



## RESEARCH PAPER

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## Effects of water deficit stress and nitrogen fertilizer on wheat varieties

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### Abstract

This field study was conducted to evaluate the effect of water deficit stress after anthesis on proline content and seed yield of four wheat genotypes during 2013 in Research Farm of Faculty of Agriculture, University of Miandoab, Iran as a factorial split plot based on randomized complete block design with three replications. Water stress levels were irrigation in 20, 40 and 60 percent field capacity depletions and used from flag leaf initiation. Nitrogen levels were 20, 40, 60 and 80 Kg ha<sup>-1</sup>. Studied genotypes were 'Alvand' and 'Shahriar' bread wheat and PGS 01-60-335 and IDW 01-61-130 durum wheat. The results showed that maximum amount of seed yield and proline content was obtained from 80 Kg ha<sup>-1</sup> nitrogen fertilizer application treatment. Result of combined analysis showed that the effect of year was significant on seed yield but it had no significant effect on proline content. Under severe water stress (60% field capacity depletion) conditions and 80 Kg ha<sup>-1</sup> nitrogen, seed yield of 'Alvand', 'Shahriar', Durum PGS 01-60-335 and Durum IDW 01-61-130 showed 72.17%, 75.37%, 54.18% and 44.81% decrease in the first year and 72.03%, 71.31%, 55.62% and 45.51% decline during second year compared to normal conditions (20% field capacity depletion). Meanwhile, proline content of flag leaf in 'Alvand', 'Shahriar', Durum PGS 01-60-335 and Durum 01-61-130 were increased by 16.17, 15.76, 19.43 and 20.39 fold respectively. In contrast, under severe water stress (60% field capacity depletion) conditions, maximum and minimum yield were recorded in Durum IDW 01-61-130 and 'Shahriar'. Under severe water stress conditions, the major decrease in seed yield and the minimum increase in proline content were documented in 'Alvand' and 'Shahriar'. However, the lowest decrease in seed yield, and the highlighted increase in proline content were measured in Durum PGS 01-60-335 and IDW 01-61-130 genotypes. In total, water deficit stress had adverse effects on yield of wheat genotypes and nitrogen fertilization had negligible potential to compensate the deteriorative effects of drought condition.

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## Introduction

Biotic and abiotic stresses are of main problems of agricultural systems (Balouchi, 2010; Hassanpouraghdam *et al*, 2009). Water stress is one of the most important abiotic stresses adversely affects crop production in many regions of the world (Secenji *et al*, 2005). In most cereal crops undergrown in water deficiency conditions, drought stress affects approximately one-third of the yield potential of plants (Blum, 2005). The main reason for a cross-over under conditions of variable water supply is an inherent difference among the tested cultivars in drought resistance, beyond difference in their yield potential (Zarei *et al*, 2007). This was also observed in international wheat variety trials where stress environments often were represented by mean yield of 4-5 t/ha as compared with a maximum yield of 8 t/ha in common wheat production areas (Blum, 2005; Secenji *et al*, 2005).

Osmotic adjustment is common physiological response of plants to most stress conditions where it is regulated by the accumulation of free amino acids, proline and sugars in the roots and shoots of stress affected plants. Proline accumulation is a widespread plant response to environmental stresses such as low water availability.

Proline has a unique role as an osmoticum under abiotic mainly water deficit conditions. In particular, because of its zwitterionic status and high hydrophilic characteristics, proline acts as a “compatible solute”, i.e. one that can accumulate to high concentrations in the cell cytoplasm without interfering with cellular structure and/or metabolism (Ali *et al*, 1994; Rauf *et al*, 2007). There is presently no clear agreement about the function of drought-induced proline accumulation, although a role in osmo-regulation seems likely (Samaras *et al*, 1995; Gupta *et al*, 2001). Other functions of proline accumulation have also been proposed, including stabilization of macromolecules, a sink of carbon and nitrogen for use after relief of water deficit conditions (Samaras *et al*, 1995; Saint *et al*, 2008), radical detoxification (Smirnoff and Cumbes, 1989) and regulation of

cellular redox status (Heuer, 1999; Farshadfar *et al*, 2002).

