Leaf water potential, photosynthetic pigments and compatible solutes alterations in four grape cultivars under salinity

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Summary

A hydroponic culture experiment was conducted to investigate the effects of different concentrations (0, 25, 50 and 100 mM) NaCl on own-rooted table grape (Vitis vinifera L.) cultivars (red 'Rishbaba', red 'Sahebi', 'Dastarchin' and red 'Sultana') under greenhouse conditions. Cultivars were evaluated for growth analysis leaf area, leaf water potential and the chlorophyll a, b and carotenoid contents in relation to proline and soluble sugars accumulation. Salinity treatments caused a growth reduction (P ≤ 0.05) in all the cultivars. Also leaf water potential and chlorophyll a, b contents decreased whereas carotenoid, proline and soluble sugars increased with increasing NaCl concentration. 'Dastarchin' and red 'Sultana' showed the salt- sensitivity, the highest loss of growth, leaf water potential and chlorophyll content and the lowest accumulation of carotenoids, proline and soluble sugars. Also salt stress significantly (P < 0.001) increased the rate of lipid peroxidation in the all cultivars particularly in 'Dastarchin' and red 'Sultana'. The increase in malondialdehyde content indicated that salinity induced oxidative stress. There was a significant negative correlation between leaf water potential and NaCl concentrations (r2: -0.781, p < 0.001). A positive correlation was also found between lamina proline contents and NaCl concentrations (r2: +0.964, p < 0.001) for all salinity treatments. Considering overall results red 'Rishbaba' and red 'Sahebi' showed higher capacity to tolerate salinity when compared to 'Dastarchin' and red 'Sultana'.

Key words: grapevine, chlorophyll a and b, lipid peroxidation, leaf area, salt stress.

Abbreviations: r-Rish: red 'Rishbaba', r-Sah: red 'Sahebi', Das: 'Dastarchin', r-Sul: red 'Sultana', MDA: Malondialdehyde.

Introduction

Salinity is a major impediment in irrigated agriculture especially in the arid and semiarid environment. Today, 20% of the world’s cultivated land and nearly half of the irrigated lands is affected by salinity (ZHU 2001). Increasing salt stress is a threat to grape growers in many regions around the world (FISARAKIS et al. 2001, WALKER et al. 2002). Salinity is known to influence grapevine growth in many ways, including reduced grape yield, reduced shoot and root vigor, reduced leaf area and appearance of leaf burns (SHANI et al. 1993, FISARAKIS et al. 2001, MUNNS 2002). Exposure of plants to salinity, drought or extreme temperatures commonly results in a water deficit. Salt stress changes the water relations of most higher plants, and salt tolerance often depends on drought tolerance (GREENWAY and MUNNS 1980, FLOWERS and YEO 1986). Salinity may decrease biomass production because it lowers plant water potential and causes specific ion toxicities or ionic imbalances in plants (MUNNS 2002). Plants achieve osmotic adjustment under saline conditions via ion uptake or synthesis of osmotica or both (PARIDA and DAS 2005). One of the most common stress responses in plants is overproduction of different types of compatible organic solutes (SERRAJ and SINCLAIR 2002). Compatible solutes are low molecular weight, highly soluble compounds that are usually non-toxic at relatively high concentrations. These organic osmolytes are most commonly carbohydrates (such as sugars), amino acids, protein and proline (YOUSSEF et al. 2003). Generally, they protect plants from stress through different processes, including via contributing to cellular water economy, detoxification of reactive oxygen species, protection of membrane integrity, and stabilisation of enzymes/proteins (ASIRAEF and FOOLAD 2006). Amino acid proline is known to occur widely in higher plants and normally accumulates in large quantities in response to environmental stresses (KAVI KISORE et al. 2005). In addition to its role as an osmolyte for water economy, proline helps stabilising sub-cellular structures (e.g., membranes and proteins), scavenging free radicals, and buffering cellular redox potential under stress conditions (ASIRAEF and DAS 2006). One of the effects of free oxygen radicals accumulation in plant cells under stress is lipid peroxidation via oxidation of unsaturated fatty acids leading to membrane damage and electrolyte leakage (LIU et al. 1987, MARSCHNER 1995). Malondialdehyde (MDA), a decomposition product of polyunsaturated fatty acids, has been utilized as a biomarker for lipid peroxidation (MITTLER 2002). In the present study, four grape cultivars, growing in hydroponic culture, were subjected to salinity. The objective was to evaluate salinity effect on the leaf water potential of cultivars. We also investigated the induction of proline (a compatible solute) accumulation in plant parts by high

