Effect of Salinity on Growth and Leaf Photosynthesis of Two Sugar Beet (Beta vulgaris L.) Cultivars

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ABSTRACT

This study investigated the variation between two sugar beet (Beta vulgaris L.) cultivars with respect to growth parameters and photosynthetic rates of individual attached leaves during salinization. Sugar beet plants grown in sand culture were gradually exposed to different levels of salinity (0, 50, 150, 250, and 350 mM, NaCl+CaCl₂ in 5:1 ratio). Salt concentrations significantly decreased all growth traits. At the highest level of salinity (350 mM), cv 7233-P29 showed a significantly higher leaf area and total dry matter than Madison after eight weeks of salt treatment commencement. Net photosynthesis (A_CO₂) and stomatal conductance (gₛ) were strongly affected by salinity. The Na⁺ and Cl⁻ concentrations in shoots significantly increased as salt concentration increased. Net photosynthesis (A_CO₂) was plotted against computed leaf internal CO₂ concentration (Cᵢ), and the initial slope of this A_CO₂-Cᵢ curve was used as a measure of photosynthetic ability. Leaves from plants exposed to 50 mM salinity showed little change in photosynthesis, whereas those treated by high levels of salinity had up to 91.5% inhibition in photosynthetic rates and an increase in CO₂ compensation point. Leaf chlorophyll content increased with increasing salinity. Although partial stomatal closure occurred with salinization, reductions in photosynthesis were partly non-stomatal at high levels of salt treatment.

Keywords: Gas exchange, Plant growth, Salt stress, Sugar beet.

INTRODUCTION

Growth of higher plants in saline soil depends on salt tolerance of the plant species. The decrease in plant growth due to salinity includes a reduction in the leaf area of the plant. The reduction in growth is a consequence of several physiological responses including modification of ion balance, water status, mineral nutrition, stomatal behaviour and photosynthetic efficiency (De-Herrale et al., 1998; Hester et al., 2001; Ghoulam et al., 2002; Al-Khateeb, 2006; Koyro, 2006).

Photosynthesis, as one of the most important physiological processes, provides 90% of the plant dry matter (Steduto et al., 2000). Several researchers have published convincing evidence showing that photosynthetic carbon metabolism is affected by reduced plant water potentials (Sultana et al., 1999; Steduto et al., 2000; Hester et al., 2001; Koyro, 2006). The photosynthetic capacity of plants grown under saline conditions is depressed depending on type of salinity, duration of treatment, species and plant age (Sultana et al., 1999; Steduto et al., 2000; Hester et al., 2001; Koyro, 2006).

Many studies have concluded that the reduction in photosynthesis in response to salinity is to some extent the result of reduced stomatal conductance and, consequently, restriction of the availability of CO₂ for carboxylation (Everard et al., 1994; Chartzoulakis et al., 1995; Koyro, 2006). Reduction in transpiration rate under salinity is another evidence for interference.
of salinity to stomatal conductance. Although reduction of photosynthesis by salinity is partly due to a reduced stomatal conductance, non-stomatal inhibition of photosynthesis, caused by direct effects of NaCl on the photosynthetic apparatus, has been reported for some species (Seemann and Sharkey, 1986; Delfine et al., 1998; Sultana et al., 1999; Steduto et al., 2000). These non-stomatal factors consist of a reduced efficiency of RuBP carboxylase, reduction of RuBP regeneration, the sensitivity of photosystem II to NaCl and a reduction in chlorophyll pigments to absorb enough light (Ball and Farquhar 1984; Ball and Anderson 1986; Seemann and Sharkey, 1986).

Since information on physiological changes occurring during salt stress is lacking for sugar beet, which is relatively tolerant of saline environments, the aim of this study was to compare the response to salinity of two cultivars of sugarbeet. The different varieties were chosen according to preliminary information on their growth under saline conditions (Dadkhah and Griffiths, 2006). In this paper, the effect of different levels of salinity treatment on gas exchange, stomatal conductance and chlorophyll content of two sugar beet cultivars are described.

