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Morphological and Physiological Responses of Six Grape Genotypes to NaCl Salt Stress

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ABSTRACT
Salt contaminated areas are considered as, a critical problem in arid and semi-arid regions that limit the yield potential of agricultural crops. In the present study, NaCl salt stress susceptibility was assessed in five Jordanian grapes landraces (Salti, Zani, Red Glob, Darawishi and SoriBaladi) and B41 root stock using two levels of salt stress (6000 and 12000 ppm NaCl) in addition to the control during 2013 and 2014 growing season under controlled condition. Plant biomass (root and shoot), physiological parameters (relative water content and total chlorophyll content) and leaves mineral content were significantly ($p<0.01$) reduced in response to salt stress. High NaCl causes more pronounced reductions in these parameters than low NaCl treatment indicating the harmful effect of NaCl on plant biomass and physiological performance of the grape. Proline accumulated two and three time more under low and high salinity treatment than their respective control. The interactive effects of genotype and salt level treatment were significant ($p<0.01$) on plant biomass, mineral content and physiological parameter indicating, high level of variability exists among studied grape genotypes in response to salt stress. The lowest reductions in plant biomass and physiological parameters were recorded in Salti and Darawishi and consequently they could be considered as potential donors for genes for salt stress tolerance.

Key words: *Vitis vinifera* L., landraces, NaCl salt stress, physiological parameters

INTRODUCTION

Salinity contaminated soils is expanding and became a world-wide constraint for increasing the productivity of agricultural crops. It was estimated that about 7% of the world's total land area is affected by salinity (Flowers *et al*., 1997). The high temperature and evaporation in arid and semi-arid region accentuate the negative effects of salinity (Pessarakli, 1999) by increasing the concentration of salts around the root zone. Two ways could be used to minimize the impact of salt stress on plants: one is based on soil reclamation by salt leaching from soil profile using fresh water and the other relies on selecting genotypes with high genetic potential to tolerate salt stress. However, selecting plant varieties is considered the best economic approach to increase yield potential of agricultural crops under salt stress.

Salinity causes serious reductions in plant growth and development and agricultural productivity as, a result of osmotic stress around the root zone (Meri, 1984). Grapes are a horticultural crop of major economic importance (FAO., 2014) and it is considered moderately sensitive to salt stress (Fisarakis *et al*., 2001). Previous studies showed that salt osmotic stress led to retardation of plant growth in grapes (Bybordi, 2012; Gomez-del-Campo *et al*., 2002; Hawker and Walker, 1978), however genetic variability exists among grape genotypes with variable sensitivity to salt stress (Antcliff *et al*., 1983; Singh *et al*., 2000; Sivritepe *et al*., 2010). High salt accumulation around the root zone retards plant growth due to the reduction in osmotic potential in surrounding root substrate, accumulation of toxic ions, nutrients deficiencies or a combination of all these factors (McEAlexander and Obbink, 1971; Al-Saidi, 1980; Shani and Ben-Gal, 2005;

Walker *et al*., 2004). When a group of genotypes is compared based on total plant biomass, the stress stability index is used in comparison to identify more tolerant genotypes for breeding purposes or in order to recommend genetic material for grape growers in the case of varieties with high economic value. Tolerance to salt stress can be determined by using, different morphological parameters and here plants displayed less reductions in plant biomass could be considered less susceptible to salt stress.

From physiological point view, grapes can tolerate salt stress by changing the osmotic potential of internal of root tissue against the surrounding environment. This mechanism is called osmoregulation by which grape is adjusting the water potential in its root tissue to be at lower level than the surrounding root substrate (Delauney and Verma, 1993). Grapes can adjust to salt stress by accumulating more free amino acids, potassium ions and dissolvable substances. In this way, less negative pressure in root tissue is ensured (Dettori, 1985). Genotypes accumulates more proline is more salt tolerant than those with less proline accumulation (Delauney and Verma, 1993; Singh *et al*., 2000).

