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The Effects of Salinity and Light on Photosynthesis, Respiration and Chlorophyll fluorescence in Salt-tolerant and Salt-sensitive Wheat (*Triticum aestivum* L.) Cultivars

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ABSTRACT

Intra-specific variation in responses of diurnal and long-term photosynthesis, stomatal conductance, chlorophyll fluorescence, and respiration in spring wheat (*Triticum aestivum* L.) to salinity was investigated using two tolerant cultivars (CR and Kharchia-65) and a sensitive one (Ghods). Plants were grown in sand culture in controlled environment at selected levels of salinity (5 as control, 100, 200, and 300 mol m^{-3} NaCl and $CaCl_2$ in 5:1 molar ratios). Photosynthesis was markedly reduced in the saline conditions, but there were no significant differences observed amongst cultivars. Stomatal conductance of both upper and lower leaf surfaces was the main factor limiting photosynthesis in the presence of salinity. However, non-stomatal limitations as indicated by reduction in variable to maximum fluorescence (F_v/F_m) showed that plants might experience some degree of photoinhibition at the highest level of salinity. Gas exchange in control and 100 mol m^{-3} did not change throughout the day in either the salt-sensitive or the salt tolerant cultivars but in the last hours of the day photosynthesis in the sensitive cultivar was markedly reduced. Respiration remained unchanged up to 200 mol m^{-3} salinity but at 300 mol m^{-3} it decreased as compared with the lower salinity levels. The most remarkable change in respiration rate was that at seven hours after light when CO_2 production was much higher than at the other times of the day. The results indicated that although photosynthesis is well correlated with stomatal conductance, wheat genotypes show different responses as regards other such aspects of photosynthesis, in different salt concentrations, as growth stage, time of the day and duration of salt exposure.

Keywords: Chlorophyll content, Chlorophyll fluorescence, C_i/C_a, Diurnal photosynthesis, Respiration, Stomatal conductance.

INTRODUCTION

Growth is known to be affected by various environmental and genetic factors to an extent which depends on species, variety as well as on plant's growing conditions. One of the main causes of reduced growth might be a reduction in the rate of photosynthesis (A) (Kasai *et al.*, 1998; Sibole *et al.*, 1998; Belkhodja *et al.*, 1999; Ouerghi *et al.*, 2000). There are two schools of thought as to the response of the photosynthetic apparatus to salinity as an environmental constraint. Firstly, photosynthesis is not significantly reduced by salinity except as a secondary effect in already heavily salt affected leaves. The main explanation for this is leaf thickening and adaptation of leaves to saline conditions, e.g. osmotic adjustment in wheat (Lewis *et al.,* 1988; Ali *et al.*, 2005). Secondly, photosynthesis is inhibited in the presence of salinity through either reduction in stomatal conductance (g) or such non-stomatal factors as a reduction in chlorophyll pigments to

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absorb enough light (De Herralde *et al., 19*98; Belkhodja *et al*., 1999; Ouerghi *et al*.**,** 2000; James *et al.*, 2002; Moradi and Esmail, 2007).

Rawson *et al.* (1983) postulated that a single measurement of photosynthesis is unlikely to bear any relationship to growth even under standard conditions, because rates of decline with leaf ageing, shading of lower leaves and time of the day when measurements were made could be different among plant materials. Sultana *et al.* (1999) reported that reduction in photosynthesis in salinized plants depends not only on reduction of available CO_2, but also on the cumulative effects of leaf water content and osmotic potential, transpiration rate, leaf relative water content, and such biochemical constituents as photosynthetic pigments, soluble carbohydrates, and protein, the cumulative effects resulting in low concentrations of assimilates in the leaves.

With regard to the balance of photosynthates within plants under salt stress, net photosynthesis is affected by respiration (Schwarz and Gale, 1981). Nieman (1962) demonstrated that respiration of leaves of twelve crop species tended to increase in both tolerant and sensitive species under saline conditions. Kasai *et al.* (1998) also reported a large increase in maintenance respiration in saline conditions.

Although the effect of salinity on photosynthesis in such plants as wheat have been studied by several investigators (Kingsbury *et al.*, 1984; Rawson, 1986; Kasai *et al.*, 1998; Ouerghi *et al*.**,** 2000, Munns *et al.*, 2006), there is a lack of information on photosynthesis in salt-tolerant and salt-sensitive Iranian wheat cultivars in short (diurnal) and long-term (weeks) exposure to salinity. Parameters which influence photosynthesis such as Photon Flux Density (PFD), intercellular (C_i) to ambient (C_a) CO_2 concentration (C_i/C_a), ratio of variable to maximum chlorophyll fluorescence (F_v/F_m) of leaves, chlorophyll content, and respiration could explain different responses of wheat genotypes to salinity. It is not clear whether salt tolerance mechanisms in wheat cultivars are similar and which physiological parameter is a limitation to growth. Therefore, the objectives of the present study were to assess photosynthesis (daily *vs*. long-term), stomatal conductance, C_i/C_a, F_v/F_m, chlorophyll content and respiration in wheat cultivars at different levels of salinity.

