ACTA
PHYSIOLOGIAE
PLANTARUM
Vol. 24. No. 4. 2002: 407-415

Water relations, gas exchange characteristics, and the level of some metabolites in two cultivars of spring wheat under different N regimes

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Key words: chlorophyll contents, photosynthesis, nitrogen fertilization, water potential, wheat

Abstract

Twenty eight-day old plants of two spring wheat cultivars differing in salinity tolerance were subjected to varying levels of nitrogen (56, 112, and 224 mg N·kg^{-1} soil) for 42 days. Both cultivars performed differently under varying soil N levels in terms of growth, and grain yield and yield components. Nitrogen levels, 112 and 224 mg·kg^{-1} soil, caused maximal growth in Sarsabz and Barani-83, respectively. Cv Sarsabz maintained higher leaf water and turgor potentials, but lower leaf osmotic potential than those of Barani-83 at all external N regimes. Sarsabz had higher Chl *a*, Chl *b* and carotenoids contents in leaves than those in Barani-83 at 56 and 112 mg N·kg^{-1} soil. Sarsabz had higher contents of leaf soluble proteins, soluble sugars, and free amino acids than those in Barani-83 at all external N levels. In Barani-83 net CO_2 assimilation rate remained almost unchanged, whereas in Sarsabz it decreased consistently with increase in external N level. The better growth performance of Sarsabaz as compared to Barani-83 under varying soil N levels except 224 mg N·kg^{-1} soil was associated with maintenance of high leaf turgor potential but not with net CO_2 assimilation rate.

Introduction

The deficiency of nitrogen causes serious physiological disorders in plants. Nitrogen deficient spring wheat had slower rates of leaf emergence (Longnecker and Robson 1994). The rate of photosynthetic CO_2 fixation often shows a strong positive correlation with leaf nitrogen content (Nevins and Loomis 1970, Marschner 1995). Thus any severe reduction in leaf N content as brought about by insufficient N supply to the plants is likely to decrease photosynthesis (Dietz and Harris 1997).

Nitrogen nutrition enhances metabolic processes that influence the physico-chemical environment at the soil root interface, modifies rhizospheric conditions, interferes with cations and anions, and enhances the activity of several enzymes (Fernandes and Rossiello 1995). But under high nitrogen rate activities of PEP carboxylase and RuBP carboxylase/oxygenase decreased thereby decreasing the rate of photosynthesis (Greef 1994).

The present study was conducted to examine the effect of different levels of nitrogen fertilization on two spring wheat cultivars (Cv. Sarsabz – salt tolerant, Cv. Barani-83 – salt sensitive) in respect to photosynthetic capacity and grain yield under non-saline conditions, as most of the cultivars developed for salt affected areas are also grown on normal non-saline soils.

Materials and Methods

The experiment was carried out in a glasshouse at the Nuclear Institute for Agriculture and Biology (NIAB, Faisalabad) during winter 1999/2000. The average daylength throughout the experiment was 11.2 ± 1.1 h, light intensity 1278 ± 156 $\mu mol \cdot m^{-2} \cdot s^{-1}$, RH 56.7 % and day/night temperature 31.8/18.6 °C. Soil used for the experiment, was analyzed for the various physico-chemical characteristics in triplicate (Table 1).

Table 1. Physico-chemical properties of the soil used in the study

Chemical properties	
NO_3^--N (mg kg^{-1} dry soil)	6.23±1.11
Ca^{2+}+Mg^{2+} (meq/L dry soil)	6.93±0.94
K^+ (mg kg^{-1} dry soil)	162±8.9
Na^+ (mg kg^{-1} dry soil)	123±6.8
Physical properties	
Saturation percentage	32.5±4.3
pH	7.84±1.3
Electrical conductivity of saturation soil extract (ECe dS/cm)	2.37±0.21
Textural class	Sandy loam

Seed of cv. Barani-83 of spring wheat (*Triticum aestivum* L.) was obtained from the Department of Botany, University of Agriculture, Faisalabad, whereas that of cv. Sarsabz from NIAB, Faisalabad. Plastic pots (24 cm diameter and 28 cm deep) were filled with air-dried soil. Twenty seeds of each variety were sown directly into each pot. Thinning was done 15 days after germination to keep 12 plants in each pot. N treatment (urea) was started 28 days after germination. The N levels applied were: 56 (low), 112 (moderate), and 224 (high) mg $N \cdot kg^{-1}$. Pots were daily maintained at field water capacity to avoid the leaching of nutrients. A completely randomized design was used in this experiment with five replications per each treatment.