Nitrogen fertilization is one of the most important and effective implements in agriculture, stimulating a lot of vital processes in plants. The amount of applied nitrogen in plants must be carefully managed to ensure that, N will be available throughout the growing season and the vegetative and reproductive development will be not restricted (Brich and Long, 1990; Zhang *et al*, 2008). Nitrogen uptake and utilization by plants and wheat are determined by genotypic differences and are linked to a variety of morphological and physiological factors, including the length and activity of the root system, the intensity of nitrate uptake, activity of nitrate reductase, sink of grains, carbohydrate production and N losses due to soil characteristics and leaching (Fathi *et al*, 1997; Shibu *et al*, 2010). Photosynthetic capacity of leaves in most crops is closely related to their nitrogen content, and chlorophyll quantity is a very stable parameter for soil nitrogen uptake estimation (Brown, 2000; Shangguan *et al*, 2000). Carbohydrate metabolism is also determined by the availability of nitrogen as the major limiting factor (Tranaviciene *et al*, 2007; Shibu *et al*, 2010). At the present time, there is no general accepted physiological model for selecting the optimal fertilization regime in field crops. The objective of the present study was to determine the effects of drought stress and supplementary nitrogen fertilizer on winter wheat species (*Triticum aestivum* and *Triticum durum*) seed yield and proline content.

## Materials and methods

This field study was conducted in Research Farm of Faculty of Agriculture, University of Miandoab, Iran during 2013. The soil texture of experimental site was sandy clay loam. The research field was located in a semi-arid region. This experiment was conducted as a factorial split plot based on randomized complete block design with three replications. Water stress and nitrogen levels were located in main plots and wheat genotypes were employed in sub-plots. Water stress levels were irrigation in 20, 40 and 60 percent field

capacity depletions and were used beyond flag leaf initiation. Nitrogen levels were application of 20, 40, 60 and 80 Kg ha<sup>-1</sup> nitrogen. Studied genotypes were 'Alvand' and 'Shahriar' bread wheat cultivars and PGS 01-60-335 and IDW 01-61-130 durum wheat cultivars. Plant density was 400 plants per m<sup>2</sup>.

#### Measurements

Proline content was measured in flag leaf, its related internode and plant root crown after anthesis. Finally at the maturing stage, seed yield was determined.

Soil water content in FC stage was measured by pressure plate set. FC was 21/1%. Related FC<sub>80%</sub>, FC<sub>60%</sub> and FC<sub>40%</sub> were calculated as 16/9%, 12/6% and 8/4% respectively. Beyond water deficit implementation, soil water content was evaluated by the present equation in two days intervals.

$$\text{Soil water content} = (W_1 - W_2 / W_2) * 100$$

Where W<sub>1</sub>: Mass of soil before drying and W<sub>2</sub>: Mass of soil after drying

#### Grain yield

After threshing, each spike was separated from the seeds and the weight of seeds was calculated based on Kg ha<sup>-1</sup>.

#### Proline content

Free proline accumulation was determined by using Bates *et al* method (1973). 0.5 g dry weight of root crown, flag leaf and flag leaf internode were homogenized with 3% sulfosalicylic acid for 72 h. Thereafter, the homogenate was centrifuged at 3000 g for 20 min. The supernatant was treated with acetic acid and ninhydrin, and boiled for one hour. Then the absorbance was read at 520 nm by a spectrophotometer (Biochrom S 2100). Content of proline was expressed as mg g<sup>-1</sup> DW of plant material.

#### Statistical analysis

The mean values of grain yield and proline content were taken from the measurements of three replicates and the "standard error" of the means was calculated. One-way ANOVA was applied to determine the significance of the results among different treatments

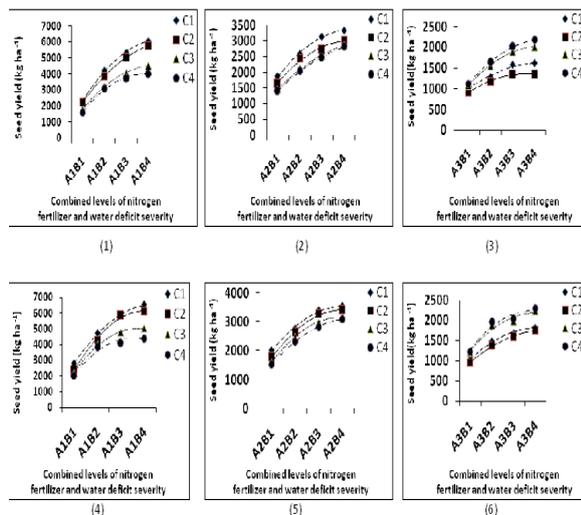
and then least significant differences (LSD<sub>5%</sub>) were evaluated. All the statistical analyses were done using the Statistical Package for Social Sciences (SPSS) for windows (version 13) and MSTATC softwares.

