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Response of wheat to drought stress: focus on root and shoot nutrients, as well as leaf chlorophyll and glycine betaine

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Abstract: Drought stress is one of the limiting factors for plant growth. To evaluate the effect of drought stress (0, 150, 250 g/L of Polyethylene glycol 6000 (PEG)) on the physiological characteristics of two wheat cultivars ('Gonbad' and 'N8720'), a hydroponic experiment was conducted. A factorial experiment was used and arranged in a completely randomized design with three replications at the Pasteur Institute of Iran (North Research Center). The experimental results showed that the main effect of cultivars was significant for all studied traits except nitrogen and phosphorus in the stems ($p \leq 0.01$). The main effect of drought stress, as well as the interaction effect of drought stress and cultivars were significant for all studied traits ($p \leq 0.01$). The highest content of elements in root and shoot and the chlorophyll content was observed in N8720 cultivar under control treatment. Moreover, in N8720 cultivar, the amount of glycine betaine increased due to drought stress, reaching its maximum at 250 g/L PEG. The results of correlation analysis showed that there is a positive and significant correlation between all traits ($p \leq 0.01$). The result of experiment showed that N8720 cultivar exhibited superior characteristics in terms of all studied traits.

Keywords: cluster analysis, cultivars, drought stress, glycine betaine, phosphorus.

Introduction

Wheat (*Triticum aestivum* L.) is one of the most important grains, serving as a dominant staple food worldwide (Guo et al., 2018; Webber et al., 2018). Wheat stands as a crucial and invaluable crop on a global scale. Any decline in its production reverberates significantly throughout the human food supply chain. Wheat plays a pivotal role in delivering nutrients and calories to millions of people worldwide (Khalvandi et al., 2023). Therefore, the reduction in wheat yield can have far-reaching consequences on food security and the overall well-being of populations, highlighting the significance of addressing issues related to wheat production and ensuring its sustainable cultivation (Barak et al., 2015; Nuttall et al., 2017; Figueroa et al., 2018).

Drought is an important abiotic stress which is reducing plant growth around the world. This is more evident in arid or semi-arid areas)(Kaur and Asthir, 2017; Khalvandi et al., 2023). The depth of the problem becomes apparent when we consider that over25% of the Earth's surface is arid or semi-arid. Drought is caused by an imbalance between water evaporation, transpiration and rainfall (Salehi et al., 2016). Drought stress leads to different biochemical and physiological reactions in plants and reduces their yield (Kumar et al., 2018). Rehabilitation of crops tolerant of environmental stresses may be a way to meet the nutritional needs of a growing population living in developing countries. Breeding for drought-tolerant crops requires knowledge of the physiological and genetic mechanisms operating at different growth stages (Osmolovskaya et al., 2018).

Cellular responses to abiotic stresses are present in the majority of plant species. One of the most common of these reactions is the overproduction of various types of compatible organic matter in plant cells. These substances have high solutes and are usually non-toxic in high concentrations (Zegaoui et al., 2017; Khalvandi et al., 2019; Keshavarz‐Mirzamohammadi et al., 2021). Organic substances commonly known as osmotic modulators include proline, sucrose, and quaternary ammonium compounds such as glycine betaine and finally proline betaine. Accumulation of glycine betaine in higher plants, which are naturally accumulators of this compound, in response to salinity, drought, and cold stress has been widely reported (Nawaz and Wang, 2020). Glycine betaine is an amphoteric compound and electrically neutral at different pH (You et al., 2019). Some plant species contain small amounts of glycine betaine; However, due to stress exposure, they accumulate larger amounts of glycine betaine (Nawaz and Wang, 2020).

Drought stress, in addition to a negative effect on plant growth, causes or exacerbates other stresses, especially nutrient deficiency stress for the plant. One of the most harmful effects of drought stress is disruption in the process of absorption and accumulation of nutrients, which in addition to fertilizer losses reduces plant growth (Liang et al., 2018; Khalvandi et al., 2021). Mechanisms of uptake and transport of nutrients in plants, such as mass flow, diffusion or uptake by osmosis are all functions of the amount of moisture in the soil and roots. If the moisture content is reduced, the intensity and amount of uptake of nutrients is affected (Bindraban et al., 2015). Although some of these nutrient transfer by shoots, such as diffusion, require less moisture to absorb nutrients, in this regard, with the reduction of moisture to the critical threshold, the process of absorption and transfer of some nutrients by the roots continues (Mitra, 2015; Kazemi et al., 2021). But others, such as mass flow, are highly dependent on the amount of moisture. If the humidity decreases, the elements transferred by this current will have a negative absorption process (Hepworth et al., 2015).

Since the wheat plant has varying needs for nutrients in different phases of growth and development, drought stress greatly affects the absorption and accumulation of nutrients (Nadeem et al., 2019). Seedling stage is one of the most important phases of plant growth and impaired absorption of nutrients can cause irreparable damage to the plant (Li et al., 2015).

