

DOI: 10.5586/aa.1788

**Publication history**

Received: 2019-06-11

Accepted: 2019-10-01

Published: 2019-12-27

**Handling editor**

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FB, MZ: designed the project, analyzed and interpreted the data; SS: carried out all experiments, collected the data, and wrote the first draft of manuscript; BA, AB: helped in study design and editing the manuscript

**Funding**

This study was supported by a grant from the Islamic Azad University, Firoozabad, Iran for the accomplishment of the Ph.D. thesis of Saeed Samsami.

**Competing interests**

No competing interests have been declared.

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**Citation**

Samsami S, Bazrafshan F, Zare M, Amiri B, Bahrani A. Effect of different rates of urea fertilization on yield and some biochemical and physiological properties of four wheat cultivars under two irrigation regimes. *Acta Agrobot.* 2019;72(4):1788. <https://doi.org/10.5586/aa.1788>

**ORIGINAL RESEARCH PAPER**

# Effect of different rates of urea fertilization on yield and some biochemical and physiological properties of four wheat cultivars under two irrigation regimes

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**Abstract**

Drought stress is one of the most serious limitations for the growth and yield of wheat worldwide. Under changing climatic conditions, understanding the urea fertilizer requirement for wheat could be helpful for improving the quality and quantity of yield. The effects of different urea fertilizer levels were evaluated for some biochemical and physiological properties of four wheat cultivars under two irrigation regimes. This experiment was conducted in a split-split plot, randomized complete block design, with three replications. The main plots were irrigation (normal irrigation and irrigation terminated at the stem elongation stage). The experimental plots employed four wheat cultivars ('Shirudi', 'Chamran', 'Chamran 2', and 'Sirvan') and four levels of urea fertilizer treatment (0, 120, 240, and 360 kg ha<sup>-1</sup>), at two locations, Khodayan (52°20' E, 29°8' N) and Nasrabad (52°64' E and 29°58' N), Fars Province, Iran. Results from the full analysis of variance across the two locations indicated considerable differences in yield parameters between irrigation, rates of urea application, and cultivars ( $p < 0.01$ ). Interaction effects of location, irrigation, cultivars, and urea levels were also significant for Chl *b*, 1,000-seed weight, and seed yield ( $p < 0.01$ ). The data indicated that termination of irrigation led to a reduction in RWC (20%), total chlorophyll content (30%), carotenoids (19%), 1,000-seed weight (18%), grain yield (29%), and a promotion in ionic leakage (17%) and proline accumulation (4%), in comparison to the controls. According to these results, 'Chamran' and 'Shirudi' showed the greatest tolerance to reduced irrigation and that using 240 kg ha<sup>-1</sup> urea may effectively moderate the adverse effects of this in these and other wheat cultivars.

**Keywords**

irrigation; proline; ionic leakage; chlorophylls; carotenoids; relative water content

**Introduction**

Water deficit is a major stress for crop production which can have substantial adverse impacts on physiological and biochemical processes, plant growth and development, and crop yield components [1]. It is important to seek new drought resistance genes from different wheat genotypes and introduce new cultivars for conventional breeding [2]. However, the nitrogen requirement of the crop may be affected by drought stress. Thus, understanding the effect of crop water status for nitrogen application is likely beneficial for ensuring the highest yield [3]. Urea fertilization is a common agricultural practice for promoting yield. However, its success depends more on the soil water status than the timing and amount of its application [4]. Early stages of vegetative growth including tiller proliferation depend on the accessibility of water and nitrogen supply

[4]. Moreover, water and nitrogen limitation at the heading stage can result in flowering failure and subsequently lead to a diminished number of grains in the head [5].

Ahmed et al. [6] have indicated that some bread wheat lines show better osmotic adjustment capacities with a high relative water content (RWC) than other lines under conditions of water deficit. They reported that a capacity for osmotic adjustment and RWC could be used for screening the drought tolerance of bread wheat genotypes. Some data reported in the literature show that rate of photosynthesis is dependent on nitrogen bioavailability. This positive and strong correlation between photosynthetic potential and nitrogen content of leaves indicates that a major part of nitrogen taken up is consumed by the photosynthetic apparatus [7–9]. Wheat fertilized with nitrogen rapidly reacts to promote drought stress by closing stomata leads which to a decline in the rate of net photosynthesis [10]. Free proline is a common compatible osmolyte and its concentration is increased in drought-stressed plants [11,12]. Proline accumulated in plant tissues during an environmental stress such as drought could therefore be used as a marker for the extent of the stress [13]. Accumulation of this amino acid is an adaptive response of plants to the stress signal [14].

