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THE RESPONSE OF WHEAT (*TRITICUM AESTIVUM* L.) TO INTEGRATING EFFECTS OF DROUGHT STRESS AND NITROGEN MANAGEMENT

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Abstract

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An experiment was conducted to study the dual effects of drought stress and nitrogen levels in wheat crop under the climatic conditions of Bahawalpur, Pakistan. For this purpose, five drought stress regimes including control (D_1) drought stress at stem elongation stage (D_2), drought stress at stem elongation + earing stage (D_3), drought stress at stem elongation + earing + milking stage (D_4), drought stress at stem elongation + earing + milking + grain formation stage (D_5) and four nitrogen levels viz. control, 130, 160, 190 kg N ha^{-1} (N_0-N_3) were involved. The exposure of plants to drought stress leads to a noticeable decrease in transpiration rate, stomatal conductance, leaf relative water contents, nitrogen use efficiency and yield of wheat, however, nitrogen application improve these parameters. Besides this, by increasing drought stress water uptake capacity was increased and significant decrease was bring about by nitrogen application. Water use efficiency increased with increasing nitrogen rate and deficit irrigation regimes and it show the capability of crop to survive under drought stress conditions and increasing wheat crop yield in study region by reasonable increasing of nitrogen fertilizer rate.

Key words: drought stress, nitrogen, *Triticum aestivum*, water relations, gas exchange, nitrogen use efficiency

Introduction

Drought is the most common factor that limits the productivity of wheat crop and it reduces plant growth by affecting various physiological and biochemical processes, such as transpiration, translocation, ion uptake and nutrient metabolism (Farooq et al., 2008). The response of plants to water deficit differ significantly at various organizational levels depending upon intensity and duration of stress as well as plant species and its growth stages (Chaves et al., 2002). Understanding of plant responses to drought stress is of great importance and a fundamental part for making the crops tolerant to stress conditions (Zhao et al., 2008).

As nitrogen (N) is often the most limiting nutrient for crop yield in many regions of the world (Giller, 2004), N fertilizer is one of the main inputs for cereals crop production systems. The increase of agricultural food production worldwide over the past four decades has been associated with a 7-fold increase in the use of N fertilizers. Therefore, the challenge for the next decades will be to accommodate the needs of the expanding world population by developing a highly productive agriculture system and at the same time preserving the quality of the environment (Hirel et al., 2007). Losses of fertilizer N have been attributed to the combined effects of volatilization and leaching (Chen et al., 2004). Reduction of applied N fertilizer rate to an optimum level can decrease soil nitrate leaching (Power et al., 2000). Worldwide, nitrogen use efficiency (NUE) for cereal production including wheat is approximately 33% (Raun and Johnson, 1999).

The most effective factor on wheat yield is proper management of N fertilizer that is design of fertilizer application regimes should combine source of application, rate, timing and splitting with a view to optimizing wheat yield (Abedi et al., 2010). As nitrogen fertilizer rates and timing of application is an important factor in the obtaining of high yields (Cui et al., 2010). Efficient nitrogen fertilization is critical for economic wheat production and valuable indicator for rational N fertilization supply is NUE of mineral nitrogen fertilizer by growing crop, winter wheat (*Triticum aestivum* L.) in this case, together with nitrogen status in soil and plant. Role of nitrogen

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in enhancing the water use efficiency (WUE) particularly under drought stress conditions is very crucial and can be described on various scales from the leaf to the field. In its simplest terms, it refers to the ratio of grain yield (GY) to the water used during crop growth. WUE 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 (Ehdaie and Waines, 1993).

Wheat (*Triticum aestivum* L.) is a staple of many people's diets, the primary source of calories for over 1.5 billion people. It has been intensively grown throughout the twentieth century and now constitutes over a part of all cereal output (Reynolds et al., 1999). In Pakistan, wheat is the first most important cereal and is grown through out the country in a wide range of climatic conditions. It contributes 2.7% to gross domestic product (GDP) and 13.1% to the value added in agriculture. Being an important "winter" crop, wheat is grown on about 8805 thousands hectares with a total yield of about 24214 thousands tones and an average yield of 2750 kg ha^{-1} (Economic Survey of Pakistan, 2010-11).

The importance of N fertilization in increasing wheat production has been well recognized but still it is difficult to determine the quantities to apply under water deficit conditions. As wheat is an irrigated crop, its production is frequently exposed to water deficits at any stage of the crop development. The high cost of fertilizer nitrogen raises the question about the feasibility of applying N fertilizer under limited soil moisture conditions. This study was intended to investigate the combined effects of drought stress and nitrogen on wheat growth and productivity and thus determine the nitrogen requirement to get maximum output.

Present study was aimed to test whether the application of irrigation water at selective growth stages and nitrogen would significantly improve WUE and NUE of wheat crop.

Materials and Methods

A field experiment was conducted during the year 2010-11 on a clay loam soil at the research area (IUB Agriculture Farm) of the University College of Agriculture and Environmental Sciences, The Islamia University of Bahawalpur, Bahawalpur Pakistan. Highly recommended wheat (*Triticum aestivum* L.) cultivar (Sehr-2006) of Bahawalpur was selected for screening the effect of drought stress and nitrogen application on crop performance and yield. Physico-chemical analysis of soil of experimental site were as soil texture were clay loam, ECe 2.50 dS m^{-1}, pH 8.2, Ca + Mg 14 meq/L, available potassium 110ppm, total nitrogen 0.035%, phosphorus 3.5ppm, organic matter 0.48%. The field capacity of the soil was 18.35% and the saturation percentage was 41%. The experiment was laid out in Randomized Complete Block Design (RCBD) with split plot arrangement with drought stress levels in main plots while nitrogen in the sub plots. Each treatment was replicated three times. Net plot size was 3m x 8m. The seed rate was used at the rate of 100 kg ha^{-1}.

Drought stress (D) was applied as control (D_1) where no drought stress was applied and drought stress at stem elongation stage (D_2), drought stress at stem elongation + earing stage (D_3), drought stress at stem elongation + earing + milking stage (D_4), drought stress at stem elongation + earing + milking + grain formation stage (D_5) and nitrogen levels were applied as control (N_0), 130 kg ha^{-1} (N_1), 160 kg ha^{-1} (N_2), 190 kg ha^{-1} (N_3). Drought stress was applied by withholding irrigation water at different stages. 1/3 N was applied at the time of sowing as side dressing with the help of single row hand drill, 1/3 N with first irrigation and remaining 1/3 N was applied at booting stage. Phosphorus and potash was applied at the time of planting at the rate of 100 kg ha^{-1}. Urea, triple super phosphate and sulphate of potash were used as a source of fertilizers. All other cultural practices were standard and uniform for all treatments including control.

