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EVALUATION OF THE TWO ROOTSTOCKS (SO_4 AND FREEDOM) FOR THE SALT STRESS *IN VITRO* CONDITIONS

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Abstract

Abiotic stresses are the major limiting factors in plant productivity. Salinity is considered as significant ecological regarding the productivity of different crops in different arid and semiarid areas all over the world, it's also the major cause of the reduction in grapevine crop yields worldwide. In the present investigation, two grapevine rootstocks (Freedom & SO_4) were evaluated to the effect of different concentrations of salinity under *in vitro* conditions. Murashige and Skoog (MS) culture medium was used at strength of ¾ in plant tissue culture technique supplemented with 2 mg/L BAP with different concentrations of NaCl (0.0, 2000, 2500 and 3000 ppm) for *in vitro* culture conditions. The results showed that the survival percentage, vegetative growth (formed shoots, shoot length, shoot number and leave numbers root growth (root number & length) and photosynthetic pigments content (chlorophyll A & B and carotenoids) significantly decreased linearly with increasing the salinity levels. Contrary, the total phenols and free proline contents increased as a result of increasing salinity. Furthermore, increased salinity levels led to high total polyphenyl oxidase expression in Freedom rootstock compared to SO_4 rootstock. In conclusion, both rootstocks (SO_4 and Freedom) showed great tolerance to high levels of salinity. Although Freedom was more tolerant to salt stress than SO_4, both of them can be used widely as salinity tolerant rootstocks in newly reclaimed soils.

***Key words*:** Grapevine; Salinity; Rootstock; Abiotic stress; SO_4; Freedom; Proline; NaCl.

Introduction

Grapevine (*Vitis vinifera L.*) is perennial woody fruit plants, which grow in most regions as tropical, subtropical and temperate regions (Anupa *et al.,* 2016). The world production of grapevine crop average 67.5 million tons each year, which confirms that it is one of the most important crops in the world (Kurmi *et al.,* 2011 and LazoJavalera *et al.,* 2016).

In Egypt, the expansion of fruit trees cultivation in the newly reclaimed soils and maximizing fruits exports are the main goals of the Egyptian Agricultural strategy. According to the statistics of FAO (2017) Egypt reached about 1.360.250 tons of grapes cultivated in 153.682 feddans. On the other hand, Ministry of Agriculture Statistics (2017) showed increase in the planted area up to 188.543 feddans in 2017, producing 1.378.815 tons.

Many grapevine rootstocks are cultivated in Egypt but vineyards SO_4 and Freedom are the best and the commonest. These rootstocks characterized by resistance against both phylloxera, nematode and suitable for viticulture (Elbotaty, 2012).

The reduction in crop yields worldwide is mainly due to the abiotic stress as drought and high soil salinity (Boscaiu *et al.,* 2008), that is why studying plant responses to abiotic stress is one of the most active research topics in plant biology, not only due to its unquestionable academic interest, but also because of its practical implications in agriculture. The high concentration of salts in the soil has been always a great obstacle for many plant species especially grapevine which is very sensitive to elevated Na^+ concentration due to its combined ion toxicity and osmotic stress (Horie *et al.,* 2011). On the other hand, salinity stress negatively affects plant pigments content.

The vegetative propagation also termed micropropagation is the common method of grape multiplication by the use of *in vitro* culture (Engelmann, 2011). *In vitro* culture is one of the main areas of biotechnologies because of its potential utilized to regeneration and conserve valuable plant genetic resources (Gaur *et al*., 2015). The importance of grape rootstocks propagation is screening for different biotic and abiotic stresses induced *in vitro* mutation (Alizadeh *et al*., 2018).

Antioxidant defenses play a major role in counteracting the negative effect of salt stress on the plants, it could be either non-enzymatic (e.g. glutathione, proline, a-tocopherols, carotenoids and flavonoids) or enzymatic (e.g. superoxide dismutase, catalase, glutathione peroxidase, Polyphenol oxidases and glutathione reductase) according to Gill and Tuteja (2010).

Regarding to the effect of salt stress, Proline content is enhanced because it plays a vital role in membrane stabilization in plant cells (Nabati *et al.,* 2011). While, Polyphenol oxidases (PPOs) are a group of Cu-containing enzymes that enhance plant immunity against salt stress (Van Gelder *et al*., 1997 and Oliveira e*t al.,* 2011).