Three plants from each pot were harvested at day 21 (1st harvest) and at day 42 (2nd harvest) after the start of urea treatments. All physiological parameters were measured just before the second harvest.

Leaf water potential

A fully expanded youngest leaf (third from the top) was excised from each plant at 8.00 a.m. and the leaf water potential measurements were made with a Scholander type pressure chamber (Arimad-2, Japan).

Leaf osmotic potential

A proportion of the leaf used for water potential determination was frozen for two weeks, thawed and the frozen sap was extracted by crushing the material with a metal rod. After centrifugation (8000 *g* x 4 min), the supernatant was used directly for osmotic potential determination in a freezing point depression osmometer (Osmolette-S, Japan).

Leaf turgor potential

It was calculated as the difference between leaf osmotic potential and water potential values.

Chlorophyll and carotenoid contents

Determination of chlorophyll content was carried out following the method described by Arnon (1949) and carotenoids according to the method of Davies (1976). One gram of fresh leaves was triturated in 80 % acetone. The optical density was measured spectrophotometrically at 480, 645, 652, and 663 nm (Hitachi U-2000).

Soluble proteins

Total soluble proteins were determined as described by Lowry *et al.*(1951). Fresh leaf material (0.2 g of third leaf) was homogenized in 4 cm^3 of 0.1 *M* sodium phosphate buffer (pH = 7.0) and centrifuged at 20,000 *g* x 5 min. The optical density was read at 620 nm.

Total free amino acids

Total free amino acids of third leaf were determined following Hamilton and Van Slyke (1943). One cm^3 of extract was treated with 1 cm^3 of 10 % pyridine and 1 cm^3 of 2 % ninhydrin solution. Optical density was read at 570 nm.

Total soluble sugars

Soluble sugars were determined following Yemm and Willis (1954). Well ground dry leaf material (0.1 g of third leaf) was homogenized in hot 80 % ethanol and centrifuged at 2,900 *g* x 10 min. The residue was retained and repeatedly washed with 80 % ethanol to remove all the traces of soluble sugars. The filtrate obtained was treated with anthrone reagent. Absorbance was read at 625 nm.

Gas exchange parameters

Measurements of net CO_2 assimilation rate (A), transpiration (E) and stomatal conductance (g_s) were made on third leaf of each plant using an open system LCA-4 ADC portable infrared gas analyzer (Analytical Development Company, Hoddesdon, England). Measurements were performed from 9:30 to 11:30 a.m. under the following conditions: molar flow of air per unit leaf area 348.4 mmol·m^{-2}, atmospheric pressure 99.8 kPa, water vapour pressure ranged from 7.6 to 8.8 mbar, PAR at leaf surface was maximum up to 1385 $\mu\text{mol·m}^{-2}\text{·s}^{-1}$, temperature of leaf ranged from 31.3 to 36.2 °C, ambient temperature ranged from 24.2 to 31.3 °C, ambient CO_2 concentration 352 $\mu\text{mol·mol}^{-1}$, temperature of leaf chamber varied from 29.4 to 36.5 °C.

Growth analysis

After all the physiological measurements, the plants were harvested. Roots were carefully removed from the soil and washed for 2-3 min in distilled water. Fresh weight of shoots and roots was recorded. Samples were kept at 65 °C for one week and dry weight was recorded. Data for grain yield and yield components were recorded at plant maturity.

The following growth parameters were calculated using the formulae listed below:

(1) Leaf area ratio (LAR) = Leaf area per plant/dry weight of shoot

(2) Specific leaf weight (SLW)

$$SLW = \frac{\text{dry wt. of leaves per plant (mg)}}{\text{leaf area per plant (cm}^2\text{)}}$$

(3) Relative growth rate (RGR) = (1/W) x (dW/dt) ($\text{g·g}^{-1}\text{·day}^{-1}$) where **W** is dry weight of shoot, **dW** is difference in dry weights of shoots at two time intervals (15 and 30 days), and **dt** is difference in time, *i.e.* days.

(4) Net assimilation rate

$$(NAR) = \frac{(W_2 - W_1)(\ln L_2 - \ln L_1)}{(t_2 - t_1)(L_2 - L_1)}$$

W_2 and W_1 are dry weights, and L_2 and L_1 are leaf areas of shoots at times t_1 and t_2 (15 and 30 days, respectively).

Statistical analysis of data

Analysis of variance was computed using COSTAT computer package (CoHort Software, Berkeley CA). The least significant differences between the mean values were calculated following Snedecor and Cochran (1980). Correlation coefficients (r) for net CO_2 assimilation rate vs. grain yield, shoot fresh weight, or shoot dry weight were worked out using the COSTAT package.

