Salinity effects on CO$_2$ assimilation and diffusive conductance of cowpea leaves

Z. Plaut, C. M. Grieve and E. V. Maas

The effect of NaCl salinity at concentrations of 43–173 mM in nutrient solution on net gas exchange of attached cowpea [Vigna unguiculata (L.) Walp cv. California Black-eyed No. 5 (CB5)] leaves was investigated under both greenhouse and growth chamber conditions.

There was a marked decrease in leaf conductance to water vapor after exposure to low salinity levels and a slighter decrease when salinity levels were higher. The decrease in net assimilation was much more gradual throughout the entire salinity range. The altered responses of net assimilation and leaf conductance to salinity were more evident at a high light intensity. A decrease in intercellular partial CO$_2$ pressure ([p(CO$_2$)]) was found at the low and intermediate salinity levels but not at the high level. These findings suggest that CO$_2$ assimilation was mainly controlled by stomatal conductance and the fixation of CO$_2$ might have been increased due to stimulated biochemical activity or to higher chlorophyll concentration per unit leaf area.

A decrease in assimilation was already found one day after salinization and proceeded up to 4 days when it was inhibited by 50% at 43 mM NaCl and up to 85% at 173 mM. The decrease in transpiration was larger than the decrease in net assimilation, and both were attributed to osmotic stress. Partial recovery was found thereafter and new steady-state rates, in the range of 55 to 100% of the control, were then obtained for salinity levels between 43 and 130 mM. Inhibition of net CO$_2$ assimilation at this stage was attributed partly to a specific sodium effect and partly to plant water status. A linear relationship between leaf sodium content and net photosynthesis was also evident at this stage. Net CO$_2$ assimilation recovered more completely than transpiration when salt stress was removed, but at 173 mM NaCl recovery was negligible.

Key words – Cowpea, leaf conductance, net CO$_2$ assimilation, osmotic adjustment, photosynthesis, salinity effects, Vigna unguiculata.

Introduction

Almost 50 years ago Hayward and Spurr (1943) reported that transpiration was reduced under saline conditions, most likely because of partial closure of stomata, and that this, in turn, led to a decrease in net photosynthesis. Many investigations have been conducted since then on the effect of salinity on photosynthesis and leaf diffusive conductance (see reviews by Greenway and Munns 1980, McCree 1986 and Pasternak 1987). Conclusions have been variable as the work has been conducted with species of different sensitivities, at different salt levels and under different environmental conditions. Limitation of photosynthesis by salinity may be attributed to two major factors: (1) limitation due to reduced leaf diffusive conductance (Gale et al. 1967, Walker et al. 1982, West et al. 1986) and (2) reduction in the rate of biochemical processes (Downton 1977, Gale et al. 1967, Longstreth et al. 1984, Seemann and Critchley 1985). At ambient CO$_2$ levels, stomatal conductance of spinach leaves is decreased by salinity much more than is photosynthesis.
The subsequent decrease in intercellular CO₂ pressure is thus associated with an increase in Water Use Efficiency (WUE). Even more striking are the findings of Kaiser et al. (1983), who showed a decrease in stomatal conductance of spinach leaves from plants grown in 350 mM NaCl, but no significant decrease in photosynthesis at saturating CO₂ levels. McCree (1986) and Richardson and McCree (1985) demonstrated that reduced carbon assimilation of some species under salinity was associated with an increase in WUE, which was due to a more severe decrease in water loss rates than in carbon gain.

Parallel responses of transpiration and photosynthesis to salinity have also been shown and have been associated with unchanged intercellular CO₂ pressure (Longstreth et al. 1984, Yeo et al. 1985). This suggests a similar quantitative response of stomata and of biochemical capacity of chloroplasts, although the two are unrelated. Lloyd et al. (1987) suggested that photosynthesis is more sensitive to salinity than is leaf diffusive conductance, when turgor is maintained. This resulted in higher intercellular CO₂ pressure under salinity. It is possible that in sensitive species, e.g. *Citrus sinensis* and *Phaseolus vulgaris*, biochemical processes may be inhibited under salinity, due to direct ion effects. Moreover, the level of salinity which was used or the time length of plant exposure to salinity may have been responsible for the observed damage to photosynthesis.

