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Alleviation Harmful Impacts of Salinity Using Some Antioxidants Substances on Thompson Seedless Grapevines Seedlings

Seham A. Ibrahim[1] and Thoraua S. A. Abo-ELwafa[2]
[1]Agric. Botany Dept., Fac. Agric., Zagazig Univ., Egypt.
[2]Viticulture Dept. Hort. Res. Inst., Agric. Res. Center, Giza, Egypt.

ABSTRACT

This work was conducted during two successive growing seasons of 2016 and 2017 in the shade house of the Viticulture Research Department, Horticulture Research Institute, Agricultural Research Center, Giza, Egypt, to evaluate the effect of foliar application of ascorbic acid at 400 ppm and salicylic acid at 200 ppm on vegetative growth, physiological characters and endogenous bio constituents, as well as anatomical structure of the leaf blade of the Thompson seedless grape rootings grown under three levels of salinity stress (1000, 2000 and 3000 ppm). The results indicated that, all parameters studied of vegetative growth of rootings significantly decreased by increasing salinity stress levels,in addition to significant decrease in photosynthetic pigments content comparison with untreated vines.In contrast, salinity stress significantlyincreasedproline, sodium, total phenols compoundand oxidative enzymes activity in leaves. Foliar spray with ascorbic acid (AA) gave the best results followed by salicylic acid (SA) to alleviate and overcome the harmful impacts of salinity stress on the aforementioned characters of both growing seasons compared to control vines. Moreover, irrigated plants with NaCl saline solution had considerable decrease in anatomical characters of Thompson seedless grape leaf. Treating rootings with ascorbic acid application at 400 ppm increased all anatomical features in comparison with control vines under salinity stress condition.

Keywords: Thompson seedless vines, ascorbic acid, salicylic acid, NaCl saline solution,endogenous bio constituents, leaf anatomy.Seedlings

INTRODUCTION

Grapevines (*Vitisvinifera* L.) are considered one of the most important fruit cropsfor local consumption and exportation in Egypt and all over the world. In Egypt, cultivation area of grapevines has developed progressively in the last decade especially in the newly reclaimed lands. Also, it takes up the second placement from the area of fruit trees planted after citrus trees and because of its precious features. In 2016, the total area of grapes reached 192,873 feddans, among them about 164,410 feddans fruitful with a total production about 1,434,686 tons according to latest the statistics of the (Ministry of Agriculture, 2016).

Salinity stress is becoming one of the major environmental problem facing grape growers. Irrigation water and/or soil salinity can damage the performance of growth, mineral content and grapevine production (Walker, 1994).Salinity stress is always as sociated with different changes in fruit plant metabolism, subsequently affect plant enzyme activity change (Liao – Xiang *et al*., 1996)growth and an increase in leaf content of proline(Eisa and Ibrahim, 2016). Also, the salinity stress exerted adverse effect on survival percent and all vegetative growth characters. Moreover, the harmful impact became more intense as salinity stress level was increased(Abo – Sayed Ahmed *et al*., 2000 a and b).Anatomical features of leaf blade reduced by increasing salinity levels (Eisa and Ibrahim, 2016).

In recent years, great attention has been paid through the application of antioxidants, such as ascorbic acid and salicylic acid which were widely present in plants and has been found to play a vital role in mitigating the adverse effects of salinity on metabolism and plant growth in many plants (Gul *et al.,* 2015).Antioxidants are a division of nutrients that protect the plants from harm caused by different stress factors, which scavengers' free radicals and save cells from the oxidative damage (Karadeniz *et al.,* 2005). Ascorbic acid (AA) presently holds an important position in plant physiology due to its ownership of antioxidant and cellular reductant properties and its various roles in plant growth, development and the regulation of a wide spectrum of plant cellular mechanisms against different environmental stresses(Taqi *et al*., 2011).Ebrahimian and Bybordi (2012) showed that ascorbic acid application into the nutrient solution can alleviate harmful effect of salinity and diminish enzyme activity by scavenging of active oxygen species. Zonouri *et al.* (2014) showed that ascorbic acid (AA) had effect on some physiological responses of grapevines.Noctor and Foyer (1998) mentioned that the protective impact of ascorbic acid (AA) is more related to reduce reactive oxygen species damage to important proteins and\or nucleic acids. Salicylic acid (SA) plays an essential role in the regulation of plant growth, development and ion uptake as well as transport and raise to tolerance against abiotic stresses (salinity). This positive impact of salicylic acid could be imputed to increment CO_2 assimilation, photosynthetic rate and raise mineral uptake for plants grown under stress (Vazirimehr and Rigi, 2014). SA reduced the Na^+ uptake of plants as comparison with control and decreased the concentration of both free radicals and the peroxidase activity (POD)(Cai *et al.*, 2006).

Therefore, the aim of this work was to evaluate the effect of foliar spray with ascorbic acid or salicylic acid, individually on growth physiological characters and endogenous bio- constituents ,as well as, the anatomical features of Thompson seedless grapevine rootings leaf grown under different levels of salinity stress conditions.

MATERIALS AND METHODS

This investigation was conducted during the two successive seasons of 2016 and 2017 in the shade house of the Viticulture Research Department, Horticulture Research Institute, Agricultural Research Center, at Giza, Egypt, to study the effect of ascorbic and salicylic acids on vegetative growth, physiological characters and endogenous bio constituents, as well as, anatomical structure of Thompson seedless grapevine rootings leaf grown under three levels of salinity stress. Cuttings of Thompson seedless grapevine were taken from one year old matured canes and were chosen 120 Uniform and healthy for grapevines of 10 years old grown in EL-mansouriyeh, Giza, Egypt. The rootings were planted at the first week of March in polyethylene bags filled with

5 kg/bag (17×30cm)of a mixture of peat moss and washing sand (1:2V/V), one per plastic bag. All bags have bottom holes to allow surplus water drainage. The rootings were irrigated with tap water twice a week with the same quantity until the end of April. Rootings were exhibited to irrigate using tap water and NaCl saline solution levels (1000, 2000 and 3000 ppm) in the first week of May till the end of September during the two growing seasons. Each bag received one liter every time. All treated rootings were irrigated by tap water once monthly in order to prevent salinity accumulation through holes at the end of the bags which are then closed well after washing. The experiment of ten treatments was arranged in a randomized complete blocks design.

