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Effect of drought Stress on some Morphological and Physiological Characteristics of tow resistance and sensitive wheat cultivars

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ABSTRACT

Water stress is one of the main abiotic stresses that reduce plant growth;this decrease is due to changes such as physiological changes and causes growth and production limitation that caused by drought stress.In order to evaluate the effects of drought stress on some morphological and physiological characteristics of tow wheat cultivars, a factorial experiment based on completely randomized design was conducted. The findings show that drought stress exacerbations result in the plant's response to stress due to increased wheat resistance. This response is due to changes in plant pigments, proline, catalase, ascorbate peroxidase, peroxidase, superoxide dismutase and malondialdehyde, glucose, galactose, rhamnose and xylose, which ultimately influence these changes effects on the morphological characteristics of wheat.Drought stress reduces the concentration of carotenoids, chlorophyll a, chlorophyll b, total chlorophylls, but glucose, galactose, rhamnose, xylose, proline, catalase, ascorbate peroxidase, peroxidase, superoxide dismutase, malondialdehyde (in leaves and roots) and the chlorophyll a and b ratios were increased.Reduction of plant height, stem height, root length, fresh and dry weight of wheat treated with 250 g / l PEG compared to non-treatment were 0.264, 0.236, 0.394, 0.183 and 0.395, respectively.From the two wheat cultivars, the morphological characteristics of the N8720 increased compared to the Gonbad cultivar. Interaction effects of cultivar and drought stress showed that N8720 cultivar without treatment had the highest morphological characteristics, carotenoid concentration, chlorophyll a, chlorophyll b, total chlorophylls a and b, and the above cultivar with 250 g / l PEG (drought stress) had the highest amount of proline, malondialdehyde, soluble sugars and enzymes in leaves and roots. Increasing activity of oxidative enzymes and soluble sugars in wheat under drought stress could be a sign of their relative tolerance to drought stress.
Keywords: Drought stress, N8720, photosynthetic pigments, enzymes

INTRODUCTION

Drought is one of the most important environmental stresses that limit production of plant products around the world and has adverse effects on plant growth and development and other metabolic processes (Lam et al., 2014; Akram et al., 2013; Mahajan and Tiotja, 2005).In agriculture, drought defined as the lack of needed moisture for plant growth and development in order to complete the life cycle (Manivan et al., 2008).The amount of water in the soil for optimum growth of the plant has an optimum level, which reduction or increasement of it, reduce plant growth. (shokouh far of Fara and Abofatilehnejad, 2013).

Water was an important molecule for all of the plant's physiological processes (Novo & Chen, 2010).When the water of soil decreases and atmospheric conditions cause continuous water loss through the plant perspiration and evaporation, it causes drought stress in the plant (Jalil et al., 2009).Iran with average precipitation (240 mm per year) is considered a part of the drought stress area (SalehiShajani et al., 1394).Iran is one of the countries where biotic and abiotic stresses (like drought stress) reduce the growth and production of crops such as wheat (Arbabian and Majd, 2010; Sabbaghpour, 2003).Plants generally have different mechanisms for respond and adapt to drought stress, by inducing a variety of physiological, biochemical and morphological responses (Mirzai et al., 2013).

The response of plants to drought stress is dependent on the nature of water deficiency and can be as follows: physiological responses to short-term drought stress (short-term response) including reduced carbon dioxide absorption,non-inheritance adoption with a certain level of drought stress (medium-term response) through osmotic regulation by the accumulation of organic salts and inherited adoption to drought (long-term response) including genetic patterns.In drought stress conditions, the plant reduces its osmotic potential in order to continue absorbing water through osmotic accumulation, including soluble carbohydrates and proline, and In other words, osmotic regulation is done. In the osmotic regulation process, turgescence and associated processes continue under water deficiency conditions. thus Osmotic regulation helps the cellular development and plant growth in drought stress (Psarkeley, 1999).

Moisture stress affects the growth and production of plant products and increases the concentration of soluble sugars and proline in sunflower leaves (Nazarli et al., 2011; Kheibari et al., 2013). Azarpanah et al. (2013) reported that proline is one of the most important osmotic regulators under environmental stresses, which, in a large number of plant species, is highly correlated with tolerance to this stress. Cladia et al. (2012) found that due to drought stress, the concentration of carbohydrates such as glucose and fructose increased in bean leaves.Singh et al. (2014) concluded that there was a direct correlation between proline and drought tolerance in wheat and the amount of proline accumulation can be as a physiological index for stress tolerance;also, high levels of proline, under stress, help the plant tolerate stress conditions easily. The amount of proline accumulation in various cultivars is different and stress resistant cultivars have is higher amounts of it.Drought stress through the formation of reactive oxygen species causes secondary stresses such as oxidative

stress, which ultimately causes changes in the biosynthesis pathways of secondary metabolites.