The aim of the present study was to evaluate the effects of different application rates of urea on some biochemical and physiological responses and grain yield of four wheat cultivars under different irrigation regimes.

## Material and methods

The experiment was conducted in a split-split plot fully randomized complete block design. The main plot included irrigation (a normal irrigation regime and irrigation terminated at the stem elongation stage). Subplots were four wheat cultivars, including three semidwarf cultivars: 'Shirudi', 'Chamran', and 'Chamran 2' and one dwarf cultivar, 'Sirvan'. The sub-subplots were four levels of urea fertilizer application: (0, 120, 240, and 360 kg ha<sup>-1</sup>) with three replications at two locations; Khodayan (52°20' E, 29°8' N) and Nasrabad (52°64' E and 29°58' N), both in Fars Province, Iran.

## Field operations

The field operations were performed according to common local methods (ploughing, disc harrowing, land leveling and furrowing). Fertilizer requirements were determined and applied according to the soil analyses (Tab. 1). The average rainfall and temperatures

during the 2016 growing season at the two locations are presented in Tab. 2. Each experimental plot comprised six rows, 4 m in length, 0.2 m apart between rows, with 450 plants m<sup>-2</sup>. Granstar (750 g kg<sup>-1</sup> Tribenuron-methyl in the form of water-dispersible granules) and Puma Super (Fenoxaprop-P-ethyl) herbicides were used to control narrow and broad leaf weeds, respectively, at the stem extension stage of the wheat plant growth. Harvesting of the crop was carried out when a 14% moisture content of the grain was attained. At the end of the growing period, seed yield, yield components, and other indices were measured or determined.

## Chlorophylls *a* and *b* and carotenoid concentrations

For the determination of photosynthetic pigment concentrations, 0.1 g of fresh leaves were extracted with 15 mL 80% acetone and centrifuged at 5,000 g for 10 min. The absorbance of the supernatant was measured at 663, 647, and 470 nm for chlorophyll *a*, chlorophyll *b*, and carotenoids, respectively [15].

**Tab. 1** Some physicochemical properties of the soil at 0–30-cm depth.

	Khodayan	Nasrabad
EC (dS m <sup>-1</sup> )	1.2	1.3
pH	7.6	7.4
OM%	2.4	2.2
C	1.65	1.63
P (mg kg <sup>-1</sup> )	13	15
K (mg kg <sup>-1</sup> )	370	380
Mn (mg kg <sup>-1</sup> )	4	3.7
Fe (mg kg <sup>-1</sup> )	7.5	7.7
Clay %	34.86	36.75
Silt %	47.23	47.55
Sand %	17.9	15.7
N (kg ha <sup>-1</sup> )	30.6	33.21

**Tab. 2** Average monthly rainfall and temperature in the 2016 growing season for the two locations investigated.

Month	Average rainfall (mm)		Average temperature (°C)	
	Khodayan	Nasrabad	Khodayan	Nasrabad
January	8	2.3	7.9	10.7
February	221.1	174.6	3	7.8
March	92.7	61.5	7.2	12.2
April	73.1	58.4	12.1	17.1
May	17.5	19.2	18.1	22.4
June	0	0	24	27.5
July	0	0	26.7	29.7
August	9.2	0	26.1	28.9
September	0	0	23.7	25.3
October	0	0	18.8	21.1
November	7.3	0.5	14.4	16.1
December	81.8	19.7	6.9	10.4

#### Free proline determination

Four leaf discs of 1-cm diameter were randomly selected for each sample. The fresh discs were weighed and immediately placed into liquid nitrogen to freeze. Then, 4 mL of deionized water were added to each sample and boiled for 30 min. Aliquots of 200  $\mu$ L of the prepared extracts were analyzed for free proline concentrations using the standard ninhydrin method [16].

#### Ionic leakage analysis

Electrolyte leakage (EL) was determined to evaluate leaf membrane damage using the method previously described by Valentovič et al. [17] with some modifications. In brief, 0.5 g of each leaf sample was washed and placed in a tube containing 20 mL deionized water and incubated for 24 h at room temperature. The initial electrical conductivity of the solution (L1) was recorded. Then, samples were autoclaved at 120°C for 20 min and after cooling to room temperature, a second conductivity measurement (L2) was made. The EL was then calculated as:  $EL (\%) = (L1/L2) \times 100$ .

#### Relative water content of leaves

The RWC values were calculated by the following equation:  $RWC = (FW - DW)/(TW - DW) \times 100$ , where *FW* and *DW* are, respectively, fresh and dry weights of the leaf and *TW* is the fully turgid weight of the leaf after 24-h floating in distilled water.