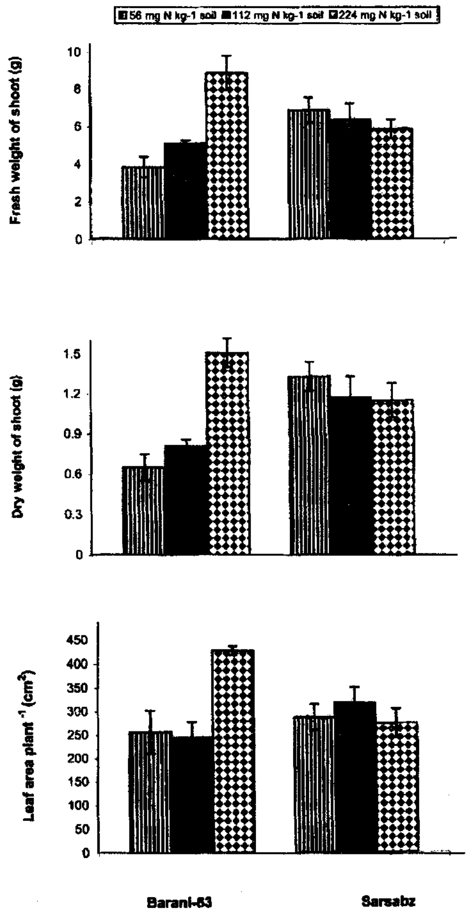

Fig. 1. Growth parameters of two cultivars of spring wheat when 28 day-old plants were subjected to varying levels of nitrogen for 42 days (n = 5).

Results

Physico-chemical properties of the soil used in the study are presented in Table 1. Shoot fresh and dry weight and leaf area increased significantly with increase in external N regime in Barani-83 and these parameters were maximal at 224 mg N·kg^{-1} of soil (Fig. 1). In contrast, in Sarsabz the first two parameters remained almost unaffected at different N levels, whereas leaf area per plant was maximal at 112 mg N·kg^{-1} of soil (Fig. 1). Relative growth rate in Barani-83 was maximal at 224 mg N·kg^{-1} of soil, whereas in Sarsabz RGR remained unaffected with increase in external N level (Table 2). Leaf area ratio decreased significantly in Barani-83 with increase in soil N, but in contrast to Sarsabz it remained almost unchanged. Specific leaf weight and net assimilation rate (NAR) increased in Barani-83 with increasing supply of soil N, but the reverse was true in Sarsabz (Table 2). Cv. Barani-83 had a maximal grain yield, 100-grain weight, and grains per spike at 224 mg N·kg^{-1} soil, whereas Sarsabz at 112 mg N·kg^{-1} soil (Table 2). Grains/spikelet in both the cultivars remained almost unchanged at all external N regimes (data not shown).

Chlorophylls a and b increased in Barani-83 with increase in N supply in the growth medium, whereas in Sarsabz there was a significant decrease in both the pigments at 224 mg N·kg^{-1} soil (Table 3). Chlorophyll a/b ratios remained unchanged in Barani-83 at varying N regimes, whereas it increased consistently in Sarsabz with increasing supply of external N (data not shown). Carotenoids in both cultivars were higher at the two higher external N levels. Although statistically non-significant, Sarsabz had higher Chl a, Chl b and carotenoids contents in leaves than those of Barani-83 at all external N regimes (Table 3).

Total leaf soluble proteins in Barani-83 remained almost unaffected at varying N regimes, but in contrast there was a marked increase in Sarsabz at higher external N regimes (Table 3). Cv. Sarsabz had markedly higher leaf soluble proteins than Barani-83 at all external N levels.

Although there was no significant effect of different soil N levels on leaf free amino acids, cultivars differed significantly in this variable (Table 3). Generally, Sarsabz had significantly higher content

Table 2. Different growth and yield parameters (±SE) of two cultivars of spring wheat when 28 day-old plants were subjected to varying levels of nitrogen for 42 days ($n = 5$).