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salinity, which could be responsible for protection against salt stress in this plant.

Material and Methods

Own-rooted vines of *Vitis vinifera* cultivars: red 'Rishbaba', red 'Sahebi', 'Dastarchin' and red 'Sultana' were grown in greenhouse from September to January 2009 for growing roots. Rooted cuttings were transferred to the hydroponics culture in 2-L pots containing aerated ¼ strength Hoagland nutrient solution containing: (1M KNO₃, 1M Ca(NO₃)₂, 1M MgSO₄.7H₂O, 1M KH₂PO₄ and micronutrients 2.85 g H₃BO₃, 1.81 g MnCl₂.7H₂O, 0.22 g ZnSO₄.7H₂O, 0.08 g CuSO₄.5H₂O, 0.05 g Na₂MoO₄.2H₂O and 0.028 g Fe-EDTA). Four weeks later, uniform plants with a new shoot of 35 cm in length were selected. The plants were treated for 2 weeks with 0, 20, 50 and 100 mM NaCl. At the end of the experiment, six plants from each treatment were sampled to determine leaf area using CompEye (leaf and symptom area) (BAKR 2005). Plants were harvested and plant parts including leaf, stem, petiole and root were weighed separately and dried at 70 °C for 48 h.

Leaf water potential (LWP): Leaf Water Potential (LWP) was determined on three to six leaves of similar age with thermocouple psychrometers (Model Wescor HR33 dew point microvoltmeter; Wescor Inc., Logan, UT, U.S.A.) and expressed in -MPa. It should be read within about 2 h of solar noon, normally in about 11:30 AM to 2:30 PM and select a leaf that is fully exposed to the light. Also leaf should be a healthy, fully expanded leaf with no insect holes, good color.

Chlorophyll a and b contents: Chlorophyll a (Chₐ) and chlorophyll b (Chₐ) concentrations were analyzed following the method of LICHTENTHALER and WELLBURN (1985). Fresh leaves (0.1 g) were used for photosynthetic pigment extraction and immersed in 5 mL of 80 % acetone. Extracts were filtered by Whatman No. 2 filter paper and absorbance was measured in a UV-visible spectrophotometer (model WPA S2100) at 646, 663 and 470 nm, Chₐ and Chₐ concentrations (mg-g⁻¹ F.W) were calculated according to the following equations:

\[ \text{Chlorophyll } a \ (Ch_a) = 12.25 \times A_{663} - 2.798 \times A_{446} \]

\[ \text{Chlorophyll } b \ (Ch_b) = 21.5 \times A_{645} - 5.1 \times A_{663} \]

Carotenoid = (1000 * A₄₇₀ – 1.82 * Chₐ – 85.02 * Chₐ) / 198

Proline content: Proline content was calculated according to BATES et al. (1973). Proline concentration was determined using calibration curve and expressed as µg proline-g⁻¹ DW. Dry plant material (0.5 g) was homogenized in 10 mL of 3 % sulfoacilic acid and the homogenate was filtered. The filtrate (2 mL) was treated with 2 mL ninhydrin reagent (1.25 mg Ninhydrin in 30 mL of Glacial acetic acid and 20 ml 6 M H₃PO₄) and incubated at 95 °C for 1 h. The reaction was terminated placing in an ice bath. The reaction mixture was vigorously mixed with 4 mL toluene. After warming at 25 °C, absorbance of the colored solutions was read at 520 nm. L-proline was used as a standard.