Following observations was recorded from the experiment during the course of study and the procedure of data collection was as under:

Flag leaf area (cm^2)

Fully expanded flag leaf area (FLA) was estimated by leaf area meter taking five samples from each plot and then average was taken.

Transpiration rate (m mol H_2O m^{-2} S^{-1}) and stomatal conductance (m mol m^{-2} S^{-1})

Transpiration rate (*E*) and stomatal conductance (C) was recorded from fully expanded flag leaf and were estimated after drought stress treatment at earing stage from the flag leaf of plant from each plot using an open system LCA-4 ADC portable infrared gas analyzer (Analytical Development Company, Hoddesdon, England) from 10:00 a.m. to 12.00 p.m.

Chlorophyll contents (SPAD-502 values)

A chlorophyll meter (SPAD-502, Minolta, Japan) was used to measure the chlorophyll contents in leaves of wheat at earing stage from the flag leaf and recorded the SPAD values. Five SPAD readings were taken around the midpoint of flag leaf from each treatment and they were averaged to get the mean SPAD values.

Leaf relative water contents (%)

Each sample consisting of three flag leaves were taken from each plot after drought stress treatment. Fresh weight of each sample was taken then the same leaves were dipped in the water for 14-16 hours and wiped with tissue paper and turgid weight was taken. After drying at 65 ± 5ºC, dry weight of each sample was taken. For each sample, leaf relative water content (LRWC) was calculated by using the formula (Karrou and Maranville, 1995) given below:

LRWC = Fresh weight – Dry weight/Turgid weight – Dry weight x 100

Water uptake capacity

Water uptake capacity (WUC) was measured by the formula given below:

WUC = Turgid weight – Fresh weight/Dry weight

Water use efficiency

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

WUE = GY/ TWA,
where

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

Nitrogen use efficiency

The nitrogen use efficiency (NUE) of mineral N fertilization was calculated according to Craswell and Godwin (1984) by the following equation:

NUE = (Grain yield $_F$ – Grain yield $_C$) / Fertilizer N applied

NUE = Nitrogen use efficiency (kg kg^{-1})
F-fertilized crop; C-unfertilized control

Biological yield

After harvesting the crop from each plot, whole material was sun dried. Then total weight of above ground portion in kilogram per plot was determined with the help of electric balance and then converted on hectare basis.

Grain yield

All the spikes from each plot were threshed, sun dried and weighed. Then weight of grains per plot was converted into kilograms per hectare.

Meteorological data

Meteorological data of growing season of crop was collected from the meteorological observatory of the Regional Agricultural Research Institute Bahawalpur, Pakistan. The mean maximum / minimum temperature, relative humidity and rainfall for the growing period of the crop have been depicted in Figure 1.

Statistical analysis

Data collected during the course of this study the data was computed for all attributes by using the MSTAT Computer

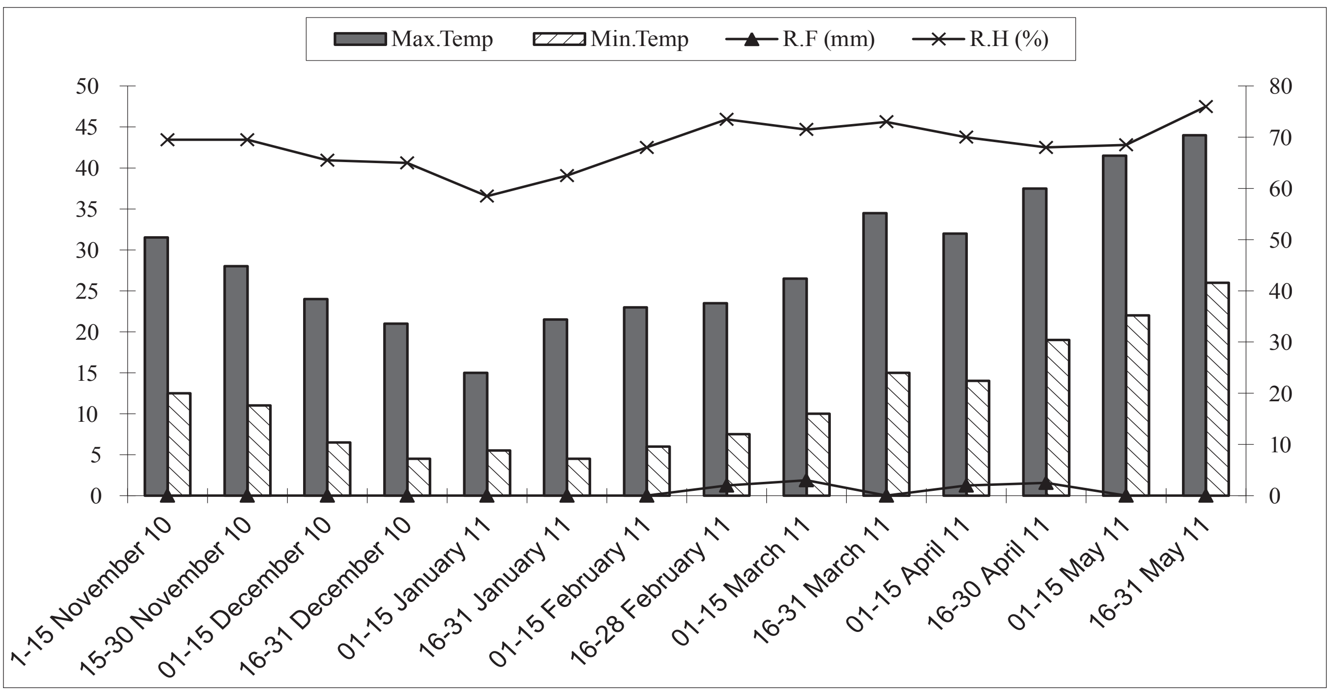

Fig. 1. Meteorological data during the growing season of crop

Program (MSTAT Development Team 1989). The treatment's means were compared using least significant difference test at 5% probability level (Steel et al., 1997).

Results

Flag leaf area: Analyzed data presented in Figure 2 indicated that drought stress decreases the flag leaf area (FLA) while nitrogen application improved it. The interaction between drought stress and nitrogen levels indicate that maximum FLA (37.09 cm^{-2}) was recorded at D_1 (no drought stress) when nitrogen was applied the rate of 190 kg ha^{-1} which is closely followed by N_2 (31.78 cm^{-2}) and N_1 (30.41 cm^{-2}) levels and minimum (12.32 cm^{-2}) was recorded at D_5 treatment in control where no nitrogen was applied (Figure 2).

