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Evalution the changes of some biomolecules of two grapevine cultivars against different NaCl levels

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Abstract

Salinity is one of the limiting factor for grape growing in arid and semi-arid areas. Hence he effect of salinity on some physiological and biochemical characteristics of two seedless cultivars of grape namely Flame Seedless and Perlette under salinity stress were investigated. The design of the experiment was factorial arrangement in a complete randomized design with four replications. Five levels of salinity (0, 25, 50,75 and 100 m molar of NaCl) in irrigation water were surveyed on rooted cuttings of both cultivars. Results indicated that with increasing salinity levels photosynthesis, amount of soluble proteins and relative leaf water content was decreased and amount of proline and soluble sugars were increased. Ion leakage of cell membrane and malondialdehyde were increased with increased salinity. Withoute salinity application Perlette cultivar produced the best values for physiological and morphological indices. In general, Perlette cultivar proved more tolerance against salinity than Flame Seedless cultivar did.

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Introduction

Salinity or increased concentration of soluble salts in cultivated soils is one of the main challenges for sustainable agriculture, with a decreasing effect on plant growth and specifically on horticultural crops yield(Bybordi, 2012). Iran, the second largest country in the middle East, has an area of 165 million ha. Approximately, 90% of the country is classified as arid and semi-arid region, most of which is faced with low rainfall, high evapotranspiration, salinization, shortage of fresh water, erosion, excessive heat and desertification. Fresh water resources are declining in the central plateau of the country as a result of overusing underground water and sever drought in recent years(Cheraghi, 2004).

Land salinization is a major limiting factor for conventional crop production in the country. Continnuos cropping together with an excessive use of chemifertilizers an ill-managed irrigation has turned hundreds of cultivated fertile fields into saline ones. These limitations have greate impacts on the welfare of the farmers whose income is soley dependent to agriculture. In recent years, increased attention has been paid to the use of saline soils and waters for crop production(Banakar and Ranjbar, 2010).

Grapevines are considered as moderately sensitive to salinity and the damage is primilarly caused by chloride ions(Walker, 1995). However, grapevine response to salinity depends on several factors, such as rootstock-scion combination, irrigation system, soil type and climate. Changing some of these factors with the same irrigation water could produce entirely different results(Fisarakis *et al*. 2001).

Estion and Harvey(Kaplan-Dalyan, 2013) conducted an in vitro experiment in order to determine the salinity tolerance in some grape cultivars and demonstrated that salinity tolerantcultivars maintain their growth rate to a relative extent, and are capable of dealing with metabolic disorders such as chlorophyll deficiency. It was found that salinity treatment caused various rate of necrosis in the samples dependent on the cultivar, NaCl concentration and treatment period. Salinity tolerance in fruit trees, particularly in grape tree, is heavily influenced by cultivar.

Results from the research revealed that the capacity of cultivars to regulate the absorption of Na+ and Cl- determines their tolerance, i.e. the higher the capacity of plant in preventing the uptake of Na+ and Cl, the higher will be its tolerance. Salinity stress produces both shortterm and long-term effects. One or two days after the plant exposure to salinity, it takes only a few hours for the short-term effects to take place, during which a complete cessation of carbon assimilation is resulted. Whereas, the long-term effects after the exposure of plant to salinity for several days and decreased carbon assimilation, happens due to salt accumulation in the leaves(Fisarakis *et al*., 2001).

The effects of salinity on both quantity and quality of grape have been researched in multitudes of investigations conducted in an out of the country. Salinity tolerance threshold for this plant reportedly is 1.5 $dS.m^{-1}$. While at 2.5 $dS.m^{-1}$ the plant growth decreased by 10%. However it·s worth consideration that cultivars of the species of a given plant vary greatly in terms of their tolerance against salinity. According to the above items the effect of salinity levels on different physiological and biochemical characteristics of two seedless cultivars of grape namely Flame Seedless and Perlette were investigated and the responses of these cultivars were compared with each other.