The ten applied treatments were as follows:

1- Irrigation with tap water without NaCl saline (control)
2- Irrigation with NaCl saline solution (1000 ppm) and spraying with distilled water
3- Irrigation with NaCl saline solution (1000 ppm) and spraying with ascorbic acid (400 ppm)
4- Irrigation with NaCl saline solution (1000 ppm) and spraying with salicylic acid (200 ppm)
5- Irrigation with NaCl saline solution (2000 ppm) and spraying with distilled water
6- Irrigation with NaCl saline solution (2000 ppm) and spraying with ascorbic acid (400 ppm)
7- Irrigation with NaCl saline solution (2000 ppm) and spraying with salicylic acid (200 ppm)
8- Irrigation with NaCl saline solution (3000 ppm) and spraying with distilled water
9- Irrigation with NaCl saline solution (3000 ppm) and spraying with ascorbic acid (400 ppm)
10- Irrigation with NaCl saline solution (3000 ppm) and spraying with salicylic acid (200 ppm)

Each treatment was replicated three times, four rootings/ replicate; i.e. 12rootings for each treatment. In both seasons, foliar spray with ascorbic acid as well as salicylic acid which was prepared to melt in organic solventon Thompson seedless grape rootings was carried out three times at 70, 100 and 130 days from planting. Control plants were foliar sprayed with distilled water. Spraying is performed using a hand atomizer with the addition wetting agent and the spraying solution was maintained just to cover completely the plant foliage till drip.

Applied antioxidant materials

Ascorbic acid ($C_6H_8O_6$)and salicylic acid ($C_7H_6O_3$)were obtained from Sigma chemical Co. USA.

Data recorded

A sample of four plants from each treatment was randomly taken from the end of May till Oct. for number of leaves/plant, main shoot length (cm) and stem thickness (mm). However, the other vegetative growth parameters and physiological properties were taken at the middle of Oct .for both seasons and the following data were recorded:

Plant survival percentage:

Number of survived rootings for each treatment was counted by the end of salinization period. Rootings of survival (%)were calculated.

Growth parameters:

The following vegetative growth aspects were measured: plant height (cm),number of leaves/plant, leaf surface area (cm^2);sixth and seventh leaves from the tip of the growing shoot were used for leaf surface area measurement according to(Montero *et al.*, 2000), main shoot length(cm),stem thickness (mm),fresh and dry weight of aerial portion and root system. The fresh material was oven dried at 70^0C for 48 h. until a constant weight then recorded in grams.

Photosynthetic pigments of leaf:

Disk samples from the 5th and the 6thupper leaf of the shoot were taken to determine chlorophyll a, b, total chlorophyll (a+b) and total arotenoids contents according to the method described by Wettestein (1957) and then calculated as mg/g fresh weight.

Endogenous Bio Constituents:

Proline content

It was determined in fresh leaves of Thompson seedless rootings using the method of Bates *et al*. (1973).

Sodium content

Sodium was estimated in the leaves by flame photometry according to Evenhuis (1978).

Total phenolscontent

Total phenols were determined in treated plants of fresh leaves with folin-Ciocalteau reagent according to Maliak and Singh (1980).

Oxidative enzymes activity

Activities of oxidative enzymes, *i.e.,* peroxidase (POX), polyphenol oxidase (PPO) were determined infreash leaves. The extraction procedure was performed as described by Biles and Martyn (1993).

Peroxidase activity

Peroxidase activity was determined according to Hammerschmidt *et al*. (1982).

Polyphenol oxidase activity

Polyphenol oxidase activity was determined according to Maliak and Singh (1980).

Anatomical Studiesof Leaf

From the results in the 1stgrowing season (2016) it was attained that foliar application with ascorbic acid gave the best values of the all studied characters comparing to salicylic acid, consequently ascorbic acid treatment was chosen for studying the anatomical features of Thompson seedless grape rootings leaf during the 2nd season (2017).Specimens from selected treatments of grapevine plants were taken from the midrib region of the upper fourth leaf on the main stem at the middle of October (in the second season). These specimens were prepared according to the method described by(Nassar and El-Sahhar, 1998). Sections were examined to detect histological manifestations of the treatments and photomicrographedusing light microscope (Olympus) provided with digital camera, (Canon power shot S80) connected to a computer. The photographs were taken by Zoom Browser Ex program. Dimensions of stems and leaves were measured using Corel Draw programver.11.

Statistical analysis

Data of the present work were statistically analyzed and the differences between the means of the treatments were significantly considered when they were more than the least significant differences (LSD) at the 5% level using computer program of Statistix version 9 (Analytical software, 2008).

RESULTS AND DISCUSSION

Plant survival percentage

Results in (Table 1) revealed that the plant survival percentage with significant differences among treatments in 2016 and 2017 seasons. The survival of plants values were ranged from (54.0 to 99.20%) in the 1st season and from (55.33 to 99.59%) in the 2nd one. The obtained data gradually reduced to (54.0and55.33%) in the two growing seasons, respectively, with increasing the tested salinity level 3000 ppm. Thus, the highest survival of plants (%) were obtained when plants were irrigated with tap water (control), whereas, the lowest percentage obtained from irrigated with NaCl saline solution(3000 ppm) and foliar sprayed with distilled water in both seasons. It is clear from the obtained results that spraying plants with ascorbic acid (AA)at 400 ppm and salicylic acid (SA) at 200 ppm alleviated the harmful effect of all salinity levels (1000, 2000 and 3000 ppm).It is clearly that ascorbic acid was more effective than salicylic acid treatments in reducing the hurtful effect of salinity stressin the two growing seasons. Irrigation with salinity stress level reduced plant survival percentage and this maybe due to increment the osmotic pressure leading to a reduction in availability of water to plants, accumulating of Na^+ and Cl^-ions in toxic concentrations of the root medium as mentioned by(Hassan and Abo - El –Azayem, 1990). Also, the salinity stress exerted adverse effect on survival %. Moreover, the harmful impact became more intense as salinity stress level was increased. Salinity stress increased (ROS) Reactive Oxygen Species which caused destruction photosynthetic protein biosynthesis, growth parameters and metabolic biosynthesis.

Table 1. Effect of foliar application with antioxidants (AA and SA) on survival (%) andplant growth parameters of Thompson seedless rooting grown under different salinity stress levels in two growing seasons(2016 and 2017)