Transpiration rate: It was observed from analyzed data presented in Figure 3 that the transpiration rate decreased by increasing the levels of drought stress. Transpiration rate at different nitrogen levels show that maximum transpiration rate (1.14 m mol H_2O m^{-2} S^{-1}) was observed at N_3 treatment

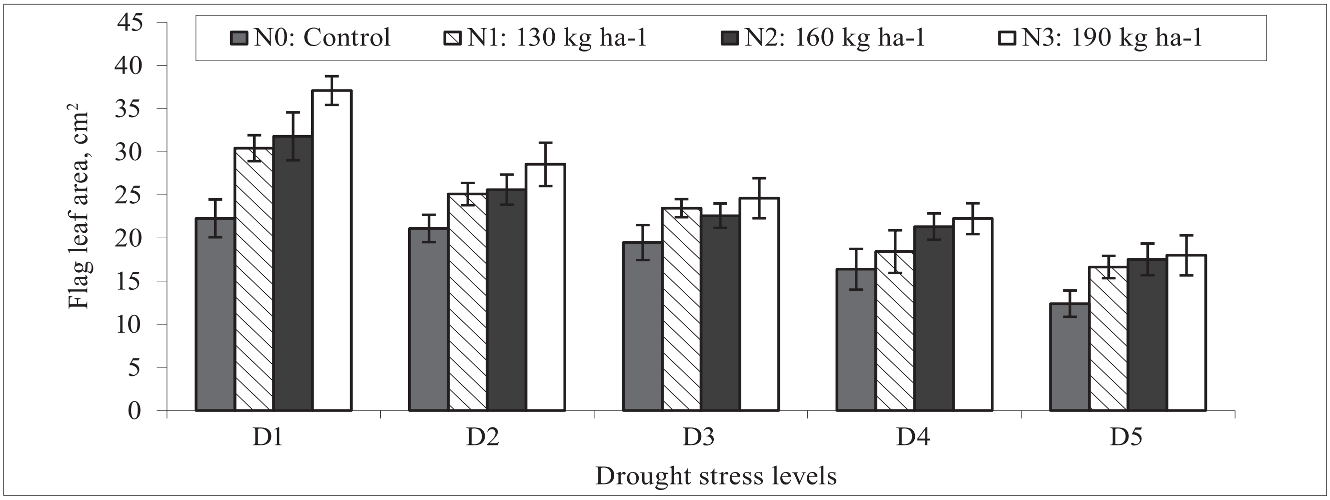

Fig. 2. Effect of different drought stress and nitrogen levels on flag leaf area in wheat

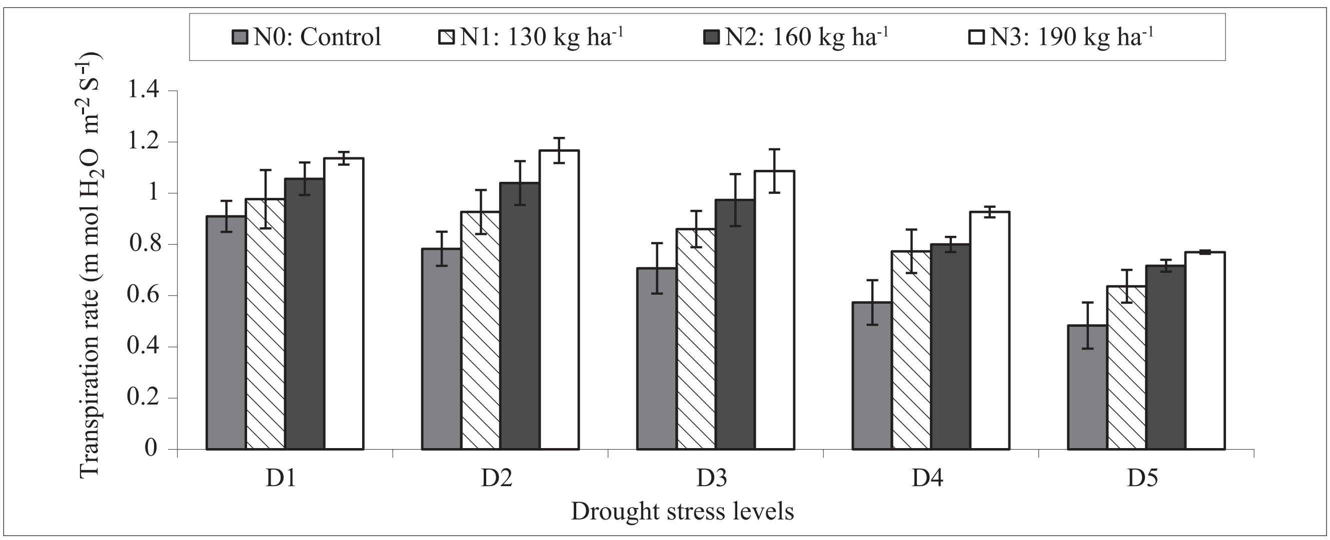

Fig. 3. Effect of different drought stress and nitrogen levels on transpiration rate in wheat

D_1 = Control (No drought stress); D_2 = Drought stress at Stem elongation stage; D_3 = Drought stress at Stem elongation + Earing stage;
D_4 = Drought stress at Stem elongation + Earing + Milking stage
D_5 = Drought stress at Stem elongation + Earing + Milking + Grain formation stage

(190 kg N ha^{-1}) when no drought stress was applied and minimum (0.48 m mol H_2O m^{-2} S^{-1}) was recorded when no nitrogen was applied under D_5 treatment.

Stomatal conductance: Data in Figure 4 depicted that statistically highly significant differences ($p \leq 0.01$) regarding stomatal conductance was observed at different drought stress and nitrogen levels. Analyzed data presented in Figure 4 also indicated that stomatal conductance decreased by increasing the levels of drought. Mean stomatal conductance at different drought stress level indicated that maximum stomatal conductance (62.53 m mol m^{-2} S^{-1}) was noted at D_1 which is closely followed by D_2 and minimum (30.90 m mol m^{-2} S^{-1}) was recorded at D_5 treatment. It was also showed that nitrogen treatment has positive effect on stomatal conductance (Figure 4).

