



Full Length Article

Morpho-physiological Responses of Cotton (*Gossypium hirsutum*) to Salt Stress

HUMA LUBNA SHAHEEN, MUHAMMAD SHAHBAZ^{1†}, IHSAN ULLAH[‡] AND MUHAMMAD ZAFFAR IQBAL[‡]

Department of Botany, Government College University, Faisalabad, Pakistan

[†]Department of Botany, University of Agriculture, Faisalabad, Pakistan

[‡]Agricultural Biotechnology Research Institute, Faisalabad, Pakistan

¹Corresponding author's e-mail: shahbazmuaf@yahoo.com

ABSTRACT

Soil salinity is a big threat for crop productivity. To assess the response of cotton (*Gossypium hirsutum* L.) to salinity stress, 10 cotton genotypes (CIM-446, CIM-506, RH-510, MNH-770, FH-2007, BH-118, FH-87, CIM-496, FH-901 & CIM-473) were grown in full strength Hoagland's nutrients solutions containing 50, 100 and 200 mM NaCl. Root-medium applied salt stress significantly reduced plant height, fresh and dry weights, and gas exchange characteristics of all cotton genotypes. This reduction was the maximum at highest level of salt stress viz. 200 mM NaCl as compared to lower i.e., 50 or 100 mM. A considerable variation in salt tolerance was observed among all ten cultivars with respect to above mentioned morphological and physiological attributes. Overall on the basis of better growth performance under saline conditions, cultivars RH-510, BH-118 and FH-87 were ranked as relatively salt tolerant; whereas CIM-446 and CIM-506 salt sensitive, while others were moderately salt sensitive. Shoot dry mass and photosynthetic rate can be used as markers of salt tolerance in cotton. © 2012 Friends Science Publishers

Key Words: Salinity; Cotton; Gas exchange; Growth; Photosystem II efficiency

INTRODUCTION

Soil salinity is the one of the most serious threats to agriculture (Munns & Tester, 2008), because it adversely affects crop growth and productivity (Afzal *et al.*, 2006; Jafar *et al.*, 2012). Soil salinity causes decrease in osmotic potential of the soil solution (Munns, 2002), deteriorates soil physical structure and increases the concentration of certain ions (Akram *et al.*, 2009).

Salinity alters almost every aspect of crops including their morphological, physiological and biochemical aspects. In addition, salinity markedly reduces water potential in cells leading to closure of stomata and limits net CO₂ assimilation rate (Munns *et al.*, 2006). Electron transport is a primary phenomenon of mechanism of photosynthesis, comprises a variety of components, the most distinctive ones are photosystem I and II. Several reports indicated that activity of photosystem II (PSII) is not significantly altered by salt stress in crops including cowpea (*Vigna unguiculata* L.), sorghum (*Sorghum bicolor* L.), barley (*Hordeum vulgare* L.) and wheat (*Triticum aestivum* L.) (Munns, 2002; Shahbaz *et al.*, 2008). However, together with the varietal difference plants response depends on salt stress intensity and duration.

Cotton is categorized as moderately salt tolerant crop (Ashraf, 2002). Salinity adversely affects cotton growth.

However, the response of the cotton plant is variable at various growth stages. For instance cultivars tolerant to salinity at seedling stage were not tolerant during later stages; however most of the cultivars show a considerable variation under saline condition during the whole life cycle (Ashraf, 2002).

Salinity tolerance is a complex phenomenon that is influenced by a wide range of morpho-physiological traits and multitude genes expression, which have some relationship with productivity under saline conditions. Development of crop plants for salinity tolerance having good combination of morpho-physiological and biochemical markers may help breeders to improve and sustain yields especially in salt prone areas. As there is considerable variation for salt tolerance exists in cotton germplasm but there is dire need to explore them for salt tolerance. Keeping in view the differential potential in cotton genotypes this study was conducted to evaluate the variation in salinity tolerance potential in cotton genotypes based on the morpho-physiological markers like growth and gas exchange characteristics.