Materials and methods

Plant material, growth conditions and treatments

Scions of grapevine(*Vitis vinifera* L.) cvs. Flame seedless and Perlette were rooted and grown in plastic pots containing sand and perlite(1:1) under natural day length in a polyhouse of Parsnarang private company at jahrom city. After rooting, nourishing the scions was done weekly by Basofoliar

solution(1%). After four months from rooting, NaCl added to the irrigation water(0, 25, 50, 75 and 100 mM). This experiment was conducted based on factorial experiment in the form of Randomized Complete Desighn(RCD) with four replication.

Determination of leaf water status

Three compound leaves were collected from each seedling. Leaf fresh weight was measured immediately and then the leaves wer submerged in distilled water at room temperature. After 24 h, the leaves were removed from the water, blotted dry with filter paper and weighted to determine saturated fresh weight. The leaves were then dried at 80°C for 24 h and weighted again. Leaf relative water content(LRWC) was calculated as follows:

LRWC=(fresh weight - dry weight)/(saturated fresh weight – dry weight)

Determination of malondialdehyde(MDA)

Malondialdehyde were determined using the methods of Zou(2000). In brief, a 0.5 g sample of fresh leaf tissue was ground in a mortar with 10 ml 10% trichloroacetic acid and a small quantity of quartz. The homogenate was centrifuged at 4,000 rpm for 10 min, then a 2-ml aliquot was removed and mixed with 2 ml 0.6% thiobarbituric acid(TBA) solution. The solution was incubated at 100°C for 15 min, allowed to cool and then again at 4,000 rpm. Absorbance values of the supernatant were recorded at 532, 600 and 450 nm and the MDA and soluble sugar content were calculated as follows:

MDA content (µmol/g FW) = $[(6.45 (A_{532} - A_{600}) - 0.56A_{450})/1{,}000]$ (µmol/ml) × volume of extract solution (ml)/fresh weight (g).

Determination of leaf electrolyte leakage

Leaves were washed with deionized water to remove surface-adhered electrolytes. These were placed in closed vials containing 10-ml deionized water and incubated at 25°C on a rotary shaker for 24 h. Subsequently, the electrical conductivity of the solution (s_1) was determined. Samples were then autoclaved at 120°C for 20 min and the final electrical conductivity (s_2) was obtained after equilibration at 25°C. The electrolyte leakage (EL) was defined as follows:

$EL(\%) = (s_1/s_2) \times 100$

Determination of soluble sugars content

A sample of the leaves was ground in a mortar with ethanol(95%). Deposits was washed again with ethanol(70%) and the upper phase was added to previous upper phase. The homogenized samples were centrifuged for 10 min at 3500 ×g. A supernatant was used to estimate the sugar content. After keeping for 10 min colour development with Anthrone and sulfuric acid, solution absorbance was read at 625 nm. The results are expressed in mg sugar g^{-1}FW.

Determination of soluble proteins content

For the quantitative determination of total saluble protein amount Bradford's(1976) Dye-binding mthod was employed. The obtained absorption values were calculated according to bovine serum albumin(BSA) protein standard which has been previously prepared and the amount of total protein was estimated as mg/gFW.

Determination of photosynthesis and transpiration rate

At the end of the experiment, portable Photosynthesis Measurement System (ADC BioscientificLCiAnalyser Serial No. 31655, UK) were used to calculate the net photosynthetic rate and transpiration rate per unit leaf area of the youngest fully expanded leaf of each plant and last but not least, the measurement was conducted between 9AM and 2PM local time under a fixed light intensity.

Determination of chlorophyll a and b

Chlorophyll a and b was measured using Arnon method, in this method, as little as a half gram of wet vegetative matter was chopped and thoroughly mashed in liquid nitrogen, in a porcelain mortar. As much as 20ml of 80% aceton was added to the sample, and then the mixture was put into centrifuge

device with 6000 rpm speed for 10 minutes. Supernatant was transferred into a glass ballon. Some of the samples in the ballon were read in spectrophotometer for chlorophyll a at 663 nm and for chlorophyll b at 645 nm in mg/g of fresh weight of the sample.

chlorophyll a = $(19.3\times A_{663}-0.86\times A_{645})$ V/100W
chlorophyll b= $(19.3\times A_{645} - 3.6\times A_{663})$ V/100W

Determination of proline content

Quantification of free proline in grape leaves was done according to Bates *et al.* (1973), using 0.1 gr of dried leaf tissues. The plant material was homogenized with 3% sulpho-salicylic acid. The homogenate was then filtered and added with glacial acetic acidand acid-ninhydrin. After stirring, the sample was incubated at 100°C for 1 h. after 1 hour, toluene was added and absorbance at 520nm was measured by using spectrofluorometer.

