vessels were in the absence or presence of the NaCl stress. Çavuşoğlu and Bilir (2015) reported that in both the upper and lower surface in comparison with the C seedlings grown in 0.25 M salinity while it decreased the epidermis cell width, stomata length and index in both surfaces. ASC application reduced the leaf thickness, but it stimulated distance between vascular bundles. In addition, it statistically demonstrated the same values as the on the epidermis cell length in both surfaces. Although this pretreatment caused a decrease on the stomata width in the upper surface, it had no effect on this parameter in the lower surface. In 0.275 M salinity, ASC pretreatment reduced the epidermis cell length, stomata index, leaf thickness and distance between vascular bundles.

Table 5: Effect of Foliar application of Salicylic acid and Ascorbic acid at 800 ppm on leaf anatomical structure of Valencia orange budded on Volkamer lemon root stock grown under saline conditions**.**

Leaf Anatomy Components	Control Volkamer	Salicylic acid 800ppm Volkamer	Ascorbic Acid 800ppm Volkamer
Upper Epidermis Thickness (μm)	11.949	9.381	9.539
Lamina thickness (μm)	270	379,255	367,76
Midven thickness (μm)	860.550	950.452	959.62
Length of Vascular Bundles (μm)	480.796	640.452	610.331
Width of Vascular Bundles (μm)	590.982	820.692	780.795
No. of xylem rows	35	75	72

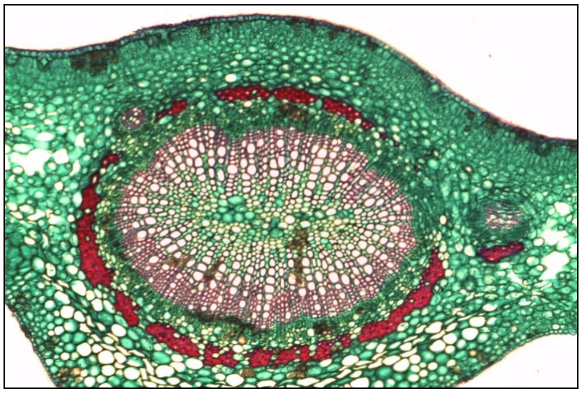


Fig. 1 : Transmission electron microscope for the effect of Foliar application of Ascorbic acid at 800 ppm on leaf anatomical structure of Valencia orange budded on Volkamer lemon root stock grown under saline conditions**.**

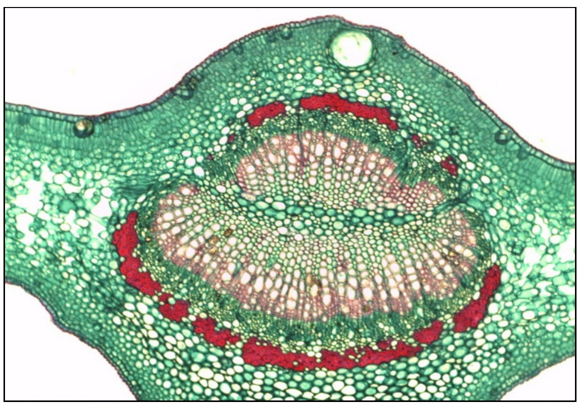
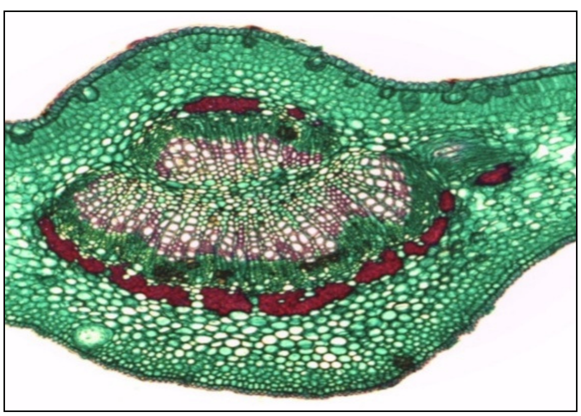


Fig. 2 : Transmission electron microscope for effect of Salicylic acid at 800 ppm on leaf anatomical structure of Valencia orange budded on Volkamer lemon root stock grown under saline conditions.



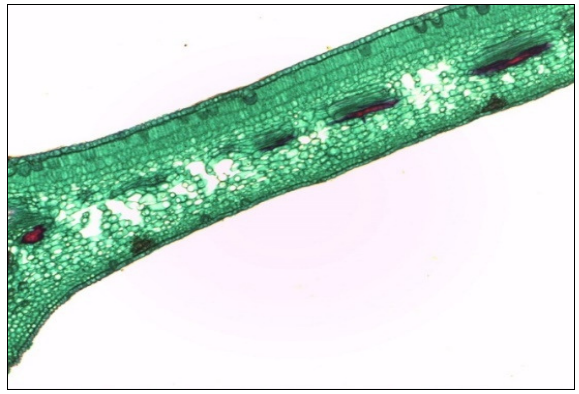


Fig. 3: Transmission electron microscope for control treesfor leaf anatomical structure of Valencia orange budded on Volkamer lemon root stock grown under saline conditions**.**

Leaf anatomical structure of Chinese tangerine young tress:

Table (6) showed effect of foliar application of ascorbic and Salicylic acids by and their interactions on leaf anatomical structure of young trees for Chinese tangerine young trees budded on (Volkamer Lemon as the best rootstock) gown under saline conditions.

