INFLUENCE OF SALINE STRESS ON GROWTH, GAS EXCHANGE, MINERAL NUTRIENTS AND NON-ENZYMATIC ANTI-OXIDANTS IN MUNGBEAN [(VIGNA RADIATA (L.) WILCZEK)]

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Abstract

The study was conducted to appraise the effect of saline stress on growth, gas exchange, chlorophyll fluorescence, mineral nutrients and non-enzymatic antioxidants in 2 mungbean [Vigna radiata (L.) Wilczek] lines (97001 and 97012). Seeds of each line were sown in sand-filled pots. When the plants were 30 day-old, 2 saline regimes [control (non-saline – full strength Hoagland’s nutrient solution) and 50mM NaCl in Hoagland’s nutrient medium] were applied and maintained 30 days, after which time data for various growth and physiological attributes were recorded. Saline stress markedly reduced shoot fresh and dry weight, shoot length, net CO2 assimilation rate, transpiration rate, stomatal conductance, water use efficiency, leaf and root N contents, and leaf ascorbic acid and phenolic contents in both mungbean lines. In contrast, salt stress significantly enhanced leaf and root Na+ and root Ca2+ contents in both mungbean lines. Saline stress did not alter leaf chlorophyll fluorescence, leaf and root K+ and P and leaf Ca2+ as well as leaf alpha-tocopherol. Overall, line 97001 showed better performance than line 97012 under both saline and non-saline conditions.

Introduction

Pulses are the best dietary source of proteins and they play a very important role to fulfill the requirements of rapidly increasing population. Mungbean [Vigna radiata (L.) Wilczek] is an important short summer season pulse crop which is grown primarily for its protein rich edible seeds. Mungbean is a very rich source of easily digestible proteins; it contains about 24.5% protein, 59.9% carbohydrates and 1.2% fat (Anon., 2000). It has a very good ability to enhance the physical, chemical and biological soil properties, therefore, it is considered as an important component of sustainable agriculture (Anon., 2001). Mungbean is a short duration crop and requires less water than other summer crops, therefore, it can be grown in rain-fed areas (Anjum et al., 2006).

Abiotic stresses severely reduce the productivity of almost all pulse crops including mungbean (Gao et al., 2007). However, stress-induced adverse effects are variable at various growth stages like in mungbean the adverse effect on grain yield is more at the reproductive stage than that at other stages (Thomas et al., 2004).

Mungbean is generally known as a salt sensitive crop (Chakrabarti & Mukherji, 2003). Salt stress causes a substantial growth reduction in mungbean. For example, salt stress was found to reduce seed germination, fresh and dry biomass, shoot and root lengths and yield attributes of mungbean (Promila & Kumar, 2000; Rabie, 2005; Ahmed, 2009). This reduction in mungbean growth increases with increase in saline regimes (Chakrabarti & Mukherji, 2003). Photosynthetic capacity is reduced in mungbean under saline regimes (Mortant-Manceau et al., 2004). The decrease in photosynthetic rate is ascribed to reduced stomatal conductance and consequently, inhibition in CO2 availability for carboxylation (Koyro, 2006).

Salt stress influences accumulation of mineral nutrients in mungbean (Raptan et al., 2001). Different researchers have reported a rapid increase in Na+ and Cl− content and a decrease in Ca2+ and K+ content in the leaves, stem and roots of mungbean plants (Kabir et al., 2004; Rashid et al., 2004; Haleem & Mohammed, 2007).

Activities of antioxidant enzymes have been reported to increase in most plants (Mittova et al., 2002; Ashraf, 2009) including mungbean under saline stress. These enhanced activities of antioxidant enzymes (CAT, SOD and POD) and non-enzymatic antioxidants (ascorbate, tocopherols and phenolic compounds) help to protect the mungbean plants from damages caused by salt-induced reactive oxygen species (ROS) (Yasar et al., 2008).

As reported earlier, as compared to most of the known pulse crops, mungbean is relatively more sensitive to saline stress. Thus, it is expected that its metabolic processes are severely affected by salt stress. Thus, the primary objective of the current study was to evaluate the salinity-induced modulation in some key morphological, physiological and biochemical attributes of mungbean.

