

RESEARCH PAPER

Water stress affect water relations, photosynthesis and oxidative defense mechanism in wheat (*Triticum aestivum* L.)

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Abstract

Water stress is a major obstacle to agricultural production, significantly impacting both yield and quality. During the 2017–18 crop year in Shahriar region near Tehran, Iran, known for its dry and cold climate, a study was conducted to examine the influence of different irrigation levels on the quantitative and qualitative characteristics of various wheat cultivars. The experiment involved three irrigation levels: normal irrigation (control), withholding irrigation at the flowering stage, and withholding irrigation at the seed-filling stage as primary factors, with 21 different cultivars as secondary factors. The analysis showed that irrigation, cultivar type, and their interactions had a significant effect on grain yield, proline, total chlorophyll, carbohydrate content in the first and second internodes during flowering and ripening, as well as on SOD, CAT, GPX, MDA, DT, and D-OH-dG levels at a one percent significance level. Withholding irrigation at the flowering stage had a more severe impact compared to the seed-filling stage. The Rakhshan and Sivand cultivars yielded the highest and lowest grain yields under normal irrigation conditions, while the drought-tolerant Ofoh cultivar showed lower yields when irrigation was withheld at the flowering stage. Water stress led to increased proline levels and higher levels of SOD, CAT, MDA, DT, and D-OH-dG in wheat cultivars. The Rakhshan and Sivand cultivars were identified as drought-tolerant in this region.

Keywords

Wheat, Irrigation, Proline, Antioxidant Enzyme, Biomarker Destruction

Introduction

Drought is one of the non-living environmental stresses that affect the quantity and quality of plant yield through changes in growth and metabolic and physiological activities (Islam et al. 2011). One basic solution to overcoming the problems caused by drought is selecting tolerant cultivars and modifying of compatible genotypes (Eyni-Nargeseh et al. 2020). Wheat (*Triticum aestivum* L.) is one of the most important and strategic crops in the world (Ghasemi and Farshad Far 2015). Wheat requires

variety amounts of water at different growth stages (Awan et al. 2017). The effects of drought on wheat plants can be observed through specific symptoms, such as reduced plant height, leaf area, seed yield, and biomass (Omidvari et al. 2020). Studies have shown significant differences in yield and yield components between drought-tolerant and sensitive wheat cultivars, particularly in the amount of proline, which plays a positive and crucial role in osmotic regulation for maintaining yield (Roozrok 2020). Drought also impacts the photosynthetic system, reducing chlorophyll, levels and stability of cell membranes, as well

as plant respiration by up to 90% (Ahmed et al. 2019). Water scarcity affects cell growth stomatal regulation, respiration, photosynthesis, and transpiration in plants (Wang et al. 2017). Enzymatic processes crucial for plant growth are also affected by water potential (Shekoofa and Sinclair 2018). Plants unable to avoid environmental stresses, rely on mechanisms to detect and respond to tensions (Liu and Hwang 2015). Osmotic regulation is a key adaptation to water deficit stress, with soluble sugars acting as osmotic regulators, cell membranes stabilizers, and maintaining cell turgor, proline accumulates rapidly under stress conditions compared to other metabolites (Lesk et al. 2016). Drought-tolerant plants exhibit increased water retention and higher levels of osmotic regulators like soluble sugars and proline to withstand drought stress (Abid 2016). In white beans, drought stress leads to decreased photosynthesis rates and chlorophyll content. While increasing proline content and ionic leakage. Researchers noted that the accelerated transfer of photosynthetic reserves during leaf aging under drought stress conditions post pollination; contribution to providing seed weight during the stress in the seed filling stage (Fokar et al. 2016). A significant ant biochemical change in plants under drought stress is the production of reactive oxygen species (ROS), which can reduce respiratory activities in mitochondria and carbon dioxide fixation in chloroplasts; increase electrolyte leakage and decrease yield (Gill and Tuteja 2010). Reduced antioxidant activity may result in hydrogen peroxide accumulation and reduce d activity of Calvin cycle enzymes impacting plant growth (Rodrigues et al. 2013). Plants with High levels of antioxidant enzymes that scavenge free radicals exhibit increased tolerance to environmental stress (Karkanis et al. 2011). For Alvand and Zarrin wheat cultivars, decreased antioxidant activity limited defense mechanisms and increased lipid peroxidation (Alimohamadi et al. 2017). Given the strategic importance of wheat crops, this research aimed to evaluate the biochemical characteristics of different wheat cultivars in Shahriar region under normal irrigation and drought conditions during a specific developmental period.

