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ASSESSMENT OF SALT TOLERANCE OF SOME NEWLY DEVELOPED AND CANDIDATE WHEAT (*TRITICUM AESTIVUM* L.) CULTIVARS USING GAS EXCHANGE AND CHLOROPHYLL FLUORESCENCE ATTRIBUTES

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Abstract

The present study was aimed to assess salt tolerance of some newly developed and candidate cultivars of wheat using gas exchange and chlorophyll fluorescence parameters. Ten wheat cultivars including five newly developed (S-24, Saher-2006, Fsd-2008, Lasani and Inqlab-91) and five candidate (P.B-18, M.P-65, S.H-20, AARI-10 and G.A-20) were grown in sand culture. Salt stress (150 m*M* NaCl in Hoagland's nutrient solution) was applied at the seedling stage. A significant reduction in plant biomass production was recorded in all wheat cultivars. Cultivars S-24, Saher-2006 and Fsd-2008 showed less reduction in biomass production as compared with the other cultivars. Different gas exchange attributes such as leaf net photosynthetic rate (*A*), transpiration rate (*E*), and stomatal conductance (g_s) were also adversely affected due to salt stress and were positively associated with the plant biomass production of the genotypes under saline stress. More negative effects in relation to these gas exchange attributes were recorded in cvs. Lasani, G.A-20 and ARRI-10 than those in the other cultivars. Leaf maximum chlorophyll fluorescence (*Fm*), maximum fluorescence at steady state (*Fms*), and photochemical fluorescence quenching (*Qp*) increased while maximum quantum yield (*Fv*/*Fm*), quantum yield of electron transport (*Qp*), electron transport rate (*ETR*) and non-photochemical quenching (*Qn*) decreased due to imposition of salt stress. The adverse effects of salt stress on these chlorophyll fluorescence attributes were minimum in cultivars S-24, Saher-2006 and Fsd-2008. A significant positive correlation was recorded between biomass production, different gas exchange attributes and chlorophyll fluorescence parameters. Overall, cvs. S-24, Saher-2006 and Fsd-2008 were ranked as salt tolerant on the basis of their performance in biomass production, gas exchange attributes and chlorophyll fluorescence parameters.

Introduction

Salt-induced inhibition in plant growth occurs due to interplay of a variety of physiological and biochemical attributes (Ashraf, 2009). Of different physiological attributes, plant gas exchange attributes are the most important one (Ashraf, 2009), and considerable alteration in these gas exchange attributes occur due to salt stress (Raza *et al*., 2007; Nawaz & Ashraf, 2010). For example, plants subjected to salt stress exhibit a marked decline in photosynthetic and transpiration rates, stomatal conductance and water use efficiency (Ashraf, 2004; Ashraf 2009). Secondly, it is known that although plant photosynthetic attributes are positively associated with plant growth and final yield in most crops, it is not the case in other crops (Ashraf, 2004; Nawaz *et al*., 2010). The variation in plants in relation to different gas exchange attributes under salt stress not only exists within species but also within the cultivars of same species. So, plant gas exchange attributes can be used as selection criteria for salt tolerance in those crops in which there is a positive relationship between growth and key gas exchange characteristics (Ashraf, 2004).

Despite causing adverse effects to stomatal features salt stress also causes a substantial damage to photosystem II. Of the two photosystems, PS II is found to be more prone to the hazardous effects of salinity (Abdeshahian *et al*., 2010). However, the functionality of PS II can be assessed by measuring different attributes of chlorophyll fluorescence. For example, salt-induced decline in *Fv*/*Fm* is a promising indicator of the functionality of photosynthetic apparatus of plants (Gomathi & Rakkiyapan, 2011). Likewise, increase in *NPQ* is an adaptive energy dissipation mechanism of plants subjected to abiotic stress (Abdeshahian *et al*., 2010). Different cultivars of important crops have been screened on the basis of their inherent potential to tolerate salinity stress in terms of response of PSII to the stress (Baker, 2008). For example Netondo *et al*. (2004) reported that in sorghum imposition of salt stress significantly decreased the quantum yield of PSII (*Fv*/*Fm*), photochemical quenching coefficient (*qP*) and electron transport rate (*ETR*), but the non-photochemical quenching increased. However, in rice salt-induced decrease in *Fv*/*Fm* was not observed in salt tolerant cultivars.