MATERIALS AND METHODS

Three wheat cultivars: CR (Iranian salt-tolerant), Ghods (Iranian salt-sensitive) and Kharchia-65 (Kharchia) (standard salt-tolerant) (Kingsbury *et al*., 1984) were grown in sand culture in controlled environment conditions. Day length was 14 hours during the experiments and relative humidity 58±2%. Temperature varied from 20°C during the day and 15°C at night. Depending on plant height, distance between leaves, and light sources, photon flux density was 100- 200 µmol m^{-2} s^{-1}. Four levels of salinity including control (5 mol m^{-3}), 100, 200 and 300 mol m^{-3} of NaCl and $CaCl_2$ in 5:1 molar ratio were imposed in modified Hoagland nutrient solution consisting of 2.5 mM $Ca(NO_3)_2$, 3.0 mM KNO_3, 0.17 mM KH_2PO_4, 1.5 mM $MgSO_4$, 50 µM Fe as ferric citrate, 23 µM H_3BO_3, 5 µM $MnSO_4$, 0.2 µM $CuSO_4$ and 0.1 µM H_3MoO_4 at two leaf stage and continued till the end of experiment (Maas and Poss, 1989).

Net photosynthesis measurements were made at the middle stage of single fully expanded attached main stem flag leaves using a LCA3 gas exchange system (Analytical Development Co., Hoddesdon, UK) with a Parkinson Leaf Chamber (PLC3) (The Analytical Development LTD, Hoddesdon, UK) at a photon flux density of 600 µmol m^{-2} s^{-1}. Youngest fully expanded flag leaf was selected for a lowest radiation disturbance by the upper leaves. Gas exchange was measured in two successive

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experiments in which the growth conditions and the treatments were identical. In the first experiment, soon after flag leaf expansion (six weeks after salinity being imposed) photosynthesis was assessed for 5 successive weeks and in one day (70 days after salinization) it was measured 6 times from 0800 hour to 1800 hour every two hours (a photoperiod 0700 to 2100 hour). In the second experiment with the same cultivars and the same levels of salinity, plants were subjected to four Photon Flux Density (PFD) regimes after flag leaf fully developed, using 600 µmol photon m^{-2} s^{-1} (high light treatment), 225 µmol m^{-2} s^{-1} (intermediate), 150 µmol m^{-2} s^{-1} (low) and 75 µmol m^{-2} s^{-1} (very low), with gas exchange rates being evaluated. The plants were light adapted 5 minutes prior to gas exchange measurements. The overall photosynthesis results are presented in three groups namely: (i) mean flag leaf photosynthesis (ii) flag leaf net photosynthesis during different times of the day, and (iii) gas exchange rates in different photon flux densities. The photosynthesis and stomatal conductance data employed for statistical analysis were the averages of four replications.

Stomatal conductance (g) is mostly measured on the upper surface of leaves, but in this experiment g on the lower surface was also measured. Although the overall stomatal conductance (g) is measurable through LCA3, but for separating g measurement on leaf sides, g an either of the upper and lower sides of the youngest fully expanded flag leaf was assessed using a digital AP4 porometer (Delta-T Devices, Burwell, Cambridge, UK). All porometer readings were made in the middle of each leaf. Stomatal conductances of six samples per replication per level of salinity and per cultivar were measured. Intercellular CO_2 concentration (C_i) is automatically calculated by LCA machine. The Equation for calculation of C_i is:

$C_i = [(g_c - E/2) \times C_a - A)]/(g_c - E/2)$ (Von Caemmerer and Farquhar, 1981),

Where g_c is the gas phase of the leaf conductance (boundary layer conductance and stomatal conductance), C_a is the partial ambient pressure of CO_2, A is net photosynthesis, and E transpiration rate (Long and Hallgern, 1993).

Leaf chlorophyll fluorescence measurements were conducted on flag leaves using the Plant Efficiency Analyser (PEA) (Hansatech, Norfolk, England). Determination of F_v/F_m ratios were made on six dark adapted leaves in all treatments. Leaves were dark adapted for 30 minutes prior to fluorescence measurements.

Respiration rate was evaluated through LCA3 after photosynthesis was measured in each leaf. The chamber was darkened by being covered with aluminum foil and respiration rate calculated using the same equation as for photosynthesis, but the CO_2 consumption was found out as negative.

Leaf chlorophyll content was measured as based on Association of Official Analytical Chemists (1975). The data were subjected to balanced analysis of variance through Minitab Statistic Software for Windows version 9.2 (Minitab Inc., 3081 Enterprise Drive, PA 16801-3008, USA). Standard error (SE) of significant treatments was calculated by dividing standard deviation by the second root of the degree of freedom (df) for each particular treatment. All regression lines were fitted by using the Scientific Figure. Processor Software (Figure P Software Corporation, Durham, NC, USA).

RESULTS

Figure 1 (a) shows that flag leaf assimilation rates of the three wheat cultivars at the four levels of salinity decreased with increasing salt concentration up to 200 mol m^{-3}. Analysis of variance data revealed significant differences in A amongst cultivars at 100 mol m^{-3} only (in CR higher than in the others).

Adverse effects of salinity on A were associated with a significant ($P < 0.001$) decrease in the stomatal conductance (g) up

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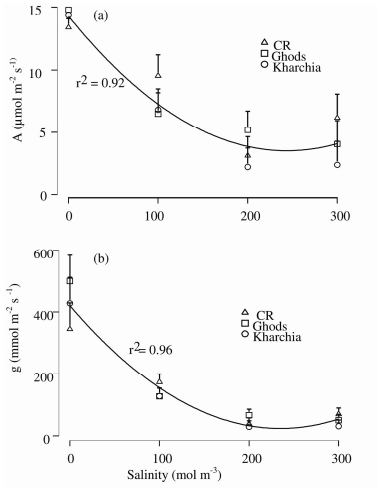

Figure 1. Photosynthesis (A) and stomatal conductance (g) of flag leaves of three wheat cultivars at four levels of salinity. Values represent the mean rate of gas exchange for 8 measurements (two measurements in each replication) made on flag leaf. Vertical lines are confidence intervals of mean values of cultivars.