#### Data analysis

Data were analyzed using the general linear model (GLM) procedure of the statistical analysis package SAS 9.2 (SAS Institute, Cary, NC, USA). When the analysis of variance indicated significant treatment effects, Duncan's multiple range tests were used to compare treatment means at  $p < 0.05$ .

## Results

### Relative water content

According to the analysis of variance (Tab. 3), irrigation regimes and urea application rates had significant effects on RWC at the 1% probability level. Comparison between treatments means showed that termination of irrigation led to a 20% reduction in RWC in comparison to the control. Furthermore, RWC increased by 5%, 8%, and 12% after application of 120, 240, and 360 kg ha<sup>-1</sup> urea, respectively. Results also showed that the highest RWC was in 'Chamran' (73%). This cultivar with 360 kg ha<sup>-1</sup> urea fertilizer application showed the highest RWC under both normal and reduced irrigation (89% and 77.5%; Tab. 4).

### Total chlorophyll and carotenoid concentrations

The effects of irrigation and urea application rates were significant on both chlorophyll and carotenoid concentrations (Tab. 3;  $p < 0.01$ ). Termination of irrigation led to 30% and 19% reduction in chlorophyll and carotenoid concentrations, respectively, when compared to normal irrigation. Moreover, 120, 240, and 360 kg ha<sup>-1</sup> urea increased the chlorophyll concentration by 40%, 33%, and 37% as well as that of the carotenoids (17%, 21%, and 22%, respectively). It was found that the highest chlorophyll (3.5 mg g<sup>-1</sup> FW) and carotenoid (0.5 mg g<sup>-1</sup> FW) concentrations were in 'Chamran'. This cultivar also showed the highest chlorophyll concentration (5 mg g<sup>-1</sup> FW) at 360 kg ha<sup>-1</sup> urea and the highest carotenoids concentration (0.547 mg g<sup>-1</sup> FW) in 240 and 360 kg ha<sup>-1</sup> urea under the normal irrigation regime. However, after terminating irrigation the highest chlorophyll concentration (4.12 mg g<sup>-1</sup> FW) was observed in 'Chamran' with 240 kg

**Tab. 3** Summarized results of the analyses of variance for RWC, chlorophyll, and carotenoids in wheat.

S.O.V	df	RWC	Chlorophyll <i>a</i>	Chlorophyll <i>b</i>	Carotenoids
Location (L)	1	1,402.92**	11.163**	0.550**	5.347**
Replication (Location) R(L)	4	790.14**	6.774**	0.036**	0.649**
Irrigation (I)	1	9,619.17**	26.981**	3.735**	33.167**
LI	1	0.130 <sup>ns</sup>	0.004 <sup>ns</sup>	0.031**	0.002 <sup>ns</sup>
RY (I)	4	56.448*	0.470*	0.003*	0.054*
Cultivar (C)	3	2,850.53**	7.090**	1.286**	41.442**
LC	3	2.727 <sup>ns</sup>	0.005 <sup>ns</sup>	0.043**	0.002 <sup>ns</sup>
IC	3	302.31**	1.302**	0.075**	3.367**
LIC	3	5.325 <sup>ns</sup>	0.006 <sup>ns</sup>	0.055**	0.002 <sup>ns</sup>
Error	24	14.8547	0.1237	0.0008	0.014
Urea (U)	3	516.84**	4.646**	0.896**	6.467**
LU	3	2.672 <sup>ns</sup>	0.003 <sup>ns</sup>	0.052**	0.002 <sup>ns</sup>
IU	3	2.672 <sup>ns</sup>	0.003 <sup>ns</sup>	0.065**	0.002 <sup>ns</sup>
LIU	3	3.380 <sup>ns</sup>	0.001 <sup>ns</sup>	0.044**	0.002 <sup>ns</sup>
CU	9	98.59**	4.360**	0.678**	0.200**
LCU	9	3.144 <sup>ns</sup>	0.002 <sup>ns</sup>	0.046**	0.002 <sup>ns</sup>
ICU	9	3.144 <sup>ns</sup>	0.002 <sup>ns</sup>	0.047**	0.002 <sup>ns</sup>
LICU	9	2.908 <sup>ns</sup>	0.002 <sup>ns</sup>	0.049**	0.002 <sup>ns</sup>
Error	96	23.008	0.198	0.001	0.019
CV%		7.3	18.8	16.0	13.4

\*, \*\*, and ns: significant at  $p < 0.01$ ,  $p < 0.05$ , and nonsignificant, respectively.

