

## INFLUENCE OF SALINE STRESS ON GROWTH, GAS EXCHANGE, MINERAL NUTRIENTS AND NON-ENZYMATIC ANTIOXIDANTS 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 CO<sub>2</sub> 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<sup>+</sup> and root Ca<sup>2+</sup> contents in both mungbean lines. Saline stress did not alter leaf chlorophyll fluorescence, leaf and root K<sup>+</sup> and P and leaf Ca<sup>2+</sup> 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 conductivity and consequently, inhibition in CO<sub>2</sub> 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<sup>+</sup> and Cl<sup>-</sup> content and a decrease in Ca<sup>2+</sup> and K<sup>+</sup> 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 50mM 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  $\text{CO}_2$  concentration ( $C_i$ ) on fully expanded leaves. Following adjustments/values of the instrument were recorded/maintained during its operation:  $403.3 \text{ mmol m}^{-2} \text{ s}^{-1}$  for molar flow of air, 99.9 kPa atmospheric pressure, 6.0 to 8.9 mbar water vapor pressure,  $1711 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$  PAR, 28.4 to 27.9°C leaf temperature and  $352 \text{ } \mu\text{mol mol}^{-1}$  ambient  $\text{CO}_2$  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  $\text{H}_2\text{SO}_4$  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  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Ca}^{2+}$ :** Leaf and root sodium ( $\text{Na}^+$ ), potassium ( $\text{K}^+$ ) and calcium ( $\text{Ca}^{2+}$ ) 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  $\text{H}_2\text{SO}_4$  until the solution turned pink.

Nitrogen was estimated using the following formula:

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

$V_1$  and  $V_2$  in the above equation show the volume of standard  $\text{H}_2\text{SO}_4$  required to titrate blank solution and sample solution, respectively, whereas N shows the normality of the  $\text{H}_2\text{SO}_4$  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  $\mu\text{l}$  of the supernatant were mixed with 1 ml of Folin-Ciocalteu's phenol. In addition, 2.0 ml distilled water and 5 ml of 20%  $\text{Na}_2\text{CO}_3$  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 & Choudhri (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 hydrazine 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  $\mu\text{l}$  of 2% 2, 2'-dipyridyl (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 ( $p \leq 0.001$ ) 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 ( $p \leq 0.01$ ), but both mungbean lines varied significantly ( $p \leq 0.01$ ) 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 ( $p \leq 0.001$ ) 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).