**MATERIALS AND METHODS**

Plant Materials and Growth Conditions

A pot experiment was carried out based on randomized complete block design with six replications to investigate the effect of salt stress on growth and gas exchange of two sugar beet (*Beta vulgaris*) cultivars, one of Iranian origin (7233-P29) and a British (Madison) cultivar, which were chosen according to preliminary information on their growth under saline conditions (Dadkhah and Griffiths, 2006).

Seeds were sown 5 mm deep in plastic containers (40x20x10 cm) filled with washed sand, with a saucer under them to prevent leaching after irrigation, and transferred to controlled conditions. The growth conditions were 26±1°C/16±1°C (day/night), while relative humidity was between 45-55%. Photon flux density (PFD) was about 350 µmol m⁻² s⁻¹ at canopy height with 16-h photoperiod. Five levels of salinity (0, 50, 150, 250, 350 mM NaCl+CaCl₂ in 5:1 molar ratio) were imposed through irrigation from the time most plants were at the 4-leaf stage and continued for 8 weeks. Salts were added to modified Hoagland nutrient solution (Maas and Poss, 1989). The pots were flushed out with saline water containing nutrients every week to ensure homogeneity of salinity and nutrient supply in the growth medium. To prevent shock to plants, irrigation started with 50 mM saline water and was increased by 50 mM every other day until reaching each salinity level.

Growth was measured as leaf area, number of leaves, and dry matter accumulation. Plants were harvested after 8 weeks of salt treatment and were separated into leaf lamina, petioles, stem, and roots. These components were dried for 48h at 70°C in a convection oven and weighed.

**Data Collection**

Gas exchange patterns in sugar beet leaves were studied in the presence of salinity under controlled environmental condition. Net photosynthesis ($A_{CO2}$) of the attached youngest fully expanded leaf (youngest leaf over 50% full size) and the oldest leaf were measured by enclosing the middle part of leaf in the cuvette of a Combined Infra Red Gas Analysis System (CIRAS-1 Portable photosynthesis system) at week 8 after commencement of salinity treatment. The area of cuvette that caught full illumination was 2.5 cm². Measurements and results were displayed on the analyzer display panel and also recorded on the data storage system. In order to study photosynthetic efficiency,
Effect of Salinity on Growth and Leaf Photosynthesis

Table 1. Mean live leaf area (cm$^2$ per plant) at different levels of salinity and percentage of reduction (compared to the control). Each number is the average of six replications.

<table>
<thead>
<tr>
<th>Salinity (mM)</th>
<th>0</th>
<th>50</th>
<th>150</th>
<th>250</th>
<th>350</th>
</tr>
</thead>
<tbody>
<tr>
<td>cvs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Madi</td>
<td>41 ± 851</td>
<td>0.0</td>
<td>31 ± 801</td>
<td>5.90</td>
<td>29 ± 499</td>
</tr>
<tr>
<td>P$_{29}$</td>
<td>35 ± 813</td>
<td>0.0</td>
<td>50 ± 813</td>
<td>0.00</td>
<td>18 ± 570</td>
</tr>
<tr>
<td>Avg.</td>
<td>832.0</td>
<td>0.00</td>
<td>807.0</td>
<td>3.00</td>
<td>534.0</td>
</tr>
</tbody>
</table>

The values are the Mean ± SE of six plants. The difference among treatments is significant at the 5% level according to Duncan’s multiple range test.
Table 2. Mean number of leaves per plant at different levels of salinity and percentage of reduction (compared to the control). Each number is the average of six replications.

<table>
<thead>
<tr>
<th>Salinity (mM)</th>
<th>0</th>
<th>50</th>
<th>150</th>
<th>250</th>
<th>350</th>
</tr>
</thead>
<tbody>
<tr>
<td>cvs</td>
<td>Actual</td>
<td>%</td>
<td>Actual</td>
<td>%</td>
<td>Actual</td>
</tr>
<tr>
<td>Madison</td>
<td>1±14</td>
<td>0.0</td>
<td>1.7±16</td>
<td>-14.3</td>
<td>1.2±13</td>
</tr>
<tr>
<td>P29</td>
<td>0.6±11</td>
<td>0.0</td>
<td>1.1±11.7</td>
<td>-6.40</td>
<td>0.6±10.5</td>
</tr>
<tr>
<td>Avg.</td>
<td>12.5</td>
<td>0.00</td>
<td>13.85</td>
<td>-10.8</td>
<td>11.75</td>
</tr>
</tbody>
</table>

The values are the Mean±SE of six plants. The difference among treatments is significant at the 5% level according to Duncan's multiple range test.