The leaf has a special role as a photosynthetic unit in the plant. Photosynthesis is the most vital process that ensures the life of plants and other living organisms (Kazemi et al., 2021). Absorption of light is done by pigments in chloroplast thylakoids such as chlorophyll. Chlorophyll is a pigment whose main responsibility is to receive light energy for use in photosynthesis (Gomes et al., 2016). However, environmental stresses affect the amount of

chlorophyll so drought stress and oxidative stress reduce the amount of chlorophyll and other pigments, this reduction will limit photosynthesis and plant growth (Salehi et al., 2016). Measurement of chlorophyll content is a typical indicator of oxidative stress observed in stressed plants (Cardona et al., 2018). Drought stress has an undeniable effect on plant growth and development by its effect on leaf chlorophyll (Salehi et al., 2016). The purpose of this experiment is to compare the response of two wheat cultivars to drought stress based on physiological and chlorophyll content.

Materials and Methods

Growth conditions

In order to evaluate the effect of drought stress on the physiological and chlorophyll contents of two wheat (*Triticum aestivum* L.) cultivars ('Gonbad' and 'N8720' as a sensitive and resistant cultivar to drought stress, respectively), an experiment was carried out using a factorial arrangement in a completely randomized design with three replications at the Pasteur Institute of Iran (North Research Center) in 2017. Wheat seeds were disinfected in 96% ethanol for 1.5 min followed by 15 min in 15 % Domestos, before being washed 4 times in sterile water. Afterward, grains germinated on wet filter paper for 3 days. Seedlings were put into plastic pots containing water with a half-strength Hoagland solution and maintained in a hydroponics culture in a greenhouse for 21 days. The hydroponic solution was aerated by air pumps. Every day, the hydroponic medium was supplemented with a fresh medium, and every week, it was completely exchanged with a fresh medium. After 21 days of growth in the control treatment (in water), seedlings were exposed to three levels of stress (0, 150, 250 g/L of Polyethylene glycol 6000 (PEG)). For these treatments, osmotic stress was applied with PEG dissolved in half-strength Hoagland. The 20 seedlings of each cultivar for each treatment were harvested 6 weeks after drought stress treatment and the following parameters were separately measured for each cultivar.

Leaf chlorophyll content

The modified Arnon (1949) used to measure chlorophyll content in leaves. According to this method, 0.25 g of 6th extended leaves were freeze-dried with liquid nitrogen, then completely homogenized with 4 ml of 96% ethanol in the dark and stored in a refrigerator at 4 °C for four hours. After centrifugation, supernatants were read at 663 and 645 wavelengths by spectrophotometer (Analytik Jena, Spekol 1300, Germany), and the concentration of chlorophyll was calculated (Equation 1, 2 and 3).

[Equation 1] Chlorophyll a = $(19.3 \times A_{663} - 0.86 \times A_{645})$ V/100W

[Equation 2] Chlorophyll b = $(19.3 \times A_{645} - 3.6 \times A_{663})$ V/100W

[Equation 3] Total chlorophyll = Chlorophyll a + Chlorophyll b

V = the volume of filtered solution (the upper solution from the centrifuge)

A = absorption of light at wavelengths of 663 and 645

W = fresh weight of the sample in gram

Glycine betaine content

Glycine betaine was measured by Grieve and Grattan (1983) method. In this method, 25 mg of leaf dry tissue was homogenized twice with 1 ml of water; the resulting solution was then stored on a shaker for 24 hours at 25 °C. The next day the samples were smooth and diluted 1: 1 with potassium iodide sulfuric acid 2 N and placed on ice for 1 hour; then 0.2 ml of cold potassium iodide reagent was added to the samples and the samples were kept at 0 to 4 °C for 16 hours. The resulting mixture was centrifuged at 10,000 rpm at 4 °C for 15 min. In the next step, the illuminating solution was carefully and gently separated, and the precipitated crystals were slightly washed with distilled water to wash off the color reagent on it; the crystals were then vortexed in one ml of solvent 1 and 2 dichloroethane to dissolve in the solvent and appear red. The resulting dye solution was stored for 2 hours and then the samples were read at 365 nm with the spectrophotometer.

Nutrient content

The content of phosphorus was measured spectrophotometrically (Analytik Jena, Spekol 1300, Germany) at a wavelength of 470 nm using a colorimetric method (molybdate vanadate yellow color) (Chapman and Pratt, 1962). The content of potassium was measured by the method of dry ash

(0.5 g) removal using a Flame photometer (PFP7 model, Jenway, UK) (Hamada and El-Enany, 1994). Moreover, nitrogen was measured by titration after distillation using an automatic Kjeldahl. For this purpose, 0.2 g of the dry matter was used to measure the amount of nitrogen in root and shoot tissue (Chapman and Pratt, 1962). Micro elements included copper, zinc, iron and manganese were measured by atomic absorption device (Cottenie, 1980).

Statistical analysis

All data obtained from the experiment were analyzed by SAS statistical software (version 9.1), and the means comparison test was performed using the least significant difference test (LSD). Mean comparison figures were drawn by Excel software. Also, the UPGMA (unweighted pair group method with arithmetic mean) method was performed using Euclidean distance to create cluster analysis. Varimax rotation was used to optimize principal component analysis.

Results

Based on the results, it was observed that the main effect of cultivars and drought stress, as well astheir interaction were significant for macro nutrients in roots ($p \leq 0.01$) (Table 1).