The reactive oxygen species accumulated in the plant are reduced through the enzymatic and non-enzymatic antioxidant mechanisms in the plant.The accumulation of reactive oxygen species in the cell causes damage to membrane lipids, proteins, and nucleic acids.During photosynthesis under different stress conditions, various types of active oxygen species such as superoxide, hydrogen peroxide, and radical hydroxyl and oxygen radicals are produced.The plants have antioxidant enzymes and non-enzymatic mechanisms to response the oxidative stress. The radical superoxide may be transformed into a hydrogen peroxide by the superoxide dismutase enzyme and then converted to the water by the ascorbate peroxidase in the chloroplasts.In addition, the propagated hydrogen peroxide is scavenged to the exterior of the chloroplast by the catalase enzyme in the leaf cells (Miller et al., 2010; Hasanuzzaman et al., 2014).

The catalase and ascorbate peroxidase enzymes play an important role in the scavenging of hydrogen peroxide. Increasing activity of catalase indicates effective inhibition of hydrogen peroxide by this enzyme, which is seen in studies on alfalfa (Van Bin et al., 2009) and Lamiaceae (Ozgen et al., 2006). Farooq et al. (2014) reported that drought stress could alter enzymes activity.

Chloroplasts and their pigments are also affected drought. For example, drought stress causes hydrolysis of thylakoid proteins and reduces the amount of chlorophyll a and b (Anjum et al., 2011; Dein et al., 2011; Tian et al., 2013).The weakening of chloroplasts caused by tension and early aging can affect the photosynthetic capacity of plants (Wang and Blumwald, 2014).Drought stress causes damage to photosynthetic pigments, thylakoid membrane decay, and a decrease in chlorophyll content (Parida et al., 2007; Arivalagan and Somasundaram, 2015; Taïbi et al., 2016). Reduction in chlorophyll content is due to oxidative stress, which is one of the consequences of drought stress (Zhang et al., 2011).

Keyvan(2010) reported that drought stress reduces total chlorophyll content in wheat but increases the amount of proline and soluble carbohydrates. HassanpourLeskoKalaye et al. (1394) stated that total chlorophyll a, b and total chlorophyll under drought stress conditions decreased in all wheat cultivars compared to wheat with non-stressed treatments. Ziaee et al. (1396) showed that with increasing drought, the rate of photosynthesis, carotenoids, chlorophyll a and b, total chlorophyll and chlorophyll a to b decreased, while the amount of proline in *Vigna radiata* leaves significantly increased.

The researchers stated that under drought stress conditions, carotenoids absorb light at certain wavelengths and send it photochemical centers to generate energy; In other words, chlorophyll b and carotenoids act as auxiliary and protective pigment of chlorophyll a, located in chloroplast photosystems, and they play an important role in absorbing and transmitting light energy to chlorophyll a (Jalil et al., 2009). Drought stress affects gene expression, growth, and products synthesis (Zheng et al., 2010; Almeselmani et al., 2011). In general, an average of more than 50 percent of many crops yields decreases due to drought stress (Zlato and Lidon, 2012).

Plant height and chlorophyll content were higher under drought stress compare to drought tolerant cultivars (Kilic and YAĞBASANLAR, 2010; Singh et al., 2014). Ali et al. (2013) stated that wheat cultivars had a different response to drought stress and stress resistant cultivars could grow successfully in dry areas without significant reduction in wheat production, and drought could reduce plant yield more than other environmental stresses.

Hamad and Ali (2014) state that drought stress significantly reduces wheat aerial biomass. Dehghan et al. (1394) stated that the root length and dry weight of tomato aerial parts were higher under the non-stress condition in comparison with drought stress. Ziaee et al. (1396) reported that increasing drought stress reduced plant height and root dry weight in Vigna radiata.

Yan and Shi (2013) showed that drought stress reduced plant height, root length, fresh and dry weight of wheat. Yavas & Unay (2016) reported that the chlorophyll content and wheat plant height were higher in control treatment than in drought stress treatment.

The aim of this study was to evaluate the effect of drought stress on soluble sugars, photosynthetic pigments, morphological characteristics, proline, catalase, ascorbate peroxidase, peroxidase, superoxide dismutase, and malondialdehyde.

MATERIALS AND METHODS

This research was carried out in Pasteur Institute of Iran (North Research Center) during 2017.Seedlings of Gonbad (sensitive cultivar) and N8720 (resistant cultivar) wheat (*Triticumaestivum* L.) were examined in the experiment.

Experimental design: Grains were disinfected in 96% ethanol for 1.5min followed by 15min in 15% Domestos, before being washed 4 times in sterile water. Afterwards, grains were germinated on wet filter paper for 3 days. Germinating seedlings were put into plastic pots containing water with a half-strength Hoagland solution and maintained in a hydroponics culture in a phytotronic 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 controltreatment (0.0 g/l Polyethylene glycol), seedlings were exposed to three levels of Polyethylene glycol (PEG) stress (0, 150g/l, 250 g/l). For these treatments, osmotic stress was applied with PEG 6000 dissolved in half-strength Hoagland. The seedlings of each genotype were grown until the fourth leaf was fully expanded.

In order to evaluate the effects of drought stress on physiological, metabolic and morphological characteristics of wheat, a factorial experiment based on completely randomized design was conducted.Finally, traits such as plant length, stem length, root length, fresh and dry weight traits in roots and leaves of wheat in each plot was measured.