Treatment	Survival percentage		plant growth parameters									
			Plant height (cm)		No. of leaves/plant		Leaf surface area (cm)^2		Shoot length (cm)		Stem thickness (mm)	
	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
Control (tap water)	99.20	99.59	110.00	123.27	19.33	20.00	91.16	91.63	101.93	103.30	5.53	5.60
1000PPM + D	83.99	83.01	82.30	91.50	15.67	16.00	80.25	81.53	74.87	76.90	4.77	4.97
1000PPM + AA	93.90	94.21	99.40	109.57	18.67	19.00	87.65	88.35	90.57	91.70	5.10	5.36
1000PPM + SA	91.21	90.25	93.40	102.33	17.33	17.67	84.49	85.35	79.63	81.27	4.90	5.20
2000PPM + D	72.94	70.38	64.13	74.90	12.33	13.00	72.03	70.76	59.90	60.84	4.16	4.47
2000PPM + AA	82.06	82.20	80.87	90.63	15.00	15.67	79.24	79.04	70.50	70.37	4.60	4.87
2000PPM + SA	79.74	78.99	73.57	82.90	14.33	14.67	75.30	75.78	67.30	68.37	4.43	4.63
3000PPM + D	54.00	55.33	47.25	53.93	9.67	10.33	66.64	66.22	39.57	41.13	3.78	3.97
3000PPM +AA	71.27	69.88	63.54	72.43	12.33	12.67	71.83	70.20	50.07	55.80	4.10	4.25
3000PPM + SA	63.04	63.66	52.10	61.73	11.00	11.33	69.77	68.96	45.67	49.80	3.97	4.01
L.S .D. 0.05	2.02	2.45	5.93	3.70	1.35	1.60	2.56	2.25	1.01	1.06	0.10	0.13

D: distilled water; AA: ascorbic acid; SA: salicylic acid

Vegetative growth parameters:

Plant height and leaf surface area (cm^2)

From Table 1, data showed that the plant height and leaf surface area had significant differences between the tested treatments in the two seasons. The same characters negatively reacted to the increment of water salinity. The significant values (110.0 and 123.27 cm) and (91.16 and 91.63cm^2) were recorded when plants were irrigated with tap water during 2016 and 2017. Irrigation with NaCl saline solution at 3000 ppm and foliar spray with distilled water resulted the lowest values of plant height (47.25 and 53.93 cm) and leaf surface area (66.64 and 66.22cm^2) for both seasons, respectively. Moreover, foliar application with both ascorbic acid and salicylic acidrecorded the highest values,respectively compared to corresponding control plants (distilled water).

The salinity stress caused hurtful effect on plant height and leaf area. They added thatthe harmful impact became more intense as salinity stress level was increased.Salinity can affect plant growth in two main waysthrough osmotic stress and ion toxicity.Osmotic stress is caused by salts (mainly Na^+ and Cl^-) in the soil solution reducing the water availability to rootscausing quick decrease in the growth ratethrough molecular damage. Ion salts toxicity occur when plant roots take up Na^+ and/or Cl^- and these ions accumulated to harmful levels in leaves. Also, mightcause ion disequilibrium, productionof active oxygen species (AOS) and nutrient deficiency particularly for ion K^+ nutrition. Subsequently, it couldleads to death of leaves and a decrease in the total leaf surface area which decrease the supply of photosynthate in plants and finally affect the yield (Tejera *et al*., 2007).Ascorbic acid (AA) increased leaf area for Thompson seedless grapevine and that might be due to the influence on cell division and elongation (Fayed, 2010).Badran *et al*. (2013) reported that salinity stress decreased plant height of treated plants, while application of ascorbic acid and salicylic acid improved it. Both AA and SA were efficient in mitigating the adverse effects of salinity on growth.

No. leaves/plant, main shoot length and stem thickness

Figs (1, 2, 3, 4, 5 and 6) showed that the No. leaves/plant, the main shoot length and stem thickness gradually increased during all examined sampling dates (from May to October). Increasing NaCl concentrations in irrigation water to 3000 ppm treatment throughout the time of the study performed the lowest number of leaves/plant (9.67and 10.33), the shoot length (39.57 and41.13cm) and stem thickness(3.78and3.97mm) in October for the two growing seasons, respectively, as comparing to control and other tested treatments. With respect to exogenously application of both ascorbic acid (AA) and salicylic acid (SA) on Thompson seedless rooting, it was clear that they reduced the harmful effect of salinity stress and the AA was more effective than SA in improving all above mentioned characteristics. These effects might be due to increase leaf area/plant, weight of roots and chlorophyll content. Tables (1, 2 and 3) of foliar applications could explain these results.

Table 1 showed that the No. leaves/plant, the shoot length and stem thickness for Thompson seedless rootings were negatively significant to the raise of NaCl saline solution concentrations. Whereas, the highest values of the No. of leaves/plant (19.33 and 20.0), the main shoot length (101.93 and 103.30 cm) and stem thickness (5.53 and5.60 mm) were recorded with irrigated with tap water during both seasons, respectively. Foliar spray with AA recorded the highest values followed by SA compared to foliar spray with distilled water (control).

Badran *et al*.(2013)found that the salinity stress showed adverse effect on number leaves and stem thickness. Furthermore, the harmful impact became more intense as salinity stress level was raised. On *Khayasenegalensis* seedlings, Badran *et al*. (2013)found that the AA and SA were effective in alleviating the hurtful effects of salinity on number of leaves and stem diameter.

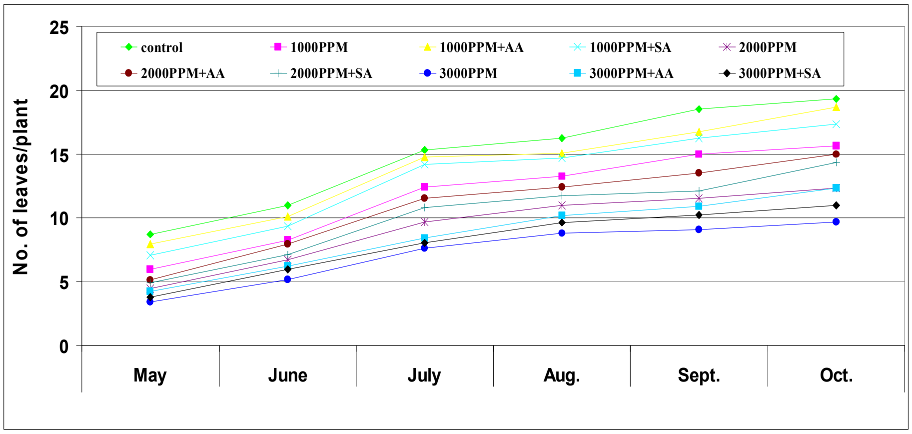

Fig. 1. Effect of foliar application with antioxidants (AA and SA) on average monthly number of leaves/plant for Thompson seedless rooting grown under different salinity stress levels in 2016 season