Furthermore, it is well known that the response of most crops to salinity stress varies at different growth stages (Ashraf *et al*., 2010). So, a crop tolerant at one stage may be sensitive at other stages. Thus, the degree of stress tolerance observed at one stage may or may not be reflected at other growth stages in most plant species. This point has been addressed comprehensively in a number of studies on stress tolerance of crops (Singh & Flowers, 2010; Ashraf & Akram, 2009). Effect of salt stress on germination and seedling growth in many cultivated crop plants have been studied (Shah *et al*., 1987; Ashraf *et al*., 1994; Hussain *et al*., 2008; Jenkins *et al*., 2010), and these early growth stages have been considered as more sensitive than the later growth stages.

Generally, wheat response to salt stress is reported to be variable at different growth stages (Ashraf, 2009). Keeping in view the above information the present research work was planned to assess the response of some newly developed (S-24, Inqlab-91, Saher-2006, Lasani and Fsd-2008) and some candidate (AARI-10, G.A-20, P.B-18, M.P-65 and S.H-20) wheat cultivars at the seedling stage using different gas exchange attributes and some chlorophyll fluorescence parameters.

Materials and Methods

The experiment described below was conducted to assess the salt stress tolerance of 10 (five newly developed and five candidate) wheat cultivars on the basis of biomass production, gas exchange characteristics and chlorophyll fluorescence parameters. The nine wheat cultivars (Inqlab-91, Saher-2006, Fsd-2008, Lasani, P.B-18, M.P-65, S.H-20, AARI-10 and G.A-20) were procured from the Wheat Section of the Ayyub Agricultural Research Institute, Faisalabad, Pakistan and the cultivar S-24 from the Department of Botany, University of Agriculture, Faisalabad, Pakistan. The experiment was performed in the old Botanic Garden (longitude 73°10 E, latitude 31°30 N and altitude 213 m), with 11/13 light/dark period at 700-1370 µmol m^{-2} s^{-1} PPFD, a day/night temperature cycle of 26/12°C and relative humidity 65 ± 5.5%. All wheat cultivars were grown in plastic pots in sand culture and salt stress applied at the seedling stage. There were two levels of salinity (control = full strength Hoagland's nutrient solution; salt stress = 150 m*M* NaCl in full strength Hoagland's nutrient solution). The whole experiment comprised 80 plastic pots filled with well washed river sand. The experiment was laid out in a Completely Randomized Design with four replications. Ten seeds per pot were sown after saturation of the sand contained in pots with full strength Hoagland's nutrient solution by supplying 2L nutrient solution per pot. After germination, the seedlings were thinned to 4 plants per pot. Salt stress was applied at the seedling stage after 15 days of germination. Data for growth, gas exchange and chlorophyll fluorescence parameters were recorded when the plants were 45 days old. Two plants per replicate were harvested for the estimation of shoot and root fresh weights.

Leaf gas exchange characteristics: For recording data for different gas exchange attributes such as net carbon dioxide (CO_2) assimilation rate *(A)*, transpiration rate (*E*), stomatal conductance (g_s) and sub-stomatal CO_2 concentration (C_i), a fully expanded second leaf of one plant from each replicate was used. The measurement of different gas exchange attributes was made using an open system LCA-4 ADC portable infrared gas analyzer (Analytical Development Company, Hoddesdon, England). The plant water use efficiency was computed as A/E and intrinsic water use efficiency as A/g_s. All gas exchange measurements were performed *in situ* from 10.30 a.m. to 12.30 p.m. when the light intensity was in range (700-900 µmoles/m^2/s) with specifications/ adjustments mentioned elsewhere (Ali *et al*., 2008).

Leaf chlorophyll fluorescence parameters: The light adapted leaves that were to be analyzed during day time in the presence of sunlight were adapted to darkness for 30 min by attaching light-exclusion clips to the leaf surface. Chlorophyll fluorescence was measured by a portable multimode chlorophyll fluorometer (Model, OS5P Optisciences, Inc. Winn Avenue Hudson, USA) following Strasser *et al*. (1995). After a dark adaptation period for 30 min using specific leaf clips, the minimal fluorescence level (F_o) was measured by a weak red light, which was sufficiently low (< 0.1 µmol m^{-2} s^{-1}) not to induce any significant variable fluorescence. The maximum fluorescence of dark-adapted leaves (F_m) was measured by applying a 0.8 s saturating pulse (8000 µmol m^{-2} s^{-1}). All measurements of F_o were performed with the measuring beam set to a frequency of 6000 Hz, whereas all measurements of F_m were performed with a measuring beam automatically switching to 20 kHz during the saturating flash. The other parameters were also measured in the same range as mentioned above.

