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Influence of Vesicular-arbuscula Mycorrhizal Fungi (*Glomus* spp.) on the Response of Grapevines Rootstocks to Salt Stress

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ABSTRACT

Mycorrhizal fungi, a symbiotic relationship between plant roots and beneficial fungi are supposed to impart the stress tolerance in the host plants. The present study was conducted to evaluate the effectiveness of AM fungi with three grapevines rootstocks under salt stress. Three grapevines rootstocks (Dogridge, 1103 Paulsen and Harmony) were irrigated at three different NaCl concentrations measured by electrical conductivity (0.65, 1.56 and 4.68 dS m^{-1}). Salinity decreased Arbuscular Mycorrhizal (AM) colonization. In both the AM and non AM, plant height, stem diameter, leaf area, total leaves number/plant and total dry weight were decreased under salinity. The plants inoculated with the AM fungus had significantly higher growth parameters compared to the non-inoculated plants. Increasing the salinity level tended to increase the proline accumulation and the concentrations of leaf N, Na and Cl while the chlorophyll content, leaf total carbohydrates, leaf P and K decreased. On the contrary, Inoculated the seedlings with AM tended to increase the levels of chlorophyll content, proline, total carbohydrates and the concentrations of leaf P and K. Whereas leaf N, Cl in the second season and Na of inoculated seedlings was significantly lower than that of un-inoculated ones.

Key words: Grape, salinity, mycorrhizal inoculation, chlorophyll, proline

INTRODUCTION

Grape (*Vitis* spp.) is one of the most commercially grown important fruit crops in Egypt. Plantation of the grape cultivars in Egypt has been progressively developed in the last few years. However, a large part of new reclaimed lands suffers from salinity.

Soil salinity in the Mediterranean basin, especially in countries in arid and semi-arid zone appears as one of the main factors limiting plant growth and biomass production (Belew *et al*., 2010). Salinity affects plant performance through the development of osmotic stress that impedes transpiration and photosynthesis (Shannon and Grieve, 1999). Toxicity, deficiency, or changes in mineral balance are specific mechanisms which in turn cause metabolic dysfunction relates to ion uptake and altered physiological processes (Hasegawa *et al*., 2000). The capability of trees to tolerate different levels of salinity varies among rootstocks. The salinity tolerance induced by rootstocks is attributed to root system restricting the movement and/or avoiding absorption and accumulation of toxic ions from the saline soils (Hepaksoy *et al*., 2006).

The benefits of Arbuscular Mycorrhizal (AM) symbiosis on plant fitness are widely known, including improved mineral nutrition in nutrient-poor soils and increased capability to overcome biotic and a biotic stresses (Smith and Read, 2008). Mycorrhizal colonization is often thought to increase the ability of salt tolerance in certain plant species (Ojala *et al*., 1983).

Grapevines are dependent on vesicular arbuscular mycorrhizae colonization and the potential of AM fungi to form symbiosis spontaneously with the roots of grapevines has been well documented. (Cheng and Baumgartner, 2004).

Therefore, the objective of this investigation was intended to study the effect of different water salinity levels on the physiology of three grape rootstocks (Dogridge, 1103 Paulsen and Harmony). Besides, the effects of the mycorrhizae (VAM) on the tolerance of grape rootstocks to salinity stress was also undertaken.

MATERIALS AND METHODS

Plant material: The study was conducted during the 2010 and 2011 growing season at private farm in Mariot region, near Alexandria city, Egypt. One-year-old of three grape rootstocks namely Dogridge (*V. champini*), 1103 Paulsen (*V. berlandieri*×*V. rupestris*) and Harmony (*V. champini*×1613) were used. The experimental plants were singly planted in black polyethylene bags filled with about two kilograms of sandy soil. The chemical composition of the experimental soil is shown in Table 1.

Arbuscular mycorrhizal fungi inoculums application: At mid of March 2010 and 2011, 90 Dogridge, 1103 Paulsen and Harmony seedlings were chosen for this study. The experimental plants were divided equally into two groups each of 45 plants. One group plants were inoculated by mycorrhizae*, Glomus intraradices* while the second was left without mycorrhizal inoculation; as control. Inoculation was achieved by adding 10 g/plant of the inoculum in the soil under the seedlings. The mycorrhizal strain *Glomus intraradices,* isolated from the Experimental station of Alexandria University at Abies, (Aboul-Nasr, 1993), was used in both experimental seasons. The inoculum consists of expanded clay aggregates (2-4 mm in diameter, Leca) which contain chlamydospores and fungus mycelium. The mycelium had been produced on *Tagetes erecta* L. (Aboul-Nasr, 2004). The control plants received the same amount of heat sterilized expanded clay. Thereafter, the plants were carefully handled in the glasshouse for about two months before the commencement of the salinity treatments. During this period of adaptation; all plants started new growth and seemed healthy, vigorous and well established. Chemical analysis of the experimental irrigation water is shown in Table 1.