MATERIALS AND METHODS

To assess salinity induced adverse effects and genetic variation in cotton an experiment was conducted in the

Botanical Garden of the University of Agriculture, Faisalabad. During the entire period of experimentation, the average day light (12 h), light intensity ($8.9 \mu\text{mol m}^{-2} \text{s}^{-1}$), relative humidity (32.2%) and temperature from 26.7 to 37.9°C were recorded. Ten cotton cultivars (CIM-446, CIM-506, RH-510, MNH-770, FH-2007, BH-118, FH-87, CIM-496, FH-901 & CIM-473) were obtained from the Cotton Research Institute, Ayub Agricultural Research Institute, Faisalabad, Pakistan. Seeds were delinted using concentrated H_2SO_4 and then washed thoroughly with distilled water to make them acid free. Seeds (10 seeds per pot) were allowed to germinate in plastic pots (29 cm height & 23.5 cm diameter) containing sand as the rooting medium. After 10 days of seed sowing, four salinity treatments [control (non-saline), 50, 100, and 200 mM NaCl] were applied in full strength Hoagland's nutrient solution. For attaining the required level of salinity, an aliquot of 50 mM NaCl was applied every day up to all desired levels. All salinity levels were applied in Hoagland's nutrient solution once a week throughout the experiment. Data for growth attributes, chlorophyll pigments, gas exchange characteristics and photosystem-II activity were determined at vegetative stage after 45 days after salinity treatment. Two plants per replicate were uprooted carefully, thoroughly washed with distilled water and their mean fresh biomass recorded 45 days after the initiation of salt treatments. Plants were oven-dried at 65°C up to their constant weight.

Relative water contents (RWC): Relative water contents were determined using the method of Jones and Turner (1978). Leaves were sampled, and after recording their fresh weight (Fw), they were soaked in de-ionized water in the dark for 24 h to re-hydrate. Leaves were wiped with tissue paper and measured their turgid weight (Tw). After that, the samples were oven dried at 65°C for 72 h and determined their dry weight (Dw). Relative water contents were calculated using the following formula:

$$\text{RWC} = [(\text{Fw} - \text{Tw}) / (\text{Fw} - \text{Dw})] \times 100.$$

Chlorophyll fluorescence: Data for chlorophyll fluorescence were recorded with a Plant Efficiency Analyzer (PEA, Handstech Instrument Ltd., King's Lynn, UK), according to Strasser *et al.* (1995). The fluorescence transients were induced by red light and catered by an array of six light inducing rays (peaks 650 nm), giving the homogenous light over the exposed area when focused on the sample surface. All samples were kept in dark for 30 min before fluorescence measurements.

Gas exchange characteristics: Gas exchange characteristics as photosynthetic capacity (A), leaf internal CO_2 concentration (C_i), transpiration rate (E), stomatal conductance (g_s) and water use efficiency (A/E) were measured using a portable infrared gas analyzer (ACD LCA-4 Analytical Development, Hoddesdon, UK). Fully expanded young intact leaf from the top of each plant was used to determine these parameters. Measurements were

made from 11:00 to 12:30 h in full day light 45 days after initiation of salinity treatment. Following specifications were at the time of data recording: leaf chamber gas flow rate (U) $251 \mu\text{mol s}^{-1}$; ambient pressure 99.8 kPa; leaf surface area 11.25 cm^2 ; water vapor pressure ranged from 7.0 to 8.9 mbar into the leaf chamber, concentration of ambient CO_2 was $351 \mu\text{mol mol}^{-1}$; leaf chamber temperature varied from 34.2 to 39.3°C; molar flow of air per unit leaf area (U_s) $403.4 \text{ mol m}^{-2} \text{s}^{-1}$; RH of the chamber 41.2%; PAR at the leaf surface was up to $1099 \mu\text{mol m}^{-2} \text{s}^{-1}$.

Statistical analysis of data: Experiment was conducted in completely randomized design with three replications. A two-way analysis of variance (ANOVA) was calculated for each variable using a COSTAT computer package (Cohort Software, Berkeley, CA). To compare the mean values, least significance difference test was applied according to Snedecor and Cochran (1980).