Soluble sugar content: Soluble sugar content in the leaf and root tissues was extracted and analyzed according to the method of DUBOIS et al. (1956). Dry plant material (0.1 g) was homogenized in 10 mL of 70 % ethanol. After one week, 2 mL of supernatant was mixed with 1 mL of 5 % phenol and 5 mL of sulfuric acid. After 30 min absorbance of the cold and colored solutions was read at 485 nm.

MDA analysis: Lipid peroxidation in the leaf tissues was determined in terms of malondialdehyde (MDA) content by thiobarbituric acid (TBA) reaction as described by NOVACKY and POHAMP (1990). Briefly, 0.2 g of the leaf tissue of plants were homogenized in 5 mL of 1 % (w:v) trichloroacetic acid (TCA), then centrifuged at 8000 g for 10 min. 1 mL of supernatant was added with 4 mL of 20 % (w:v) TCA containing 0.5 % (w:v) thiobarbituric acid (TBA), and the solution was heated for 30 min at 95 °C in the warm water bathroom. The samples were cooled on ice for 5 min and recentrifuged for 5 min at 8000 g. Absorbance was measured at 532 nm. For the MDA calculation, an extinction coefficient of 155mM⁻¹cm⁻¹ was used at 532 nm. The results were expressed in µmol of malondialdehyde (MDA) equivalent per gram fresh weight.

Statistical analysis: Analysis of variance was performed by the statistical program SpSS version 18 and one-way-ANOVA was used to compare the main effects and interactions between cultivars and salinity levels using GLM.

Results

The results indicated that the growth rate of shoot and root decreased under salt stress. The accumulation of dry matter decreased more in shoots than in roots, resulting in nearly 40 % increase in root/shoot ratio (data not presented). r-Rish showed higher dry matter production than all cultivars. Also r-Rish showed higher shoot/root fresh weight ratio than all cultivars (Tab. 1). In addition salinity significantly affected leaf area (P < 0.001). The decrease of leaf area in Das was higher than that of r-Rish and r-Sah cultivars. The reduction of leaf area at 100 mM NaCl was 22.59 and 52.24 % respectively for the r-Sah and Das when compared to their controls (Tab. 2). The chlorophyll a and b contents of leaves decreased with increasing salinity levels (Tab. 2). The decrease in r-Sul and Das cultivars were higher than r-Rish and r-Sah cultivars. The reduction in chlorophyll a content due to increased salt treatments from 0 to 100 mM NaCl was 33.97, 37.71, 52.22 and 45.55 % in leaves for the r-Rish, r-Sah, r-Sul and Das respectively (Tab. 2). Also, the chlorophyll b content was significantly decreased. The decreased values were calculated as 63.12, 51.93, 66.99 and 75.9 % in r-Rish, r-Sah, r-Sul and Das respectively (Tab. 2). Also, the chlorophyll b content was significantly decreased. The decrease in carotenoids content in four cultivars increased, but the increase in carotenoids content in r-Rish and r-Sah cultivars was higher than that of Das and r-Sul (Tab. 2).

Salinity markedly decreased leaf water potential of all the cultivars (Fig. 1). As a result, r-Rish showed a lower reduction leaf water potential than other varieties after 14 d
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Leaf water potential was lower for 100 mM salt-treated plants than for plants in the other treatments (Fig. 1). There was a significant negative correlation between leaf water potential and NaCl concentrations ($r^2$: -0.781, $p < 0.001$). Fourteen days of salinization were sufficient to increase the proline and soluble sugar contents in both lamina and roots of four cultivars, and this increase was more evident in plants at the 50 and 100 mM NaCl treatment. The proline and soluble sugar increased more in r-Rish and r-Sah than in r-Sul and Das when four cultivars were exposed to increased salt concentrations. This increase was greater in lamina than in roots (Figs 2 and 3).