		N fertilization level (mg kg^{-1})		
		56	112	224
Relative growth rate (g·g^{-1} day^{-1})	Barani-83	0.032±0.002a	0.036±0.002a	0.046±0.003b
	Sarsabz	0.046±0.001b	0.046±0.002b	0.045±0.001b
Leaf area ratio (cm^{-2}·g^{-1} shoot)	Barani-83	406.7±27.7a	307.4±12.8b	282.4±17.0b
	Sarsabz	220.9±21.3c	290.1±55.4b	246.9±38.4bc
Specific leaf weight (mg leaf dry wt.·cm^{-2} leaf area)	Barani-83	1.60±0.120a	2.29±0.245b	2.15±0.109b
	Sarsabz	2.63±0.268c	2.43±0.293b	2.43±0.219b
Net assimilation rate (mg dry wt.·cm^{-2} leaf area·day^{-1})	Barani-83	0.160±0.049a	0.219±0.021b	0.363±0.009c
	Sarsabz	0.413±0.051d	0.337±0.006c	0.328±0.016c
Grain yield (g)	Barani-83	1.50±0.17a	1.67±0.15a	2.96±0.30b
	Sarsabz	1.95±0.16a	2.67±0.23b	2.43±0.31bc
100 grain weight (g)	Barani-83	2.75±0.17a	3.11±0.16a	3.86±0.15b
	Sarsabz	3.44±0.21b	3.80±0.30b	3.47±0.17b
Grains spike^{-1}	Barani-83	37.27±2.78a	39.31±2.04a	44.09±2.04b
	Sarsabz	36.36±1.25a	41.48±1.70ab	39.77±2.15a

Means with the same letters within each parameter do not differ significantly at the 5 % level.

Table 3. Chlorophyll content and different organic solutes (±SE) of two cultivars of spring wheat when 28 day-old plants were subjected to varying levels of nitrogen for 42 days (*n* = 5).

		N fertilization level (mg·kg^{-1})		
		56	112	224
Chlorophyll *a* (mg·g^{-1} fresh weight)	Barani-83	0.283±0.023a	0.299±0.018a	0.328±0.015b
	Sarsabz	0.321±0.009b	0.322±0.046b	0.296±0.023a
Chlorophyll *b* (mg·g^{-1} fresh weight)	Barani-83	0.234±0.025a	0.247±0.037a	0.289±0.027b
	Sarsabz	0.309±0.054b	0.276±0.027b	0.224±0.043a
Carotenoids (μg·g^{-1} fresh weight)	Barani-83	7.13±0.59a	8.63±0.20b	8.32±0.28b
	Sarsabz	8.03±0.33b	9.05±0.35c	9.01±0.17c
Total soluble proteins (mg·g^{-1} fresh weight)	Barani-83	1.18±0.06a	1.23±0.02a	1.18±0.06a
	Sarsabz	1.84±0.14b	2.45±0.13c	2.36±0.19c
Free amino acids (mg·g^{-1} fresh weight)	Barani-83	1.14±0.24a	1.05±0.19a	0.89±0.09a
	Sarsabz	2.25±0.21b	2.10±0.27b	2.13±0.21b
Soluble sugars (mg·g^{-1} fresh weight)	Barani-83	7.85±0.84a	7.09±0.89a	6.32±0.64a
	Sarsabz	11.16±0.77b	8.74±0.71a	10.90±2.25b

Means with the same letters within each parameter do not differ significantly at the 5 % level.

of free amino acids as compared to Barani-83 at all external N regimes.

There was no-significant effect of different soil N levels on leaf soluble sugars, but cultivars differed significantly in response to N levels with respect to soluble sugars. Cv Sarsabz again had significantly greater leaf soluble sugars compared with Barani-83 at all N regimes except 112 mg N·kg^{-1} (Table 3).

Leaf water potential in Barani-83 had a slightly increasing trend with increasing supply of external N. However, leaf osmotic potential and turgor potential in both cultivars remained unaffected at varying N regimes (Table 4). However, generally, Sarsabz had lower osmotic potential and higher leaf water and turgor potentials than those of Barani-83 at all external N regimes (Table 4).

Addition of nitrogen to the rooting medium of two spring wheat cultivars had a significant effect on net CO_2 assimilation rate ($p \leq 0.001$) (Table 4). Net CO_2 assimilation rate was maximal in Barani-83 at 224 mg N·kg^{-1} soil, whereas that in Sarsabz at 56 mg N·kg^{-1} soil. In the latter cultivar there was a sharp decline in net CO_2 assimilation rate at 224 mg N·kg^{-1} soil compared with Barani-83. Stomatal conductance decreased in both cultivars with increase in N supply, but the decrease in stomatal conductance was more drastic in Sarsabz than in Barani-83 (Table 4). Transpiration remained unaffected in Barani-83, but there was a marked decrease in this variable in Sarsabz with increase in external N supply. Water use efficiency (WUE) remained unaffected in Barani-83 at varying N regimes, whereas in Sarsabz the maximal WUE was observed at 112 mg N·kg^{-1} soil. However, WUE was higher in Barani-83 than that in Sarsabz at 56 and 224 mg N·kg^{-1} soil. Intrinsic water use efficiency (A/gs) increased consistently in both cultivars with increasing supply of N, but Sarsabz had generally greater A/gs values than those in Barani-83. Relative intercellular CO_2 concentration [intercellular CO_2/ambient CO_2 (Ci/Ca)] remained almost unchanged in Barani-83 at all external nitrogen regimes (Table 4), whereas in contrast, in Sarsabz it increased consistently with increase in external N supply.

