PHYSIOLOGICAL RESPONSES TO WATER STRESS AND NITROGEN MANAGEMENT IN WHEAT (*Triticum aestivum* L.): EVALUATION OF GAS EXCHANGE, WATER RELATIONS AND WATER USE EFFICIENCY

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ABSTRACT

A field experiment was conducted over 2 years to evaluate the gas exchange, water relations and water use efficiency (WUE) of wheat under different water stress and nitrogen management practices at Crop Physiology Research Area, University of Agriculture, Faisalabad. Pakistan. Four irrigation levels, i.e. one irrigation (Irrigation at tillering stage), two irrigations (Irrigations at tillering and anthesis stages), three irrigations (Irrigations at tillering, anthesis and grain development stages), four irrigations (Irrigations at tillering, stem elongation, anthesis and grain development stages) and four nitrogen levels i.e., 0, 50, 100 and 150 kg N ha^{-1} were applied in this study. The photosynthetic gas exchange parameters (Net CO2 assimilation rate, transpiration rate and stomatal conductance) are remarkably improved by water application and nitrogen nutrition. The exposure of plants to water and nitrogen stress lead to noticeable decrease in leaf water potential, osmotic potential and relative water content. Relative water content (RWC) of stressed plants dropped from 98 to 75 % with the decrease in number of irrigation and nitrogen nutrition The higher leaf water potential, and relative water contents were associated with higher photosynthetic rate. Water use efficiency (WUE) reduced with increasing number of irrigations and increased with increasing applied nitrogen at all irrigation levels.

Keywords: Gas exchange, Water relations, Water use efficiency, Water stress, Nitrogen nutrition and Wheat

INTRODUCTION

The shortage of water resources is a worldwide problem and the phenomenon of water stress is very obvious. Plant water deficit resulting from shortage of water (rainfall or supplemental irrigation) lead many plants not to meet their physiological activities. Therefore, the studies on plants including identification and selection of physiological traits that increase plant WUE and drought tolerance under water-limited conditions are important for well understanding plant physiological characters and taking physiological water saving measures.

Water stress and nitrogen deficiency are major constraints to wheat production and yield stability in Pakistan. Similar problems are encountered worldwide (Lawlor, 1995). An efficient use of limited water resources and better growth under both limited water and nitrogen supply are desirable traits for crops in water limited environments. Photosynthetic capacity in crop plants is a primary component of dry matter productivity. The final biological or economical yield can be increased either by increasing the rate of photosynthesis, by reducing wasteful respiration or by optimizing assimilate partitioning (Lawlor, 1995), because in some studies a positive correlation has been found between rate of photosynthesis and ultimate yield. Photosynthesis is one of the major physiological phenomena contributing to growth and grain yield, it is presumed that rate of photosynthesis of the flag leaf from anthesis to grain filling may contribute to a great extent to grain development and hence ultimate yield as compared to the other leaves of a plant.

Water use efficiency provides a simplest mean of assessing whether yield is limited by water supply or other factors and is considered an important component of adaptation to water deficit conditions (Edhaie and Waines, 1993). As the amount of water used is strongly affected by crop management, a good understanding of crop management effects on WUE may provide researchers opportunities to identify and select appropriate crop management practices for improved water use efficiency. Some studies (Li *et al*., 2001 b; Parsand and Knosla, 1999; Stephens and Hess, 1999) suggest that limited supplemental irrigation and fertilization during the growth season can significantly increase WUE and wheat yield.

The present study was designed to test whether modifications to recommended crop management practices could significantly improve WUE and to measure physiological and yield parameters with a view to understand the basis of improved performance and to identify the potential characteristics contributing to improved WUE. As water use efficiency could be improved by the nitrogen nutrition supply. For this purpose wheat was grown under different combinations of nitrogen and water supply levels and the study was focused on the irrigation and nitrogen management impacts on leaf gas exchange, water relation parameters and water use efficiency.

MATERIALS AND METHODS

A field experiment was conducted during November-April 2002-03 and 2003-04 on a sandy loam soil at the research area of the Department of Crop Physiology, University of Agriculture, Faisalabad, Pakistan (31.250 N latitude, 73.090 E longitude and 184 m altitude). The climate of the experimental site is characterized by a semi-arid with hot dry summer and severe cold winter seasons. Mean maximum temperature for the wheat-growing season was 26 °C in 2002-03 and 29 °C in 2003-04 (Fig. 1 and 2). Before sowing soil test showed organic matter content from 0.60 to 0.73% and N 0.031- 0.037%, P_2O 5.0-5.6 ppm and K_2O 161-175 ppm in both the seasons. All the analysis was done according to the method given in Hand Book No. 60 (US Salinity Lab. Staff, 1954) except available P and soil texture, which were determined by methods described by Watanabe and Olsen, (1965) and Moodie et al. (1959), respectively.