Statistical analysis: Data for each attribute were subjected to CoStat Computer Program (version 6.303, PMB 320, Monterey, CA, 93940 USA) for calculating ANOVA. Mean values were compared with an LSD worked out following Steel & Torrie (1986).

Results

The present study was conducted to assess the response of some newly developed wheat cultivars to salt stress applied at the seedling stage in relation to growth, gas exchange characteristics and chlorophyll fluorescence attributes. Data presented in Fig. 1 show that imposition of salt stress significantly reduced the shoot and root fresh weights of all wheat cultivars. The cultivars differed significantly in relation to all these attributes. While comparing different wheat cultivars in relation to shoot fresh weight it was evident that maximum reduction in shoot fresh weight due to salt stress took place in cvs. P.B-18 and M.P-65 and the minimum in cvs. S-24 and S.H-20. However, in relation to root fresh weight this salt-induced reduction was greater in cultivars G.A-20, S.H-20 and S-24 than that in the other cultivars, however, the minimum reduction being in cvs. Lasani and AARI-10 (Fig. 1).

Of different gas exchange attributes, leaf photosynthetic rate (*A*) and transpiration rate (*E*) in all wheat cultivars were also adversely affected due to imposition of salt stress. A maximal salt-induced reduction in *A* was recorded in cv. M.P-65 and S.H-20 followed by Inqlab-91 and the minimum being in cvs. Saher-2006, Fsd-2008 and S-24. However, in relation to *E* this reduction was minimal in cv. S-24 and maximal in cvs. Lasani, P.B-18 and G.A-20 (Fig. 1).

A significant reduction in leaf intrinsic CO_2 concentration (C_i) and stomatal conductance (g_s) was also recorded in all wheat cultivars due to salt stress, but the reduction being cultivar-specific. More reduction in C_i due to salt stress was recorded in cvs. S.H-20, AARI-10 and G.A-20 followed by cv. Lasani, but the minimum being in cvs. S-24, Saher-2006 and Fsd-2008. However, in relation to g_s this salt-induced reduction was almost similar in all wheat cultivars (Fig. 1).

Plant water use efficiency (A/E) and C_i/C_a of all wheat cultivars also reduced significantly due to salt stress applied at the seedling stage. The cultivars differed significantly in these attributes. A maximal reduction in A/E due to salt stress took place in cvs. Lasani, P.B-18 and G.A-20 and a minimal in cvs. S-24 and Inqlab-91. However, in relation to C_i/C_a the minimum salt-induced reduction was recorded in cvs. Saher and S-24 and the maximum in cvs. SH-20, AARI-10 and G.A-20. Furthermore, salt-induced effects were also recorded in relation to intrinsic water use efficiency (A/g_s) in all wheat cultivars but the increasing or decreasing effect was cultivar-specific. A significant increase in A/g_s was recorded in cvs. S-24, Saher-2006, Fsd-2008, AARI -10 and G.A-20, but in the other cultivars it decreased significantly and the maximum reduction took place in cvs. Lasani and M.P-65. So, on the basis of different gas exchange attributes it can be concluded that cvs. S-24, Fsd-2008 and Saher-2006 showed better results as compared to the other cultivars and the adverse was true for cvs. Lasani, AARI-10 and G.A-20 (Fig. 1).