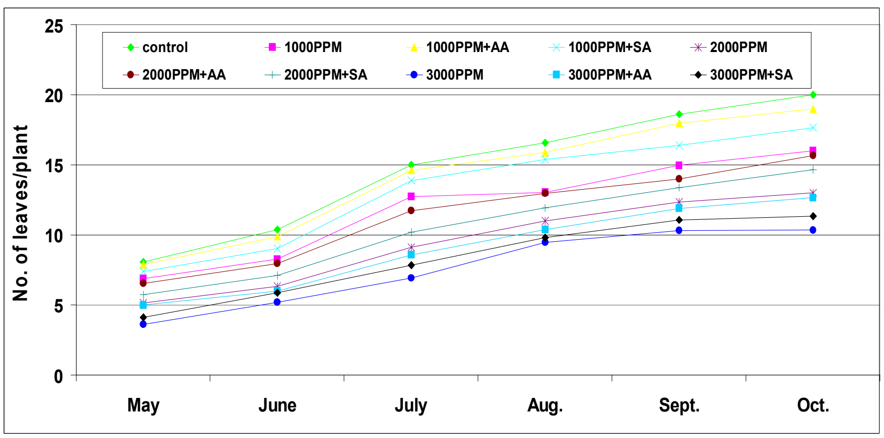

Fig. 2. Effect of foliar application with antioxidants (AA and SA) on average monthly number of leaves/plant for Thompson seedless rooting grown under different salinity stress levels in 2017 season

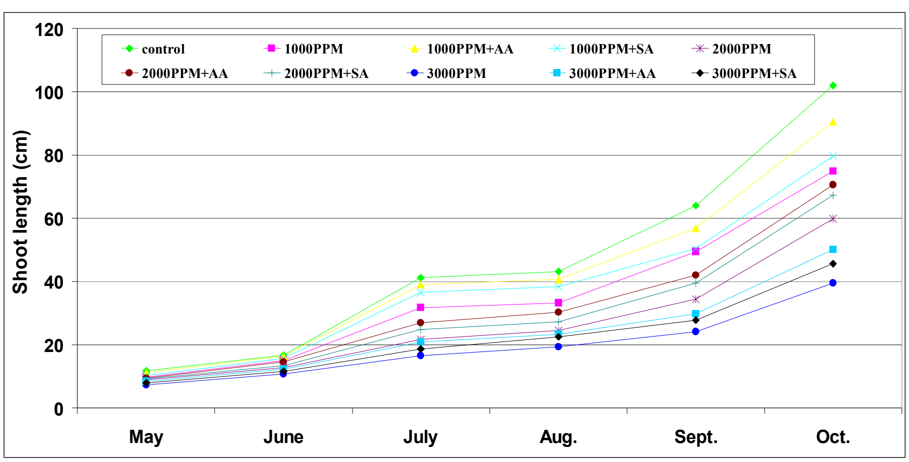

Fig. 3. Effect of foliar application with antioxidants (AA and SA) on average monthly shoot length (cm) for Thompson seedless rooting grown under different salinity stress levels in 2016season

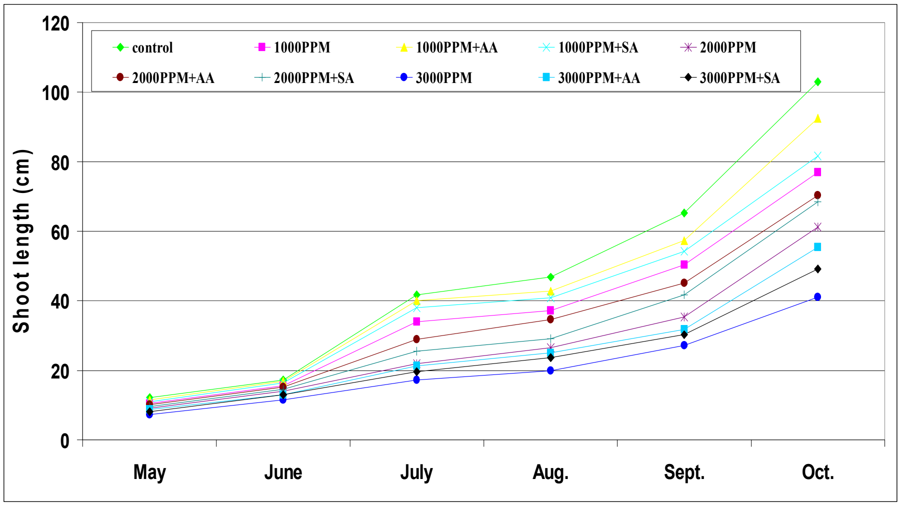

Fig. 4. Effect of foliar application with antioxidants (AA and SA) on average monthly shoot length (cm) for Thompson seedless rooting grown under different salinity stress levels in 2017season

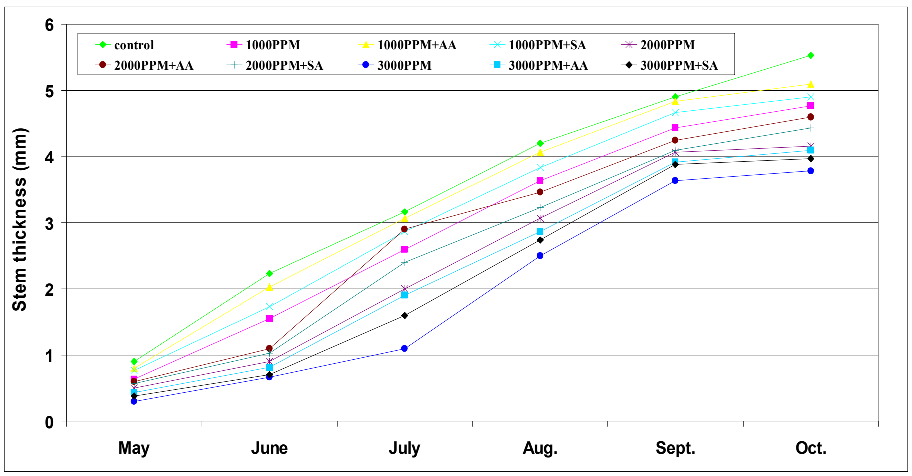

Fig. 5. Effect of foliar application with antioxidants (AA and SA) on average monthly stem thickness (mm) for Thompson seedless rooting grown under different salinity stress levels in 2016season

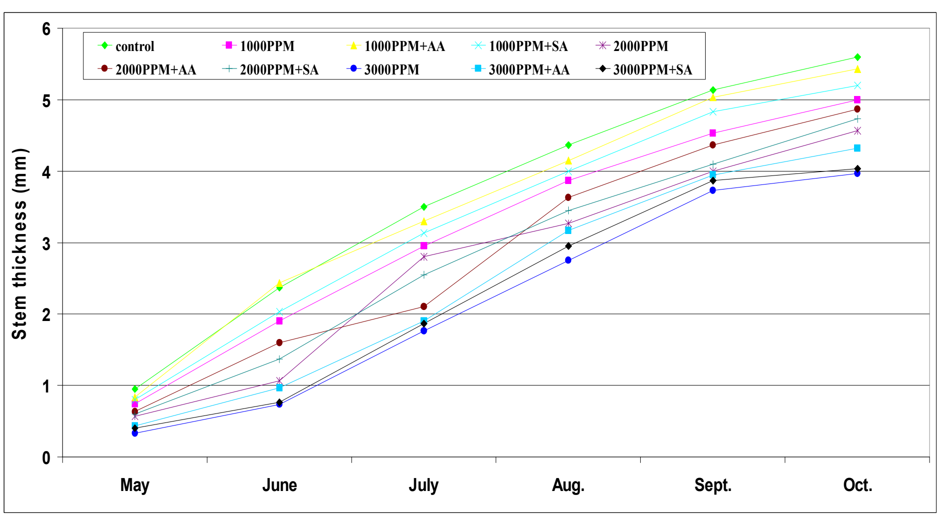

Fig. 6. Effect of foliar application with antioxidants (AA and SA) on average monthly stem thickness (mm) for Thompson seedless rootings grown under different salinity stress levels in 2017season