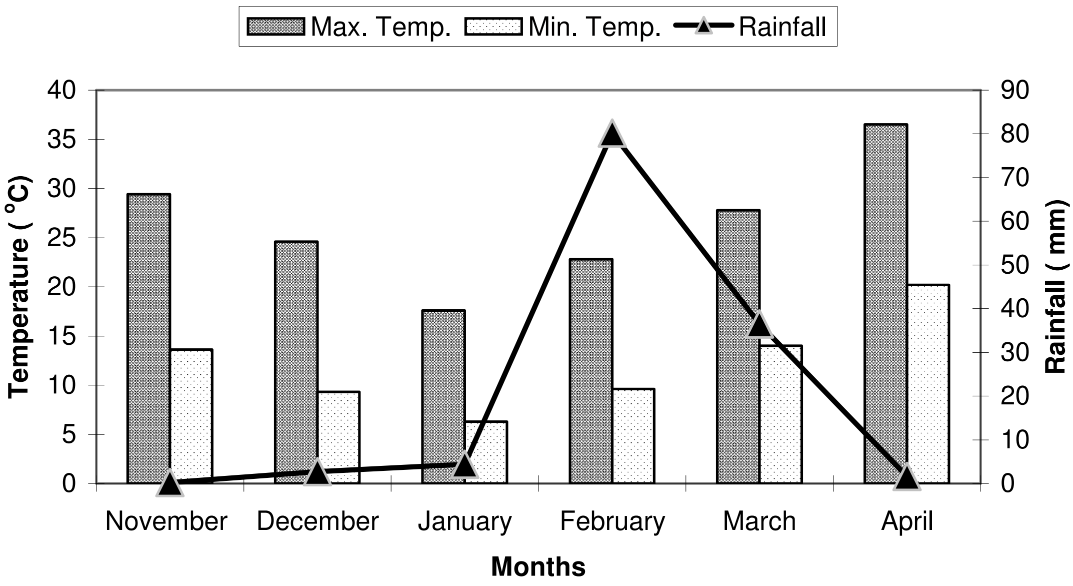

Figure 1. Meteorological data for the year 2002-03

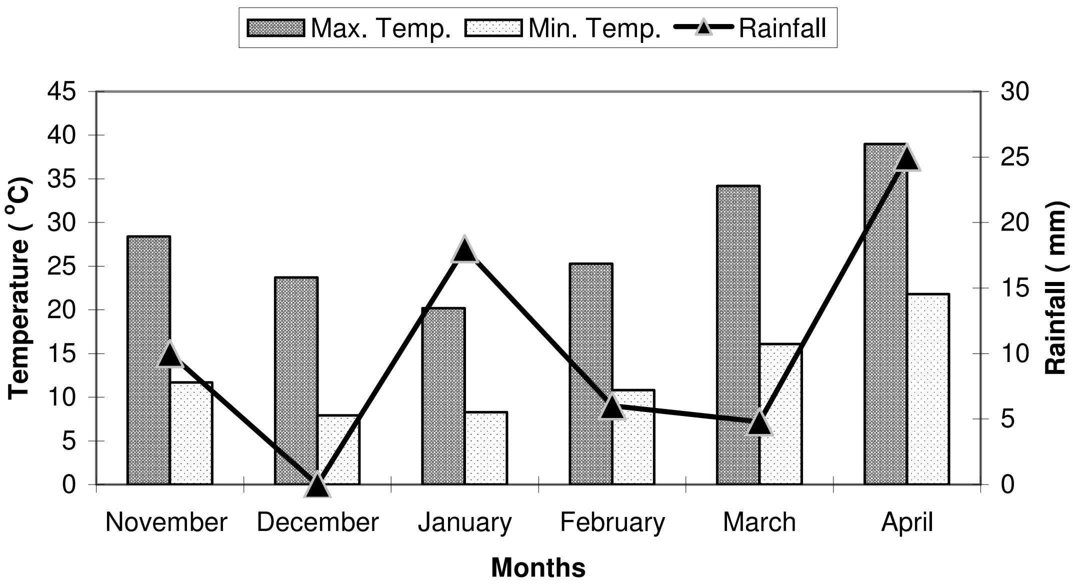

Figure. 2. Meteorological data for the year 2003-04

Locally recommended spring wheat (*Triticum aestivum* L.) cultivar "Inqlab-91" was used as the experimental crop. The crop was planted on 14th November 2002 and 3rd December 2003 at a seeding rate of 100 kg ha^{-1} with 25 cm row spacing. Four irrigation and nitrogen treatments were applied for the study. Irrigation treatments included: One irrigation at tillering stage (I_1); two irrigations each at tillering and anthesis stages (I_2); three irrigations each at tillering, stem elongation and grain development stages (I_3); four irrigations each at tillering, stem elongation, anthesis and grain development stages (I_4). Irrigation water was applied by flooding at a rate of 76.4 mm/irrigation using cut-throat flume (90 cm x 20 cm). The total seasonal water applied (Irrigation + Rainfall) at different growth stages in the 2002-03 and 2003-04 is presented in Table 1. Four nitrogen rates (0, 50, 100, 150 kg ha^{-1}) were applied to each irrigation treatment. Half nitrogen was applied as side dressing with the help of a single row hand drill at the time of planting and remaining half was applied with first irrigation. Phosphorus at 100 kg ha^{-1} was applied to all plots at the time of planting. Urea and triple super phosphate were used as a source of fertilizers. The experiment was laid out in randomized complete block design in a split-plot arrangement with irrigation in main plots while nitrogen in sub plots. Every treatment was replicated three times for a total of 48 plots of 1.5 m x 6 m (9 m^2). All other cultural practices were standard and uniform for all treatments.

Table 1. Seasonal total water applied (Irrigation + Rainfall) to wheat at different growth stages in the 2002-03 and 2003-04

Growth stages	Irrigation Regimes (I)			
	I-1	I-2	I-3	I-4
Tillering	x	x	x	x
Stem elongation	-	-	x	x
Anthesis	-	x	x	x
Grain development	-	-	-	x
Total Seasonal 2002-03 (mm)	278.4	354.8	431.2	507.6
Total Seasonal 2003-04 (mm)	120.2	196.0	273.0	349.4

"X" stage where irrigation was applied

Measurements of net CO_2 assimilation rate (A), transpiration rate (E) and stomatal conductance (gs) were made on flag leaves of three plants, selected randomly from each plot. Measurements were made from 9:00 to 11:00 am using an open system LCA-4 ADC portable infrared gas analyzer (Analytical Development Company, Hoddesdon, England). The LCA-4 analyzer is used in conjunction with portable leaf chambers, to measure various parameters associated with photosynthesis. Such measurements are carried out in an open system configuration in which fresh air is passed through the chamber on continuous basis. Measurements are carried out on the state of incoming gas (the reference air) and after passing the leaf specimen (the analysis level); the gas is then vented away. This arrangement tolerates some outward gas leakage and adsorption/absorption by the material used in the gas path. This chamber also provides sensors for the measurement of other parameters (a total of 40 different parameters can be measured), and it therefore forms an integral part of the system for photosynthesis measurement. It was supposed that the data recorded from this leaf could represent optimum gas exchange characteristics of whole plant.

Following adjustments/specifications for these measurements were made during the year 2002-03: leaf surface area 6.25 cm^2, ambient CO_2 concentration (Cref) 326 $\mu molmol^{-1}$, temperature of the leaf chamber ranged from 31.5 to 37.8°C, chamber gas flow rate (V) 408 ml min^{-1}, molar flow of air per unit leaf area (Us) 409.5 $molm^{-2}s^{-1}$, ambient pressure (P) 98.2 kPa, water vapor pressure in the chamber (eref.) ranged 21.2-24 mbar, PAR (Q leaf) at leaf surface was maximum up to 1181 μ mol $m^{-2}s^{-1}$.