A positive correlation was found between net CO_2 assimilation rate and grain yield (r = 0.982ns) and plant biomass (with F.wt r = 0.947ns and with D.wt r = 0.966ns) in Barani-83 at different N levels in the

Table 4. Water relations and gas exchange parameters (±SE) of two cultivars of spring wheat when 28 day-old plants were subjected to varying levels of nitrogen for 42 days ($n = 5$).

		N fertilization level ($mg \cdot kg^{-1}$)		
		56	112	224
		Water relation parameters		
Water potential (–MPa)	Barani-83	1.18±0.23a	0.58±0.15b	0.75±0.12ab
	Sarsabz	0.61±0.12ab	0.38±0.07c	0.59±0.14b
Osmotic potential (–MPa)	Barani-83	1.39±0.14a	1.48±0.15a	1.55±0.15a
	Sarsabz	1.81±0.02b	1.91±0.09b	1.86±0.11b
Turgor potential (MPa)	Barani-83	0.43±0.16a	0.69±0.12b	0.68±0.11b
	Sarsabz	1.25±0.13c	1.55±0.11c	1.29±0.19c
		Gas exchange parameters		
CO_2 assimilation rate (μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$)	Barani-83	10.40±0.45a	10.27±0.38a	11.67±0.64b
	Sarsabz	13.32±1.29c	11.93±1.02b	6.07±1.41d
Stomatal conductance (gs) ($mmol \cdot m^{-2} \cdot s^{-1}$)	Barani-83	100.36±7.64a	88.36±3.27b	93.81±12.54ab
	Sarsabz	80.72±3.28b	52.36±4.91c	32.72±10. 91d
Transpiration rate (E) ($mmol \cdot m^{-2} \cdot s^{-1}$)	Barani-83	2.90±0.20a	3.09±0.68a	3.18±0.32a
	Sarsabz	4.68±0.11b	3.55±0.14c	2.41±0.57d
WUE (μmol CO_2 /mmol H_2O)	Barani-83	3.73±0.25a	3.38±0.16a	3.85±0.43a
	Sarsabz	2.93±0.50b	3.54±0.39a	2.93±0.91b
A/g_s (μmol $CO_2 \cdot mmol^{-1}$)	Barani-83	0.105±0.008a	0.121±0.003a	0.140±0.018b
	Sarsabz	0.168±0.021c	0.229±0.009d	0.216±0.050d
C_i/C_a	Barani-83	0.229±0.024a	0.232±0.005a	0.261±0.016a
	Sarsabz	0.160±0.010b	0.218±0.019a	0.241±0.019a

Means with the same letters within each parameter do not differ significantly at the 5 % level.

present study (Table 5). In contrast, in the salt tolerant cv. Sarsabz there was a negative correlation ($r = -0.363$ns) between net CO_2 assimilation rate and grain yield.

Discussion

Based on data presented here for plant biomass and growth analysis at the vegetative stage and on seed yield, the response of salt tolerant Sarsabz to varying N levels differed at both growth stages, but in contrast, the performance of salt sensitive Barani-83 remained almost unchanged at both growth stages. The different response of the two spring wheat cultivars to different N levels can be partially related to the earlier findings showing considerable variation in responses among cultivars/lines to N deficiency in wheat (Chandler 1970, Haeder *et al.* 1977), rice (Kemmler 1972) and sorghum (De 1974). In an earlier study, Ashraf and Zafar (1996) found that a salt tolerant line ILL-6793 a leguminous crop lentil (*Lens culivaris* Medic.) maintained considerably higher growth at low N regimes com-

Table 5. Correlation coefficients (r) between net CO_2 assimilation rate (A) and grain yield, shoot fresh weight or shoot dry weight of two wheat cultivars.