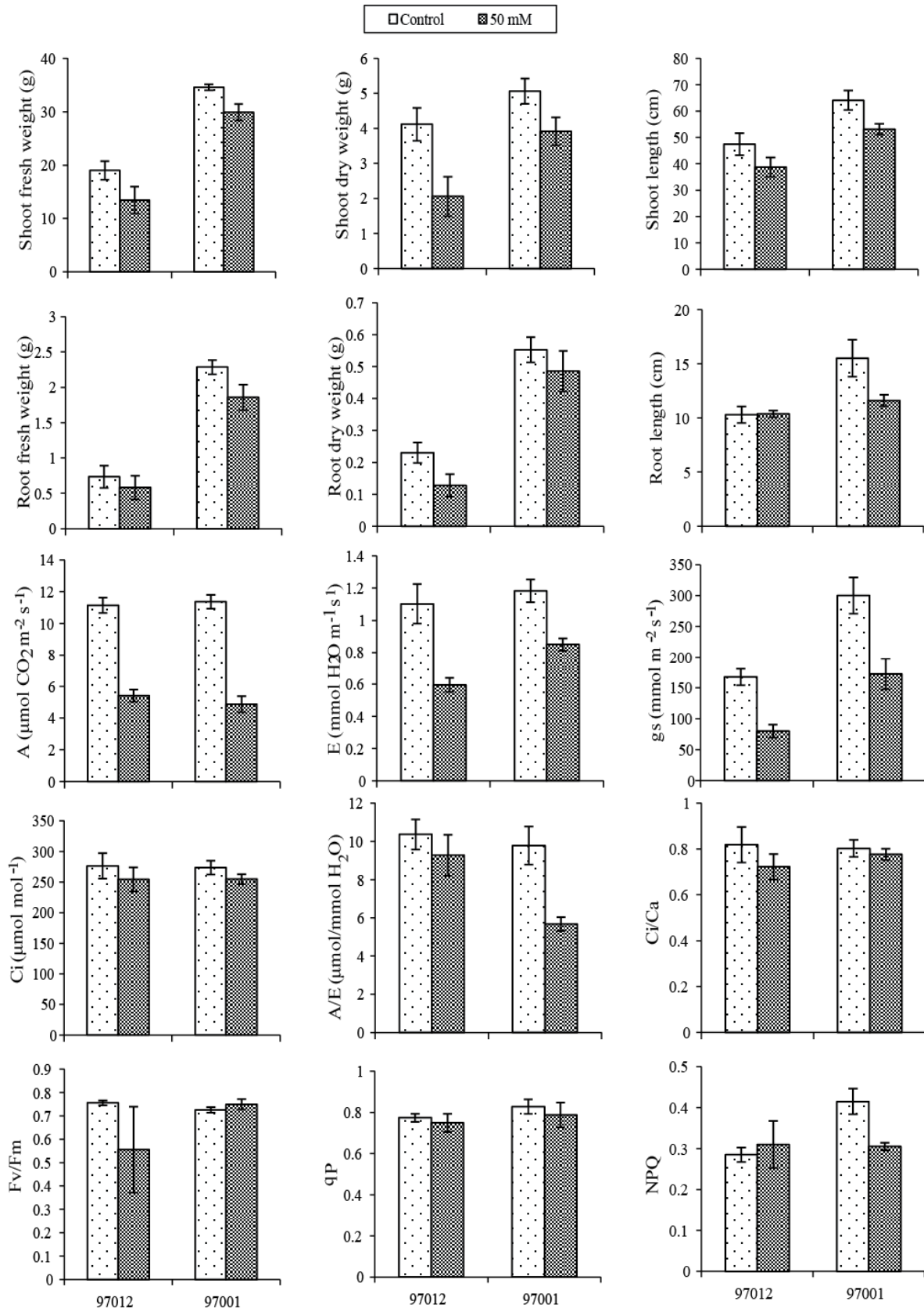


Fig. 1 Growth attributes, gas exchange and chlorophyll fluorescence in two lines of mungbean [*Vigna radiata* (L.) Wilczek] when 30 days old plants were subjected for 30 days to saline or non-saline conditions.