The purposes of the present study were to (1) evaluate the role of stomatal vs non-stomatal responses to NaCl salinity, (2) study the time course in the response of CO₂ assimilation and transpiration to salinity, (3) assess the potential of recovery after salinity removal and (4) to determine the rate of osmotic vs ion effects on CO₂ assimilation. This was attained by simultaneous measurements of net CO₂ assimilation, leaf water vapor conductance or transpiration, and intercellular CO₂ pressure at a range of salinity levels. Measurements of net CO₂ assimilation and transpiration were conducted from the time of exposure until a steady state in their responses was obtained and their recovery upon removal of salinity. Net assimilation was plotted against salt ions content in the leaf to determine the role of specific ion effects.

**Materials and methods**

Cowpea [*Vigna unguiculata* (L.) Walp. cv. California Blackeye No. 5 (CB-5)] seeds were coated with 96% (v/v) tetrachloro-p-benzoquinine Spergon seed protectant and germinated in rolled paper towels saturated with CaSO₄ solution (0.5 mM). Five-day-old seedlings were transplanted into containers with 28 l of nutrient solution and grown hydroponically. The composition, in mM, was: 2.5 Ca(NO₃)₂, 3 KNO₃, 1.5 MgSO₄, 0.17 KH₂PO₄, 0.050 Fe as sodium ferre diethylenetriamine pentaacetate, 0.023 H₂BO₃, 0.10 MnSO₄, 0.0004 ZnSO₄, 0.0002 CuSO₄ and 0.0001 H₂MoO₄. Each pot contained 12 cowpea seedlings. The pH of the cultures was maintained between 5.5 and 6.5 with H₂SO₄ and KOH, and the solutions were aerated continuously. Several cycles of plants were grown in a naturally illuminated glasshouse located in Riverside, CA, USA, between March and July, a mostly cloudless period. A rise in temperature above 27°C and a drop below 17°C in the greenhouse were prevented with pad and fan and heating systems, respectively.

After 3 weeks of growth under identical conditions, plants of similar size and leaf area were selected for salinization. At this age the primary leaves were almost fully expanded; the first trifoliated leaf was partly expanded and the second was at an initial expanding stage. NaCl was added to the nutrient solution over a 3-day period to achieve final NaCl concentrations of 0, 43, 87, 130 and 173 mM. This procedure of salinization was preferred over the more common daily additions of equal and small amounts of NaCl, which would have resulted in exposure of plants to the final concentration for different time periods. The salt was added shortly before sunset, as this has been found to cause no visible damage. All solutions were changed every 5 days. Preliminary studies have shown that in contrast to some other species (Maas and Grieve 1987), the growth of salinized CB-5 seedlings was not adversely affected by the Na/Ca ratio in the root media prevailing in these experiments. No indications of nutrient deficiency or ion imbalance were detected when the plants were stressed with NaCl.

Net CO₂ assimilation, leaf diffusive conductance and transpiration were measured between 7 and 10 days after salinization using the second trifoliate leaf (unless noted otherwise), which was the youngest fully expanded leaf. Measurements were made with a LiCor 6000 portable photosynthesis system equipped with a 1.0 l chamber. (LiCor, Lincoln, NE, USA). Air was constantly stirred in the chamber at two opposite points. A fixed leaf area, between 12.3 and 15.2 cm², was confined by insets and the flow rate was 14 ml s⁻¹. The CO₂ analyzer was calibrated daily with a series of standard CO₂-air mixtures. Ten consecutive measurements of all parameters were taken at 5-s intervals and were replicated 6–8 times on different plants. All experiments, excluding those which served to determine diurnal patterns, were conducted between 10:30 h and 14:30 h, only on bright days, when photosynthetically active radiation (PAR) intensity at the leaf surface was 1200–1300 μmol m⁻² s⁻¹. Light saturation of control plants was around 1 100–1 200 μmol m⁻² s⁻¹, and somewhat lower for the salinity-grown plants. The ambient p(CO₂) was 37±0.2 Pa and vapor pressure deficit (VPD) was 1.98±0.42 KPa.