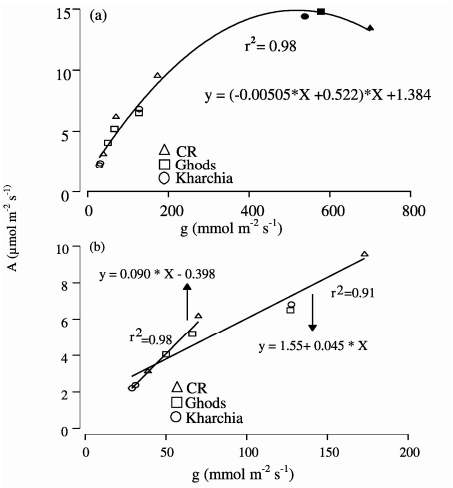

Figure 2. Relationship between net photosynthesis (A) and stomatal conductance (g), in wheat cultivars at different levels of salinity. Part (a) includes control and all other levels of salinity, while part (b) shows just three levels of salinity. Short line in part (b) shows the data of 200 and 300 mol m^{-3}. Each point represents the average of 8 measurements (two measurements in each replication). Solid markers refers to control and while ones to saline conditions.

to 200 mol m^{-3} salinity but as with *A*, no significant differences in *g* were observed between 200 and 300 mol m^{-3} salinity (Figure 1 (b)). Stomatal conductance in control plants was almost three times that at the low level of salinity (100 mol m^{-3}). At higher levels of salinity, stomatal conductance was very low (Figure 1(b)). The fitness of line fitted by least squares quadratic regression for stomatal conductance against salinity levels was highly significant (Figure 1(b)).

The nearly similar trend of reduction in stomatal conductance and in photosynthesis in parts (a) and (b) of Figure 1 suggests a strong correlation between *A* and *g* (r= 0.98). Quadratic equations fitted to the rate of CO_2 uptake and g in Figure 2 (a), indicates that A increased linearly up to 500 mmol m^{-2} s^{-1} of *g*, after which the slope of the regression line was not significantly different from zero. The data for gas exchange in control conditions were the cause of this type of curve for *A* and *g* relationships. Thus, when gas exchange data of salinity treatments only, were plotted against stomatal conductance (Figure 2 (b)) the regression line became linear and as the level of salinity increased, the slope of regression line between *A* and *g* increased too.

As shown in Figure 3, for both sides of the leaves g was strongly reduced by salinity from control values. It was reduced from more than 500 mmol m^{-2} s^{-1} to less than 100 mmol m^{-2} s^{-1} at 300 mol m^{-3} of salinity. The mean lower side stomatal conductance of the leaf was at least four times that at the upper surface of the leaf (Figure 3). CR cultivar had the highest upper g in control conditions and with increasing salt concentration, it still had the greatest *g* (but not significant) amongst all the three cultivars. The ranking of cultivars with regard to leaf lower surface stomatal conductance was different from that of the upper, e.g. salt-tolerant cultivar, Kharchia, which had the least upper *g*, showed the greatest lower g in control as well as in 100 mol m^{-3} as salinity compared with other

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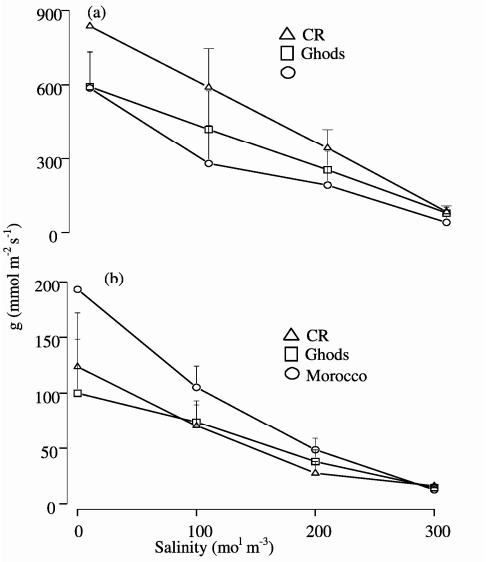

Figure 3. Stomatal conductance (g) of upper (a) and lower (b) sides of the flag leaf in three wheat cultivars at four levels of salinity. Each point represents the average of 12 measurements (three measurements in each replication). Vertical lines represent confidence intervals of mean values of cultivars.

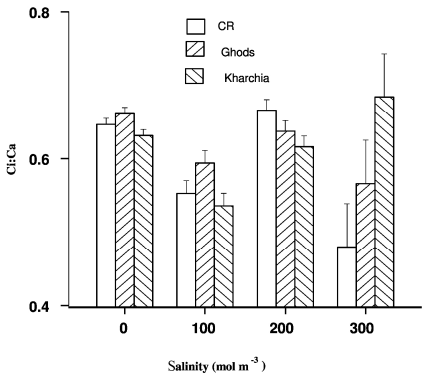

Figure 4. Ratio of intercellular to ambient CO_2 concentration (C_i/C_a) of flag leaves of wheat cultivars at different levels of salinity. Each column is the average of 8 measurements (two measurements in each replication). Vertical lines are confidence intervals of mean values of cultivars.

cultivars. At 100 mol m^{-3} salinity, g_s on the lower side of the leaf in CR, Ghods and Kharchia were 70, 74 and 105 mol m^{-2} s^{-1}, respectively. These results indicated that g in the lower surface of the leaf in Kharchia was higher in control as well as in the low level of salinity treatment (Figure 3).