Chlorophyll contents (SPAD-502 values): Chlorophyll contents differ significantly ($p \leq 0.01$) at different drought stress and nitrogen levels (Figure 5). Analyzed data present-

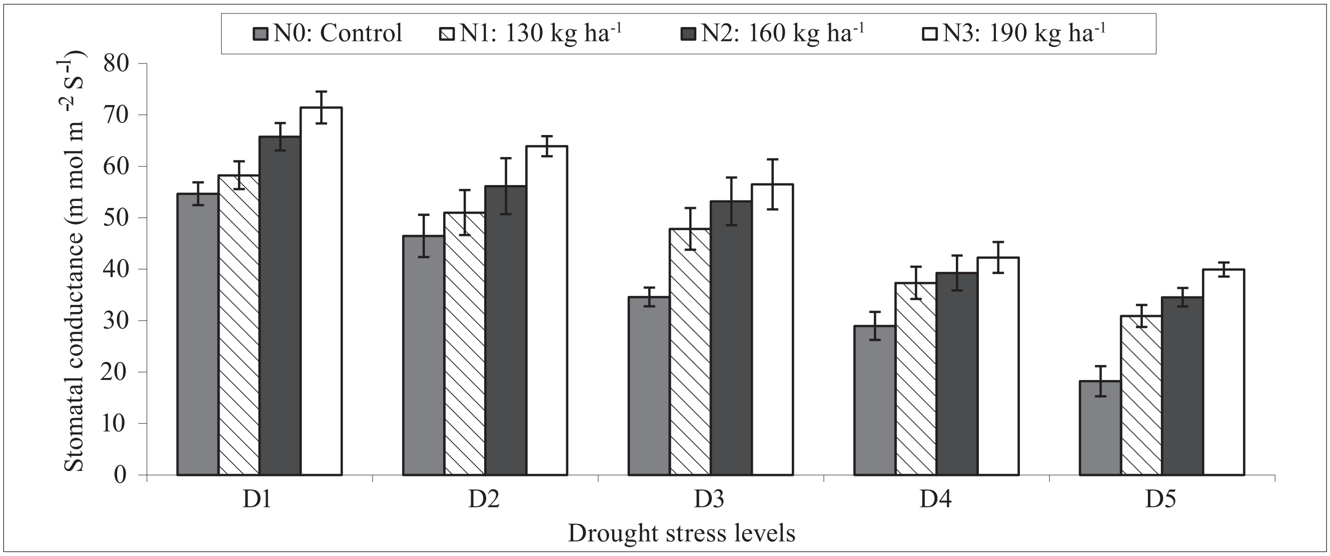

Fig. 4. Effect of different drought stress and nitrogen levels on stomatal conductance in wheat

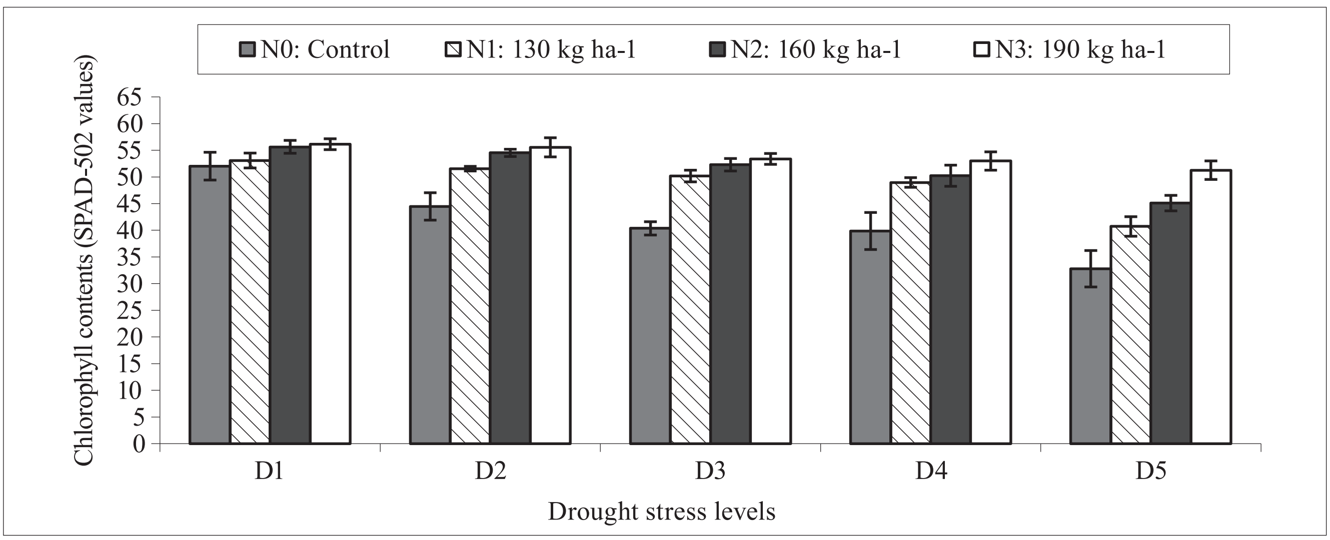

Fig. 5. Effect of different drought stress and nitrogen levels on chlorophyll contents (SPAD-502 values) in wheat

D_1 = Control (No drought stress); D_2 = Drought stress at Stem elongation stage; D_3 = Drought stress at Stem elongation + Earing stage; D_4 = Drought stress at Stem elongation + Earing + Milking stage

D_5 = Drought stress at Stem elongation + Earing + Milking + Grain formation stage

ed in Figure 5 also showed that by increasing the nitrogen levels the chlorophyll contents also increased. Maximum chlorophyll contents (53.86) were noted at N_3 level where nitrogen was applied at the rate of 190 kg ha^{-1} and minimum (41.90) was recorded at N_0 treatment (control). The interaction between drought stress and nitrogen levels also indicated that maximum chlorophyll contents was recorded at D_1 when nitrogen was applied at the rate of 190 kg ha^{-1}.

Leaf relative water contents: It is evident from data presented in Figure 6 that statistically highly significant ($p \leq 0.01$) differences were found among different drought stress levels regarding leaf relative water contents (LRWC). Analyzed data in Figure 6 revealed that LRWC decreased with increase in the drought stress levels while the effect of application of nitrogen is totally opposite to the drought stress. The interaction among different drought stress and nitrogen levels indicate that maximum LRWC (71.11%) was recorded at D_1 with nitrogen application at the rate of 190 kg ha^{-1} which was closely followed by D_2, D_3 and D_4 levels and minimum (30.16%) was recorded at D_5 in control treatment where no nitrogen was applied (Figure 6).

Water uptake capacity: It is clear from Figure 7 that statistically significant variation ($p \leq 0.01$) was recorded for water uptake capacity (WUC) at different drought stress levels and