	Sarsabz	Barani-83
A vs. Grain yield	-0.363 ns	0.982ns
A vs. Shoot fresh wt.	0.722ns	0.966ns
A vs. Shoot dry wt.	0.942ns	0.947ns

ns = non-significant

pared with the salt sensitive line ILL-6439. In the present study the higher growth of the salt tolerant line in comparison with the salt sensitive line at low N levels was ascribed to the higher N use efficiency. A similar situation was found in the two spring wheat cultivars examined here because the salt tolerant line Sarsabz showed relatively better growth as compared to the salt sensitive Barani-83 under low N levels. The salt tolerant cultivar Sarsabz has a great adaptability to saline conditions (Anonymous 1997) so its adaptation to low nitrogen levels might also be expected in view of the fact that saline soils, in particular sodium saline soils, are deficient in N among the others essential inorganic nutrients (Ponnamperuma 1976, Seemann and Sharkey 1986, Ashraf and McNeilly 1994). In contrast, Barani-83 was originally bred for drought-hit areas (Ashraf *et al.*1994) where nitrogen deficiency is also encountered (Bidinger *et al.* 1987, Van Oosterom *et al.* 1995) but poor growth of this cultivar at low N regimes is not easy to explain.

Every cultivar/line of a crop has its own specific requirement of N or other nutrients for the optimal growth (Mengel and Kirkby 1987, Marschner 1995), so is the case of the two wheat cultivars under test. Barani-83 grew well at 224 mg·kg^{-1} soil, a nitrogen level suitable for the optimal growth of other cereals as suggested by Epstein (1972). In contrast, the growth of Sarsabz at the vegetative stage was almost the same at all different nitrogen levels, but at the adult stage this cultivar showed maximal growth at 112 mg·kg^{-1} soil.

A decrease in net CO_2 assimilation rate has been ascribed to the decline in stomatal conductance, inhibition of specific metabolic processes in carbon uptake, suppression of photochemical capacity, or a combined effect of all these processes (Baker 1996, Taiz and Zeiger 1998). In the present study, the CO_2 assimilation rate and stomatal conductance of both lines were positively related and the former variable also had a positive association with transpiration. If the rate of carbon assimilation was entirely dependent on the stomatal closure, Ci would be expected to be lower in leaves of plants subjected to low or high nitrogen levels. But it is evident from the data of relative intercellular CO_2 concentration (Table 3) that in Barani-83, none of the N levels affected intercellular CO_2 concentration. Similarly, in Sarsabz no consistent pattern of increase or decrease in relative intercellular CO_2 concentration was found. This indicates a decrease in carbon fixation by the chloroplasts (Yeo *et al.*1985) and also suggests that stomatal conductance was not the principal factor affecting photosynthesis. Constable and Rawson (1980) reported that similar changes occur in stomatal and internal resistances during leaf aging thereby causing Ci to remain constant despite changes in net CO_2 assimilation rate. Likewise, such changes in net CO_2 assimilation rate and stomatal resistance were found in water stressed sunflower plants with Ci remaining unchanged (Krampitz *et al.*1984).

The reduction in WUE in Barani-83 under low N supply was primarily due to the decreased carbon assimilation rate. The intrinsic water use efficiency in Barani-83 increased at the highest N level, whereas in Sarsabz this variable was higher at the higher two nitrogen levels. In both the cultivars the increase in intrinsic water use efficiency was due to simultaneous changes in both net CO_2 assimilation rate and stomatal conductance. The intrinsic water use efficiency and net CO_2 assimilation rate were negatively correlated (r = -0.481ns) in Sarsabz. This is in contrast to what was earlier found in C_4 dicot *Amaranthus retroflexus* (Sage and Pearcy 1987) and sugar cane (Ranjith and Meinzer 1997).

Data for leaf water potential had a negative relationship with those of stomatal conductance (Table 3) in two spring wheat cultivars. These results can be related to some earlier studies in which it was found that reduction in stomatal conductance did not affect leaf water potential under water deficit conditions in cowpea (Bates and Hall 1981, Osonubi 1985) and maize (Blackman and Davies 1985). Nonetheless, the better growth of Sarsabz compared with Barani-83 at varying N regimes can be related to its relatively higher leaf turgor potential since it is known that turgor potential is one of the major factors maintaining plants growth under various abiotic stresses (Hsiao 1973, Subbarao *et al.*1995, Greenway and Munns 1980, Ashraf 1994).

The salt tolerant cultivar had lower leaf osmotic potential as compared to that in the salt sensitive cultivar at all external N levels. The differential leaf osmotic potentials in the two lines can be easily related to their organic compounds such as soluble sugars, soluble proteins and free amino acids since the salt tolerant line accumulated these organic compounds considerably higher than the salt sensitive line at almost all N regimes.