Table 1. Analysis of variance related to the macro-nutrients in roots under drought stress conditions in two wheat cultivars.

SOV	Df	Mean square		
		Nitrogen	Phosphorus	Potassium
Cultivar (C)	1	0.6498**	0.0072**	0.0007**
Drought stress (Ds)	2	1.4384**	0.0225**	5.7534**
C × Ds	2	0.0341**	0.0012**	1.2365**
Residual	12	0.0170	0.0007	0.0679
Coefficient of variation (%)		4.55	12.09	6.38

* and ** are significant at 0.05 and 0.01 levels of probability, respectively

Table 2. Mean comparison related to the macro nutrients in root under drought stress conditions in two wheat cultivars.

Cultivar	Nitrogen (%)	Phosphorus (%)	Potassium (%)
Gonbad	2.67±0.15 b	0.20±0.02 b	3.93±0.29 b
N8720	3.05±0.15 a	0.24(±0.02 a	4.23±0.29 a
Drought stress (g/L)			
0	3.40±0.09 a	0.28±0.01) a	5.16±0.09 a
150	2.74±0.10 b	0.20±0.01 b	3.83±0.12 b
250	2.45±0.10 c	0.16±0.01 c	3.25±0.14 c
C × Ds			
Gonbad×0	3.21±0.06 b	0.26±0.01 ab	5.01±0.12 a
Gonbad×150	2.55±0.03 d	0.18±0.01 cd	3.68±0.06 bc
Gonbad×250	2.26±0.10 e	0.14±0.02 d	3.10±0.20 d
N8720×0	3.59±0.04 a	0.30±0.01 a	5.31±0.09 a
N8720×150	2.93±0.10 c	0.22±0.02 bc	3.98±0.20 b
N8720×250	2.64±0.09 d	0.18±0.02 cd	3.40±0.17 cd

Results are represented as mean ± standard error. Means in a column followed by the different letters are significantly different at $p \leq 0.05$ using LSD test

Macro and micro nutrients in the roots

The results of the interaction effect of drought stress and cultivar showed that the highest amount of root nitrogen was related to the control condition in the N8720 cultivar (3.59%) (Table 2). In contrast, the lowest amount of root nitrogen related to the application of 250 g/L PEG in the Gonbad cultivar (2.26%). Based on the results, the highest amount of phosphorus and potassium in the root was related to drought stress treatment in the N8720 cultivar by 0.30 and 5.31%, respectively (Table 2).

Based on the results, the content of micro nutrients in roots were significantly affected by cultivars and drought stress levels ($p \leq 0.01$). The interaction (C×Ds) was also statistically significant ($p \leq 0.01$) (Table 3). The highest content of copper, zinc, iron, and manganese in root was related to control treatment in N8720 cultivars by 31.1, 34.1, 83.1, and 55.3 mg/kg DW, respectively. In contrast, the lowest content of elements mentioned in the root was related to the application of 250 g/L PEG in Gonbad cultivar with 22.2, 24.4, 67.6 and 42.8 mg/kg DW, respectively (Table 4).

Table 3. Analysis of variance related to the micro nutrients in roots under drought stress conditions in two wheat cultivars.

SOV	Df	Mean square			
		Copper	Zinc	Iron	Manganese
Cultivar (C)	1	42.9665**	68.0945**	154.0013**	37.3248**
Drought stress (Ds)	2	51.7806**	51.7806**	143.8350**	143.8350**
C × Ds	2	5.0214**	8.0255**	12.6584**	25.3256**
Residual	12	6.7917	6.7917	24.5000	3.0179
Coefficient of variation (%)		9.9	9.01	6.62	3.58

* and ** are significant at 0.05 and 0.01 levels of probability, respectively

Table 4. Mean comparison related to the micro nutrients in roots under drought stress conditions in two wheat cultivars.

Cultivar	Copper (mg/kg DW)	Zinc (mg/kg DW)	Iron (mg/kg DW)	Manganese (mg/kg DW)
Gonbad	24.75±1.09 b	26.97±1.09 b	71.84±1.99 b	47.03±1.49 b
N8720	27.84±1.17 a	30.86±1.17 a	77.69±2.03 a	49.91±1.51 a
Drought stress (g/L)				
0	29.54±0.95 a	32.16±1.08 a	80.17±1.84 a	53.87±0.77 a
150	25.55±1.17 b	28.17±1.28 b	73.52±2.55 b	47.22±0.90 b
250	23.81±1.38 b	26.43±1.47 b	70.62±2.25 b	44.32±1.02 c
C × Ds				
Gonbad×0	27.99±1.15 ab	30.21±1.15 ab	77.24±1.73 ab	52.43±0.77 a
Gonbad×150	24.00±0.58 bc	26.22±0.58 bc	70.59±3.46 bc	45.78±0.39 bc
Gonbad×250	22.26±2.02 c	24.48±2.02 c	67.69±2.89 c	42.88±1.35 c
N8720×0	31.08±0.87 a	34.10±0.87 a	83.09±2.31 a	55.31±0.58 a
N8720×150	27.09±2.02 ab	30.11±2.02 ab	76.44±3.46 a-c	48.66±1.35 b
N8720×250	25.35±1.73 bc	28.37±1.73 bc	73.54±2.89 bc	45.76±1.15 bc

Results are represented as mean ± standard error. Means in a column followed by the different letters are significantly different at $p \leqslant 0.05$ using the LSD test.