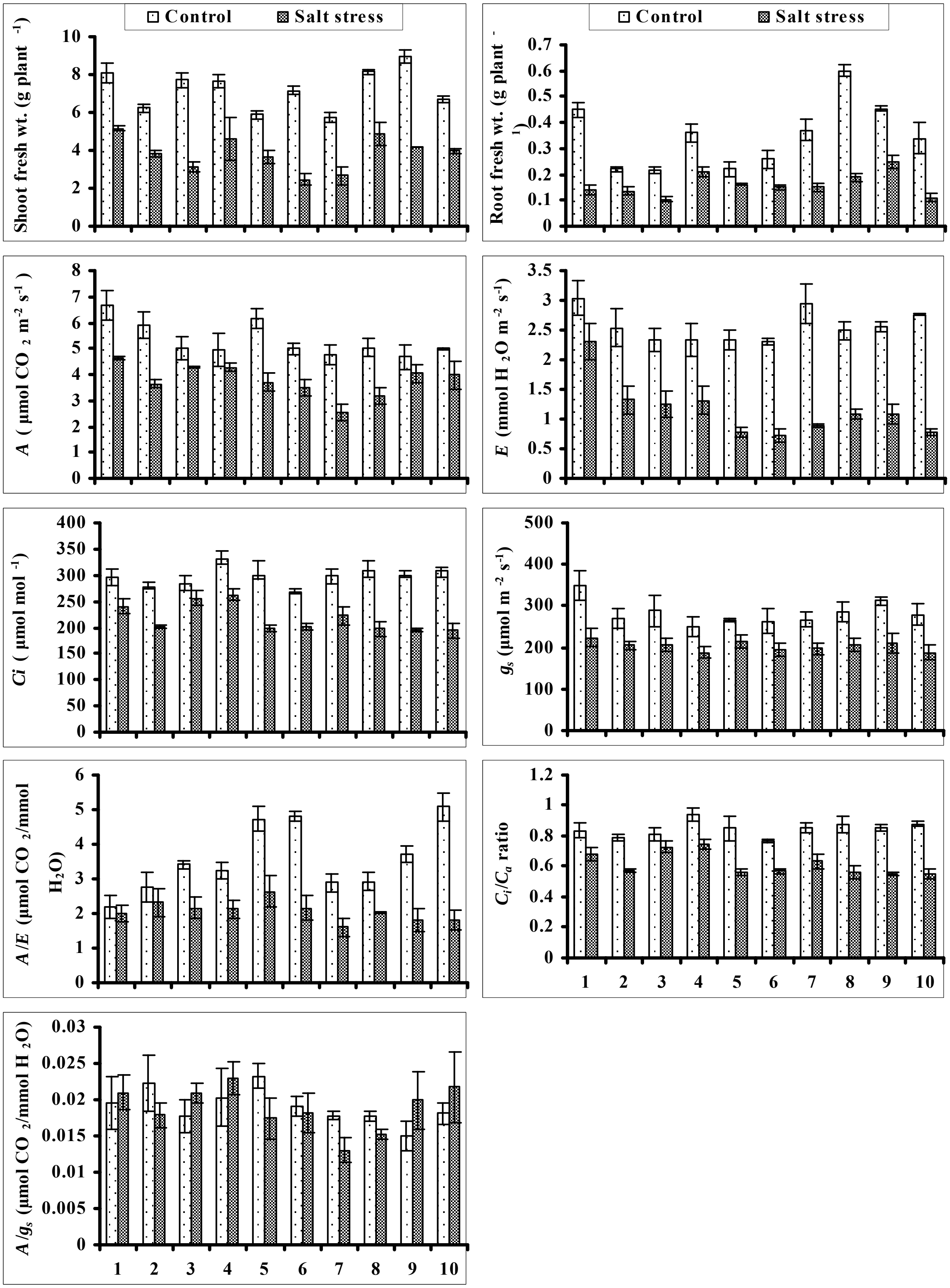

Fig. 1. Biomass production and gas exchange attributes of some newly developed wheat (*Triticum aestivum* L.) cultivars under salt stress applied at seedling stage (mean ± *S.E.*).

1= S-24; 2= Inqlab-91; 3= Saher-2006; 4= Fsd-2008; 5= Lasani; 6= P.B-18; 7= M.P-65; 8= S.H-20; 9= AARI-10
10= G.A-20

A= photosynthetic rate; *E*= transpiration rate; C_i= intrinsic CO_2 concentration; g_s= stomatal conductance; *A*/*E*= water use efficiency; A/g_s= intrinsic water use efficiency; C_i/C_a= ratio of intrinsic CO_2 to ambient CO_2 concentration

The adverse effects of salt stress were also observed on chlorophyll fluorescence parameters. Minimum fluorescence in light (*Ft*) and minimum fluorescence in dark (*Fo*) of all wheat cultivars increased significantly due to imposition of salt stress applied at the seedling stage. Comparison of different cultivars shows that the maximum increase in *Ft* had been in cvs. Saher-2006, P.B-18 and AARI-10 of all cultivars. However, in relation to *Fo* this increase was maximum in cvs. S-24, Inqlab-91 and AARI-10 but no change occurred in cv. Saher-2006 (Fig. 2).