For second year (2003-04) the adjustments/specifications were as follows: Leaf surface area 11.35 cm^2, ambient CO_2 concentration (Cref) 405.87 μ mol mol^{-1}, temperature of the leaf chamber ranged from 31.8 to 38.5°C, chamber gas flow rate (V) 408 ml min^{-1}, molar flow of air per unit leaf area (Us) 251.43 μ mol s^{-1}, ambient pressure (P) 992 kPa, water vapor pressure in the chamber (eref.) ranged 21.2-24 mbar, PAR (Q leaf) at leaf surface was maximum up to 1122 μ mol m^{-2} s^{-1}.

Randomly selected three flag leaves (fully expanded) from each treatment plot were used to measure the leaf water potential. The measurements were made from 8:00-9:00 a.m. with Scholander type pressure chamber available in the Department of Botany, University of Agriculture, Faisalabad, Pakistan. The same leaf as used for water potential was frozen in a freezer below -20°C for seven days, then the frozen leaf material was thawed and cell sap was extracted by crushing it into a 1.5 mL tube with the help of a blue pestle. The sap so extracted was directly used for the determination of osmotic potential using an osmometer (Wescor 5500).

A sample consisting of five flag leaves was taken from each sub plot and recorded the fresh weight. Leaves were immersed in distilled water for 14-16 hours, and saturated weight was recorded after blotting off the excess water. Then the dry weight of the same leaves was recorded after drying in an oven at 80°C for forty eight hours. The relative water contents were calculated using the formula:

$$RWC\ (\%) = (\text{Fresh weight - Dry weight / Saturated weight - Dry weight})\ 100 \quad (1)$$

Water use efficiency was calculated using the following formula given by Hussain and Al-Jaloud (1995):

$$WUE = GY / TWA \quad (2)$$

where

WUE = Water use efficiency (kg ha^{-1} mm^{-1})
GY = Grain yield (kg ha^{-1})
TWA = Total water applied (mm)

Data collected during this study were statistically analyzed using MSTAT statistical package (Michigan State University, USA 1989). The Duncan's new Multiple Range (DMR) test at 5% probability level was used to test the differences among mean values (Steel and Torrie, 1984).

RESULTS

Irrigation treatments significantly affected the net CO_2 assimilation rate in flag leaf of wheat in both the years 2002-03 and 2003-04 (Table 2) .Net CO_2 assimilation rate increased with increasing number of irrigations (Table 2). Maximum and significantly higher rates of net CO_2 assimilation (13.02 µ mol $m^{-2}s^{-1}$ and 7.75 µ mol $m^{-2}s^{-1}$) were recorded under the treatment where four irrigations (irrigation at tillering, stem elongation, anthesis and grain development) were applied in both the years. The minimum net CO_2 assimilation rate (9.33 µ mol $m^{-2}s^{-1}$ during 2002-03 and 3.99 µ mol $m^{-2}s^{-1}$ in 2003-04) was recorded under the treatment where only one irrigation was applied at tillering. Nitrogen application during the year 2002-03 and 2003-04 significantly ($P \leq 0.01$) increased net CO_2 assimilation rate at all irrigation levels (Table 2). Maximum net CO_2 assimilation rates (11.92 µ mol $m^{-2}s^{-1}$ during 2002-03 and 8.00 µ mol $m^{-2}s^{-1}$ in 2003-04) were recorded under the nitrogen treatment of 150 kg N ha^{-1} followed by 100, 50 and 0 kg N/ ha. The minimum net CO_2 assimilation rates (9.33 µ mol m^{-2} s^{-1} during 2002-03 and 4.22 µ mol $m^{-2}s^{-1}$ in 2003-04) were recorded under the treatment where nitrogen was applied at 0 kg / ha.

Table 2. Gas exchange parameters of wheat under water stress and nitrogen levels in the year 2002-03 and 2003-04

Treatments	Net CO_2 assimilation rate (µ mol $m^{-2}s^{-1}$)		Transpiration rate (µ mol $m^{-2}s^{-1}$)		Stomatal Conductance (µ mol $m^{-2}s^{-1}$)	
	2002-03	2003-04	2002-03	2003-04	2002-03	2003-04
Irrigation regimes						
I-1	9.33 c	3.99 d	1.43 d	1.39 d	0.15 d	0.18 c
I-2	10.14 b	6.25 c	1.61 c	1.51 c	0.17 c	0.19 c
I-3	9.67 bc	7.21 b	1.72 b	1.88 b	0.22 b	0.24 b
I-4	13.02 a	7.75 a	2.11 a	2.02 a	0.46 a	0.33 a
Nitrogen levels (kg ha^{-1})						
0	9.33 d	4.22 d	1.36 d	1.56 c	0.21 d	0.19 d
50	10.00 c	6.01 c	1.68 c	1.61 c	0.24 c	0.21 c
100	10.91 b	6.96 b	1.84 b	1.72 b	0.27 b	0.25 b
150	11.92 a	8.00 a	1.99 a	1.93 a	0.29 a	0.28 a

Data within columns followed by different letters are significantly different at $P < 0.05$.

Data regarding the transpiration rate showed that in both the years (2002-03 and 2003-04) the irrigation treatments significantly ($P \leq 0.01$) increased the transpiration rate (Table 2). Maximum transpiration rate (2.11µ mol m-$^{2}s^{-1}$ during 2002-03 and 2.02 µ mol $m^{-2}s^{-1}$ in 2003-04) was recorded under the treatment where four irrigations (irrigation at tillering, stem elongation, anthesis and grain development) were applied, followed by three (irrigation at tillering, anthesis and grain development), two (irrigation at tillering and anthesis) and one (irrigation at tillering) irrigations, respectively. The minimum transpiration rate (1.43 µ mol m-$^{2}s^{-1}$ during 2002-03 and 1.39 µ mol m-^{2}s-^{1} in 2003-04) was recorded under the treatment where only one irrigation was applied at tillering (Table 2). Nitrogen application during the years 2002-03 and 2003-04 significantly ($P \leq 0.01$) increased transpiration rate at all irrigation levels (Table 2). Maximum transpiration rate (1.99 µ mol $m^{-2}s^{-1}$ during 2002-03 and 1.93 µ mol m-$^{2}s^{-1}$ in 2003-04) was recorded under the nitrogen treatment 150 kg N ha^{-1} followed by 100, 50 and 0 kg N ha^{-1}. The minimum transpiration rate (1.36 µ mol $m^{-2}s^{-1}$ during 2002-03 and 1.56 µ mol $m^{-2}s^{-1}$ in 2003-04) was recorded under the treatment where nitrogen was applied at 0 kg ha^{-1} (Table 2). In general, transpiration rate increased with increasing levels of irrigation and nitrogen.