Material and Methods

An experiment was conducted to assess the effect of salt stress on mungbean [Vigna radiata (L.) Wilczek]. Seeds of two mungbean lines i.e. 97012 and 97001 were obtained from the Ayub Agricultural Research Institute, Faisalabad. Fifteen seeds of each line were sown in each of the plastic pots, filled with equal weight thoroughly washed river sand. After 15 days of sowing, thinning of plants was done to maintain 5 plants per pot. Full strength Hoagland’s nutrient solution was used to provide sufficient nutrients to the plants. Two salinity treatments [control (0 mM NaCl in full strength Hoagland’s nutrient solution) and 50 mM NaCl + full strength Hoagland’s nutrient solution] were applied to 30-day old plants. The experiment was laid down in a completely randomized design with 4 replications. After 30 days of salinity treatment, 2 plants were harvested from each of replicate, washed with distilled water and recorded data for shoot and root fresh weights and shoot and root lengths. The samples were oven-dried at 65°C up to their constant weight and then dry weights recorded. In addition, data for following attributes were also recorded:
Gas exchange characteristics: A portable infra-red gas analyzer (IRGA) (ACD LCA-4 Analytical Development, Hoddesdon, UK) was used to determine the net photosynthetic rate ($A$), transpiration rate ($E$), stomatal conductance ($g_s$), water use efficiency ($A/E$), and internal CO$_2$ concentration ($Ci$) on fully expanded leaves. Following adjustments/values of the instrument were recorded/maintained during its operation: 403.3 mmol m$^{-2}$ s$^{-1}$ for molar flow of air, 99.9 kPa atmospheric pressure, 6.0 to 8.9 mbar water vapor pressure, 1711 µmol m$^{-2}$ sec$^{-1}$ PAR, 28.4 to 27.9°C leaf temperature and 352 µmol mol$^{-1}$ ambient CO$_2$ concentration.

Chlorophyll fluorescence: Polyphasic rise of fluorescence transients was determined using an OS5p Modulator Fluorometer (ADC BioScientific Ltd, Great Amwell Herts, UK) according to Strasser et al. (1995). All the samples were dark-adapted for 30 min before the fluorescence measurements.

**Determination of mineral ions**

**Digestion method:** The dried ground leaf or root material (100 mg) was digested with 2 ml H$_2$SO$_4$ following the method of Wolf (1982). The volume of the extract was brought up to 50 ml with distilled water, filtered and used determining mineral elements.

**Determination of Na$^+$, K$^+$ and Ca$^{2+}$:** Leaf and root sodium (Na$^+$), potassium (K$^+$) and calcium (Ca$^{2+}$) contents were determined using a flame photometer (Jenway, PFP-7, UK).

**Nitrogen estimation:** Nitrogen was determined following the Kjeldahl method as described by Bremner et al. (1965). Five ml of acid digested sample with 20 ml of 40% NaOH solution were taken in Kjeldahl tubes and placed all tubes in the Kjeldahl Ammonium Distillation Unit. Boric acid solution was taken in conical flasks with a few drops of mixed indicator of bromocresol green and methylene red. When the distillate was approximately 40 ml, the distillation stopped. The distillate was cooled and titrated against 0.01 N H$_2$SO$_4$ until the solution turned pink.

Nitrogen was estimated using the following formula:

$$% \text{N} = \frac{(V_2-V_1 \times N \times 0.014 \times 100)}{W}$$

$V_1$ and $V_2$ in the above equation show the volume of standard H$_2$SO$_4$ required to titrate blank solution and sample solution, respectively, whereas N shows the normality of the H$_2$SO$_4$ used and W the weight of sample.

**Determination of phosphorus:** Phosphorus was determined spectrophotometrically following method of Jackson (1962).

**Determination of non-enzymatic antioxidants**

**Total phenolics:** Julkenen-Titto (1985) proposed a method which was used to determine total phenolics. Leaf fresh material (50 mg) was homogenized in 80% acetone. The homogenized material was centrifuged at 10,000 $\times$ g for 10 min, removed the pellet and the supernatant was used for the determination of phenolics. Then 100 µl of the supernatant were mixed with 1 ml of Folin-Ciocalteau's phenol. In addition, 2.0 ml distilled water and 5 ml of 20% Na$_2$CO$_3$ were also added. The mixture was vortexed and absorbance read at 750 nm using a UV-Visible spectrophotometer (IRMECO U2020).