Identifying grape genotypes for salt tolerance is a major objective to adapt to salt contaminated soils. Such material once detected, can be utilized in salt tolerance improvement. In the present investigation, Jordanian grape landraces and B41 root stock were evaluated to recommend potential gene donors for salt tolerance for grapes breeding. The specific objectives of the current research were to; (1) Assess the level of tolerance of five local grape varieties and B41 rootstock to salinity using salinity susceptibility index, (2) Study the effect of salinity levels on some morphological traits, leaves proline accumulation, leaves relative water contents, K^+ and Na^+ in leaf tissue and leaves chlorophyll content and (3) Correlate the level of tolerance in grapes with some biochemical and physiological traits in grapes.

MATERIALS AND METHODS

Pot experiment: Two pot experiments in two successive seasons (2013 and 2014 growing seasons) were carried out in sand-clay soil, which found in the agricultural fields at Rabba Agricultural Station, Mutah University. Physical and chemical properties of the soil, were determined according to the standard procedures. Five Jordanian grape landraces (Salti, Zani, Red Glob, Darawishi and Sori Baladi) widely grown by local farmers in addition to B41 rootstock were planted in two replications in each season in split plot arrangement, where the salt treatments were the main plots and varieties constituted the sub-plots. Each replicate was represented with 4 plants grown in 10 L pot. One year old grape seedling that vegetatively propagated by stem cutting were planted in spring (15 April, 2013 and 20 April, 2014). One month after seedling transplanting, seedlings were imposed to four NaCl treatments (tap water or control, 6000, 12000 and 18000 ppm). Macro and microelements were added in split doses at the 0.5-strength Hoagland nutrient concentration (Dunn and Arditti, 1968).

All seedlings were irrigated day and after day with salt solutions and tap water to the amount sufficient to achieve 80% of the field capacity. The duration of the experiment was extended to three months after transplanting. The high concentration treatment (18000 ppm) led to complete death of grape plants after one month salanization and consequently excluded from any statistical analysis and any biochemical analysis.

Relative Water Content (RWC): The level of water stress in leaves was assessed based on Relative Water Content (RWC) according to the procedure used by Weatherley (1950). Leaf disks (1.5 cm^2) were weighed immediately after collection to determine Fresh Weight (FW) and placed in a Petri dish containing wet filter paper and kept at 4°C in the dark for 24 h. After words, the Turgid Weight (TW) was recorded. For the Dry Weight (DW), leaf disks were oven-dried for 24 h at 80-90°C and weighed. The RWC was determined using the following formula:

$$RWC = \frac{FW - DW}{TW - DW} \times 100\%$$

Chlorophyll content: One gram of fresh leaf material was homogenized in 20 mL of 80% acetone and filtered. After filtration, the extraction was filtered again with additional 20 mL of 80% acetone. At the end, the volume of filtered extracts was completed making up 50 mL with acetone. Total chlorophyll content was measured spectrophotometrically according to Harborne (1973) as follows:

$$\text{Total chlorophyll (mg } L^{-1}) = 17.3\ A_{646} + 7.18\ A_{663}$$

Extraction and determination of proline: Proline accumulation in the leaves was estimated according to the method used by Bates *et al*. (1973). One gram of leaf material was homogenized in 10 mL of 3% aqueous sulfosalicylic acid. The homogenate was filtrated and then, a 2 mL aliquot of the filtrate was mixed with an equal volume of acetic acid and acid ninhydrin (1.25 g ninhydrin in 30 mL acetic acid and 20 mL of 6M H_3PO_4) and incubated for 1 h boiled water (100°C). Consequently, the reaction was cooled in an ice bath and extracted with 4 mL of toluene. The extract was vortexed for 20 sec. The chromatophore-containing toluene was then aspirated from the aqueous phase and its absorbance was determined spectrophotometrically at 520 nm (Beckman 640 D, USA) using, toluene as a blank.

Morphological parameters: At the end of the experiment, the shoots and roots were separated after cleaning the roots from the soil. Thereafter, roots and shoots were oven dried at 70°C for 24 h to determine dry weight.

Leaves mineral content: Replicated dried leaf samples were ground for chemical composition determination. One gram of dried leaf samples was digested in 5.1% HNO_3 to determine Na^+ and K^+ concentrations. Leaves' nitrogen (N) content was determined using standard macro-Kjedahl method from one gram leaf samples. Phosphorus was determined by Olsen method.

