TOLERANCE TO SALINITY STRESS IN PEANUT (ARACHIS HYPOGAEA L.) THROUGH OSMOTIC ADJUSTMENT AND UNDAMAGED CHLOROPLAST

Mohammad Abul Kalam Azad¹, Mohammad Mozammel Haque¹, Mohammad Abdul Hamid, Fahmina Yasmine and Mohammad Abdul Wahab Golder²

Plant Breeding Division, Bangladesh Institute of Nuclear Agriculture
Bangladesh Agricultural University Campus, Mymensingh-2202, Bangladesh

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ABSTRACT

A pot experiment was conducted under glasshouse condition to study the mechanism of salt tolerance in peanut at Bangladesh Institute of Nuclear Agriculture, Mymensingh, during February to July 2006. Two Spanish type varieties, Dacca-1 and Binachinabadam-3, and 1 Valencia type variety, Zhingabadam with unknown tolerance were exposed to 0.4 (unstressed), 3, 5, 7 and 9 dS/m doses of salinity at vegetative and flowering stages. The experiment was laid out in a factorial completely randomized design. It appeared that Binachinabadam-3 allocated higher assimilate to kernel at both vegetative and flowering stages through maintaining total sugar and chlorophyll ‘a’ contents close to unstressed treatment, particularly at 3.5 dS/m salinity doses.

Keywords: Peanut, Salinity tolerance, Growth stage, Assimilate translocation, Osmotic adjustment, Chloroplast.

INTRODUCTION

Salinity is certainly one of the most detrimental environmental factors limiting crop productivity (Ashraf, 1999). This stress is complex and causes a number of detrimental effects: (i) reduces the ability of plants to absorb water, called water or osmotic stress, (ii) causes ionic imbalance, (iii) imposes hyper osmotic shock by decreasing chemical activity of water and causing loss of cell turgidity and (iv) reduces chloroplast stromal volume and generates reactive oxygen species (ROS). Globally, nearly 100 million ha of land is affected by salinity which accounts for 6-7% of the total arable land (Munns and James, 2003). In Bangladesh, in the coastal belt, 1.02 million ha of cultivated land is affected by different degrees of soil salinity, 2.0 to >16 dS/m (Karim and Iqbal, 2001; SRDI, 2003). Soil salinity in these areas remains lower during June to November. Thereafter, it starts increasing and accentuates in May (Islam et al., 1997).

Cultivation of high water demanding crop is not possible in the saline areas of Bangladesh because of unavailability of suitable irrigation water. Peanut (Arachis hypogaea L.) requires only 350 mm water for completing life cycle (Field, 1995) and thus mostly grown under rainfed condition during December -May. The climatic and edaphic conditions of a large part of saline area are quite suitable for growing peanut except the salinity problem. The effective reclamation of these soils in that area is difficult and complex. Therefore, salt tolerant variety is the best alternative to combat this problem. But it is mostly unknown how peanut tolerate salinity stress. Therefore, it is an urgent need to assess mechanism of salinity tolerance before initiating any breeding program. This study, thus, intends to elucidate the mechanism of salt tolerance in peanut.

MATERIALS AND METHODS

The experiment was conducted in glass house condition at Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh, during February to July, 2006. The experiment followed factorial completely randomized design with 3 replications.

¹Corresponding author e-mail: mazazad.bina@bpbd.gov
²Department of Crop Botany, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh.
³Soil Resources Development Institute, Farmgate, Dhaka-1215, Bangladesh.
Two Spanish type varieties, Dacca-1 and Binachinabadam-3, and one Valencia type Zhingabadam with unknown tolerance were subjected to 3, 5, 7 and 9 dS/m salinity doses at vegetative and flowering stages. Five pre-germinated seeds of the selected varieties were sown on 27 February, 2006 in each earthen pots of size 27 × 22 cm. The pots were lined with polyethylene sheet and filled with 8.0 kg soil mixture, prepared with sandy loam soil and rotten farm yard manure (FYM) in 1:1 ratio. Nitrogen, phosphorus, potassium, sulphur and zinc were applied in the form of urea, triple super phosphate, muriate of potash, gypsum and zinc sulphate following Fertilizer Recommendation Guide (BARC, 2005). When the plants were established, only three healthy plants were kept in each pot. The pots were kept free from weeds and protected from insect pest and diseases by spraying appropriate insecticides, fungicides and acaricide as and when required.