Two experiments were conducted in a growth cham-
Tab. 1. Effect of salinity on area, fresh and dry weights, chlorophyll and protein contents of the second trifoliate leaf of cowpeas. Plants were exposed to salinity for 8 days during the expansion of this leaf. Values are means ± SE of 6 replicates.

<table>
<thead>
<tr>
<th>NaCl (mM)</th>
<th>Area (cm²)</th>
<th>Fresh weight (g)</th>
<th>Dry weight (g)</th>
<th>Chlorophyll (mg)</th>
<th>Protein (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>264±30</td>
<td>8.28±1.02</td>
<td>0.97±0.11</td>
<td>7.69±0.71</td>
<td>211±17</td>
</tr>
<tr>
<td>43</td>
<td>252±30</td>
<td>8.45±1.00</td>
<td>1.21±0.12</td>
<td>9.61±0.90</td>
<td>255±18</td>
</tr>
<tr>
<td>87</td>
<td>245±28</td>
<td>7.12±0.90</td>
<td>1.07±0.12</td>
<td>9.06±0.77</td>
<td>226±18</td>
</tr>
<tr>
<td>130</td>
<td>226±25</td>
<td>5.48±0.76</td>
<td>0.85±0.08</td>
<td>6.08±0.51</td>
<td>213±17</td>
</tr>
<tr>
<td>173</td>
<td>187±19</td>
<td>4.10±0.61</td>
<td>0.63±0.07</td>
<td>4.65±0.42</td>
<td>188±14</td>
</tr>
</tbody>
</table>

Results

Fresh weight and area of the second trifoliated leaf, which was in its active growing stage during exposure to salinity, were unchanged up to 87 mM NaCl and decreased at higher salinity levels (Tab. 1). Leaf dry weight, chlorophyll and protein content increased in 43 mM NaCl, decreased to about the control value at 87 mM and were below this level at higher NaCl concentrations. The largest fluctuations due to salinity were obtained in leaf chlorophyll content and the smallest in protein content. Net assimilation and transpiration rates were determined throughout this study on a leaf area basis, which showed the least changes at different salinity levels.

Leaf diffusive conduction and net assimilation were inhibited by salinity throughout the day, but maximal effects were found during midday in both greenhouse and growth chamber (Tab. 2). Leaf conductance in the greenhouse was decreased markedly by the first increments of salinity followed by smaller decreases at the higher salinity levels. The rate of net CO₂ assimilation decreased more gradually over the entire range of salinity. The difference was even more marked in the growth chamber, where the decrease in leaf conductance was 46% and that of net assimilation only 12% as a result of the initial rise in NaCl up to 43 mM. Intercellular p(CO₂) decreased up to the salinity level of 87 mM NaCl, which was the highest level to influence leaf conductance markedly. At 130 mM NaCl, intercellular p(CO₂) was not much changed and at 173 mM it was increased. The low PAR in the growth chamber, which was below saturation, and the higher ambient p(CO₂) were probably responsible for the lower net photosynthesis and leaf conductance and the higher intercellular p(CO₂) as compared with the greenhouse.

An evaluation of stomatal vs nonstomatal factors to control photosynthesis under salinity was made by plotting net photosynthesis rates against leaf conductance (Fig. 1). The values are from measurements taken between 0900 and 1600 h in the greenhouse on fully light-saturated leaves of plants grown under salinity between 0 and 130 mM for 9 days. A linear regression of net assimilation (A) against leaf conductance (gₘ) up to a conductance of 0.25 mol m⁻² s⁻¹ was A = 78.87 gₘ −3.65, while for leaf conductances above 0.25 mol m⁻²
were transferred to nonsaline nutrient solution following 10 days of exposure to salinity. In plants that had been stressed with up to 130 mM NaCl, net assimilation returned to the control levels within 2 days after stress removal (Fig. 3). Even plants that had been stressed with 173 mM NaCl showed a 90% recovery after 4 days in the nonsaline medium. Transpiration showed a similar pattern, but recovered more slowly and incompletely after stress removal even after 4 days.