The irrigation treatments significantly ($P \leq 0.01$) affected the stomatal conductance in both the years (Table 2). Maximum stomatal conductance values (0.46 mol $m^{-2}s^{-1}$ during 2002-03 and 0.33 mol $m^{-2}s^{-1}$ in 2003-04) were recorded under the treatment where four irrigations (irrigation at tillering, stem elongation, anthesis and grain development) were applied, followed by three (irrigation at tillering, anthesis and grain development), two (irrigation at tillering and anthesis) and one (irrigation at tillering) irrigation, respectively. The minimum stomatal conductance values (0.15 mol $m^{-2}s^{-1}$ during 2002-03 and 0.18 mol $m^{-2}s^{-1}$ in 2003-04) were recorded under the treatment where only one irrigation was applied at tillering (Table 2). Nitrogen application significantly ($P \leq 0.01$) increased stomatal conductance at all irrigation levels (Table 2). Maximum stomatal conductance (0.29 mol $m^{-2}s^{-1}$ during 2002-03 and 0.28 mol $m^{-2}s^{-1}$ in 2003-04) was recorded under the nitrogen treatment of 150 kg N ha^{-1} followed by 100, 50 and 0 kg N ha^{-1}. The minimum stomatal conductance (0.21 mol $m^{-2}s^{-1}$ during 2002-03 and 0.19 mol $m^{-2}s^{-1}$ in 2003-04) was recorded under the treatment where nitrogen was applied at 0 kg ha^{-1}.

The water potential and osmotic potential of flag leaves were measured after every 10 days starting from anthesis stage. Water potential and osmotic potential had a decreasing trend with time in both the years. The effect of irrigation and nitrogen on these parameters during both the seasons is shown in Figs. 3 and 4. Increasing number of irrigations significantly ($P \leq 0.01$) increased the values of water potential and osmotic potential. The maximum values (less negative) of water potential and osmotic potential were observed after 10 days of anthesis during both the years. After 10 days of anthesis, the progressive decreases in the values of these two parameters were observed and the minimum values were recorded 30 days after anthesis in both the years. Nitrogen application significantly increased the values of water potential and osmotic potential at all harvesting dates. In general the maximum values of these parameters were observed in the treatment combination of four irrigations and nitrogen at 150 kg ha^{-1} after 10 days of anthesis and the minimum were recorded in the treatment combination of one irrigation and 0 kg N ha^{-1} at all harvesting dates in both the years.

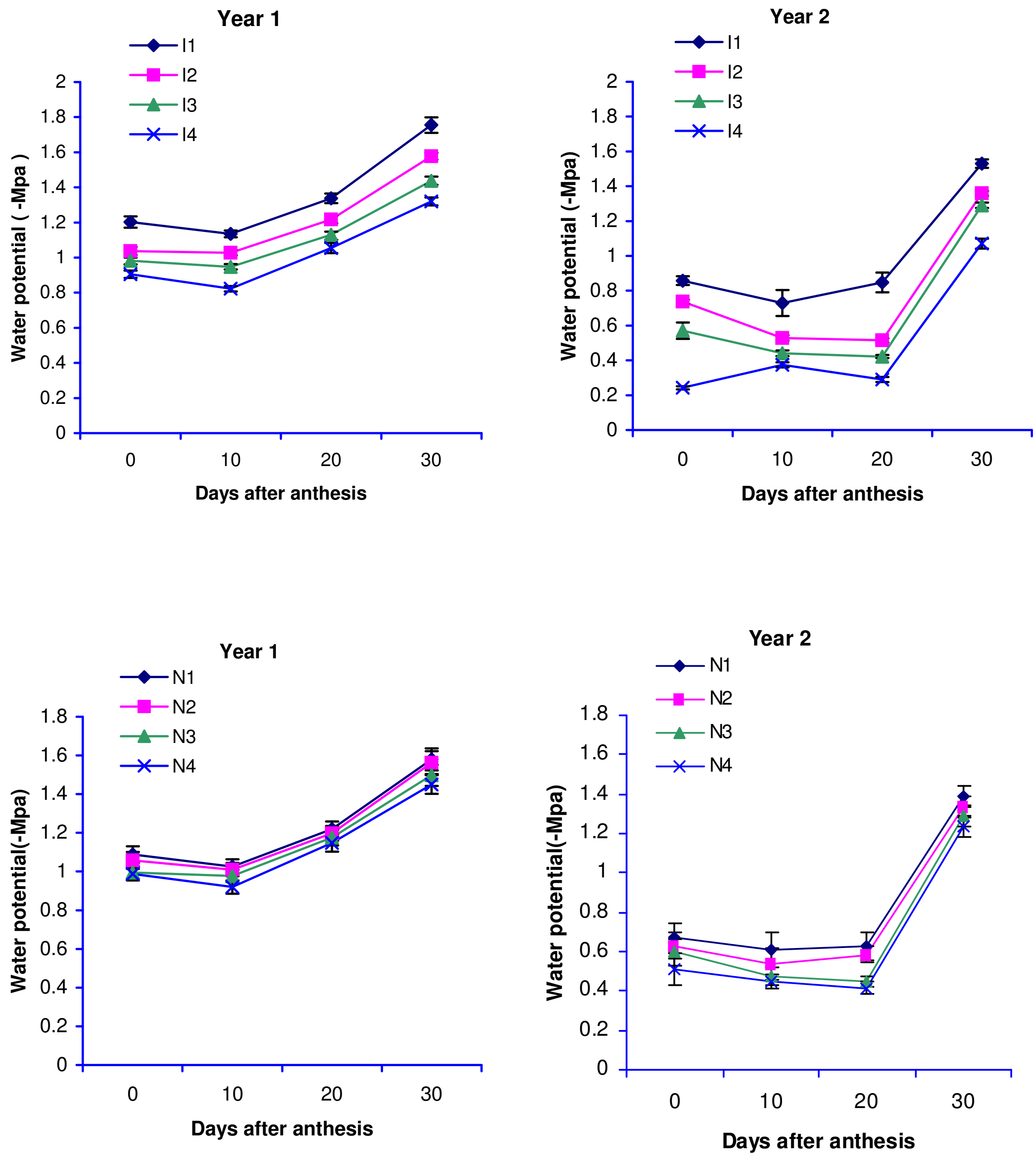

Figure 3. Irrigation and nitrogen management impacts on water potential of wheat during the years 2002-03 and 2003-04