Materials and methods

Materials and experiment treatments

This study aimed to evaluate the effect of water deficit stress on the yield of wheat cultivars in Shahriar region

and to identify tolerant and sensitive varieties. The field experiment took place during the 2017–2018 crop year. The research utilized a complete randomized block experimental design with a split-plot arrangement and three replications. The main plots were assigned three levels of water deficit: conventional irrigation (S0), irrigation withheld at the flowering stage (S1), and irrigation withheld at the seed filling stage. The sub-factors included 21 wheat cultivars: Chamran (hot and dry climate, south, V1), Stare (hot and dry climate, south, V2), Sirvan (hot and humid, V3), Sivand (hot and humid, V4), Arg (hot and humid, V5), Parsi (hot and humid, V6), Barat (hot and humid, V7), Narin (hot and humid, V8), Heidari (hot and humid, V9), Ofoq (hot and humid, V10), Rakhshan (hot and humid, V11), Baharan (hot and humid, V12), Sepahan (moderate, V13), Aflak (hot and dry-south, V14), Miham (cold, V15), Zaree (Caspian Sea Margin-cold, V16), Gonbad (Caspian Sea margin-moderate, V17), Erum (cold, V18), Sisson (cold, V19), Gascogen (cold, V20), and Mehregan (hot and dry-south, V21).

Growth parameters

The cultivars used in initial experiment were chosen based on the climatic conditions of the region Cultivation was done in November of 2017 with a Jang API cereal seeding machine made in South Korea. Each experimental plot had 6 rows, was 6 meters in length, with a 20 cm gap between rows and a 5 cm gap between seeds. Irrigation treatments were applied based on the growth period of wheat in the flowering and ripening stages during in the growing stage, broadleaf weeds were destroyed with the use of the 2, 4-D herbicide. At the physiological maturity stage and after stress, the following traits were measured in the lab. The grain yield of each plot per square meter was calculated in Kg. Ha⁻¹. Total chlorophyll was measured with a chlorophyll meter on the leaf with a SPAD unit.

Enzyme activities

Superoxide dismutase enzyme (SOD) was assayed according to Misra and Fridovich 1972 (Misra and Fridovich 1972). Catalase (CAT) and glutathione peroxidase (GPX) enzymes were estimated using the Paglia method with a spectrophotometer (Model u – Shimadzu – u - 100 z) and the protein amount was determinedmg.mlit-1 Catalase en-

Table 1. The amount of rainfall in the crop year 2017–2018 (mm).

September	August	July	June	May	April	March	February	January	December	November	October
0	0	0	0	0	1.7	10	55	44	46	28	12

Table 2. The temperature of the crop year 2017–2018 (C).

	September	August	July	June	May	April	March	February	January	December	November	October
Max	21	37	29	24	21	18	11.7	4	3.2	4.6	6.8	16
Min	12	16	14	11.6	11	9	3	-2.1	-1.3	0	2	5.2

zyme activity unit is defined as the conversion rate of oxygenated water in 1 minute during a first-order reaction (Paglia 1997). Malon Di Aldehyde (MDA) and Di Tyrosine (DT) assays were performed according to Steven (1978) using HPLC chromatography and the MDA peak was identified in the spectrophotometer with a visible detector at a wavelength of 532 nm measured based on the area under the curve of the peak (Steven and Joseph 1978). Di hydroxyl guanosine assay was conducted according to Bogdanov and Bical (1999) by solution absorption in the spectrophotometer at wavelengths of 280 and 260 nm, respectively and analyzed for 8-OH-dG after centrifugation at 300 rpm for 15 minutes (Bogdanov and Bical 1999). To determine the difference in soluble carbohydrates of the first internode (peduncle) and the second (penultimate) in the flowering and ripening stages of wheat cultivars, from the phenol-sulfuric acid method was used (AOAC 1995). Leaf proline was measured in the laboratory following the method of Irigoyen et al. 1993. Initially, 0.5 grams of leaves were crushed in 5 ml of 0.95 ethanol. Finally, the absorption intensity was read with a spectrophotometer at a wavelength of 515 nm and a standard curve was prepared with concentrations of 0–0.4 micrograms of proline per gram (Tatar et al. 2016).