RESULTS

Data for shoot fresh and dry weights showed that root medium applied salinity markedly reduced both these growth attributes in all 10 cultivars (Table I; Fig. 1a & b). However, the response of cultivars was also variable under saline or non-saline conditions. Of various salt levels, 200 mM NaCl caused a maximum reduction in biomass production in all cultivars. Among various cultivars, BH-118 and FH-87 were higher and CIM-446 and CIM-506 lower in shoot fresh and dry biomass than the other cultivars (Fig. 1a & b).

Root fresh and dry mass of ten cultivars significantly reduced under saline conditions. Variation among various cultivars was also prominent, showing a tremendous genetic diversity in all cultivars (Table I; Fig. 1c & d). Cultivars BH-118 and FH-87 were higher in root fresh and dry biomass as compared to the other cultivars. Maximum salt-induced reduction in root fresh and dry weights was observed in CIM-506.

Reduction in shoot length was prominent in all cotton cultivars due to saline stress (Table I; Fig. 1e). The cultivars varied significantly in shoot length, while such variation in cultivars was not prominent with respect to root length (Table I; Fig. 2f). Overall, FH-87 was the highest of all cotton cultivars in shoot length.

Root medium salinity slightly reduced the relative water content of cotton cultivars, but the cultivars did not differ significantly in this attribute (Table I; Fig. 2a). Data recorded for quantum yield of PSII (F_v/F_m) in the cotton cultivars showed that salt stress significantly reduced F_v/F_m . However, cultivars variation in quantum yield of PSII was not prominent under both non-stressed and salt-stressed conditions (Table I; Fig. 2b).

Root-medium applied NaCl significantly reduced net CO_2 assimilation and transpiration rates in all cotton cultivars. This reduction was highest at the highest salt level i.e., 200 mM. Of 10 cotton cultivars, BH-118 and FH-87

Table I: Growth attributes, RWC, activity of photosystem II and gas exchange characteristics of salt-stressed and non-stressed cotton plants when grown under different salt levels

Source of variation	df	Shoot FW	Shoot FW	Root FW	Root DW	Shoot length
Cultivars (Cvs)	9	130.2***	5.600***	0.800***	0.090***	965.4***
Salinity (S)	3	452.6***	28.30***	6.800***	1.300***	6932***
Cvs × S	27	15.70**	1.500***	0.200ns	0.020ns	130.1ns
Error	80	6.900	0.600	0.200	0.020	86.60
Source of variation	df	Root length	RWC	Fv/Fm	A	E
Cultivars (Cvs)	9	10.70ns	208.6***	0.005ns	17.80***	0.290***
Salinity (S)	3	177.5***	646.9***	0.049***	124.1***	2.980***
Cvs × S	27	7.800ns	17.09ns	0.004ns	2.160ns	0.074ns
Error	80	7.500	28.88	0.004	1.550	0.066
Source of variation	df	g_s	C_i	C_i/C_a	A/E	
Cultivars (Cvs)	9	6442.6**	1338***	0.017***	4.220ns	
Salinity (S)	3	145694***	10342***	0.075***	9.120*	
Cvs × S	27	4581.2*	342.6ns	0.004ns	4.240ns	
Error	80	2386.4	226.03	0.003	2.680	

*' **' *** = significant at 0.05, 0.01, and 0.001 levels respectively; ns = non-significant; df = degrees of freedom; RWC= relative water contents; Fv/Fm = maximal quantum yield of PSII; FW = Fresh weight; DW = Dry weight; A = net CO₂ assimilation rate; E = transpiration rate; g_s = Stomatal conductance; C_i = sub-stomatal CO₂ concentration; C_i/C_a = Relative sub-stomatal CO₂ concentration

were higher, while CIM-446 and FH-901 low in both gas exchange attributes as compared to other cultivars examined (Table I; Fig. 3a & b).

Salt stress markedly decreased the stomatal conductance of all cotton cultivars. The salt level 200 mM NaCl was more lethal in all cultivars except in BH-118 where reduction at 200 mM was almost similar to that of 100 mM NaCl (Table I; Fig. 3c).

Sub-stomatal CO₂ concentration and C_i/C_a ratio slightly reduced in all cultivars under saline conditions (Table I; Fig. 3d & e). Cultivars RH-510, BH-118 and FH-2007 were better in both attribute under both saline and non-saline conditions than the other cultivars.