**Tab. 4** Mean comparisons for RWC, chlorophyll *a*, chlorophyll *b*, and carotenoid concentrations (mg g<sup>-1</sup> FW) in response to the experimental treatments.

Irrigation	Cultivar	Urea (kg ha <sup>-1</sup> )	RWC (%)	Chll <i>a</i>	Chll <i>b</i>	Carotenoids
Normal	'Shirudi'	0	72.4 <sup>b-e</sup>	1.615 <sup>j-m</sup>	0.425 <sup>l</sup>	0.427 <sup>e</sup>
		120	74.5 <sup>bcd</sup>	3.515 <sup>abc</sup>	1.195 <sup>b</sup>	0.497 <sup>c</sup>
		240	75.3 <sup>bc</sup>	2.515 <sup>fgh</sup>	0.695 <sup>h</sup>	0.507 <sup>bc</sup>
		360	76.5 <sup>bc</sup>	3.215 <sup>a-d</sup>	0.955 <sup>d</sup>	0.507 <sup>bc</sup>
	'Sirvan'	0	67.1 <sup>efg</sup>	2.615 <sup>e-h</sup>	0.595 <sup>i</sup>	0.367 <sup>gh</sup>
		120	70.6 <sup>c-f</sup>	3.115 <sup>b-e</sup>	0.305 <sup>no</sup>	0.417 <sup>e</sup>
		240	72.5 <sup>b-e</sup>	2.915 <sup>def</sup>	0.755 <sup>g</sup>	0.447 <sup>d</sup>
		360	71.9 <sup>b-e</sup>	2.315 <sup>ghi</sup>	0.615 <sup>i</sup>	0.457 <sup>d</sup>
	'Chamran'	0	72.5 <sup>b-e</sup>	2.215 <sup>g-j</sup>	0.465 <sup>k</sup>	0.447 <sup>d</sup>
		120	75.2 <sup>bc</sup>	2.615 <sup>e-h</sup>	0.615 <sup>i</sup>	0.537 <sup>a</sup>
		240	77.5 <sup>b</sup>	3.715 <sup>a</sup>	0.955 <sup>d</sup>	0.547 <sup>a</sup>
		360	90.3 <sup>a</sup>	3.64 <sup>ab</sup>	1.375 <sup>a</sup>	0.547 <sup>a</sup>
	'Chamran2'	0	63.5 <sup>g</sup>	2.715 <sup>d-g</sup>	0.695 <sup>h</sup>	0.337 <sup>j</sup>
		120	66.4 <sup>efg</sup>	2.756 <sup>d-g</sup>	0.895 <sup>e</sup>	0.377 <sup>fg</sup>
		240	67.5 <sup>efg</sup>	2.215 <sup>g-j</sup>	0.585 <sup>i</sup>	0.377 <sup>fg</sup>
		360	68.3 <sup>d-g</sup>	2.250 <sup>g-j</sup>	0.765 <sup>g</sup>	0.387 <sup>f</sup>
Reduced irrigation	'Shirud'	0	62.5 <sup>gh</sup>	1.115 <sup>mn</sup>	0.225 <sup>p</sup>	0.377 <sup>fg</sup>
		120	64.7 <sup>fg</sup>	3.015 <sup>c-f</sup>	0.995 <sup>c</sup>	0.447 <sup>d</sup>
		240	65.5 <sup>fg</sup>	2.015 <sup>h-k</sup>	0.495 <sup>jk</sup>	0.457 <sup>d</sup>
		360	66.2 <sup>efg</sup>	2.715 <sup>d-g</sup>	0.755 <sup>g</sup>	0.457 <sup>d</sup>
	'Sirvan'	0	52.5 <sup>ij</sup>	1.715 <sup>i-l</sup>	0.195 <sup>p</sup>	0.267 <sup>l</sup>
		120	55.5 <sup>i</sup>	2.215 <sup>g-j</sup>	0.045 <sup>q</sup>	0.317 <sup>k</sup>
		240	57.3 <sup>hi</sup>	2.015 <sup>h-k</sup>	0.355 <sup>m</sup>	0.347 <sup>ij</sup>
		360	56.9 <sup>hi</sup>	1.415 <sup>lmn</sup>	0.215 <sup>p</sup>	0.357 <sup>hi</sup>
	'Chamran'	0	62.4 <sup>gh</sup>	1.765 <sup>i-l</sup>	0.365 <sup>m</sup>	0.417 <sup>e</sup>
		120	65.5 <sup>fg</sup>	2.165 <sup>g-j</sup>	0.515 <sup>j</sup>	0.507 <sup>bc</sup>
		240	67.3 <sup>efg</sup>	3.265 <sup>a-d</sup>	0.855 <sup>f</sup>	0.517 <sup>b</sup>
		360	77.5 <sup>b</sup>	3.165 <sup>a-e</sup>	0.775 <sup>g</sup>	0.507 <sup>bc</sup>
	'Chamran2'	0	40.5 <sup>l</sup>	1.550 <sup>k-n</sup>	0.3317 <sup>mn</sup>	0.187 <sup>n</sup>
		120	46.3 <sup>k</sup>	1.615 <sup>j-m</sup>	0.595 <sup>i</sup>	0.227 <sup>m</sup>
		240	47.5 <sup>jk</sup>	1.115 <sup>mn</sup>	0.285 <sup>o</sup>	0.227 <sup>m</sup>
		360	47.8 <sup>jk</sup>	1.009 <sup>n</sup>	0.425 <sup>l</sup>	0.237 <sup>m</sup>