Different studies suggested the chlorophyll A & B content tends to decrease with increasing NaCl doses in two grape (Soltanin and Fakhri) cultivars of (Bybordi A., 2012) and in *P. alba* of (Husaini and Abdin, 2008; Ouyang *et al*., 2010; Rahimi and Biglarifard, 2011 and Amr S., 2018). On the other hand, the toxic effect of Na^+ and Cl^- ions could inhibit carotenoids synthesis and accumulation (Garriga *et al*., 2015).

The aim of this research work is to establish a protocol to evaluate salinity tolerance for two rootstocks Freedom and SO4 by tissue culture and to utilize this regeneration system for improving the efficiency of transgenic salinity resistant grapevine rootstocks by using *Agrobacterium tumefaciens*-mediated transformation system.

Material and Methods

Plant Materials

Two rootstocks SO_4 and Freedom were used in this investigation. The explants were collected from the greenhouse-grown plants from Horticulture Research Institute, Agricultural Research Center, Egypt. The axillary-buds (1.0 cm to 1.5 cm length) of the two rootstocks (Freedom & SO_4) transplants, which were aged 6-12 month-old were used as the culture explants.

Methods

***In vitro* culture and sterilization**

The axillary-buds were thoroughly washed under running tap water three times to remove all dust and debris adhered to its surface ,then soaked in savlon plus drops of Tween 20 for 5min. in as a surfactant on a shaker. The axillary-buds were rinsed thoroughly under running tap water for 35 min. Cleansed explants were dipped in 70 % ethanol solution for one min. under laminar flow hood conditions. The explants were soaked separately in the sodium hypochlorite (NaOCL) solution or commercial Clorox (5.25 % available Chlorine) concentration of 20 for 30min., followed the explants were shacked in a Mercuric chloride (0.25 % $HgCl_2$) solution for 8min. Subsequent to the previous surface sterilization treatments, explants had been rinsed three times in sterile distilled water 3 min. for each rinsing (Sim, 2006).

Sterilized explant were then cultured on establishment media ¾ MS (Murashige & Skoog, 1962) containing 3.5 % sucrose, 0.7 % agar supplemented with 2 mg/l Benzyl aminopurine (BAP) and 0.5 mg/l naphthaline acetic acid (NAA) at pH 5.8 (Lee *et al.,* 2002). After cutting their edges, the explants were cultured as four explants in each glass jar. Cultures were incubated in a temperature controlled chamber at 23–26 °C with a 16/8 hours dark / light and 3000 lux light intensity, provided by cool-white fluorescent light. Relative humidity varied from 55 to 60 %.

Axillary-buds were cultured for four weeks on ¾ MS medium supplemented with 1 mg/l BAP for shoot initiation. While, in multiplication stage were cultured for four weeks on ¾ MS medium supplemented with of 2 mg/l (BAP) and 0.5 mg/l (NAA) and two weeks of elongation stage were cultured on ¾ MS medium with concentrations of 1.5 mg/l GA_3 + 0.5 mg/l NAA. ¾ MS medium without growth regulators was used for rooting stage of three weeks to get plantlets. On the other hand, a plant were subjected to various sodium chloride salinity levels (zero, 2000, 2500 and 3000 ppm) during all tissue culture stages to evaluate the effect of salinity tolerance.

After successful rooting, the rooted plantlets were carefully washed with warm H_2O to remove agar and traces of medium and then they were transplanted to plastic pots containing a 1:1 (v / v) mixture of sterile peat moss and sand. The top of the pots was covered with transparent plastic inside the greenhouse.

Estimation of Proline contents: Contents of free proline (mg/100g of dry w.t.) were estimated according to Bates *et al*. (1973).

Antioxidant Isoenzymes: The activity of polyphenol oxidase isoenzymes was determined according to the method described by Ros Barcelo *et al.* (1987).

Determination of pigments: Quantitative determination of pigments was estimated according to Vernon and Selly (1966).

Polyphenolic components: Determination of phenolic compounds (mg/100g of dry wt.) was carried out according to that method described by Daniel and George (1972).

Statistical Analysis: Experimental data were subjected to one-way analysis of variance (ANOVA) and the differences between means were separated using Duncans multiple range test and the (L.S.D) at 5 % level of probability using Co-state software (Snedecor and Cochran, 1982) .Experiments were designed as factorial in a completely randomized design (CRD). Number of explants survived, the number of shoots, nodes, roots per explants, shoots height and root length was recorded).