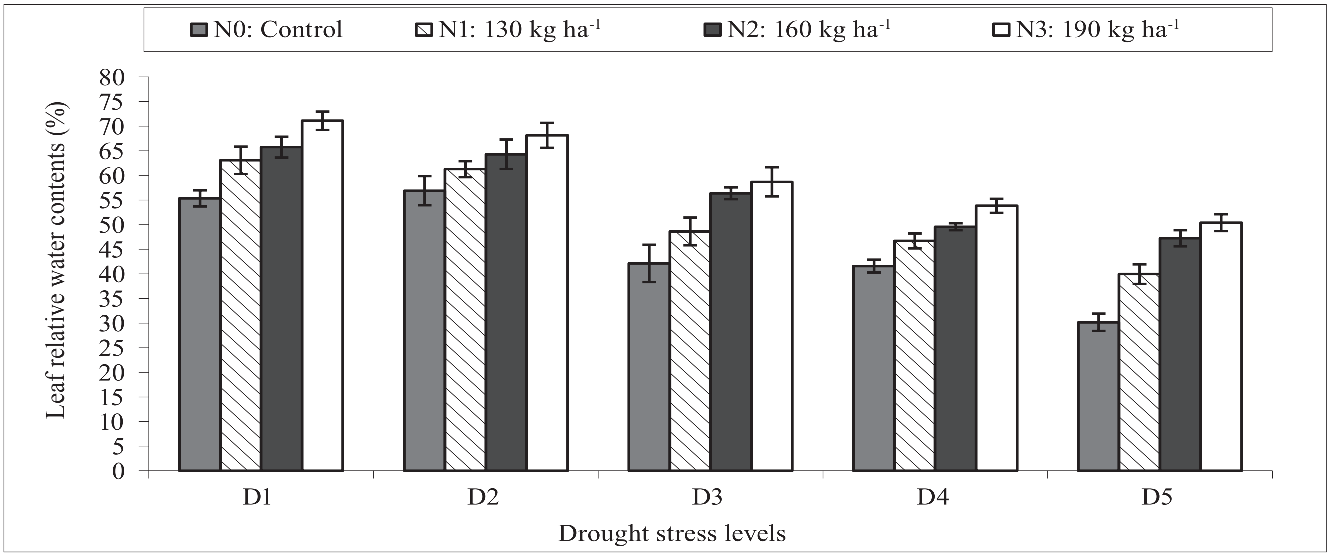

Fig. 6. Effect of different drought stress and nitrogen levels on leaf relative water contents in wheat

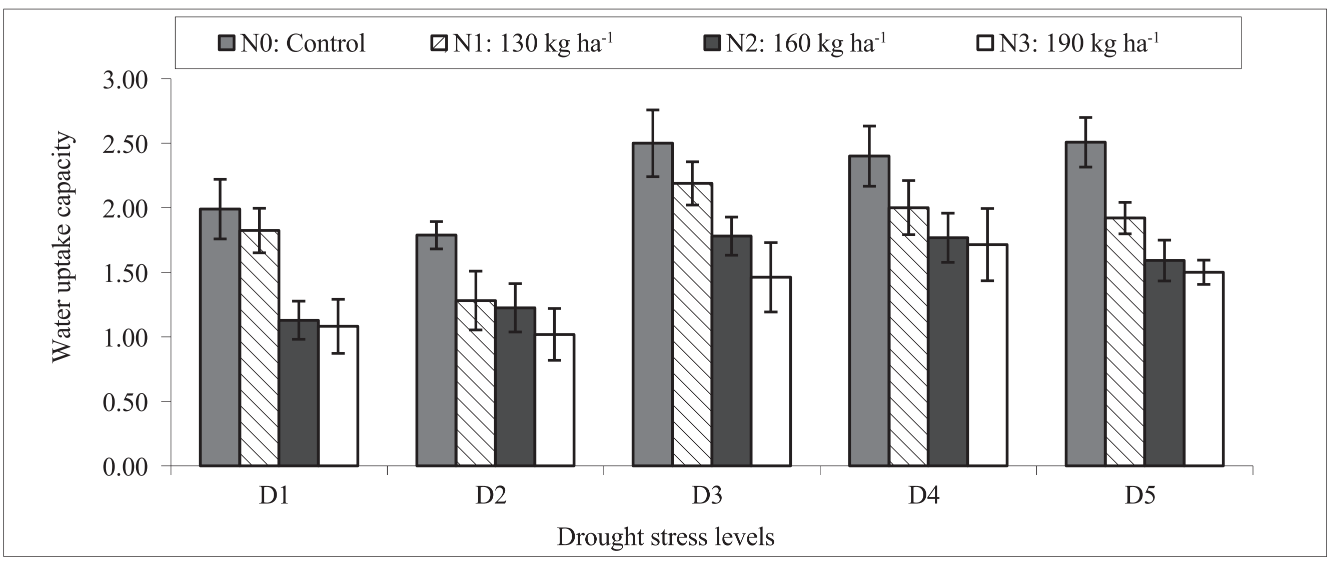

Fig. 7. Effect of different drought stress and nitrogen levels on water uptake capacity in wheat

application of nitrogen. Figure 7 indicates that WUC decease by increasing the rate of nitrogen application. Mean WUC at different nitrogen treatment was noted and minimum WUC (1.33) was observed at D_2 treatment. At different nitrogen treatment, maximum WUC was noted at N_0 treatment where no nitrogen was applied and by increasing the levels of nitrogen the WUC decrease (Figure 7).

Water use efficiency: Water use efficiency (WUE) increased with increase in the drought stress levels and differed significantly ($p \leq 0.01$) from each other (Figure 8). Application of nitrogen significantly improved the WUE. Interaction between different drought stress and nitrogen levels indicate that both the drought stress and nitrogen levels have positive and significant effect in improving the WUE (Figure 8).

Nitrogen use efficiency: Analyzed data (Figure 9) indicate the nitrogen use efficiency (NUE) under different drought stress treatment and maximum NUE (12.84 Kg kg^{-1}) was recorded in N_3 treatment, which is statistically at par with

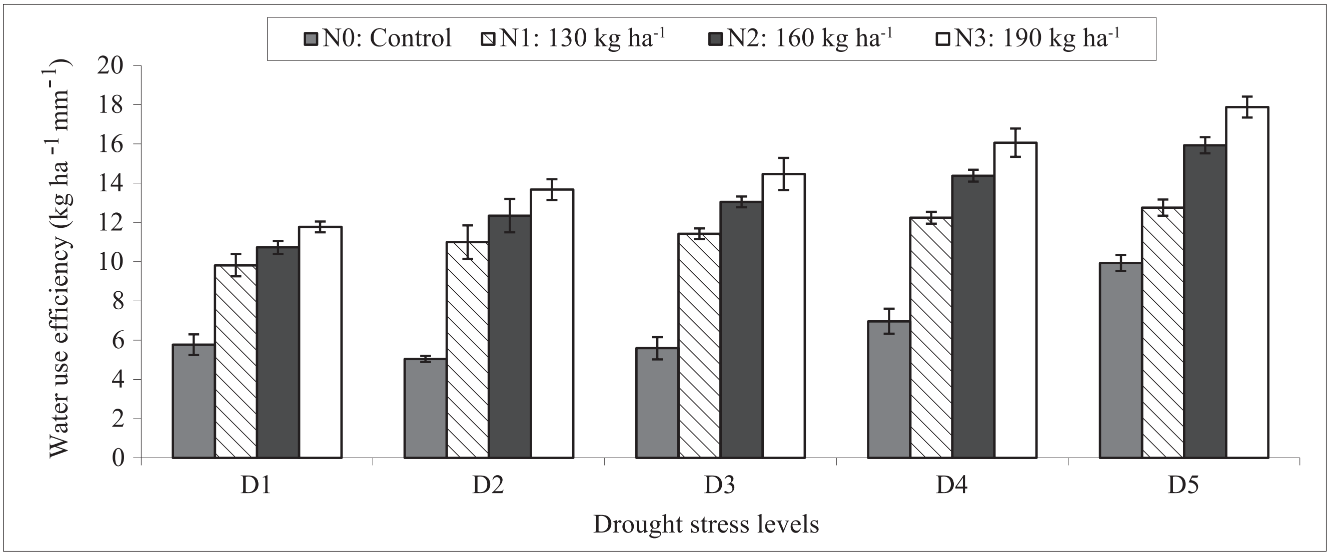

Fig. 8. Effect of different drought stress and nitrogen levels on water use efficiency in wheat