a–n: mean values within a column with the same letters are not significantly different at  $p < 0.05$ .

ha<sup>-1</sup> urea and the highest carotenoids (0.517 mg g<sup>-1</sup> FW) concentration was also in this cultivar at this rate of urea application (Tab. 4).

#### Proline accumulation

As shown in Tab. 5, irrigation and rates of urea application had significant effects on proline concentrations ( $p < 0.01$ ). Termination of irrigation reduced proline in comparison to the normal conditions. Applications of 120, 240, and 360 kg ha<sup>-1</sup> urea led to an increase in free proline (30%, 32%, and 47%, respectively). Cultivar 'Chamran' showed the highest proline concentration under both normal irrigation and reduced irrigation (6.67 and 7.175 mg g<sup>-1</sup> FW, respectively). Proline was at the highest concentration in this cultivar at 360 kg ha<sup>-1</sup> urea (Fig. 1).

**Tab. 5** Summarized analyses of variance for proline, ionic leakage, 1,000-seed weight, and seed yield.

S.O.V	df	Proline	Ionic leakage	Thousand-seed weight	Seed yield
Location (L)	1	1.153**	657.5**	61.36**	551,672**
Replication Location) R(L)	4	4.168**	67.812**	0.986**	27,326**
Irrigation (I)	1	1.555**	2,136**	69.41**	584,271**
LI	1	11.761**	0.962 <sup>ns</sup>	22.03**	15.66 <sup>ns</sup>
RY(I)	4	0.285	5.510*	0.017	486.4
Cultivar (C)	3	110.346**	1,085.5**	12.50**	103,578**
LC	3	0.032 <sup>ns</sup>	1.109 <sup>ns</sup>	8.157**	23,944**
IC	3	0.910**	101.3**	11.10**	91,787**
LIC	3	0.120 <sup>ns</sup>	1.256 <sup>ns</sup>	11.50**	43,966**
Error	24	0.163	1.4500	0.018	875.8
Urea (U)	3	26.153**	112.3**	5.472**	66,571**
LU	3	0.090 <sup>ns</sup>	0.468 <sup>ns</sup>	8.316**	68,903**
IU	3	0.130 <sup>ns</sup>	0.468 <sup>ns</sup>	6.226**	65,780**
LIU	3	0.140 <sup>ns</sup>	3.336 <sup>ns</sup>	10.14**	41,222**
CU	9	0.946**	10.613**	8.990**	49,753**
LCU	9	0.040 <sup>ns</sup>	2.380 <sup>ns</sup>	9.793**	47,004**
ICU	9	0.054 <sup>ns</sup>	2.380 <sup>ns</sup>	9.280**	83,607**
LICU	9	0.120 <sup>ns</sup>	1.424 <sup>ns</sup>	8.978**	42,878**
Error	96	0.118	1.952	0.018	786.3
CV%		12.1	13.3	13.080	10.75

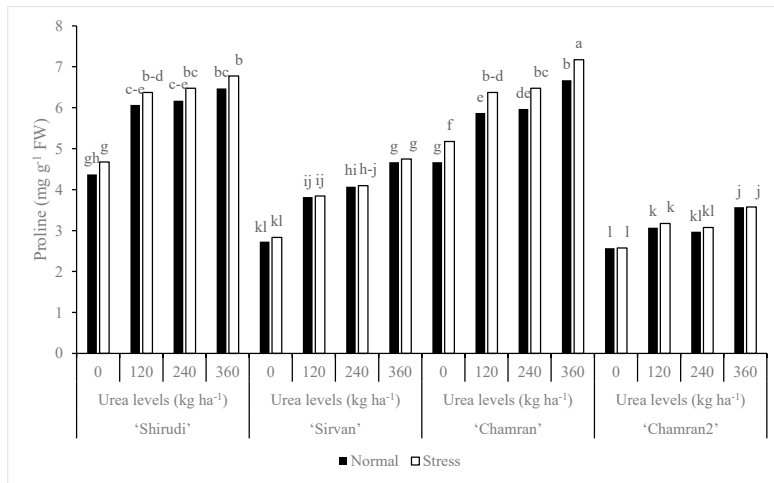
\*, \*\*, and ns: significant at  $p < 0.01$ ,  $p < 0.05$ , and nonsignificant, respectively.