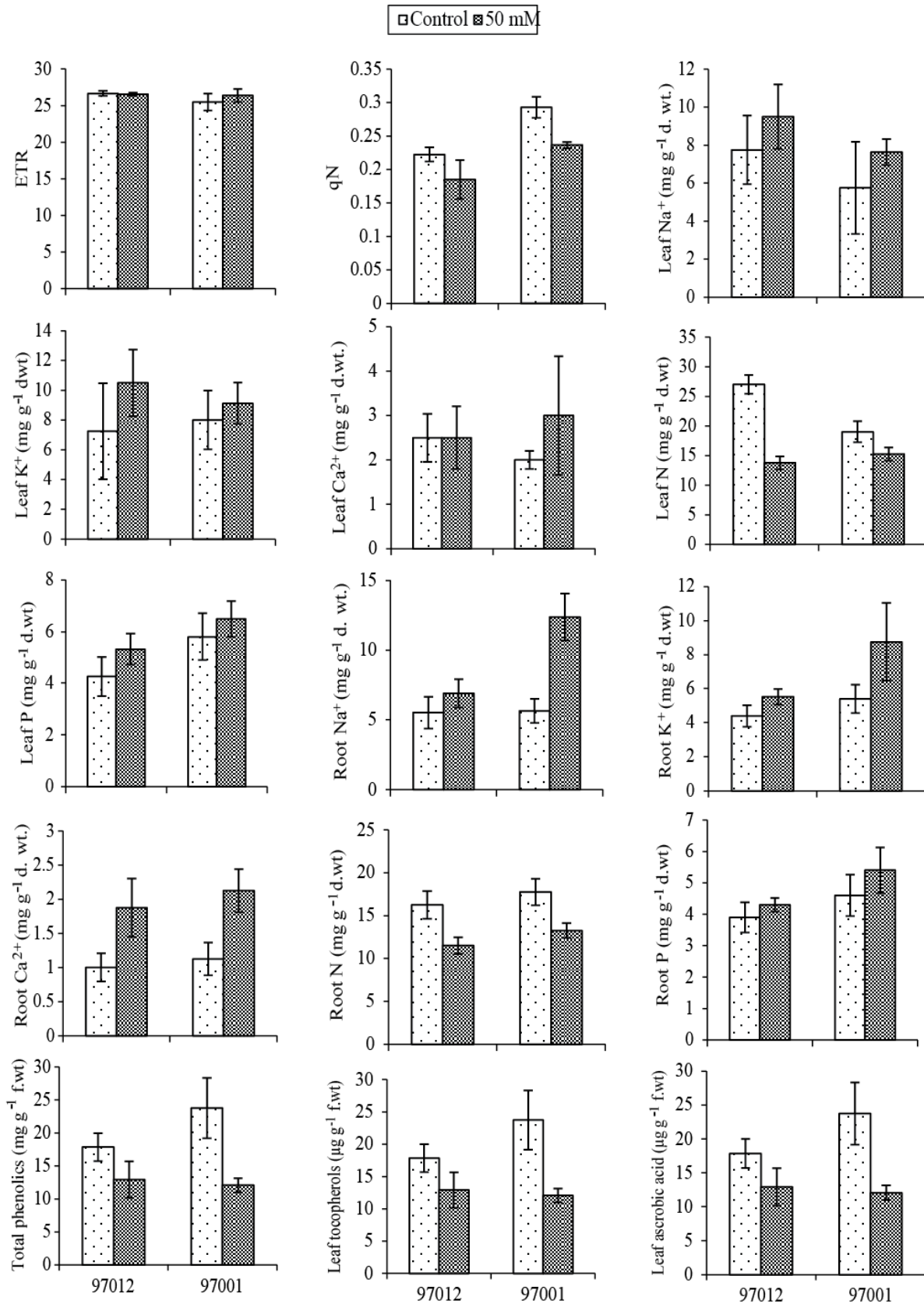


Fig. 2. Chlorophyll fluorescence, mineral nutrients and non-enzymatic antioxidants in two lines of mungbean [*Vigna radiata* (L.) Wilczek] when 30 days old plants were subjected for 30 days to saline or non-saline conditions.

**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.**

Source of variation	df	Shoot fresh weight	Shoot dry weight	Shoot length	Root fresh weight	Root dry weight
Lines (L)	1	1025.9***	7.86**	957.9***	7.98***	0.462***
Salinity (S)	1	105.16*	10.30**	388.09*	0.3364ns	0.028ns
L × S	1	0.748ns	0.83ns	5.64ns	0.076ns	0.0012ns
Error	12	12.077	0.824	48.88	0.094	0.0079
Source of variation	df	Root length	A	E	$g_s$	$C_i$
Lines (L)	1	41.60**	0.104ns	0.112*	50625***	4.86ns
Salinity (S)	1	14.82ns	150.001***	0.71***	46225***	1674.9ns
L × S	1	15.80ns	0.566ns	0.031ns	1600ns	9.59ns
Error	12	3.85	0.817	0.023	1762.5	1013.8
Source of variation	df	A/E	C <sub>i</sub> /C <sub>a</sub>	F <sub>v</sub> /F <sub>m</sub>	NPQ	q <sub>N</sub>
Lines (L)	1	17.49*	0.0014ns	0.026ns	0.015ns	0.014**
Salinity (S)	1	27.11**	0.014ns	0.031ns	0.0072ns	0.008*
L × S	1	9.11ns	0.0049ns	0.050ns	0.018ns	3.51ns
Error	12	2.89	0.011	0.034	0.005	0.0012
Source of variation	df	q <sub>p</sub>	ETR	Leaf Na <sup>+</sup>	Leaf K <sup>+</sup>	Leaf Ca <sup>2+</sup>
Lines (L)	1	0.0086ns	1.76ns	15.01ns	0.39ns	0.001ns
Salinity (S)	1	0.0042ns	0.601ns	13.14ns	19.14ns	1.0ns
L × S	1	2.402ns	1.05ns	0.015ns	4.51ns	1.0ns
Error	12	0.0071	2.33	12.47	21.28	2.62
Source of variation	df	Leaf N	Leaf P	Root Na <sup>+</sup>	Root K <sup>+</sup>	Root Ca <sup>2+</sup>
Lines (L)	1	42.25*	7.42ns	31.64*	18.06ns	0.14ns
Salinity (S)	1	289***	3.15ns	66.01**	20.25ns	3.51*
L × S	1	90.25**	0.14ns	28.89*	5.06ns	0.015ns
Error	12	8.125	2.25	5.83	6.55	0.38
Source of variation	df	Root N	Root P	Total phenolics	Leaf tocopherols	Leaf ascorbic acid
Lines (L)	1	10.56ns	3.24ns	0.307ns	14.06**	25.50ns
Salinity (S)	1	85.56**	1.44ns	0.512ns	0.302ns	275.6*
L × S	1	0.062ns	0.16ns	2.335**	1.690ns	45.56ns
Error	12	6.604	1.23	0.147	0.780	34.15