Fernandez drought tolerance coefficient

Using this formula, the varieties were evaluated based on the Fernandez Drought Tolerance Index.

$$Y = (Y_p + Y_s) / 2$$

$$Ti_{(a)} = \{Yp_{(a)} \times Ys_{(a)}\} / (Y)^2$$

Y = Average yield of all varieties in all conditions

Y_p = Yield index of all varieties under normal water conditions

Y_s = Average yield of all varieties under stress conditions

$Yp_{(a)}$ = Average yield of variety a in normal conditions

$Ys_{(a)}$ = Average yield of variety a under stress conditions

$Ti_{(a)}$ = Fernandez drought tolerance coefficient for variety A

Statistical analysis

At the end, the amount of soluble sugars was determined in milligrams per gram of dry weight. The data were subjected to analysis of variance using Mstat-c computer software. Duncan's multiple range tests ($p < 0.05$) was applied for mean separation when F values. The graph was drawn using Excel software.

Abbreviations

S_0	Normal irrigation
S_1	Cut irrigation at the flowering stage
S_2	Cut irrigation at the seed filling stage
%	Percent
ANOVA	Analysis of variance
Chl	Total Chlorophyll
mg.gFw	Milligrams per gram fresh weight
µg.g	microgram per gram
Kg.ha	Kilograms per hector
ns	not significant
SOD	EC 1.15.1.1 Superoxide dismutase
CAT	EC 1.11.1.6 Catalase
GPX	EC 1.11.1.9 Glutathione peroxidase
MDA	Malondialdehyde
DT	Dityrosine
D-OH-dG	Dihydroxyguanosine
µmol.mg ⁻¹	protein nano mol per Milligram protein
U.mg protein	Unit per Milligram protein

Results and discussion

The results of the variance analysis table showed the simple effects of irrigation and variety and the mutual effects of the treatments had a significant effect on Grain yield, proline, total chlorophyll, the difference of soluble carbohydrates of the first and second internodes in the flowering and ripening stages, and antioxidant enzymes SOD, CAT, GPX and degradation biomarkers MDA, DT,

Table 3. Composite analysis on the traits of Shariar region.

S.O.V	df	Grain yield	Proline	Total Chlorophyll	M.s Differences in soluble carbohydrates of the first intermediate at the flowering and ripening stages	Differences in soluble carbohydrates of the second intermediate at the flowering and ripening stages.	SOD	CAT	GPX	M.D.A	Di-Ty	D-OH-dG
Replication	2	ns	ns	ns	*	*	ns	ns	ns	ns	ns	ns
Irrigation	2	**	**	**	**	**	**	**	**	**	**	*
Error (a)	4		0.5210	0.0258	197.4200	28.6000	0.0086	0.0628	0.00297	0.0079	0.089	4.82
Varity (Ir*V)	20	**	**	**	**	**	**	**	**	**	**	**
Error (b)	40	**	**	**	**	**	**	**	**	**	**	**
(C.v)	120		0.314	0.0042	249.04	45.63	0.0051	0.0421	0.00182	0.0051	0.048	2.46
			5.92	8.09	7.31	5.17	4.89	5.14	5.63	6.82	4.97	6.37

ns, ** and * are not significant, significant at 1% and 5% probability level respectively.

D-OH-dG and the differences were significant at the level of one percent ($P < 0.01$).

Grain yield

The average comparison results showed that withholding irrigation reduced grain yield. There were no statistically significant differences in grain yield when irrigation was withheld during the flowering and seed filling stages. The highest seed yield observed in Rakhshan cultivar treatment with regular irrigation ($10857.2 \text{ kg.ha}^{-1}$), which was not significantly different from the Heydari variety treatment, both falling in to statistical class A. The lowest seed yield recorded in the Ofogh cultivar treatment with irrigation cut during the flowering stage ($1467.9 \text{ kg.ha}^{-1}$) (Fig. 1). Heidari and Rakhshan cultivars, which were more tolerant to drought conditions, experienced less reduction in yield. These genotypes showed less reduction in photosynthetic assimilate production during photosynthesis, resulting in less reduction in 1000 seed weight and grain yield (Ohe et al. 2015). Water deficit stress is the main factor causing growth and yield reduction in plant in arid and semi-arid regions, leading to plant stimulation and defense responses at various molecular, cellular and physiological levels (Bhatt and Roa 2017). The decrease in biomass and dry weight of the whole plant under drought stress is attributed to reduced plant growth, decreased photosynthesis intensity, and premature leaf aging (Yang