The ratio of intercellular to ambient CO_2 concentrations, C_i/C_a, decreased at 100 mol m^{-3} salinity as compared to control, but in spite of decreasing A and g with increasing levels of salinity (Figure 1), C_i/C_a did not shows significant reduction at 200 mol m^{-3} salinity as compared with control (Figure 4). In control conditions Kharchia showed a lower C_i/C_a, while at 100 mol m^{-3} Ghods showed a higher C_i/C_a ratio than the other cultivars, whilst at 200 mol m^{-3} salinity, C_i/C_a did not significantly differ amongst cultivars. At 300 mol m^{-3} of salinity, Kharchia and CR showed the highest and lowest C_i/C_a ratios, respectively. At 300 mol m^{-3}, Kharchia cultivar carried a higher C_i/C_a ratio than CR and Ghods. The relationships between C_i/C_a and photosynthesis, and between C_i/C_a and stomatal conductance are shown in Figure 5. A positive correlation was found between C_i/C_a and photosynthesis (r= 0.85), between C_i/C_a and g (r^2= 0.86).

The ratio of variable to maximum fluorescence (F_v /F_m) was significantly reduced with increase in salinity levels, it did but not however varybetween control and 100 mol m^{-3}. Maximum level of fluorescence (Fm) and the time at which maximum fluorescence occurs were also different in different salinity treatments than in control F_m being lower in salt treated cultivars (Table 1). In almost all levels of salinity, cultivars Kharchia and CR carried the highest and the lowest ratios of F_v /F_m, respectively.

Figure 6 shows gas exchange measurement in three levels of salinity in salt-sensitive (Ghods) and salt-tolerant (CR) cultivars at different times of the day (1 hour after illumination to 3 hours before lighting

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Table 1. Salinity levels and different genotypes vs. ratio of variable to maximum chlorophyll fluorescence (Fv/Fm), minimum fluorescence level (Fo), maximum level of fluorescence (Fm), variable level of fluorescence (Fv) and life time (T) of fluorescence. Salinity is in mol m-3 in the nutrient solution, and 0 indicating control. LSD refers to Least Significant Difference of means, in 95% probability.

Salinit	Cultivar	F_o	F_m	F_v	T	F_v/F_m
0	CR	571.8	3628	3055	352.2	0.842
0	Ghods	582.3	3909	3327	405.0	0.850
0	Kharchia	513.1	3748	3236	360.8	0.862
	LSD	37.3	140.99	102.00	28.3	0.010
100	CR	579.8	3773	3193	300.0	0.845
100	Ghods	571.3	3857	3290	314.3	0.852
100	Kharchia	507.3	3545	3038	328.8	0.855
	LSD	39.6	217.70	127.00	14.4	0.005
200	CR	592.8	3621	3026	289.0	0.835
200	Ghods	582.0	3756	3174	303.7	0.844
200	Kharchia	528.0	3502	2974	298.0	0.849
	LSD	34.7	131.70	103.70	3.3	0.007
300	CR	596.0	3646	3049	266.0	0.838
300	Ghods	611.8	3777	3166	256.0	0.837
300	Kharchia	535.1	3536	3001	269.0	0.848
	LSD	40.5	120.65	84.90	6.8	0.006

off). In both CR and Ghods, rates of gas exchange in control and in 100 mol m^{-3} did not change during the early hours of the day length, whilst during the last hours of the day, photosynthesis in Ghods (the sensitive cultivar) was markedly reduced. At 200 mol m^{-3} salinity, overall rate of gas exchange was slightly less than those in control and in 100 mol m^{-3}, (parts a, b and c of Figure 6) while the sensitive cultivar again showed a marked reduction in gas exchange rate during the last hours of the day.

Leaf content of chlorophyll a, chlorophyll b, and total chlorophyll increased with increasing salt concentration above 50 mol m^{-3} in all wheat cultivars, but there were no significant differences observed among 100, 150 and 200 mol m^{-3} salinity levels (Figure 7). Amongst the cultivars, Ghods was of the highest chlorophyll content at high salinity level (Figure 7). The ratio of chlorophyll a/b was significantly greater in control than in saline conditions but it did not differ between control and the low level of salinity. Leaf total chlorophyll and photosynthesis were negatively correlated in the presence of salinity. This might be because of the negative effect of salinity on rate of photosynthesis (A), *vs.* its positive effect on leaf thickness which results in a higher chlorophyll content per unit leaf area.