Table 1: Chemical analysis of irrigation water and soil used at experimental planting

Characteristics	Soil	Water
pH	7.75	7.98
E.C (mmohs/cm)	1.35	0.55
Chemical properties: (meq/100 g) (meq/L)		
Sodium (Na^+)	6.28	1.55
Potassium (K^+)	0.39	0.19
Calcium (Ca^+)	1.39	1.23
Magnesium (Mg^{++})	5.45	1.55
Bicarbonates (HCO_3)	2.62	1.70
Chloride (Cl^-)	4.90	1.12
Sulfates (SO_4)	3.40	1.10
Organic matter (%)	0.15	0.00

Application of salinity treatments: At mid-May of each season, each group, with or without mycorrhizae (VAM) were irrigated at three different NaCl concentrations measured by electrical conductivity 0.65 (control), 1.56 and 4.68 dS m^{-1}. Each plant was irrigated twice a week by 500 mL from the experimental salt solution. All experimental plants were irrigated with tap water once every month to avoid salt accumulation in the root zone. Thirty plants from each rootstock were used in either year of study (3 salinity levels x 2 mycorrhizae treatments (with or without AM)×3 rootstocks×5 replicates) = 90 plants, 30 for each rootstock in each experimental season). The treatments were terminated when 75% of the leaf blades of the seedlings irrigated by the highest salinity level were irreversibly injured. This lasted for 125 days, in 2010 season and 147 days, in 2011 season.

Response measurements

Mycorrhizal root colonization percentage: At the end of each experimental season the percentage of mycorrhizal root colonization was estimated by the observation of cleared and stained 1 cm root segments under a research microscope for every treatment the procedure was done according to Gianinazzi (2004).

Determination of vegetative growth: Vegetative growth parameters included plant height; the number of leaves was recorded at zero time and at the end of each season. Besides, the stem diameter and leaf area was estimated. The stem diameter of each replicate was measured at the soil surface. For leaf area determination, five leaves from the middle of one shoot per plant were collected at the end of the experimental and their areas were measured using a planimeter. Leaf total chlorophyll content was determined in the fresh leaf samples according to the method described by Yadava (1986), using a Minolta SPAD chlorophyllmeter. Five readings were taken for each plant at the end of either season. The results were expressed as SPAD units.

Determination of biochemical status: At the termination of the experiment, the plants were carefully excavated from the polyethylene bags. The leaves, stem and roots of each experimental plant were separated, washed several times with tap water and then with distilled water. The different tissues of each seedling were then oven dried at 70°C until a constant weight and the dry weights of the leaves, stems and roots of each plant were recorded. The proline estimation was done using dry plant samples (leaves) according to Bates *et al.* (1973). While the total carbohydrates estimation were done by the Nelson-Somogyi method using oven dried samples as described by Thimmaiah (2004).

Determination of nutritional parameters: For mineral elements determination, 0.1 g from the dried ground materials of the leaf tissues of each experimental plant was digested with diacid solution (HNO_3 and $HCLO_4$, 9:4). This extract was used for the estimation of phosphorus, potassium, sodium and chloride. The nitrogen estimation was done by micro Kjeldahl method. All the nutrient analyses were done as per the procedures given by Bhargava and Raghupathi (1998). The concentration of nitrogen, phosphorus, potassium, sodium and chloride were expressed as percent on dry weight basis.

Statistical analysis: Data were analyzed as a 3×3×2 factorial design with five replications. The factors were three rootstocks (R: R1 = Dogridge, R2 = 1103 Paulsen and R3 = Harmony), three salinity levels (S: S1 = 0.65, S2 = 1.56 and S3 = 4.68 dS m^{-1}) and Mycorrhizae (Myco: M0 = uninoculated and M1 = inoculated). The analysis of variance of treatments differences was performed according to Steel and Torrie (1980). Statistical analysis was done by ANOVA, F-test and LSD procedures available within the SAS software package (Version 9.13, 2008). Data for the percentage of mycorrhizal colonization were analyzed after angular transformation (Steel and Torrie, 1980). Simple regression and determination coefficients were done using GLM model with statistica release 7.