et al. 2011). Drought stress negatively impacts the grain yield of wheat cultivars after flowering. Researchers have shown that the genetic potential of high-yielding wheat cultivars is maximized in the absence of stress, particularly from flowering to maturity, increasing the possibility of achieving higher grain yield. Drought stress reduces seed yield by affecting photosynthetic level. The timing intensity and duration, of stress significantly affect seed the performance (Liang et al. 2013).

Proline

The comparison of mean interaction effects showed that the interruption of irrigation caused an increase in proline in different cultivars. The greatest effect was seen when irrigation was withheld during the flowering stage, resulting in an increase in this amino acid. The highest amount of proline was found in the Ofogh cultivar when irrigation was withheld at the flowering stage ($0.994 \mu\text{g.g}$). The lowest proline levels were observed in the Rakhshan cultivar with regular irrigation of treatment at a rate of $0.416 \mu\text{g.g}$ (Fig. 2).

It appears that tolerant genotypes (such as Heidari, Rakhshan, etc.) were better able to withstand stress conditions by upregulating certain genes, as demonstrated in this study. The accumulation of proline is considered as an indicator of drought tolerance. Drought stress led to higher levels of soluble proline in tolerant genotypes compared

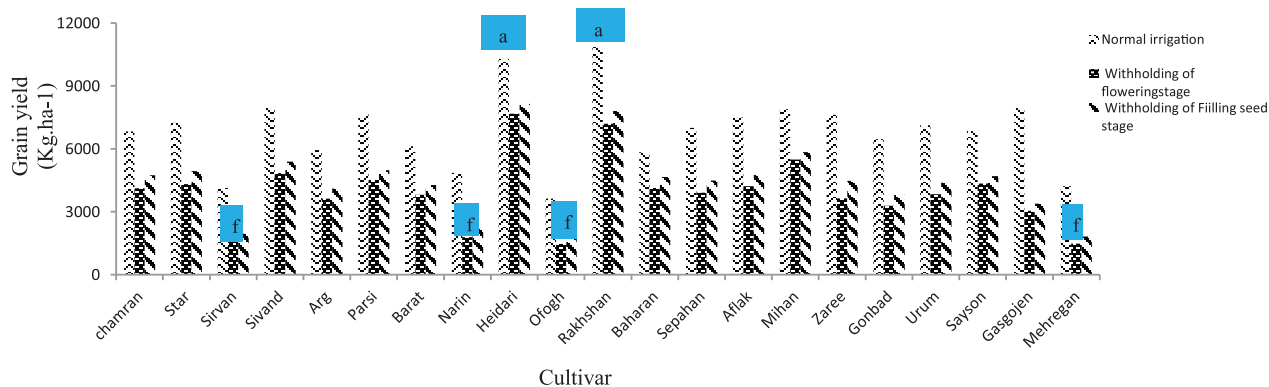


Figure 1. Interaction effects of cut irrigation and cultivars on grain yield of wheat.