Macro and micro nutrients in shoot

Based on the results, it was observed that the main effect of cultivars was significant for potassium in shoot ($p \leq 0.01$) (Table 5). Nitrogen, phosphorous, and potassium of the shoot were affected significantly by the main effect of drought stress ($p \leq 0.01$). Also, the results showed that macro nutrients in shoots were affected by the interaction of cultivars and drought stress ($p \leq 0.01$). The highest content of nitrogen, phosphorus, and potassium in the shoot were related to the control condition in N8720 cultivar with 4.16, 0.23 and 5.03%, respectively (Table 6).

Table 5. Analysis of variance related to the macro nutrients in the shoot under drought stress conditions in two wheat cultivars.

SOV	Df	Mean square		
		Nitrogen	Phosphorus	Potassium
Cultivar (C)	1	0.0761^{NS}	0.0018^{NS}	0.1152**
Drought stress (Ds)	2	5.7534**	0.0225**	5.7534**
C × Ds	2	0.0021**	0.0013**	0.0002**
Residual	12	0.2717	0.0007	0.0170
Coefficient of variation (%)		17.28	16.77	3.37

* and ** are significant at 0.05 and 0.01 levels of probability, respectively

Table 6. Mean comparison related to the macro nutrients in the shoot under drought stress conditions in two wheat cultivars.

Cultivar	Nitrogen (%)	Phosphorus (%)	Potassium (%)
Gonbad	2.95±0.31 ^{a}	0.15±0.02 ^{a}	3.79±0.28 ^{b}
N8720	3.08±0.33 ^{a}	0.17±0.02 ^{a}	3.95±0.29 ^{a}
Drought stress (g/L)			
0	4.10±0.13 ^{a}	0.22±0.01 ^{a}	4.95±0.05 ^{a}
150	2.77±0.19 ^{b}	0.14±0.01 ^{b}	3.62±0.06 ^{b}
250	2.19±0.24 ^{b}	0.10±0.01 ^{c}	3.04±0.07 ^{c}
C × Ds			
Gonbad×0	4.03±0.23 ^{a}	0.21±0.01 ^{a}	4.87±0.06 ^{a}
Gonbad×150	2.70±0.12 ^{b}	0.13±0.01 ^{bc}	3.54±0.03 ^{b}
Gonbad×250	2.12±0.40 ^{b}	0.09±0.02 ^{c}	2.96±0.10 ^{c}
N8720×0	4.16±0.17 ^{a}	0.23±0.01 ^{a}	5.03±0.04 ^{a}
N8720×150	2.83±0.40 ^{b}	0.15±0.02 ^{b}	3.70±0.10 ^{b}
N8720×250	2.25±0.35 ^{b}	0.11±0.02 ^{bc}	3.12±0.09 ^{c}

Results are represented as mean ± standard error. Means in a column followed by the different letters are significantly different at $p \leqslant 0.05$ using LSD test.

The results showed that the main effect of cultivars and drought stress was significant for micro nutrients in shoot ($p \leq 0.01$) (Table 7). Also, the results showed that micro nutrients in shoot were affected by the interaction of cultivars and drought stress ($p \leq 0.01$). The content of copper, zinc, iron and manganese in shoot were affected by cultivar and drought stress interaction (Table 7). The highest content of shoot copper was related to drought stress treatment in N8720 cultivar (22.47 mg/kg DW), while the lowest content of it was observed when 250 g/L PEG was applied in Gonbad cultivar (14.53 mg/kg DW) (Table 8).

Table 7. Analysis of variance related to the micro nutrients in the shoot under drought stress conditions in two wheat cultivars.

SOV	Df	Mean square			
		Copper	Zinc	Iron	Manganese
Cultivar (C)	1	21.9785**	45.2201**	71.2818**	37.4545**
Drought stress (Ds)	2	51.7806**	51.7806**	143.8350**	143.4244**
C × Ds	2	6.8746**	6.8745**	9.5874**	15.3254**
Residual	12	1.3419	6.7917	5.5000	6.7917
Coefficient of variation (%)		6.38	6.74	3.35	3.71

* and ** are significant at 0.05 and 0.01 levels of probability, respectively.

Table 8. Mean comparison related to the micro nutrients in the shoot under drought stress conditions in two wheat cultivars.