Fresh and dry weight of aerial portion and root system

Irrigation with NaCl saline solution significantly affected both fresh and dry weights of aerial portion and root system. Increasing salinity levels gradually diminished these measurements for Thompson seedless rootings (Table 2). Irrigation with NaCl saline solution 3000 ppm recorded the minimum values for fresh and dry weights of aerial portion and root system for the two growing seasons. However, the maximum values were gained with plants irrigated via tap water(control). Foliar applications of antioxidants were shown to have their specific effect on fresh and dry weights of aerial portion and root system in both seasons. The highest increase in these characters was observed with ascorbic acid application followed by salicylic acid for alleviating the harmful effect of salinity stress compared to foliar spray with distilled water.

Table 2. Effect of foliar application with antioxidants (AA and SA) on fresh and dry weight of aerial portion and root system of Thompson seedless rooting grown under different salinity stress levels in twogrowing seasons (2016 and 2017)

Treatment	Fresh weight of aerial portion (g)		Dry weight of aerial portion (g)		Fresh weight of root System (g)		Dry weight of root System (g)	
	2016	2017	2016	2017	2016	2017	2016	2017
Control (tap water)	79.63	80.07	26.34	26.58	54.32	54.87	21.90	22.28
1000PPM + D	60.61	61.51	19.87	20.17	48.55	48.76	19.72	19.81
1000PPM + AA	71.91	72.81	23.30	23.94	51.89	52.66	20.95	21.20
1000PPM + SA	67.20	66.87	21.40	20.96	50.23	50.80	20.09	20.32
2000PPM + D	45.90	46.90	14.97	15.63	40.43	41.23	16.17	16.83
2000PPM + AA	58.72	60.39	18.91	19.46	47.00	46.67	18.90	18.80
2000PPM + SA	55.22	55.88	17.41	18.63	43.94	44.28	17.58	17.65
3000PPM + D	34.73	35.28	11.91	12.09	35.13	35.61	14.03	14.24
3000PPM +AA	44.09	46.09	14.36	15.36	39.44	40.24	15.71	16.10
3000PPM + SA	40.86	41.19	13.62	13.86	37.80	38.13	15.05	15.25
L.S .D. 0.05	3.43	2.44	1.09	1.76	1.44	1.75	0.67	0.76

D: distilled water; AA: ascorbic acid; SA: salicylic acid

Salinity stress doing adverse effect on fresh and dry weights of both the aerial portion and root system. These growth parameters reduced the components of yield these inhibitory effect of salinity may be due to number of physiological processes such as a decrease in meristic activity and/or cell enlargement (Sakr *et al*., 2012) or a perturbation of functioning of vital components of photosynthesis (Yang and Birtton, 1990) and these was some evidence for this in decreasing levels of chlorophyll measured. The exact mechanism of the effect of these antioxidants (SA, AA) is not known but may be due to osmotic protection (Arteca, 1996) or promotion of the uptake of essential macro-nutrients which then facilitated normal growth and development (Foyer and Spencer, 1986).The foliar application of ascorbic acid and salicylic acid improved dry weight of shoots and roots of plants grown under salinity stress (Badran *et al*., 2013).Ascorbic acid treatment increased fresh and dry weight of leaves for Thompson seedless seedlings and this may be due to its encourage effect on cell division and elongation under salinity conditions (Fayed, 2010).

Leaf Photosynthetic pigments

Results of (Table 3)tabulated the photosynthetic pigment concentration of chlorophyll a, b, total chlorophyll (a+b) and carotenoids of Thompson seedless rootings leaves. Pigmentsconcentration was significantly affected by different salinity stress levels. Irrigation of rootings by tap water (control)recorded the highest values [(1.152 and 1.172), (0.496 and 0.504), (1.648 and 1.676) and (1.120 and 1.145) mg/g fw]for chlorophyll a, b, total chlorophyll and carotenoids in both 2016 and 2017 seasons, respectively. Meanwhile, the rootings irrigated with NaCl saline solution (3000 ppm) presented the lowest values [(0.721 and 0.736), (0.186 and 0.198), (0.907 and 0.934) and (0.713 and 0.721) mg/g fw] for all mentioned photosynthetic pigments for both seasons, respectively. It is evident from the results in (Table 3)that the effect of spraying rootings with ascorbic acid (AA)or salicylic acid (SA) led to increment photosynthetic pigments as compared with plants sprayed only with distilled water. Ascorbic acid was more effective than salicylic acid in raising the photosynthetic pigments in the two growing seasons. In addition, these results mean that ascorbic acid (AA) or salicylic acid (SA) reduced the harmful effect of salinity on photosynthetic pigments formation. Increasing salinity stress levels reduced photosynthetic pigments Thompson seedless rootings in leaves and this decrease might be related to promote activity of the chlorophyll degrading enzyme chlorophyllase as submitted by Rizk-Alla (2010)it found that increasing saline decreased concentration of photosynthetic pigments. This decrease might be related to the reduction of plant growth which attributed to the decrease of the water availability for tissues development and reduction of inorganic nutrient supply. Photosynthesis is inhibited with high concentration of Na^{+} accumulated in chloroplasts as mentioned by Sudhir and Murthy(2004). Soil salinity decreased chlorophyll a,b and carotenoids, while the foliar application with AA and SA were effective in alleviating the adverse effects of salinity on all photosynthetic pigments (Badran *et al*., 2013).