Result and Discussions

Most plants species are sensitive to the high concentration of Na^+, which causes a combined ion toxicity and osmotic stress (Horie *et al.,* 2011). Thus, plants have evolved intricate tolerance mechanism for dealing with the salt stress (Zhu, 2003).

The effect of salinity levels on vegetative growth:

In the present investigation, the effect of different concentrations of salt stress on vegetative growth of two rootstocks (SO_4 and Freedom) was evaluated. Full understanding of the physiological and molecular foundation of salt tolerance may help us in producing plants that better cope with salinity.

(a) Establishment stage

The results in Table (1) show the effect of salinity treatments on survival percentage in (SO_4 and Freedom) explants compared with control. It was noticed that increasing salinity from (2000 to 3000 ppm) is accompanied by a significant reduction in the survival rate (%) of two rootstocks. However, Freedom rootstock showed the highest percentage of survival rate (85.25 %) compared to SO_4 rootstock (81.95 %). Also, increasing of salt concentration (2000, 2500 and 3000 ppm) resulted in a decrease for mean percentage of survival rate of two rootstocks i.e., (89, 83.1 and 72.3 %), respectively compared with the control (90 %). Meanwhile, the Freedom rootstock revealed the highest mean percentage of survival rate (91.4, 84.4 and 73.2 %) compared with the SO_4 (86.6, 81.8 and 71.4%) in the different concentrations (2000, 2500 and 3000 ppm) respectively. On the other hand, the highest percentage of survival (92 %) of Freedom rootstock free salt followed by (91.4 and 86.6 %) at salt concentration (2000 ppm) of Freedom and SO_4 rootstocks, respectively. Then, explant survival (84.4 and 81.8 %) at salt concentration (2500 ppm) of Freedom and SO_4 rootstocks, respectively. On the other hand, explants under salt concentration (3000 ppm) of produced the lowest of survival (73.2 and 71.4%) of Freedom and SO_4 rootstocks, respectively.

On the other side, salinity stress resulted in a clear stunting of shoot length in two rootstocks as observed from Table (1). Increasing salinity (from 2000 to 3000 ppm) is accompanied by a significant reduction in shoot length (from 2.15 to 1.58 cm) of Freedom rootstock and from (1.76 to 1.25 cm) of SO_4 rootstock. Several authors reported a reduction in the shoot length due to salinity stress in grapevines (Hawker

and Walker, 1978; Alsaidi *et al.,* 1985; Alsaidi *et al.,* 1988 and Fisarakis *et al.,* 2001).

It is obvious that the new leaves formation mean was influenced by the salt stress concentrations with a greater reduction of mean leave numbers from (2.34 to 1.41) by increasing salinity stress from (2000 to 3000 ppm) respectively. These results of mean leave numbers were significant when compared with that of untreated-salt explants (2.6) as observed from Table (1).

(b) Multiplication stage

Effects of the BAP concentration and different concentrations of salinity on shoot multiplication are presented in Table) 2). By using concentration of 2 mg/l of BAP, 0.5 mg of NAA and different concentrations of salinity, their effect on average number of multiplied shoots, shoot length and average number of leaves was significant. These treatments produced significantly maximum mean number of shoots (4.8) for Freedom rootstock at control cambered with lowest number of shoots (1.55) in SO_4 in (3000 ppm) salt concentration. On the other hand, Abido *et al.* (2013) found the highest mean number of shoots at 2 mg/L of BAP and the least number was found in the absences of BAP. These results were in compatible with previous studies which confirmed that BAP is the most effective on inducing shoot proliferating among all other cytokinins in Vitis (Abido *et al.,* 2013). Also, Zuraida *et al.* (2011) treated Maspine pineapple by with BAP 0, 0.5, 1.0, 2.0 and 5.0 mg/l they found that the highest number of shoots was observed on the medium containing 5 mg/l BAP.

Regarding shoot length, the results showed that different concentrations of salinity have a negative effect on shoot length in each rootstock, where the shoot height decreases with increased concentrations of salt stress. The highest (2.69 cm) of Freedom rootstock in control, followed by (2.3 cm) of SO_4 in the same condition. While the lowest of shoot length (1.29 cm) at a salt concentration of (3000 ppm) of SO_4 rootstock. On the other hand, the average of effect salt concentration on shoot length in two rootstocks was significantly higher compared with control. Data showed that average shoots' length (2.08, 1.84 and 1.46 cm) were observed at ¾MS medium with salinity (2000, 2500 and 3000 ppm) respectively. While, maximum average of shoot length (2.49 cm) observed at ¾MS medium without salinity. These results correlate with those obtained by Be and Debergh (2006).