Salinity had a significant effect on MDA content in shoots ($F_{1,4} = 32.07$, $p < 0.001$). It is clear from the Fig. 4 that a sharp increase in the accumulation of MDA content was observed in all cultivars at all stress regimes, however the increase in r-Sul and Das being higher than in r-Rish and r-Sah. The levels of accumulation were 195.67, 84.5, 465.51 and 226.48 % in r-Rish, r-Sah, r-Sul and Das cultivars respectively, indicating a high rate of lipid per oxidation in r-Sul due to salt stress.

### Table 1

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Root/shoot dry weight ratio</th>
<th>Root/shoot fresh weight ratio</th>
<th>Dry/fresh root weight ratio</th>
<th>Dry/fresh shoot weight ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>r-Rish</td>
<td>0.256 a</td>
<td>0.566 a</td>
<td>0.0601 a</td>
<td>0.134 b</td>
</tr>
<tr>
<td>r-Sah</td>
<td>0.22 b</td>
<td>0.508 b</td>
<td>0.0613 a</td>
<td>0.144 ab</td>
</tr>
<tr>
<td>r-Sul</td>
<td>0.19 c</td>
<td>0.467 c</td>
<td>0.0618 a</td>
<td>0.154 a</td>
</tr>
<tr>
<td>Das</td>
<td>0.225 b</td>
<td>0.520 b</td>
<td>0.0629 a</td>
<td>0.148 ab</td>
</tr>
</tbody>
</table>

### Analysis of variances (F-values)

- Salinity: $F_{1,4} = 32.07$, $p < 0.001$
- Cultivar: $F_{3,4} = 23.71$, $p < 0.001$
- Salinity × Cultivar: ns

*Means within a column followed by the same letter are not significantly different at $p = 5\%$ level according to the tuky, ($n = 3$): **$p < 0.01$. ***$p < 0.001$.

### Table 2

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Leaf area (cm²)</th>
<th>Chla (mg g⁻¹ fw)</th>
<th>Chlb (mg g⁻¹ fw)</th>
<th>Carotenoids (mg g⁻¹ fw)</th>
</tr>
</thead>
<tbody>
<tr>
<td>r-Rish</td>
<td>42.50 a</td>
<td>10.049 b</td>
<td>4.71 b</td>
<td>0.3 a</td>
</tr>
<tr>
<td>r-Sah</td>
<td>43.18 a</td>
<td>10.91 a</td>
<td>5.74 a</td>
<td>0.284 a</td>
</tr>
<tr>
<td>r-Sul</td>
<td>-</td>
<td>9.74 c</td>
<td>4.7 b</td>
<td>0.237 b</td>
</tr>
<tr>
<td>Das</td>
<td>36.43 b</td>
<td>9.54 c</td>
<td>4.31 c</td>
<td>0.193 c</td>
</tr>
</tbody>
</table>

### Analysis of variances (F-values)

- Salinity: $F_{1,4} = 22.156$, $p < 0.001$
- Cultivar: $F_{3,4} = 13.96$, $p < 0.001$
- Salinity × Cultivar: ns

*Means within a column followed by the same letter are not significantly different at $p = 5\%$ level according to the tuky, ($n = 3$): ns: non-significance at $p < 0.05$. **$p < 0.01$. ***$p < 0.001$.