Measuring of leaf chlorophyll and carotenoidscontent: The modified Arnon method (1967) also used to measure chlorophyll content.According to this method, 0.25 g of

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, 646, and 470 wavelengths, and the concentration of chlorophyll a, chlorophyll b andcarotenoids were calculated.

Determining the proline content, soluble sugar content, and antioxidant enzyme activity: Dry roots and leaf (0.15 g) were homogenized in 5 mL of 3% sulfosalicylic acid at 100 °C for 20 min in a water bath and then filtrated. The filtrate (2 mL) was mixed with acid-ninhydrin (2 mL) and glacial acetic acid (2 mL) in a test tube. The reaction mixture was incubated in a water bath at 100 °C for 30 min, cooled to room temperature, and then extracted with toluene (5 mL). The toluene, which contains chromophores, was aspirated, and the absorbance was then measured at 520 nm. The dry leaf and roots (0.1 g) were homogenized in 5 mL of 80% ethanol at 80 °C for 30 min in a water bath, and then the supernatant was collected. The above steps were repeated twice. The filtrate (2 mL) was mixed with anthrone-sulfuric acid (2 mL) in ice water, and the reaction mixture was then incubated in a water bath for 10 min at 100 °C and then cooled to room temperature. Afterwards, the absorbance was measured at 620 nm.

Fresh root and leaf tissues (0.3 g) were ground with 5 mL of 50 mM phosphate buffer (pH 7.0), which was prepared by mixing NaH2 PO4·H2O and Na2HPO4·7H2O and then centrifuging at 12000g for 20 min at 4 °C. Then, the supernatant was collected to measure the antioxidant enzyme activity and the soluble sugar content. The superoxide dismutase (SOD) activity was determined using reagent kits. The peroxidase (POD) and catalase (CAT) activities were measured using the guaiacol method and the ultraviolet absorption method (Li et al., 2000a). The activity of the ascorbate peroxide enzyme was measured by Nakano and Asada (1981). The activity of the ascorbate peroxide enzyme was measured by Mac Adam (2013).Measurements of lipid peroxidation (the concentration of malondialdehyde, MDA) were taken due to Dhindsa et al. (1981). Sugar content was measured spectrophotometrically due to Dubois et al. (1951). Proline content was evaluated spectrophotometrically due to Ting and Rouseff (1979)

Statistical analysis

The experiment was performed according to a completely randomized design. Standard errors of means were calculated for all parameters. SAS software was used for data analysis. The mean comparison was done by the LSD test at a probability level of 1% and 5%.

Results:

Effect of drought stress on plant height, stem height, root length, fresh and dry weight of wheat

The results showed that wheat cultivar and drought stress had a significant effect at 1% level on plant height, stem height, root length, fresh and dry weight of wheat,however, their interaction has only a significant effect on the dry weight of the plant at a 1% level. Interaction effects of cultivar and drought stress on plant height, root length and wheat fresh weight at 5% level had a significant effect but no significant effect on stem height observed.Plant height, stem height, root length, fresh and dry weight of wheat were higher in N8720 than Gonbad cultivar. With increasing drought stress (due to increased concentration of PEG), the above characteristics of the plant decreased, so that the highest and lowest amount of these plant characteristics were observed in non-stress and drought stress treatments (250 g /lPEG), respectively.reduction of Plant height, stem height, root length, fresh and dry weight of wheat treated with 250 g / l PEG compared to non-treatment were 0.264, 0.236, 0.394, 0.183 and 0.395, respectively.Interaction effects of cultivar and drought stress indicate that the non-stress treatment with N8720 has the highest amount of these characteristics and the lowest amount was related to the Gonbad cultivar with a concentration of 250 g / L of PEG (Table 1).

The effect of drought stress on photosynthetic pigments

Table 2 shows that wheat cultivars, drought stress and their interactions have a significant effect on carotenoid, chlorophyll a, chlorophyll b, total and chlorophyll a and b ratios at 1% level. Between two varieties, the highest amount of the above characteristics related to the N8720. The concentration of carotenoids, chlorophyll a, chlorophyll b, total chlorophylls a and b decreased with increasing drought stress, but the chlorophyll a and b ratio increased.The reduction in the concentration of carotenoids, chlorophyll a, chlorophyll b, total chlorophylls a and b under drought stress at a concentration of 250 g / l of PEG were 0.312, 0 and 0.23, respectively.The reduction in the concentration of carotenoids, chlorophyll a, chlorophyll b, total chlorophylls a and b under drought stress at a concentration of 250 g / l of PEG were 0.312, 0 and 0.23, respectively. Interaction effects of cultivar and drought stress indicate that the highest and lowest amounts of carotenoids, chlorophyll a, chlorophyll b, total chlorophylls a and b were related to non-stress treatment in N8720 and drought stress with concentration of 250 g/l PEG in Gonbad cultivar, but the highest and lowest ratio of two chlorophylls were observed in N8720 cultivar under drought stress with a concentration of 250 g/L of PEG and in Gonbad cultivar without stress, respectively.

Effect of drought stress on production of proline, catalase, ascorbate peroxidase, peroxidase, superoxide dismutase and malondialdehyde in leaf

The results of this section indicate that wheat cultivars, drought stress and their interactions had significant effects on proline, catalase, ascorbate peroxidase, peroxidase, superoxide dismutase and malondialdehyde.