Regarding salinity effect on leave numbers at two rootstocks as shown in Table (2), there was no significant difference in leave number per adventitious shoot between different rootstocks in same salt concentration. But significant difference was observed between different salt concentrations on same rootstock. Results showed that the number of leaves per adventitious shoot decreased by increasing the concentration of salts. On the ¾ MS medium, the highest number of leaves (3.14) was observed at Freedom rootstock in control and the lowest number of leaves (1.49) at 3000 ppm in SO_4 rootstocks.

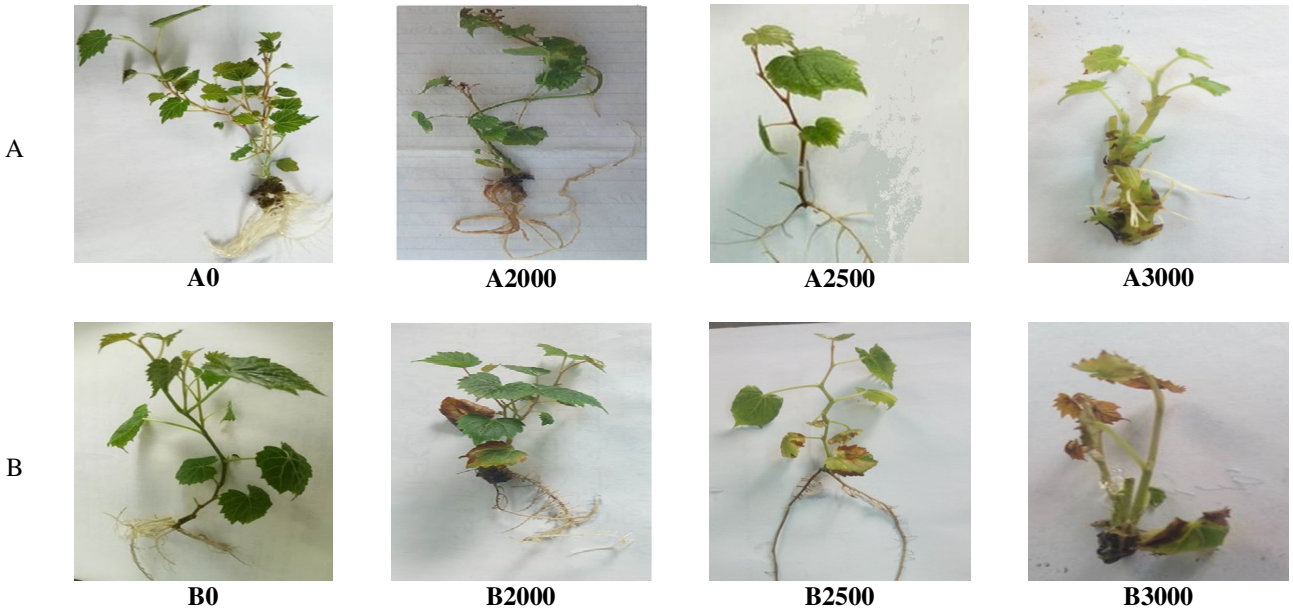

Fig 1. : Effects of NaCl stress on Freedom and SO_4 rootstocks in plantlet stage, from left to right: 0, 2000, 2500 and 3000 ppm, concentrations. A= Freedom & B=SO_4 rootstock.

(c) Plantlet stage:

After successful shoot multiplication the elongated shoots were excised and transferred to free ¾ MS medium supplemented with different concentrations of salt. Data represented in Table (3) and Fig.(1) Revealed that two rootstocks subjected to salinity stress treatments were significantly influenced. The results in Table (3) revealed that the effect of salinity treatments on survival percentage in (SO_4 & Freedom) plantlet compared with control, it was observed that increasing salinity from (2000 to 3000 ppm) led to a significant reduction in plantlet survival rate (%) of both rootstocks. However, data showed that the mean value of the survival percentage was decreasing with increasing of salt concentrations (80, 66.2 and 47.9 %) survival percentage at salinity (2000, 2500 and 3000 ppm) respectively,

compared with the highest percent of survival (86.98 %) at control.