RESULTS AND DISCUSSION

Mycorrhizal colonization: All three rootstocks responded positively to the mycorrhizal inoculation. The mycorrhizal colonization was observed at all developed salt levels. With increasing salt stress, the root colonization decreased significantly, in 2010, from 55.11-39.18% in Dogridge, from 57.70-44% in 1103 Paulsen and from 43.15-27% in Harmony. The highest values were observed in non-saline mycorrhizal treatment (55.11, 59.13%) for Dogridge rootstock, (57.70, 58.16%) for 1103 Paulsen and (43.15, 43.12%) for Harmony rootstock. Figure 1 declared the colonized root length percentage of Dogridge, 1103 Paulsen and Harmony rootstocks in case of inoculated plants with *Glomus intraradices* and grown under different salinity levels. Significant regression coefficients were observed between salinity and mycorrhizal root colonization percentages when the overall data of both experimental seasons was statistically analyzed. The decline in colonization under stress could be caused by adverse conditions for soprulation and development of spores under unfavorable rhizosphere conditions (Murkute *et al.* 2006).

Growth parameters: Data presented in Table 2 and 3 generally indicated that the seedlings height, stem diameter, leaf area and total dry weight tended to respond negatively to any increase in salinity stress of the growth media. Nevertheless, the magnitude of reduction in these growth indices greatly varied with three rootstocks. For example, in 2010 season, Dogridge seedlings irrigated with 1.56 and 4.68 dS m^{-1} salinity levels showed a reduction in leaf area (53.18 and 45.12 cm^2), respectively, in comparison with those grown under control. The corresponding values for 2011 season were 47.16 and 30.47 cm^2. There seemed to be a marked relationship between salinity and growth parameters studied. The regression analysis between salinity and plant height, stem diameter, leaf area and total dry weight might support such notation. Significant regression coefficients were observed between these variables when the overall data of both experimental seasons was statistically analyzed (Table 2, 3). Similar results were also reported by numerous investigators; such as: Anjum (2008), Derbew *et al.* (2010) and Upreti and Murti (2010). They all concluded that the growth of grape was decreased by increasing salinity levels in the growing media.

Concerning the effect of rootstocks on growth indices, in both seasons, although all growth parameters values of Dogridge were higher than those of 1103 Paulsen and Harmony rootstocks except for leaves numbers per plant, 1103 Paulsen rootstock has the highest values in the first season. No significant differences were detected between Dogridge and Harmony rootstocks except