In Sarsabz total soluble proteins were higher at 112 and 224 mg N·kg^{-1} soil. This is similar to Helal *et al.* (1975) who reported that osmotic stress generally enhances incorporation of N into proteins. But such pattern of increase in soluble proteins was not found in the salt sensitive Barani-83.

Chlorophyll *a* and chlorophyll *b* had a positive association with net CO_2 assimilation rate in both cultivars. It is now known that some special chlorophyll *a* molecules directly take part in the light reactions of photosynthesis, whereas the remaining chlorophyll *a* and all chlorophyll *b* molecules along with carotenoids and some other compounds act as antennae for light harvesting in higher plants (Salisbury and Ross 1992, Taiz and Zeiger 1998). It means in the present study that pigments may have been affected due to changes in soil N levels thereby affecting the net CO_2 assimilation rate (Taiz and Zeiger 1998).

The optimal N levels were 112 and 224 mg·kg^{-1} soil for Sarsabz and Barani-83, respectively. The better growth performance in terms of biomass and grain yield of the salt tolerant Sarsabz as compared to the salt sensitive Barani-83 was associated with maintenance of relatively high leaf turgor potential at different N levels but not with net CO_2 assimilation rate.

References

Anonymous 1997. Silver Jubilee of NIAB, Fifth Five Year Report. p. 126. Nuclear Institute for Agriculture and Biology, Faisalabad, Pakistan.

Arnon D.T. 1949. Copper enzyme in isolated chloroplast polyphenoloxidase in *Beta vulgaris*. Plant Physiol. 24, 1-15.

Ashraf M. 1994. Breeding for salinity tolerance in plants. CRC Crit. Rev. Plant Sci. 13: 17-42.

Ashraf M., McNeilly T. 1994. Responses of three arid zone grasses to N deficiency: A greenhouse study. Arid Soil Res. Rehab. 6: 125-136.

Ashraf M., Zafar Z.U. 1996. Effect of nitrogen deficiency on growth and some biochemical characteristics in salt-tolerant and salt-sensitive lines of lentil (*Lens culinaris* Medic.). Arch. Acker. Pfl. Boden. 40: 231-239.

Ashraf M.Y., Azmi A.R., Khan A.H., Naqvi S.S.M. 1994. Water relations in different wheat (*Triticum aestivum* L.) genotypes under soil water deficits. Acta Physiol. Plant. 16: 231-240.

Baker N.R. 1996. Photosynthesis and the environment. Kluwer, Dordrecht, Netherlands.

Bates L.M., Hall A.E. 1981. Stomatal closure with soil water depletion not associated with changes in bulk leaf water status. Oecologia 50: 62-65.

Bidinger F.R., Mahalakshmi V., Rao G.D.P. 1987. Assessment of drought resistance in pearl millet (*Pennisetum americanum* (L.) Leeke). II. Estimation of genotype response to stress. Aust. J. Agric. Res. 38: 49-59.

Blackman P.G., Davies W.G. 1985. Root to shoot communication in maize plants of the effects of soil drying. J. Exp. Bot. 36: 39-48.

Chandler R.F. 1970. Overcoming physiological barriers to higher yield through plant breeding. In: Role of Fertilization in the Intensification of Agricultural Production. Int Potash Inst Bern, pp. 421-434.

Constable G.A., Rawson H.M. 1980. Effect of leaf position, expansion and age on photosynthesis, transpiration and water use efficiency of cotton. Aust. J. Plant Physiol. 7: 89-100.

Davies B.H. 1976. Carotenoids.In: Chemistry and biochemistry of plant pigments, ed. by T.W. Goodwin. 2nd ed. Academic Press, London, vol 2, pp. 38-165.

De R. 1974. Cultural practices for maize, sorghum and millets. First FAO/SIDA seminar for plant scientists from Africa and Near East, Cairo, FAO Rome, pp 440-451.

Dietz K.J., Harris G.C. 1997. Photosynthesis under nutrient deficiency. In: Handbook of photosynthesis, ed. by M. Pessarakli. Marcel Dekker Press Inc. New York, pp. 951-975.

Epstein E. 1972. Mineral Nutrition of Plants: Principles and Perspectives. Wiley, New York.

Fernandes M.S., Rossiello R.O.P. 1995. Mineral nitrogen in plant physiology and plant nutrition. Crit. Rev. Plant Sci. 14: 111-148.

Greef J.M. 1994. Productivity of maize in relation to morphological and physiological characteristics under varying amount of nitrogen supply. J. Agron. Crop Sci. 172: 317-326.