In return, it was found that the effect of salt concentrations compared with control on survival percentage of both rootstocks was significant, the highest percent of survival (90.16 %) of Freedom rootstock followed by (83.8) of SO_4 rootstocks at control. Also, the survival percentage was (82.4 and 77.6 %) at the salt concentration (2000 ppm) of Freedom and So4 rootstocks, respectively. Then, plantlet survival (68.6 and 63.8 %) at salt concentration (2500 ppm) of Freedom and SO_4 rootstocks, respectively. On the other hand, plantlet under salt concentration (3000 ppm) of produced the lowest of survival (49.2 and 46.6 %) of Freedom and SO_4 rootstocks, respectively.

Regarding the interaction between plant height and salinity in ¾ MS medium, Salinity stress resulted in the clear stunting of plant height. Increasing salinity from (2000, 2500 and 3000 ppm) is accompanied by the signification reduction in average plant height from (9.99, 7.41 and 5.42 cm) respectively, compared with highest average of plant height (15.31 cm) in control. Besides, show that the plantlets without salt had the highest plantlet height (16.48 and 14.13 cm) of Freedom and SO_4 respectively. On the other hand, the lowest significant plantlets the height (5.11 cm) was measured with the greater salt concentrations at SO_4 rootstock.

Regarding salinity effect on leave numbers of both rootstocks, there are significant differences between control and salinity concentrations, these results proved that different salt concentrations had significantly affected the average of leave numbers / plantlet as observed in Table (3) and Fig. (1). It was found that increasing salinity from (2000, 2500 and 3000 ppm) led to a significant reduction in average of leave numbers / plantlet of two rootstocks from (10.30, 5.45 and 3.84) leaves, respectively. While, the plantlets at control had the highest average of leave numbers / plantlet (12.73) leaves. On the other hand, the plantlets without salt had the highest average of leave numbers / plantlet (13.38) leaves of Freedom. While, the lowest significant average of leave numbers / plantlet (3.49) leaves was measured with the greatest salt concentrations (3000 ppm) at SO_4 rootstock.

It was noticed that, the primary roots became visible after two weeks of culture in control of each rootstocks. Shoots were rooted in all ¾ MS media during four to five week after subculture. Similarly, Ali-akbar (2016) and Lu (2005) observed root initiation in grape species of *Vitis thunbergii* 11 days after plantlets cultivation. With increasing time of the establishment of explants in the rooting stage, number of rooted decreased in each rootstock with increasing salts.

However, the higher number of roots were observed in Freedom rootstock in ¾ MS media without salt concentration (4.95) roots, compared with the lowest at SO_4 (0.79) roots, at (3000 ppm) salt concentration.

These results indicated that salt stress had a significant effect on root initiation and rooting rate. In this application there are many investigators discussed this work, Pierik *et al.* (1984) and Danso *et al.* (2008) reported that in vitro rooting of pineapples can be enhanced by an addition of auxins such as NAA, IBA or combination of NAA and IBA in the medium. On the other side, root length was negatively affected by salinity stress on each Freedom & SO_4 plantlets with higher reduction values more than the control. However, from the obtained results Freedom rootstock proved higher resistance to salinity than SO_4 as observed from Table (3) and Fig. (1).

The effect of salinity levels on photosynthetic pigments:

Data in Table (4) reveal that salinity stress negatively affected plant pigments content. It can be observed that chlorophyll A content tended to decrease with increasing NaCl doses in both rootstock plants. However, significant higher content can be seen in Freedom compared to SO_4 rootstocks, especially by increasing NaCl as shown in Fig. (1).

The concentration of chlorophyll B showed the same trend by increasing salinity stress of NaCl. In terms of the total chlorophyll concentration, the same tendency is observed. However, there is a highly significant difference between total chlorophylls at the highest NaCl concentration (0.0 and 3000 ppm) in each rootstocks (10.8 and 4.29 mg/g) in Freedom plants, (10.07 and 3.83 mg/g) in SO_4, respectively.

Besides, with increasing salt stress, the carotenoids tended to diminish with no significant difference, in both rootstock plants. However, a significant decrease is observed of the carotenoids at the highest NaCl concentration (3000 ppm) with value of (0.13 and 0.12 mg/g) for Freedom and SO_4 plants, respectively.

Chlorophylls are pigments whose concentrations are reduced by salt stress (Husaini and Abdin, 2008; Rahimi and Biglarifard, 2011 and Ouyang *et al.,* 2010) the reduction in leaf chlorophylls concentration could be due to the interference of Na and Cl^- ions with the activity of enzymes associated with chlorophyll biosynthesis or disturbance in the integration of the chlorophyll molecules in stable complexes (Garriga *et al.,* 2015). Additionally, the salt stress could increase chlorophyllase activity, which may be caused by a depressive effect of salinity on up take of Mg^2 and Fe^2 ions which are involved in the formation of chloroplasts (Hanafy Ahmed *et al.,* 2002). On the other hand, the toxic effect of Na and Cl^- ions could inhibit carotenoids synthesis and accumulation (Garriga *et al.,* 2015).