The possibility of interaction between the effects of salinity and PAR intensity on both leaf conductance and net CO₂ assimilation was studied in plants transferred to different PAR intensities in the growth chamber (Fig. 4). The decrease in net assimilation was almost linear up to 130 mM NaCl at all PAR intensities. In contrast, the decrease in leaf conductance (excluding PAR of 200 μmol m⁻² s⁻¹) was much larger at the initial rise in NaCl compared with additional increments. The lower the PAR intensity, the more extended was the linear response of leaf conductance to salinity and at PAR of 200 μmol m⁻² s⁻¹ it became linear over the entire salinity range.

The content of Cl⁻ in cowpea leaves grown at 43 mM NaCl for 13 days increased markedly (Tab. 3). Similar Cl⁻ contents were found in leaves of all ages and at all salt levels up to 130 mM. The contents of sodium in leaves were less than those of Cl⁻ at the two lowest

s⁻¹ it was \( A = 11.07 g_c + 15.06 \). The larger slope at low leaf conductances clearly indicates the control of assimilation by conductance, found at the higher salinity levels. In the control and low salinity grown plants the slope was much less, due to a more marked effect of salinity on leaf conductance than on assimilation, as also concluded from Tab. 2. There was probably also a transition range, which was difficult to detect.

The most substantial inhibition of the rates of net assimilation and transpiration occurred between 2 and 4 days after the plants were at final salinity levels (Fig. 2). Thereafter, the rates of both net assimilation and transpiration increased and ultimately reached new steady-state levels. These rates were expressed as relative values because of day-to-day fluctuations even in the control which occurred even though measurements were always taken at the same time. The decrease in relative transpiration rate was 15–50% greater than that in relative assimilation at 2, 4 and 7 days after exposure of plants to 43, 87 or 130 mM NaCl. Even after 12 days, when the differences were smaller, the decrease in transpiration exceeded that in assimilation. At 173 mM, neither net assimilation nor transpiration recovered appreciably and the differences between rates of transpiration and assimilation were rather small.

Another approach to interpret the inhibitory effects of salinity on net assimilation and on leaf conductance was to determine the rate and extent of their recovery from salinity. Net assimilation and transpiration rates were determined during a 4-day period on plants that

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\text{Fig. 1. Relationship between net assimilation and leaf diffusive conductance of cowpea leaves grown in a greenhouse under a series of NaCl concentrations between 0 and 130 mM. Net assimilation and leaf conductance were measured simultaneously at different hours of the day on fully light-exposed leaves. Each value is the average of 6 replicates. Linear regressions of net assimilation (A) against leaf conductance (g_c) were determined separately for conductances below and above 0.25 mol m⁻² s⁻¹, marked as (a) and (b) respectively.}
\]

\[
\text{Fig. 2. Daily response of net assimilation (a) and of transpiration rate (b) to NaCl in the nutrient solutions. Plants were grown in a greenhouse under a series of NaCl concentrations between 0 and 173 mM. Measurements were taken between 1200 and 1400 h. Values are the average percentage (6 replicates) of controls measured on the same day. Control values varied from day to day, and were 18.3–23.3 μmol CO₂ m⁻² s⁻¹ for photosynthesis and 19.3–26.7 mol H₂O m⁻² s⁻¹ for transpiration.}
\]
Fig. 3. Relative rate of net assimilation and of transpiration of cowpea leaves after removal of salinity. Plants were grown in a greenhouse under a series of NaCl concentrations between 0 and 173 mM for 10 days and then transferred to non-salinized nutrient solutions for 4 days. Measurements were conducted shortly before transfer from salinity, and 2 and 4 days after transfer. Values are the average percentages (6 replicates) of controls measured on the same day. Control values varied from day to day, and were 22.4-25.6 µmol CO₂ m⁻² s⁻¹ for photosynthesis and 18.6-20.5 mol H₂O m⁻² s⁻¹ for transpiration.

Fig. 4. Response of net assimilation (a) and leaf diffusive conductance (b) to salinity at different PAR intensities. Plants were grown in a greenhouse under a series of NaCl concentrations between 0 and 173 mM and transferred to growth chambers 48 h prior to measurements. Values are means of 6 replicates; the SE of the means are indicated by vertical bars.

