



Salt-induced effects on some key morpho-physiological attributes of cotton (*Gossypium hirsutum* L.) at various growth stages

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Abstract

Salinity is a multidimensional stress affecting crop yield and productivity at various levels of plant organization. To assess salt induced adverse effects on cotton (*Gossypium hirsutum* L.), ten cultivars were grown in sand culture supplemented with full strength Hoagland's nutrients solutions and different salt concentrations (0, 50, 100 and 200 mM NaCl). Salt stress markedly reduced growth attributes, relative water contents, efficiency of photosystem II, net CO₂ assimilation rate (A), transpiration rate (E) and stomatal conductance in all cultivars. Reduction was maximum at the highest level of salt stress i.e. 200 mM. However, response of cotton cultivars was variable to various levels of salinity and even at various developmental stages. Cultivars RH-510, BH-118 and MNH-770 were ranked as relatively salt tolerant on the basis of their better growth performance and net CO₂ assimilation rate whereas cvs. CIM-496, CIM-473 and FH-901 were relatively salt sensitive. Cultivars RH-510, BH-118 and MNH-770 exhibited high shoot fresh and dry weights, photosynthetic rate (A), and Photosystem II (Fv/Fm) efficiency at both seedling and maturity growth stages. Results suggest that selection of plants having high photosynthetic rate and biomass at seedling stage may be a good source of high yield at mature stage of growth.

Keywords: Salt stress, cotton, relative water content, gas exchange, photosystem II activity

Introduction

Salinity problem is becoming a big threat for agricultural crops and is common in arid and semi-arid regions. It is the major limiting factor in terms of growth and productivity. Demand of crops is becoming high due to rapid increase in human population (Munns *et al.*, 2010). Although population is increasing but cultivated land is decreasing day by day due to abiotic stresses including salt stress (Kchaou *et al.*, 2010).

It could adversely affect plant growth and productivity (Akram and Ashraf, 2011). It markedly reduces the relative water contents leading to significant reduction in plant growth attributes. Perturbation in various plant physiological mechanisms such as chlorophyll fluorescence and gas exchange attributes is dilemma of saline environment due to higher accumulation of NaCl salt (Ashraf, 2004; Moradi and Ismail, 2007; Ashraf and Ashraf, 2012). However, response of plants varies with salt concentration and development stages. Seedling stage is known to be more predictive to salt response as compared to other growth stages (Nawaz *et al.*, 2010).

Photosynthesis is the primary determinant of plant growth and yield. It is evident that reduced rate of net CO₂ assimilation, transpiration and water use efficiency under salt stress is attributed to limitation of stomatal and non-stomatal function (Shafi *et al.*, 2011; Ashraf and Ashraf,

2012). This consideration is true for many productive crops like rice (Moradi and Ismail, 2007), wheat (Kanwal *et al.*, 2011), sunflower (Noreen and Ashraf, 2008), common bean (Stoeva and Kaymakanova, 2008), chickpea (Singla and Garg, 2005) etc.

Under saline conditions, reduction in photosynthesis can also be attributed to change in chlorophyll fluorescence, photoinhibition of photosystem II (PS-II), conformational changes in membrane-bound ATPase enzyme complex, as well as decrease in both concentration and activity of Rubisco enzyme (Lawlor, 2002). Severe saline conditions induce reduction in chlorophyll fluorescence efficiency (electron transports phenomenon). In higher plants, salinity inhibits activity of PS-II (Kao *et al.*, 2003; Perveen *et al.*, 2010), while other reports illustrate that salt stress has no major effect on PS-II in different crops like sorghum, wheat and barley (Hasegawa *et al.*, 2000; Shahbaz *et al.*, 2008; Mehta *et al.*, 2010) etc.

