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Photosynthetic parameters at the vegetative stage and during grain development of two hexaploid wheat cultivars differing in salt tolerance

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Abstract

Response of two spring wheat (*Triticum aestivum* L.) cultivars, salt tolerant SARC-I and salt sensitive Potohar, to different concentrations of NaCl was examined under glasshouse conditions. Eighteen-day-old plants of both the lines grown in sand culture were irrigated with 0 (control), 80, 160 or 240 mM NaCl in full strength Hoagland's nutrient solution. Shoot fresh and dry masses, and leaf area per plant of SARC-I at the vegetative stage, were significantly greater than those of cv. Potohar at higher salt concentrations, however, relative growth rate (RGR) of cv. Potohar was significantly higher than that of SARC-I. SARC-I had higher net photosynthetic rate (P_N), stomatal conductance (g_s) and transpiration rate (E) than cv. Potohar at the vegetative stage, but the cultivars did not differ significantly in water-use efficiency (P_N/E), intrinsic water use efficiency (P_N/g_s), and intercellular/ambient CO_2 concentration ratio. At the grain development stage, SARC-I had significantly higher P_N and g_s in the flag leaf than cv. Potohar under salinity. SARC-I was superior to cv. Potohar with respect to number of grains per spike, number of grains per spikelet, mean grain mass, and grain yield per plant at all NaCl concentrations.

Additional key words: salinity, stomatal conductance, transpiration, *Triticum aestivum*, water use efficiency.

Introduction

Photosynthesis is a primary process in plant productivity and a positive relationship between photosynthesis and yield under saline conditions has been found in different crops, *e.g.*, *Zea mays* (Crosbie and Pearce 1982), *Spinacia oleracea* (Robinson *et al.* 1983), *Phaseolus vulgaris* (Seemann and Critchley 1985), *Vigna mungo* (Chandra Babu *et al.* 1985), *Gossypium barbadense* (Cornish *et al.* 1991), *Gossypium hirsutum* (Pettigrew and Meredith 1994), and *Asparagus officinalis* (Faville *et al.* 1999). In contrast, there are many species in which no or little association between growth and photosynthesis has been reported, *e.g.*, *Hibiscus cannabinus* (Curtis and Läuchli 1986), *Trifolium repens* (Rogers and Noble 1992), *Triticum aestivum* (Hawkins and Lewis 1993, Ashraf and O'Leary 1996).

Decline in photosynthesis due to salinity stress could be due to lower stomatal conductance, depression in carbon uptake and metabolism, inhibition in photochemical capacity, or a combination of all these factors (Seemann and Critchley 1985, Dubey 1997). Varying relationships between photosynthesis and growth or yield of salt tolerant and salt sensitive lines of some species have been reported. For example, Kumar *et al.* (1999) found that under salt stress, net CO_2 assimilation rate, substomatal conductance and transpiration rate was declined more in salt sensitive mulberry cultivar than in salt tolerant one. Recently, Ashraf (2001) has reported that two amphidiploid salt tolerant species, *Brassica napus* and *B. carinata* had higher net photosynthetic rate, water use efficiency and intrinsic water use efficiency than those of their diploid relatives but there was little association of the tolerance of these species with stomatal conductance, leaf water potential or transpiration rate.

In view of the contrasting reports it was hypothesized that the growth of two spring wheat lines differing in salt tolerance may exhibit differing responses with respect to their photosynthetic capacity. Thus the present study was conducted to elucidate whether higher growth of salt

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Abbreviations: c_a - ambient CO_2 concentration; c_i - internal CO_2 concentration; E - transpiration rate; g_s - stomatal conductance; LAR - leaf area ratio; NAR - net assimilation rate; P_N - net photosynthetic rate; RGR - relative growth rate; WUE - water use efficiency.

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tolerant wheat cultivar compared to salt sensitive one under salinity was due to its higher water use efficiency and gas exchange related characteristics.

Flag leaf is known to contribute to a large extent to grain development as compared to the other leaves of the same plant (Tripathi and Purohit 1971, Joshi *et al.* 1982, Mahmood *et al.* 1991). It is presumed that different rate of photosynthesis of the flag leaf from anthesis to gram filling in these cultivars may also contribute to their different yield under NaCl stress.