\*, \*\*, and \*\*\* = Significant at 0.05, 0.01, and 0.001 levels, respectively  
ns = Non-significant

Shoot length of both mungbean lines decreased considerably ( $p \leq 0.05$ ) due to saline regimes. There was a significant ( $p \leq 0.001$ ) 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 ( $p \leq 0.01$ ) in this growth attribute. Line 97001 performed significantly better than the other line in root length under saline regimes (Table 1; Fig. 1).

Net CO<sub>2</sub> 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).

Saline stress applied through root growing medium caused a significant ( $p \leq 0.001$ ) decrease in transpiration rate (E) of both lines; and this reduction was considerable ( $p \leq 0.05$ ) in both lines, although the former being better than the latter.

Stomatal conductance ( $g_s$ ) also decreased significantly ( $p \leq 0.001$ ) in both lines of mungbean due to salt stress. Both lines differed significantly ( $p \leq 0.001$ ) 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 ( $p \leq 0.05$ ) in this attribute, particularly, line 97012 performed slightly better than 97001 under both control and salt stress conditions (Table 1; Fig. 1). Sub-stomatal CO<sub>2</sub> concentration ( $C_i$ ) and ( $C_i/C_a$ ) 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 ( $F_v/F_m$ ), non-photochemical quenching ( $NPQ$ ), Photochemical quenching efficiency ( $q_p$ ) 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 ( $q_N$ ) 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 ( $p \leq 0.01$ ) of both lines and these lines also differed considerably ( $p \leq 0.05$ ) 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  $Ca^{2+}$  content in mungbean lines, but significantly ( $p \leq 0.05$ ) enhanced root  $Ca^{2+}$  content in them. However, both lines showed a uniform behavior in both these attributes (Table 1; Fig. 2).

Saline stress caused a significant ( $p \leq 0.001$ ) 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 ( $p \leq 0.01$ ) 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 lined 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  $CO_2$  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  $CO_2$  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 ( $g_s$ ) and water use efficiency (WUE) (Chartzoulakies *et al.*, 2002). Loss in turgidity of guard cells may cause stomatal closure which could be responsible for low availability of  $CO_2$  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  $F_v/F_m$ ,  $NPQ$ ,  $qP$  and  $ETR$  while reduced only  $qN$ . It is already reported that maximum quantum yield of PSII ( $F_v/F_m$ ) and  $qN$  are related to rate of photosynthesis and therefore  $qN$ ,  $qP$  and  $F_v/F_m$  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  $\text{Na}^+$  contents in both mungbean lines. This increase in leaf or root  $\text{Na}^+$  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  $\text{Na}^+$  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  $\text{K}^+$  and P contents. In contrast to our findings, Dar *et al.*, (2007) observed decrease in shoot and root  $\text{K}^+$  mungbean due to imposition of salt stress. Shahbaz *et al.*, (2011) also observed a considerable decrease in leaf and root  $\text{K}^+$  content in sunflower and according to El-bassiouny & Bekheta (2005),  $\text{Na}^+$  ion accumulation leads to reduced  $\text{K}^+$  ion absorption in plants. However, non-significant effect of salinity on  $\text{K}^+$  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  $\text{Ca}^{2+}$ , while increased root  $\text{Ca}^{2+}$  contents. Contrary to these findings it has been reported by some researchers that salinity causes a significant reduction in  $\text{Ca}^{2+}$  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 alpha-tocopherol 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  $\text{CO}_2$  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  $\text{Na}^+$ , decreased leaf and root N, and did not alter leaf and root  $\text{K}^+$  and P. Overall, line 97001 performed better than line 97012 in most of the attributes appraised in this study under saline stress.