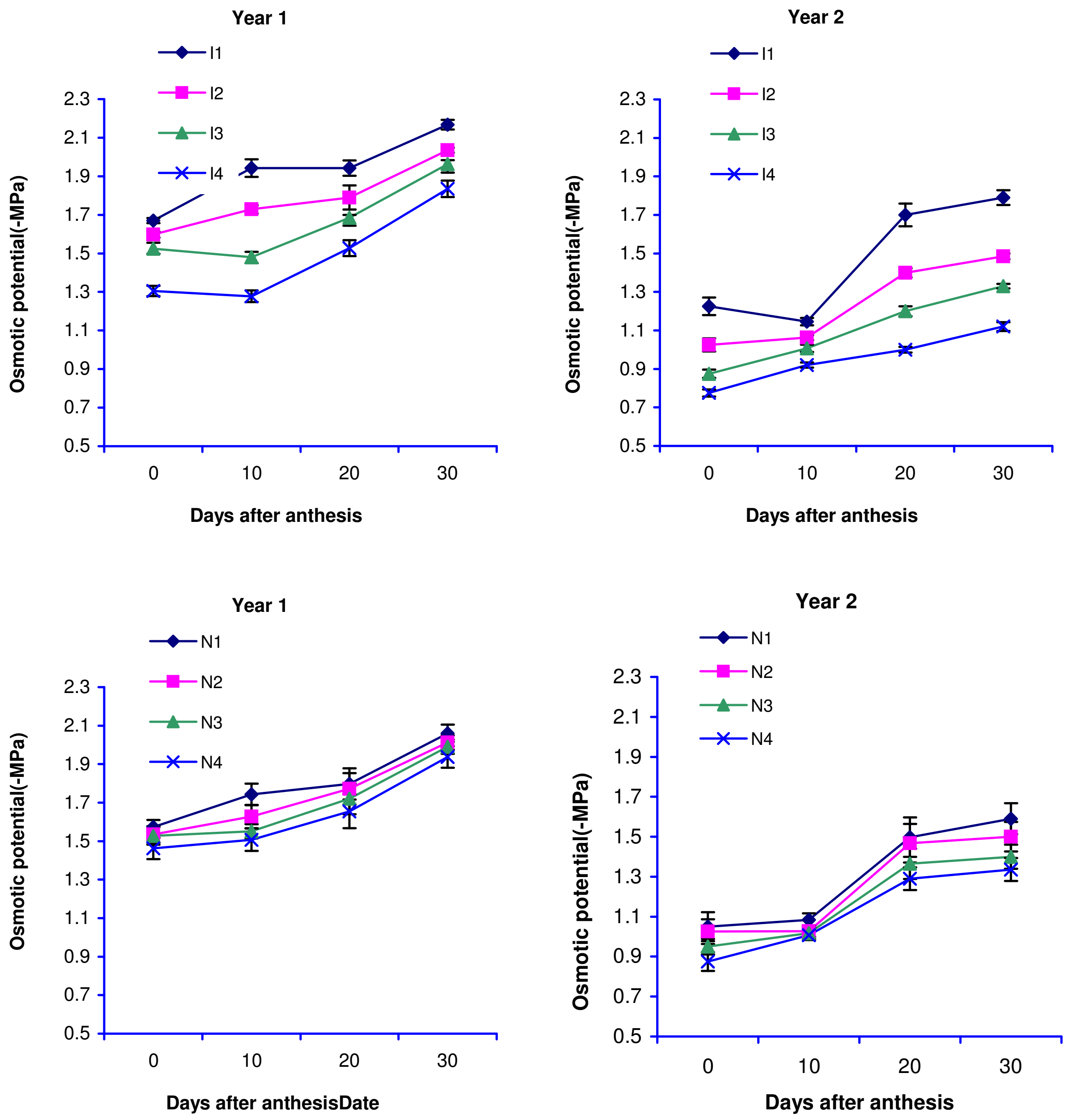

Figure 4. Irrigation and nitrogen management impacts on osmotic potential of wheat during the years 2002-03 and 2003-04

The RWC of flag leaves was measured after every 10 days from booting stage to grain development stage. RWC generally had a decreasing trend with time in both the years. The effect of irrigation and nitrogen on RWC during both the seasons is shown in the Fig. 5. Increasing number of irrigations significantly ($P \leq 0.01$) increased RWC. RWC values in four irrigation treatments at all harvests were significantly higher than all other irrigation treatments. The maximum RWC value 92% and 98% were recorded at anthesis stage during both the years. After anthesis, the progressive decrease in RWC was observed and the minimum RWC values were recorded 30 days after anthesis (80% and 77% during the year 2002-03 and 2003-04, respectively). RWC increased significantly with increasing levels of nitrogen at all harvesting dates. The maximum RWC value 93% during the year 2002-03 and 92% in 2003-04 was recorded under the nitrogen treatment of 150 kg ha^{-1}. The minimum RWC values 83 and 87 % were recorded after 30 days of anthesis during year 2003 and 2004 respectively. In general the maximum relative water content values were observed in the treatment combination of four irrigations and nitrogen at 150 kg ha^{-1} and the minimum were recorded in the treatment combination of one irrigation and 0 kg N ha^{-1} at all harvesting dates in both the years.