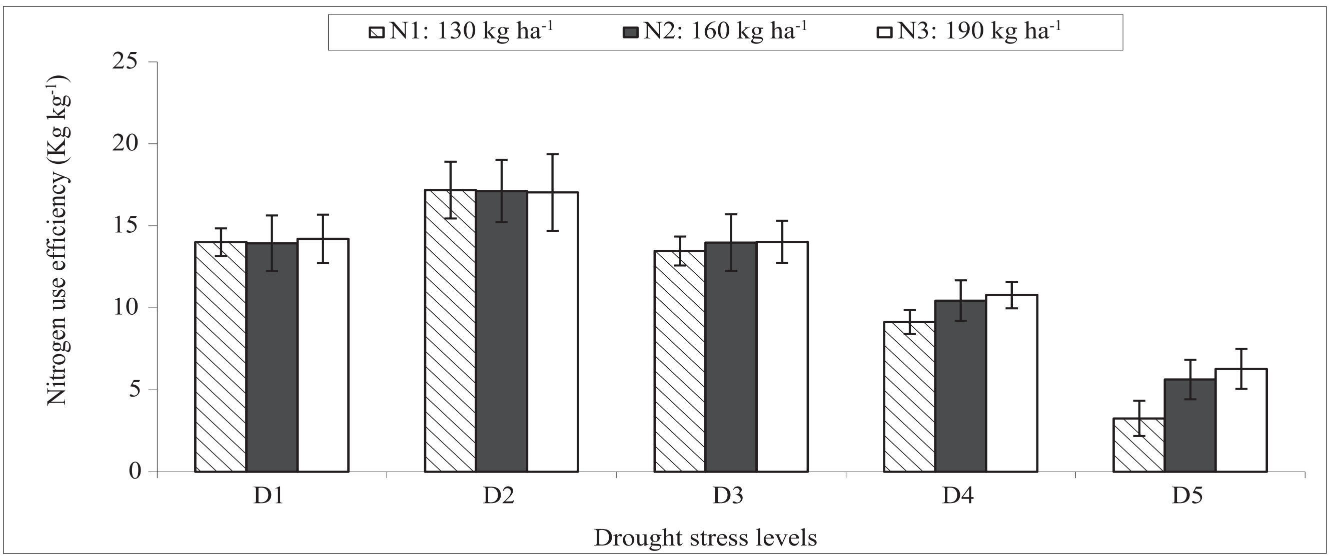

Fig. 9. Effect of different drought stress and nitrogen levels on nitrogen use efficiency in wheat

D_1 = Control (No drought stress); D_2 = Drought stress at Stem elongation stage; D_3 = Drought stress at Stem elongation + Earing stage; D_4 = Drought stress at Stem elongation + Earing + Milking stage

D_5 = Drought stress at Stem elongation + Earing + Milking + Grain formation stage

N_2 and N_1. NUE at D_3 treatment in N_3, N_2, N_1 levels is statistically at par with the D_1 (Control) in N_3, N_2, N_1 treatments. Mean NUE under different levels of nitrogen also differ significantly ($p \leq 0.05$) at D_4 and D_5 drought stress treatments. Interaction between different drought stress and nitrogen levels indicate that NUE differ significantly ($p \leq 0.01$) among different drought stress levels (Figure 9).

Biological yield: The data for biological yield at different drought stress and nitrogen application levels is presented in Figure 10. It indicated that biological yield of plants decreases with increase in the drought stress levels while the application of nitrogen improves the biological yield in wheat crop (Figure 10). Interaction between drought stress and nitrogen treatment indicate that maximum biological yield was recorded in N_3 treatment at D_1 level and minimum was recorded N_0 (control) and same trend was observed at D_3, D_4 and D_5 treatment (Figure 10).

Grain yield: Data regarding grain yield (Figure 11) indicated that statistically significant differences ($p \leq 0.01$) were present among different drought stress levels. Analyzed data shown in Figure 11 also indicated that grain yield increase with the increase in application of nitrogen but drought stress has inverse effect on grain yield. Mean maximum grain yield (4284 kg ha^{-1}) was obtained at D_1 and minimum (2119 kg ha^{-1}) at D_5 treatment. Application of nitrogen significantly improves the grain yield however, statistically non-significant

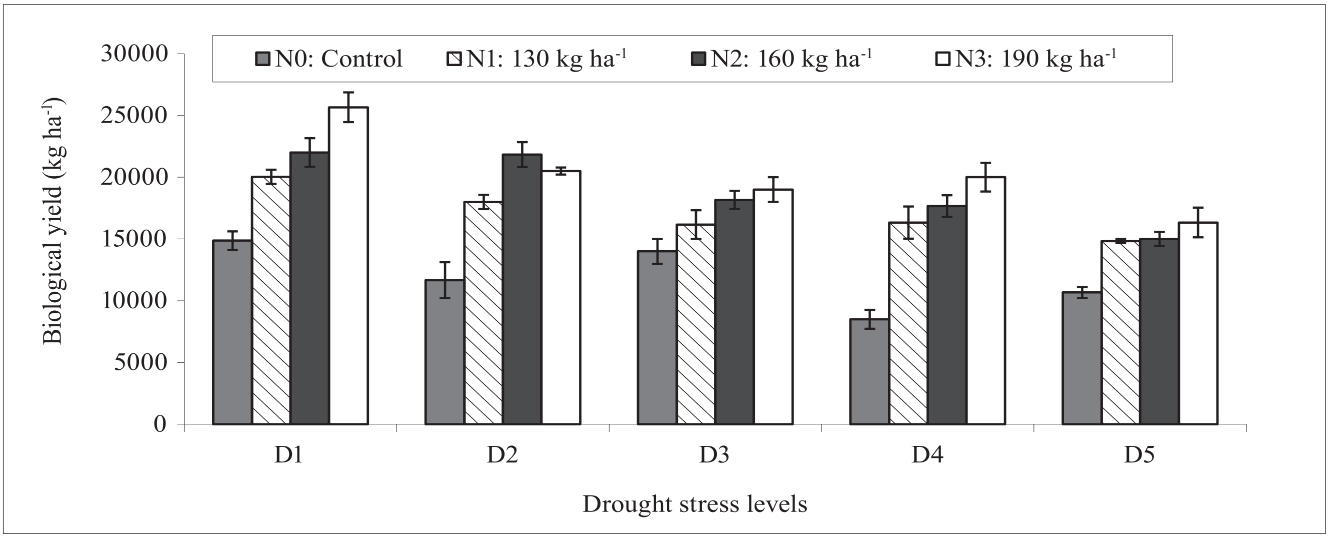

Fig. 10. Effect of different drought stress and nitrogen levels on biological yield in wheat