Statistical analysis

The analysis of variance was performed using MSTAT-C software. Duncan،s Multiple Range- Test was used to determine differences among treatment means at a significance level of $p\leq 0.05$.

Results

Effect of salinity on leaf water status

Increasing salinity level had a significantly decreasing effect on percentage of relative water content of grape leaf while the lowest value(48.25%) was found at 100mM NaCl level wherease the highest value(82.15%) was produced at without salinity application treatment(control) (Table 3).

Table 1. Analysis of variance on different characteristic of grape affected by salinity and cultivars.

Sources Of variation	Degree Of freedom	Proline (mg/gr FW)	Chlorophyll a (mg/gr FW)	Chlorophyll b (mg/gr FW)	Transpiration rate (mmolm-2s-1)	Photosynthesis rate (µmolm-2s-1)	RWC (%)	MDA (µmol/gFW)	EL (%)	Soluble sugars	Soluble proteins
Cultivars	1	0.223 ^{ns}	101.731**	38.571****	730.11**	52.45**	1268****	0.000** **	530.4**	14.10**	1391**
Salinity	4	16.205**	98.436 **	7.501**	613.23**	902.0**	7510** ** **	0.007 **	93640**	333**	331**
Salinity × cultivars	4	0.454^{ns}	2.397*	0.343 ^{ns}	38.37 **	13.69**	23.24 ^{ns}	0.000 ^{ns}	11.67 ^{ns}	1.38 ^{ns}	9.57 ^{ns}
Error	150	0.316 ^{ns}	0.721 ^{ns}	0.333 ^{ns}	2.00**	2.78**	144.7 ^{ns}	0.000 ^{ns}	14840 ^{ns}	0.640 ^{ns}	13.36 ^{ns}
C.V (%)	---	20.11%	23.47%	16.69%	19.94%	19.38%	17.94%	16.06%	18.73%	13.89%	12.25%

*, **, ns: significant at 0.05 , 0.01 probability level and no significant respectively.

Effect of salinity on malondialdehyde(MDA) and leaf electrolyte leakage(EL)

Our results showed that MDA content and EL percentage was increased in NaCl treatments while the maximum value of MDA(0.038µmol/gFW)was observed for the 100mm NaCl treatment and the minimum value(0.004µmol/gFW)was observed for the control treatment (Table 3). More ever, results showed that MDA value and EL percentage of flame cultivar(0. 021µmol/gFW and 54.73% respectively) was significantly higher than MDA value and EL percentage of perlette cultivar(0.019µmol/gFW and 51.47% respectively).

Effect of salinity on soluble sugars content

The cntent of soluble sugars was increased up to 75 mM NaCl but that decreased with higher level of salinity. The content of soluble sugars in 50 and 75 mM NaCl treatment, was 2.97 and 3.31-fold compared to control, but in perlette variety the content of soluble sugars, in 50, 75 and 100 mM NaCl treatments was 2.05 and 2.26-fold compared to control (Table 3). In general the content of soluble sugars in Perlette variety was higher than that amount in Flame Seedless variety (Table 2).

Effect of salinity on soluble proteins content

In both cultivars salinity reduced the content of soluble proteins. In Perlette variety, the content of soluble proteins reduced 9.41%(in contrast with

control) and in 75 and 100 mM NaCl treatment, decreased 11.13 and 14.4% respectively, but that amount in Flame Seedless variety, in 50, 75 and 100 mM NaCl treatments increased 6.61, 9.33 and 16.86% respectively(in contrast with control) (Table 3). In general the content of soluble proteins in Perlette variety was higher than that amount in Flame Seedless variety (Table 2).