Statistical analysis: Data from the two successive seasons were combined to perform statistical analysis. Salinity and variety effects on different recorded variables were determined by analysis of variance (ANOVA). Treatment means was separated by calculating the least significant value (LSD). A stress intensity index, D_i, was calculated according to Fischer and Maurer (1978) as follows:

$$D_i = 1 - \frac{X_d}{X_P}$$

where, X_d and X_p are average grain yield over all entries under stress (i.e., the highest salt stress treatment) and non-stress (control treatment). The D_i, was used to estimate the a Stability Index (SI) for each genotype (Fischer and Maurer, 1978) as follows:

$$SI = \frac{\left(1 - \frac{Y_d}{Y_p}\right)}{D_i}$$

where, Y_d and Y_p, are grain yield under stress and non-stress treatments, respectively.

The growth inhibition as a result of salinity stress was calculated according to the following formula:

$$GI(\%) = \frac{(Wl - Sl)}{WI} \times 100\%$$

where, Wl and Sl are the value of recorded parameter under stress and non-stress environments, respectively.

RESULTS

Salinity effect on shoot and root weight: NaCl salinity treatments and genotypes were significant ($p<0.01$) on shoot and root fresh weight (Table 1). Significant ($p<0.01$) genotype×NaCl treatment interactions were also observed. Salt stress led to significant reductions in plant biomass (Table 2). Salinity had a more pronounced effect on shoot fresh weight than on root fresh weight. The low NaCl salt treatment (6000 ppm NaCl) decreased plant biomass (shoot and root) to a lesser degree than high NaCl effect (12000 ppm). At 6000 and 12000 ppm, shoot fresh weight was decreased by 14.10% and 45.54% and root weight decreased by 16.31 and 27.47% as compared with their respective control, respectively.

The interactions between genotypes and NaCl treatments on root and shoot biomass were significant ($p<0.01$) (Table 3). The maximum reduction in shoot mass at low NaCl concentration was observed in B41, while the minimum reduction was observed in Zani (6.44%). In other genotypes, the reductions in shoot weight ranged from 11.14-19.63%. The minimum effect of high NaCL treatment was observed in Salti,

Table 1: Mean squares of analysis of variance on different morphological and physiological characteristic of grape as affected by salinity and genotypes

Source of variance	Shoot fresh weight	Root fresh weight	RWC	Total chlorophyll content	Proline
Replication	219.2	280.6*	28.13	0.28	102.72
S	3422.2**	1704.5**	328.38**	6.25**	236.12**
G	13184.0**	2608.9**	5609.68**	204.38**	3632.10**
G×S	927.9**	132.5*	121.66**	1.06	92.58**
Error	69.2	70.0	36.65	1.06	18.59

S: Salinity, G: Genotype, RWC: Relative water content, *Significant at 0.05 and **Significant at p<0.01

Table 2: Mean separation of the effect of different salinity levels and genotypes on some morphological and physiological traits of the six grape genotypes

Source of variance	Shoot fresh weight	Root fresh weight	Relative water content (RWC)	Total chlorophyll content	Proline
Genotype (G)					
Salti	95.56^{b}	69.67^{a}	74.91^{a}	8.19^{b}	25.50^{a}
Zani	103.41^{a}	72.77^{a}	66.16^{b}	7.20^{c}	15.58^{b}
Red Glob	72.26^{d}	72.48^{a}	68.58^{b}	7.23^{c}	16.75^{b}
Darawishi	75.59^{cd}	70.31^{a}	77.66^{a}	8.00^{bc}	24.66^{a}
Sori Baladi	80.01^{c}	59.06^{b}	77.83^{a}	9.10^{a}	22.75^{a}
B41	56.40^{e}	42.41^{c}	67.91^{b}	7.51^{bc}	16.91^{b}
$LSD_{0.05}$	6.81	6.85	4.95	0.84	3.53
Salinity (S)					
Control	100.53^{a}	75.46^{a}	87.88	11.06	7.12^{c}
6000 ppm NaCl	86.36^{b} (14.10%)	63.15^{b} (16.31%)	71.33 (18.83%)	7.25 (34.44%)	22.54^{b} (-216.57%)
12000 ppm NaCl	54.75^{c} (45.54%)	54.73^{c} (27.47%)	57.33 (34.76%)	5.33 (51.80%)	31.42^{a} (-339.86%)
$LSD_{0.05}$	4.81	4.84	3.51	0.60	2.50
Interactive effect (G×S)	**	*	**	Ns	**