The response of the cotton cultivars towards water use efficiency was not consistent under non-stressed or salt stressed conditions. Cultivars CIM-446, CIM-496, and CIM-473 showed higher water use efficiency at 200 mM NaCl level, while CIM-506 and RH-510 lower in water use efficiency at this level (Table I; Fig. 3f). Overall, the cultivars did not differ significantly in water use efficiency.

DISCUSSION

Growth of all cotton genotypes reduced considerably with increase (50 to 200 mM NaCl) in saline regimes, and reduction was maximum at 200 mM level. The results are in agreement to what has been already reported in different crops like wheat (Ashraf *et al.*, 2012), rice (Shahabaz & Zia, 2011), sunflower (Shahbaz *et al.*, 2011), eggplant (Abbas *et al.*, 2010). In order to improve salinity tolerance in crops, inter-cultivar/genotype/line variation should be observed to select the potential cultivars/lines/genotypes for salt affected areas (Sabir *et al.*, 2009). Differential behavior in salinity tolerance was observed in cotton to various saline conditions i.e. 50, 100 and 200 mM. Variation in growth in cotton cultivars might be associated with variation in other physiological attributes like photosynthetic rate, water use efficiency etc. Differential growth responses of cotton

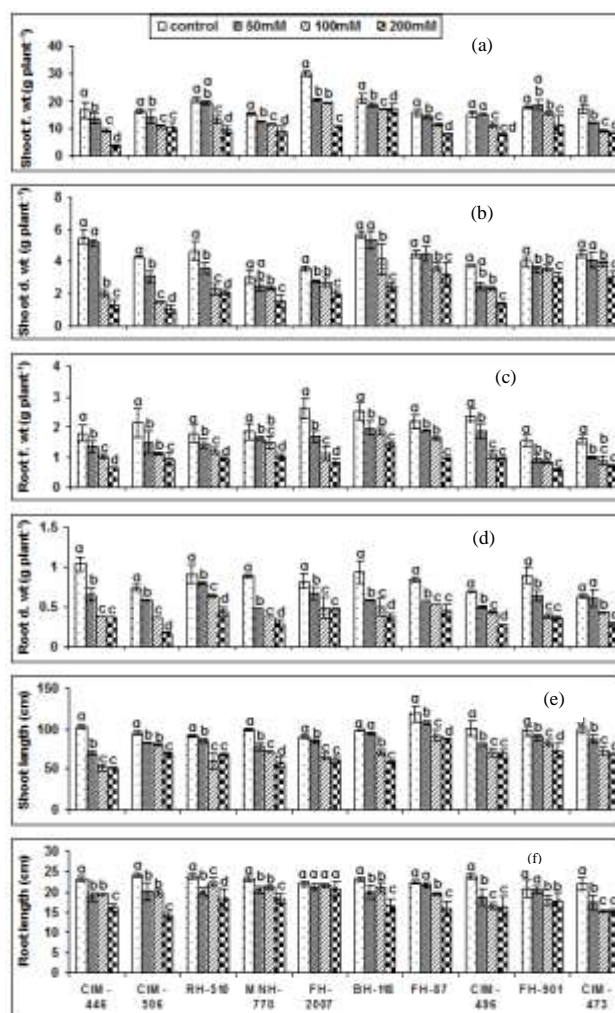
Fig. 1: Influence of salt stress on (a) shoot fresh weight, (b) shoot dry weight, (c) root fresh weight, (d) root dry weight, (e) shoot length and (f) root length of cotton (*Gossypium hirsutum* L.) genotypes

Fig. 2: Influence of salt stress on (a) relative water contents and (b) activity of photosystem II of cotton (*Gossypium hirsutum* L.) genotypes