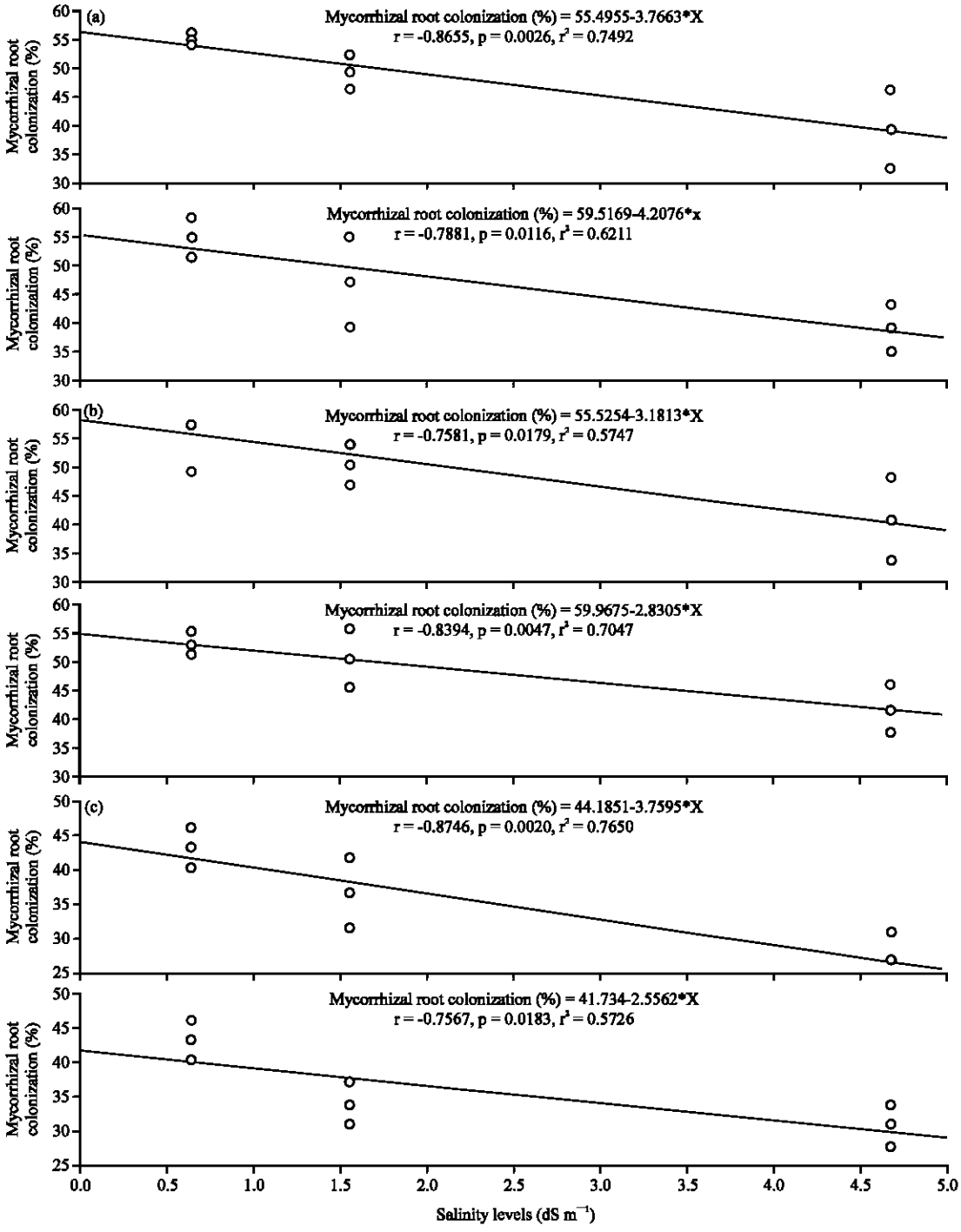

Fig. 1(a-c): Effect of salinity stress on AM root colonization (%) of Dogridge, 1103 Paulsen and Harmony rootstocks in the first and second season, (a) Linear regression between salinity levels and mycorrhizal root colonization% of Dogridge rootstock in 2010 and 2011 seasons, (b) Linear regression between salinity levels and mycorrhizal root colonization% of 1103 Paulsen rootstock in 2010 and 2011 seasons and (c) Linear regression between salinity levels and mycorrhizal root colonization% of Harmony rootstock in 2010 and 2011 seasons

for total dry weight in both seasons, stem diameter in the first season and leaf area in the second season. Sahnity stress had strong effects on growth in all the rootstocks tested. However, the extent of the reduction differed among the rootstocks. Derbew *et al.* (2010); working on Dogridge, Salt creek, 1613 and St.George. They reported that Dogridge was generally the best rootstock in growth and salt tolerance than other tested rootstocks. The effect of Mycorrhizal (AM) inoculation on the growth indices of the experimental seedlings is shown in Table 2. The result generally indicated that the growth indices of all rootstocks obviously increased as a result of AM inoculation in comparison with those grown without AM. This general trend was observed in all rootstocks during both experimental seasons. For example, in 2010 season, 1103 Paulsen seedlings under 1.56 dS m^{-1} salinity level with mycorrhizal inoculation had the highest value of total dry weight (61.76 g) while the seedlings without mycorrhizal inoculation had the lowest one (91.72 g).

Table 2: Effect of salinity and mycorrhizal inoculation on the growth parameters of grape rootstocks during 2010 season

		Plant height (cm)			Stem diameter (mm)			Leaf area (cm^2)			Total leaf number/plant			Total dry weight (g)		
Treatments		Salinity levels			Salinity levels			Salinity levels			Salinity levels			Salinity levels		
Mycorrhizae	Rootstocks	S0	S1	S2	S0	S1	S2	S0	S1	S2	S0	S1	S2	S0	S1	S2
M0	R1	112.6	95.0	85.4	10.19	7.38	6.11	56.34	47.16	30.48	81.33	64.67	44.67	110.28	86.33	73.67
	R2	77.5	72.6	54.5	7.17	6.45	5.05	33.16	15.02	11.12	86.67	70.67	68.00	88.75	61.76	56.07
	R3	100.6	93.0	80.5	10.76	8.92	6.96	51.42	45.74	32.31	72.33	61.33	51.00	31.37	27.39	14.48
M1	R1	143.36	124.5	99.9	9.51	8.81	7.72	71.48	58.82	52.78	69.33	75.67	62.00	135.11	114.24	86.62
	R2	87.3	86.3	76.4	6.96	7.88	5.79	41.62	24.69	19.40	97.00	91.33	84.33	105.38	91.72	27.89
	R3	122.7	108.4	99.4	11.39	10.52	7.80	75.12	55.46	50.45	75.00	61.33	54.67	46.77	39.00	27.89
Mean salinity (S)																
	S0	107.39			9.32			54.83			80.27			85.72		
	S1	96.65			8.32			41.15			70.83			70.07		
	S2	82.64			6.72			32.75			60.77			55.39		
LSD (0.05)		6.43			6.57			6.21			7.17			7.48		
Mean Myco.(M)																
	M0	85.75			7.66			35.84			66.74			61.12		
	M1	105.35			8.48			49.98			74.51			80.03		
LSD (0.05)		14.42			1.28			12.07			20.48			12.60		
Mean Rootstock (R)																
	R1	110.15			8.28			52.84			66.27			101.04		
	R2	75.79			6.55			24.17			83.00			79.55		
	R3	100.73			9.39			51.73			62.61			30.59		
L.S.D.(0.05)		10.95			0.95			5.91			8.36			6.38		
Interactions	S×M	ns			ns			ns			ns			ns		
	S×R	ns			ns			ns			ns			ns		
	M×R	ns			ns			ns			ns			ns		
	S×M×R	ns			ns			ns			ns			ns		
Regression Coefficient (R^2)																
(r)	R1	0.80**			0.68**			0.65**			0.91**			0.71**		
	R2	0.70**			0.88**			0.76**			0.63*			0.70**		
	R3	0.76**			0.68**			0.79**			0.76**			0.73**		