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### References

- Abbas, W., M. Ashraf and N.A. Akram. 2010. Alleviation of salt-induced adverse effects in eggplant (*Solanum melongena* L.) by foliar-applied natural and synthetic glycinebetaine. *Sci. Hortic.*, 125: 188-195.
- 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.
- Ahmed, S. 2009. Effect of soil salinity on the yield and yield components of mungbean. *Pak. J. Bot.*, 41: 263-268.
- Akram, M.S., M. Ashraf, M. Shahbaz and N.A. Akram. 2009. Growth and photosynthesis of salt-stressed sunflower (*Helianthus annuus*) plants as affected by foliar-applied different potassium salts. *J. Plant Nutr. Soil Sci.*, 172: 884-893.
- Akram, N.A., M. Shahbaz and M. Ashraf. 2007. Relationship of photosynthetic capacity and proline accumulation with the growth of differently adapted populations of two potential grasses (*Cynodon dactylon* (L.) Pers. and *Cenchrus ciliaris* L.) to drought stress. *Pak. J. Bot.*, 39: 777-786.
- Anjum, M.S., Z.I. Ahmed and C.A. Rauf. 2006. Effect of rhizobium inoculation and nitrogen fertilizer on yield and yield Components of mungbean. *Int. J. Agric. Biol.*, 8: 238-240.
- Anonymous. 2000. Statistical yearbook of Bangladesh. Bur. Stat. Div. Min. Plan. Govt. People's Repub. Bangladesh, Dhaka. pp. 165.
- Anonymous. 2001. Statistical yearbook of Bangladesh. Bur. Stat. Div. Min. Plan. Govt. People's Repub. Bangladesh, Dhaka. pp. 372.
- Anwar, F., S.N. Zafar and U. Rashid. 2006. Characterization of *Moringa oleifera* seed oil from drought and irrigated regions of Punjab, Pakistan. *Grasas Aceites*, 57: 160-168.
- Ashraf, M. 2004. Some important physiological selection criteria for salt tolerance in plants. *Flora*, 199: 361-376.
- Ashraf, M. 2009. Biotechnological approach of improving plant salt tolerance using antioxidants as markers. *Biotechnol. Adv.*, 27: 84-93.
- Ashraf, M. and A. Khanum. 1997. Relationship between ion accumulation and growth in two spring wheat lines differing in salt tolerance at different growth stages. *J. Agron. Crop Sci.*, 178: 39-51.
- Ashraf, M. and S. Ahmad. 2000. Influence of sodium chloride on ion accumulation, yield components and fibre characteristics in salt-tolerant and salt-sensitive lines of cotton (*Gossypium hirsutum* L.). *Field Crops Res.*, 66: 115-127.
- Ashraf, M.A., M. Ashraf and M. Shahbaz. 2012. Growth stage-based modulation in antioxidant defense system and proline accumulation in two hexaploid wheat (*Triticum aestivum* L.) cultivars differing in salinity tolerance. *Flora*, 207: 388-397.