Table 3. Effect of foliar application with antioxidants (AA and SA) on photosynthetic pigments (mg/g fw)of Thompson seedless rooting grown under different salinity stress levels in twogrowing seasons (2016 and 2017)

Treatment	chl a		chl b		chl a+ b		Carotenoides	
	2016	2017	2016	2017	2016	2017	2016	2017
Control (tap water)	1.152	1.172	0.496	0.504	1.648	1.676	1.120	1.145
1000PPM + D	0.995	1.019	0.388	0.403	1.383	1.421	0.973	0.998
1000PPM + AA	1.073	1.073	0.458	0.461	1.531	1.534	1.049	1.058
1000PPM + SA	1.012	1.037	0.423	0.433	1.435	1.470	1.014	1.034
2000PPM + D	0.845	0.860	0.292	0.305	1.137	1.165	0.870	0.889
2000PPM + AA	0.969	0.988	0.361	0.367	1.330	1.355	0.935	0.953
2000PPM + SA	0.926	0.946	0.344	0.356	1.270	1.302	0.914	0.932
3000PPM + D	0.721	0.736	0.186	0.198	0.907	0.934	0.713	0.721
3000PPM +AA	0.806	0.822	0.262	0.267	1.068	1.089	0.832	0.845
3000PPM + SA	0.786	0.799	0.227	0.238	1.014	1.037	0.769	0.776
L.S .D. 0.05	0.025	0.032	0.014	0.011	0.028	0.034	0.012	0.019

D: distilled water; AA: ascorbic acid; SA: salicylic acid

Endogenous Bio Constituents

Total phenol compound, proline and sodium in leaves of Thompson seedless grape rootings were significantly influenced by different salinity stress levels (Table 4).The concentrations of the above mentioned characters were gradually increased with increasing the salinity stress levels, whereas the highest values were recorded when rootings irrigated with NaCl saline solution (3000 ppm) compared to control and other tested treatments. However, the lowest values were recorded when plants were irrigated with tap water (control). Furthermore, these parameters of Thompson seedless rootings were decreased after foliar application with the antioxidant ascorbic acid (AA) and salicylic acid (SA) compared to foliar spray with distilled water. Moreover, AA was more effective than SA in decreasing the harmful effect of salinity stress, whereas AA reduced phenols, proline and sodium content over SA followed by plants foliar sprayed with distilled water.

Proline is one of the important components of the adaptation plants to salinity (Kuznetsov and Shevyakova, 1999) and its increasing with increasing water salinity could be due to the increment of hydrolytic enzymes caused by salinity(Salem *et al.,* 2011).Moreover, there are several functions for the accumulation of proline in plant tissues under salinity stress including osmotic adjustment, stability and increased protection of proteins and membranes, improvement of the stabilization of some mitochondrial and cytoplasmic enzymes and a scavenger of free radicals. Sakr *et al.* (2007) found that the increase in sodium content mainly in the vacuole supply an osmotic regulation for plants under salinity stress, this accumulation might be related to the essential role of sodium in increment the osmotic pressure. Irrigation with 1000, 2000 and 3000 ppm saline water levels increase proline content in the leaves as compared to untreated plants Abd EL Aziz *et al.* (2006). Noctor and Foyer (1998) argued that additional ascorbic acid significantly reduced the undesirable accumulation of Na^+ in the plants under salinity stress. Badran *et al.* (2013) mentioned that plants grown under salinity increased Na and proline concentrations in leaves, but the foliar application with AA and SA decreased both Naand proline and were effective in alleviating the adverse effects of salinity. Waśkiewicz *et al.*(2013) found that salt stress causes a restriction of photosynthesis process, which results in excrescent ROS production. To adapt with the harmful salt stress, plants induced the synthesis of different secondary metabolites, such as phenolic compounds.

Table 4. Effect of foliar application with antioxidants (AA and SA) on total phenols (mg/g fw) , (proline and Na %), polyphenol oxidase and peroxidase (Unit/g fw) of Thompson seedless rooting grown under different salinity stress levels in two growing seasons (2016 and 2017)

Treatment	Endogenous Bio Constituents						Oxidative enzymes activity			
	Total phenols		Prolin		Na		PPO		POX	
	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
Control (tap water)	0.947	0.935	0.077	0.074	0.243	0.234	0.306	0.282	0.953	0.958
1000PPM + D	2.119	1.940	0.181	0.176	0.395	0.388	0.713	0.779	1.182	1.177
1000PPM + AA	1.366	1.075	0.101	0.092	0.306	0.266	0.426	0.329	1.007	1.036
1000PPM + SA	1.685	1.153	0.125	0.105	0.335	0.286	0.516	0.400	1.061	1.061
2000PPM + D	3.338	3.324	0.392	0.385	0.507	0.503	1.326	1.312	1.665	1.658
2000PPM + AA	2.628	2.475	0.259	0.253	0.422	0.414	0.935	0.919	1.306	1.304
2000PPM + SA	3.013	3.003	0.302	0.298	0.469	0.467	1.147	1.066	1.435	1.331
3000PPM + D	4.547	4.530	0.512	0.507	0.608	0.604	2.040	2.020	1.925	1.921
3000PPM +AA	3.898	3.892	0.422	0.417	0.531	0.516	1.658	1.665	1.784	1.780
3000PPM + SA	4.259	4.086	0.445	0.443	0.569	0.526	1.787	1.685	1.787	1.833
L.S .D. 0.05	0.110	0.101	0.011	0.012	0.005	0.014	0.039	0.043	0.046	0.057

D: distilled water; AA: ascorbic acid; SA: salicylic acid; PPO:Polyphenol oxidase;POX: Peroxidase.

Oxidative enzymes activity

Results presented in (Table 4)indicated the effect of exogenous application with antioxidants (AA and SA) on oxidative enzymes activity of Thompson seedless rootings leaves grown under salinity stress. The concentrations of polyphenol oxidase (PPO) and peroxidase (POX) were significantly increased with increasing the NaCl saline solution levels. The least values for PPO and POX activity [(0.306 and 0.282) and (0.953 and 0.958) Unit/g fw] were registered with plants that irrigated with tap water (control) for both seasons, respectively. It is clear from such results in Table 4 that the impact of foliar application with ascorbic acid (AA) and salicylic acid (SA) led to a decrease in PPO and POX activity compared with plants foliar sprayed with distilled water. Moreover, the ascorbic and salicylic acid decreased the hurtful effect of salinity stress on PPO and POX activity for Thompson seedless rootings. Also, AA was more efficient than SA in reducing the oxidative enzymes activity, where ascorbic acid when used in combination with 3000, 2000 and 1000 ppm gained[(1.658 and 1.665), (0.935 and 0.919) and (0.426 and 0.329) Unit/g fw for PPO] and [(1.784 and1.780), (1.306 and 1.304) and 1.007 and 1.036) Unit/g fw for POX] during the two growing seasons, respectively. On the other hand, the highest concentrations [(2.040 and 2.020), (1.326 and 1.312) and (0.713 and 0.779) Unit/g fw for PPO] and [(1.925 and1.921), (1.665 and 1.658) and 1.182 and 1.177) Unit/g fw for POX] were recorded in the combination treatments between foliar spray with distilled water and the same salinity levels (3000, 2000 and 1000 ppm) for both seasons, respectively.