Table 3: Interactive effect of salinity level and genotypes on shoot and root fresh weight, and their drought stability index (DSI)

	Shoot fresh weight					Root fresh weight					
		6000 (ppm)		12000 (ppm)			6000 (ppm)		12000 (ppm)		
Genotype	Control	No.	%	No.	%	Control	No.	%	No.	%	DSI
Salti	113.1	90.90	19.63	82.7	26.88	75.91	72.5	4.49	60.6	20.17	0.64
Zani	135.0	126.30	6.44	48.9	63.78	95.00	62.5	34.21	60.8	36.00	1.38
Red glob	97.7	85.60	12.38	33.4	68.81	84.40	72.5	14.10	60.6	28.20	1.27
Darawishi	87.3	75.60	13.40	63.9	29.41	77.50	73.3	5.42	60.2	22.32	0.65
Sori baladi	92.5	82.20	11.14	65.3	29.41	71.10	56.7	20.25	49.4	30.52	0.82
B41	77.5	57.50	25.81	34.2	55.87	48.90	41.5	15.13	36.8	24.74	1.15
LSD (0.05)		9.90			3.73						

Table 4: Mean squares of analysis of variance on leaf mineral contents of grape as affected by salinity and genotypes

Source of variance	Nitrogen	Phosphorus	Potassium	Sodium
Replication	0.022	0.002	0.518*	0.014
Salinity (S)	1.940**	0.413**	0.341**	0.055**
Genotype (G)	23.100**	1.446**	2.629**	0.537**
G×S	2.890**	0.586**	0.093	0.017
Error	0.098	0.004	0.090	0.015

Table 5: Mean separation of the effect of different salinity levels and genotypes on leaf mineral content of the six grape genotypes

Source of variance	Nitrogen	Phosphorus	Potassium	Sodium
Genotype (G)				
Salti	3.49^{a}	0.49^{a}	2.48^{b}	0.23^{b}
Zani	2.73^{b}	0.31^{cd}	2.69^{ab}	0.23^{b}
Red glob	2.54^{b}	0.38^{b}	2.91^{a}	0.29^{b}
Darawishi	3.37^{a}	0.46^{a}	2.66^{b}	0.41^{a}
Sori baladi	3.35^{a}	0.35^{bc}	2.45^{b}	0.30^{b}
B41	2.80^{b}	0.28^{d}	2.56^{b}	0.29^{b}
LSD (0.05)	0.27	0.05	0.25	0.10
Salinity (S)				
Control	4.09^{a}	0.55^{a}	2.94^{a}	0.15^{c}
6000 (ppm) NaCl	2.90^{b} (29.10%)	0.39^{b} (29.09%)	2.66^{b} (9.52%)	0.28^{b} (-86.67%)
12000 (ppm) NaCl	2.15^{c} (47.43%)	0.20^{c} (63.64%)	2.28^{c} (22.45%)	0.45^{a} (-200.0%)
LSD (0.05)	0.18	0.04	0.17	0.07
Interactive effect (G×S)		**	Ns	Ns

Darawishi and Sori Baladi (reductions ranged from 26.88-26.88%), while maximum reductions were detected in Zani, Red Glob and B41 (reductions ranged from 55.87-68.81%). The minimum stability indices values were observed in Salti, Darawishi and Sori Baladi (values = 0.64, 0.65 and 0.82, respectively), whereas Zani, Red Glob and B41 exhibited comparatively higher values (values = 1.38, 1.27 and 1.15, respectively) (Table 3).

Effect of salinity on leaf mineral content: Nitrogen, phosphorus and potassium leaf content were significantly decreased ($p<0.01$) with increasing NaCl concentration (Table 4). The NaCl treatment decrease nitrogen, phosphorus and potassium leaf contents to a lesser extent than high NaCl treatment (12000 ppm) (Table 5). At 6000 and 12000 ppm, nitrogen was reduced by 29.10 and 47.43%, phosphorus was reduced by 29.09 and 63.64% and potassium decreased by 9.52 and 22.45%. Sodium concentration at high salinity (12000 ppm) was two times higher than that of under no stress condition.