Rootstocks (R): R1: Dogridge, R2: 1103 Paulsen, R3: Harmony: Mycorrhizal treatment (M): M0: Uninoculated, M1: Inoculated, Salinity levels (S): S0: 0.65 dS m^{-1} control, S1: 1.56 dS m^{-1}, S2: 4.68 dS m^{-1}, (d.w. = dry weight). Different letters in each column indicate a significant difference ($p<0.05$), ns: No significant, *Significant at $p<0.05$, **Significant at $p<0.01$

Table 3: Effect of salinity and mycorrhizal inoculation on the growth parameters of grape rootstocks during 2011 season

Treatments		Plant height (cm)			Stem diameter (mm)			Leaf area (cm^2)			Total leaf number/plant			Total dry weight (g)		
		Salinity levels			Salinity levels			Salinity levels			Salinity levels			Salinity levels		
Mycorrhizae	Rootstocks	S0	S1	S2	S0	S1	S2	S0	S1	S2	S0	S1	S2	S0	S1	S2
M0	R1	151.40	122.10	89.90	13.84	11.35	9.22	79.71	53.18	45.12	91.00	85.67	58.00	148.50	141.83	122.40
	R2	93.60	74.60	53.90	10.28	7.40	6.70	22.12	13.21	10.82	93.67	84.33	67.67	141.47	124.88	87.10
	R3	161.00	116.80	109.60	11.43	8.78	6.54	41.15	30.50	23.94	91.33	79.33	65.00	40.56	29.10	19.05
M1	R1	173.6	155.0	142.2	12.79	12.72	8.78	89.84	83.22	50.56	93.33	80.67	74.33	165.09	150.09	133.04
	R2	102.6	90.5	92.6	9.69	7.98	6.73	31.22	18.15	14.22	119.33	90.33	77.00	159.56	129.07	70.67
	R3	154.9	143.5	121.0	15.55	15.35	11.44	56.94	50.78	38.83	92.67	84.00	75.00	54.45	41.50	26.16
Mean salinity (S)																
	S0	139.52			12.26			53.49			96.88			118.27		
	S1	117.07			10.43			41.50			84.05			102.74		
	S2	101.54			8.23			30.56			69.60			76.40		
LSD (0.05)		14.32			0.55			6.50			9.44			8.75		
Mean Myco.(M)																
	M0	108.11			9.50			35.51			79.55			94.99		
	M1	130.65			11.22			48.19			87.40			103.29		
LSD (0.05)		9.43			0.24			11.21			20.11			8.28		
Mean rootstock (R)																
	R1	139.04			11.45			66.94			80.50			143.49		
	R2	84.62			8.13			18.29			88.72			118.79		
	R3	134.48			11.35			40.34			81.22			35.13		
LSD (0.05)		18.90			0.71			5.75			10.48			9.25		
Interactions	S×M	N.S			1.50			N.S			N.S			N.S		
	S×R	N.S			1.34			N.S			N.S			N.S		
	M×R	N.S			1.73			N.S			N.S			N.S		
	S×M×R	N.S			N.S			N.S			N.S			N.S		
Regression coefficient (R^2)																
(r)	R1	0.84**			0.91**			0.76**			0.65**			0.88**		
	R2	0.75**			0.73**			0.58**			0.69**			0.82**		
	R3	0.60**			0.86**			0.50**			0.72**			0.83**		

Rootstocks (R): R1: Dogridge, R2: 1103 Paulsen, R3: Harmony: Mycorrhizal treatment (M): M0: Uninoculated, M1: Inoculated, Salinity levels (S): S0: 0.65 dS m^{-1} control, S1: 1.56 dS m^{-1}, S2: 4.68 dS m^{-1}, (d.w. = dry weight). Different letters in each column indicate a significant difference ($p<0.05$), ns: No significant, *Significant at $p<0.05$, **Significant at $p<0.01$