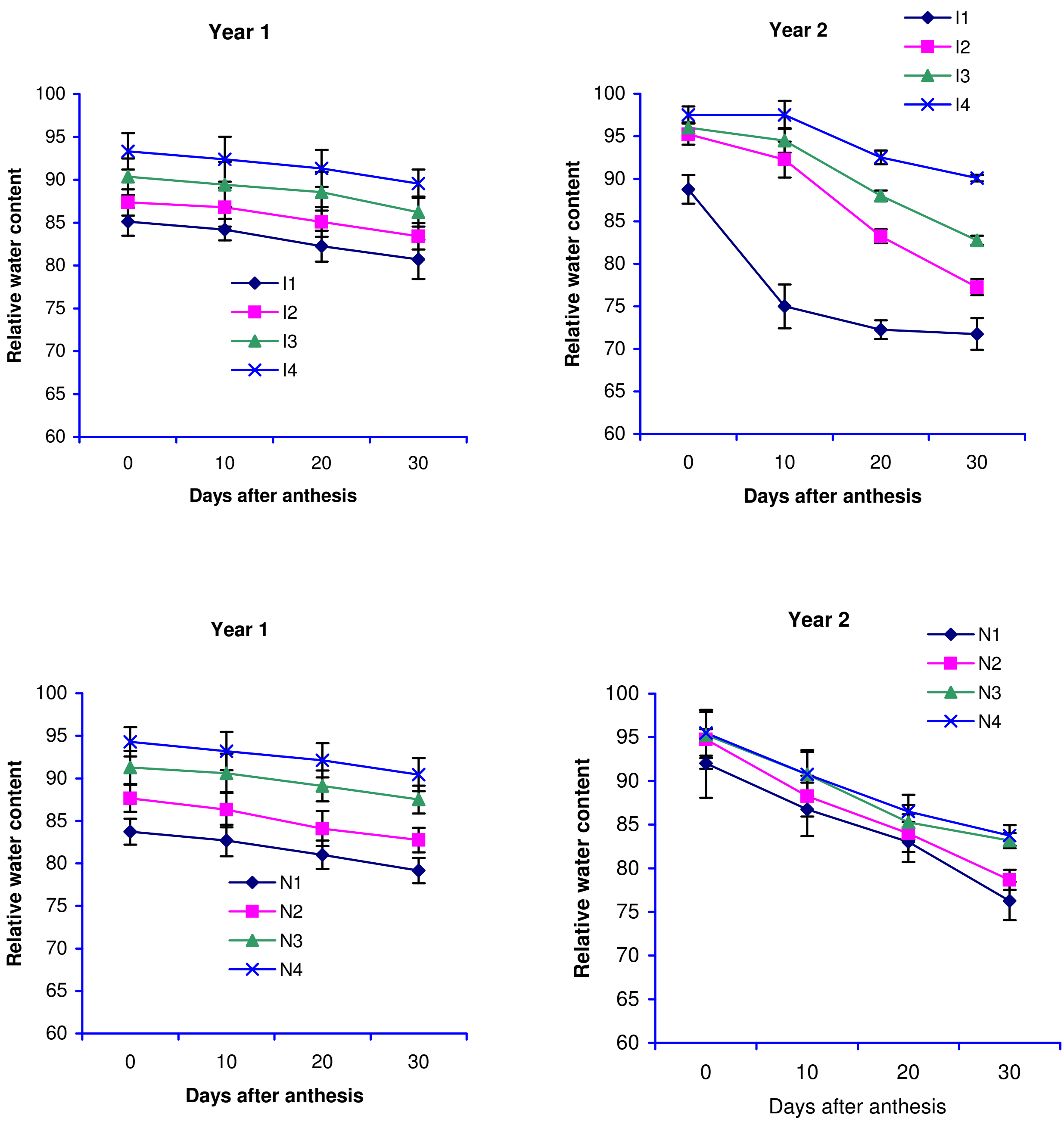

Figure 5. Irrigation and nitrogen management impacts on RWC (%) of wheat during the years 2002-03 and 2003-04

Productivity in crop plants may be increased by improving water use efficiency (WUE). Data regarding water use efficiency showed that in both the years 2002-03 and 2003-04 the irrigation treatments had significant ($P \leq 0.01$) effect on water use efficiency (Table 3). WUE at all irrigation levels, recorded for the year 2003-04 was higher than the year 2002-03. In general WUE decreased with increasing irrigation supply in both the years of study. The maximum WUE (15.88 kg ha^{-1} mm^{-1} during 2002-03 and 21.19 kg ha^{-1} mm^{-1} in 2003-04) was recorded under the treatment where only one irrigation was applied at tillering followed by two (irrigation at tillering and anthesis) three (irrigation at tillering anthesis and grain development) and four irrigations (irrigation at tillering, stem elongation, anthesis and grain development) respectively. The minimum WUE (10.97 kg ha^{-1} mm^{-1} during 2002-03 and 15.36 kg ha^{-1} mm^{-1} in 2003-04) was recorded under the treatment where four irrigations (irrigation at tillering, stem elongation, anthesis and grain development) were applied (Table 3). Nitrogen application during the years 2002-03 and 2003-04 significantly ($P \leq 0.01$) increased water use efficiency at all irrigation levels (Table 3). WUE of 15.08 kg ha^{-1} mm^{-1} during 2002-03 were recorded under the nitrogen treatment 150 kg N ha^{-1} followed by 100, 50 and 0 kg N ha^{-1}. In the year 2003-04, the maximum WUE (20.39 kg ha^{-1} mm^{-1}) was observed in the treatment where nitrogen was applied at 150 kg ha^{-1} (Table 3).

Table 3. Grain yield and water use efficiency (kg ha^{-1} mm^{-1}) of wheat under different irrigation regimes and nitrogen levels in the year 2002-03 and 2003-04

Treatments	Grain yield (kg ha^{-1})		WUE (kg ha^{-1} mm^{-1})	
	2002-03	**2003-04**	**2002-03**	**2003-04**
Irrigation regimes				
I-1	3208.25 d	2970.68 d	15.889	21.19 a
I-2	3527.75 c	3664.13 c	12.676	18.64 b
I-3	3952.83 b	5461.34 b	11.12 c	16.92 c
I-4	4729.25 a	5672.58 a	10.97 c	15.36 d
Nitrogen levels (kg ha^{-1})				
0	3093.75 d	3939.54 d	10.01 d	15.96 d
50	3619.58 c	4293.49 c	11.90 c	17.40 c
100	4156.25 b	4546.93 b	13.65 b	18.35 b
150	4548.50 a	4988.76 a	15.08 a	20.39 a

Data within columns followed by different letters are significantly different at $P < 0.05$.

Pearson correlations of yield and WUE with several physiological parameters are presented in Table 4. Different physiological traits in spring wheat as affected by limited water and nitrogen supply showed interdependence of these traits on each other. Water potential is important for its relation to cell turgor pressure which is important in stomatal opening and providing access of photosynthesis apparatus to CO_2.