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Tab. 3. Chloride and sodium accumulation (mmol kg\(^{-1}\) dry weight) in cowpea leaves of different ages (in days). Leaves were analyzed 13 days after transfer of plants to salinity. Values are means ± se of 6 replicates. Plants were transferred to NaCl at leaf ages of 8, 2, -3 and -7 days, respectively. (No growth of L4 at 173 mM NaCl.)

<table>
<thead>
<tr>
<th>NaCl concentration (mM)</th>
<th>Leaf number and age</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L1=21 days</td>
</tr>
<tr>
<td>Chloride</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>3± 2</td>
</tr>
<tr>
<td>43</td>
<td>414± 25</td>
</tr>
<tr>
<td>87</td>
<td>463± 38</td>
</tr>
<tr>
<td>130</td>
<td>478± 39</td>
</tr>
<tr>
<td>173</td>
<td>2305± 205</td>
</tr>
<tr>
<td>Sodium</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>1± 2</td>
</tr>
<tr>
<td>43</td>
<td>196± 20</td>
</tr>
<tr>
<td>87</td>
<td>313± 35</td>
</tr>
<tr>
<td>130</td>
<td>542± 48</td>
</tr>
<tr>
<td>173</td>
<td>2548± 242</td>
</tr>
</tbody>
</table>

inhibition of assimilation at this NaCl level can thus be expected.

Discussion

The present study demonstrates that different responses of net assimilation and of stomatal conductance to salinity can be found in the same species at different salinity levels and not merely in different species, as has been shown earlier (compare, for instance, Kaiser et al. 1983 vs Yeo et al. 1985 and vs Lloyd et al. 1987). At a low salt level (up to ca 87 mM NaCl) leaf diffusive conductance was markedly decreased and net CO\(_2\) assimilation was only slightly reduced. This can be concluded from at least two observations: (1) The decrease in net assimilation as a function of increasing salt concentrations was gradual, while the decrease in diffusive conductance was more drastic (Tab. 2 and Fig. 4) and (2) the decrease in leaf conductance was associated with a decrease in intercellular p(CO\(_2\)) (Tab. 2). A similar conclusion can also be drawn from the work of West et al. (1986) who showed for field-grown cowpeas that CO\(_2\) assimilation is less sensitive to remaining salinity in soil as compared with water vapor conductance.

At higher salinity levels the decrease in net assimilation became more drastic (Tab. 1 and Fig. 4 at high intensities). As leaf diffusion conductance did not respond in this way, the relationship between net assimilation and diffusive conductance was not similar throughout the salinity range (Fig. 1). For wheat leaves Rawson (1986) has shown a larger increase in photosynthesis for a given rise in stomatal conductance under salinity as compared with non-saline conditions, also suggesting that photosynthesis was less sensitive to salinity than transpiration. Our results, as well as those of others (McCree 1986; Rawson et al. 1988, Richardson and McCree 1985), thus suggest that WUE is increased by salinity, at least under low salt levels.

Maintaining similar intercellular p(CO\(_2\)) under both saline and non-saline conditions would imply a similar inhibition of stomatal conductance and of biochemical activity, as shown by Longstreth et al. (1984) and Yeo et al. (1985). An inhibition of biochemical processes would lead to a rise in intercellular p(CO\(_2\)), which could cause partial stomatal closure and this, in turn, could prevent changes in intercellular p(CO\(_2\)). On the other hand, a more marked decrease in stomatal conductance than the inhibition of biochemical processes would result in a drop in intercellular p(CO\(_2\)). This may result in
decreased CO₂ fixation rates and, finally, in a new steady-state of unchanged intercellular p(CO₂). Our findings that intercellular p(CO₂) was decreased at a low and intermediate salinity level (Tab. 2), and that the decrease in CO₂ assimilation was less sensitive to salinity than leaf conductance (Tab. 2, Figs 1 and 4) show that CO₂ fixation processes were quite salt tolerant at these salt levels.