Organic components: The data listed in (Table 4 and 5) indicated that, seedlings irrigated with 1.56 and 4.68 dS m^{-1} salinity levels showed significant decrease in leaf chlorophyll content and leaf total carbohydrates during both years of study, whereas, that of leaf free proline content tended to increase. In 2010 season, seedlings irrigated with 4.69 dS m^{-1} salinity level showed increase in leaf free proline content reached as much as 23.7%, in comparison with those grown under control. The corresponding value for 2011 season was 21.7%. Murkute *et al.* (2006) reported that proline and various betaines can function as osmoprotectants and cryprotectants, when accumulated in cell. The reduction in leaf total carbohydrates in seedlings grown under 4.68 dS m^{-1} salinity level, reached as much as 20.3 and 23% in the first and second season, respectively as compared with control. The corresponding values for leaf chlorophyll content were 16.8 and 18.9%. These results were in harmony with those found by Arbona *et al.* (2005). They attributed the low carbohydrates content of salt treated plant to decreases in CO_2 assimilation. The adverse effects of water salinity on total chlorophyll content in the leaves can be attributed to the suppression of specific enzymes that are responsible for the synthesis of photosynthetic pigments Murkute *et al.* (2006).

Table 4: Effect of salinity and mycorrhizal inoculation on the organic components and mineral composition of grape rootstocks during 2010 season

		Chlorophyll content (SPAD unit)			Proline (mg/100 g d.w.)			Leaf total carbohydrates (% on d.w. basis)			N %			P%			K%			Na%			Cl%		
Treatments		Salinity levels			Salinity levels			Salinity levels			Salinity level			Salinity levels			Salinity levels			Salinity levels			Salinity levels		
Mycorrhizae	Rootstocks	S0	S1	S2	S0	S1	S2	S0	S1	S2	S0	S1	S2	S0	S1	S2	S0	S1	S2	S0	S1	S2	S0	S1	S2
M0	R1	27.13	25.05	21.73	51.27	53.81	68.97	12.03	10.12	8.24	1.61	2.02	2.70	0.39	0.33	0.28	1.15	0.89	0.81	0.29	0.30	0.38	0.20	0.24	0.27
	R2	25.62	24.05	19.04	61.17	61.96	86.22	12.00	10.91	10.09	1.08	1.70	1.92	0.50	0.39	0.35	0.89	0.79	0.67	0.31	0.39	0.50	0.24	0.30	0.40
	R3	26.29	25.20	20.17	70.43	75.81	86.14	14.25	13.19	12.05	2.03	2.57	3.30	0.64	0.55	0.48	0.76	0.67	0.58	0.55	0.68	0.71	0.41	0.61	0.73
M1	R1	30.33	29.70	27.15	58.45	67.32	104.47	12.63	11.35	10.18	1.37	1.79	2.49	0.45	0.41	0.34	1.34	0.95	0.70	0.26	0.27	0.36	0.19	0.22	0.26
	R2	28.16	26.00	24.20	71.71	81.09	97.89	13.25	11.79	10.14	1.06	1.44	1.17	0.61	0.50	0.42	1.12	0.87	0.78	0.32	0.38	0.47	0.22	0.29	0.37
	R3	30.19	31.19	28.46	75.38	85.23	49.50	16.04	15.77	12.99	1.80	1.99	2.82	0.75	0.65	0.50	0.81	0.72	0.62	0.51	0.59	0.67	0.43	0.60	0.79
Mean salinity (S)																									
	S0	27.95			64.73			13.36			1.49			0.56			1.01			0.37			0.28		
	S1	26.86			70.87			12.18			1.91			0.47			0.81			0.43			0.37		
	S2	23.45			89.69			10.61			2.39			0.39			0.69			0.51			0.47		
LSD (0.05)		1.46			7.00			0.44			0.20			0.03			0.05			0.02			0.02		
Mean Myco.(M)																									
	M0	23.81			68.40			11.43			2.10			0.43			0.80			0.46			0.37		
	M1	28.37			81.78.			12.68			1.76			0.51			0.87			0.42			0.37		
LSD (0.05)		1.68			21.15			0.40			0.07			0.06			0.01			0.02			0.06		
Mean rootstock (R)																									
	R1	26.84			62.72			10.75			1.99			0.37			0.97			0.31			0.23		
	R2	24.51			76.56			11.36			1.39			0.46			0.85			0.39			0.30		
	R3	26.91			81.25			14.04			2.41			0.59			0.69			0.61			0.59		
LSD (0.05)		2.03			8.37			0.50			0.14			0.05			0.08			0.03			0.03		
Interactions	S×M	ns			ns			ns			ns			ns			0.14			ns			ns		
	S×R	ns			ns			ns			0.47			ns			0.14			ns			0.05		
	M×R	ns			ns			ns			ns			ns			ns			ns			ns		
	S×M×R	ns			ns			ns			ns			ns			ns			ns			ns		
Regression coefficient (R^2)																									
(r)	R1	0.92**			0.69**			0.94**			0.88**			0.84**			0.84**			0.82**			0.67**		
	R2	0.99**			0.63*			0.89**			N.S			0.73**			0.76**			0.86**			0.94**		
	R3	0.69**			0.64**			0.88**			0.92**			0.84**			0.56**			0.87**			0.84**		