Peroxidase activity increased with increasing salt concentrations. Rizk -Alla(2010).Maurice *et al.* (2000) found that antioxidants play an important role in the decrease or prevention of enzymatic browning through inhibiting polyphenol oxidase.SA decreases the concentration of both free radicals and the peroxidase activity (POD)Cai*et al.*(2006).

Anatomical Studies of Leaf

Effect of salinity

Microscopic counts and measurements of some features in transverse sections through the blade of upper fourth leaf on the main stem of Thompson grape and those grown under salinity stress of 1000 or 3000 ppm are presented in (Table 5). Microphotographs illustrating these treatments in transverse sections are shown in (Fig. 7). It is realized that salinity stress at the level of 1000 ppm reduced the thickness of both midve in and lamina by 42.25 and 41.04 % less than of the control plants, respectively. The thinner of lamina induced by salinity stress could be refered to the decrease in thickness of epidermis as well as in thickness of mesophyll tissues. The decrements below the control were (32.00 and 34.78 %) for thickness of upper and lower epidermis, growing and (40.34 and 44.55 %) for thickness of palisade and spongy tissues, respectively. Such treatment decreased the dimensions of midvein vascular bundle below the control by 48.75 in length and by 49.28 % in width. Also, low salinity level decreased the thickness of xylem and phloem tissues in comparison with the control by 47.76 and 33.96 %, respectively. Likewise, it is noted that high salinity reduced the thickness of both lamina and midvein of blades; the reduction was more pronounced than that induced by low salinity level, being 52.24 % less than the control for thickness of lamina and 54.01 % less than the control for thickness of midvein. It is clear that the thinner blade induced by 3000 ppm salinity could be imputed to the decrements in thickness of palisade and spongy tissues and thickness of upper and lower epidermis by (54.62 and56.44 %) and (40.00 and 39.13 %) less than those of the control, respectively. It was obvious that vascular bundles of midvein showed prominent decrease in length by 61.29 % and in width by 61.87 % less than the control. As a result, thickness of xylem and phloem were decreased less than of the control by 56.72 and 50.94 %, respectively.

Table 5. Effect of foliar application with ascorbic acid (AA) on counts and measurements of certain anatomical features in transverse sectionsof the main stem for Thompson seedless rootings grown under two salinity stress levels during the second growing season (2017)

Anatomicalfeatures	Treatments								
	Control	1000 ppm		3000 ppm		1000 ppm + AA		3000 ppm+ AA	
	µ	µ	± % to control	µ	± % to control	µ	± % to 1000 ppm	µ	± % to 3000 ppm
Thickness of midvein	1326.45	766.08	-42.25	610.03	-54.01	812.19	+6.02	730.61	+19.77
Thickness of lamina	212.61	125.35	-41.04	101.55	-52.24	142.01	+13.29	109.48	+7.81
Thickness of upper epidermis	19.83	13.49	-32.00	11.90	-40.00	15.87	+17.65	12.69	+6.67
Thickness of lower epidermis	18.25	11.90	-34.78	11.11	-39.13	14.28	+20.00	11.90	+7.14
Thickness of palisade tissue	94.41	56.33	-40.34	42.84	-54.62	61.88	+9.86	47.60	+11.11
Thickness of spongy tissue	80.13	44.43	-44.55	34.91	-56.44	51.57	+16.07	39.67	+13.64
Dimensions of midvein bundle:									
Length	989.52	507.17	-48.75	383.04	-61.29	567.47	+11.89	450.43	+17.59
Width	985.97	500.08	-49.28	375.95	-61.87	553.28	+10.64	443.33	+17.92
Thickness of xylem tissue	237.63	124.13	-47.76	102.85	-56.72	159.60	+28.57	117.04	+13.79
Thickness of phloem tissue	187.97	124.13	-33.96	92.21	-50.94	127.68	+2.86	109.95	+19.23