**Leaf ascorbic acid contents:** The amount of ascorbic acid in the mungbean leaves was determined following Mukherjee & Choudhuri (1983). Fresh leaves (0.25 g) were ground in 10 ml of 6% TCA. The mixture was centrifuged for 10 min at 4°C at 1000 $\times$ g. An aliquot of 2 ml of 2% dinitrophenyl hydrizone solution was added to 4 ml of supernatant. One drop of thiourea (10% thiourea prepared in 70% ethanol) was added to the mixture, and boiled the mixture for 20 min in a water bath. The mixture was placed in ice to reduce the temperature to about 25°C, then added 5 ml of 80% sulphuric acid (v/v) at 0°C and the absorbance read at 530 nm. The ascorbic acid content was quantified against a standard curve which was prepared by known concentrations of ascorbic acid.

**Estimation of leaf tocopherol content:** The method of Baker et al., (1980) was used for the determination of leaf alpha-tocopherol concentration. A mixture of 20 ml of petroleum ether and ethanol (2:1:6, v/v) was used to grind the fresh leaves (1.0 g each sample). The mixture was centrifuged at 10,000 $\times$ g for 20 min. An aliquot of 200 µl of 2% 2, 2- dipiridyl (prepared in ethanol) was added to 1 ml of supernatant, mixed the mixture and placed it in the dark for 5 min. The absorbance was read at 520 nm using a spectrophotometer.

**Statistical analysis:** A two-way analysis of variance (ANOVA) of data for all attributes was calculated using the COSTAT computer program. The least significant difference was used to compare the mean values of all treatments (Snedecor & Cochran, 1980).

**Results**

Salt stress (50mM) applied through root growing medium significantly (p<0.05) decreased the shoot fresh weight of both mungbean lines. Both mungbean lines showed significant (p<0.001) difference in response to salt stress, particularly line 97001 performed better than the other line under control and saline conditions (Table 1; Fig. 1). Salt stress decreased shoot dry weight of both mungbean lines (p<0.01), but both mungbean lines varied significantly (p<0.01) in their response to salt stress (Table 1; Fig. 1). The line 97001 showed better performance than 97012 in terms of shoot dry weight under both non-saline and saline conditions.