Rootstocks (R): R1: Dogridge, R2: 1103 Paulsen, R3: Harmony: Mycorrhizal treatment (M): M0: Uninoculated, M1: Inoculated, Salinity levels(S): S0: (0.65 dS m^{-1}) control, S1: (1.56 dS m^{-1}), S2: (4.68 dS m^{-1}); (d.w. = dry weight). Different letters in each column indicate a significant difference ($p<0.05$), ns: No significant, *Significant at $p<0.05$, **Significant at $p<0.01$

Table 5: Effect of salinity and mycorrhizal inoculation on the organic components and mineral composition of grape rootstocks during 2011 season.

Treatments		Chlorophyll content (SPAD unit) Salinity levels			Proline (mg/100 g d.w.) Salinity levels			Leaf total carbohydrates (% on d.w. basis) Salinity levels			N % Salinity level			P% Salinity levels			K% Salinity levels			Na% Salinity levels			Cl% Salinity levels		
Mycorrhizae	Rootstocks	S0	S1	S2	S0	S1	S2	S0	S1	S2	S0	S1	S2	S0	S1	S2	S0	S1	S2	S0	S1	S2	S0	S1	S2
M0	R1	29.71	27.62	24.41	55.27	67.28	74.70	13.04	11.40	10.34	1.48	1.80	2.22	0.57	0.50	0.42	0.88	0.80	0.73	0.25	0.28	0.37	0.20	0.27	0.35
	R2	26.09	24.06	19.83	67.87	78.54	87.82	13.70	11.72	10.29	1.49	2.08	2.91	0.51	0.46	0.45	1.23	1.06	0.91	0.27	0.33	0.39	0.21	0.28	0.39
	R3	33.47	30.35	26.97	69.92	77.49	87.32	15.97	14.40	11.22	1.85	2.47	2.96	0.66	0.55	0.48	0.73	0.62	0.49	0.50	0.58	0.65	0.39	0.58	0.70
M1	R1	32.40	30.09	27.19	59.36	75.41	94.10	14.35	13.47	12.00	1.24	1.81	2.41	1.83	0.57	0.45	1.00	0.83	0.91	0.27	0.28	0.34	0.18	0.21	0.29
	R2	28.59	25.30	23.04	89.49	97.29	109.70	14.30	13.15	11.43	1.37	2.15	2.42	0.67	0.60	0.46	1.23	1.10	0.97	0.30	0.30	0.36	0.20	0.25	0.32
	R3	35.23	32.97	30.43	75.05	85.53	96.00	16.17	15.01	12.48	1.58	2.03	2.05	0.74	0.58	0.52	0.78	0.75	0.61	0.46	0.50	0.57	0.40	0.55	0.69
Mean salinity (S)																									
	S0	30.91			69.49			14.58			1.50			0.83			0.97			0.34			0.26		
	S1	28.39			30.25			13.19			2.05			0.54			0.86			0.37			0.35		
	S2	25.31			91.60			11.29			2.49			0.46			0.77			0.44			0.45		
LSD (0.05)		1.23			6.72			0.49			0.26			0.05			0.08			0.02			0.02		
Mean Myco.(M)																									
	M0	26.94			74.02			12.45			2.13			0.51			0.82			0.40			0.37		
	M1	29.49			86.88			13.59			1.89			0.71			0.90			0.37			0.34		
LSD (0.05)		2.39			11.37			0.63			0.29			0.05			0.31			0.01			0.01		
Mean rootstock (R)																									
	R1	28.57			71.02			12.43			1.82			0.72			0.85			0.29			0.25		
	R2	24.48			88.45			12.43			2.07			0.52			1.08			0.32			0.27		
	R3	31.57			81.88			14.20			2.15			0.58			0.66			0.54			0.55		
LSD (0.05)		1.70			5.39			--------			0.27			0.05			0.10			0.02			0.02		
Interactions	S×M	N.S			N.S			N.S			N.S			N.S			N.S			N.S			N.S		
	S×R	N.S			N.S			N.S			N.S			N.S			N.S			N.S			0.05		
	M×R	N.S			13.24			1.09			N.S			N.S			N.S			0.04			N.S		
	S×M×R	N.S			N.S			1.21			N.S			N.S			N.S			N.S			N.S		
Regression coefficient(R^2)																									
(r)	R1	0.92**			0.80**			0.87**			0.84**			0.69**			N.S			0.68**			0.83**		
	R2	0.67**			0.68**			0.87**			0.78**			0.79**			0.49*			0.92**			0.86**		
	R3	0.94**			0.61*			0.97**			0.53*			0.77**			0.75			0.82**			0.88**		