- Ashraf, M.A., M. Ashraf and Q. Ali. 2010. Response of two genetically diverse wheat cultivars to salt stress at different growth stages: leaf lipid peroxidation and phenolic contents. *Pak. J. Bot.*, 42: 559-565.
- Baker, H., O. Frank, B. De Angelis and S. Feingold. 1980. Plasma tocopherol in man at various times after ingesting free or acetylated tocopherol. *Nutr. Rep. Int.*, 21: 531-536.
- Bano, S., M. Ashraf, N.A. Akram and F. Al-Qurainy. 2012. Regulation in some vital physiological attributes and antioxidative defense system in carrot (*Daucus carota* L.) under saline stress. *J. Appl. Bot. Food Qual.*, 85: 105-115.
- Batool, A., M. Ashraf, N.A. Akram and F. Al-Qurainy. 2013. Salt-induced changes in growth, some key physio-biochemical attributes, activities of enzymatic and levels of non-enzymatic antioxidants in cauliflower (*Brassica oleracea* L.). *J. Hortic. Sci. Biotechnol.*, 88(2): 231-241.
- Bremner, J.M. 1965. Total nitrogen. In: *Methods of Soil Analysis*. Part 2. C.A. Black (Ed.), *Agronomy* 9: 1238-1255. American Society of Agronomy, Madison, Wisconsin, U.S.A.
- Chakrabarti, N. and S. Mukherji. 2003. Effect of Phytohormone pretreatment on nitrogen metabolism in Vigna radiata under salt stress. *Biol. Plant.*, 46: 63-66.
- Chartzoulakis, K., M. Loupassaki, M. Bertaki and I. Androulakis. 2002. Effect of NaCl salinity on growth, ion content and CO<sub>2</sub> assimilation rate of six olive cultivars. *Sci. Hortic.*, 96: 235-247.
- Cha-Um, S. and C. Kirdmance. 2009. Effect of salt stress on proline accumulation, photosynthetic ability and growth characters in two maize cultivars. *Pak. J. Bot.*, 41: 87-98.
- Chedlia, B.A., B.R. Bechi and M. Boukhris. 2007. Effect of water deficit on olive trees cv. Chemlali under field conditions in arid region in Tunisia. *Sci. Hortic.*, 113: 267-277.
- Dar, Z.M., A. Hemantaranjan and S.K. Panday. 2007. Antioxidative response of mungbean (*Vigna radiata* L.) to salt stress. *Legume Res.*, 30: 57-60.
- Dubey, R.S. 2005. Photosynthesis in plants under stressful conditions. In: *Hand book photosynthesis*, (Ed.): M. Pessaraki. 2nd edn. C.R.C., Press, New York, USA, pp. 717-718.
- El-Bassiouny, H.M.S. and M.A. Bekheta. 2005. Effect of salt stress on relative water content, lipid peroxidation, polyamines, amino acids and ethylene of two wheat cultivars. *Int. J. Agric. Biol.*, 7: 363-368.
- Falleh, H., R. Ksouri, K. Chaieb, N. Karray-Bouraoui, N. Trabelsi, M. Boulaaba and C. Abdelly. 2008. Phenolic composition of *Cynara cardunculus* L. organs, and their biological activities. *Comp. Rend. Biol.*, 331: 372-379.
- Fisarakis, I., K. Chartzoulakis and D. Stavrakas. 2001. Response of sultanavines (*V. vinifera* L.) on six rootstocks to NaCl salinity exposure and recovery. *Agric. Water Manage.*, 51: 13-27.
- Gao, J.P., C. Dai-Yin and H.X. Lin. 2007. Understanding Abiotic stress tolerance mechanisms: Recent studies on stress response in rice. *J. Integrate. Plant Biol.*, 49: 742-750.
- Haleem, A. and M.A. Mohammed. 2007. Physiological aspects of mungbean plant (*Vigna radiata* L. wilczek) in response to salt stress and gibberellic acid treatment. *Res. J. Agric. Biol. Sci.*, 3: 200-213.
- Hatami, E., M. Esna-Ashari and T. Javadi. 2010. Effect of salinity on some gas exchange characteristics of grape (*Vitis vinifera*) cultivars. *Int. J. Agric. Biol.*, 12: 308-310.
- Jackson, M.L. 1962. Soil Chemical Analysis. Constable and Co. Ltd., England.
- Julkenen-Titto, R. 1985. Phenolics constituents in the leaves of northern willows: methods for the analysis of certain phenolics. *Agric. Food Chem.*, 33: 213-217