The light response curve of *A* showed no significant differences between control and the treatment 100 mol m^{-3}, but at 200 mol m^{-3} *A* was markedly lower than control at all photon flux densities (Figure 8). There were no significant differences observed between cultivars to photon flux density at non saline conditions, while at 100 mol m^{-3} salinity; Kharchia had a significantly lower net photosynthesis than the other two cultivars (Figure 8). In control and low level of salinity, light saturation point was 600 µmol m^{-2} s^{-1} or more, while at 200 mol m^{-3} salinity, leaves approached their light saturation point at 225 µmol m^{-2} s^{-1} except in CR cultivar in which net photosynthesis

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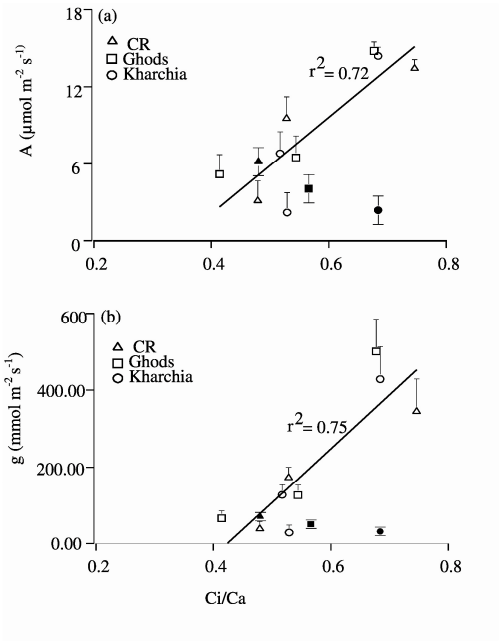

Figure 5. The relationship between intercellular to ambient CO_2 concentration (C_i/C_a), photosynthesis (A) and stomatal conductance (g) of three wheat cultivars in the presence of salinity. Each point is the average of 8 measurements (two measurements in each replication). Solid symbols represent high level of salinity and open symbols are control, 100 and 200 mol m^{-3}. Vertical lines represent confidence intervals of mean values of cultivars.

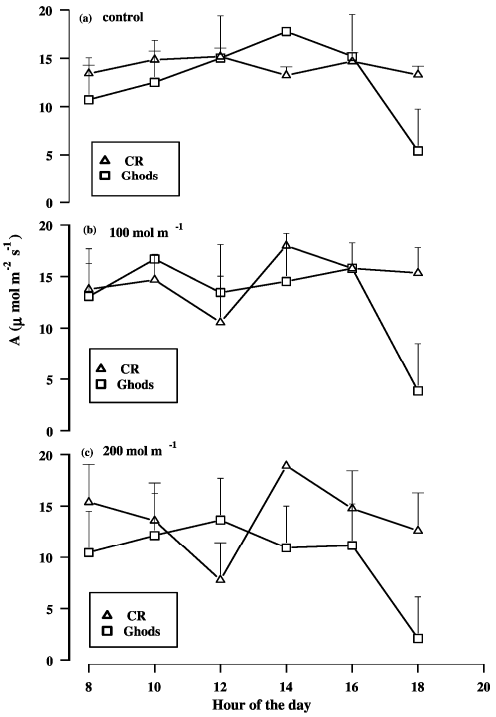

Figure 6. Photosynthesis (A) of salt tolerant (CR) and salt-sensitive (Ghods) wheat cultivars in control, 100 and 200 mol m^{-3} salinity during different hours of the day. Each point represents 12 measurements (three measurements in each replication) and vertical lines representing confidence intervals of mean values of cultivars.

increased up to 600 µmol m^{-2} s^{-1}. At the high level of salinity CR exhibited a better light use efficiency than the other cultivars (Figure 8).

Respiration remained unchanged up to 200 mol m^{-3} salinity but decreased markedly at 300 mol m^{-3} (Figure 9 (a)). Cultivars showed different rates of respiration in the presence of salinity. Cultivar Kharchia had the lowest and CR the highest respiration rates amongst cultivars at all the salt treatments. Respiration was quite different at different hours of the day. The most remarkable change in respiration rate was at 1400 hour, (7 hours after light) when CO_2 production was much higher than at the other times of the day in all the three cultivars (Figure 9 (b)). The amplitude of changes in respiration, during the day in CR, was less than those in Ghods and Kharchia (Figure 9 (b)).

DISCUSSION

Long-term exposure of wheat plants to salinity depressed the rate of net CO_2 assimilation (A) and decreased stomatal conductance (g). These observations are in agreement with those of Ouerghi *et al.* (2000) who reported that in wheat and at 100 mM (100 mol m^{-3}) of NaCl salinity, decreases in stomatal conductance led to limited photosynthesis. Also Kasai *et al.* (1998) reported a reduction in net photosynthesis in wheat (cultivar Chinese Spring) at 0.4 M (400 mol m^{-3}) of NaCl salinity.

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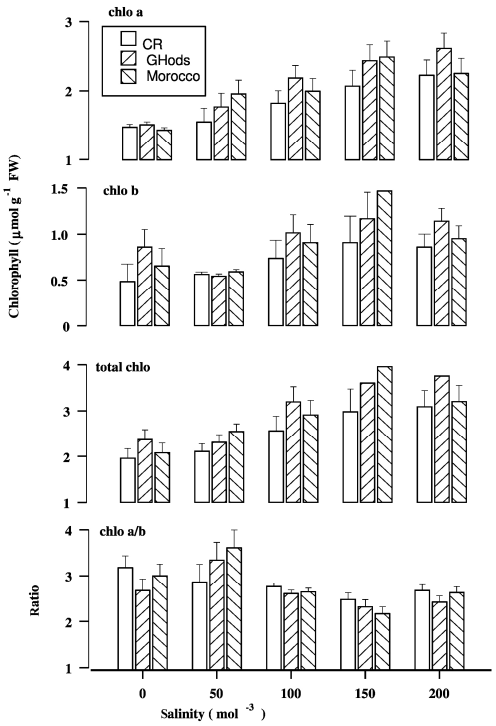

Figure 7. Chlorophyll a, b, total, and a/b ratio in wheat cultivars and in different levels of salinity. Each bar represents 6 measurements. Lines above the bars represent the confidence intervals of mean values of cultivars. Symbols are the same for all parts of the Figure.