The amount of enzymes in N8720 leaf was higher than the Gonbad. With increasing drought stress (increasing concentration of PEG in water), the amount of produced enzymes in the leaf increased, so that the highest and lowest amounts of the above enzymes observed under drought stress at 250 g /l PEG and without stress, respectively.Interaction effects of cultivar and drought stress showed that the highest amount of enzymes, produced in the leaf, related to N8720 and drought with a concentration of 250 g /l PEG, respectively (Table 3).

Effect of drought stress on production of proline, catalase, ascorbate peroxidase, peroxidase, superoxide dismutase and malondialdehyde in root

Analysis of variance showed that wheat cultivar and drought stress had a significant effect on the production of

proline, catalase, ascorbate peroxidase, peroxidase, superoxide dismutase and malondialdehyde in root at 1% level.Their interactions were significant in the production of catalase, superoxide dismutase and malondialdehyde at 1% level.In addition, their interaction on proline and ascorbate peroxidase enzymes was significant at 5% level but had no significant effect on peroxidase enzyme production.The results show that the amounts of the enzymes in the N8720 were higher than the enzymes produced in the Gonbad. With increasing drought stress on wheat, the amounts of these enzymes increased in the roots and the highest and lowest amounts of these enzymes produced in wheat under drought stress with a concentration of 250 g/L PEG treatment and non-treatment, respectively.The effects of drought stress and wheat cultivars showed that the highest amount of enzymes produced in the root belonged to N8720 and drought stress at a concentration of 250 g / L PEG (Table 4).

Effect of drought stress on glucose, galactose, rhamnose xylose in leaf and root: Table 5 showed that wheat cultivars, drought stress and their interactions had a significant effect on glucose, galactose, rhamnose xylose in leaf and root at 1% level.The N8720 cultivar had a higher glucose, galactose, rhamnose and xylose content in the leaf and roots in comparison with Gonbad. With increasing drought stress, the amount of these compounds increased in leaf and root of wheat.The highest and lowest amounts of these compounds were in the leaf and roots treated with 250 g/l PEG and without treatment, respectively.Interaction effects of cultivar and drought showed that the highest and lowest amounts of these compounds were observed in N8720 treated with 250 g/L PEG and Gonbad without drought stress (Table 5 and 6).

Table 1. Analysis of variance and mean comparison for plant length, stem length, root length, fresh and dry weight traits

Treatments		Plant length (cm)	Stem length (cm)	Root length (cm)	Fresh weight (mg)	Dry weight (mg)
Cultivar	Gonbad	16.558 ± 0.668	10.929 ± 0.393	5.629 ± 0.300	429.444 ± 21.577	37.857 ± 2.281
	N8720	20.124 ± 0.971	13.368 ± 0.588	6.757 ± 0.400	618.444 ± 12.200	62.048 ± 4.910
	LSD value	0.5751	0.5285	0.2158	21.6520	2.5432
Stress(gram/Liter)	0	21.133 ± 1.031	13.658 ± 0.745	7.475 ± 0.309	575.167 ± 35.197	60.808 ± 7.350
	150	18.332 ± 0.860	12.358 ± 0.539	5.973 ± 0.323	527.000 ± 41.919	52.292 ± 5.809
	250	15.558 ± 0.575	10.428 ± 0.438	5.130 ± 0.164	469.667 ± 52.162	36.757 ± 3.297
	LSD value	0.7044	0.6473	0.2643	26.5180	3.1148
C × S	Gonbad×0	18.897 ± 0.133	12.103 ± 0.113	6.793 ± 0.023	501.333 ± 5.812	44.583 ± 1.156
	Gonbad ×150	16.470 ± 0.137	11.190 ± 0.104	5.280 ± 0.075	433.667 ± 6.692	39.393 ± 0.948
	Gonbad ×250	14.307 ± 0.219	9.493 ± 0.283	4.813 ± 0.064	353.333 ± 4.372	29.593 ± 1.501
	N8720×0	23.370 ± 0.538	15.213 ± 0.587	8.157 ± 0.107	649.000 ± 26.627	77.033 ± 2.347
	N8720×150	20.193 ± 0.462	13.527 ± 0.274	6.667 ± 0.191	620.333 ± 5.487	65.190 ± 1.218
	N8720×250	16.810 ± 0.199	11.363 ± 0.078	5.447 ± 0.174	586.000 ± 7.211	43.920 ± 0.885
	LSD value	0.9962	0.9155	0.3738	37.5020	4.4050
	C	**	**	**	**	**
	S	**	**	**	**	**
	C × S	*	ns	*	*	**
	CV (%)	3.05	4.23	3.39	4.02	4.95

*, **, ns Significant at *P*< 0.05, P< 0.01 and non-significant, respectively. LSD means least significant differences between means.