Greenway H., Munns R. 1980. Mechanism of salt tolerance in non-halophytes. Annu. Rev. Plant Physiol. 31: 149-190.

Haeder H.E., Beringer H., Mengel K. 1977. Redistribution of ^{14}C into the grains of two spring wheat cultivars. Z. Pflanzenern. Boden. 140: 409-419.

Hamilton P.B., Van Slyke D.D. 1943. Amino acid determination with ninhydrin. J. Biol. Chem. 150: 231-233.

Helal M., Koch K., Mengel K. 1975. Effect of salinity and potassium on the uptake nitrogen and on nitrogen metabolism in young barley plants. Physiol. Plant. 35: 310-313.

Hsiao T.C. 1973. Plant response to water stress. Annu. Rev. Plant Physiol. 24: 519-570.

Kemmler G. 1972. Fertilizer application to modern rice and wheat cultivars in developing countries. In: Proc. 7th Fertilizer World Congress, Vienna, pp. 545-563.

Krampitz M.J., Klug K., Fock H.P. 1984. Rates of photosynthetic CO_2 uptake, photorespiratory CO_2 evolution and dark respiration in water-stressed sunflower and bean leaves. Photosynthetica 18: 329-337.

Longnecker N., Robson A. 1994. Leaf emergence of spring wheat receiving varying nitrogen supply at different stages of development. Ann. Bot. 74: 1-7.

Lowry O.H., Rosebrough N.J., Farr A.L., Randal R.J. 1951. Protein measurement with Folin phenol reagent. J. Biol. Chem. 193: 265-275.

Marschner H. 1995. Mineral Nutrition of Higher Plants. 2nd edition, Academic Press, London.

Mengel K., Kirkby E.A. 1987. Principles of Plant Nutrition. International Potash Institute, Bern, Switzerland.

Nevins D.J., Loomis R.S. 1970. Nitrogen nutrition and photosynthesis in sugar beet (*Beta vulgaris* L.). Crop Sci. 10: 21-25.

Osonubi, O. 1985. Responses of cowpeas (*Vigna unguiculata* (L.) Walp) to progressive soil drought. Oecologia 66: 554-557.

Ponnamperuma F.N. 1976. Screening rice for tolerance to mineral stresses. In: Plant adaptation to mineral stresses in problem soils, ed. by M.J. Wright. Cornell University, Ithaca, New York, pp. 341-354.

Ranjith S.A., Meinzer F.C. 1997. Physiological correlates of variation in nitrogen-use efficiency in two contrasting sugarcane cultivars. Crop Sci. 37: 818-825.

Sage R.F., Pearcy R.W. 1987. The nitrogen use efficiency of C_3 and C_4 plants. II. Leaf nitrogen effects on the gas exchange characteristics of *Chenopodium album* (L.) and *Amaranthus retroflexus* (L.). Plant Physiol. 84: 959-963.

Salisbury F.B., Ross C.W. 1992. Plant physiology. Fourth edition. Wadsworth Publ. Co., Belmont, California.

Seemann J.R., Sharkey T.D. 1986. Salinity and nitrogen effects on photosynthesis, ribulose-1,5-bisphosphate carboxylase and metabolite pool size in *Phaseolus vulgaris* L. Plant Physiol. 82: 555-560.

Snedecor G.W., Cochran W.G. 1980. Statistical methods. 7th Edition. Iowa State University Press, Ames, Iowa.

Subbarao G.V., Johansen C., Slinkard A.E., Nageswara Rao R.C., Saxena N.P., Chauhan Y.S. 1995. Strategies for improving drought resistance in grain legumes. Crit. Rev. Plant Sci. 14: 469-523.

Taiz L., Zeiger E. 1998. Plant Physiology, 2nd edition, Sinauer Associates Inc. Publishers, Sunderland, Massachusetts.

Van Oosterom E.J., Mahalakshmi V., Arya G.K., Dave H.R., Gothwal B.D., Joshi A.K., Joshi P., Kapoor R.L., Sagar P., Saxena M.B., Singhania D.L., Vyas K.L. 1995. Effect of yield potential, drought escape and drought tolerance on yield of pearl millet (*Pennisetum glaucum*) in different stress environments. Indian J. Agric. Res. 65: 629-635.

Yemm E.W., Willis A.J. 1954. The estimation of carbohydrates in plant extracts by anthrone. Biochem. J. 57: 508-514.

Yeo A.R. 1998. Molecular biology of salt tolerance in the context of whole-plant physiology. J. Exp. Bot. 49: 915-929.

Received February 05, 2002; accepted September 06, 2002