The effect of salinity levels on total phenols and free proline

Data generated in Table (5) showed that the salt stress, cause a marked significant increase in total phenols of shoots & roots of the plants. Concerning the effect of salt concentrations on total phenols of the rootstock plants, it was found that SO_4 in the salt concentration (3000 ppm) was the highest (3.13 mg GAE/g). In contrast, the lowest total phenols (1.59 and 1.55 mg GAE/g) in SO_4 and Freedom respectively, at free salt are non-significant.

Also, it can be observed from data in Table (5) that a significant accumulation of proline in leaf tissues took place as affected by salt stress increase. The proline content of plants exposed to high levels of NaCl was greater than plants exposed to low levels of NaCl. While, under the highest salt stress (3000 ppm) the results were (2.23 and 2.07 mg/100 mg) to Freedom and SO4 rootstocks, respectively. On the contrary, the proline content of plants was decreased (0.98 and 0.88 mg/100 mg) under the low salt stress (0.0 ppm) of Freedom and SO_4 rootstocks, respectively. Increasing proline

content in the leaves with increasing water salinity might be attributed to the increase of hydrolytic enzymes caused by chloride salts and salinity (Klyskov and Rakova, 1964). Furthermore, leaf proline content has been used as an evaluation parameter for selecting salinity and drought resistant varieties (Bates *et al*., 1973). In addition, plants build up proline in the tissues to maintain osmotic balance with the soil solution (Salisbury and Ross, 1992). In this connection, El-Said *et al*. (1995) and Abbas (1999) suggested that proline functions as a source of solute for intera-cellular osmotic adjustments under saline condition. The obtained results confirm those previously discussed by Derbew (2006), free proline accumulation in the leaves of grape rootstocks, commercial varieties and rootstock-scion combinations progressively increased with the increase in salt concentration.

The effect of salinity levels on polyphenol oxidase

Isozymes merely represent different structure configuration of the same polypeptide chain of an enzyme (Morsy, 2007). For this reason polyphenyl oxidase (ppo) were used to study the effect of different salt concentration on each rootstocks. Polyphenyl oxidase electrophoretic patterns in Fig. (2 and 3) and Table (6 and 7) exhibit a maximum of four consisted bands very well visible on the gel to each rootstock, though tightly associated with relative mobility range (from 0.343 to 0.935) of Freedom in Table (6) and (0.345 to 0.894) in Table (7) of SO_4, characterized by a clear inter and intra polymorphism in bands presence and absence and variable densities among different concentration of salt stress and control samples.

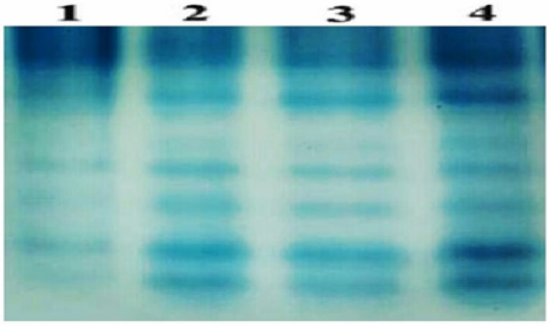

Fig. 2 : Patterns of polyphenyl oxidase (PPO) isozymes in Freedom rootstock under salt stress lanes 1-4 (1=0, 2=2000, 3=2500 and 4=3000 ppm NaCl respectively).

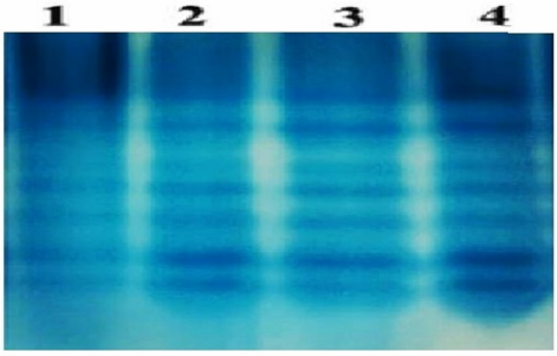

Fig. 3 : Patterns of polyphenyl oxidase (PPO) isozymes in SO_4 rootstock under salt stress lanes 1-4 (1=0, 2=2000, 3=2500 and 4=3000 ppm NaCl respectively).