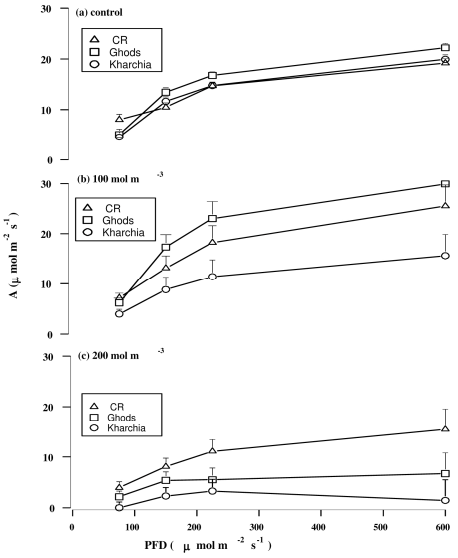

Figure 8. Photosynthesis (A) in flag leaves of plants of wheat cultivars grown at control, 100 and 200 mol m^{-3} salinity and different Photon Flux Densities (PFDs). Each point represents the average of eight measurements (two measurements in each replication). Vertical lines are confidence intervals of mean values of cultivars. Symbols are applicable for all parts of the Figure.

The lack of significant effect of salinity on rate of photosynthesis *A*, between 200 and 300 mol m^{-3}, was possibly due to the face that *g* at 200 mol m^{-3}, is almost equal to *g* at 300 mol m^{-3}, and this degree of stomatal closure is quite enough to reduce photosynthesis to its very low rates. Net photosynthesis in control conditions (Figure 1) revealed that genotypes do not have significant differences in gas exchange charactersistics. Results also indicated that at the low level of salinity (100 mol m^{-3}) the impact of salinity on *A* of cultivar CR was lower than that on the other cultivars.

The relationship between *A* and *g* was curvilinear in control and salinity treatment measurements (Figure 2 (a)). The initial rapid increase in *A* with increase in *g* indicates that non-stomatal parameters did not limit the gas exchange rate (Ouerghi *et al.*, 2000, Netondo *et al.*, 2007)) but in the inflection to the slower rise the non-stomatal parameters might have acted as limiting factor. In stressed plants, particularly at high levels of salinity, the relationship between *A* and *g* changed to linear which means that non-stomatal parameters did not limit net photosynthesis (Figure 2 (b)) (Kasai *et al.*, 1998). Several authors have found that when concentration of CO_2 in the substomatal cavity is low (low g), RuBP carboxylase was at its maximum activity (Munns *et al.*, 2006; Netondo *et al.*, 2007).

Stomatal conductance of the upper and lower leaf surfaces of wheat cultivars was markedly different. Kharchia, which had the lower *g* on the flag leaf upper surface, had the greatest *g* on the lower surface. Having higher *g* in the lower surface of the leaf

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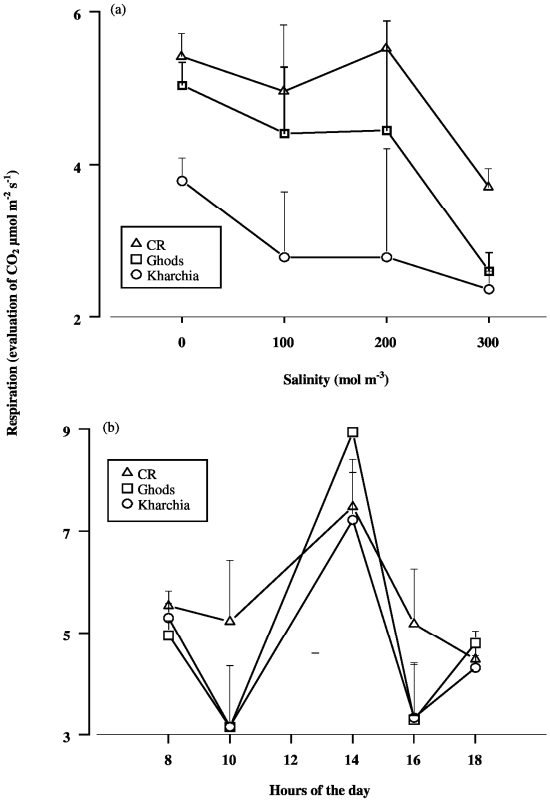

Figure 9. Respiration (R), in flag leaves of three wheat cultivars at four levels of salinity (a) and hours of the day (b). Each point represents 8 measurements (two measurements in each replication). Vertical lines are representative of confidence intervals of mean values of cultivars.

might be a beneficial mechanism to cope with stresses like salinity and drought, because in field conditions boundary layer conductance and temperature on the upper surface are higher (due to ventilation and to direct illumination by sunlight) than those on the lower surface. So the leaf has to spend more energy and water to lower its temperature on the upper side, while gas exchange on the lower side does not meet these limitations to the same extent. Reduction in *A* as a consequence of non-stomatal inhibition of *A* by salt has also been observed (Munns *et al.*, 2006; Netondo *et al.,* 2007). These alterations in capacity must be the result of either a change in the leaf content of photosynthesis operating system and/or an alteration in the efficiency with which this system is operated.