Materials and methods

Seeds of salt tolerant cultivar SARC-I and salt sensitive Potohar of spring wheat (*Triticum aestivum* L.) were sown in November 1999 in wet sand contained in plastic trays. After 10 d of germination twelve 10-d-old seedlings of uniform size of each cultivar were transplanted equidistantly into each pot measuring 22 cm in diameter and 22.5 cm in depth. Each pot contained 7.52 kg sand. Hoagland's nutrient solution was applied weekly to each pot until day 8 of transplantation, after which time varying NaCl treatments were started stepwise in aliquots of 80 mM every day. There were four NaCl treatments, *i.e.*, 0 (control), 80, 160 and 240 mM in full strength Hoagland's nutrient solution. The appropriate treatment solution was applied every week (2 dm^3 to each pot). This volume was sufficient to flush through the salts already present in the sand. During the week distilled water (200 - 300 cm^3) was applied to each pot daily to keep the sand moist and hence to maintain the salt level.

Two plants were harvested from each pot sequentially after every two weeks after the start of salt treatment, and four such harvests were taken. Every time plants were uprooted carefully, washed with distilled water and fresh mass of shoots was recorded. Plant samples were dried in an oven at 65 °C to constant dry mass. The remaining plants were maintained until maturity to record grain yield. After measuring leaf area, leaf area ratio (LAR) was determined as the ratio of leaf area per plant to dry mass of shoot. Relative growth rate (RGR) was determined using the formula of Shennan *et al.* (1987): $RGR = (1/M) \times (\Delta M/\Delta t)$, where M was dry mass of shoots at initial harvest, ΔM was the difference between M at final harvest and M at initial harvest, and Δt was number of days between initial and final harvest. Net assimilation rate (NAR) was calculated using the formula: $(M_2 - M_1) \times (\lg^\circ L_2 - \lg L_1)/(t_2 - t_1) \times (L_2 - L_1)$, where M_2 and M_1, and L_2 and L_1 were dry masses of shoots and leaf areas at second and first harvests, respectively, and $t_2 - t_1$ was the number of days between first and second harvest. At maturity grain yield and yield components were recorded.

Just before each harvest during the vegetative growth, measurements of net photosynthetic rate, transpiration rate and stomatal conductance were made on a fully expanded 3rd leaf of each plant using a gas exchange open system (*Analytical Development Company*, Hoddesdon, England). These measurements were made from 10:00 to 13:00 under following conditions: ambient temperature ranged from 25.6 to 32.7 °C, temperature of leaf ranged from 26.2 to 31.9 °C, ambient CO_2 concentration (c_a) was 343.5 μmol mol^{-1}, leaf chamber gas flow rate 385.49 cm^3 min^{-1}, photosynthetically active radiation (PAR) at leaf surface was up to 1332 μmol m^{-2} s^{-1}. The gas exchange characteristics were also recorded on alternate days from anthesis to grain filling. However, the conditions were slightly different: mean ambient temperature 34.7 °C, c_a 324.2 μmol mol^{-1}, temperature varied from 32.5 to 40.5 °C, gas flow rate 396 cm^3 min^{-1}, PAR up to 1431 μmol m^{-2} s^{-1}.

The data of each variable were subjected to a two-way analysis of variance using a *COSTAT* computer package (*Cohort Software*, Berkeley, USA) and Duncan's Multiple Range Test was used to compare the mean values following Steel and Torrie (1980).

Results

There was a marked reduction in shoot fresh and dry masses, and leaf area per plant at the higher two NaCl concentrations in both cultivars at the vegetative stage (Table 1). Comparison of the cultivars showed that SARC-I had significantly greater shoot fresh and dry masses, and leaf area per plant than cv. Potohar at the higher two NaCl concentrations. In contrast, RGR was higher in cv. Potohar than that in SARC-I under all salt concentrations. The cultivars did not differ significantly in LAR and NAR.