Table 4: Simple correlation coefficients of water use efficiency(WUE) and grain yield with net CO2 assimilation rate (PN), transpiration rate (E), stomatal conductance (g_s), water potential (Ψ_w), osmotic potential (Ψ_s) and relative water contents (RWC) grown under different levels of irrigation and nitrogen (n = 48)

	2002-2003		2003-2004	
Traits	**GY**	**WUE**	**GY**	**WUE**
Pn	0.857**	-0.045	0.520**	-0.224
E	0.871**	0.019	0.833**	-0.116
g_s	0.788**	-0.266	0.713**	-0.199
Ψ_w	0.799**	0.340*	0.862**	0.417**
Ψ_s	0.818**	0.345*	0.894**	0.431**
RWC	0.783**	0.119	0.773**	0.443**

*,** Significant at 0.05 and 0.01probability levels, respectively
WUE = grain yield (kg ha^{-1})/ total water applied (mm); GY = grain yield (kg ha^{-1})

DISCUSSION

The major factor for enhanced productivity is the net CO_2 assimilation rate. CO_2 assimilation rate in plants is controlled by stomatal conductance, specific metabolic processes in carbon uptake, photochemical capacity or a combination of all these factors (Taiz and Zeiger, 1998). In this study, the net CO_2 assimilation rate was more during the year 2002–03 than the year 2003-04. The possible reason of this variation in photosynthetic rate may be the differences in weather conditions. The year 2003-04 was a dry year, resulting more water stress to the crop than the stress level during the year 2002-03. Net CO_2 assimilation rate decreased with increase in water deficit developed due to limited irrigation in the present study. Decrease in stomatal conductance as a result of water deficit could be the main reason of reduced CO_2 assimilation rate. These results are in conformity with the findings of Passioura, (1994) who also reported reduction in expansion of leaves and stomatal conductance a reason of reduced photosynthetic rate in wheat under water stress. Positive correlations (0.80 and 0.52) in the years 2002-03 and 2003-04, respectively between flag leaf photosynthetic rate and biomass production were observed in this study which indicate contribution of flag leaf in producing plant biomass (Table 4). These findings are well supported by the early reports (Patterson and Ohm, 1975; Mahmood *et al.*, 1991) who stated that flag leaf being the main site of photosynthesis during grain development possessed a close relationship to the plants grain yield capacity as it contributes its major proportion of assimilates to grain development, compared with other leaves of the same plant. Another reason of this decreased photosynthetic rate may be the decreased leaf water potential and relative water content under water stress due to limited irrigation, which has a pronounced effect on photosynthetic rate. Changes in leaf water potential might be attributable to a change in osmotic pressure, the osmotic component of water potential (Siddique et al., 2000). Results of our experiment are in line with the findings of Radin (1981) and Radin and Boyer (1982) who reported that N concentration in plants alters water relations of plants under water stress conditions. The results of the present study clearly revealed that net CO_2 assimilation rate was significantly higher at nitrogen level of 150 kg N ha^{-1} as compared to all other nitrogen treatments (0, 50 and 100 kg N ha^{-1}). Results of present study are also in line with the findings of Shangguan *et al*. (2000 b), who reported increased gas exchange parameters in wheat with N application.

Limiting irrigation in this study from four (control) to three; two and one irrigation resulted in decrease in transpiration rate ranging from 12-33 %. Reduced stomatal conductance due to water deficit imposed by limited irrigation is the main reason of this reduction in transpiration rate. These results are in good agreement with those of Javis and McNaughton, (1986) who reported enhanced transpiration rate per unit leaf area with an increase in stomatal conductance. Several other reports (Cooper *et al*., 1987 and Siddique *et al*., 1990) also indicate the increase in the rate of transpiration by the crop with increasing water applied as irrigation. Reduction in transpiration rate usually is followed by smaller reduction in incoming CO_2, which results in enhanced WUE (Grashoff and Verrerk, 1991; Pereira *et al*., 1992). Transpiration rate at 150 kg N ha^{-1} was 34 % higher than the transpiration rate observed at 0 kg N ha^{-1}. The result of this study are in conformity with those of Cooper *et al.,* (1987) and Oweis *et al.,* (1998), who reported increase in transpiration rate in wheat with increasing application of nitrogen fertilizers. However, contradictory to our results Cooper *et al*. (1983) and Pilbeam *et al*. (1995) reported that transpiration was not influenced by differences in nitrogen. Water stress due to limited irrigation resulted in decrease in water absorption at root level, thus reducing the transpiration rate. Increasing rate of transpiration with increasing supply of nitrogen in this study indicates that nitrogen application might have promoted the absorption of soil moisture (Zi-Zhen *et al.,* 2004) which ultimately increased the transpiration rate.

The results of this study indicate significant decrease (about 50 % on an average) in stomatal conductance in limited-irrigation treatments (three, two and one irrigation) as compared to control (four irrigations) which could be attributed to the partial closure of stomata due water deficit developed in water-limited treatments. These observations are well supported by the early findings of Heaton and Tallman (1987) who also reported decrease in stomatal conductance due to water deficit. Reduction in stomatal conductance limited CO_2 intake into leaf thereby reducing photosynthesis rates which in turn resulted in decreased biomass and low crop yield. This observation can be well related to Pereira et al. (1992) who reported that reduction in stomatal conductance limits the access of the photosynthetic apparatus to CO_2 before the photosynthetic apparatus is damaged by water deficit. In the present study, the stomatal conductance increased with increasing levels of nitrogen in all irrigation treatments, possibly due to more water uptake by the roots (Zi-Zhen *et al*., 2004). Radin and Mathews (1989); and

Radin (1990) also reported that increase in stomatal conductance with increasing nitrogen application was due to improved root's hydraulic conductance and water flux through the plant.