Cotton is one of the potential crops that plays a pivotal role in the economic development of the country in agriculture and industry. It is a major cost-effective crop known as backbone of Pakistan's agriculture. Cotton and cotton products are the two-third of the total earnings made by export (ASA, 2010). Salinity induces change in plant characteristics from the time of salt imposition until they get mature (Munns, 2002). Most of crops show a

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considerable variation under saline conditions throughout their life cycle (Ashraf, 2002). Thus, it is of vital importance to assess salt tolerance and its consistency at various growth stages of crop specie. So, in the present study, salt tolerance potential of 10 cotton (*Gossypium hirsutum* L.) cultivars were assessed at different phases of growth, which may provide an insight into the mechanism of salt tolerance.

Materials and Methods

An experiment was carried out in Botanical Garden, University of Agriculture, Faisalabad, Pakistan to assess the response of ten cotton (*Gossypium hirsutum* L.) cultivars to four salinity levels developed in sand filled pots using morpho-physiological attributes as criteria. Seeds of the ten cultivars (CIM-446, CIM-506, RH-510, MNH-770, FH-2007, BH-118, FH-87, CIM-496, FH-901 and CIM-473) were obtained from Ayub Agricultural Research Institute, Faisalabad, Pakistan. Cotton seeds were delinted using concentrated H_2SO_4 and rinsed extensively with d_3H_2O water to eliminate acid contents. Ten seeds of each cultivar were sown in plastic pots (29 cm depth and 23.5 cm diameter) containing 8 kg of well washed river sand as the rooting medium, and four plants per pot were maintained by thinning practice and plants were allowed to grow under natural photoperiod (12 h) and temperature 26.9 to 37.9 °C. Four salinity treatments i.e. control (non-saline), 50, 100, and 200 mM NaCl, along with full strength Hoagland's nutrient solution, were applied at 10 days after sowing. To minimize the salt shock and attaining the required level of salinity, salts were applied at increment of 50 mM NaCl per day. In addition to regular irrigation, each plastic pot was irrigated once a week with 2 liters of full strength Hoagland's nutrient solution throughout the experiment.

Data for growth attributes, relative water contents, chlorophyll fluorescence and gas exchange attributes were recorded at seedling and maturity stages of development. Two plants from each pot were uprooted, thoroughly washed with distilled water and their fresh shoot and root weights were recorded. In addition, data for following attributes were recorded before harvesting plants.

Relative water contents

Relative water contents were determined following the Jones and Turner (1978) protocol. A fully developed and young leaf of uniform size was selected from each of the selected plant. Immediately after detachment of leaf, samples were covered with the polythene bags and each sample fresh weight was recorded. After water immersion for 24 h, turgid weight (Tw) of leaf samples was obtained. The leaf samples of plant were oven dried at 75 °C till constant weight and dry weights (Dw) were measured.

Then leaf relative water contents (RWC) were determined using the following formula:

$$\text{Relative water content (\%)} = [(\text{Leaf Fw} - \text{Leaf Dw}) / (\text{Leaf Tw} - \text{Leaf Dw})] * 100$$

Chlorophyll fluorescence

Plant Efficiency Analyzer (PEA, Handstech Instrument Ltd., King's Lynn, UK), was used to record the data for chlorophyll fluorescence as described by Strasser *et al.* (1995). Leaf samples were kept in dark for half an hour before these measurements.

Gas exchange parameters

Gas exchange characteristics as rate of transpiration (E), photosynthetic capacity (A), conductance of stomata (g_s), leaf internal CO_2 concentration (C_i), and water use efficiency (A/E) were determined using a portable (ADC LCA-4 Analytical Development, Hoddesdon, UK) infrared gas analyzer with following adjustments: gas flow rate in leaf chamber (U), 251 $\mu\text{mol s}^{-1}$; leaf chamber temperature, 34.2 to 39.3 °C; ambient pressure, 99.8 kPa; molar air flow per unit leaf area (U_s), 403.4 $\text{mol m}^{-2} \text{s}^{-1}$; vapor pressure, 7.0 to 8.9 mbar into the leaf chamber; surface area of leaf, 6.25 cm^2 ; ambient CO_2 concentration, 351 $\mu\text{mol mol}^{-1}$; relative humidity (RH) of the chamber, 41.2% and photosynthetic active radiation (PAR) at leaf surface, up to 1099 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

Statistical analysis

The experimental design was CRD (completely randomized design) with four replicates. For all attributes, data were analyzed by three way analysis of variance (ANOVA) using COSTAT (Cohort Software, Berkeley, California) statistical software. Standard error was applied to compare means to determine significant differences.