Table 2. Analysis of variance and mean comparison for chlorophyll a, b, a+b, a/b and carotenoid traits

Treatments		Chlorophyll a (µg/mL)	Chlorophyll b (µg/mL)	Chlorophyll a+b (µg/mL)	Chlorophyll a/b	Carotenoid (µg/mL)
Cultivar	Gonbad	4.551 ± 0.256	1.836 ± 0.119	6.387 ± 0.370	2.492 ± 0.053	1.396 ± 0.129
	N8720	6.201 ± 0.119	2.406 ± 0.088	8.607 ± 0.205	2.593 ± 0.053	2.073 ± 0.061
	LSD value	0.0995	0.0394	0.1279	0.0413	0.0690
Stress (gram/Liter)	0	5.893 ± 0.288	2.433 ± 0.078	8.327 ± 0.366	2.416 ± 0.043	2.040 ± 0.098
	150	5.575 ± 0.330	2.180 ± 0.168	7.755 ± 0.498	2.576 ± 0.049	1.760 ± 0.152
	250	4.660 ± 0.494	1.748 ± 0.138	6.408 ± 0.632	2.637 ± 0.075	1.403 ± 0.211
	LSD value	0.1218	0.0483	0.1566	0.0506	0.0845
C × S	Gonbad×0	5.253 ± 0.023	2.260 ± 0.017	7.513 ± 0.019	2.325 ± 0.025	1.827 ± 0.015
	Gonbad×150	4.840 ± 0.061	1.807 ± 0.038	6.647 ± 0.098	2.680 ± 0.029	1.423 ± 0.035
	Gonbad×250	3.560 ± 0.046	1.440 ± 0.015	5.000 ± 0.059	2.472 ± 0.020	0.937 ± 0.022
	N8720×0	6.533 ± 0.073	2.607 ± 0.015	9.140 ± 0.087	2.506 ± 0.015	2.253 ± 0.050
	N8720×150	6.310 ± 0.038	2.553 ± 0.012	8.863 ± 0.035	2.471 ± 0.022	2.097 ± 0.034
	N8720×250	5.760 ± 0.075	2.057 ± 0.024	7.817 ± 0.094	2.801 ± 0.026	1.870 ± 0.059
	LSD value	0.1723	0.0683	0.2215	0.0715	0.1195
	C	**	**	**	**	**
	S	**	**	**	**	**
	C × S	**	**	**	**	**
	CV (%)	1.8	1.8	1.66	1.58	3.87

*, **, ns Significant at *P*< 0.05, P< 0.01 and non-significant, respectively. LSD means least significant differences between means.

Table 3. Analysis of variance and mean comparison for proline, catalase, ascorbate peroxidase, superoxide dismutase and malondialdehyde traits in leaf

Treatments		Proline (mg/g fresh weight)	Catalase (U/mg of protein)	Ascorbate peroxidase (U/mg of protein)	Peroxidase (U/mg of protein)	Superoxide dismutase (U/mg of protein)	Malondialdehyde (mmol/gFW)
Cultivar	Gonbad	1.138 ± 0.193	228.889 ± 15.482	43.333 ± 6.322	237.556 ± 19.658	486.000 ± 40.189	230.333 ± 9.433
	N8720	1.317 ± 0.218	286.000 ± 24.899	51.667 ± 7.792	271.333 ± 25.119	554.222 ± 51.235	232.778 ± 15.462
	LSD value	0.0896	4.6567	2.5042	6.7525	16.3300	8.1200
Stress (gram/Liter)	0	0.457 ± 0.014	190.667 ± 4.856	25.667 ± 0.422	184.667 ± 3.602	374.167 ± 11.845	189.167 ± 6.188
	150	1.392 ± 0.086	252.000 ± 14.201	43.333 ± 3.106	241.333 ± 9.301	499.000 ± 13.658	232.167 ± 3.458
	250	1.833 ± 0.049	329.667 ± 19.653	73.500 ± 2.872	337.333 ± 11.453	687.167 ± 24.843	273.333 ± 5.426
	LSD value	0.1097	5.7033	3.0670	8.2701	20.0000	9.9449
C × S	Gonbad×0	0.437 ± 0.018	180.333 ± 2.603	25.667 ± 0.882	179.333 ± 1.764	356.000 ± 9.452	201.000 ± 6.245
	Gonbad×150	1.223 ± 0.058	220.333 ± 1.453	36.667 ± 1.202	221.000 ± 2.082	470.000 ± 5.859	227.000 ± 4.726
	Gonbad×250	1.753 ± 0.054	286.000 ± 4.619	67.667 ± 1.764	312.333 ± 1.453	632.000 ± 4.726	263.000 ± 6.083
	N8720×0	0.477 ± 0.018	201.000 ± 2.082	25.667 ± 0.333	190.000 ± 5.774	392.333 ± 16.796	177.333 ± 3.528
	N8720×150	1.560 ± 0.075	283.667 ± 1.856	50.000 ± 1.528	261.667 ± 3.844	528.000 ± 7.572	237.333 ± 3.283
	N8720×250	1.913 ± 0.052	373.333 ± 1.764	79.333 ± 2.028	362.333 ± 5.364	742.333 ± 4.485	283.667 ± 1.856
	LSD value	0.1552	8.0656	4.3374	11.6960	28.2840	14.0640
	C	**	**	**	**	**	ns
	S	**	**	**	**	**	**
	C × S	*	**	**	**	**	**
	CV (%)	7.1	1.76	5.13	2.58	3.05	3.41

*, **, ns Significant at *P*< 0.05, P< 0.01 and non-significant, respectively. LSD means least significant differences between means.