A significant increase in leaf maximum chlorophyll fluorescence (*Fm*) of all wheat cultivars was recorded due to salt stress when applied at the seedling stage. Of all wheat cultivars, the minimum increase in *Fm* was recorded in cvs. M.P-65 and S.H-20 and the maximum in cvs. Saher-2006 and Lasani. However, the maximum quantum yield of primary photochemical reaction in dark adapted leaves (*Fv*/*Fm*) decreased significantly due to imposition of salt stress applied at the seedling stage. This decrease in *Fv*/*Fm* due to salt stress was maximum in cvs. Inqlab-91, Saher-2006 and Fsd-2008 and minimum in cvs. M.P-65 and S.H-20 (Fig. 2).

The value of fluorescence at steady state in light adapted leaf (*Fs*) and maximum fluorescence at steady state in dark adapted leaves (*Fms*) also increased significantly due to salt stress applied at the seedling stage. Of all wheat cultivars, the maximum increase in *Fs* and *Fms* due to salt stress was recorded in cvs. S-24, Inqlab-91 and Saher-2006 and the minimum in cv. M.P-65 (Fig. 2).

Data presented in Fig. 2 show that quantum yield of electron transport (*Y*) and electron transport rate (*ETR*) of all wheat cultivars decreased significantly due to imposition of salt stress applied at the seedling stage. The maximum reduction in these attributes was observed in cvs. Fsd-2008, AARI-10 and G.A-20 and the minimum in cvs. S-24, M.P-65 and Inqlab-91.

A significant increase in leaf photochemical fluorescence quenching (*Qp*) and a decrease in nonphotochemical quenching (*Qn*) of all wheat cultivars was observed due to salt stress applied at the seedling stage. In case of *Qp* this increase was recorded only in cvs. S-24, Inqlab-91, Saher-2006, Fsd-2008 and G.A-20 but in the other cultivars this increase in *Qp* due to salt stress was not significant. However, in case of *Qn*, the minimum decrease resulted in cv. S-24 and the maximum in cvs. Saher-2006, P.B-18 and M.P-65 (Fig. 2).

Discussion

Salt stress is known to cause a number of adverse physiological and biochemical changes in plants that in turn result in reduced plant biomass and ultimately reduced crop yield. Of different salt-induced physiological changes, plant photosynthetic capacity and other gas exchange characteristics are the most important one because plant growth and vigor mainly depends on these attributes (Ashraf *et al*., 2010; Carpici *et al*., 2010; Mahmood, 2011).

The present study was aimed to assess comparative salt tolerance of some newly developed wheat cultivars and candidate varieties yet to be released after formal registration using some potential physiological selection criteria such as gas exchange characteristics and chlorophyll fluorescence. From the results of the study it is evident that different wheat cultivars responded differently to saline stress in terms of biomass production at the seedling stage. All wheat cultivars were adversely affected due to salt stress and maximal reduction in plant biomass was recorded in cvs. Lasani, P.B-98, AARI-10 and G.A-20 and the minimal in cvs. S-24, Saher-2006 and Fsd-2008. Thus, the latter three cultivars could be ranked as salt tolerant in terms of biomass production at the seedling stage under saline stress. This differential response of different wheat cultivars to salt stress on plant biomass production might have been due to their differential genetic potential to salt stress. Such a cultivar variation in plant biomass production under salt stress has already been reported in different crops such as maize (Carpici *et al*., 2010), barley (Mahmood, 2011), sunflower (Shahbaz *et al*., 2011), and soybean (Tuncturk *et al*., 2008).