Results

Data for various attributes were recorded at varying time period of growth (seedling and maturity) after initiation of salinity. Data presented in (Table 1; Figure 1) for shoot and root fresh and dry weights of 10 cotton cultivars (*Gossypium hirsutum* L.) showed that salt stress significantly reduced shoot and root fresh and dry weights of all cultivars. Cotton cultivars were markedly inconsistent from each other with respect to these growth attributes. Among tested cotton cultivars, BH-118, MNH-770, FH-2007 and RH-510 were high and CIM-506, CIM-473 and FH-901 low in biomass under saline conditions at seedling stage of growth (Table 1; Figure 1). In contrast, at maturity, cotton cultivars CIM-446, RH-510, FH-2007, BH-118 and CIM-496 showed higher biomass under control conditions but a significant reduction was found in biomass under

saline conditions (Table 1; Figure 1). Although, these cultivars exhibited lower shoot and root fresh weights at highest level of salinity, but they performed better when grown under non-stress conditions as compared to cvs. CIM-496, CIM-473 and FH-901. It is imperative to mention here that FH-2007, FH-87 and BH-118 were consistently high in their fresh and dry weights of shoots particularly under saline environment at seedling as well as maturity growth stages (Figure 1).

Net CO₂ assimilation rate (*A*) of all cultivars reduced significantly ($P \leq 0.001$) due to imposition of salt stress to the growth medium (Table 1; Figure 2). The cultivars responded differently under salt stress by means of this photosynthetic characteristic, but most of them were uniform with respect to their net CO₂ assimilation rate under moderate (50 and 100 mM NaCl) saline conditions (Figure 2), whereas at the highest salt level (200 mM NaCl treatment) cvs. MNH-770, RH-510 followed by BH-118

Table 1: Mean squares from analyses of variance of data for growth attributes, relative water contents, efficiency of PS-II and gas exchange attributes of salt-stressed and non-stressed cotton (*Gossypium hirsutum* L.) plants grown under different salt regimes

Source of variation	df	Shoot f. wt.	Shoot d. wt.	Root f. wt.	Root d wt.	RWC	Fv/Fm
Cultivars (Cvs)	9	286.3**	34.98***	7.099***	0.960***	240.6***	0.004*
Salinity (S)	3	5191.9***	615.2***	76.06***	11.87***	1267.8***	0.063***
Growth stages (G.S.)	1	20284***	1269***	2735.8***	277.4***	18.70ns	0.004ns
Cvs x S	27	112.3ns	19.35***	2.530***	0.326ns	35.61ns	0.001ns
Cvs x G.S.	9	273.2**	31.37***	6.483***	0.996***	78.97ns	0.006***
S x G.S.	3	4957.1***	585.3***	69.30***	11.98***	11.90ns	0.013***
Cvs x S x G.S.	27	113.9ns	19.09***	2.493***	0.320ns	12.77ns	0.001ns
Error	160	96.80	5.888	0.996	0.234	41.82	0.001
Source of variation	df	<i>A</i>	<i>E</i>	<i>g_s</i>	<i>A/E</i>	<i>C_i</i>	<i>C_i/C_a</i>
Cultivars (Cvs)	9	36.74***	1.248***	61741.1***	8.382**	1095.5ns	0.0086ns
Salinity (S)	3	177.9***	9.782***	177884***	5.277ns	2325.1*	0.018*
Growth stages (G.S.)	1	674.8***	271.4***	1467970***	404.9***	1844***	0.147***
Cvs x S	27	2.123ns	0.116ns	17621.0***	3.760ns	645.4ns	0.005ns
Cvs x G.S.	9	6.931***	0.583**	66929.6***	2.992ns	1091.4ns	0.008ns
S x G.S.	3	2.0361ns	1.9276***	21991.528**	6.368ns	6560.0***	0.053***
Cvs x S x G.S.	27	1.572ns	0.104ns	18984.1***	3.306ns	825.3ns	0.006ns
Error	160	1.848	0.179	5564.1	2.605	686.7	0.005

* ** *** = 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; *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.