Table 2. Main effect of Cultivars on different characteristics of grape.

parameter Cultivars	Proline (mg/gr FW)	Chlorophyll a (mg/gr FW)	Chlorophyll b (mg/gr FW)	Transpiration Rate (mmolm-2s-1)	Raet of Photosynthesis (µmolm-2s-1)	RWC (%)	MDA (µmol/gFW)	EL (%)	Soluble sugrs mg/gFW	Soluble proteins mg/gFW
flame seedless	2.760 A	2.905 B	3.019 B	5.183 B	8.095 B	64.57 B	0.02108 A	54.73 A	5.49B	41.66B
perlette	2.827 A	4.332 A	3.897 A	9.005 A	9.120 A	69.61 A	0.01941 B	51.47 B	6.02A	46.94A

Values within the each column and followed by the same letter are not different at P<0.005 by an ANOVA protected Duncan´s Multiple Range- Test.

Effect of salinity on photosynthesis and transpiration rate

Transpiration and photosynthesis declined significantly in the face of increasing salinity levels while the lowest value of transpiration(2.120mmolm-2s-1) and photosynthesis(2.387 µmolm-2s-1) was seen at 100mM sodium chloride level (Table 3). The increasing salinity levels caused a significant decrease in transpiration and photosynthesis rate of two cultivars but the transpiration value in perlette cultivar(9.005mmolm-2s-1) was significantly more than transpiration value of flame cultivar(5.183mmolm-2s-1) (Table 2).

The content of chlorophylle a and b was reduced significantly with NaCl treatments although maximum reduction was induced by 100mM NaCl (Table 3).

Table 3. Main effect of salinity on different characteristics of grape.

Parameter salinity	Proline (mg/gr FW)	Chlorophyll a (mg/gr FW)	Chlorophyll b (mg/gr FW)	Transpiration Rate (mmolm-2s-1)	Raet of Photosynthesis (µmolm-2s-1)	RWC (%)	MDA (µmol/gFW)	EL (%)	Soluble sugrs mg/gFW	Soluble proteins mg/gFW
0NaCl	2.248 D	5.290 A	3.941 A	12.30 A	14.21 A	82.15 A	0.0047 E	27.13 E	3.29I	40.15H
25mM	2.761 C	4.661 B	3.717 AB	9.135 B	11.92 B	77.20 A	0.011 D	33.95 D	4.35G	84.59G
50mM	3.258 B	4.007 C	3.560 B	7.137 C	8.919 C	68.77 B	0.020 C	49.75 C	5.51F	44.46FG
75mM	3.592 A	2.774 D	3.24 C	4.782 D	5.601 D	59.07 C	0.027 B	69.83 B	7.18D	46.25DEF
100mM	2.109 D	1.360 E	2.832 D	2.12 E	2.387 E	48.25 D	0.0382 A	84.86 A	8.43B	48.36CD

Values within the each column and followed by the same letter are not different at P<0.005 by an ANOVA protected Duncan´s Multiple Range- Test.

Perlette cultivar exhibited more efficiency with respect to qualitative factors and highest value for traits such as proline content, photosynthesis and transpiration rate, chlorophylle a and b content and RWC percentage were more in perlette cultivar than flame seedless cultivar. In addition, perlette cultivar had the lowest percentage of EL and MDA content (Table 2).

The increasing salinity caused a significant increase in proline content of grape leaf (Table 1). While the highest value(3.592 mgr/grFW) was measured in 75mM treatment, but there wasn't any significant difference between proline content in 100mM sodium chloride(2.109 mgr/grFW) and control treatment(2.248 mgr/grFW) (Table 3).

Discussion

Photosynthesis and transpiration rate

Increasing salinity level causes a rise in leaf temperature and consequently the stomatas are closed duo to water limitation stress caused by salinity, at the same time duo to synthesis of abscisic acid in the root and its translocation to the stomatas.

In addition, shrinking of the mezophyllic cells contribute to synthesis of abscisic acid and its translocation to stomatal cells. A drastic decline of photosynthesis and transpiration was caused by salt stress in cowpea, kidney bean(Murillo-Amador *et al*., 2007), and bush bean(Montero *et al*, 1997) were in tune with our results.