Table 4. Analysis of variance and mean comparison for glucose, galactose, rhamnosus and xylose traits in leaf

Treatments		Glucose (mg/gFW)	Galactose (mg/gFW)	Rhamnosus (mg/gFW)	Xylose (mg/gFW)
Cultivar	Gonbad	73.444 ± 0.915	126.000 ± 1.732	71.000 ± 1.732	43.000 ± 0.866
	N8720	77.778 ± 1.985	135.333 ± 4.069	80.333 ± 4.069	53.778 ± 4.870
	LSD value	1.3907	3.0622	3.0622	1.6419
Stress (gram/Liter)	0	70.833 ± 0.307	121.333 ± 0.667	66.333 ± 0.667	40.667 ± 0.333
	150	75.500 ± 1.232	130.333 ± 2.704	75.333 ± 2.704	45.167 ± 1.352
	250	80.500 ± 1.821	140.333 ± 4.014	85.333 ± 4.014	59.333 ± 6.020
	LSD value	1.7033	3.7504	3.7504	2.0110
C × S	Gonbad×0	70.667 ± 0.333	121.333 ± 0.667	66.333 ± 0.667	40.667 ± 0.333
	Gonbad×150	73.000 ± 0.577	124.667 ± 0.667	69.667 ± 0.667	42.333 ± 0.333
	Gonbad×250	76.667 ± 0.667	132.000 ± 2.309	77.000 ± 2.309	46.000 ± 1.155
	N8720×0	71.000 ± 0.577	121.333 ± 1.333	66.333 ± 1.333	40.667 ± 0.667
	N8720×150	78.000 ± 1.000	136.000 ± 2.000	81.000 ± 2.000	48.000 ± 1.000
	N8720×250	84.333 ± 1.202	148.667 ± 2.404	93.667 ± 2.404	72.667 ± 1.453
	LSD value	2.4088	5.3039	5.3039	2.8439
	C	**	**	**	**
	S	**	**	**	**
	C × S	**	**	**	**
	CV (%)	1.79	2.28	3.94	3.3

*, **, ns Significant at *P*< 0.05, P< 0.01 and non-significant, respectively. LSD means least significant differences between means.

Table (5). Analysis of variance and mean comparison for proline, catalase, ascorbate peroxidase, superoxide dismutase and malondialdehyde traits in root

Treatments		Proline (mg/g fresh weight)	Catalase (U/mg of protein)	Ascorbate peroxidase (U/mg of protein)	Peroxidase (U/mg of protein)	Superoxide dismutases (U/mg of protein)	Malondialdehyde (mmol/gFW)
Cultivar	Gonbad	1.094 ± 0.183	213.000 ± 14.033	37.222 ± 5.552	234.667 ± 18.741	242.111 ± 19.428	533.667 ± 9.792
	N8720	1.223 ± 0.203	262.333 ± 22.221	43.556 ± 6.254	258.444 ± 18.132	276.778 ± 24.570	536.333 ± 16.091
	LSD value	0.0683	7.8297	3.5580	9.0225	6.7699	7.7997
Stress (gram/Liter)	0	0.430 ± 0.011	179.167 ± 4.643	22.667 ± 0.667	193.167 ± 5.582	190.167 ± 3.535	490.833 ± 6.091
	150	1.315 ± 0.065	230.833 ± 12.424	36.500 ± 2.952	230.167 ± 6.949	247.667 ± 9.397	535.333 ± 3.756
	250	1.732 ± 0.035	303.000 ± 17.301	62.000 ± 2.338	316.333 ± 6.307	340.500 ± 11.514	578.833 ± 5.192
	LSD value	0.0837	9.5893	4.3576	11.0500	8.2914	9.5526
C × S	Gonbad×0	0.420 ± 0.017	170.333 ± 4.177	22.667 ± 1.202	182.000 ± 1.528	183.333 ± 2.333	502.667 ± 4.807
	Gonbad×150	1.190 ± 0.040	203.667 ± 4.177	30.333 ± 2.028	215.000 ± 1.732	227.667 ± 3.283	529.667 ± 3.756
	Gonbad×250	1.673 ± 0.039	265.000 ± 4.726	58.667 ± 2.186	307.000 ± 3.606	315.333 ± 0.882	568.667 ± 3.528
	N8720×0	0.440 ± 0.015	188.000 ± 3.512	22.667 ± 0.882	204.333 ± 5.364	197.000 ± 3.215	479.000 ± 4.726
	N8720×150	1.440 ± 0.064	258.000 ± 4.041	42.667 ± 1.202	245.333 ± 2.906	267.667 ± 5.548	541.000 ± 4.933
	N8720×250	1.790 ± 0.032	341.000 ± 5.508	65.333 ± 3.383	325.667 ± 9.939	365.667 ± 5.364	589.000 ± 4.359
	LSD value	0.1183	13.5610	6.1626	15.6270	11.7260	13.5090
	C	**	**	**	**	**	ns
	S	**	**	**	**	**	**
	C × S	*	**	*	ns	**	**
	CV (%)	5.73	3.2	8.57	3.56	2.54	1.41

*, **, ns Significant at *P*< 0.05, P< 0.01 and non-significant, respectively. LSD means least significant differences between means.