Root fresh and dry weights of both mungbean lines did not alter by imposition of saline stress to the root growing medium. However, the lines differed markedly (p<0.001) in both root fresh and dry weights. Line 97001 performed better than 97012 in both the earlier-mentioned attributes under both control and salt stress conditions (Table 1; Fig. 1).
Fig. 1 Growth attributes, gas exchange and chlorophyll fluorescence in two lines of mungbean [Vigna radiata (L.) Wilczek] when 30 days old plants were subjected for 30 days to saline or non-saline conditions.
Fig. 2. Chlorophyll fluorescence, mineral nutrients and non-enzymatic antioxidants in two lines of mungbean [(Vigna radiata (L.) Wilczek] when 30 days old plants were subjected for 30 days to saline or non-saline conditions.
Table 1. Mean squares from analyses of variance of data for growth attributes, gas exchange, chlorophyll fluorescence, mineral nutrients and biochemical attributes of mungbean ([*Vigna radiata* (L.) Wilczek] when 30 days old plants subjected for 30 days to saline or non-saline conditions.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>Shoot fresh weight</th>
<th>Shoot dry weight</th>
<th>Shoot length</th>
<th>Root fresh weight</th>
<th>Root dry weight</th>
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<tbody>
<tr>
<td>Lines (L)</td>
<td>1</td>
<td>1025.9***</td>
<td>7.86**</td>
<td>957.9***</td>
<td>7.98***</td>
<td>0.462***</td>
</tr>
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<td>Salinity (S)</td>
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<td>105.16*</td>
<td>10.30**</td>
<td>388.09*</td>
<td>0.3364ns</td>
<td>0.028ns</td>
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<tr>
<td>L × S</td>
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<td>0.748ns</td>
<td>0.83ns</td>
<td>5.64ns</td>
<td>0.076ns</td>
<td>0.0012ns</td>
</tr>
<tr>
<td>Error</td>
<td>12</td>
<td>12.077</td>
<td>0.824</td>
<td>48.88</td>
<td>0.094</td>
<td>0.0079</td>
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<tr>
<th>Source of variation</th>
<th>df</th>
<th>Root length</th>
<th>A</th>
<th>E</th>
<th>g_s</th>
<th>Ci</th>
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<td>41.60**</td>
<td>0.104ns</td>
<td>0.112*</td>
<td>50625***</td>
<td>4.86ns</td>
</tr>
<tr>
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<td>1</td>
<td>14.82ns</td>
<td>150.001***</td>
<td>0.71***</td>
<td>46225***</td>
<td>1674.9ns</td>
</tr>
<tr>
<td>L × S</td>
<td>1</td>
<td>15.80ns</td>
<td>0.566ns</td>
<td>0.031ns</td>
<td>1600ns</td>
<td>9.59ns</td>
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<tr>
<td>Error</td>
<td>12</td>
<td>3.85</td>
<td>0.817</td>
<td>0.023</td>
<td>1762.5</td>
<td>1013.8</td>
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<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>A/E</th>
<th>Ci/Ca</th>
<th>Fv/Fm</th>
<th>NPQ</th>
<th>q_N</th>
</tr>
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<tr>
<td>Lines (L)</td>
<td>1</td>
<td>17.49*</td>
<td>0.0014ns</td>
<td>0.026ns</td>
<td>0.015ns</td>
<td>0.014**</td>
</tr>
<tr>
<td>Salinity (S)</td>
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<td>27.11**</td>
<td>0.014ns</td>
<td>0.031ns</td>
<td>0.0072ns</td>
<td>0.008*</td>
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<tr>
<td>L × S</td>
<td>1</td>
<td>9.11ns</td>
<td>0.0049ns</td>
<td>0.050ns</td>
<td>0.018ns</td>
<td>3.51ns</td>
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<tr>
<td>Error</td>
<td>12</td>
<td>2.89</td>
<td>0.011</td>
<td>0.034</td>
<td>0.005</td>
<td>0.0012</td>
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<tr>
<th>Source of variation</th>
<th>df</th>
<th>q_P</th>
<th>ETR</th>
<th>Leaf Na⁺</th>
<th>Leaf K⁺</th>
<th>Leaf Ca²⁺</th>
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<td>Lines (L)</td>
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<td>0.0086ns</td>
<td>1.76ns</td>
<td>15.01ns</td>
<td>0.39ns</td>
<td>0.001ns</td>
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<tr>
<td>Salinity (S)</td>
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<td>0.601ns</td>
<td>13.14ns</td>
<td>19.14ns</td>
<td>1.0ns</td>
</tr>
<tr>
<td>L × S</td>
<td>1</td>
<td>2.402ns</td>
<td>1.05ns</td>
<td>0.015ns</td>
<td>4.51ns</td>
<td>1.0ns</td>
</tr>
<tr>
<td>Error</td>
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<td>0.0071</td>
<td>2.33</td>
<td>12.47</td>
<td>21.28</td>
<td>2.62</td>
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<table>
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<th>Source of variation</th>
<th>df</th>
<th>Leaf N</th>
<th>Leaf P</th>
<th>Root Na⁺</th>
<th>Root K⁺</th>
<th>Root Ca²⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lines (L)</td>
<td>1</td>
<td>42.25*</td>
<td>7.42ns</td>
<td>31.64*</td>
<td>18.06ns</td>
<td>0.14ns</td>
</tr>
<tr>
<td>Salinity (S)</td>
<td>1</td>
<td>289***</td>
<td>3.15ns</td>
<td>66.01**</td>
<td>20.25ns</td>
<td>3.51*</td>
</tr>
<tr>
<td>L × S</td>
<td>1</td>
<td>90.25**</td>
<td>0.14ns</td>
<td>28.89*</td>
<td>5.06ns</td>
<td>0.015ns</td>
</tr>
<tr>
<td>Error</td>
<td>12</td>
<td>8.125</td>
<td>2.25</td>
<td>5.83</td>
<td>6.55</td>
<td>0.38</td>
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<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>Root N</th>
<th>Root P</th>
<th>Total phenolics</th>
<th>Leaf tocopherols</th>
<th>Leaf ascorbic acid</th>
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<tr>
<td>Lines (L)</td>
<td>1</td>
<td>10.56ns</td>
<td>3.24ns</td>
<td>0.307ns</td>
<td>14.06**</td>
<td>25.50ns</td>
</tr>
<tr>
<td>Salinity (S)</td>
<td>1</td>
<td>85.56**</td>
<td>1.44ns</td>
<td>0.512ns</td>
<td>0.302ns</td>
<td>275.6*</td>
</tr>
<tr>
<td>L × S</td>
<td>1</td>
<td>0.062ns</td>
<td>0.16ns</td>
<td>2.335**</td>
<td>1.690ns</td>
<td>45.56ns</td>
</tr>
<tr>
<td>Error</td>
<td>12</td>
<td>6.604</td>
<td>1.23</td>
<td>0.147</td>
<td>0.780</td>
<td>34.15</td>
</tr>
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* *, **, and *** = Significant at 0.05, 0.01, and 0.001 levels, respectively  
ns = Non-significant