Cultivar Madison had greater reduction in leaf numbers due to salinity after 8 weeks of salt treatment (Table 2). However, leaf number of Madison was higher than cv P29 at all levels of salinity but its total leaf area was less than P29. Therefore, it can be concluded that Madison genetically produces more, but smaller, leaves. The results show that the leaf number was less affected than leaf area by salinity. It suggested that most of the reduction in plant leaf area due to salinity was caused by the inhibition of leaf expansion. This is consistent with the result of previous research, which showed that high levels of salinity decreased leaf area due to a combination of a decrease in cell number and cell size (De-Herralde et al., 1998). Witkowski and Lamont (1991) reported that plants might reduce water loss by reducing their evaporation surface. Therefore, leaves tend to be smaller and thicker in saline conditions.

Salt stress significantly reduced dry matter production (Table 3). Total plant dry matter at the highest salinity (350 mM) was decreased by 50.6% (mean of both cvs) compared to dry matter of non-stressed plants after 8 weeks of salinity treatment. At the highest salinity, cv P29 had greater dry weight than Madison. Shoot dry weight was significantly reduced by salinity. Shoot dry weight at the highest salinity (350 mM) decreased by 43.55% (mean of 2 cvs).

Table 3: Total dry weight, shoot dry weight, and root dry weight (gr per plant) and percentage of reduction (compared to the control) of sugar beet cultivars at different levels of salinity after 8 weeks of salinization. Each number is the mean of six replications.

<table>
<thead>
<tr>
<th>Salinity (mM)</th>
<th>0</th>
<th>50</th>
<th>150</th>
<th>250</th>
<th>350</th>
</tr>
</thead>
<tbody>
<tr>
<td>cvs</td>
<td>Actual</td>
<td>%</td>
<td>Actual</td>
<td>%</td>
<td>Actual</td>
</tr>
<tr>
<td>Madison</td>
<td>0.02±9.1</td>
<td>0.0</td>
<td>0.2±7.99</td>
<td>12.2</td>
<td>0.4±5.8</td>
</tr>
<tr>
<td>P29</td>
<td>0.4±7.9</td>
<td>0.0</td>
<td>1.0±8.00</td>
<td>-1.3</td>
<td>0.9±6.4</td>
</tr>
<tr>
<td>Avg.</td>
<td>8.5</td>
<td>0.00</td>
<td>8.0</td>
<td>5.90</td>
<td>6.1</td>
</tr>
<tr>
<td>Shoot dry weight (gr per plant)</td>
<td>3.13</td>
<td>0.50±4.4</td>
<td>31.25</td>
<td>0.4±3.5</td>
<td>45.31</td>
</tr>
<tr>
<td>Madison</td>
<td>0.07±6.4</td>
<td>0.0</td>
<td>0.3±6.2</td>
<td>3.13</td>
<td>0.50±4.4</td>
</tr>
<tr>
<td>P29</td>
<td>0.2±6.0</td>
<td>0.0</td>
<td>1.0±6.8</td>
<td>-1.3</td>
<td>0.8±5.4</td>
</tr>
<tr>
<td>Avg.</td>
<td>6.20</td>
<td>0.00</td>
<td>6.50</td>
<td>-4.84</td>
<td>4.90</td>
</tr>
<tr>
<td>Root dry weight (g per plant)</td>
<td>33.3</td>
<td>0.1±1.4</td>
<td>48.1</td>
<td>0.04±1.07</td>
<td>60.4</td>
</tr>
<tr>
<td>Madison</td>
<td>0.09±2.7</td>
<td>0.0</td>
<td>0.1±1.8</td>
<td>33.3</td>
<td>0.1±1.4</td>
</tr>
<tr>
<td>P29</td>
<td>0.2±1.9</td>
<td>0.0</td>
<td>0.4±1.2</td>
<td>36.8</td>
<td>0.1±1.0</td>
</tr>
<tr>
<td>Avg.</td>
<td>2.30</td>
<td>0.00</td>
<td>1.50</td>
<td>34.80</td>
<td>1.20</td>
</tr>
</tbody>
</table>