Water use efficiency indicates the performance of a crop growing under any environmental condition. Plant biomass production depends on the amount of water used for growth as well as on WUE. At leaf level water use efficiency is defined as the ratio of photosynthetic rate (Pn) to transpiration rate (Shangguan *et al*., 2000 b) whereas on crop level WUE is calculated as the ratio of grain yield (kg ha^{-1}) to the amount of total water applied in mm (Hussain and Al-Jaloud, 1995) or used (Ehdaie, 1995). Water use efficiency at all irrigation levels, recorded for the year 2003-04 was higher than the year 2002-03 which is obviously the result of variation in the total amount of water applied in two study years. In both years, WUE was significantly higher in limited irrigation treatments as compared to control. The maximum WUE (15.88 kg ha^{-1} mm^{-1} during 2002-03 and 21.19 kg ha^{-1} mm^{-1} in 2003-04) was recorded under the treatment where only one irrigation was applied at tillering followed by two (irrigation at tillering and anthesis) three (irrigation at tillering anthesis and grain development) and four irrigations (irrigation at tillering, stem elongation, anthesis and grain development). The results of our study confirms the findings of Zhang *et al*., (2002b), who also reported decrease in WUE with increasing amount of irrigation but yield increased. However, some other reports (Ehdai, 1995; Li *et al*., 1999; HoWell *et al*., 1998; Deng *et al*., 2002 and Zhang *et al.*, 2004) do not support results of this study. These reports indicate that irrigation can increase crop yield by improving soil water conditions and WUE significantly. The higher values of WUE observed in limited irrigation treatments as compared to control was mainly due to less water applied for these treatments. Nitrogen application increased water use efficiency at all irrigation levels in our study. The maximum values of WUE (15.08 kg ha^{-1} mm^{-1} during 2002-03 and 20.39 kg ha^{-1} mm^{-1} in 2003-04 were recorded under the nitrogen treatment of 150 kg N ha-1 followed by 100, 50 and 0 kg N ha^{-1} treatments. Results of present study are in good agreement with Shan and Chen, (1993), who also reported increased yield and water use efficiency in wheat with nitrogen application. Increased yield and WUE due to application of nitrogen in this study can be attributed to increase in yield components such as number of tillers, number of grains per spike, 1000-grain weight and harvest index. All these yield components were improved with nitrogen application with significantly higher values recorded from plots fertilized with 150 kg N ha^{-1}. The better performance of wheat fertilized with nitrogen at 150 kg ha^{-1} than the recommended practice for wheat (100 kg N ha^{-1}) could possibly be due to better development of root system by fertilizer application (Liu et al., 1998) which in turn is a desired character for improved crop water use and nutrient absorption especially under water deficit conditions. In addition, large and earlier canopy covers resulting from improved nitrogen uptake due to enhanced supply under water deficit developed due to limited irrigations was helpful in reducing soil water evaporation and making more water available for crop growth and improve WUE (Zhang *et al.*, 1998). The results of the present study are in good agreement with finding of some other researchers (Persand and Knosla, 1999; Stephens and Hess, 1999; Li *et al.,* 2001a; Li *et al*., 2001b) who reported that limited supplemental irrigation and nitrogen fertilizer application in wheat during the growth season can significantly increase water use efficiency and yields.

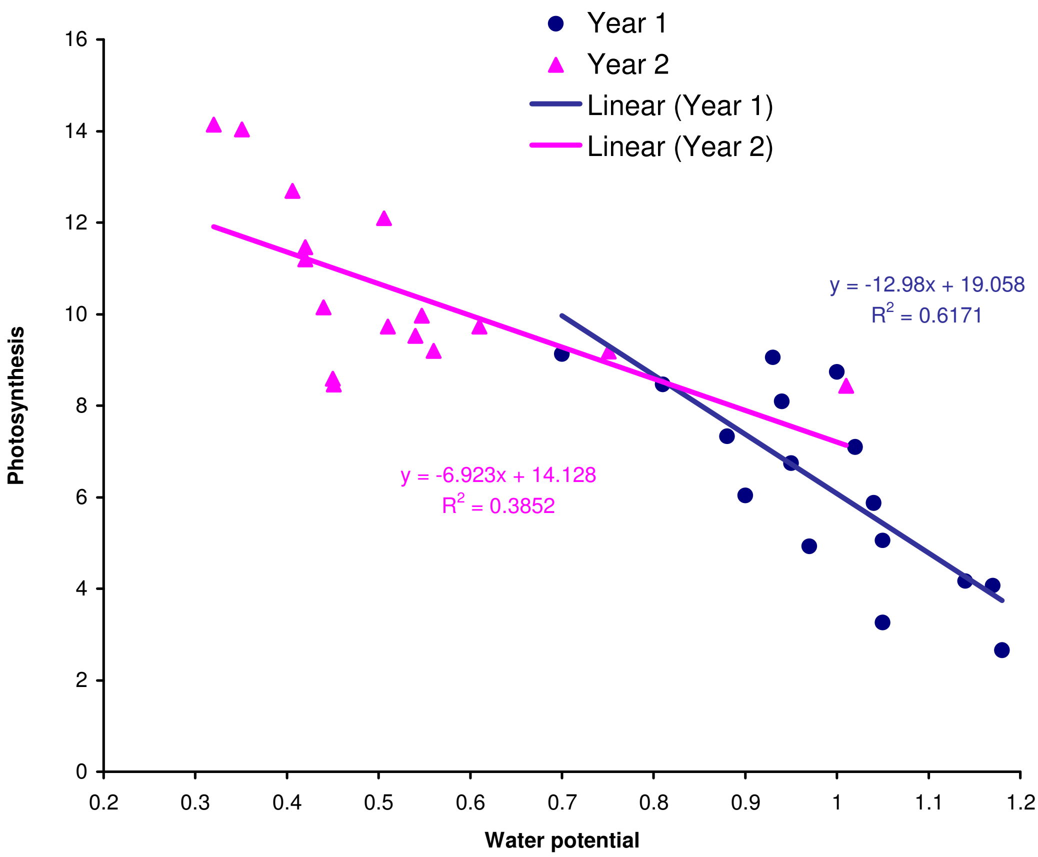

Figure 6. Relationship between water potential and photosynthesis

CONCLUSION

In this study, soil water deficit induced by limited irrigation at different stages of crop growth, significantly reduced flag leaf water potential and relative water contents. The reduction in water potential and relative water contents reduced the stomatal conductance, decreased transpiration which ultimately limited access of photosynthetic apparatus to CO_2; consequently, decreased rate of photosynthesis, dry matter production and final grain yield. The irrigation and nitrogen application improved the Net CO_2 assimilation rate, transpiration rate and stomatal conductance. The water use efficiency (WUE) increased with nitrogen application at all irrigation treatments.

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