## Results and discussion

### Seed yield

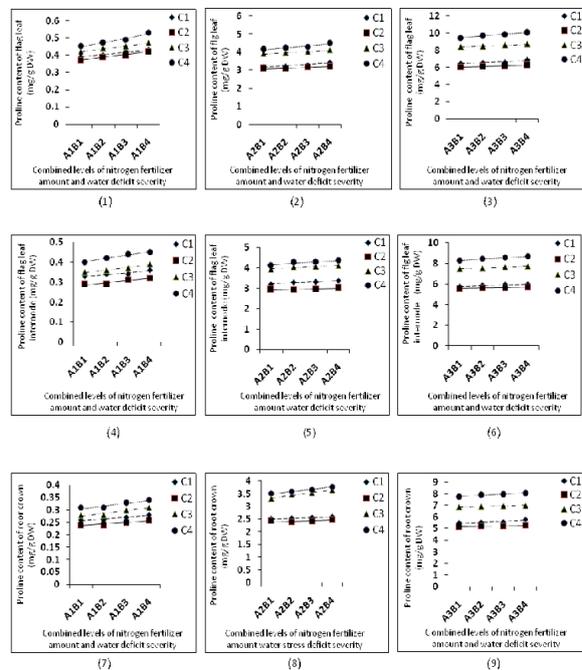
The results showed that the effects of irrigation levels, nitrogen fertilizer and genotypes were significant on seed yield. The effect of year was meaningful on seed yield as well. This increase was due to the enlargement of grain filling period in consequence of decreased temperature during maturity period. As mentioned, by increasing of fertilizer application, seed yield was increased with a descending pattern in all levels of watering level. The maximum seed yield was obtained in 80 Kg ha<sup>-1</sup> nitrogen (fig 1). The maximum amount for seed yield under non-stressed (20 percent field capacity depletion) conditions and 80 Kg ha<sup>-1</sup> nitrogen was belonged to 'Alvand', 'Shahriar', Durum PGS 01-60-335 and Durum IDW 01-61-130 respectively with 6141, 5852, 4605 and 4114 Kg ha<sup>-1</sup> in the first year and 6532, 6121, 5028 and 4402 Kg ha<sup>-1</sup> in the second year (fig 1.1, 1.4). The average amount for seed yield under severe water stress (60 percent field capacity depletion) conditions and 80 Kg ha<sup>-1</sup> nitrogen possessed by Durum IDW 01-61-130, Durum PGS 01-60-335, 'Alvand' and 'Shahriar' with 2270, 2110, 1709 and 1441 kg ha<sup>-1</sup> in the first year and 2398, 2231, 1826 and 1755 kg ha<sup>-1</sup> in the second year respectively (fig 1.3, 1.6). Under condition with severe stress compared with normal un-stressed situations and in the application of same amounts of nitrogen fertilizer, decrease of seed yield in 'Alvand', 'Shahriar', Durum PGS 01-60-335 and Durum IDW 01-61-130 were 72%, 75%, 54% and 44% in the first year and 72%, 71%, 55% and 45% in second year of production respectively. Therefore, under suitable situations with balanced watering and fertilizer levels, maximum seed yield was related to 'Alvand' and 'Shahriar'. Meanwhile, under severe water stress with the same fertilizer levels, maximum seed yield was related to Durum IDW 01-61-130 and Durum PGS 01-60-335. It seems that under suitable circumstances regarding nitrogen and water use

efficiency, bread wheat was more suitable ones with higher seed yield compared to durum wheat. However, under water stress conditions, water and nitrogen use efficiency and seed yield of durum wheat was more than bread wheat. Meanwhile, between two genotypes of bread wheat, ‘Shahriar’ was more sensible than ‘Alvand’ to the drought stress. Between durum wheat, Durum IDW 01-61-130 was resistant one (Fig 1.3, 1.6). Agarwal and Sinha (1984) reported the negative effects of drought on the seed yield of different wheat genotypes. In their research any decreases in seed yield was closely related to water deficiency about 10-69 days before pollination. Recorded decrease was 37-86% compared to the control treatment. Demir and haw (1990) pointed out that, water stress encountered in pollination stage of corn plant decreased the seed yield up to 50 percent. The seed yield of wheat similar to other crops has positive responses following chemical fertilizer application. Some factors such as climate and management practices such as crop rotation, drainage and watering are of main significance in response of field crops to chemical fertilizers (Bohnert *et al*, 1995; Fathi *et al*, 1997). It has been reported that increasing application of nitrogen fertilizer up to 80 Kg h<sup>-1</sup> has been increased the number of claws, length of stems and seed yield in wheat and barley crops (Belanger and Richards, 1997; Turk, 1998).