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Figure 1. Net photosynthesis rates of the youngest fully expanded (a) and old leaves (b) of two sugar beet cultivars at different levels of salt concentration. Each bar is the average of six measurements. Vertical lines are standard error of the means.

compared to non-stressed plants. Shoot dry weight of cv Madison decreased compared to its control at the same time (Table 3). Salt stress also had a significant negative effect on root dry weight. The effect of salinity on root dry weight was greater than shoot dry weight because root dry weight of both cvs decreased even at the lowest level of salinity. Root dry weight at the lowest level (50 mM) of salinity decreased by 34.8% compared to root dry weight of non-stressed plants (Table 3). This might be due to the type of sugar beet root (storage root) and also water deficiency caused by concentration of salt in the growth medium. Abdollahian-Noghabi (1999) found that shoot/root ratio of Beta vulgaris increased under drought stress condition. The root percentage reduction of cv P29 was less than that of Madison at high level of salinity (Table 3), although both were severely influenced. A possible reason for dry matter reduction could be the greater reduction in uptake and utilization of mineral nutrients by plants under salt stress. Pessarakli and Tucker (1985, 1988) reported that total nitrogen uptake of cotton plants decreased with increasing salinity, reflecting primarily a dry matter reduction. The uptake of nitrogen (N) in salt stressed plant might be competitively limited by Cl⁻ (Aslam et al., 1984; Ward et al., 1986).

Leaf Gas Exchange

Net Photosynthesis \( (A_{\text{CO}_2}) \) significantly \( (P \leq 0.001) \) decreased with increasing salt concentration (Figure 1-a). Plants grown at low and high levels of salinity had rates that were, respectively, 80% and 8.3% of the controls. Analysis of variance revealed that there were no significant differences between the two cultivars in the rates of net photosynthesis, but cv Madison had higher net photosynthesis than P29. Net photosynthesis rate generally decreased with leaf age (Figure 1-b) and this was paralleled by a lower stomatal conductance. Net photosynthesis rate of cv Madison was significantly decreased at the lowest level of salinity (50 mM), while P29 had a higher net photosynthesis rate compared to its control at the same salt concentration but this was not significant. The photosynthetic rates of old leaves of Madison plants grown at 250 mM salt concentration were below the compensation point, while P29 still maintained rates above the compensation point. The photosynthetic rates of old leaves of plants grown at 350 mM of salt concentration were at the compensation point and below it for, respectively, P29 and Madison cultivars (Figure 1-b). Therefore, old leaves of these plants were effectively parasitic, whereas old leaves of non-stressed plants and those grown at low levels of salinity still made a positive contribution to the carbon economy of the plant.

The adverse effects of salinity on net photosynthesis \( (A_{\text{CO}_2}) \) was associated with a significant \( (P \leq 0.001) \) decrease in stomatal
conductance ($g_s$) (Figure 2). Stomatal conductance of plants irrigated with low level of salinity (50 mM) decreased by 31% compared with the controls. At high level of salinity, stomatal conductance was very low (91.3% decrease compared to the controls).

The ratio of intercellular to ambient $CO_2$ concentration ($C_i:C_a$) decreased up to 150 mM salinity compared to the controls (Figure 3). In spite of decreasing $A_{CO_2}$ and $g_s$ with increasing levels of salinity, the $C_i:C_a$ ratio not only showed no significant reduction at 250 mM but also increased at 350 mM. In the control conditions, there was no significant difference in $C_i:C_a$ ratio between the two cultivars.