Cultivar	Copper (mg/kgDW)	Zinc (mg/kgDW)	Iron (mg/kgDW)	Manganese (mg/kgDW)
Gonbad	17.02±0.90 b	37.06±1.09 b	67.81±1.59 b	68.70±1.57 b
N8720	19.23±0.92 a	40.23±1.17 a	71.79±1.54 a	71.59±1.63 a
Drought stress (g/L)				
0	21.37±0.57 a	41.88±0.96 a	75.20±1.29 a	75.54±0.91 a
150	17.38±0.65 b	37.89±1.18 b	68.55±1.41 b	68.89±1.14 b
250	15.64±0.72 c	36.15±1.39 b	65.65±0.96 b	66.00±1.35 b
C × Ds				
Gonbad×0	20.26±0.51 b	40.30±1.15 ab	73.21±1.73 ab	74.10±1.15 ab
Gonbad×150	16.27±0.26 de	36.31±0.58 bc	66.56±1.73 cd	67.44±0.58 cd
Gonbad×250	14.53±0.90 e	34.57±2.02 c	63.66±0.58 d	64.57±2.02 d
N8720×0	22.47±0.39 a	43.47±0.87 a	77.19±1.15 a	76.98±0.87 a
N8720×150	18.48±0.90 bc	39.48±2.02 ab	70.54±1.73 bc	70.35±2.02 bc
N8720×250	16.74±0.77 cd	37.74±1.73 bc	67.64±0.58 cd	67.44±1.73 cd

Results are represented as mean ± standard error. Means in a column followed by the different letters are significantly different at $p \leqslant 0.05$ using LSD test.

The highest content of zinc, iron and manganese in the shoot were 43.47, 77.19 and 76.98 mg/kg DW, respectively, related to drought stress treatment in N8720 cultivar. In contrast, the lowest content of zinc, iron and manganese in the shoot (34.57, 63.66 and 64.57 mg/kgDW) were related to the 250 g/L PEG treatment in Gonbad cultivar, respectively (Table 8).

Total chlorophyll and glycine betaine

Based on the results, it was observed that the main effect of cultivars and drought stress was significant for total chlorophyll and glycine betaine ($p \leq 0.01$). Also, the results of the analysis of variance showed that total chlorophyll and glycine betaine were affected by the interaction of cultivars and drought stress ($p \leq 0.01$) (Table 9). The highest content of total chlorophyll was observed in the control treatment in both N8720 and Gonbad cultivars (12.9 and 13 µmol/g FW, respectively) (Figure 1A). Based on the results, it was observed that 150 and 250 g/L PEG levels had the lowest content of total chlorophyll.

Table 9. Analysis of variance and mean comparison related to the glycine betaine and total chlorophyll in leaves under drought stress conditions in two wheat cultivars.

S.O.V	Degree of freedom	Glycine betaine	Total chlorophyll
Cultivar (A)	1	0.3362**	0.0456**
Drought stress (B)	2	0.7511**	10.1516**
A × B	2	0.0028**	0.0033**
Residual	12	0.0027	0.6113
Coefficient of variation (%)	-	8.04	6.79

* and ** represent significant levels of probability at 5 and 1%, respectively.

On the other hand, the results of the mean comparison showed that the amount of glycine betaine in both studied cultivars increased significantly with increasing drought stress (Figure 1B). The results showed that the highest content of glycine betaine was observed in the application of 250 g/L PEG in the N8720 cultivar (1.23 µmol / g). In contrast, the lowest content of glycine betaine was related to non-stress treatment in Gonbad and N8720 cultivars.

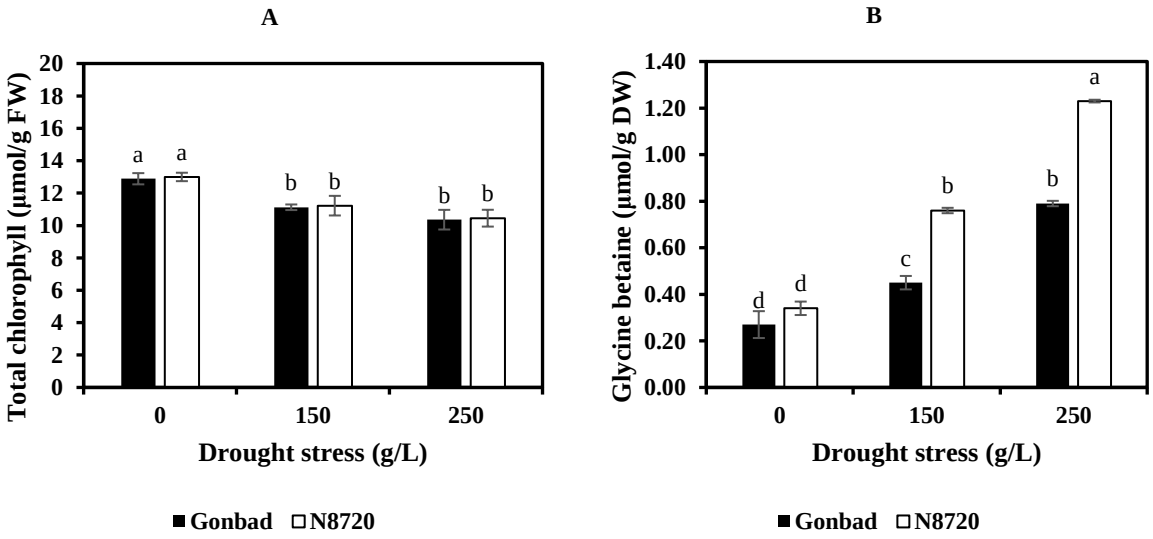

Figure 1. Mean comparison related to the interaction effects of cultivar and drought stress for glycine betaine and total chlorophyll. Results are represented as mean ± standard error.