### Fig. 1: Leaf water potential of r-Rish, r-Sah, r-Sul and Das treated with different concentrations of NaCl for 2 weeks. Bars are ± SE of the means ($n = 3$) tuky $p \leq 0.05$. Different letters indicate significant differences between varieties at each salt concentration.
Discussion

Growth reduction is an early phenomenon and a common response in woody plants to salt stress both in vitro and in vivo (Vijayan et al. 2003). Growth response to salinity is often regarded as a basis of evaluation for tolerance (Kuiper et al. 1988). High salinity due to indirect effect on uptake of other nutrients probably resulted in reduction in growth and disturbance of several other physiological processes (Prior et al. 1992). In the few seconds or minutes period of time for plants exposed to salinity, cells lose water and shrink. Over hours, cells regain their original volume but cell elongation rates are reduced, leading to lower rates of leaf and root growth. Over days, changes in cell elongation and cell division lead to slower leaf appearance and smaller final size, and leaf growth is usually more affected than root growth (Hasegawa et al. 2000, Hsiao and Xu 2000). According to Sotiropoulos et al. (2006 b) explants are stressed in two ways under in vitro salinity: by the increase in osmotic potential of culture media as a result of high solute content, and by the toxic effects of high concentrations of ions. The negative effect of salinity on plant growth and water content may be due to the occurrence of defect metabolism in plant cells. Since high osmotic pressure resulted from high salinity restricted plant cells to uptake water and some mineral nutrients dissolved in the

Fig. 2: Proline content in shoot (A) and root (B) of r-Rish, r-Sah, r-Sul and Das treated with different concentrations of NaCl for 2 weeks. Bars are ± SE of the means (n = 3) tuky p ≤ 0.05. Different letters indicate significant differences between varieties at each salt concentration.

Fig. 3: Soluble Sugar content in shoot (A) and root (B) of r-Rish, r-Sah, r-Sul and Das treated with different concentrations of NaCl for 2 weeks. Bars are ± SE of the means (n = 3) tuky p ≤ 0.05. Different letters indicate significant differences between varieties at each salt concentration.