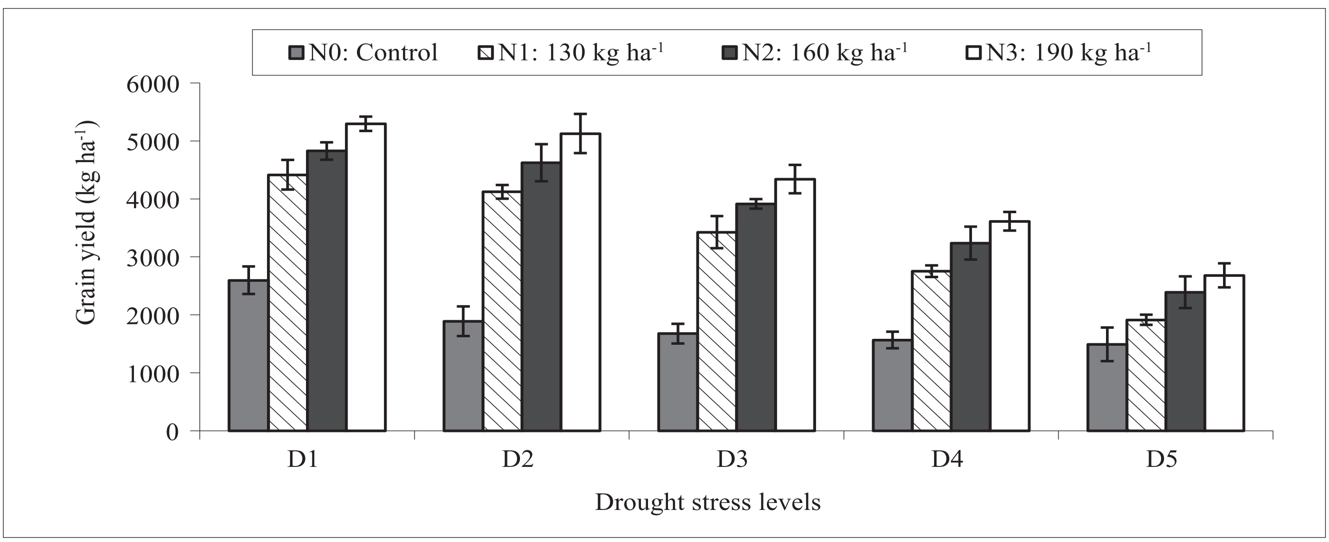

Fig. 11. Effect of different drought stress and nitrogen levels on grain yield in wheat

difference was recorded regarding grain yield under N_2 (160 kg ha^{-1}) and N_3 (130 kg ha^{-1}) levels at all the drought stress levels (Figure 11).

Correlation and linear regression: The correlation coefficients (*r*) between different parameters (Table 1) clearly show a significant positive relationship ($r \geq 0.60$, $P > 0.001$) of wheat crop grown under drought stress conditions and under different levels of nitrogen (Table 1). However, the correlations between the water relation indices (LRWC, WUC and WUE) with other parameters were insignificant. The correlations between water relation attributes like LRWC and WUC, LRWC and WUE, WUC and WUE were also non-significant (Table 1).

A linear regression analysis was performed between various physiological and yield parameters. The R^2 values were larger than 0.50 ($P < 0.01$), indicating a linear regression between flag leaf area and grain yield (Figure 12), Chlorophyll contents and grain yield (Figure 13), transpiration rate and stomatal conductance (Figure 15) and biological yield and grain yield (Figure 14).

Discussion

The flag leaf area (FLA) decrease under water deficit relative to the well-watered treatment. The observed decrease in FLA under water deficit condition is in agreement with the previous reports on durum wheat (Araus et al., 1997a), barley (Araus et al., 1997b). In the present study, decrease in FLA under water deficit condition may be due to the loss of weight of leaf (Figure 2). Relationship between flag leaf

Table 1
Correlations coefficients (r) of flag leaf area, water relation parameters, nitrogen use efficiency, physiological and yield attributes of wheat grown under drought stress and nitrogen treatments

	LRWC	WUC	WUE	NUE	Chl	*E*	C	BY	GY
FLA	0.91**	-0.59 NS	0.02 NS	0.71*	0.80*	0.89*	0.94**	0.86*	0.89*
LRWC	-	-0.68 NS	0.12 NS	0.73*	0.89*	0.95**	0.96**	0.82*	0.89*
WUC	-	-	-0.31 NS	-0.67 NS	-0.58 NS	-0.66 NS	-0.62 NS	-0.63 NS	-0.74 NS
WUE	-	-	-	0.50 NS	0.38 NS	0.23 NS	0.04 NS	0.44 NS	0.36 NS
NUE	-	-	-	-	0.80*	0.80*	0.71*	0.84*	0.92**
Chl	-	-	-	-	-	0.93**	0.89*	0.86*	0.89*
E	-	-	-	-	-	-	0.96**	0.88*	0.94**
C	-	-	-	-	-	-	-	0.82*	0.89*
BY	-	-	-	-	-	-	-	-	0.93**

*, **, *** = significant at 0.05, 0.01, and 0.001 levels, respectively; NS = non-significant
FLA = Flag leaf area; LRWC = Leaf relative water contents; WUC = Water uptake capacity; WUE = Water use efficiency
NUE = Nitrogen use efficiency; Chl = Chlorophyl contents; *E* = Transpiration rate; C = Stomatal conductance;
BY = Biological yield; GY = Grain yield