The interaction between genotype and NaCl treatment was significant ($p<0.01$) on nitrogen and phosphorus (Table 6). Salti, Darawishi and Sori Baladi displayed the minimum reductions in nitrogen leaf content with reductions ranged from 16.87-22.46 and 33.50-36.50% at 6000 and 12000 ppm NaCl, respectively, while other genotypes displayed high reductions ranging from 35.52-38.71% under 6000 ppm and reductions ranging from 55.56-60.72% under 12000 ppm treatments. Salti genotype showed a minimum reduction in leaf phosphorus content at low and high NaCl concentration (reductions were 23.81 and 42.86%). Other genotypes displayed medium reduction in phosphorus content at low NaCl concentration (37.68-51.11%) but high reductions at high NaCl concentration (60.00-82.69%).

Effect of salt treatments on physiological parameters: Proline accumulation in leaves was significantly increased ($p<0.01$) with increasing salinity, a significant genotype×salt interaction (Table 1). Accumulated proline at low (6000 ppm NaCl) and high salt stress concentration (12000 ppm NaCl) was two and three times higher than of that

Table 6: Interactive effect of salinity level and genotypes on nitrogen and phosphorus content

	Nitrogen					Phosphorus				
		6000 (ppm)		12000 (ppm)			6000 (ppm)		12000 (ppm)	
Genotype	Control	No.	%	No.	%	Control	No.	%	No.	%
Salti	4.33	3.40	21.48	2.75	36.50	0.63	0.48	23.81	0.36	42.86
Zani	4.15	2.43	41.45	1.63	60.72	0.52	0.30	42.31	0.09	82.69
Red glob	3.72	2.28	38.71	1.65	55.65	0.65	0.35	46.15	0.14	78.46
Darawishi	4.23	3.28	22.46	2.60	38.53	0.69	0.43	37.68	0.25	63.77
Sori baladi	4.03	3.35	16.87	2.68	33.50	0.58	0.34	41.38	0.14	75.86
B41	4.11	2.65	35.52	1.63	60.34	0.45	0.22	51.11	0.18	60.00
LSD (0.05)		0.55			0.25					

Table 7: Interactive effect of salinity level and genotypes on leaf relative water content and proline accumulation in leaves

	RWC					Proline				
		6000 (ppm)		12000 (ppm)			6000 (ppm)		12000 (ppm)	
Genotype	Control	No.	%	No.	%	Control	No.	%	No.	%
Salti	86.25	75.25	12.75	63.25	26.67	4.75	30.00	-531.58	41.75	-778.95
Zani	87.75	65.75	25.07	45.00	48.72	8.00	16.00	-100.00	22.75	-184.38
Red glob	87.25	64.75	25.79	53.75	38.40	6.75	19.30	-185.93	24.00	-255.56
Darawishi	90.25	80.25	11.08	62.50	30.75	8.25	27.50	-233.33	38.25	-363.64
Sori baladi	85.75	79.50	7.29	68.25	20.41	6.50	25.50	-292.31	36.25	-457.69
B41	90.00	62.50	30.56	51.25	43.06	8.50	16.75	-97.06	25.50	-200.00
LSD (0.05)		35.80			3.12					

Interactive effects of varieties and salt levels on number of days to heading, number of days to maturity and grain- ling. period (days). The horizontal bar indicates the least significant difference (LSD, $p<0.05$) for comparison of treatment combinations, RWC: Relative water contents

under control or tap water (Table 2). In contrast, leaf chlorophyll and RWC were significantly reduced under salt stress. The reductions ranged from 18.83-34.76% and from 34.44-51.80% for RWC and chlorophyll content, respectively (Table 2).

The interactions were significant ($p<0.01$) on RWC and proline accumulation in leaves (Table 7). Under low NaCl treatment, Salti landrace accumulated 5 time more proline than its respective control, while other genotypes accumulated one time to two times higher proline than that under control condition. Under high salt stress (12000 ppm NaCl), proline was eight, four and five times higher in Salti, Darawishi and Sori Baladi than of that under control. Other genotypes accumulated less proline (i.e., two time higher than the control).