**Salinity and Leaf Ion Concentration**

The concentration of salt in the rooting medium induced a substantial increase in Na$^+$ concentration in leaves ($P \leq 0.001$) (Figure 4-a). There were significant differences in leaf sodium concentration between the two cultivars at different levels of salinity. Cultivar P$_{29}$ had significantly higher Na$^+$ content in its leaves at the lowest salinity (50 mM) than cv Madison (Figure 4-a). However, at the highest salinity level, cv P$_{29}$ had significantly lower leaf sodium content compared to Madison. Leaf K$^+$ concentration decreased significantly by increasing salinity to around half of the control values at 350 mM (Figure 3-b). Chloride (Cl$^-$) contents in leaves of both cultivars increased with increasing salt concentration (Figure 3-c). There were no significant differences in leaf Cl$^-$ concentration between the two cultivars in the control plants. At high levels of salinity, cv P$_{29}$ had significantly lower Cl$^-$ content in leaves than Madison. There is substantial evidence that accumulation of inorganic ions, predominantly Na$^+$ and Cl$^-$, has an important role in the process of osmotic adjustment. (Ashraf and Ahmad, 2000; Ghoulam et al., 2002). The increase in Na$^+$ and Cl$^-$ concentrations in salinized expanded leaves of the two cultivars was not similar. Cultivar P$_{29}$ (salt tolerant) accumulated less Na$^+$ and Cl$^-$ in its cells at high levels of salinity compared to cv Madison. Schachtman and Munns (1992) reported that lower rate of Na$^+$ accumulation in expanding leaves of Triticum species might be due to higher leaf expansion and/or a mechanism that limits the transport of roots uptake of Na$^+$. The lower Na$^+$ and Cl$^-$ contents in P$_{29}$ at high levels of salinity could be attributed to the higher rates of leaf expansion at high
level of salinity in this cultivar. Another possibility might be that cv P29 absorbs Na\(^+\) and Cl\(^-\) up to a threshold level and, then, acts as a salt excluder.

**Leaf Chlorophyll Content and Chlorophyll Fluorescence Analysis**

Leaf chlorophyll a, chlorophyll b and total chlorophyll content generally increased with increasing salinity (data not shown). The ratio of chlorophyll a/b was unaffected by salinity up to 150 mM in both cultivars. The amount of leaf total chlorophyll and photosynthetic rate showed a weak negative correlation in the presence of salinity. This could be due to the inverse effect of salinity on photosynthesis and its positive effect on chlorophyll content.

The ratio of variable to maximum fluorescence (\(F_v/F_m\)) was significantly reduced by increasing salt concentration, but not between the controls and the 50 mM salt treatment (Figure 5). The maximum level of fluorescence (\(F_m\)) was lower under salinity treatments than in the controls, which suggests that there might have been some damage due to salinity to photosystem II reaction centres, or, in transfer of excitation energy from the antenna to the reaction centres.

**Photosynthesis and Stomatal Parameters**

The present study indicated substantially lower rates of net photosynthesis and stomatal conductances with increasing salinity. Similar results have been obtained...
with other plant species (Everard et al., 1994; Delfine et al., 1998; Sultana, 1999; Steduto et al., 2000).

Everard et al. (1994) reported that, at intermediate salinity (100 mM), photosynthesis in celery (Apium graveolens L.) was limited by a decrease in stomatal conductance, while at the highest level of salinity (300 mM), carboxylation capacity (by measuring C\textsubscript{i}) and electron transport (fluorescence) were the apparent prevailing limitations to photosynthesis.

Comparing rates of net photosynthesis under the control condition (Figure 1a) revealed that the two cultivars did not have significant differences in gas exchange characteristics. Lower rates of net photosynthesis at higher levels of salinity were associated with a decreased stomatal conductance (Figure 2). Decreased stomatal conductance had relatively little effect on intercellular CO\textsubscript{2} concentration, which responded in a complex manner. If stomatal closure was the main reason for decreased rates of net photosynthesis at high levels of salinity (250 and 350 mM), it would be expected that there would be lower intercellular CO\textsubscript{2} concentrations. The absence of such an observation in plants exposed to salinities above 150 mM suggests that a non-stomatal effect limited photosynthesis at higher salinities.

**Photosynthesis and Non-stomatal Parameters**

A reduction in the rate of photosynthesis as a consequence of non-stomatal inhibition of photosynthesis by salt has also been observed previously (Seeman and Sharkey, 1986; Lloyd et al., 1987; Delfine et al., 1998). Such alterations in photosynthetic capacity must be the result of either a change in the leaf content of photosynthetic machinery and/or alteration in the efficiency with which this machinery operates.