**Preparation of saline water**

The saline water was prepared by mixing different salts with Na⁺, Mg²⁺ and Ca²⁺ in a ratio of 15:3:2 and Cl⁻, SO₄²⁻, and HCO₃⁻ in ratio of 7:2:1 so that their compositions were almost alike to ground water of saline areas of Bangladesh (SRDI, 2003). Fifty gram of such salt was dissolved per liter tap water to prepare the stock solution with salinity 80 dS/m.

**Imposition of salinity in pot soil**

The amount of stock solution required to attain desired salinity levels of the soil mixture was estimated with the following equation:

\[ V_1 S_1 = V_2 S_2 \]

Where,

- \( V_1 \) = volume of soil mixture in a pot
- \( S_1 \) = desired salinity - initial salinity of the soil mixture
- \( V_2 \) = volume of water at 70-80% plant available water
- \( S_2 \) = salinity of stock solution

Again, volume of soil mixture \((V_1)\) was determined using the following formula

\[ V_1 = \frac{\text{Weight of oven dried soil}}{\text{Bulk density of soil}} \]

Volume of water \((V_2)\) was determined by dividing the weight of water with its density (0.98 g/cc).

The estimated amount of stock solution was then diluted to 3, 5, 7 and 9 dS/m by adding tap water and then imposed at 2 installments of 17 and 23 days after sowing (DAS) during vegetative and one installment at 45 DAS during flowering stage. For the control treatments, same amount of tap water (0.4 dS/m) was applied. After imposition of salinity, 70-80% plant available water (PAW) was maintained till harvest. The pots were saturated with tap water only before imposition of salinity when the plants showed symptoms of water requirement.

For determination of leaf area, specific leaf weight and biochemical parameters, the first fully opened compound leaf from the apex of main shoot in a plant was used.

**Determination of leaf area and specific leaf weight**

Leaf area was measured after 66 and 22 days of salinity imposition at the vegetative and flowering stages, respectively. Leaf area was measured with a Licor leaf area meter. After recording the area, the leaf was oven dried at 70 °C for 72 hours. Finally, specific leaf weight was determined by dividing the oven dry weight with leaf area.

**Determination of different chlorophyll contents**

Chlorophyll reading was recorded with a chlorophyll meter (SPAD Konica Minolta Sensing Inc) after 84 and 67 days of salinity imposition at the vegetative and flowering stages, respectively. For calibration of the reading the method of Shabala et al. (1998) was used with some modifications. Compound leaves of 10 plants (1 from each salinity level/stage) were cut into pieces and 0.05 g was dipped in 10 ml 80% ethanol separately and kept in dark. After 7 days when the colors of the solution become green, absorbance readings were gathered using a spectrophotometer at 645 and 663 nm wave lengths. Finally, chlorophyll ‘a’, ‘b’ and total chlorophyll were estimated following Yoshida et al. (1976):

Total Chlorophyll = \((20.2 \times D_{645} + 8.20 \times D_{663}) \times DF\)

Chlorophyll ‘a’ = \((12.7 \times D_{663} - 2.69 \times D_{645}) \times DF\)

Chlorophyll ‘b’ = \((22.9 \times D_{645} - 4.68 \times D_{663}) \times DF\)

Where,

\( D_{645} = \) Absorbance at 645 nm wave length.
\( D_{663} = \) Absorbance at 663 nm wave length.
Thereafter, three standard curves were fitted using the SPAD reading against the respective chlorophyll 'a', 'b' and total chlorophyll data at each stage. From these standard curves, three curve factors were obtained for chlorophyll 'a', 'b' and total chlorophyll at each stage. At the end, chlorophyll 'a', 'b' and total chlorophyll contents in each treatment were estimated from the respective SPAD reading as follows:

\[ \text{CF (Curve factor)} \times \text{DF (dilution factor)} \times \text{SPAD reading} \]

**Determination of total and reducing sugar contents**

Total and reducing sugars were determined following the method of Dubois et al. (1956) and Nelson (1944), respectively. Exactly 0.1 g pieces of leaf was dipped in 10 ml 80% ethanol and kept in dark. When the green color of the solution disappeared they were extracted in a hot water bath at 80-90°C temperature. The extracts were then volume to 10 ml with distilled water and readings were recorded with a spectrophotometer.

**Collection of data on different shoot and root characters, and yield attributes**

Finally, all plants in a pot were uprooted at full maturity and washed with running tap water. After sun drying, plant height, number of branch, mature and total pod, pod and kernel weight per plant and kernel number per pod were recorded. Thereafter, leaves, stems, and roots were separated. The additional roots left in the growth medium were also collected. The leaves, stems and roots were oven dried at 70°C for 72 hours and weighed after cooling. The collected data were then analyzed following the design used.