Data for quantum yield of PSII (*Fv/Fm*) showed that all cotton cultivars were suppressed significantly at high level (200 mM NaCl) of salt. Low level salinity did not affect the cotton photosystem II efficiency. However, cultivars RH-510, FH-87 and BH-118 were uniform in response to efficiency of PS-II under both control and salt-stressed conditions at maturity (Table 1; Figure 2).

Leaf relative water content (RWC) of all cotton cultivars decreased substantially under salt treatment. Significant difference was observed among the 10 cotton cultivars, but salt-induced reduction was higher at seedling stage as compared to maturity stage of growth (Table 1; Figure 2). Leaf water contents were maximum in cv. CIM-506 followed by cv. FH-2007 under saline conditions, while cvs. CIM-446 and FH-87 showed the lowest value of RWC under highest salt level (200 mM).

exhibited maximum photosynthetic rate during seedling as well as maturity growth stages. In contrast, cotton cvs. CIM-506, CIM-446, FH-2007, FH-87 and CIM-496 were considered moderately salt tolerant owing to minimum photosynthetic rate under high (200 mM) saline conditions (Table 1; Figure 2).

Transpiration rate (*E*) reduced significantly in all cotton cultivars with increasing level of salt stress. Although cultivars differed significantly in this attribute, their behavior to varying salt levels was uniform (Table 1; Figure 2). Moderate salt levels (50 and 100 mM NaCl) did not reduce the transpiration rate of all cultivars except MNH-770, FH-87, CIM-473 and CIM-901. However, highest level of salt (200 mM) reduced transpiration rate of all cotton cultivars as compared to non-saline environment. However, cvs BH-118 and FH-2007 excelled in transpiration rate under non-saline conditions.