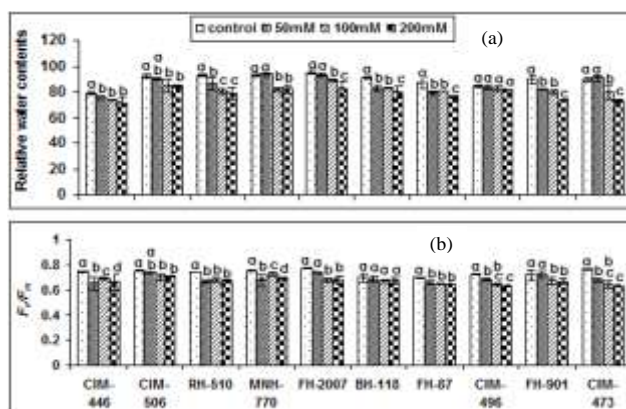
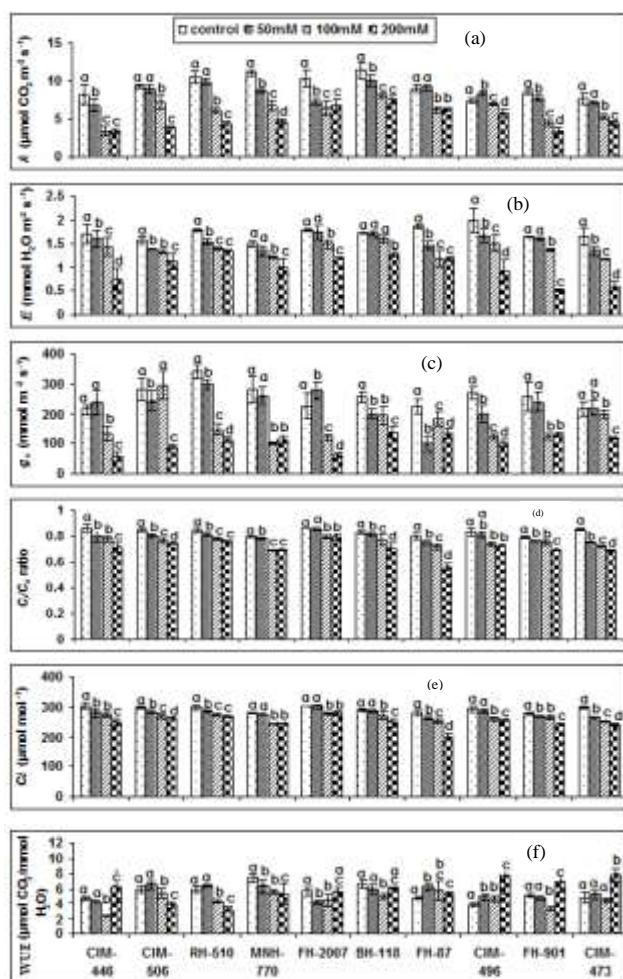


Fig. 3: Influence of salt stress on (a) net CO₂ assimilation rate (A), (b) transpiration rate (E), (c) stomatal conductance (g_s), (d) relative sub-stomatal CO₂ concentration (C_i/C_a), (e) sub-stomatal CO₂ concentration (C_i) and (f) water use efficiency of cotton (*Gossypium hirsutum* L.) genotypes



genotypes might have been due to their differential genetic behavior in absorption of water and change in biochemical mechanisms under saline conditions (Ziaf *et al.*, 2009).

In many studies positive correlation between growth and photosynthetic rate has been observed and reduction in growth in most of the crops under saline conditions is attributed to be due to decline in photosynthetic rate (Sabir *et al.*, 2009; Shahbaz *et al.*, 2011). However, a significant salt-induced reduction in photosynthetic rate in current study might be due to deficiency of photoassimilates necessary for plant growth and imbalance in water contents. One of the reasons for inter-genotypes might be due to variation in photosynthetic efficiency. Such type of relationship has already observed in various crops like sunflower (Shahbaz *et al.*, 2011), canola (Ulfat *et al.*, 2007), wheat (Kanwal *et al.*, 2011) etc. Generally, it is believed that stomatal regulation and non-stomatal mechanisms are the major cause of decline in photosynthetic rate and then in plant biomass also.