**Computation of relative performance (RP)**

Relative performances for different traits were computed with the mean data following Azad (2006) as follows:

\[
\text{Relative performance} \times 100 = \frac{\text{Performance under saline condition}}{\text{Performance under control condition}}
\]

**Statistical analysis**

Analysis of variance was computed following a 3-factor experiment using completely randomized design (CRD) as suggested by Gomez and Gomez (1984). Mean separation was made following Duncan's Multiple Range Test (DMRT) and Least Significant Difference (LSD), particularly for the interaction effects.

**RESULTS AND DISCUSSION**

**Influence of varieties on different shoot and root characters, yield attributes and biochemical parameters as influenced by salinity doses and stage of salinity imposition**

Varieties had shown significant differences for all the shoot and root characters, yield attributes, and biochemical parameters except chlorophyll 'b'. This result is in conformity with many workers (Azad, 2006; Vadez et al., 2005; Joshi et al., 1990) who reported significant variations for shoot and root characters, yield attributes and biochemical parameters in their experiments with peanut exposed to salinity stress. The means of different shoot and root characters, yield attributes, and biochemical parameters of 3 varieties, averaged over stress and unstressed treatments imposed at vegetative and flowering stages are presented in Tables 1, 1a and 1b. The figures in parentheses indicate relative

<table>
<thead>
<tr>
<th>Variety</th>
<th>Plant height (cm)</th>
<th>Leaf area (cm²)</th>
<th>Dry weight of leaves/plant (g)</th>
<th>SLW (cm³/g)</th>
<th>Branch/plant (number)</th>
<th>Stem/leaf ratio</th>
<th>Dry weight of shoot/plant (g)</th>
<th>Dry weight of roots/plant (g)</th>
<th>Total dry weight plant (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daica-1</td>
<td>25.7±b</td>
<td>31.5±b</td>
<td>16.9±a (85.4±a)</td>
<td>2.7±b</td>
<td>6.2±a</td>
<td>0.75±b</td>
<td>30.5±a (93.18±a)</td>
<td>1.1±b</td>
<td>31.6±a</td>
</tr>
<tr>
<td></td>
<td>(90.7%±)</td>
<td>(112.8%±)</td>
<td>(86.22%±)</td>
<td>(104.88%±)</td>
<td>(92.46%±)</td>
<td>(88.80%±)</td>
<td>(86.40%±)</td>
<td>(86.40%±)</td>
<td></td>
</tr>
<tr>
<td>Birmohalbadum-3</td>
<td>19.4±c</td>
<td>34.1±a</td>
<td>14.2±b (80.9±b)</td>
<td>3.1±a</td>
<td>6.3±a</td>
<td>0.6±a</td>
<td>20.0±c (76.83±c)</td>
<td>1.5±a</td>
<td>25±6±c</td>
</tr>
<tr>
<td></td>
<td>(86.09%±)</td>
<td>(92.32%±)</td>
<td>(90.64%±)</td>
<td>(104.17%±)</td>
<td>(90.17%±)</td>
<td>(73.2%±)</td>
<td>(65.65%±)</td>
<td>(65.65%±)</td>
<td></td>
</tr>
<tr>
<td>Zhingabadum</td>
<td>30.2±a</td>
<td>32.2±a</td>
<td>14.7±b (83.6±b)</td>
<td>2.9±a</td>
<td>5.0±b</td>
<td>0.7±b</td>
<td>25.6±b (60.0±b)</td>
<td>1.4±b</td>
<td>28.2±a</td>
</tr>
<tr>
<td></td>
<td>(78.5%±)</td>
<td>(90.24%±)</td>
<td>(102.0%±)</td>
<td>(106.10%±)</td>
<td>(137.93%±)</td>
<td>(61.41%±)</td>
<td>(62.45%±)</td>
<td>(62.45%±)</td>
<td></td>
</tr>
</tbody>
</table>

*Same letters in a column do not differ significantly at P< 0.05*
performance (RP), performance under salinity stress in percentage of unstressed treatments. Most of the shoot and root characters, yield attributes, and biochemical parameters of all 3 varieties had reduced performance under salinity stress than means over stress and unstressed treatments except leaf area in Dacca-1, branch number in all varieties, SLW and stem/leaf ratio in Zhingabadam (Table 1), chlorophyll 'b' and reducing sugar in Binachinabadam-3 and Zhingabadam and total sugar in all varieties (Table 1b). In contrast, immature pod number in Dacca-1 increased and that in Binachinabadam-3 remained mostly unchanged (Table 1a). Chlorophyll 'a' and total chlorophyll remained mostly unchanged in Binachinabadam-3 and chlorophyll 'b' in Dacca-1 (Table 1b).