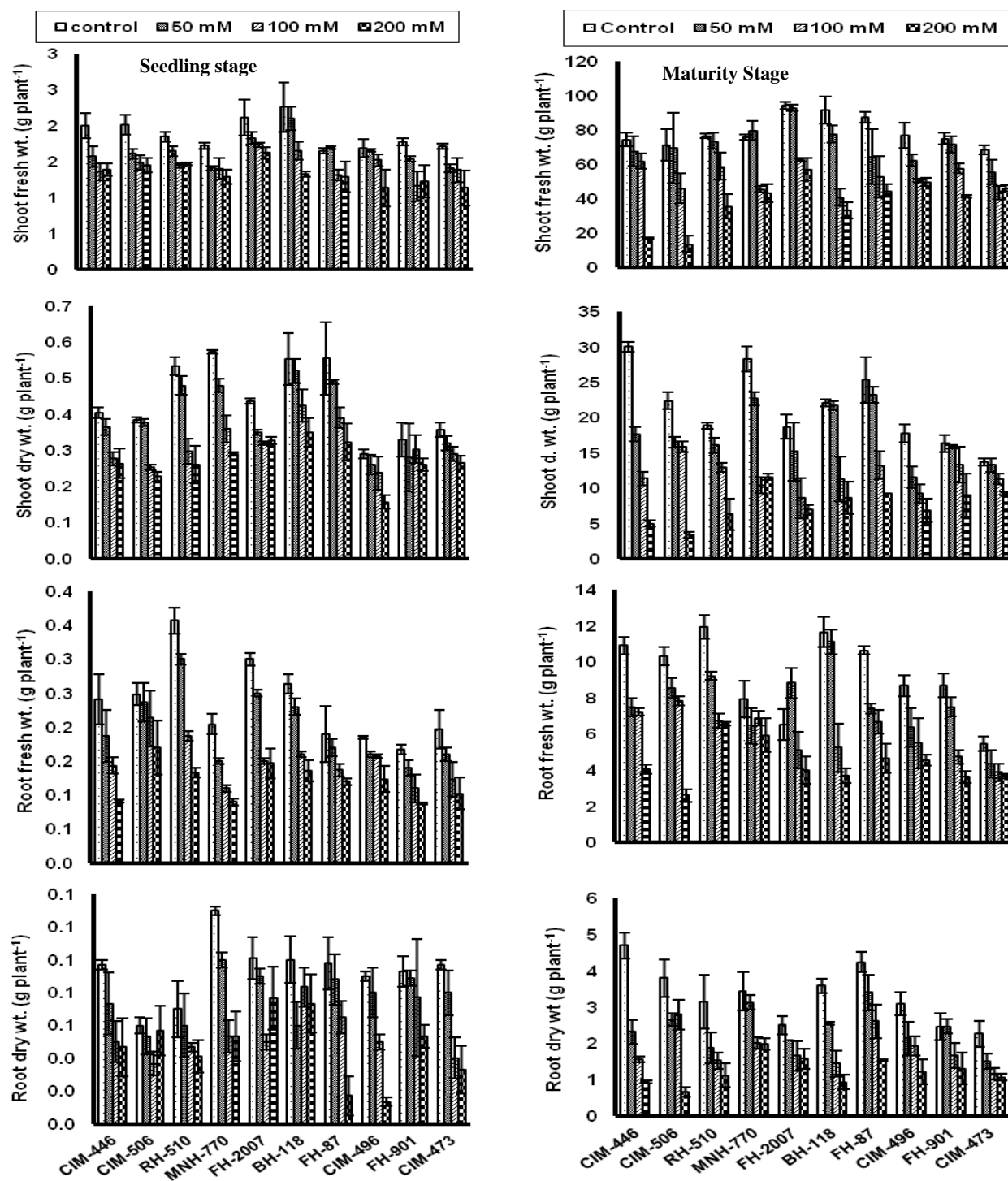


Figure 1: Shoot and root fresh and dry weights of ten cotton (*Gossypium hirsutum* L.) cultivars when 10 days old plants were subjected to salt stress at varying time period of growth stages.