Biomass production is the interplay of multiple physiological and biochemical attributes. In this regard, plant gas exchange attributes are the most important that significantly contribute to plant biomass production. Under salt stress these gas exchange attributes are inhibited (Ashraf & Ali, 2008; Ashraf *et al*., 2010) that result in reduced biomass production. Generally, it is believed that salt-induced decline in plant photosynthetic rate takes place due to stomatal regulation and non-stomatal processes (Raza *et al*., 2007; Shahbaz *et al*., 2011). Of these two factors, the stomatal closure is the most important factor which results in reduced plant photosynthetic rate under moderate and severe salt stress conditions (Ashraf, 2009). These adverse effects of salt stress on plant photosynthetic rate have been reported to occur due to a number of factors including plant respiration system, electron transport system, the synthesis of ATP in the mitochondria, accumulation of stress metabolites (Zhang *et al*., 1999), and gene expression and protein synthesis (Lawlor & Tezara, 2009). However, different plant species and cultivars have different potential in relation to these physiological attributes under salt stress conditions. In the present study, different wheat cultivars responded differently to salt stress in relation to plant photosynthetic rate. The lower biomass production of wheat cultivars was positively associated with the reduced plant photosynthetic rate (*A*), transpiration rate (*E*), intrinsic CO_2 concentration, and plant water use efficiency (*A*/*E*) that were positively associated with reduced plant stomatal conductance (g_s). More reduction in photosynthetic capacity was observed in cvs. Lasani and AARI-2010 and minimum in cvs. S-24, Fsd-2008 and Saher-2006. In relation to plant intrinsic water use efficiency (A/g_s), a significant increase was recorded in cvs. S-24, Saher-2006 and Fsd-2008, whereas in the remaining cultivars a significant reduction in plant intrinsic water use efficiency was recorded. The high intrinsic water use efficiency recorded in the three cvs. S-24, Saher-2006 and Fsd-2008 might have contributed to their enhanced salt tolerance as did the other gas exchange characteristics.