Leaf area in Dacca-1 of the uppermost fully unfolded new compound leaf was increased under salinity stress than that of unstressed treatment despite decreased in the remainder two varieties (Table 1). This was due to its maintenance of relatively higher leaf area at 5-9 dS/m salinity doses at vegetative and all salinity doses at flowering stages (Fig. 1b). Vadez et al., (2005) observed tolerant plants maintain leaf size under salinity stress close to its unstressed treatment. This indicates Dacca-1 is tolerant to all salinity doses at vegetative and flowering stages. But tolerance should be assessed based on the ultimate product, kernel yield (kernel weight).

Interestingly, branch number in all 3 varieties increased under salinity stress over unstressed treatment. Increased branch number might be a tolerance mechanism in peanut when exposed to salinity stress; compartmentalizes the excess Na⁺ by forming new branches. This result is in conformity with that of Vadez et al. (2005) who observed higher stem/leaf ratios in peanut due to salinity stress. The increase in stem/leaf ratio and SLW under salinity stress to that of unstressed treatment in Zhingbadam indicate when exposed to salinity stresses the older leaves become thicker and senesced at a higher rate than the remainder two varieties which was also confirmed by its lowest percent of dry weight of leaves under salinity stress (Table 1).

Increased number of immature pod in Dacca-1 under salinity stresses was attributed to its

<table>
<thead>
<tr>
<th>Variety</th>
<th>Pod: peg</th>
<th>Number of immature pods/ plant</th>
<th>Number of mature pods/ plant</th>
<th>Total pod weight/plant</th>
<th>Kernel weight/plant</th>
<th>Shelling percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dacca-1</td>
<td>0.79a</td>
<td>79.6a</td>
<td>79.6a</td>
<td>12.46a</td>
<td>12.46a</td>
<td>61.4a</td>
</tr>
<tr>
<td>Binachinabadam-3</td>
<td>0.79b</td>
<td>79.6b</td>
<td>79.6b</td>
<td>12.46b</td>
<td>12.46b</td>
<td>61.4b</td>
</tr>
<tr>
<td>Zhingabadam</td>
<td>0.80c</td>
<td>80.7c</td>
<td>80.7c</td>
<td>12.47c</td>
<td>12.47c</td>
<td>61.5c</td>
</tr>
</tbody>
</table>

Same letters in a column do not differ significantly at P < 0.05; figures in the parentheses indicate relative performance (RP).
TABLE 1b: Means of different biochemical parameters of the varieties, averaged over the salinity doses and stages of salinity imposition.

<table>
<thead>
<tr>
<th>Variety</th>
<th>Chlorophyll 'a' content in leaves (mg/gfw)</th>
<th>Chlorophyll 'b' content in leaves (mg/gfw)</th>
<th>Total chlorophyll content in leaves (mg/gfw)</th>
<th>Total sugar content in leaves (mg/gfw)</th>
<th>Reducing sugar content in leaves (mg/gfw)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dacca-1</td>
<td>1.86b (91.89%)</td>
<td>0.43a (69.37%)</td>
<td>1.86b (93.37%)</td>
<td>4.1a (121.2%)</td>
<td>2.3b (58.63%)</td>
</tr>
<tr>
<td>Binachinabad-3</td>
<td>1.58a (99.30%)</td>
<td>0.44a (103.7%)</td>
<td>1.58a (99.22%)</td>
<td>3.91b (103.75%)</td>
<td>1.9c (130.69%)</td>
</tr>
<tr>
<td>Zhingabadam</td>
<td>1.42c (87.09%)</td>
<td>0.46a (114.19%)</td>
<td>1.88c (92.20%)</td>
<td>3.82c (114.92%)</td>
<td>2.47a (122.28%)</td>
</tr>
</tbody>
</table>