In photosynthesis, photosystem II (PSII) plays a key role in electron transport (Baker, 1991), and its function is considered as an important tool for discriminating species/cultivars for stress tolerance (Cha-um *et al.*, 2010; Mehta *et al.*, 2010). The reduction in the actual quantum yield of PSII had been associated with both the efficiency of excitation energy capture and photochemical quenching (Tavakkoli *et al.*, 2011). Furthermore, regeneration of RuBP could have been interrupted by salt-induced decrease in *Fv/Fm* (Kafi, 2009). Salinity effects on chlorophyll fluorescence (*Fv/Fm*) have been investigated in barley, rice (*Oryza sativa*), sorghum (*Hordeum vulgare*) and wheat (Shahbaz *et al.*, 2008; Perveen *et al.*, 2010, 2011, 2012; Kanwal *et al.*, 2011; Ashraf *et al.*, 2012) and found that there was non-significant alteration under both saline and non-saline conditions, while in rice (Amirjani, 2011) and wheat (Ashraf & Ashraf, 2012) a marked reduction in *Fv/Fm* was observed under stress. It may have been due to the effect of salt on a number of physiological processes as the nature and intensity of the emission bands, quantum yield, and induction kinetics, reflect the properties of the chlorophyll molecules and their interaction with the external environment (Megdich *et al.*, 2008). Cotton genotypes also showed variable response in chlorophyll fluorescence (*Fv/Fm*) as it markedly decreased in cultivars CIM-473 and CIM-496 under high salt concentration. While, the response of the remaining cultivars to salt stress in terms of quantum yield of PSII (*Fv/Fm*) was non-significant. Higher concentration of salt (200 mM NaCl) showed the slight reduction in fluorescence similar to a previous report on wheat by Kanwal *et al.* (2011). On the other hand, it has also been reported that salt stress has no significant effect on PSII efficiency as in wheat (Perveen *et al.*, 2010) and tomato (Zribi *et al.*, 2009) cultivars etc.

In conclusion, saline regimes markedly reduced growth of cotton and high reduction was observed at highest salinity level i.e., 200 mM. Cultivars RH-510, BH-118 and

FH-87 were ranked as relatively salt tolerant on the basis of their growth performance whereas CIM-446, CIM-506 and FH-901 as salt sensitive. Better growth performance is positively correlated with photosynthetic rate. Shoot dry mass and photosynthetic rate can be used as morpho-physiological markers for salinity tolerance in cotton.

Acknowledgment: The data presented in this manuscript is a part of Ph.D. research of first author.