- Kabir, M.E., M.A. Karim and M.A.K. Azad. 2004. Effect of potassium on salinity tolerance of mungbean (*Vigna radiata* L. Wilczek). *J. Biol. Sci.*, 4: 103-110.
- Kanwal, H., M. Ashraf and M. Shahbaz. 2011. Assessment of salt tolerance of some newly developed and candidate wheat (*Triticum aestivum* L.) cultivars using gas exchange and chlorophyll fluorescence attributes. *Pak. J. Bot.*, 43: 2693-2699.
- Kausar, F. and M. Shahbaz. 2013. Interactive effect of foliar application of nitric oxide (NO) and salinity on wheat (*Triticum aestivum* L.). *Pak. J. Bot.*, 45: 67-73.
- Koyro, H.W. 2006. Effect of salinity on growth, photosynthesis, water relations and solute composition of the potential cash crop halophyte *Plantago coronopus* (L.). *Environ. Exp. Bot.*, 56: 136-146.
- Mittova, V., M. Tal, M. Volokita and M. Guy. 2002. Salt stress induces upregulation of an efficient chloroplast antioxidant system in the salt-tolerant wild tomato species *Lycopersicon pennellii* but not in the cultivated species. *Physiol. Plant.*, 115: 393-400.
- Morant-Manceau, A., E. Pradier and G. Tremblin. 2004. Osmotic adjustment, gas exchange and chlorophyll fluorescence of a hexaploid triticale and its parental species under salt stress. *J. Plant Physiol.*, 161: 25-33.
- Mukherjee, S.P. and M.A. Choudhri. 1983. Implications of water stress-induced changes in the leaves of indigenous ascorbic acid and hydrogen peroxide in *Vigna* seedlings. *Physiol. Plant.*, 58: 166-170.
- Munns, R. and Tester, M. 2008. Mechanism of salinity tolerance. *Annu. Rev. Plant Biol.*, 59: 651-681.
- Naheed, G., M. Shahbaz, A. Latif and E.S. Rha. 2007. Alleviation of the adverse effects of salt stress on rice (*Oryza sativa* L.) by phosphorus applied through rooting medium: growth and gas exchange characteristics. *Pak. J. Bot.*, 39: 729-737.
- Naheed, G., M. Shahbaz, N.A. Akram and M. Ashraf. 2008. Interactive effect of rooting medium application of phosphorus and NaCl on plant biomass and mineral nutrients of rice (*Oryza sativa* L.). *Pak. J. Bot.*, 40: 1601-1608.
- Noreen, S. and M. Ashraf. 2010. Modulation in salt (NaCl)-induced effects on oil-composition and fatty-acid profile of sunflower (*Helianthus annuus* L.) by exogenous application of salicylic acid. *J. Sci. Food Agric.*, 90: 2608-2616.
- Parida, A.K., A.B. Das and B. Mittra. 2004. Effects of salt on growth, ion accumulation, photosynthesis and leaf anatomy of the mangrove, *Bruguiera parviflora*. *Trees Struct. Funct.*, 18: 167-174.
- Pereira, W.E., D.L. De-Siqueira, C.A. Martinez and M. Puiatti. 2000. Gas exchange and chlorophyll fluorescence in four citrus rootstocks under aluminum stress. *J. Plant Physiol.*, 157: 513-520.
- Perveen, S., M. Shahbaz and M. Ashraf. 2010. Regulation in gas exchange and quantum yield of photosystem II (PSII) in salt-stressed and non-stressed wheat plants raised from seed treated with triacontanol. *Pak. J. Bot.*, 42: 3073-3081.
- Perveen, S., M. Shahbaz and M. Ashraf. 2012. Changes in mineral composition, uptake and use efficiency of salt stressed wheat (*Triticum aestivum* L.) plants raised from seed treated with triacontanol. *Pak. J. Bot.*, 44: 27-35.
- Promila, K. and S. Kumar. 2000. *Vigna radiata* seed germination under salinity. *Biol. Plant.*, 43: 423-426.
- Rabie, G.H. 2005. Influence of arbuscular mycorrhizal fungi and kinetin on the response mungbean plants to irrigation with seawater. *Mycorrhiza*, 15: 225-230.
- Raptan, P.K., A. Hamid, Q.A. Khaliq, A.R.M. Solaiman, J.U. Ahmed and M.A. Karim. 2001. Salinity tolerance of blackgram and mungbean: II- Mineral ions accumulation in different plant parts. *Korean J. Crop Sci.*, 46: 387-394.