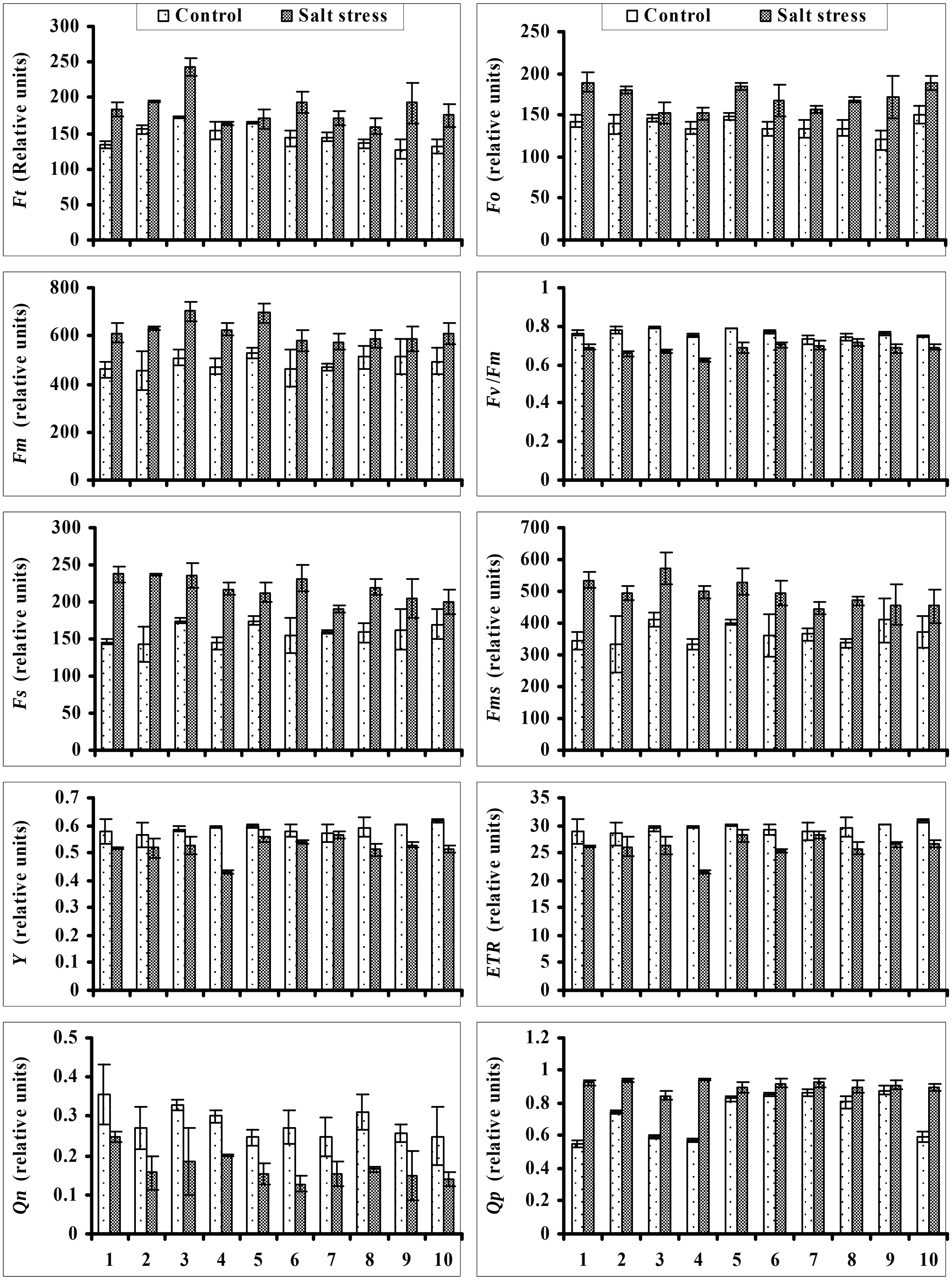

Fig. 2. Chlorophyll fluorescence attributes of some newly developed wheat (*Triticum aestivum* L.) cultivars under salt stress applied at seedling stage (mean±*S.E.*).

1= S-24; 2= Inqlab-91; 3= Saher-2006; 4= Fsd-2008; 5= Lasani; 6= P.B-18; 7= M.P-65; 8= S.H-20; 9= AARI-10
10= G.A-20

Ft= minimum fluorescence in light; *Fo*= minimum fluorescence in dark; *Fm*= maximum fluorescence; *Fv*/*Fm*= quantum yield of primary photochemical reaction of dark adopted leaves; *Fs*=fluorescence at steady state; *Fms*=maximum fluorescence at steady state; Y=quantum yield of electron transport; *Qp*=leaf photochemical fluorescence quenching; *Qn*=non-photochemical quenching; *ETR*=electron transport rate

Under salt stress when there is reduced photosynthetic rate, excess light excitation energy results in photo-oxidative damage to the photosynthetic machinery that results in suppressed plant photosynthesis coupled with a progressive decrease in plant carbon assimilation rate (Cornic, 2000). How far stress can induce damage to PSII reaction centers can be assessed by a non-invasive measurement of photosynthesis by chlorophyll *a* fluorometry. By this technique plant viability and performance in response to any stress including salt stress can be easily appraised (Woo *et al*., 2008). A number of studies on photosystem II performance have been conducted on different plant species in the field (Bacelar *et al*., 2009), on cultivated plants under salt stress (Sofo *et al*., 2009), and on detached leaves (Catala *et al*., 2007). These studies have reported a good correlation between plant growth and changes in the chlorophyll fluorescence parameters in response to salt stress conditions. Of different chlorophyll fluorescence parameters the most important one is the leaf maximum quantum yield of PSII (*Fv*/*Fm*) that results in a severe reduction under stress conditions. Using this parameter it was possible to easily discriminate among the wheat cultivars as this parameter was found to be positively associated with photosynthetic capacity and hence growth of all wheat cultivars. Earlier Akram *et al*., (2006) and Abdeshahian *et al*., (2010) attributed the chlorophyll fluorescence as a direct and good selection criterion for plant salt tolerance. In another study, Jiang *et al*., (2006) assessed salt tolerance in different cultivars of barley on the basis of chlorophyll fluorescence and photosynthetic attributes. They reported a significant reduction in photochemical quenching coefficient, electron transport rate (*ETR*), estimated plastoquinone pool and maximum quantum efficiency of photosystem II. This reduction in plant chlorophyll fluorescence parameters was positively correlated with a decrease in different photosynthetic parameters and biomass production. Furthermore, the barley cultivars with some higher values were found to be salt tolerant. These findings can be correlated with those reported in the present study in which salt stress resulted in decreased PS II quantum yield (*Fv*/*Fm*) with an increase in some other parameters.

From the results of the present study it can be concluded that salt tolerance of the wheat cultivars examined in the present study was associated with their higher photosynthetic rate and PS II efficiency. So the newly developed wheat cultivars including S-24, Saher-2006 and Fsd-2008 were found to be salt tolerant particularly at the seedling stage on the basis of plant gas exchange and chlorophyll fluorescence parameters. However, to ascertain whether the high salt tolerance observed in the three newly developed cultivars is maintained at the later growth stages further research is essential.

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