Total chlorophyll content (in all cultivars under saline conditions) demonstrated alterations that were directly proportional to changes in both chlorophyll a and b. This result is in agreement with that in the findings of Krishnaraj *et al.* (1993) in two wheat cultivars and two levels of salinity, while Yeo *et al.* (1985) did not observe any change in concentrations of chlorophyll at an Na^+ concentration which reduced photosynthesis up to 50%. Observations by De Herralde *et al*. (1998) indicated a reduction in the photosynthetic rate and chlorophyll content in *Agyranthemum coronopifolium* under 140 mmol NaCl.

A negative relationship (r^2= 0.77) was observed between *A* and total chlorophyll in all wheat cultivars and at all levels of salinity, probably because photosynthesis decreases while chlorophyll content increases with increase in salt concentration. Thus, because assimilation rate is expressed per leaf area, and chlorophyll concentration per unit leaf area increased in plants in saline conditions, chlorophyll content could not be considered as a limiting factor on photosynthesis in the presence of salinity. Other non-stomatal factors responsible for photosynthesis reduction in the presence of salinity could be RuBP carboxylation (mesophyll conductance) and regeneration (Belkhojda *et al.*, 1999; Ouerghi *et al.,* 2000).

A lower F_v/F_m in the salt stressed conditions as compared to control indicates that RuBP regeneration, which needs adequate electron translocation from PS II to electron acceptors, might be disturbed by salinity. However, in this work although there were significant differences in F_v/F_m in different levels of salinity, values of F_v/F_m were relatively high for both control and salt stress treatments (0.83-0.86) (Table 1). Similar values of F_v/F_m were found by Belkhodja, *et al.* (1994; 1999) for barley, grown in the presence of salinity. Although cultivar Kharchia had a significantly higher F_v/F_m than the other cultivars, this difference

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was mainly because of its lower F_m (Table 1).

A part of growth reduction due to salinity is a direct consequence of stomatal closure and a reduction in the C_i (Ouerghi *et al.*, 2000; Munns *et al.*, 2006; Netondo *et al.*, 2007). The reduction in g under salt stress is estimated to be substantial in spinach and *Phaseolus vulgaris* with the intercellular CO_2 concentration reduced by up to 30% (Seemann and Critchly, 1985). In this experiment C_i/C_a in up to 200 mol m^{-3} of salinity had a reasonable negative correlation with net photosynthesis and stomatal conductance. However at 300 mol m^{-3}, the C_i/C_a actually increased, despite reduction in *A* and *g*. Similar results were observed by other researchers (personal communication with G. D. Farquhar). The possible explanation for increasing C_i/C_a at this level of salinity might be higher C_a and lower *A* leading to overestimation of C_i in the presence of high salinity. The assumption in the calculation of C_i is that conductance is uniform across the leaf, but in severe water stress, closure of stomata in patches has been observed (Downton *et al.,* 1988). In this experiment (because of severe physiological water stress) this phenomenon may have occurred at high levels of salinity, for clarification of which further work needs to be done in the area.

One might expect that *A* in the control conditions would not significantly change during the day. But in this study the results of gas exchange measurement in different hours of the day from 0800 to 1800 hour showed that *A* changed even in quite similar growth conditions in control as well as in salinity treatments. In the later hours of the day gas exchange rate was depressed by salinity particularly in the salt-sensitive cultivar (Figure 6). Temporal gas exchange variation might be due to carbohydrate content in the leaves at different hours of the day. For instance carbohydrate content of wheat leaves was raised in the last hours of the day (Kafi, 1996)

The results of measurement of *A* at different Photon Flux Densities (PFDs) revealed that at high salinity the light saturation point for wheat cultivars decreased. It might be that at high salinity, photon flux density more than 300 µmol m^{-2} s^{-1} in cultivars Kharchia and Ghods might not have been a main photosynthetic limiting factor, therefore, the reduction in photosynthesis might have been due to other limiting factors. In contrast, in photon flux density lower than 150 µmol m^{-2} s^{-1}, light could be the main limiting factor and even at high salinity level, genotypes may not be able to show their capability of CO_2 fixation (Figure 8). However, at low PFD, plants in non saline environment could have some carbon fixation, but at a high level of salinity (because of high respiration) the rate of net photosynthesis was limited (Figure 8 (c)). For instance photosynthesis of Ghods at 75 µmol photon m^{-2} s^{-1} was 5 µmol of CO_2 m^{-2} s^{-1} in control, while it was only 2 µmol CO_2 m^{-2} s^{-1} at 200 mol m^{-3} salinity (Figure 8).

The rate of respiration decreased at the high level of salinity, which is not in agreement with McCree's (1986) results who reported that in many species respiratory rate, at zero growth rate, increased with increasing salt concentrations. Kasai *et al.* (1998) also reported an increase in wheat respiration under salinity stress. At 300 mol m^{-3}, growth respiration might be lower than that in control and than those at low levels of salinity, but in low and medium levels of salinity the excess energy may be spent on ion influx to maintain cell turgor, secretion, and/or repair of cellular damage caused by salt toxicity, which needs a supply of respiratory energy (Schwarz and Gale, 1981; McCree, 1986; Munns *et al.*, 2006; Netondo *et al.*, 2007).