Principal component analysis results

The results of principal components analysis showed that the first and second components had an eigenvalue higher than one and were selected as the most effective components among all the components (Figure 2A). Based on the results of the principal component analysis, it was shown that the first and second components with 95.93 and 3.67%, respectively, had the highest relative variance and gathered 99.60% of the total variance. Biplot obtained from the first and second components showed that Gonbad+0 g/L PEG, N8720+0 g/L PEG, and N8720+150 g/L PEG treatments had a stronger relationship with all traits.

Cluster analysis

The dendrogram obtained from cluster analysis based on the UPGMA method and Euclidean distance is shown in Figure 2B. Based on the results, cluster analysis divided the different treatments into three separate groups.

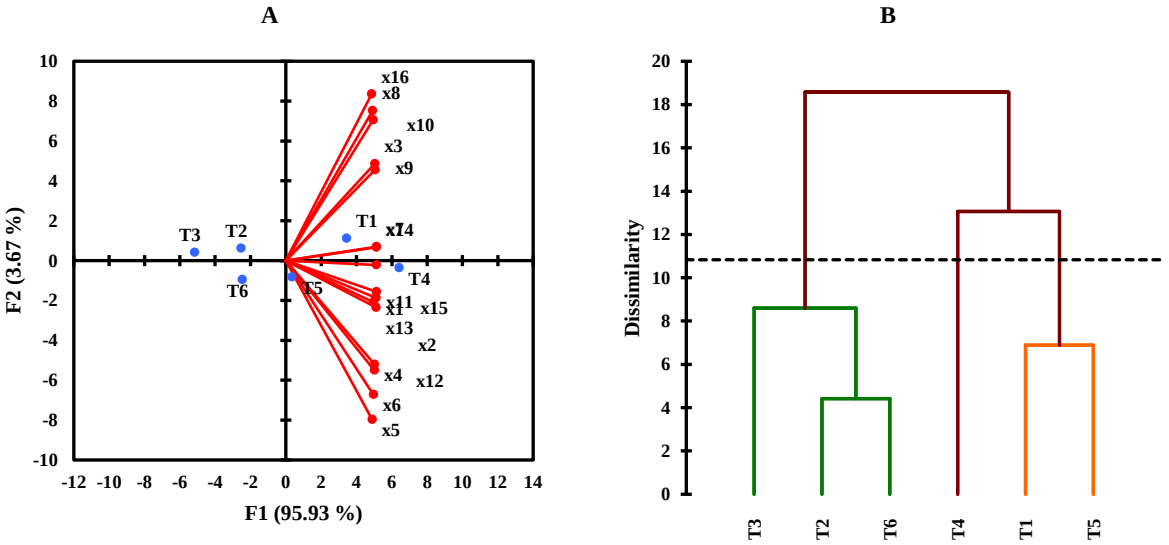

Figure 2. A) Biplot obtained from principal components analysis based on the first and second components related to all studied traits in two wheat cultivars under drought stress. (X1: nitrogen of root; X2: phosphorus of root; X3: potassium of root; X4: copper of root; X5: zinc of root; X6: iron of root; X7: manganese of root; X8: nitrogen of shoot; X9: phosphorus of shoot; X10: potassium of shoot; X11: copper of shoot; X12: zinc of shoot; X13: iron of shoot; X14: manganese of shoot; X15: glycine betaine; X16: total chlorophyll), B) Dendrogram obtained from cluster analysis based on all studied traits in two wheat cultivars under drought stress. (T1: Gonbad+0 (g/L); T2: Gonbad+150(g/L); T3: Gonbad+250(g/L); T4: N8720+0(g/L); T5: N8720+150(g/L); T6: N8720+250(g/L)).

Table 10. Pearson correlation coefficients based on all traits in two wheat cultivars under drought stress conditions.

Traits	x_1	x_2	x_3	x_4	x_5	x_6	x_7	x_8	x_9	x_{10}	x_{11}	x_{12}	x_{13}	x_{14}	x_{15}	x_{16}
x_1	1															
x_2	0.99**	1														
x_3	0.97**	0.98**	1													
x_4	0.91**	0.95**	0.87**	1												
x_5	0.91**	0.94**	0.84**	0.99**	1											
x_6	0.88**	0.91**	0.82**	0.97**	0.97**	1										
x_7	0.99**	0.99**	0.99**	0.93**	0.92**	0.89**	1									
x_8	0.91**	0.96**	0.97**	0.90**	0.87**	0.86**	0.96**	1								
x_9	0.96**	0.99**	0.99**	0.92**	0.89**	0.87**	0.99**	0.99**	1							
x_{10}	0.94**	0.93**	0.99**	0.79**	0.76**	0.74**	0.95**	0.94**	0.96**	1						
x_{11}	0.99**	0.99**	0.97**	0.95**	0.94**	0.91**	0.99**	0.94**	0.97**	0.93**	1					
x_{12}	0.91**	0.95**	0.87**	0.99**	0.99**	0.97**	0.93**	0.90**	0.92**	0.78**	0.95**	1				
x_{13}	0.97**	0.97**	0.93**	0.92**	0.92**	0.93**	0.96**	0.90**	0.94**	0.89**	0.97**	0.92**	1			
x_{14}	0.97**	0.99**	0.97**	0.97**	0.95**	0.93**	0.99**	0.97**	0.99**	0.92**	0.99**	0.97**	0.96**	1		
x_{15}	0.95**	0.92**	0.91**	0.81**	0.81**	0.78**	0.93**	0.83**	0.89**	0.90**	0.93**	0.81**	0.92**	0.89**	1	
x_{16}	0.89**	0.94**	0.95**	0.90**	0.86**	0.85**	0.94**	0.99**	0.98**	0.92**	0.92**	0.90**	0.88**	0.96**	0.80**	1