Net photosynthetic rate (P_N), stomatal conductance (g_s), and transpiration rate (E) declined significantly in both cultivars with increase in NaCl concentration. At the vegetative stage, SARC-I had higher P_N, g_s, and E than cv. Potohar, but the cultivars did not differ significantly for water-use efficiency (WUE = P_N/E), intrinsic water use efficiency (P_N/g_s) and relative intercellular CO_2 concentration (c_i/c_a) (Fig. 1). There was a significant increase in P_N/g_s and a decrease in c_i/c_a in both cultivars with increase in NaCl concentration, but pattern of increase or decrease in WUE was not consistent.

Data for different photosynthetic characteristics for

leaves of varying age were also recorded during grain development, *i.e.*, from anthesis to grain filling. P_N of flag leaf and leaf 1 of SARC-1 were significantly higher than those of Potohar from day 6 to day 8 of grain development at the salt treatment (Fig. 2). However, the lines did not differ significantly with respect to P_N of leaf 2 throughout the entire period of grain development. A similar pattern of P_N of leaf 2 was observed in the control treatment. At salt treatment, g_s of two wheat cultivars varied only for leaf 1 since this leaf of SARC-1 had significantly higher g_s from anthesis up to day 8 of grain development (Fig. 2). In contrast, g_s of the other two leaves of the two cultivars had no consistent pattern. At control treatment, g_s of flag leaf of SARC-I was

Table 1. Different growth attributes of two spring wheat cultivars when 18-d-old plants were subjected to varying NaCl concentrations (0 to 240 mM) for 56 d. Means ± SE, $n = 4$. Means with the same letters within each column do not differ significantly at $P = 0.05$.

Parameter	Cultivar	0 mM	80 mM	160 mM	240 mM
Shoot fresh mass	SARC-I	14.12 ± 2.78 a	11.91 ± 2.36 a	9.81 ± 2.11 a	5.96 ± 1.12 a
[g plant^{-1}]	Potohar	15.83 ± 2.44 b	13.64 ± 1.76 b	8.76 ± 1.37 b	5.21 ± 1.22 b
Shoot dry mass	SARC-I	2.18 ± 0.47 a	1.98 ± 0.28 a	1.72 ± 0.24 a	1.22 ± 0.11 a
[g plant^{-1}]	Potohar	2.08 ± 0.36 a	1.96 ± 0.19 a	1.29 ± 0.11 b	0.93 ± 0.21 b
Leaf area	SARC-I	20.92 ± 2.02 a	22.94 ± 8.12 a	19.87 ± 2.36 a	16.14 ± 3.01 a
[cm^2 plant^{-1}]	Potohar	18.74 ± 1.33 b	20.28 ± 6.41 b	17.12 ± 2.29 b	13.21 ± 1.42 b
RGR	SARC-I	0.15 ± 0.01 a	0.12 ± 0.02 a	0.11 ± 0.02 a	0.12 ± 0.06 a
[g g^{-1}(d.m.) d^{-1}]	Potohar	0.21 ± 0.04 b	0.18 ± 0.05 b	0.14 ± 0.02 b	0.15 ± 0.08 b
LAR	SARC-I	10.87 ± 2.97 a	12.02 ± 1.16 a	12.62 ± 1.82 a	12.46 ± 1.87 a
[cm^2 g^{-1}(shoot d.m.)]	Potohar	10.91 ± 3.01 a	12.11 ± 1.06 a	9.22 ± 1.26 b	14.28 ± 2.16 b
NAR	SARC-I	6.54 ± 0.46 a	5.12 ± 0.92 a	5.02 ± 1.12 a	4.28 ± 0.86 a
[mg(d.m.) cm^2(leaf) d^{-1}]	Potohar	8.46 ± 1.96 b	6.62 ± 1.01 b	4.81 ± 0.88 a	4.83 ± 0.98 a