- Rashid, P., J.L. Karmoker, S. Chakraborty and B.C. Sarker. 2004. The effect of salinity on ion accumulation and anatomical attributes in mungbean (*Phaseolus radiata* L. cv. BARI-3) seedlings. *Int. J. Agric. Boil.*, 6: 495-498.
- Shahbaz, M. and B. Zia. 2011. Does exogenous application of glycinebetaine through rooting medium alter rice (*Oryza sativa* L.) mineral nutrient status under saline conditions? *J. Appl. Bot. Food Qual.*, 84: 54-60.
- Shahbaz, M. and M. Ashraf. 2007. Influence of exogenous application of brassinosteroid on growth and mineral nutrients of wheat (*Triticum aestivum* L.) under saline conditions. *Pak. J. Bot.*, 39: 513-522.
- Shahbaz, M. and M. Ashraf. 2013. Improving salinity tolerance in cereals. *Crit. Rev. Plant Sci.*, 32: 237-249.
- Shahbaz, M., M. Ashraf and H.R. Athar. 2008. Does exogenous application of 24-epibrassinolide ameliorate salt induced growth inhibition in wheat (*Triticum aestivum* L.)? *Plant Growth Regul.*, 55: 51-64.
- Shahbaz, M., M. Ashraf, F. Al-Qurainy and P.J.C. Harris. 2012. Salt tolerance in selected vegetable crops. *Crit. Rev. Plant Sci.*, 31: 303-320.
- Shahbaz, M., M. Ashraf, N.A. Akram, A. Hanif, S. Hameed, S. Joham and R. Rehman. 2011. Salt-induced modulation in growth, photosynthetic capacity, proline content and ion accumulation in sunflower (*Helianthus annuus* L.). *Acta Physiol. Plant.*, 33: 1113-1122.
- Shahbaz, M., N. Noreen and S. Perveen. 2013. Triacetonol modulates photosynthesis and osmoprotectants in canola (*Brassica napus* L.) under saline stress. *J. Plant Interac.* DOI:10.1080/17429145.2013.764469.
- Shaheen, H.L., M. Shahbaz, I. Ullah and M.Z. Iqbal. 2012. Morpho-physiological responses of cotton (*Gossypium hirsutum* L.) to salt stress. *Int. J. Agric. Biol.*, 14: 980-984.
- Shaheen, S., S. Naseer, M. Ashraf and N.A. Akram. 2012. Salt stress affects water relations, photosynthesis and oxidative defense mechanisms in *Solanum melongena* L. *J. Plant Interac.*, 8: 85-96.
- Siddiqi, E.H., M. Ashraf, F. Al-Qurainy and N.A. Akram. 2011. Salt-induced modulation in inorganic nutrients, antioxidant enzymes, proline content and seed oil composition in safflower (*Carthamus tinctorius* L.). *J. Sci. Food Agric.*, 91: 2785-2793.
- Snedecor, G.W. and W.G. Cochran. 1980. Statistical methods. 7<sup>th</sup> Edition Iowa State University Press, Ames, Iowa.
- Stepien, P. and G. Klobus. 2006. Water relations and photosynthesis in *Cucumis sativus* L. leaves under salt stress. *Biol. Plant.*, 50: 610-616.
- Stirbet, A. and Govindjee. 2011. On the relation between the kautsky effect (chlorophyll a fluorescence induction) and Photosystem II: basics and applications. *J. Photochem. Photobiol. B: Biol.*, 104: 236-257.
- Strasser, R.J., A. Srivastava and Govindjee. 1995. Polyphasic chlorophyll a fluorescence transients in plants and cyanobacteria. *Photochem. Photobiol.*, 61: 32-42.
- Thomas, M.J. Robertson, S. Fukai and M.B. Peoples. 2004. The effect of timing and severity of water deficit on growth, development, yield accumulation and nitrogen fixation of mungbean. *Field Crops Res.*, 86: 67-80.
- Turan, M.A., A.H.A. Elkarim, N. Taban and S. Taban. 2010. Effect of salt stress on growth and ion distribution and accumulation in shoot and root of maize plant. *Afr. J. Agric. Res.*, 5: 584-588.
- Turan, M.A., N. Turkmen and N. Taban. 2007. Effect of NaCl on stomatal resistance and proline, chlorophyll, Na, Cl and K concentrations of lentil plants. *J. Agron.*, 6: 378-381.
- Wolf, B. 1982. A comprehensive system of leaf analysis and its use for diagnosing crop nutrient status. *Commun. Soil Sci. Plant Anal.*, 13: 1035-1059.
- Yasar, F., S. Ellialtioglu and K. Yildiz. 2008. Effect of salt stress on antioxidant defence systems, lipid peroxidation, and chlorophyll content in green bean. *Russ. J. Plant Physiol.*, 55: 782-786.

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