** is significant at 0.01 levels of probability. (Nitrogen of root (x_1), phosphorus of root (x_2), potassium of root (x_3), copper of root (x_4), zinc of root (x_5), iron of root (x_6), manganese of root (x_7), nitrogen of shoot (x_8), phosphorus of shoot (x_9), potassium of shoot (x_{10}), copper of shoot (x_{11}), zinc of shoot (x_{12}), iron of shoot (x_{13}), manganese of shoot (x_{14}), glycine betaine (x_{15}) and total chlorophyll (x_{16})).

Results showed that Gonbad+250 g/L PEG, Gonbad+150 g/L PEG, and N8720+250 g/L PEG treatments were located in one group. On the other hand, Gonbad+0 g/L PEG and N8720+150 g/L PEG treatments were placed in a similar group and N8720+0 g/L PEG treatment was placed in an independent group alone

Pearson correlation analysis

The results of Pearson correlation coefficients were shown in Table 10. The results showed that there is a positive and significant correlation between all traits ($p \leq 0.01$)

Discussion

Drought stress not only reduces the growth and development of plants but also changes the direction of some metabolic processes (Salehi et al., 2016). These changes can make the plant tolerant to stress. Adaptation to drought depends on reactions through which the initial metabolic processes continue and prepare the plant to deal with it (Sallam et al., 2019). In this experiment, the cultivars reacted similarly to other plants in the face of drought stress and coped with changes in physiological characteristics and changes in the absorption of elements under stress conditions.

Photosynthetic contents

The results of this experiment showed that the total chlorophyll content decreased with increasing drought stress. According to research, drought stress causes the destruction of chloroplasts and the instability of pigment and protein compounds (Gomes et al., 2016; Salehi et al., 2016; Cardona et al., 2018). The researchers indicated that the amount of chlorophyll decreased due to drought stress, it is found that due to higher chlorophyllase activity under drought stress conditions (Siddiqui et al., 2015; Salehi et al., 2016; Guo et al., 2018). In addition, many researchers showed that there is a significant correlation between nutrients absorbed by plants and chlorophyll content (Sohrabi et al., 2012; Rabiei et al., 2020; Khalvandi et al., 2021). However, some growth regulators, such as abscisic acid and ethylene, which increase in drought stress, stimulate the activity of this enzyme (Wang et al., 2019a). According to researchers, the decrease in chlorophyll content may be due to changes in nitrogen metabolism in connection with the production of compounds such as proline, which is used in osmotic regulation (Filstrup and Downing, 2017; Hosseini et al., 2021; Hosseini et al., 2023). Glutamine kinase is one of the key enzymes in proline synthesis, which is the first enzyme in the proline biosynthesis pathway and is found in the cytoplasm and chloroplasts (Bashir et al., 2020). Drought stress has a stimulating effect on the activity of this enzyme. In contrast, glutamate ligase is the first important enzyme in chlorophyll synthesis, which drought stress inhibits its activity. Therefore, under stress conditions, chlorophyll production decreases due to a decreased in glutamate-ligase activity and more glutamate consumption by the activated glutamine kinase enzyme (Ahmad et al., 2016).

Other symptoms presented by the researchers include increased stomatal resistance due to stomatal obstruction, increased mesophilic resistance due to increased cell wall thickness and cell thickness, increased plant respiration, ion toxicity and disruption of plant growth metabolic processes, decreased mineral uptake, reduced RUBP and regeneration efficiency, all of which directly or indirectly reduce photosynthesis (Jangid and Dwivedi, 2016). Drought stress hydrolyzes thylakoid proteins and reduces the content of chlorophyll a and b (Wang et al., 2019b). On the other hand, drought stress disrupts enzymatic activations, reduces the activity of reactive oxygen species, and increases the peroxidation of fats, resulting in damage to cell membranes and the destruction of chlorophyll a and b (Salehi-Lisar and Bakhshayeshan-Agdam, 2016). The researchers reported that increasing drought stress significantly reduced the content of chlorophyll a, b, and total in wheat, which was consistent with the results of this experiment (Abbas et al., 2018; Ahmad et al., 2018).