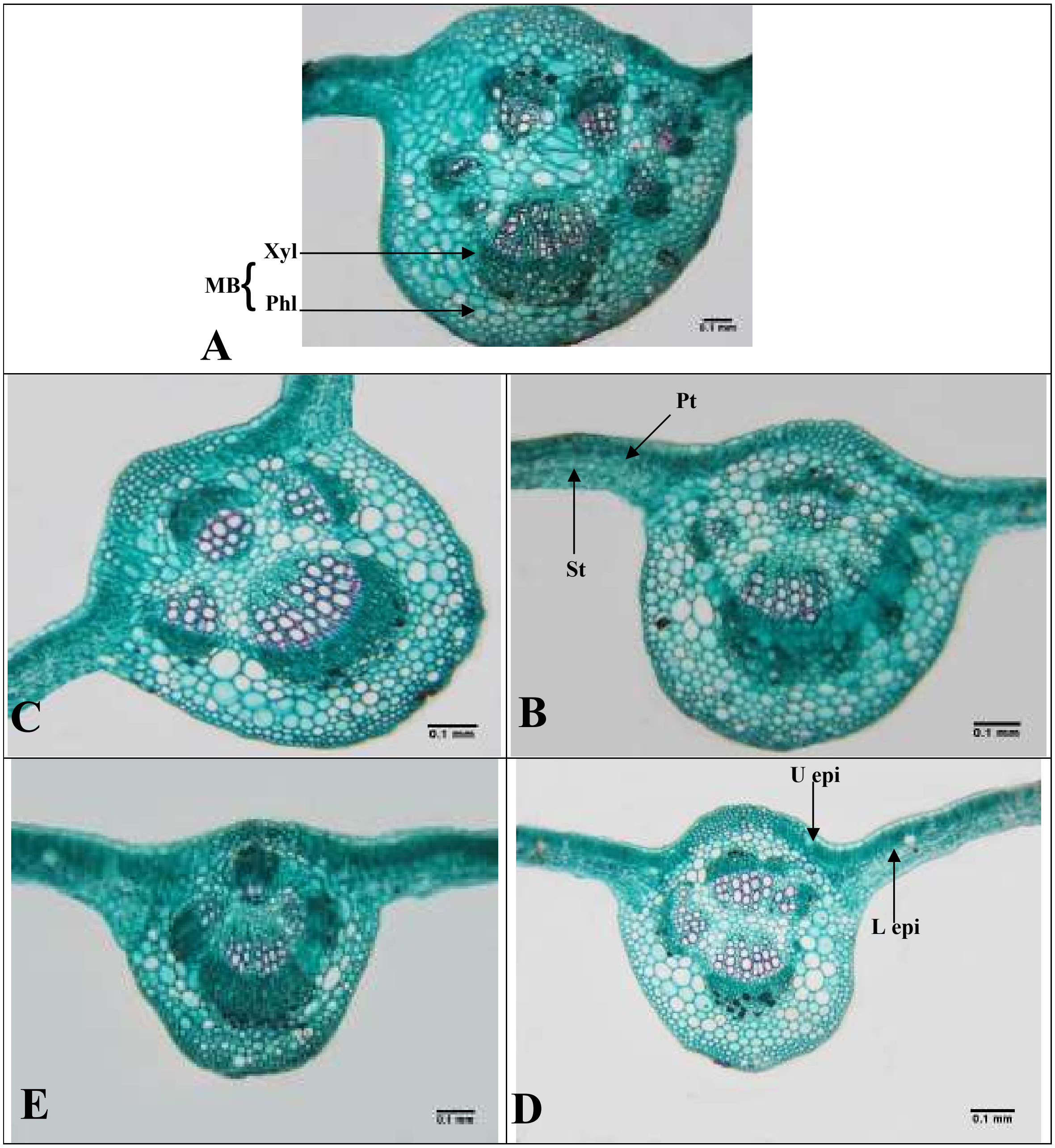

Fig 7.Changes in transverse sections through the blade of upper fourth leaf on the main stem for Thompson seedless rootings affected with foliar application of ascorbic acid (AA) at 400 ppm grown under different salinity stress levels during the growing season (2017)

A: Irrigation with tap water (control); B: Irrigation with NaCl saline solution (1000 ppm) and spraying with distilled water; C: Irrigation with NaCl saline solution (1000 ppm) and spraying with (AA); D: Irrigation with NaCl saline solution (3000 ppm) and spraying with distilled water; E: Irrigation with NaCl saline solution (3000 ppm) and spraying with (AA)
U epi: Upper epidermis; Pt: Palisade tissue; St: Spongy tissue; L epi: Lower epidermis; MB: Midvein bundle; Xyl: Xylem; Phl: Phloem

Effect of combined treatments among salinity levels and ascorbic acid

It is obvious from (Table 5) and (Fig. 7) that spraying ascorbic acid at concentration of 400 ppm on Thompson grape plants grown under salinity stress at 1000 ppm increased in thickness of lamina and midvein by 13.29 and 6.02% more than stressed plants (foliar sprayedwith distilled water), respectively. The increase in lamina thickness due to spraying of ascorbic acid could be referred to the increase in thickness of upper and lower epidermis by (17.65 and 20.00%) and thickness of palisade and spongy tissues by (9.86 and 16.06%) more than stressed plants, respectively. However, such treatment caused an increment of 11.89 and 10.64% in length and width of midvein bundle over those of stressed plants, respectively. Also, the increments over than stressed plants were 28.57% for thickness of xylem tissue and 2.86% for thickness of phloem tissue.

It was clear that the foliar spray of rootings with ascorbic acid under salinity level 3000 ppm diminished the harmful effects of salinity stress and increased thickness of midvein and lamina by 19.77 and 7.81% more than plants grown under salinity level 3000 ppm, respectively. This increment in lamina thickness due to the increase in thickness of upper, lower epidermis by (6.67 and 7.14%), thickness of palisade and spongy tissues by (11.11 and 13.64%) are more than stressed plants, respectively. Furthermore, such treatment caused an increase of 17.59 and 17.92% in length and width of midvein bundle of those stressed plants, respectively. As well as, the increase over stressed plants was 13.79 and 19.23% for thickness of xylem and phloem tissues, respectively.

Anatomical features of leaf blade reduced by increasing salinity levels (Eisa and Ibrahim, 2016).The inhibition impact of salinity on leaf construction due to the growth inhibiting of vascular elements and/or commitment with an inhibiting the formation of the procambial activity, initial vascular tissues and/or reduce in the size and number of mesophyll cells(Rashid *et al.,*2004). On basil plants, Azoz *et al.* (2016) found that application of foliar spray with ascorbic acid (AA) at 300 ppm increased the thickness of lamina, palisade, spongy, midvein and the main vascular bundle.

CONCLUSION

It could be concluded that all studied vegetative growth characters, leaf photosynthetic pigments, total phenols, proline, sodium andoxidative enzymes activity of Thompson seedless grape rootings significantly reduced when plant grown under different salinity stress levels. Foliar application of ascorbic acid and salicylic acid alleviated the undesirable effects of salinity stress of the aforementioned features. Ascorbic acid was the most effective than salicylic acid in avoiding the hurtful effects of salinity on Thompson seedless rootings. Finally, it could be recommended by using ascorbic acid or salicylic acid to partially reduce the harmful effects of Thompson seedless grape rootings growing under salinity stress.

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تخفيف الآثار الضارة للملوحة باستخدام بعض المواد المضادة للأكسدة علي شتلات العنب الطومسون سيدليس

سهام عبدالعال إبراهيم[1] و ثريا صابر على ابوالوفا[2]

[1]قسم النبات الزراعى- كلية الزراعة- جامعة الزقازيق- مصر

[2]قسم العنب- معهد بحوث البساتين- مركز البحوث الزراعية- جيزة- مصر

تم إجراء هذا البحث خلال موسمين متعاقبين هما 2016 و 2017م في صوبة قسم بحوث العنب، معهد بحوث البساتين، مركز البحوث الزراعية، الجيزة، مصر، تهدف الدراسة لتقييم تأثير الرش الورقى بحمض الأسكوربيك بتركيز 400 جزء في المليون وحمض الساليسيليك بتركيز 200 جزء في المليون تحت ثلاثة مستويات من الإجهاد الملحى (1000، 2000 و 3000 جزء في المليون) على صفات النمو الخضري، والصفات الفسيولوجية والمركبات الداخلية بالنبات وكذلك التركيب التشريحى لنصل ورقة شتلات العنب طومسون سيدلس. إتضح من النتائج إنخفاض معنوى فى جميع صفات النمو الخضري للشتلات تحت الدراسة وأيضا تسجيل إنخفاض معنوي في صبغات التمثيل الضوئي بزيادة مستويات الإجهاد الملحى مقارنة بالنباتات غير المعاملة بينما زاد بشكل ملحوظ فى الاوراق كلا من البرولين والصوديوم والفينولات الكلية ونشاط الإنزيمات المؤكسدة مع إجهاد الملوحة. كماأعطي الرش بحمض الاسكوربيك أفضل النتائج لتخفيف الآثار الضارة للإجهاد الملحى يليه حمض الساليسيليك على الصفات المذكورة أعلاه لكلا موسمى النمو مقارنة بنباتات الكنترول. وعلاوة على ذلك، أظهرت النباتات انخفاض كبير في الصفات التشريحية لورقة شتلات العنب طومسون سيدلس عند المعاملة بمحلول ملحي من كلوريد الصوديوم, وحيث زادت جميع الصفات التشريحية برش الشتلات بحمض الاسكوربيك بتركيز 400 جزء في المليون مقارنة بالكنترول تحت ظروف الإجهاد الملحى.