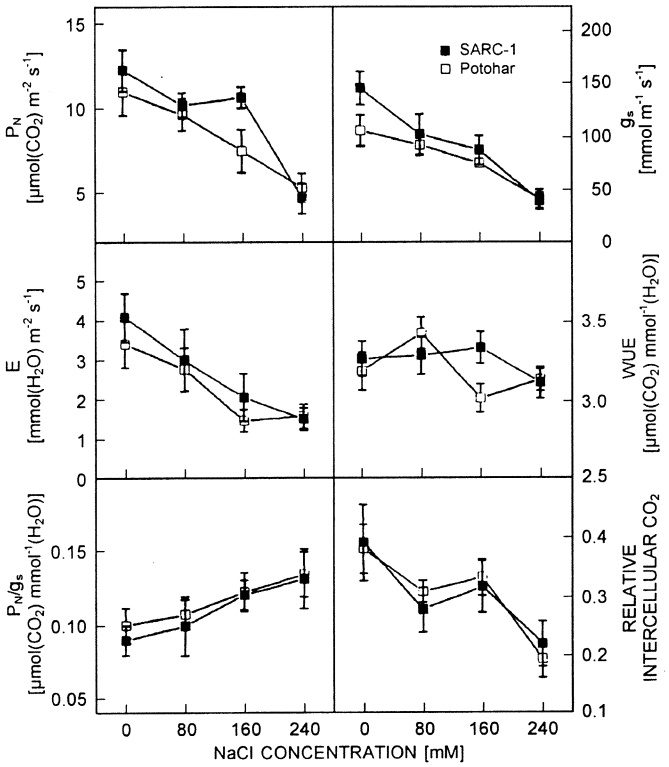

Fig. 1. Gas exchange characteristics in two spring wheat cultivar when 18-d-old plants were subjected to different NaCl concentrations for 56 d. Means ± SE, $n = 4$.

consistently higher than that of cv. Potohar throughout the entire period of grain development, but g_s of leaf 1 of SARC-I was higher than that of cv. Potohar only at the initial stages of grain development, whereas the cultivars did not differ significantly with respect to g_s of leaf 2 throughout the grain development. E of flag leaf and leaf 1 of cv. Potohar at salt treatment was higher than that of SARC-1 from day 8 to day 12, whereas the difference between the cultivars with respect to E of leaf 2 under salt stress was not consistent (Fig. 2). However at control treatment, E of flag leaf and leaf 1 of cv. SARC-1 was higher than that of cv. Potohar at most of the time intervals.

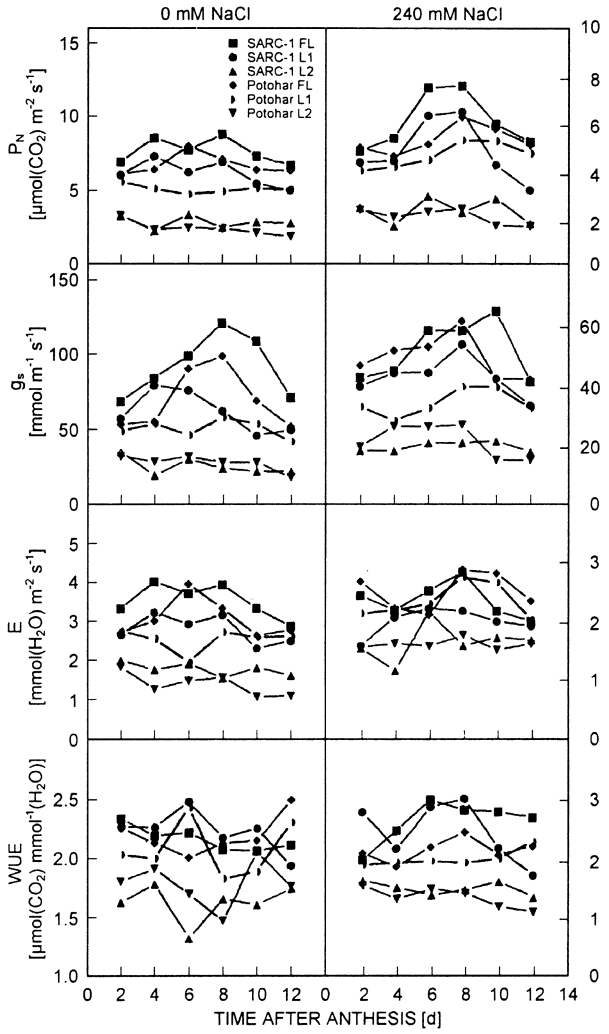

Fig. 2. Time course of P_N, g_s, E, and WUE of different leaves (FL - flag leaf, L1 - first leaf, L2 - second leaf) of two spring wheat cultivars from anthesis to grain filling.