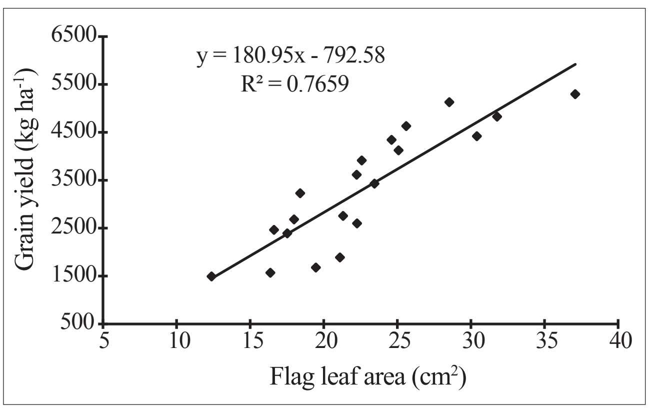

Fig. 12. Regression between flag leaf area and grain yield in wheat

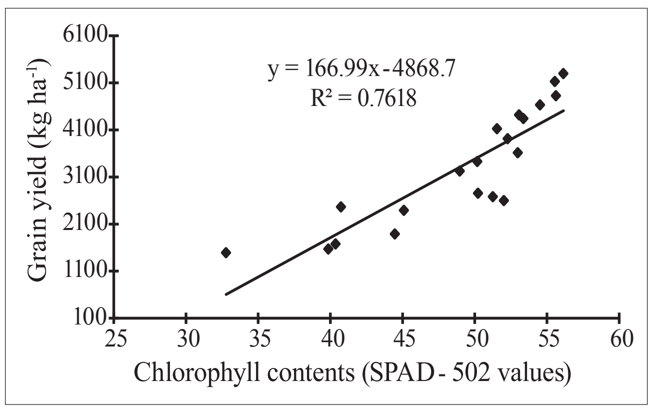

Fig. 13. Regression between chlorophyll contents (SPAD-502 values) and grain yield in wheat

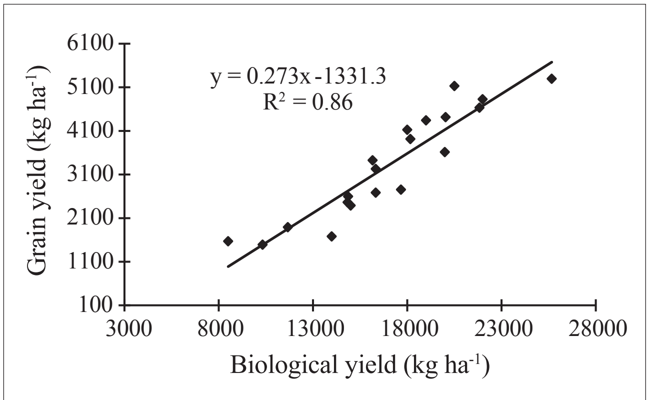

Fig. 14. Regression between biological yield and grain yield in wheat

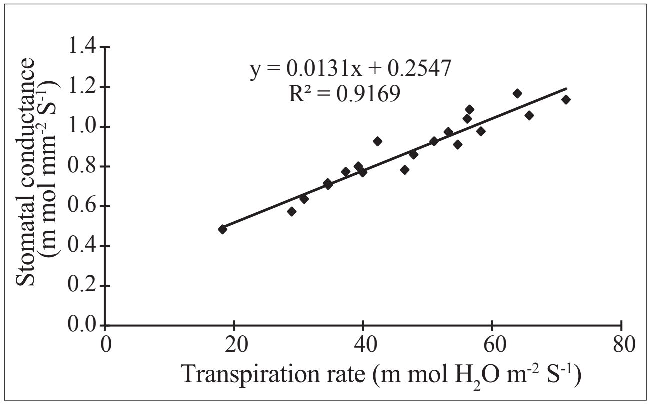

Fig. 15. Regression between transpiration rate and stomatal conductance in wheat

area and grain yield was found to be significant in the present study and it show the contribution of flag leaf in producing plant biomass and grain yield (Figure 12). These findings are well supported by 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.

In the present study, it was observed that there is strongly correlation of transpiration rate with grain yield (Table 1) and selection for higher rates of leaf transpiration has improved yield most probably because the source provides the assimilate to the sink (Araus et al., 2001). Increase in the rate of transpiration by the crop with increasing water applied as irrigation (Siddique et al., 1990). The result of present study are in conformity with those of Oweis et al. (1998) who reported that transpiration rate in wheat crop increased with the application of nitrogen fertilizers. In the present experiment, the stomatal conductance increased with increasing levels of nitrogen in all drought stress treatments (Figure 4) and possibly it may be due to more water uptake by the roots (Zi-Zien et al., 2004).

Chlorophyll contents values is a sensitive indicator of the tolerance of the photosynthetic apparatus to environmental stress (Maxwell and Johnson, 2000). Chlorophyll contents values in this study were sensitive to water deficit at tillering and grain-filling stages. The patterns of changes in chlorophyll contents values observed in this study are supported by the pattern of change under drought stress conditions (Zlatev and Yordanov, 2004). Leaf RWC is considered as a sensitive indicator of drought stress and more useful integrator of plant water balance (Clavel et al., 2005). Drought stress cause disruption in water balance of a plant and as a result of which the RWC of leaves decreased (Bajjii et al., 2001).

NUE was defined by Moll et al. (1982) as grains produced per unit of available N (soil + fertilizer or as fertilizer N). In the present experiment, NUE was calculated as grain production per unit of applied N fertilizer and Figure 9 showed that NUE was found to increase with increasing N level. Campbell and Davison (1979) suggested that, inefficient use of N is associated with increased drought stress induced by excessive vegetative growth. Part of the decrease in NUE can be attributed to decreased light intensity or increased evapotranspiration that could result from excessive vegetation (Pearman et al., 1977).

Radin and Boyer (1982) reported that N concentration in plants alters water relations of plants under drought stress conditions. WUE indicates the performance of a crop growing or plant biomass production per unit of amount of water used for growth. WUE was significantly higher in limited irrigation treatments as compared to control and maximum WUE in the present investigation (Figure 8) was recorded under the treatment where only two irrigations (D_5) followed by three (D_4) four (D_3) and five irrigations (D_2). The results of our study also confirms the findings of Zhang et al. (2002b) who reported that WUE decreased with increasing the amount of irrigation but yield increased. However, findings of Zhang et al. (2004) do not support results of this study. They indicate that irrigation can significantly increase WUE and crop yield by improving soil water conditions and 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 drought stress levels in our study. 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 and significantly higher values recorded from plots fertilized with 190 kg N ha^{-1}. The 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. The results of the present study are in good agreement with finding of Li et al. (2001b) who reported that limited irrigation and nitrogen fertilizer application in wheat during the growth season could significantly increase water use efficiency and yields.

Conclusions

In this study, soil water deficit induced by limited irrigation at different stages of crop growth significantly reduced the flag leaf area and leaf relative water contents. The reduction in leaf relative water contents reduced the stomatal conductance, decreased transpiration that ultimately limits the access of photosynthetic apparatus to CO_2, dry matter production and final grain yield. N application improved all the physiological, water relation and yield parameters. The WUE increased with nitrogen application under the drought stress treatments. It is also concluded that application of nitrogen is important to improve WUE and enables the plants to survive under drought stress conditions.

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