#### Ionic leakage level

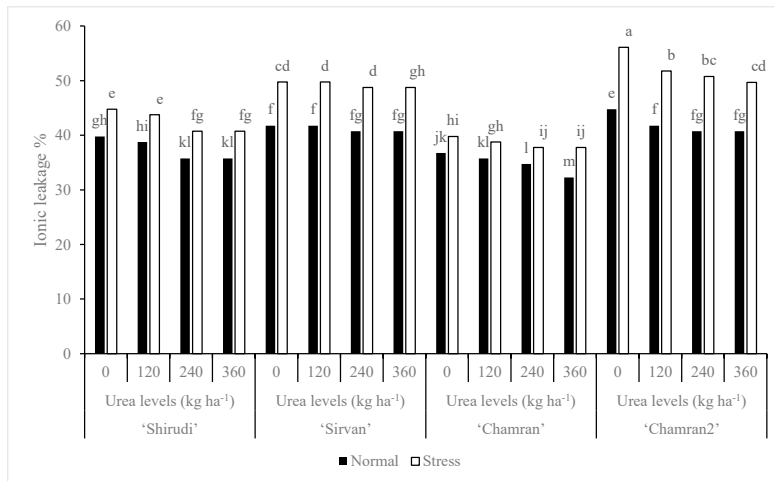
The effect of irrigation regimes, rates of urea application and their interaction had significant effect on ionic leakage between wheat cultivars (Tab. 5;  $p < 0.01$ ). Termination of irrigation increased the ionic leakage by 17%. Likewise, applications of 120, 240, and 360 kg ha<sup>-1</sup> urea decreased the ionic leakage by 4%, 7%, and 8%, respectively. The greatest ionic leakage (47%) was observed in 'Chamran 2'. With terminated irrigation, the lowest ionic leakage (37%) was in 'Chamran' at 240 and 360 kg ha<sup>-1</sup> urea. Under normal irrigation, the lowest ionic leakage (32%) was observed in 'Chamran' at 360 kg ha<sup>-1</sup> urea (Fig. 2).

#### Thousand-seed weight and seed yield

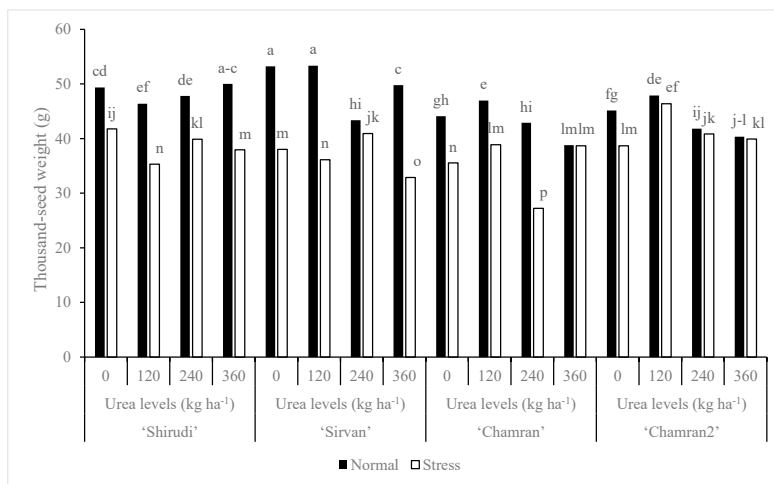
Comparison of treatment means between cultivars for the two types of irrigation showed that the highest 1,000-seed weight (43.56 g) and seed yield (267.81 g m<sup>-2</sup>) were in 'Shirudi'. With terminated irrigation, 'Chamran 2' supplied with 120 kg ha<sup>-1</sup> urea and 'Shirudi' supplied with 240 kg ha<sup>-1</sup> urea gave higher 1,000-seed weights (46.38 g; Fig. 3) and seed yield (322 g m<sup>-2</sup>; Fig. 4), respectively. Under normal irrigation, the greatest 1,000-seed weight and seed yield were produced by 'Sirvan' at 120 kg ha<sup>-1</sup> urea (53.3 g; Fig. 3) and 'Chamran' at 240 kg ha<sup>-1</sup> urea (441.7 g m<sup>-2</sup>; Fig. 4). However, with reduced irrigation the highest 1,000-seed weight and seed yield were in 'Chamran 2' supplied with 120 kg ha<sup>-1</sup> urea (46.38 g; Fig. 3) and 'Shirudi' supplied with 240 kg ha<sup>-1</sup> urea (322.0 g m<sup>-2</sup>; Fig. 4).