Shoot length of both mungbean lines decreased considerably (p ≤ 0.05) due to saline regimes. There was a significant (p ≤ 0.001) difference between the 2 lines in response of shoot length to salt stress. Line 97001 showed better performance than Line 97012 (Table 1; Fig. 1).

Salt stress treatment had no effect on root length of both mungbean lines. However, the lines varied significantly (p ≤ 0.01) in this growth attribute. Line 97001 performed significantly better than the other line in root length under saline regimes (Table 1; Fig. 1).

Net CO₂ assimilation rate (A) was negatively affected in both mungbean lines by salt stress. However, both lines showed a uniform behavior in this attribute (Table 1; Fig. 1).

Stomatal conductance (g_s) also decreased significantly (p ≤ 0.001) in both lines of mungbean due to salt stress. Both lines differed significantly (p ≤ 0.001) in this attribute. Line 97001 showed higher values of stomatal conductance behavior than 97012 in both stressed and non-stressed conditions (Table 1; Fig. 1).

Water use efficiency (WUE) was adversely affected in both lines under saline regimes. These lines also differed significantly (p ≤ 0.05) in this attribute, particularly, line 97012 performed slightly better than 97001 under both control and salt stress conditions (Table 1; Fig. 1). Sub-stomatal CO₂ concentration (C_i) and (C_i/Ca) did not change in both lines due to imposition of saline stress to the growth medium. Both mungbean lines showed uniform behavior with respect to these both attributes (Table 1; Fig. 1).
Salt stress applied through the root growing medium showed non-significant effect on efficiency of photosystem II (Fv/Fm), non-photochemical quenching (NPQ), photosynthetic efficiency (qP) and electron transport rate (ETR) of both mungbean lines. Both lines did not show significant difference in these attributes (Table 1; Figs. 1, 2).

Various saline regimes markedly reduced the coefficient of non-photochemical quenching (qN) of both mungbean lines. Mungbean line 97001 showed better performance than 97012 in this attribute under both non-stress and salt-stress conditions (Table 1; Fig. 2).

Salt stress significantly increased the leaf Na⁺ content of both mungbean lines. A non-significant difference was observed between these 2 lines with respect to these mineral ions under saline conditions (Table 1; Fig. 2). Salinity also enhanced root Na⁺ content (p≤0.01) of both lines and these lines also differed considerably (p≤0.05) in this attribute. Line 97001 showed higher accumulation of root Na⁺ as compared to that in 97012 (Table 1; Fig. 2). Leaf and root K⁺ and P contents did not change in both lines due to imposition of saline stress to the growth medium. Non-significant difference was also found between the two lines with respect to these attributes (Table 1; Fig. 2).

Salt stress did not affect leaf Ca²⁺ content in mungbean lines, but significantly (p≤0.05) enhanced root Ca²⁺ content in them. However, both lines showed a uniform behavior in both these attributes (Table 1; Fig. 2).

Saline stress caused a significant (p≤0.001) reduction in shoot N content of both mungbean lines. However, both lines did not show a significant difference in shoot N contents (Table 1; Fig. 2). Root N content also decreased significantly (p≤0.01) in both mungbean lines under saline regimes. However, this reduction was non-significant in both lines with respect to this attribute (Table 1; Fig. 2).