REFERENCES

- Abbas, W., M. Ashraf and N.A. Akram, 2010. Alleviation of salt-induced adverse effects in eggplant (*Solanum melongena* L.) by glycinebetaine and sugarbeet extracts. *Sci. Hortic.*, 125: 188–195
- Afzal, I., S.M.A. Basra, M. Farooq and A. Nawaz, 2006. Alleviation of salinity stress in spring wheat by hormonal priming with ABA, salicylic acid and ascorbic acid. *Int. J. Agric. Biol.*, 8: 23–28
- Akram, M.S., M. Ashraf and N.A. Akram, 2009. Effectiveness of potassium sulfate in mitigation salt-induced adverse effects on different physio-biochemical attributes in sunflower (*Helianthus annuus* L.). *Flora*, 204: 471–483
- Amirjani, M.R., 2011. Effect of salinity stress on growth, sugar content, pigments and enzyme activity of rice. *Int. J. Bot.*, 7: 73–81
- Ashraf, M., 2002. Salt tolerance of cotton: some new advances. *Crit. Rev. Plant Sci.*, 21: 1–30
- Ashraf, M.A. and M. Ashraf, 2012. Salt-induced variation in some potential physiological attribute of two genetically diverse spring wheat (*Triticum aestivum* L.) cultivars: photosynthesis and photosystem II efficiency. *Pakistan J. Bot.*, 44: 53–64
- 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
- Baker, N.R., 1991. A possible role for photosystem II in environmental perturbations of photosynthesis. *Physiol. Plant.*, 81: 563–570
- Cha-um, S., M. Ashraf and C. Kirdmanee, 2010. Screening upland rice (*Oryza sativa* L. sp. indica) genotypes for salt-tolerance using multivariate cluster analysis. *African J. Biotechnol.*, 9: 4731–4740
- Jafar, M.Z., M. Farooq, M.A. Cheema, I. Afzal, S.M.A. Basra, M.A. Wahid, T. Aziz and M. Shahid, 2012. Improving the performance of wheat by seed priming under saline conditions. *J. Agron. Crop Sci.*, 198: 38–45.
- Jones, M.M. and N.C. Turner, 1978. Osmotic adjustment in leaves of sorghum in response to water deficits. *Plant Physiol.*, 61: 122–126
- Kafi, M., 2009. The effects of salinity and light on photosynthesis, respiration and chlorophyll fluorescence in salt-tolerant and salt-sensitive wheat (*Triticum aestivum* L.) cultivars. *J. Agric. Sci. Technol.*, 11: 535–547
- 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. *Pakistan J. Bot.*, 43: 2693–2699
- Megdich, W., F. Gharbi, C. Galeel, R. Ksouri and C. Abdely, 2008. Photosynthesis and photosystem 2 efficiency of two salt-adapted halophytic seashore *Cakile maritima* ecotypes. *Photosynthetica*, 46: 410–419
- Mehta, P., A. Jajoo, S. Mathur and S. Bharti, 2010. Chlorophyll fluorescence study revealing effects of high salt stress on photosystem II in wheat leaves. *Plant Physiol. Biochem.*, 48: 16–20
- Munns, R., 2002. Comparative physiology of salt and water stress. *Plant Cell Environ.*, 25: 239–250
- Munns, R. and M. Tester, 2008. Mechanisms of salinity tolerance. *Annu. Rev. Plant Biol.*, 59: 651–681
- Munns, R., R.A. James and A. Lauchli, 2006. Approaches to increasing the salt tolerance of wheat and other cereals. *J. Exp. Bot.*, 5: 1025–1043
- 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. *Pakistan J. Bot.*, 42: 3073–3081
- Perveen, S., M. Shahbaz and M. Ashraf, 2011. Modulation in activities of antioxidant enzymes in salt stressed and non-stressed wheat (*Triticum aestivum* L.) plants raised from seed treated with triacontanol. *Pakistan J. Bot.*, 43: 2463–2468
- 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. *Pakistan J. Bot.*, 44: 27–35
- Sabir, P., M. Ashraf, M. Hussain and A. Jamil, 2009. Relationship of photosynthetic pigments and water relations with salt tolerance of proso millet (*Panicum miliaceum* L.) accessions. *Pakistan J. Bot.*, 41: 2957–2964
- 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., 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, N.A. Akram, A. Hanif, S. Hamid, 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
- Snedecor, G.W. and W.G. Cochran, 1980. *Statistical Methods*, 7th edition. Iowa State University Press, Ames, Iowa, USA
- Strasser, R.J., A. Srivastava and Govindjee, 1995. Polyphasic chlorophyll 'a' fluorescence transients in plants and cyanobacteria. *Photochem. Photobiol.*, 61: 32–42
- Tavakkoli, E., F. Fatehi, S. Coventry, P. Rengasamy and G.K. McDonald, 2011. Additive effects of Na⁺ and Cl⁻ ions on barley growth under salinity stress. *J. Exp. Bot.*, 422: 1–15
- Ulfat, M., H.R. Athar, M. Ashraf, N.A. Akram and A. Jamil, 2007. Appraisal of physiological and biochemical selection criteria for evaluation of salt tolerance in canola (*Brassica napus* L.). *Pakistan J. Bot.*, 39: 1593–1608
- Ziaf, K., M. Amjad, M.A. Pervez, Q. Iqbal, I.A. Rajwana and M. Ayub, 2009. Evaluation of different growth and physiological traits as indices of salt tolerance in hot pepper (*Capsicum annum* L.). *Pakistan J. Bot.*, 41: 1797–1809
- Zribi, L., G. Fatma, R. Fatma, R. Salwa, N. Hassan, and R.M. Nejib, 2009. Application of chlorophyll fluorescence for the diagnosis of salt stress in tomato *Solanum lycopersicum* (variety Rio Grande). *Sci. Hortic.*, 120: 367–372

(Received 19 July 2012; Accepted 06 September 2012)