Salt-tolerant wheat cultivar Kharchia had a lower Respiration (R) than the rest, suggesting that this cultivar had a lesser utilisation of respiratory substrates because of less need for cellular repair. Total respiration (R) may be divided into two components: (i) growth *R* which represents the spending associated with the production of new biomass and (ii) maintenance *R* which represents the spending associated

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with maintaining the existing tissues. Because *R* measurements were made in fully expanded flag leaf, it might reflect maintenance *R* rather than growth *R*. However, CR which is a salt-tolerant cultivar and its net photosynthesis rate more than the other cultivars, had the highest *R* rate amongst cultivars. A possible explanation for this result might be that the developmental stage of CR lagged behind the other cultivars. Therefore, at sampling time, its flag leaf might still have been growing and the *R* measurement being the sum of both growth and maintenance *Rs*. A similar result was observed by Averill and Rees (1995) in wheat plants.

Respiration rate of wheat leaves was appreciably higher after 7 hours of photosynthesis than any time during the day, which agrees with the results obtained by Azcon-Bieto *et al.* (1983). They also found that in wheat, after 6 hours of photosynthesis, CO_2 production in the dark was 160% of that at the end of the night but for O_2 uptake the figure was only 33%. They observed that leaf sugar content was higher following 6 hours of photosynthesis as compared with that at the end of the night. Averill and Rees (1995) also reported an increasing respiration rate after 7 hours of respiration in wheat plants, accompanied by an increase in glucose 6-phosphate.

The results showed that: of the cultivars investigated, Kharchia exhibited a higher performance at the highest level of salinity, but at low salinity CR had a higher photosynthesis rate along with the related parameters. CR also exhibits a better photosynthetic performance in interaction effects of light and salinity. Changes in relationship amongst *A*, *g*, C_i/C_a, and F_v/F_m, beyond a salinity threshold (200 mol m^{-3} in these experiments) might be because of breakdown of some kind of tolerance mechanisms in different wheat cultivars. Photosynthesis in the sensitive cultivar significantly declined in the later hours of the day, which suggests that even with the same photosynthetic rate in the first hours of the day, dry matter production in this cultivar would be reduced in the presence of salinity. Therefore when measuring rates of photosynthesis in saline conditions, method of calculation of *A*, time of measurement during the day, duration of salinity exposure, level of salinity, PFD and growth stage should be taken into consideration.

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اثرات تنش شوری و شدت نور بر فتوسنتز، تنفس و فلورسنس کلروفیل ارقام مقاوم و حساس به شوری گندم (*Triticum aestivum*)

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چکیده

در این پژوهش تنوع داخل گونه ای در پارامترهایی مانند فتوسنتز، هدایت روزنه ای، فلورسنس کلروفیل و تنفس کوتاه مدت و دراز مدت در گندم بهاره (*Triticum aistivum*) در پاسخ به سطوح مختلف تنش شوری بررسی شد. در این آزمایش دو رقم گندم مقاوم به شوری (یک کراس روشن و خارچیا) و یک رقم حساس به شوری (قدس) به عنوان کرت فرعی و چهار سطح شوری شاهد، ۱۰۰، ۲۰۰ و ۳۰۰ مول در متر مکعب از ترکیب کلرور سدیم و کلرور کلسیم به نسبت مولی ۵ به یک به عنوان کرت اصلی در محیط کشت ماسه و تغذیه با محلول هوگلند در گلخانه در قالب یک طرح کرتهای خرد شده با چهار تکرار اعمال شدند. نتایج نشان داد که سرعت فتوسنتز در پاسخ به تنش شوری کاهش یافت ولی بین ارقام تفاوت معنی داری در این صفت مشاهده نشد. هدایت روزنه ای سطح بالا و سطح زیر برگ مهمترین عامل کاهش فتوسنتز در شرایط تنش شوری بود. هر چند محدودیت های غیر روزنه ای مانند نسبت فلورسنس متغیر به فلورسنس بیشینه نشان داد که گیاهان ممکن است در سطوح بالای شوری در معرض درجاتی از ممانعت نوری نیز قرار گرفته باشند. فتوسنتز در گیاهان شاهد و تحت تنش شوری در طول ساعات مختلف یک روز در هیچکدام از ارقام تفاوت معنی داری نشان نداد ولی در ساعات پایانی روز فتوسنتز رقم حساس به شوری کاهش یافت. تنفس نیز تا سطح شوری ۲۰۰ مول در متر مکعب بدون تغییر باقی ماند ولی در سطح شوری ۳۰۰ مول به طور معنی داری کاهش یافت. مهمترین تغییر در سرعت تنفس ۷ ساعت بعد از شروع تابش روزانه رخ داد که در این ساعت میزان گاز کربنیک تولیدی دارای بیشترین میزان بود. نتایج نشان داد که هر چند سرعت فتوسنتز به خوبی با تغییرات هدایت روزنه ای همبستگی دارد ولی ارقام گندم در سطوح مختلف شوری به جنبه های دیگر مرتبط با فتوسنتز نظیر مراحل مختلف رشد، زمان روز و مدت زمان قرار گرفتن در معرض تنش خشکی نیز واکنش نشان می دهند.

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