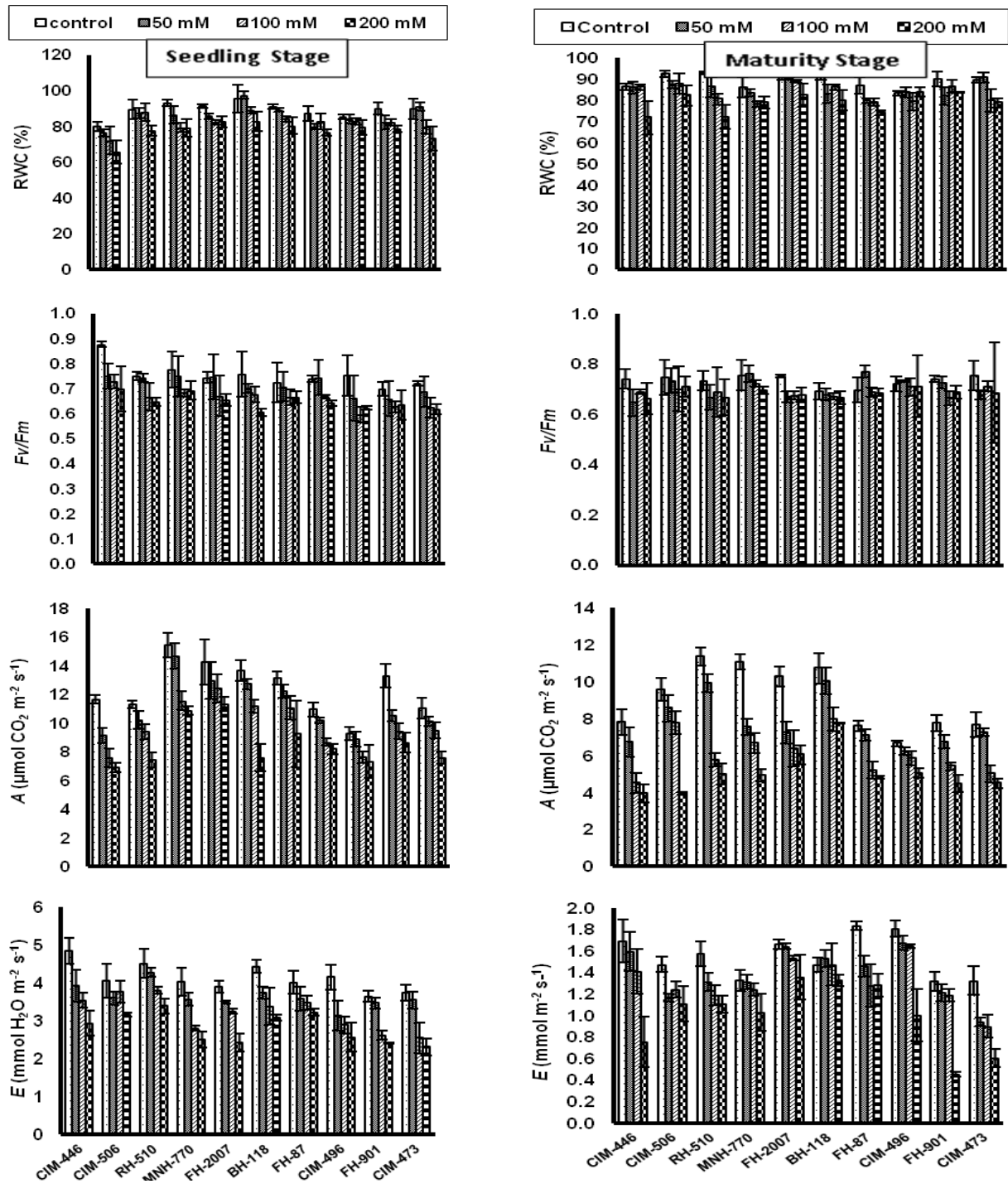


Figure 2: Relative water contents, chlorophyll fluorescence and gas exchange parameters of ten cotton (*Gossypium hirsutum* L.) cultivars when 10 days old plants were subjected to salt stress at varying time period of growth stages.

Application of salt stress significantly ($P \leq 0.001$) decreased stomatal conductance (g_s) of all ten cotton cultivars (Table 1; Figure 3). The response of cultivars also differed significantly ($P \leq 0.001$). CIM-506, RH-510, MNH-770, CIM-496 and FH-901 cotton cultivars showed significant reduction in this attribute at high level (100 and 200 mM) of NaCl salt as compared to control. Maximum value for (g_s) was recorded in cultivar FH-2007. However, of all saline regimes 200 mM NaCl was most effective in reducing the stomatal conductance of cotton cultivars.

Sub stomatal CO_2 concentration (C_i) decreased consistently in all cultivars due to increasing concentration of salt. However, the extent of salt-induced reduction in C_i in different cultivars varied at different salt levels. The C_i/C_a ratio reduced considerably in all ten cotton cultivars due to salt stress application and the cultivars varied significantly in this gas exchange characteristic (Figure 3). Furthermore, C_i/C_a ratio among cotton cultivars was not observed consistent under varying levels of salt (Table 1; Figure 3). Cultivar FH-87 followed by cv. MNH-770 showed maximal C_i/C_a ratio under saline conditions, whereas other cultivars responded uniformly at varying levels of salinity (Table 1; Figure 3).

Water-use-efficiency (A/E) was not consistent under non-stressed or salt stressed conditions. Cultivars CIM-446, CIM-496, FH-901 and CIM-473 showed significant increase in water-use-efficiency at 200 mM NaCl level, while cvs. CIM-506 and RH-510 were lower in A/E ratio at this level (Table 1; Figure 3). Overall, the cotton cultivars did not differ significantly in this attribute.