Leaf anatomical structure such as thickness of upper epidermis of lamina, thickness of mid rib, length of vascular bundle, width of vascular bundle and number of xylem were studied for leaf of Chinese tangerine trees.

Cross section of Chinese tangerine leaves showed that, there were changes in leaf anatomical characters induced by both antioxidants application and the control young trees. Application of either salicylic and ascorbic acids showed a decrement in epidermis thickness compared to the untreated young trees, whereas application of Salicylic acid decreased thickness of epidermis by about 9.21% while for ascorbic acid reached by about 9.34%.

As for thickness of lamina salinity decreased it, while young tress treated with ascorbic acid were more favorable than Salicylic acid in increasing thickness of lamina, the increment reached 26.63% while the increment for Salicylic acid reached 21.89%.

In addition, the thickness of leaf blade through mid-rib area also decreased under saline conditions. Antioxidants application was effective in increasing mid rib thickness over the untreated young tress, the increments were relatively equal where Salicylic acid gave 65.94% and ascorbic acid gave 65.93% over the control young tress.

Moreover, under saline conditions the length and width of vascular bundle decreased compared with the foliated young tress. Salicylic acid increased length and width of vascular bundles 27.92% and 38.91% respectively over the control young tress.

As for ascorbic acid was more effective in this concern, the increment in both length and width of vascular bundle reached 27.89% and 38.89% respectively over the control young tress.

Thus its means that the increase in length and width of vascular bundle resulted in increasing the accumulation of necessary water required for photosynthesis.

Ascorbic acid application to salinized young tress was effective in increasing number of xylem rows reached 30.50% over the control plants for ascorbic acid compared with 30.595 for Salicylic acid. Xylem row number increased in Chinese tangerine affected by ascorbic acid at 800 ppm followed by salicylic acid at 800 ppm compared with control young trees.

These results agreed with those reported by Sakhabutdinova *et al.,* (2003) indicated that Treatment of wheat plants with 0.05mMSalicylic acid SA increased the level of cell division within the apical meristem of seedling roots, which caused an increase in plant growth.

Table 6 : Effect of Foliar application by Salicylic acid and Ascorbic acid Leaf anatomical structure of Chinese tangerine budded on Volkamer lemon rootstock under saline conditions season 2017.

Leaf Anatomy Components	Control Volkamer	Salicylic acid 800ppm Volkamer	Ascorbic Acid 800ppm Volkamer
Upper Epidermis Thickness (µm)	12.330	10.210	10.340
Lamina thickness (µ)	220.120	255.202	267.310
Midven thickness (µ)	660.550	780.404	782.330
Length of Vascular Bundles (µm)	280.796	440.452	510.239
Width of Vascular Bundles (µm)	390.170	415.540	460.720
No. of xylem rows	32	45	48

Fig. 4: Effect of Foliar application by Ascorbicacidon Leafanatomical structure of Chinese tangerine budded on Volkamer lemon rootstock under saline conditions season 2017.

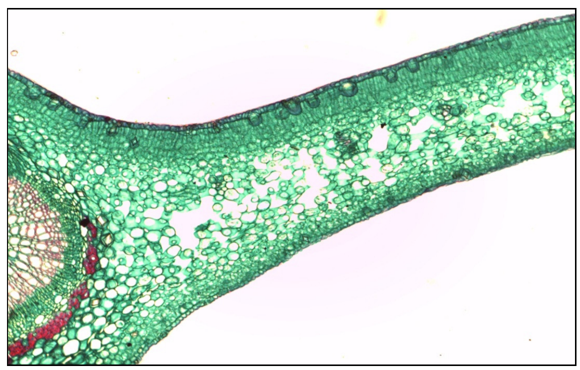
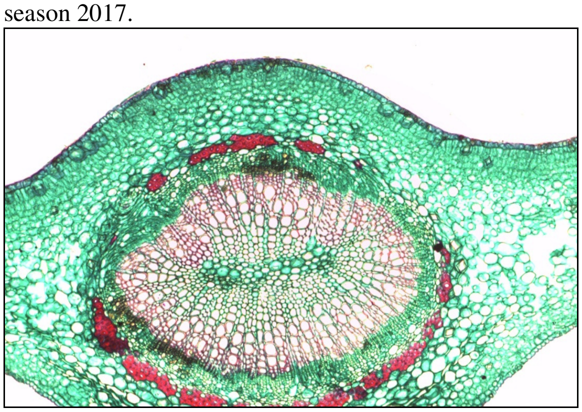


Fig. 5: Effect of Foliar application by Salicylic acid on Leaf anatomical structure of Chinese tangerine budded on Volkamer lemon rootstock under saline conditions season 2017.



Conclusion

It could be recommended that: the treated trees showed a decrement effect on upper epidermis, thickness of lamina as well as blade through midrib area .However ascorbic acid with 800 ppm was more effective in this respect, the cross sections showed also that, length and width of vascular bundles as well as xylem rows showed apparent increase compared with untreated trees. In this respect ascorbic acid was more effective thansalicylic acid.

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