WUE of flag leaf and leaf 1 of SARC-I under salt stress was higher than that of cv. Potohar, whereas the difference between the two lines was not possible to discern in relation to WUE of leaf 2 (Fig. 2). In contrast, in control treatment, it was not possible to discriminate between the cultivars with respect to WUE of either leaf.

Intercellular CO_2 concentration (c_i) of cv. Potohar was significantly higher than that of SARC-I for all leaves differing in age at control treatment (Fig. 3). A similar pattern was observed at salt treatment. In cv. Potohar c_i of leaf 2 was markedly higher than those of the remaining leaves. The ratio c_i/c_a of all leaves of cv. Potohar was considerably higher than that of SARC-I throughout the entire period of grain development under both saline and non-saline conditions (Fig. 3). In addition, leaf 2 of cv. Potohar had markedly greater c_i/c_a than the other leaves.

SARC-I was superior to cv. Potohar at all NaCl concentrations with respect to number of grains per spike, number of grains per spikelet, grain mass and grain yield per plant (Table 2). Cv. Potohar had greater spike length, number of tillers per plant, and number of spikelets per spike than those of SARC-I. However, the two lines did not differ significantly with respect to number of fertile tillers per plant.

Table 1. Grain yield and yield components of two spring wheat cultivars when 18-d-old plants were subjected to varying NaCl concentrations (0 to 240 mM). Means ± SE, $n = 4$. Means with the same letters within each column do not differ significantly at $P = 0.05$.

Parameter	Cultivar	0 mM	80 mM	160 mM	240 mM
Number of tillers	SARC-I	2.75 ± 0.72 a	2.08 ± 0.42 a	1.71 ± 0.54 a	1.03 ± 0.06 a
[plant^{-1}]	Potohar	2.81 ± 0.41 a	2.58 ± 0.34 b	2.23 ± 0.32 b	1.05 ± 0.08 a
Fertile tillers	SARC-I	2.75 ± 0.72 a	2.08 ± 0.42 a	1.71 ± 0.54 a	1.03 ± 0.06 a
[plant^{-1}]	Potohar	2.43 ± 0.15 a	2.38 ± 0.21 a	1.96 ± 0.16 a	1.05 ± 0.08 a
Spike length	SARC-I	11.38 ± 0.46 a	9.96 ± 0.69 a	9.85 ± 0.62 a	9.08 ± 0.69 a
[cm]	Potohar	12.39 ± 1.15 a	12.77 ± 0.46 b	11.86 ± 0.54 b	10.92 ± 0.47 b
Number of spikelets	SARC-I	13.29 ± 0.86 a	13.15 ± 0.68 a	12.29 ± 0.64 a	11.29 ± 0.57 a
[spike^{-1}]	Potohar	14.98 ± 1.14 b	14.07 ± 0.79 a	13.02 ± 0.71 a	10.93 ± 1.07 a
Number of grains	SARC-I	2.91 ± 0.29 a	2.71 ± 0.18 a	2.68 ± 0.13 a	2.31 ± 0.24 a
[spikelet^{-1}]	Potohar	2.02 ± 0.28 b	1.88 ± 0.17 b	1.53 ± 0.18 b	1.29 ± 0.20 b
Number of grains	SARC-I	39.23 ± 1.92 a	36.15 ± 3.08 a	33.85 ± 1.93 a	26.16 ± 1.46 a
[spike^{-1}]	Potohar	30.77 ± 2.69 b	26.92 ± 2.31 b	19.23 ± 1.35 b	13.85 ± 1.15 b
Grain mass	SARC-I	58.10 ± 2.90 a	55.20 ± 3.10 a	45.70 ± 3.00 a	36.20 ± 3.00 a
[mg]	Potohar	47.60 ± 3.00 b	43.80 ± 8.60 b	36.20 ± 2.90 b	24.80 ± 3.80 b
Grain yield	SARC-I	6.61 ± 0.95 a	4.22 ± 0.91 a	2.82 ± 0.92 a	1.01 ± 0.07 a
[g plant^{-1}]	Potohar	5.20 ± 0.99 b	2.95 ± 0.52 b	1.81 ± 0.41 b	0.51 ± 0.03 b