The results indicated that with increasing salt stress concentration, it showed that increased of polyphenol expression evidence of affected of salinity stress on plant cells. This is in agreement with (Qin *et al.,* 1998 and Sairam *et al.,* 2005) they stated that increase in toxic that effect on membrane and that it damaged induced among plants under salt stress.

Conclusion

Our results indicated that SO_4 and Freedom grape rootstocks had good growth and salt stress tolerance, as well as had higher free proline accumulation and potassium contents especially Freedom grape rootstock. Contrary, SO_4 grape rootstock they had lowest in salt stress tolerance. Also, the high contents of phenolic compounds indicated that these compounds contribute to the antioxidant activity. But these advantages will eventually to conclude that SO_4 and Freedom can be used as suitable rootstocks for tolerance of salinity stress.

Authors' Contributions

All authors contributed effectively. The Prof. Soliman, M.H. and Prof. Basita, A.H. designed the experiments, Supervised the research and then revised the paper with Prof. Nahla, A.A. Also, Barakat, A.A. conducted the experiments, analyzed the data and wrote the paper.

Table 1: Effect of NaCl treatments on Survival %, Shoot length and Leave number of SO_4 and Freedom rootstocks during establishment stage.

Treatments	Survival %		Means (B)	Shoot length (cm)		Means (B)	Leave number		Means (B)
	SO_4	Freedom		SO_4	Freedom		SO_4	Freedom	
Control	88 a	92 a	90 a	1.82 a	2.32 a	2.07 a	2.4 a	2.8 a	2.6 a
2000 ppm	86.6 a	91.4 a	89 a	1.76 ab	2.15 ab	1.96 a	2.3 a	2.38 a	2.34 ab
2500 ppm	81.8 ab	84.4 ab	83.1 a	1.49 ab	1.89 bc	1.69 ab	1.94 a	2.04 ab	1.99 b
3000 ppm	71.4 b	73.2 b	72.3 b	1.25 b	1.58 c	1.42 b	1.39 b	1.44 b	1.41 c
MEANS (A)	**81.95**	**85.25**		**1.58**	**1.99**		**2.01**	**2.17**	

Numbers connected by the same letters in the same column are not significantly different. While, Different letters in the same column denote significant differences at 5% probability level.

Table 2: Effect of NaCl treatments on Shoot length, Shoot number, Leave number of SO_4 and Freedom rootstocks during multiplication stage.

Treatments	Shoot number per explant		Means (B)	Shoot length (cm)		Means (B)	Leave number		Means (B)
	SO_4	Freedom		SO_4	Freedom		SO_4	Freedom	
Control	4.12 a	4.8 a	4.46 a	2.3 a	2.69 a	2.49 a	2.84 a	3.14 a	2.99 a
2000 ppm	3.14 b	3.72 b	3.43 b	1.9 ab	2.25 b	2.08 ab	2.5 ab	2.64 ab	2.57 ab
2500 ppm	2.2 c	2.76 c	2.48 c	1.69 bc	1.99 bc	1.84 bc	1.99 bc	2.19 bc	2.09 bc
3000 ppm	1.55 d	1.96 c	1.75 d	1.29 c	1.62 c	1.46 c	1.49 c	1.72 c	1.60 c
MEANS (A)	**2.75**	**3.31**		**1.79**	**2.14**		**2.21**	**2.42**	

Numbers connected by the same letters in the same column are not significantly different. While, Different letters in the same column denote significant differences at 5% probability level.

Table 3: Effect of NaCl treatments on plantlet length, Leave number, Root number, Root length of SO_4 and Freedom rootstocks during rooting stage.

Treatments	Survival %		Means (B)	Average plantlet length (cm)		Means (B)	Average leave number		Means (B)	Average root number		Means (B)	Average primary root length (cm)		Means (B)
	SO_4	Freed		SO_4	Freedom		SO_4	Freedom		SO_4	Freedom		SO_4	Freedom	
Control	83.8 a	90.16 a	86.9 a	14.1 a	16.48 a	15.3 a	12.1 a	13.38 a	12.7 a	4.88 a	4.95 a	4.92 a	13.6 a	15.46 a	14.5 a
2000 ppm	77.6 a	82.4 ab	80 a	9.5 b	10.48 b	9.99 b	9.72 b	10.88 a	10.3 b	3.02 b	3.67 b	3.35 b	10.5 b	11.41 b	10.9 b
2500 ppm	63.8 b	68.6 b	66.2 b	6.8 c	7.97 c	7.41 c	4.96 c	5.94 b	5.45 c	1.9 bc	2.63 c	2.25 c	5.47 c	7.29 c	6.38 c
3000 ppm	46.6 c	49.2 c	47.9 c	5.1 c	5.73 c	5.42 d	3.49 d	4.19 b	3.84 c	0.79 c	1.52 d	1.16 d	2.86 d	3.98 c	3.42 d
MEANS (A)	**67.95**	**72.59**		**8.88**	**10.17**		**7.57**	**8.59**		**2.65**	**3.19**		**8.11**	**9.54**	