**Fig. 1.** Effects of nitrogen (Nitrogen levels were B1=20, B2=40, B3=60 and B4=80 Kg ha<sup>-1</sup> nitrogen application) and water deficit (Irrigation in A1=20, A2=40 and A3=60 percent field capacity depletions) on seed yield of wheat genotypes (C1=Alvand,

C2=Shahriar, C3=PGS 01-60-335 and C4=IDW 01-61-130). Figures 1-1, 1-2 and 1-3 relates to the first year and figures 1-4, 1-5 and 1-6 relates to the second year of experiment. Least significant difference based on LSD at 5%.



**Fig. 2.** Effects of nitrogen (Nitrogen levels were B1=20, B2=40, B3=60 and B4=80 Kg ha<sup>-1</sup> nitrogen application) and water deficit (Irrigation in A1=20, A2=40 and A3=60 percent field capacity depletions) on proline content of defferent organs of wheat genotypes (C1=Alvand, C2=Shahriar, C3=PGS 01-60-335 and C4=IDW 01-61-130). Least significant difference based on LSD at 5%.

*Proline content*

Results indicated that the effects of irrigation levels and genotype were significant on proline content. Meanwhile, effect of nitrogen fertilizer levels and year were not significant. The maximum and minimum levels of proline were observed under severe water stress and minimum level of water deficit conditions respectively (Fig.2). The highest amount for proline content was contained in flag leaf beyond it placed flag leaf, internode and root crown which relates with osmotic adjustment and slope of water potential from roots to leaves. Under normal irrigation (20 percent field capacity depletion) conditions, mean proline content in ‘Alvand’, ‘Shahriar’, Durum PGS 01-60-335

and Durum IDW 01-61-130 were 0.41, 0.39, 0.44 and 0.48 mg/g dry weigh respectively (Fig 2.1). However, under severe water deficit conditions (60 percent field capacity depletion), mean proline content in 'Alvand', 'Shahriar', Durum PGS 01-60-335 and Durum IDW 01-61-130 were 6.63, 6.15, 8.55 and 9.79 mg/g dry weight (Fig 2.3). Therefore, the higher proline content under severe water stress conditions belonged to Durum IDW 01-61-130, Durum PGS 01-60-335, 'Alvand' and 'Shahryar' with 20.39, 19.43, 16.17 and 15.76 fold increase compared to low water deficit conditions respectively. High levels of proline could be related to osmosis regulation and furthermore it can be a source of carbon and nitrogen available to use after stress alleviation. As mentioned above, proline content was affected by watering levels (Fig.2). High proline content under water deficit conditions in Durum cultivars especially Durum IDW 01-61-130 compared with bread wheat indicates the high capacity of durum wheat in osmotic regulation and keeping the slope of water potential and at last more resistance to the drought stress. Kuznetsov and Shevyakova (1999) have been pointed out the role of proline in adaptation of plants to the stress conditions due to its diverse biological effects such as osmosis regulation, antioxidant action, energy transfer and, carbon and nitrogen source. Usually, the amount of proline in plants under normal growing conditions is very low (0.2 to 0.6 mg/g dry weight). The absolute amount of this compound beyond drought stress may be up to 50 mg/g dry weight based on severity of water deficit severity and plant type (Rajinder,1987). Although proline accumulates in any organ of plants, its major accumulation occurs in leaves (Heuer, 1999; Ali *et al*, 1994). Increased proline accumulation acts as an osmoticum for lessening of osmotic potential and increases water availability for many of fundamental biochemical pathways ongoing in plants and hence induces drought resistance (Ramond and Smirnoff, 2002).

### Conclusion

In the present experiment data showed that under 80 Kg per hectare nitrogen fertilizer and lack of water stress (20 percent field capacity depletion), the

maximum and minimum seed yield were obtained in 'Alvand' and Durum IDW 01-61-130 cultivars in both years. Under severe water stress (60 percent field capacity depletions) situations, the highest and the lowest seed yield belonged to Durum IDW 01-61-130 and 'Sharyar' during two successive years. Under severe water stress conditions, the highest decrease in seed yield as well as concomitant increase in proline content were seen in 'Alvand' and 'Shahryar'. However, the highest amount of seed yield and proline content were observed in Durum PGS 01-60-335 and IDW 01-61-130.

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