Same letters in a column do not differ significantly at P < 0.05;
decreased translocation of dry matter to kernel from leaves and shoots when exposed at flowering stage particularly at 7 and 9 dS/m (Fig. 4b). On the contrary, immature pod number in Binachinabad-3 mostly remained unchanged under salinity stresses might be due to reverse phenomenon to that of Dacca-1 i.e. increased dry matter translocation to kernel (Fig. 4b). More insight in this regard is that increased total and reducing sugar contents in Binachinabad-3 under salinity stresses to that of unstressed control (Table 1) might help maintaining turgor of guard cell and intake of CO₂ through opened stomata. This CO₂ in presence of undamaged chloroplast (Table 1b) helped maintaining photosynthesis and mobilization of assimilates to reproductive organs, particularly kernel. As a result this variety had the highest relative kernel yield and number (Table 1a) which made it as tolerant. Chlorophyll 'b' content in Dacca-1 remained mostly unchanged while in Zhingabadam increased than the control treatments despite having decreased contents of chlorophyll 'a' and total chlorophyll. This means chlorophyll 'b' is not a determining factor for salinity tolerance in peanut. This result corroborates with that of Azad (2008) who also observed chlorophyll 'b' was less sensitive to salinity stress. Zhingabadam accumulated larger amounts of total and reducing sugar than control (Table 1b) indicative of this variety has ability to maintain turgor through osmotic adjustment and thus intake of CO₂ through opened stomata like Binachinabad-3. This CO₂ with the damaged chloroplast (Table 1) could not maintain photosynthesis and mobilization of assimilates to reproductive organs, particularly kernel, at a rate as Binachinabad-3. This phenomenon obligated this variety to produce lower relative yield and number of kernel than Binachinabad-3 (Table 1a).

Effect of salinity dose on different shoot and root characters, yield attributes and biochemical parameters as influenced by variety and stage of salinity imposition

Salinity doses had also shown significant differences for all the shoot and root characters, yield attributes, and biochemical parameters except leaf area, branch number and chlorophyll 'b'. Effect of salinity doses, averaged over the varieties and stage of salinity imposition, on different shoot and root characters, yield attributes and biochemical parameters are presented in Tables 2, 2a and 2b. Plant height, dry weight of roots, total dry weight (Table 2) mature pod number, pod and kernel weights (Table 2a) were gradually decreased with gradual increase in salinity, doses showing either significant or not significant differences. In contrast, leaf area and branch number (Table 2), chlorophyll 'b' (Table 2b) had not shown significant differences amongst the salinity doses. Dry weight of leaves and shoots, stem/leaf ratio, SLW (Table 2), number of immature and total pods, pod/peg ratio (Table 2a) and the biochemical parameters (Table 2b) did not follow linear trend. Interestingly, number of kernels/pod was increased at 3dS/m salinity and then decreased gradually (Table 2a).

With gradual increase in salinity all the shoot and root characters, yield and yield attributes also decreased gradually except for leaf area of younger leaves and branch number (Table 2). These results corroborate with that of many others (Patel et al., 1992; Janila et al., 1999; Joshi et al, 1990; Vadez et al., 2005; Hernandez et al., 1995; Cherian et al., 1999; Takemura et al., 2000; Azad, 2006; Gupta and Yadav, 1986; Silberbush and Lips, 1988; Lauter et al., 1988). The insight is that plants when subjected to salt stress cannot absorb water for low water potential in the soil medium, this message is transmitted to the leaf possibly via ABA signaling routes. This is reasonably proper since the increased
production of abscisic acid (ABA) results in salt stressed condition (Munns and Cramer, 1996). Therefore, ABA is considered as the potent candidate of signal transduction pathway that forces stomata to close thereby reduce water expense via transpiration (Cramer and Quarry, 2002; Sauter et al., 2001; Ren et al., 2006). The closure of stomata also limits parallelly CO₂ intake by the plant and results in reduced growth via reduced photosynthesis. Interestingly, leaf area of younger leaves and branch number remained unreduced at all salinity doses despite leaf dry weight, shoot dry weight and total dry weight decreased almost gradually with increasing salinity doses (Table 2). This means although area of newly formed leaves and number of branches in a plant do not reduce with increasing salinity doses but the older leaves shed at a higher rate and the branches become smaller which is confirmed by the gradual increase in stem/leaf ratio and decrease in plant height with increasing salinity doses (Table 2). The insight is that when peanut plants are exposed to salinity stresses they absorb excess Na⁺ in the cytosol that make it toxic. Therefore, as an adaptation mechanism, they push off the excess Na⁺ from newly formed leaves to the older leaves and consequently the older leaves die and shed. Moreover, plants compartmentalize the excess Na⁺ to branches which in turn detoxify the cytosol of newly formed leaves and sustain its expansion.