Our recent work on the effect of salinity on photosynthesis of isolated mesophyll cells supports this view and shows that only at 130 mM NaCl did cowpea cells exhibit a significant decrease in CO₂ fixation (Plaut et al. 1989). Moreover, earlier work on the effect of salinity on the activity of Rubisco indicated a stimulation of the enzyme isolated from sugar beet, a much more salt tolerant plant, after 7 days of exposure to 180 mM NaCl (Heuer and Plaut 1981). Such stimulation may also explain the reports that stomatal diffusion was decreased by salinity in various crop plants while photosynthesis was unaffected (Curtis and Läuchli 1986, Rawson et al. 1988, Taleisnik 1987).

The increase in CO₂ fixation at low salinity levels can also be attributed to an increase in chlorophyll concentrations per unit leaf area. These concentrations were increased by 31 and 27% at 43 and 87 mM NaCl, respectively, calculated on the basis of the data in Tab. 1. The increase in chlorophyll per unit leaf area may thus explain the different responses of net assimilation and leaf diffusive conductance to salinity in both Tab. 2 and Fig. 4. In fact, only at the high light intensity (Fig. 4) will higher chlorophyll concentrations enhance photosynthesis when calculated per leaf area, but it will have no effect on diffusive conductance. The decrease in leaf conductance at low as compared with high light intensity was thus gradually reduced at the high salinity levels, while the relative decrease in net assimilation at low light was even slightly enhanced up to 130 mM NaCl. For spinach grown under rate-limiting light intensity, Downton et al. (1985) found a more drastic inhibition of photosynthesis by salinity under high rather than under low light. This is the opposite of our observation, but our plants were grown at high light intensity.

It was shown in our earlier work that full osmotic adjustment was obtained when cowpea plants were exposed to different salinity levels up to a ψₛ of 0.6 MPa, which is equivalent to about 130 mM NaCl (see Fig. 1 in Plaut et al. 1989). The similar decrease in ψₛ and ψₑ maintained a level of turgor similar to that in the control plants. During the first 3–4 days after salinization, however, adjustment was only partial and leaf turgor was not full. This is probably the reason for the sharp decrease in net assimilation and transpiration during the first 4 days after final salt levels were reached (Fig. 2). While osmotic effects were the main factor in controlling assimilation and transpiration shortly after salinization, a direct ion effect is probably controlling these processes at the later steady-state stage when plants were already adjusted osmotically. This is in agreement with the data of Fig. 5 showing that net assimilation was more dependent on leaf Na⁺ than on leaf Cl⁻ content during steady state (10 days after salinization). This different response of assimilation to both ions was in fact seen in expanded as well as in expanding leaves, although they were dissimilar in their assimilation potential. Yeo et al. (1985) have also shown that photosynthesis in rice is inversely proportional to leaf Na⁺ content. In contrast to cowpeas, Phaseolus vulgaris L. seems to exclude sodium but accumulate chloride in the leaves and at 100 mM external NaCl concentration, leaf Na⁺ content was only about one-tenth that of chloride (Seemann and Critchley, 1985).

One of the most interesting findings is the recovery of net assimilation and transpiration after removal of salinity (Fig. 3). This can be caused by the dilution of leaf ion content and by restoration of leaf water status, which would certainly be completed after a short time. Dry weight content of the second trifoliate leaf was 11.7, 14.3, 15.0 and 15.5% at 0, 43, 87 and 130 mM NaCl, respectively (calculated from Tab. 1). Provided there was no displacement of ions during restoration of leaf water content in the salinized leaves to the level of controls, sodium concentrations in the leaf (shown in Tab. 3) would be reduced at the 3 salinity levels to 153, 246 and 439 mmol kg⁻¹ dry weight respectively. This would have relieved the inhibition of assimilation at these salinity levels respectively, from 20, 35 and 63 to 15, 26 and 51% of the control (based on L1 in Fig. 5). The dilution of leaf Na⁺ can thus account only partially for the full recovery of assimilation after salinity removal (Fig. 3). The remaining recovery must therefore be attributed to the increase in leaf ψₑ, which was −0.77, −0.90 and −1.137 MPa at these 3 salinity levels prior to salinity removal, to the control value of −0.5 MPa. This implies that the decrease in net assimilation during steady state cannot be attributed solely to a direct ion effect but that plant water status plays an important role also when plants are already osmotically adjusted.

References


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