Total chlorophyll contents in both cultivars increased under saline conditions. However, a negative relationship (R\textsuperscript{2} = -0.52) was observed between photosynthetic rate and total chlorophyll content in both sugar beet cultivars under salinity. This negative correlation may be due to the contrasting effects of salinity on photosynthesis (photosynthesis decreased with increasing salt concentration). In contrast, chlorophyll content was increased with increasing salinity. This increase in chlorophyll can be due to an inverse effect of salinity on specific leaf area (SLA), which is considered...
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Figure 6. Correlation between the initial slope of the A/C_i curve and different levels of salt treatment in two sugar beet cvs.

a measure of leaf density or thickness (Hunt, 1982; Cramer et al., 1994). As leaves of stressed plants became thicker than non-stressed plants, and thicker leaves contain more cells per unit leaf area, both photosynthesis and chlorophyll concentration were expressed per unit leaf area. Thus, chlorophyll content could not be a limiting factor for photosynthesis in the presence of salinity. An increase in chlorophyll content with salinity is not sufficient to counter the adverse effects of decreasing g_s and non-stomatal factors that may have influenced net carbon gain.

The initial slope of the A-C_i curve is a convenient measure of photosynthetic efficiency and is often defined as the carboxylation efficiency (CE). In this study, greater decreases in carboxylation efficiency were observed under increased salinity levels (Figure 6). Changes in carboxylation efficiency with salinization have been reported previously (Bethke and Drew, 1992) and may indicate a lower activity of Rubisco, because the initial slope of the A-C_i curve is thought to be controlled mainly by the ability of the enzyme to fix CO_2 (von Caemmerer and Farquhar; 1986). The Madison cultivar grown at 350 mM had the lowest initial slope. Commensurate with the change in carboxylation efficiency, the CO_2 compensation point also increased with severity of salt treatment. Comparable changes in CO_2 compensation point have been reported previously for salinized barley (Rawson, 1986). Another limiting factor could be RuBP regeneration, which could limit electron capture and transport for quinone reduction (Long and Hallgren, 1993). Chlorophyll fluorescence measurements could represent the differences in the efficiency of photosystem II in sugar beet cultivars in the presence of salinity.

The fluorescence data suggested that the rate of energy translocation or light capture might be limited by salinity (Long and Hallgren, 1993). Relating fluorescence and gas exchange data in stressed plants suggested that these plants might be experiencing some degree of photoinhibition (Figure 5). Salt stress is accompanied by oxidative damage in plants (Hernandez et al., 1993; Streb et al., 1993; Gossett et al., 1994). Lower F_v/F_m in the salt stressed compared to the control plants indicated that RuBP regeneration, which needs adequate electron translocation from photosystem II to electron acceptors, might be disturbed by salinity. However, in this study, although there were significant differences in F_v/F_m in different salt treatments, values of F_v/F_m were relatively high for both the control and salt stress treatments (0.80-0.91) (Figure 5).
Similar values of $F_/F_m$ were found by Belkhodja et al. (1994) and Kafi (1996) for barley and wheat, respectively, grown in the presence of salinity.

In this experiment, $C_i/C_a$ ratio decreased up to 150 mM salinity. However, at 250 and 350 mM, despite reduction in $A_{CO2}$ and $g_s$, the $C_i/C_a$ ratio actually increased. It is likely that the increased intercellular CO$_2$ concentration ($C_i$) was mainly associated with the decreased CO$_2$ fixation of mesophyll cells due to the decreased CO$_2$ assimilation capacity (Lu and Zhang 1998).

**CONCLUSIONS**

The present assessment of the effect of salinity on performance of two sugar beet cultivars allows us to conclude that increasing the levels of salinity decrease most of the growth and physiological parameters. Salinity had greater effect on root dry weight than shoot dry weight. Data also revealed that salinity significantly decreased photosynthesis of leaves in both cultivars. The ratio of intercellular to ambient CO$_2$ concentration ($C_i/Ca$) decreased up to 150 mM salinity compared to non-stressed plants. However, $C_i/Ca$ ratio increased at higher levels of salt treatment. This shows that at high levels of salt concentrations (250 and 350 mM) non-stomatal parameters have a greater effect on photosynthesis.

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