Discussion

A great genetic variability for salt tolerance is found among species. Like many other crops, cotton also shows variable behavior at different developmental stages (Sattar *et al.*, 2010). Literature reveals that cultivars producing good vegetative growth under non-saline conditions also perform better under salt stress conditions (Basal, 2010; Higbie *et al.*, 2010). Similarly, it was also reported that salt-tolerant varieties of cotton (*G. hirsutum* L.) produce more shoot biomass than that produced by salt-sensitive cultivars (Ashraf and Ahmad, 2000). In the present study, 10 cotton cultivars showed reduction in growth with the increasing levels of NaCl. Cotton cultivars also varied significantly in response to saline condition at different growth stages. Cultivars CIM-446 and CIM-506 behaved as tolerant at seedling growth stage with higher biomass in comparison of maturity. These findings are similar to some previous literature in which significant variations among cultivars were found in various crop species in relation to salt tolerance at varying developmental stages like canola

(Bybordi, 2010) and wheat (Benderradji *et al.*, 2011; Ashraf and Ashraf, 2012).

Relative water contents reflect the maintenance of plant water status (Flowers *et al.*, 2010) and are considered as one of the key factor for salt tolerance under salinity. In the present study, salt application significantly reduced leaf water contents in all cotton cultivars at high level of salt (100 and 200 mM), it might be due to little uptake and loss of water under highly saline conditions (Heidari *et al.*, 2011; Amirjani, 2011). However, together with varietal differences salt induced reduction was relatively high in comparison to mature stage of growth.

To discriminate the cultivars tolerance to a variety of abiotic stresses, photosystem-II (PS-II) chlorophyll fluorescence is a key mechanism (Akram and Ashraf, 2011; Saleem *et al.*, 2011). A key role of PS-II in photosynthesis under saline conditions has been observed in plants (Perveen *et al.*, 2010). In the present study, significant decrease in PS-II efficiency was observed in all cotton cultivars with the increasing level of salt (100-200 mM) particularly at seedling stage. In fact, enhanced concentration of Na^+ in rooting medium is responsible for decrease in chlorophyll fluorescence (Liu and Shi, 2010). The destruction of chloroplast structure by NaCl toxicity may lead to stomatal closure caused by oxidative stress (Ashraf, 2009). At maturity stage, chlorophyll fluorescence (F_v/F_m) significantly reduced only in cultivars CIM-446, CIM-473 and CIM-496 under high (200 mM) concentration of salt treatment. However, the response of other cotton cultivars in terms of F_v/F_m to NaCl stress was found to be non-significant. Similarly, salt-induced non significant effect on quantum yield of PS II has been observed in wheat (Shahbaz *et al.*, 2008; Perveen *et al.*, 2010), while in rice a marked reduction in F_v/F_m was observed under stressed conditions (Moradi and Ismail, 2007; Amirjani, 2011).

Photosynthetic capacity is an important aspect for plant growth. Under salt stress, reduction in plant growth is often attributed to inhibition in photosynthetic rate as observed in many studies (Noreen and Ashraf, 2008; Kanwal *et al.*, 2011; Shahbaz *et al.*, 2011). Current study showed that salt stress induced a significant reduction in photosynthetic rate (A), transpiration rate (E), and stomatal conductance (g_s) in all 10 cotton cultivars. But cotton cultivars showed a marked variation in this attribute at various growth stages. Such variable response has also been observed at various growth stages under saline conditions in various plants like *Brassica* species (Kumar *et al.*, 2009), canola (Ulfat *et al.*, 2007; Kazemnia, 2008) and sunflower (Shahbaz *et al.*, 2011).

In conclusion, in the present study, cultivars RH-510, BH-118, and MNH-770 were ranked as relatively salt tolerant on the basis of their better growth performance