Of the biochemical parameters, chlorophyll 'a' and total chlorophyll contents between unstressed and 3 dS/m salinity was statistically at par despite reduced to the lowest at 5 dS/m (Table 2b). Reduction in chlorophyll 'a' and total chlorophyll contents at 5 dS/m was mostly contributed by Zhuangbadam at flowering stage (Fig. 3b). The unreduced chlorophyll 'b' contents at all salinity doses as like unstressed treatment were attributed to its significant reduction at vegetative and increase at flowering stages in all varieties and salinity doses (Table 3b and Fig. 3b). Total sugar increased mostly gradually with increasing salinity doses up to 7 dS/m while reducing sugar followed reverse trend (Table 2b). The insight is that when plants face salt stress they cannot uptake water due to osmotic stress and thus accumulate compatible solutes such as proline (Khatkar and Kuhad, 2000; Singh et al., 2000), glycinebetaine (Rhodes and Hanson, 1993; Khan et al., 2000;
TABLE 2a: Means of yield and yield attributes for the salinity doses, averaged over the varieties and stages of salinity imposition.

<table>
<thead>
<tr>
<th>Salinity dose (dS/m)</th>
<th>Pod : peg</th>
<th>Number of immature pods/ plant</th>
<th>Number of mature pods/ plant</th>
<th>Number of total pods/ plant</th>
<th>Pod weight/ plant (g)</th>
<th>Kernel weight/ plant (g)</th>
<th>Number of kernel/ pod</th>
<th>Shelling percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.76a</td>
<td>9.94a</td>
<td>24.17a</td>
<td>34.78a</td>
<td>17.61a</td>
<td>11.49a</td>
<td>1.87c</td>
<td>67.29a</td>
</tr>
<tr>
<td>3</td>
<td>0.71b</td>
<td>6.39b</td>
<td>22.56b</td>
<td>28.89c</td>
<td>14.44b</td>
<td>9.87b</td>
<td>1.99a</td>
<td>68.39a</td>
</tr>
<tr>
<td>5</td>
<td>0.75a</td>
<td>10.94a</td>
<td>17.44c</td>
<td>31.61b</td>
<td>12.72c</td>
<td>7.72c</td>
<td>1.95b</td>
<td>54.88b</td>
</tr>
<tr>
<td>7</td>
<td>0.59d</td>
<td>6.56b</td>
<td>11.83e</td>
<td>18.28d</td>
<td>7.39d</td>
<td>3.86d</td>
<td>1.42d</td>
<td>53.86b</td>
</tr>
<tr>
<td>9</td>
<td>0.63c</td>
<td>6.00b</td>
<td>12.89d</td>
<td>13.94e</td>
<td>5.55e</td>
<td>2.14e</td>
<td>1.43d</td>
<td>42.92c</td>
</tr>
</tbody>
</table>

Same letters in a column do not differ significantly at P < 0.05; figures in the parentheses indicate relative performance (RP).

TABLE 2b: Means of different biochemical parameters for the salinity doses, averaged over the varieties and stages of salinity imposition.

<table>
<thead>
<tr>
<th>Salinity dose (dS/m)</th>
<th>Chlorophyll &quot;a&quot; content in leaves (mg/g fw)</th>
<th>Chlorophyll &quot;b&quot; content in leaves (mg/g fw)</th>
<th>Total chlorophyll content in leaves (mg/g fw)</th>
<th>Total sugar content in leaves (mg/g fw)</th>
<th>Reducing sugar content in leaves (mg/g fw)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.56a</td>
<td>0.44a</td>
<td>2.00a</td>
<td>3.68c</td>
<td>2.18bc</td>
</tr>
<tr>
<td>3</td>
<td>1.52a</td>
<td>0.46a</td>
<td>1.98a</td>
<td>4.21ab</td>
<td>2.40ab</td>
</tr>
<tr>
<td>5</td>
<td>1.37c</td>
<td>0.41a</td>
<td>1.78c</td>
<td>4.09ab</td>
<td>2.19c</td>
</tr>
<tr>
<td>7</td>
<td>1.44b</td>
<td>0.46a</td>
<td>1.90ab</td>
<td>4.31a</td>
<td>1.75d</td>
</tr>
<tr>
<td>9</td>
<td>1.45b</td>
<td>0.47a</td>
<td>1.92ab</td>
<td>3.95bc</td>
<td>2.73a</td>
</tr>
</tbody>
</table>

Same letters in a column do not differ significantly at P < 0.05;

Wang and Nil, 2000), polyol (Ford, 1984; Popp et al., 1985; Orthen et al., 1994; Bohnert et al., 1995) and sugar (Kerepesi and Galiba, 2000; Bohnert and Jensen, 1996; Plon-Smits et al., 1995). They protect plants from stress by turgor maintenance, detoxification of reactive oxygen species (ROS), and by stabilization of quaternary protein structure (Yancey et al., 1982; Bohnert and Jensen, 1996; Hasegawa et al., 2000).