Table 6. Analysis of variance and mean comparison for glucose, galactose, rhamnose and xylose traits in root

Treatments		Glucose (mg/gFW)	Galactose (mg/gFW)	Rhamnosus (mg/gFW)	Xylose (mg/gFW)
Cultivar	Gonbad	33.000 ± 0.866	48.000 ± 0.866	38.000 ± 0.866	25.000 ± 0.866
	N8720	37.667 ± 2.034	52.667 ± 2.034	42.667 ± 2.034	29.667 ± 2.034
	LSD value	1.5311	1.5311	1.5311	1.5311
Stress (gram/Liter)	0	30.667 ± 0.333	45.667 ± 0.333	35.667 ± 0.333	22.667 ± 0.333
	150	35.167 ± 1.352	50.167 ± 1.352	40.167 ± 1.352	27.167 ± 1.352
	250	40.167 ± 2.007	55.167 ± 2.007	45.167 ± 2.007	32.167 ± 2.007
	LSD value	1.8752	1.8752	1.8752	1.8752
C × S	Gonbad×0	30.667 ± 0.333	45.667 ± 0.333	35.667 ± 0.333	22.667 ± 0.333
	Gonbad×150	32.333 ± 0.333	47.333 ± 0.333	37.333 ± 0.333	24.333 ± 0.333
	Gonbad×250	36.000 ± 1.155	51.000 ± 1.155	41.000 ± 1.155	28.000 ± 1.155
	N8720×0	30.667 ± 0.667	45.667 ± 0.667	35.667 ± 0.667	22.667 ± 0.667
	N8720×150	38.000 ± 1.000	53.000 ± 1.000	43.000 ± 1.000	30.000 ± 1.000
	N8720×250	44.333 ± 1.202	59.333 ± 1.202	49.333 ± 1.202	36.333 ± 1.202
	LSD value	2.6520	2.6520	2.6520	2.6520
	C	**	**	**	**
	S	**	**	**	**
	C × S	**	**	**	**
	CV (%)	4.21	2.96	3.69	5.45

*, **, ns Significant at *P*< 0.05, P< 0.01 and non-significant, respectively. LSD means least significant differences between means.

DISCUSSION

Since moisture plays an important role in the plant, reducing moisture absorption has adverse effects on plant physiological properties.Studies have shown that drought stress, plant pigments such as carotenoids, chlorophyll a, chlorophyll b, total chlorophyll concentration significantly decreased, which was lower in pigment content in N8720 compare to the Gonbad.

Previous studies on plants such as beans, chickpea and wheat showed that drought stress reduced the amount of chlorophyll a and b, which was consistent with the results of this experiment (Mathobo et al., 2017; Mafakheri et al., 2010; Lonbani and Arzani, 2011).Total chlorophyll concentration decreases with increasing drought stress and increasing the concentration of PEG (Pratap& Sharma, 2010; Guoet al., 2013).Chlorophyll b decreased compared to chlorophyll a due to drought stress, which increased the chlorophyll b / a ratio, which was consistent with the results of Ashraf et al. (1994).Navabpour et al. (1394) reported that increasing amounts of carotenoids under drought stress conditions were anticipated due to their role in the antioxidant defense system to protect photosynthetic pigments (chlorophyll). The significant increase in the amounts of carotenoids in the grain filling stage as well as its increase under drought stress indicates its role in regulating the amounts of active oxygen radicals.Sharifi and Mohammad Khani (2016) stated that long-term drought stress reduced total chlorophyll content, which was higher in susceptible wheat cultivars than drought resistant cultivars.

Khayyatnezhadet al. (2011) reported that drought-tolerant cultivars had high levels of chlorophyll content than drought tolerant cultivars due to increased levels of superoxide dismutase enzymes in drought tolerant cultivars.Shivakrishna et al. (2018) showed that the concentration of chlorophyll a and b in almonds decreased with increasing concentration of PEG.The reduction of chlorophyll content in the plant under drought stress is due to the production of reactive oxygen species, such as O2 and H2O2, Which can lead to lipid peroxidation and ultimately chlorophyll degradation and as a result, the plant leaves become yellow.Plants have several mechanisms for controlling drought stress, one of which is the enzymatic defense system.Due to drought stress, the amount of proline, catalase, ascorbate peroxidase, peroxidase, superoxide dismutase and malondialdehyde in wheat roots and leaves has increased.Drought stress leads to the production of free oxygen radicals in wheat and the plant enhances the enzymes to cope with oxidative stress.The accumulation of proline in plants under stress is due to the proline synthesis and inactivation of its degradation. Proline content in stress conditions protects cell membranes, proteins, cytoplasmic enzymes, and inhibits reactive oxygen species and removes free radicals (Gorbanli et al., 2013; Liang et al., 2013).Allah Moradi et al. (2013) showed that drought stress significantly increases the amount of proline in lentils.Catalase and ascorbate peroxidase play an important role in converting hydrogen peroxide into water to prevent the toxic effects of hydrogen peroxide.The enzyme peroxidase used to collect reactive oxygen species in order to prevent excessive damage to the active plasmid membrane. Superoxide dismutase enzyme regulates the concentration of hydrogen peroxide and superoxide in the cell and is an important factor in the plant's defense system against antioxidant stress (Sharma et al., 2012).Hassanpour and Niknam (2014) stated that drought stress increased the activity of ascorbite peroxidase, superoxide dismutase and peroxidase.Malick et al. (2011) observed that proline increased in some wheat genotypes due to drought stress, which was consistent with the results of this study.