Rootstocks (R): R1: Dogridge, R2: 1103 Paulsen, R3: Harmony: Mycorrhizal treatment (M): M0: Uninoculated, M1: Inoculated, Salinity levels(S): S0: (0.65 dS m^{-1}) control, S1: (1.56 dS m^{-1}), S2: (4.68 dS m^{-1}); (d.w. = dry weight). Different letters in each column indicate a significant difference ($p<0.05$), ns: No significant, *Significant at $p<0.05$, **Significant at $p<0.01$

Regarding the variation in the response of the all rootstocks to increasing salinity stress of the media, the data in Table 4 and 5 indicated that the Harmony rootstock had significantly higher total carbohydrates, leaf free proline content and leaf chlorophyll content in their leaves than that of Dogridge and 1103 Paulsen rootstocks in 2010 and 2011 seasons. With exception of leaf chlorophyll content in the first season and leaf proline content in the second season, they did not statically vary in this concern.

Quite aside from salinity, the data indicated that, the total carbohydrates, leaf free proline content and total leaf chlorophyll content of inoculated seedlings was significantly higher than that of the uninoculated ones. This trend was observed in the all rootstocks during both experimental seasons with exception of leaf proline content in the first season. For example, in 2010 season, increased in leaf total carbohydrates were noticed (10.91 and 11.79%) in seedlings inoculated with AM, in comparison with un-inoculated. The corresponding values for 2011 season were 11.72 and 13.15% in seedling inoculated with AM. As for leaf proline, in 2010 season, 1103 Paulsen seedlings under 1.56 dS m^{-1} salinity level with mycorrhizal inoculation had the highest value (81.09 mg/100 g dry weight) while the seedlings without mycorrhizal inoculation had the lowest value (61.96 mg/100 g dry weight). It has been reported that mycorrhizal fungi markedly enhanced the photosynthesis of inoculated plants and increased its stomatal conductance might provide a reasonable explanation for the general increase in the carbohydrate content of mycorrhizal plants observed herein. Nawar *et al.* (1988) as well as Nylund and Wallander (1989). The mycorrhizal inoculation significantly enhanced chlorophyll content in grape leaves. Preinoculated seedlings had greater leaf chlorophyll content compared with non mycorrhizal ones. This can further be attributed to increased Mg and Fe uptake (Krishna *et al.*, 2005).

Nutritional parameters: The nutrient uptake by all the rootstocks was analyzed in the leaf. The most noticeable features were increased leaf content in N, Na and Cl and a decrease in P and K (Table 4, 5). In 2010 season, Dogridge seedlings irrigated with 1.56 and 4.68 dS m^{-1} salinity levels showed an increase in leaf Na concentration (0.30 and 0.38%), respectively, in comparison with those grown under control. The corresponding values for 2011 season were 0.28 and 0.37%. It was not surprising that irrigating the seedlings with elevated levels of salinity was associated with an apparent increase in leaf Na and Cl contents. On the other hand, mycorrhizal infection, tended to decrease the concentrations of Na and Cl in the leaves of the seedlings whereas, the concentration of leaf Cl in the first season seemed not to differ statistically in response to mycorrhizal inoculation. Concerning leaf N, the reduction in growth of salt affected plants consumed little amount of N in comparison with those absorbed. These results are supported by Khalil (2009) and Khalil *et al.* (2011).

A strong relationship was observed between salinity and leaf mineral composition. Significant regression coefficients were found when the overall data of both experimental seasons was statistically analyzed (Table 4, 5).

Concerning the influence of mycorrhizal fungus on leaf P and K the data of the present study showed that mycorrhizal caused an increase in leaf P and K content of all rootstocks in both seasons. In the first season, for example, this increment in the leaf P content reached as much as 16.9% in the inoculated seedlings, in comparison with those un-inoculated ones. Cress *et al.* (1979) demonstrated that VAM were able to absorb P more efficiently by greater affinity for phosphate carrier systems than those of root alone.

CONCLUSION

It seems that grape rootstocks exhibited considerable variations in the salinity tolerance as evident from growth, total dry matter accumulation, proline, chlorophyll, carbohydrates and Na and Cl contents. The tolerant rootstocks exhibited high growth and high total dry matter accumulation so for alleviating the adverse affects of water salinity on growth and nutritional status of grape, it is suggested to use Dogridge rootstock and inoculation the soil with mycorrhizae *Glomus intraradices*.

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