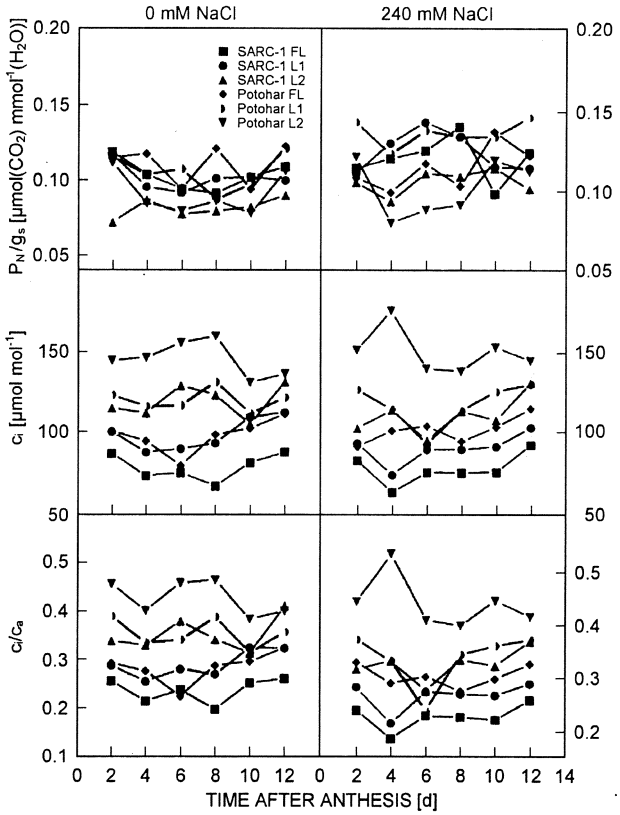

Fig. 3. Time course of P_N/g_s, c_i, and c_i/c_a of different leaves (FL - flag leaf, L1 - first leaf, L2 - second leaf) of two spring wheat cultivars from anthesis to grain filling.

Discussion

In view of the results reported above, it is evident that the SARC-I had higher salt tolerance as compared to cv. Potohar in terms of shoot fresh and dry masses, and leaf area produced at the vegetative stage, as well as grain

yield. The high salt tolerance of SARC-I was found to be associated with its relatively higher P_N under salt stress. These results are in agreement with some earlier studies on different crops in which a positive relationship between degree of salt tolerance and P_N was reported (see Introduction). The positive relationship between P_N and g_s, found here is quite parallel to what has been earlier reported in sunflower (Rawson and Constable 1980), faba bean (Melesse and Caesar 1992), and alfalfa (Anand *et al.* 2000).

Salt stress is known to inhibit photosynthesis due to either suppressed g_s or perturbance in carbon fixation or combination of both (Robinson *et al.* 1983, Bowman 1988). From the data for c_i/c_a presented here, it is evident that salinity caused a decline in c_i/c_a, uniformly in both the cultivars at the vegetative stage. This suggests that the decline in photosynthesis in both the lines at the vegetative stage occurred partially through stomatal closure.

If the two wheat cultivars are compared with respect to post-anthesis photosynthetic rate of individual leaves, it is clear that higher yield of salt tolerant SARC-I was associated with higher P_N of the first two leaves of SARC-I as compared to cv. Potohar. In leaves of different age of both the cultivars c_i/c_a remained unchanged. This suggests that stomatal conductance was not the primary factor limiting photosynthesis at the grain development stage. Constable and Rawson (1980) reported that parallel changes occur in stomatal and internal resistances during leaf ageing thereby causing c_i to remain constant despite changes in photosynthesis. Likewise, parallel changes in P_N and g_s was found in water stressed sunflower plants with c_i remaining unchanged (Krampitz *et al.* 1984).

Taken together, the higher salt tolerance of SARC-I was found to be associated with its higher photosynthetic capacity and stomatal conductance at both the vegetative and grain development stages as compared to salt sensitive cv. Potohar.

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