**Fig. 1** Interaction effects of location, irrigation, cultivar, and urea levels on the free proline concentrations. Mean values with the same letters are not significantly different at  $p \leq 0.05$  according to Duncan's test.

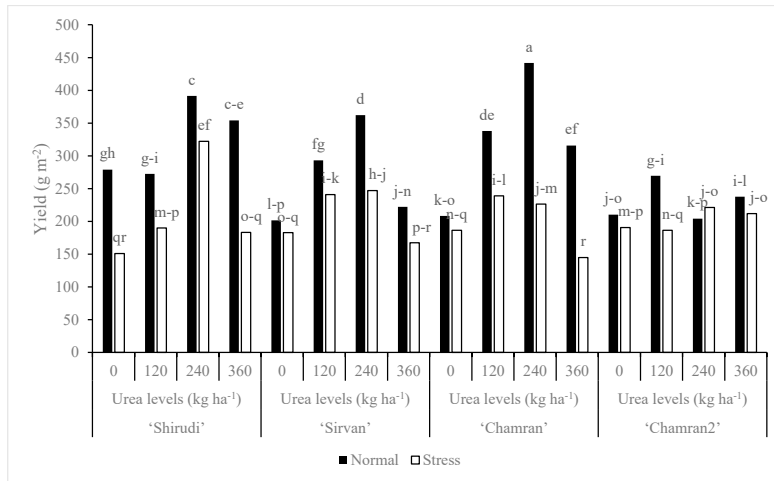


**Fig. 2** Interaction effects of location, irrigation, cultivar, and urea levels on ionic leakage from leaves. Mean values with the same letters are not significantly different at  $p \leq 0.05$  according to Duncan's test.



**Fig. 3** Interaction effects of location, irrigation, cultivar and urea levels on 1,000-seed weight. Mean values with the same letters are not significantly different at  $p \leq 0.05$  according to Duncan's test.





**Fig. 4** Interaction effects of location, irrigation, cultivar, and urea levels on seed yield. Mean values with the same letters are not significantly different at  $p \leq 0.05$  according to Duncan's test.

## Discussion

Drought stress conditions can induce harmful effects on crop plants which result in a reduction in RWC and an increase in electrolyte leakage [18]. Evidence from data reported in the literature demonstrates that a higher RWC is associated with a greater resistance to water deficit stress [19,20]. In the present study, the leaf RWC was greatly diminished by the termination of irrigation. However, after adding urea fertilizer at an appropriate rate, the RWC was improved. Reduction in RWC can reduce water availability to the roots and subsequently, transpiration processes will be affected by the water deficit. Evaluation of RWC in leaf tissues is therefore a suitable indicator of water status in crop plants [21] and is routinely used for this purpose [22]. Our results showed that termination of irrigation led to a 20% reduction in RWC. This is agreement with two previous studies which showed that the RWC of bean leaves under drought stress was significantly lower when compared to control plants [23,24].

It is well documented that a reduction in chlorophyll concentrations during water deficit conditions is correlated with diminished photochemical activities in the chloroplasts; photosynthetic performance is thus negatively affected. Nitrogen is a major component of proteins, chlorophyll, and the key photosynthetic carboxylating enzyme, rubisco. Limitation in nitrogen availability during water deficit has also been shown to have a negative influence on both chlorophyll concentrations and rubisco activity [25]. A considerable proportion of plant nitrogen is accumulated in rubisco [26] and an adequate supply of nitrogen can potentially aid the recovery of the photosynthetic biochemistry under water deficit conditions. However, after intense drought stress this recovery is limited [27]. Given the fact that about half of the nitrogen in the green part of crops participates in the light-dependent phase of photosynthesis, an additional nitrogen supply may improve photosynthetic potential and stomatal control in normal irrigation and water deficit conditions [28].