Fig. 4: MDA content of r-Rish, r-Sah, r-Sul and Das treated with different concentrations of NaCl for 2 weeks. Bars are ± SE of the means (n = 3) tuky p ≤ 0.05. Different letters indicate significant differences between varieties at each salt concentration.
culture medium (CICEK and CAKIRLAR 2002). The chemical potential of the saline media initially establishes a water potential imbalance between the apoplast and symplast that leads to decrease in pressure potential, which might cause growth reduction (BOHNERT et al. 1995). On the other hand, the cellular response to water potential reduction is osmotic adjustment. It involves the transport, accumulation and compartmentation of organic solutes and inorganic ions (BOHNERT et al. 1995). Under high salt environment, higher plants maintain their water content by accumulation of compatible organic solute in their cytoplasm. Plant cells decrease their osmotic potential by the accumulation of inorganic and organic solutes or by loss of water. The accumulation of organic solutes might be of importance for the adjustment of the cellular water potential under conditions of reduced water availability (YOUSSEF and ALFREDAN 2008). In organisms ranging from bacteria to higher plants there is a strong correlation between increased cellular proline levels and the capacity to survive both water deficit and the effects of high environmental salinity (AHMAD and JHON 2005). Proline plays an adaptive role in mediating osmotic adjustment and protecting the sub-cellular structures in stressed plants. Apart from protection of macromolecules from denaturation and carbon and nitrogen reserve for stress relief, proline has several other functions during stress; e.g. osmotic adjustment (VOETBERG and SHARP 1991), osmoprotection (KISHOR et al. 2005), free radical scavenger and antioxidative activity (SHARMA and DIPETZ 2006). In many studies a positive correlation between the accumulation of proline and stress tolerance in plants has been found (LUTTS et al. 1996, KUMAR et al. 2003). Also proline content have been reported to increase under NaCl stress in Phaseolus aureus (MISRA and GUPTA 2005), Morus alba (AHMAD et al. 2007), Sesamum indicum (KOCA et al. 2007). Plant cells growing in saline media must adjust osmotically, since a positive turgor is required for cell expansion and most biochemical, physiological, and developmental processes (GREENWAY and MUNNS 1980). Increase in sugar content only in tolerant cvs. (DOWNTON 1985) help them in osmotic adjustment (REUVENI et al. 1991). Proline content increased significantly in the leaves of all the cultivars as the salt concentration increased (Fig. 2 A). This increase in salt-tolerant cultivars was higher than that of the salt-sensitive cultivars. The increase of proline in lamina at 100 mM NaCl was 468.941 and 313.26 % respectively for the r-Sah and r-Sul when compared to their controls. However, increasing in proline content in r-Rish and r-Sah cultivars was higher than that of Das and r-Sul, also there were no significant differences between r-Sul and Das cultivars. Our results revealed that the leaf water potential is affected by an increase in leaf proline content. That is, an increase in proline content caused lower reduction in leaf water potential. There was a positive correlation between proline and leaf water potential. R-Rish showed a lower reduction in leaf water potential than other cultivars, whereas Das had a higher reduction in leaf water potential. The decreased values were calculated as 20.19, 27.29, 31.96 and 33.86 % in r-Rish, r-Sah, r-Sul and Das respectively at 100 mM NaCl (Fig. 1). There were no significant differences between r-Sul and Das cultivars. Chlorophyll content reduction was observed with increasing salinity in all the cultivars (Tab. 2). PARIDA and DAS (2005) suggested that such a decrease in chlorophyll content in response to salt stress is a general phenomenon. The reduction of chlorophyll contents in abiotic stress plants might possibly be due to changes in the lipid protein ratio of pigment-protein complexes or increased chlorophyllase activity (PARIDA et al. 2004). Our results are consistent with several reports in a number of plant species (AGASTIAN et al. 2000, HAMADA and EL-ENANY 1994). In addition to chlorophyll degradation, salt-induced necroses on leaf and shoot tissues were observed in grape explants (SIVETEPE and ERIS 1999). Moreover, reduction in chlorophyll concentrations is probably due to the inhibitory effect of the accumulated ions of various salts on the biosynthesis of the different chlorophyll fractions. Salinity affects the strength of the forces bringing the complex pigment-protein-liquid, in the chloroplast structure. As the chloroplast is surrounded by a membrane its stability is dependent on the membrane stability (YEo et al. 1990, ALI et al. 2004). Increase of carotenoids content in r-Rish cultivar was higher than Das cultivar. Therefore r-Rish has a better ability to protect chlorophyll from photo oxidation. Considering MDA content in shoots, lipid peroxidation was significantly higher under salt stress than in control plants (Fig. 4). Determining the MDA content and hence, the extent of membrane lipid peroxidation, has often been used as a more reliable tool than anti-oxidative scavenging systems to assess the degree of plant sensitivity to oxidative damage (BLOKHINA et al. 2003). KOCA et al. (2007) also showed that lipid peroxidation was higher at 100 mM NaCl treatment in a salt sensitive cultivar of Sesamum indicum than in a salt tolerant one. Our data showed remarkable increase in shoot MDA content for Das and r-Sul at different concentration of NaCl than other cultivars. HONG et al. (2000) found that, under salt stress, MDA production in tobacco cell cultures was enhanced.

**Conclusion**

The present study was conducted to determine alterations of leaf water potential status, proline, soluble sugar and chlorophyll contents in four own-rooted grapevine cultivars under salinity stress. Parameters such as root/shoot ratio and leaf area were significantly decreased by salinity. Das and r-Sul showed the highest growth reduction. Proline and soluble sugars contents increased while there was a reduction in leaf water potential and chlorophyll a and b contents under different levels of salinity. In comparison to the other cultivars, red ‘Rishbaba’ and red ‘Sahebi’ accumulated high amounts of proline and soluble sugars in leaf blade and root particularly at 50 and 100 mM NaCl. Compared to others, these cultivars showed a slight reduction in leaf water potential. Increasing in MDA content in Das and r-Sul were higher than that of others. The results showed that red ‘Rishbaba’ and ‘DaStarchin’ had respectively a higher and a lower capacity to tolerate salt stress when compared to the other cultivars. However, all the cultivars studied seem to be relatively sensitive when exposed to salinity.
References


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