Saline stress caused a significant reduction in total phenolics of line 97001, however, such reduction was not prominent in line 97012 (Table 1; Fig. 2). Leaf tocopherols did change in both lines due to imposition of salt stress to the growth medium, while lines showed a significant variation in leaf tocopherols. Line 97001 was higher than line 97012 in leaf tocopherols (Table 1; Fig. 2). Saline stress markedly reduced leaf ascorbic acid in both lines but the difference between the lines was not prominent (Table 1; Fig. 2).

Discussion

Salt stress is one of the most harmful environmental stresses which reduce growth and development of plants (Perveen et al., 2012; Shahbaz et al., 2012, 2013). Although plants have the ability to cope with these conditions, this ability depends upon species or cultivar types (Ashraf & Khanum, 1997; Ashraf, 2004; Shahbaz & Ashraf, 2013). In the present study, imposition of salt stress (50 mM NaCl) caused a significant reduction in growth and development of both mungbean lines (97012 and 97001). Our findings are in agreement with some previous studies in which salt-induced reduction in growth attributes have been observed in different crops like wheat (Ashraf & Khanum, 1997; Shahbaz et al., 2008, 2013; Kanwal et al., 2011; Ashraf et al., 2012; Kausar & Shahbaz, 2013), rice (Shahbaz & Zia, 2011), various vegetables (Shahbaz et al., 2012), eggplant (Abbas et al., 2010), carrot (Banot et al., 2012), cauliflower (Batool et al., 2012), Solanum melongena (Shaheen et al., 2012), Haleem & Mohammed (2007) also reported reduction in growth attributes such as fresh and dry weights and shoot and root lengths of mungbean plants under saline stress. However, the reduction was more prominent in shoot growth as compared to that in root growth. It has already been observed that usually reduction in shoot growth is higher than that in root growth because roots have higher ability of osmotic adjustment under salt stress conditions as compared to shoots (Fisarakis et al., 2001).

Gas exchange attributes substantially contribute to plant growth and development. These attributes are adversely affected in many crop plants due to root-applied saline stress (Perveen et al., 2010; Shahbaz et al., 2013). In our study, net CO₂ assimilation rate, transpiration rate, stomatal conductance and water use efficiency decreased considerably in both mungbean lines under saline stress. Similar findings were observed by various researchers in many crops like wheat (Shahbaz et al., 2008; Kanwal et al., 2011), grasses (Akram et al., 2007), rice (Naheed et al., 2007), sunflower (Akram et al., 2009; Shahbaz et al., 2011), cotton (Shaheen et al., 2012), etc. Salt-induced negative effects on gas exchange characteristics have also been observed in mungbean by Hatami et al., (2010). They reported that salt stress caused reduction in photosynthetic rate, transpiration rate as well as stomatal conductance in mungbean plants. Photosynthetic rate decreased in both mungbean lines significantly which can be associated with decreased utilization efficiency of light, photoinhibition of photosystem and reduction in stomatal conductance with a subsequent reduction in CO₂ availability at the site of its fixation (Chedlia et al., 2007). Low transpiration rate might have been due to turgidity loss of guard cells, which commonly occurs under almost all abiotic stresses (Stepin & Klobus, 2006). Salt stress causes some physiological and anatomical alterations which might be responsible for reduction in stomatal conductance (gs) and water use efficiency (WUE) (Chartzoulakis et al., 2002). Loss in turgidity of guard cells may cause stomatal closure which could be responsible for low availability of CO₂ in mesophyll cells ultimately leading to decreased photosynthetic efficiency (Dubay, 2005).