Amini et al. (1393) stated that the amount of malondialdehyde and proline in olive increased with the increase of drought stress.

The amounts of antioxidant enzymes produced in wheat N8720 were higher than the Gonbad cultivar.Hojjati et al. (2011) reported that high levels of enzymes in plants indicate increased tolerance to drought stress.SeyyedEbrahimi et al. (1394) stated that the activity of superoxide dismutase and peroxidase enzymes in drought resistant cultivars was higher than susceptible cultivars.One of the other mechanisms to deal with drought stress is osmotic regulation, which, through the accumulation of soluble materials in cells, can lead to the preservation of the cells' turgescence and its dependent processes during moisture stress; so, due to drought stress the amount of glucose, galactose, rhamnose, xylose in leaf

and root of wheat increased.Increased soluble sugars in wheat under drought conditions were reported (Johari-Pireivatlou, 2010). Under drought stress conditions, osmotic regulators can increase water absorption through plant cells (Hassanpour et al., 2013; Ahsngar et al., 2013).

Borujerdnia et al. (1395) concluded that drought stress leads to increased soluble sugars, glucose and proline in beans. Lokhande et al. (2010) reported that under drought stress, organic molecules with lower molecular weight, such as soluble sugars, proline and proteins, act as osmotic regulators in the plants' root and aerial organs.AfsharMohammadian et al. (1395) stated that drought stress increased the amounts of soluble sugars such as glucose, galactose, rhamnose and xylose in *Menthapulegium*and the activity of catalase and peroxidase enzymes increased in root and aerial organs.

Wheat responds to drought stress through change in enzymatic and non-enzymatic activity, which ultimately reveals these changes in morphological characteristics.Due to drought stress, in order to reduce the cellular water potential, a large number of photosynthetic compounds are used to produce osmotic regulatory compounds.These compounds are not cost-effective for the plant and the plant compensates this cost by reducing the morphological characteristics including plant height, stem height, root length, fresh and dry weight.

The researchers expressed the cause of the decrease in stem and plant height related to reduced cell division and vegetative growth due to drought stress (Zabet and Hosseinzadeh, 2011).

Increasing dry biomass production in plants under favorable irrigation conditions can be due to the greater leaf area, which by generating an efficient physiological source for the further use of light and photosynthesis, increases the production of dry biomass (Lak et al., 2007).Chegah et al. (2013) argued that moisture deficiency reduced root growth, chlorophyll a and b, and total chlorophyll but increased the soluble sugars content. Other researchers said that increased drought stress reduced root length in chickpea and cane (Mafakheri et al., 2011, Jangpromma et al., 2012).Stem length, root length, fresh and dry weight decreased with increasing drought stress (increasing the concentration of PEG) in *Trachyspermumammi* and *Foeniculumvulgare* (Fakheri et al., 1396). The fresh weight of the plant at all levels of drought compared to the control showed a significant decrease, which may be due to a decrease in cell division and its growth, resulting in low turgescence pressure (Mohammadi et al., 1394).The use of sunflower resistant varieties increased root length (Manivannan et al., 2014). Plant and stem height in corn under moisture deficit conditions is significantly reduced (Khan et al., 2015).This study showed that one of the methods of coping with drought stress is using resistant cultivars that N8720 increased the plant height, stem height, root length, fresh and dry weight of wheat compared to Gonbad cultivar.The total weight of the wheat plant and plant height decreased with drought stress and selection of drought-tolerant varieties was very effective in increasing the height and weight of the whole-wheat plant compared to sensitive cultivars (Khayyatnejad et al., 2011).Under drought stress conditions, root length in drought-tolerant genotype increased and it decreased in drought-sensitive genotypes (Bibi et al., 2009).Previous studies to cope with drought stress showed that Manage tools such as the using suitable wheat cultivars (Asifa et al., 2015), the using growth regulators such as cyclocell (Sedaghat and Imam, 1395) and the application of Zn spray on wheat leaves (Hera et al., 2018) increased plant resistance to stress.

CONCLUSION

Plants use various mechanisms to reduce the negative effects of drought stress and respond to stress through morphological, physiological and metabolic changes.Due to drought stress, characteristics including plant height, stem height, root length, fresh and dry weight, carotenoid content, chlorophyll a and b, and their total reduced in the plant, but the amounts of proline, catalase, ascorbate peroxidase, peroxidase, superoxide dismutase, malondialdehyde and soluble sugars such as glucose, galactose, rhamnose and xylose in wheat increased.The enhancement in the amounts of photosynthetic pigments, oxidant enzymes, proline, malondialdehyde and soluble sugars in N8720 cultivar compared to the Gonbad cultivar related to increased expression of resistance genes in N8720, Which increases the resistance of wheat to drought stress.

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