Uptake of micro and macro elements

Drought stress has a great impact on the absorption of micro and macro elements, so finding the effect of drought stress on the absorption of elements in the seedling stage is very important (Bista et al., 2018). The results of this experiment showed that the adsorption of the micro and macro elements was affected by drought stress and their adsorption decreased with increasing stress. Absorption of nutrients by plant roots and water availability are

closely related. Water availability affects all physiological processes associated with the solubility and availability of nutrients (Bowles et al., 2016). Drought stress reduces active transmission in the shoots, membrane permeability, plant root absorption, and nutrient uptake through transpiration (Nadeem et al., 2019). The responses of nutrient accumulation in plants to drought stress are different. The efficiency of the plant root in order to absorb the nutrients may also be reduced due to lack of moisture (Bista et al., 2018). A number of researchers have shown that the uptake of nitrogen, phosphorus and potassium decreased in the face of drought stress (Delshadi et al., 2017; Tadayyon et al., 2018). Absorption of iron, zinc, copper, and manganese also decreased in the two cultivars, but the amount of uptake in the resistant cultivar was higher than in the sensitive cultivar. Several researchers have suggested that the uptake of iron, copper, zinc and manganese should be reduced under drought stress (Rizwan et al., 2015; Abbas et al., 2018; Liang et al., 2018).

Glycine betaine

The results of the assessment of glycine betaineshowed that drought stress increased the amount of glycine betaine in both cultivars. Also, based on the results, it was observed that more glycine betaine was produced in the resistant cultivar. Depending on the type of stress, plants in their evolutionary path, have found ways to reduce the adverse effects of stress (Seleiman et al., 2021). One of the common reactions that occurs in plant cells as a result of increased accumulation of organic solutions in the cytoplasm is osmotic regulation (Choudhary et al., 2023). Osmotic regulation helps plants to increase the osmotic balance between the cytoplasm and various cell components (Zivcak et al., 2016). Osmotic regulation in plants occurs through the production of various types of compatible organic solutions and some inorganic ions (Aslam et al., 2015). In general, these substances protect plants from stress during different periods by participating in osmotic balance and maintaining membrane fluidity and the stability of proteins or enzymes (Thalmann et al., 2016). Their main role is to insulate plant cells from stress-induced damage by osmotic moderation, to stabilize the structure of key proteins such as Rubisco, and to maintain the photosynthetic apparatus (Singh et al., 2015). Most halophytes increase glycine betaine as an osmotic moderator in their cells when exposed to stress (Pardo-Domènech et al., 2016). Glycine betaine is also increased in response to stress in many other crops, including spinach, barley, wheat, and sorghum (Osman, 2015; Roychoudhury and Banerjee, 2016; Kurepin et al., 2017; Annunziata et al., 2019).

Conclusion

The results of this experiment showed that N8720 cultivar had superior characteristics across all studied traits. Also, severe drought stress had a very negative effect on all characteristics, highlighting the importance of a tolerant cultivar like N8720, which can demonstrate better physiological characteristics in severe stress conditions. Due to the favorable attributes of N8720 cultivar including its efficient nutrient uptake as well as its optimal efficiency of the photosynthetic system, and high content of glycine betaine for combating drought stress, this cultivar holds promise for inclusion in future wheat breeding endeavors.

Supplementary Materials

No supplementary material is available for this article.

Author contributions

Conceptualization, N.M. and S.A.; methodology, N.M.; software, S.A.; validation, B.R., M.E. and S.A.; formal analysis, S.A.; investigation, S.A.; resources, N.M.; data curation, S.A.; writing—original draft preparation, S.A.; writing—review and editing, N.M.; visualization, B.R.; supervision, N.M.; project administration, M.E.; funding acquisition, N.M. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest statement

The authors declare no conflict of interest.

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مقاله پژوهشی

پاسخ ارقام گندم به تنش خشکی با تمرکز بر عناصر مغذی ریشه و بخش هوائی، کلروفیل و گلاسئین‌بتائین برگ

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چکیده: تنش خشکی یکی از عوامل محدودکننده رشد گیاه است. به منظور بررسی اثر تنش خشکی (۰، ۱۵۰، ۲۵۰ گرم در لیتر پلی‌اتیلن گلیکول ۶۰۰۰ (PEG) بر خصوصیات فیزیولوژیکی دو رقم گندم (گنبد و N8720)، آزمایش هیدروپونیک به صورت فاکتوریل در قالب طرح کاملاً تصادفی با سه تکرار در انستیتو پاستور ایران (مرکز تحقیقات شمال) در سال ۱۳۹۶ اجرا شد. نتایج آزمایش نشان داد که اثر ساده ارقام برای تمامی صفات مورد مطالعه به جز نیتروژن و فسفر ساقه معنی‌دار بود. همچنین اثر ساده تنش خشکی و اثر متقابل تنش خشکی و ارقام برای تمامی صفات مورد مطالعه معنی‌دار بود. بیشترین مقدار عناصر در ریشه و اندام هوایی و میزان کلروفیل در رقم N8720 تحت تیمار شاهد مشاهده شد. همچنین در رقم N8720 میزان گلیسین‌بتائین به دلیل تنش خشکی افزایش یافت و در تیمار ۲۵۰ گرم در لیتر PEG به حداکثر خود رسید. نتایج تحلیل همبستگی نشان داد که بین تمامی صفات همبستگی مثبت و معنی‌داری وجود دارد. نتایج آزمایش نشان داد که رقم N8720 از نظر کلیه صفات مورد مطالعه از ویژگی‌های برتر برخوردار بود.

کلمات کلیدی: ارقام، تجزیه خوشه‌ای، تنش خشکی، فسفر، گلیسین بتائین.

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