Chlorophyll fluorescence characteristics are helpful tools used to determine the influences of various biotic and abiotic stresses on the process of photosynthesis (Stirbet & Govindjee, 2011). However, in our findings saline stress did not alter chlorophyll fluorescence parameters including Fv/Fm, NPQ, qP and ETR while reduced only qN. It is already reported that maximum quantum yield of PSII (Fv/Fm) and qN are related to rate of photosynthesis and therefore qN, qP and Fv/Fm reduce in leaf tissues under saline conditions (Cha-Um & Kirdmanee, 2009). In another study, Pereira et al. (2000)
observed decrease in ETR which was ascribed to impairment in electron transport. Non-significant effect of saline stress on \( Fv/Fm \) has also been observed in wheat (Perveen et al., 2010). Availability of essential nutrients in most plants is generally reduced under saline stress (Perveen et al., 2012). In the present study, saline stress significantly increased both leaf and root Na\(^+\) contents in both mungbean lines. This increase in leaf or root Na\(^+\) contents had also been observed in earlier studies on different crops such as rice (Ahmad et al., 2007; Naheed et al., 2008; Shahbaz & Zia, 2011), wheat (Ashraf & Khanum, 1997; Shahbaz & Ashraf, 2007; Perveen et al., 2012), sunflower (Shahbaz et al., 2011), cotton (Ashraf & Ahmad, 2000), etc. Increase in leaf or root Na\(^+\) may be due to high concentration of sodium in root growing medium causing its high uptake through roots (Munns & Tester, 2008). In our study, saline stress did not alter leaf or root K\(^+\) and P contents. In contrast to our findings, Dar et al., (2007) observed decrease in shoot and root K\(^+\) content due to imposition of salt stress. Shahbaz et al., (2011) also observed a considerable decrease in leaf and root K\(^+\) content in sunflower and according to El-bassyouny & Bekheta (2005), Na\(^+\) ion accumulation leads to reduced K\(^+\) ion absorption in plants. However, non-significant effect of salinity on K\(^+\) contents might have been due to non-antagonistic effect of these two ions (Ashraf, 2004). Similarly, in our study, salt stress caused a non-significant effect on leaf Ca\(^{2+}\), while increased root Ca\(^{2+}\) contents. Contrary to these findings it has been reported by some researchers that salinity causes a significant reduction in Ca\(^{2+}\) ion accumulation in plants (Haleem & Mohammed, 2007). Turan et al., (2007) observed a slight increase in shoot P in mungbean. In our study, both leaf and root N decreased under saline stress. Similar, pattern of accumulation in these nutrients was observed by Turan et al., (2010). They reported a decreased accumulation of N in the roots of mungbean plants under saline stress.

Of a variety of non-enzymatic antioxidants, leaf phenolic contents play a very protective role in plant cells. Various abiotic stresses are known to adversely affect the synthesis of phenolics (Parida et al., 2004). In our study, saline stress reduced the phenolic contents in both mungbean lines. Falleh et al. (2008) also observed salt-induced reduction in phenolic contents in \( Cynara cardunculus \) leaves. This reducing trend was also observed in wheat by Ashraf et al., (2010). Tocopherols are non-enzymatic antioxidants which protect plants from oxidative stress. In our study, salt stress did not affect alphatocopherol content which was in agreement with the findings of Anwar et al., (2006) who observed a non-significant effect of salt stress on tocopherol contents in \( Moringa \) seed oils, while in contrast, in safflower (Siddiqi et al., 2011) and sunflower (Noreen & Ashraf, 2010) seed oils, saline stress significantly increased tocopherols. Saline stress also reduced the leaf ascorbic acid in mungbean plants, which is similar to what has been observed in wheat plants under saline stress (Ashraf et al., 2012).

In conclusion, saline stress significantly reduced shoot fresh and dry weights, shoot length, net CO\(_2\) assimilation rate, transpiration rate, stomatal conductance, water use efficiency and leaf ascorbic acid, while a non-significant effect of salt stress was observed on chlorophyll fluorescence attributes. Saline stress markedly enhanced leaf and root Na\(^+\), decreased leaf and root N, and did not alter leaf and root K\(^+\) and P. Overall, line 97001 performed better than line 97012 in most of the attributes appraised in this study under saline stress.

Acknowledgements

The second author gratefully acknowledges the funding from the Pakistan Academy of Sciences (PAS) (Grant No. 5-9/PAS/7536). The results presented in this paper are a part of M. Phil. studies of Miss Sobia Kanwal.

References


Ahmad, M.S.A., F.J. Khan and M. Ashraf. 2007. Iso-osmotic effect of NaCl and PEG on growth, cations and free proline accumulation in callus tissue of two indica rice (\( Oryza sativa \) L.) genotypes. \( Plant Growth Regul. \), 53: 53-63.


(Received for publication 8 January 2012)