Malondialdehyde(MDA) and leaf electrolyte leakage(EL)

Temperature, drought or salinity stress can result in oxidative damage to plant cell membranes, MDA is one of the end products of lipid peroxidation(Zlatev *et al*., 2006). The treatment with EBR and MeJA resulted in decrease in MDA and electrolyte leakage.Several researchers have found that increased proline levels can protect plants from damage duo to mild or severe water stress. More importantly, proline seems to have a protective effect on plants under sever water stress(Ain-Lhout *et al*., 2001). Saradhi *et al.* (1995) reported that proline protects protein structure and membranes from damage and reduce enzyme denaturation; this could minimize damage caused by dehydration. A decrease in protein content in tomato plants grown under water stress was reported by Rahman *et al.* (2004). They postulated that water stress reduces the synthesis of protein, because of a possible suppression of the energy supply owing to reductions in photosynthesis and the overall adverse effects of the stress on the biochemical processes.

Salt stress adversely affected plant development and the results of the corrent study confirmed the negative effects of NaCl treatments on all physiological and biochemical traits.

Proline and soluble sugars content

Increasing salinity stress had a significantly increasing effect on proline content of the leaves, while this was more evident in perlette cultivar than flame seedless cultivar. Accumulation of solutes especially proline, glycin e-betain and sugars is a common observation under stress conditions(Ashraf *et al*., 1994). Proline is an important osmolyte which synthesizing in many micro organisms and plants exposed to salinity and drought stress, thus it as a osmoses protector in plant. Proline accumulating in plants exposed salinity stress is duo to low activity of oxidant enzymes(Sudhakar, 2001). Increasing proline is important for osmosis compatibility but also to preserving carbohydrates sink in chloroplasts. It is known that salinity stress reduces chlorophyll content, because the glutamate which is the primary constituents of chlorophyll and proline is consumed in favor of proline production. Furthermore, salinity stress induce glutamate ligase enzyme to transform glutamate into proline. Another reason for chlorophyll reduction is the increased use of nitrogen for proline synthesis. Proline plays a key part in maintaining the osmotic pressure and cytoplasmic enzymes and protects cell membrane from any damage through absorbing free radicals. Our results were similar to earlier reports that proline content significantly increased in common bean(Khadri *et al.* 2006) and corn(Yoon *et al.* 2005) under salt stress.

Increasing salinity level had a decreasing effect on chlorophyll content of the leaf, while this was more evident in the leaves of flame seedless variety than in perlette variety. Many environmental factors control chlorophyll synthesis in plant. Existing there factors as limiting factors cause to disordering synthesizing chlorophyll and appearing chlorosis in plant. Nacl stress decreased total chlorophyll content of the plant by increasing the activity of the chlorophyll degrading enzyme: chlorophyllase(Rao and Rao, 1981), inducing the destruction of the chloroplast structure and the instability of pigment protein complexes(Dubey, 1997). The decrease in chlorophyll content under saline cinditions is reported by Iqbal *et al.* (Iqbal *et al.* 2006) and Ashraf *et al.* (Ashraf and Foolad, 2005) and in several plants such as pea(Ahmad and Jhon, 2005), wheat (Ashraf and foolad, 2005), rice (Anuradha and Rao, 2003) and tomato(Al-Aghabary *et al*., 2004). Chlorophyll reduction can attributed to changing Nitrogen metabolism direction to forming compounds such as proline which used to regulating

osmoses(Dela-Roza and Maiti, 1995). Forming protolityc enzymes such as chlorophyllase which responsible to decompose chlorophyll and damaging photosynthetic structure, is other cause at this reduction(Sabater and Rodriguez, 1978). Different researcher also believe that decreased chlorophyll content may be duo to inhibitory effect of ions accumulated in chloroplast, chlorophyll degradation by oxidative stress caused by salt, activation of chlorophyllase enzyme by salinity ions and its negative effect on protophyzine. Furthermore increasing salinity level leads to decreased chlorophyll biosynthesis through increased salt.

Soluble proteins content

Contrary to our results the stability of soluble proteins was also observed by Dalio *et al.* (2013) and Ashraf (1994). It has been suggested that the maintenance of soluble protein levels reflects an increase in stress-specific proteins(younis *et al.*, 2009).

Conclusion

Results revealed that perlette cultivar was more tolerant against salinity than flame seedless variety, because mechanisms including RWC and proline concentration and lower lipid peroxidation makes it a tolerant variety for overcoming salinity stress, wherease flame seedless could not potentially employ this mechanism as efficiently as perlette could, duo to lower accumulation of proline.

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