Enzymatic and nonenzymatic antioxidants including free proline and carotenoids are needed to maintain the balance between ROS production and radical scavenging in plants [29]. Enhancement of free proline accumulation by additional applied nitrogen may lead to a greater capability in amino acid synthesis [30]. Carotenoids with structural functions and antioxidant activity not only prevent cells from lipid peroxidation and improve cell membrane stabilization [31], but also have critical roles in photosynthesis and photoprotection [32]. Any changes in plant membranes under abiotic stresses are often related to enhancement in permeability and damage to their integrity [33]. Thus, the ability of membranes to regulate ion transfer between the inside and outside of cells could be used to evaluate injury to plant tissues. Our results showed that water deficit increased the ionic leakage and applied urea mitigated this effect. Similarly, one report has indicated that electrolyte leakage in maize decreased after nitrogen fertilization



[17] and there is a further report of a higher electrolyte leakage in drought-stressed plants of this crop [34].

Our research has shown that termination of irrigation led to a decline in the 1,000-seed weight and overall seed yield, and that urea fertilization at an optimum rate of application moderated the negative effects of water deficit in the wheat cultivars tested. Other workers have reported a negative influence of water deficit stress on the 1,000-seed weight, and that by using a higher nitrogen level a depletion in grain weight resulted during drought stress in wheat crops [35,36].

Total chlorophyll and carotenoid concentrations were reduced by terminating irrigation, but urea application reduced this depletion. It was found that under normal irrigation conditions, the greatest 1,000-seed weight and the seed yield were in 'Shirudi', and under reduced irrigation, the highest mean values for these yield components were shown by 'Chamran 2' and 'Shirudi'.

## Conclusion

Different wheat cultivars may have different reactions and potential to adapt to reduced irrigation based on their genotype. Urea (a common source of nitrogen fertilization) is very soluble in water and so at low concentrations, the roots of wheat need to absorb more water to take up the optimal nitrogen requirement. Thus, with any reduced irrigation conditions, prediction of this optimal dosage of urea could play a pivotal role in mitigating the negative effects of this stress. Based on our results, wheat 'Chamran' and 'Shirudi' showed the greatest tolerance to a reduction in irrigation, and an application of 240 kg ha<sup>-1</sup> urea was effective in alleviating the adverse effects of terminating irrigation at the stem elongation stage.

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### **Wpływ zróżnicowanego poziomu nawożenia mocznikowego na wybrane parametry biochemiczne i fizjologiczne czterech odmian pszenicy w warunkach dwóch systemów nawadniania**

#### **Streszczenie**

Stres spowodowany suszą stanowi jedno z najpoważniejszych ograniczeń dla wzrostu i plonowania pszenicy na świecie. W obecnych warunkach klimatycznych poznanie zapotrzebowania pszenicy na nawozy mocznikowe może być pomocne w poprawie jakości i wielkości jej plonu. W tym celu oceniono wpływ zróżnicowanego poziomu nawożenia mocznikowego na podstawie niektórych właściwości biochemicznych i fizjologicznych czterech odmian pszenicy w dwóch systemach nawadniania. Doświadczenie przeprowadzono w całkowicie zrandomizowanym układzie blokowym typu split-split plot z trzema powtórzeniami. Poletka główne były nawadniane (nawadnianie normalne lub ograniczenie nawadniania na etapie wydłużania łodygi), a podpoletka eksperymentalne obejmowały cztery odmiany pszenicy ('Shirudi', 'Chamran', 'Chamran 2', i 'Sirvan') oraz cztery poziomy nawożenia mocznikowego (0, 120, 240 i 360 kg ha<sup>-1</sup>) w dwóch lokalizacjach Khodayan (52°20' E, 29°8' N) i Nasrabad (52°64' E i 29°58' N), prowincja Fars, Iran. Wyniki analizy wariancji z obu badanych lokalizacji wykazała istotną różnicę pomiędzy zastosowanymi poziomami nawadniania, nawożenia mocznikiem oraz odmianami ( $p < 0,01$ ). Wpływ interakcji lokalizacji, nawadniania, odmiany i poziomu mocznika był istotny w odniesieniu do Chl**l**b, masy 1000 nasion oraz plonu nasion ( $p < 0,01$ ). Dane wskazują, że ograniczenie nawadniania doprowadziło do zmniejszenia RWC (20%), całkowitej zawartości chlorofilu (30%), karotenoidów (19%), masy 1000 nasion (18%), plonu ziarna (29%) oraz wpływało na podwyższenie wycieku jonów (17%) i akumulacji proliny (4%) w porównaniu z kontrolą. Uzyskane wyniki wskazują, że odmiany 'Chamran' i 'Shirudi' wykazywały najwyższą tolerancję na ograniczenie nawadniania, a zastosowanie 240 kg ha<sup>-1</sup> mocznika może skutecznie złagodzić niekorzystny wpływ suszy na plonowanie pszenicy.