**Stages of salinity imposition effect on different shoot and root characters, yield attributes and biochemical parameters as influenced by variety and salinity doses**

Stages of salinity imposition had shown significant differences for all the shoot and root characters, yield attributes, and biochemical parameters except branch number, dry weight of leaves and shoots, immature and mature pod number. Means of different shoot and root characters, yield attributes and biochemical parameter at the stages of salinity imposition, averaged over unstressed and stress treatments, are presented in Tables 3, 3a and 3b. All shoot and root characters (Table 3), yield attributes (Table 3a), and biochemical parameters (Table 3b) reduced under salinity stress to that of their unstressed treatments at vegetative and flowering stages except branch number and stem/leaf ratio at vegetative stage and leaf area (younger leaves) and SLW at flowering stage (Table 3), number of immature pod at vegetative stage (Table 3a), chlorophyll ‘b’ and reducing sugar at vegetative stage and total sugar at both vegetative and flowering stages (Table 3b).

Of the two growth stages, vegetative stage was more sensitive than flowering stage because of higher reduction of ultimate product, kernel number and weights (Table 3a). This reduction in kernel number and weight was caused by lower leaf area of younger leaves, SLW and dry weight of roots (Table 3) and increased number of immature pods (Table 3a) than flowering stage. Moreover, total sugar content was also lower than flowering stage although reducing sugar and chlorophyll 'b' contents were higher (Table 3b). On the contrary, the relatively tolerant flowering stage had shown only increased total sugar content. This means total sugar content
### TABLE 3: Means of different shoot and root characters at the stages of salinity imposition, averaged over the varieties and salinity doses.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Plant height (cm)</th>
<th>Leaf area plant (cm²)</th>
<th>Number of branch/plant</th>
<th>Specific leaf weight (cm²/g)</th>
<th>Stem:leaf</th>
<th>Dry weight of leaves/plant (g)</th>
<th>Dry weight of shoot/plant (g)</th>
<th>Dry weight of roots/plant (g)</th>
<th>Total dry weight/plant (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vegetative</td>
<td>23.89a (88.79%)</td>
<td>31.36a (96.62%)</td>
<td>6.02a (111.13%)</td>
<td>2.83a (89.99%)</td>
<td>0.73b (119.13%)</td>
<td>15.49a (88.29%)</td>
<td>27.02a (85.62%)</td>
<td>12.6a (80.95%)</td>
<td>28.97a (88.15%)</td>
</tr>
<tr>
<td>Flowering</td>
<td>24.33b (81.81%)</td>
<td>33.91a (101.83%)</td>
<td>5.64a (97.23%)</td>
<td>3.06a (98.02%)</td>
<td>0.76a (94.56%)</td>
<td>15.16b (70.39%)</td>
<td>26.47b (72.60%)</td>
<td>14.6a (90.82%)</td>
<td>28.02b (61.87%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 3a: Means yield attributes at the stages of salinity imposition, averaged over the varieties and stag salinity doses.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage of salinity imposition</td>
</tr>
<tr>
<td>-----------------------------</td>
</tr>
<tr>
<td>Vegetative</td>
</tr>
<tr>
<td>Flowering</td>
</tr>
</tbody>
</table>

*Same letters in a column do not differ significantly at P < 0.05.*
FIG. 1: Interaction effect of variety, salinity dose and stage of salinity imposition on (a) plant height, (b) leaf area, (c) leaf dry weight and (d) shoot dry weight. The vertical bar indicates LSD value at P<0.05.

TABLE 3b: Means of different biochemical parameters at the stages of salinity imposition, averaged over the varieties and stag salinity doses.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Chlorophyll ‘a’ content in leaves (mg/gfw)</th>
<th>Chlorophyll ‘b’ content in leaves (mg/gfw)</th>
<th>Total chlorophyll content in leaves (mg/gfw)</th>
<th>Total sugar content in leaves (mg/gfw)</th>
<th>Reducing sugar content in leaves (mg/gfw)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vegetative</td>
<td>1.36b (92.17%)</td>
<td>0.28b (113.6%)</td>
<td>1.53b (95.28%)</td>
<td>4.27a (101.96%)</td>
<td>2.86a (111.48%)</td>
</tr>
<tr>
<td>Flowering</td>
<td>1.58a (93.3%)</td>
<td>0.61a (98.32%)</td>
<td>2.25a (94.58%)</td>
<td>3.83b (124.66%)</td>
<td>1.65b (96.62%)</td>
</tr>
</tbody>
</table>

Same letters in a column do not differ significantly at P< 0.05; figures in the parentheses indicate relative performance (RP).
FIG. 2: Interaction effect of variety, salinity dose and stage of salinity imposition on (a) immature pod, (b) total pod, (c) pod weight and (d) kernel weight/plant. The vertical bar indicates LSD value at $P<0.05$.