Numbers connected by the same letters in the same column are not significantly different. While, Different letters in the same column denote significant differences at 5% probability level

Table 4: Plant pigments content (chlorophyll A, B and carotenoids mg/g f.wt.) in SO_4 and Freedom plant leaves subjected to different NaCl levels.

Treatments	Chlorophyll (a) mg/g f.wt		Means (B)	Chlorophyll (b) mg/g f.wt		Means (B)	Total (a + b) mg/g f.wt.		Means (B)	Carotenoid mg/g fresh weight		Means (B)
	SO_4	Freedom		SO_4	Freedom		SO_4	Freedom		SO_4	Freedom	
Control	6.33 a	6.93 a	6.63 a	3.7 a	3.87 a	3.8 a	10.1 a	10.8 a	10.4 a	0.54 a	0.55 a	0.54 a
2000 ppm	5.4 b	5.56 b	5.48 b	2.5 b	2.53 b	2.5 b	7.9 b	8.09 b	7.99 b	0.42 b	0.43 b	0.42 b
2500 ppm	3.8 c	3.96 c	3.88 c	1.8 bc	2.02 bc	1.9 bc	5.57 c	5.98 c	5.78 c	0.2 c	0.22 c	0.21 c
3000 ppm	2.83 d	3.09 c	2.96 d	0.99 c	1.19 c	1.1 c	3.83 d	4.29 d	4.06 d	0.12 d	0.13 d	0.12 d
MEANS (A)	**4.59**	**4.89**		**2.25**	**2.4**		**6.84**	**7.29**		**0.32**	**0.33**	

Numbers connected by the same letters in the same column are not significantly different. While, Different letters in the same column denote significant differences at 5% probability level

Table 5: Effect NaCl treatments on total phenol (mg/100g d.wt (g) and free proline (mg/100g d.w) in SO_4 and Freedom rootstocks.

Treatments	Total Phenol mg/100g d.wt (g)		Means (B)	Free Proline mg/100g d.w		Means (B)
	SO_4	Freedom		SO_4	Freedom	
Control	1.59 c	1.55 c	1.57 c	0.88 c	0.98 b	0.93 c
2000 ppm	2.19 bc	2.13 bc	2.16 bc	1.21 bc	1.44 ab	1.32 bc
2500 ppm	2.67 ab	2.58 ab	2.62 ab	1.68 ab	1.91 a	1.79 ab
3000 ppm	3.13 a	3.05 a	3.09 a	2.07 a	2.23 a	2.15 a
MEANS (A)	2.4	2.33		1.46	1.64	

Numbers connected by the same letters in the same column are not significantly different. While, Different letters in the same column denote significant differences at 5% probability level.

Table 6: Patterns of polyphenyl oxidase (PPO) isozymes in Freedom rootstock under salt stress lanes 1-4 (0, 2000, 2500 and 3000 ppm) NaCl, respectively.

Treatments (NaCl / RF)	Lane 1	Lane 2	Lane 3	Lane 4
0.343	+	++	++	+++
0.388	+	+	+	+
0.685	+	++	++	++
0.782	+	+	++	++
0.894	++	++	+++	+++
0.935	+	++	++	+++

(+) low density, (++) moderate density and (+++) high density.

Table 7: Patterns of polyphenyl oxidase (PPO) isozymes in SO_4 rootstock under salt stress lanes 1-4 (0, 2000, 2500 and 3000 ppm) NaCl, respectively.

Treatments (NaCl / RF)	Lane 1	Lane 2	Lane 3	Lane 4
0.345	+	+	++	+++
0.417	+	+	+	+
0.437	+	++	++	++
0.663	+	+	+	+
0.859	++	++	+++	+++
0.894	+	++	++	+++

(+): low density, (++) moderate density and (+++) high density.

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