The minimum reductions in RWC were observed in Salti, Darawishi and Sori Baladi, ranging from 7.29-12.75 and 20.41-26.67% under low and high salt stress treatments, respectively. The reductions in RWC were maximal in other genotypes, ranging from 25.07-30.56 and 38.40-48.72% under low and high salt stress treatments, respectively.

DISCUSSION

Salt stress caused prominent reductions in plant biomass (shoot and root fresh weight). Reductions in plant biomass were higher when the salt stress was elevated from 6000-12000 ppm. Increasing salt stress to 18000 ppm NaCl stopped plant growth and caused complete necrosis. The reductions in plant biomass were mainly due to reductions in plant height, total number of plant leaves, stem number and stem length (data is not shown). Increasing salt stress caused more reductions in RWC and chlorophyll content of leaves and an increase in accumulated proline in the leaves.

The minimum SI values were observed in Salti, Darawishi and Sori Baladi indicating their high potential for salt tolerance.

One of the pronounce effect of salt stress is clear reduction vegetative plant related traits such as plant height and leaf area (Bybordi, 2012; Gomez-del-Campo *et al*., 2002; Hawker and Walker, 1978). Even though salt stress caused reductions in plant growth, the genetic variability exits among studied genotypes (Antcliff *et al*., 1983; Singh *et al*., 2000; Sivritepe *et al*., 2010). In our study, different grape varieties displayed various responses to salt stress. On the basis of plant biomass Salti, Darawishi and Sori Baladi could be declared as tolerant, whereas Zani, Red Glob and B41 could be considered as sensitive. Tolerant genotypes displayed the minimum reductions in plant biomass, maintained high RWC and chlorophyll content and accumulated more proline in leaf tissue. They also maintained high N, P and K levels in their tissue.

The reduction in plant biomass under salinity in the current experiment could be attributed to the toxic effects of Na^+ and Cl^- in plant tissues (McEAlexander and Obbink, 1971; Al-Saidi, 1980; Shani and Ben-Gal, 2005; Walker *et al*., 2004). Reduction in plant growth is mainly due to reduction in leaf chlorophyll content that leads to reductions of dry weights

of leaves, stems and roots (Gomez-del-Campo *et al*., 2002). In the current study, leaf Relative Water Content (RWC) decreased by increasing salinity. This is mainly due to increasing the rate of transpiration under stress as a result of osmotic stress in the growing medium (Dettori, 1985). Reduction in plant growth is mainly due to negative osmotic pressure around the root zone and due to the toxic effects of sodium and chloride ions (Flowers, 2004; Munns, 2002). Salt stress hampers the uptake of essential nutrients such as potassium and NO^-_3. Sodium is competing essential nutrients during absorption which lead to inhibition of essential nutrient uptake (Gorham and Wyn Jones, 1993; Meri, 1984).

Grapes can tolerate salt stress by decreasing internal tissue osmotic potential by accumulation of inorganic (such as K^+) (Troncoso de Arce *et al*., 1999; Walker *et al*., 2004) and organic compounds, such as; proline (Delauney and Verma, 1993). Maintaining Na^+ below its toxic level is an a mechanism also to minimize the impact of salt stress, sodium is accumulated in vacuoles for osmotic adjustment (Singh *et al*., 2000; Troncoso de Arce *et al*., 1999). Salt tolerance in grape is also relate to low transport rate of Na^+ and consequently enhance K^+/Na^+ ratio. Maintaining high RWC in leaves is one mechanism to tolerate the salt stress that maintain to the turgid state of plant cell (Walker *et al*., 2003). Furthermore, selection genotypes with leaf chlorophyll content might be considered as reliable indicator for salt stress (Divate and Pandey, 1981; Singh *et al*., 2000).

CONCLUSION

Three grape genotypes (Salti, Darawishi and Sori Baladi) relatively displayed, a potential for salt tolerance. These three varieties showed low reductions in plant biomass and plant physiological parameters. Therefore, these genotypes could be considered as, salt tolerant and they are suitable in improving grapes for salt tolerance. Moreover, further research is required to confirm these results under field conditions.

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