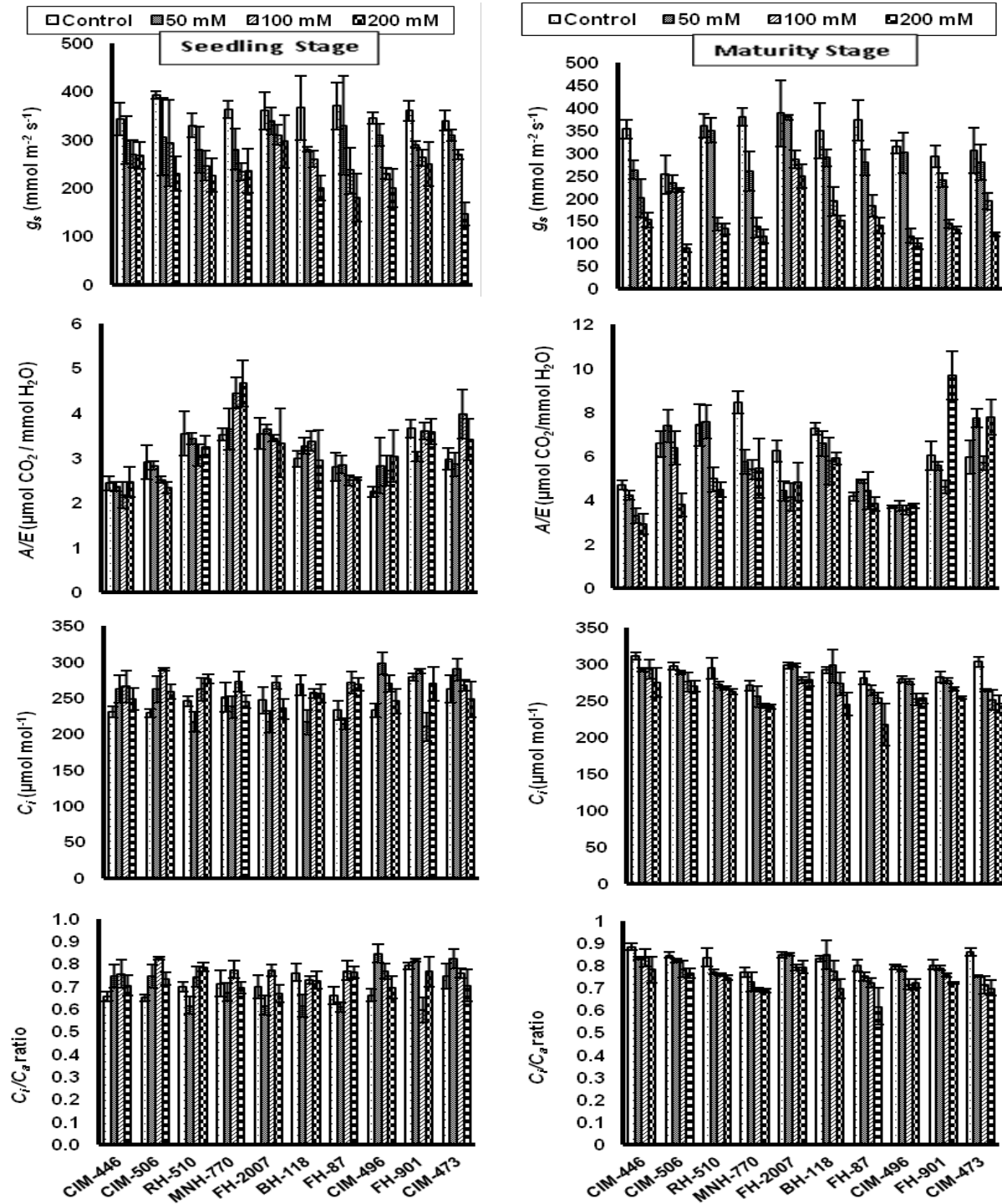


Figure 3: Gas exchange parameters of ten cotton (*Gossypium hirsutum* L.) cultivars when 10 days old plants were subjected to salt stress at varying time period of growth stages.

whereas cvs. CIM-496, CIM-473 and FH-901 as relatively salt sensitive, as cultivars RH-510, BH-118 and MNH-770 were better in growth (fresh and dry weight), photosynthetic rate (A), and Photosystem II (Fv/Fm) efficiency at both stages (seedling and maturity) of growth. Identification of salt tolerance is of considerable value because they can be used for cultivation on moderately saline soils with some amendments, or for use in breeding programs aimed at improving salt tolerance in cotton. Furthermore, the cultivars using good genetic potential can be used to develop new lines/cultivars with high growth and yield on salt affected soils.

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