association with total dry weight at different salinity doses (Vadez et al., 2005). At vegetative stage, leaf dry weight of Dacca-1 decreased significantly than that of unstressed treatment at only 9 dS/m, in Binachinabadam-3 at 7.9 dS/m and in Zhingabadam at all salinity doses except 5 dS/m (Fig. 1c). At flowering stage, it reduced significantly in all varieties. At vegetative stage, shoot dry weight of Dacca-1 and Binachinabadam-3 decreased significantly at 9 and 7.9 dS/m doses, respectively. At flowering stage, it decreased significantly than that of unstressed treatment in Zhingabadam at all salinity doses, in Binachinabadam-3 at 5.9 dS/m and in Dacca-1 at 3 dS/m.

The interactions of variety × salinity dose on immature and total pod numbers, pod and kernel
FIG. 3: Interaction effect of variety, salinity dose and stage of salinity imposition on (a) chlorophyll 'a', (b) chlorophyll 'b', (c) reducing sugar and (d) total sugar contents in leaves. The vertical bar indicates LSD value at P<0.05.

weights at vegetative and flowering stages are presented in Figs. 2a, 2b, 2c and 2d. Zhingabadam produced significantly higher number of immature pod than the remainder two varieties under unstressed treatments at both vegetative and flowering stages (Fig. 2a). In contrast, its immature pod number mostly decreased significantly when exposed to salinity stress irrespective of salinity doses and growth stages. Dacca-1 and Binachinabadam-3 appeared with reverse trend to that of Zhingabadam. This means immature pod number mostly increased for salinity stresses to that unstressed treatments in Dacca-1 and Binachinabadam-3 at both vegetative and flowering stages with some exceptions. Total pod number mostly decreased in all varieties irrespective of salinity...
doses and growth stages to that of respective unstressed treatments except Binachinabadam-3 at 3 and 5 dS/m salinity doses and Dacca-1 at 3 dS/m at vegetative stage.

Pod weight reduced gradually and significantly than respective unstressed treatments in all varieties with increasing salinity doses up to 5-9 dS/m at vegetative stage (Fig. 2c). Similarly, pod weight of all varieties reduced at all salinity doses than that of respective unstressed treatments at flowering stage but with relatively lower rate, particularly in Binachinabadam-3.

Kernel weight of Dacca-1 decreased significantly and gradually with increased salinity doses at vegetative stage while that of Binachinabadam-3 at 5-9 dS/m doses (Fig. 2d). At flowering stage, kernel weight of Dacca-1 and Binachinabadam-3 decreased significantly and gradually with increasing salinity doses except 3 dS/m for Dacca-1 and 5 dS/m for Binachinabadam-3. Kernel weight of Zhingabadam decreased significantly than unstressed treatment only at 7 and 9 dS/m doses.

The interaction of variety × salinity dose on chlorophyll ‘a’ chlorophyll ‘b’, reducing and total sugar contents in leaves when salinity imposed at vegetative and flowering stages are presented in Figs. 3a, 3b, 3c and 3d. Chlorophyll ‘a’ content of Binachinabadam-3 and Zhingabadam remained statistically indifferent with unstressed treatments at all salinity doses at vegetative and flowering stages except Zhingabadam at 5 dS/m at flowering stage (Fig. 3a). In contrast, it decreased significantly in Dacca-1 at 3, 5 and 9 dS/m at vegetative and 7 dS/m at flowering stage. Chlorophyll ‘b’ content was significantly lower at vegetative than flowering stage in all the varieties at all salinity doses (Fig. 3b). It only decreased significantly with unstressed treatment in Dacca-1 at 9 dS/m at vegetative stage. Reducing sugar content increased significantly than that of unstressed treatment in Binachinabadam-3 at 3 and 9 dS/m, in Zhingabadam at 7 and 9 dS/m doses at vegetative stage (Fig. 3c). At flowering stage, reducing sugar contents increased significantly in Zhingabadam while decreased in Dacca-1 at all salinity doses and in Binachinabadam-3 at 5 dS/m. Total sugar content increased significantly in Zhingabadam at 3 and 7 dS/m doses at vegetative stage (Fig. 3d). In contrast, it decreased significantly in Binachinabadam-3 at 5 and 7 dS/m salinity doses at flowering stage.

Finally, it could be concluded that tolerance to salinity stress in peanut is conferred by higher allocation of assimilate to kernel through maintaining total sugar and chlorophyll ‘a’ contents close to unstressed treatment.
REFERENCES