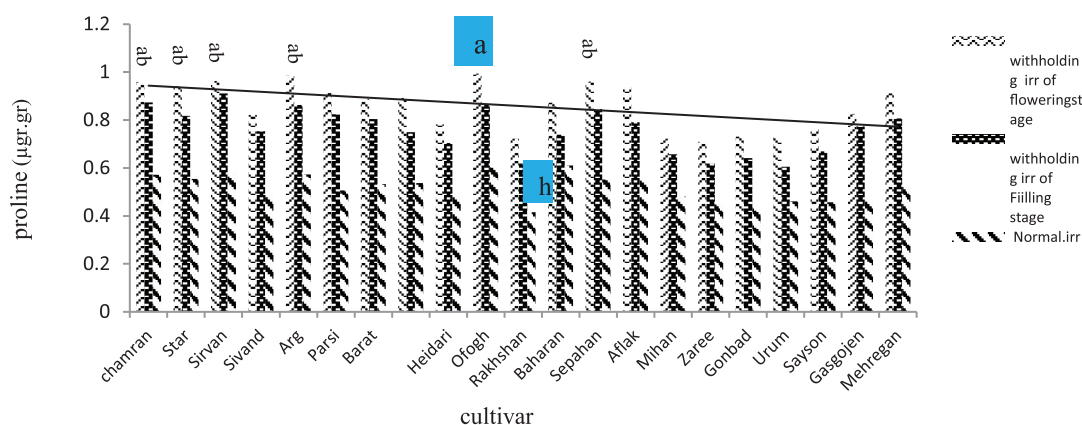


Figure 2. Interaction effects of cut irrigation and cultivars on proline.

to sensitive ones. The impact of drought stress on proteins varies depending on plant species and tissue (Jdey et al. 2014). Increased proline content under stress conditions helps protect the cell membrane, proteins, cytoplasmic enzymes and inhibits reactive oxygen species while removing free radicals (Reddy et al. 2004). In this study, the Ofogh cultivar, which had less tolerance to stress conditions, produced the highest amount of proline to preserve its structure but did not achieve significant yield. Tolerant cultivars like Rakhshan were able to maintain cell structure, produce more photosynthetic materials, and transfer nutrients from stem to seed, resulting in higher seed weight and yield when in irrigation was interrupted during flowering stage at the end of the growing season.

Total chlorophyll

The results of the analysis of variance table showed that the total chlorophyll of wheat leaves was affected by the main effects of irrigation, variety and the interaction effects of the treatments, with statistically significant at the 1% level (Table 1). The highest total chlorophyll content of wheat leaves was obtained from the normal irrigation and Zaree cultivar (52.607 SPAD) while the lowest was from the Ofogh cultivar and withholding irrigation at the seed filling stage (29.812 SPAD) treatments.

During periods of drought stress, a reduction in leaf chlorophyll content leads to a decrease in the photosynthesis rate (Chaves et al. 2014). Researchers have reported a positive and significant relationship between leaf photosynthesis rate and leaf chlorophyll concentration (Alberte and Troner 2017). The results of this research showed that chlorophyll decreased more during stress in the seed filling period compared to stress in the flowering period treatments. This could be attributed to the reduction in leaf thickness. Resources being redirected back to the seed. As the plant approaches the final growth stage, ATP used for photosynthesis and leaf maintenance is redirected to grain filling. This is considered one of the key factors contributing to the decreasing chlorophyll concentration during drought stress. Other factors include a decrease in membrane protein concentration in dry conditions

(Ashraf et al. 2014), as well as increase in the activity of Chlorophyllase and Peroxidase enzymes (Ezzat Ahmadi et al. 2009), which also contribute to the reduction in chlorophyll concentration during drought stress.

Differences in soluble carbohydrates of the first intermediate at the flowering and ripening stages

The highest amount of soluble carbohydrates in the first internode in the flowering and physiological ripening stage was found in the usual irrigation and Sivand variety treatment (31.87 mg.gFw⁻¹). The lowest amount of difference in soluble carbohydrates in the first internode during the flowering and physiological ripening stages was observed in the treatment with withholding irrigation in the seed filling stage and the Baharan cultivar (9.28 mg.gFw⁻¹) (Fig. 3).

Under drought stress condition, the rate of reserve utilization for seed filling increased from 28% to 38% moisture stress had an adverse effect on the of absorption of wheat material in the stage after flowering. Therefore, the yield has a relatively dependent on moisture access and the genotypes behavior in transferring reserves in aerial organs during the seed filling stage. Reconditions of water scarcity.

There was Rapid decline in photosynthesis after flowering; leading to a limitation in the share allocation of current assimilates to the grain. This resulted in an increase, in the share of seed dry matter obtained from stem reserves (Ma et al. 2013). On average, genotypes under control and drought stress conditions showed that the maximum weight between nodes was reached in 7 to 21 days after flowering. Endosperm cells begin filling approximately two weeks after flowering. Before the activation of strong reservoirs of photosynthetic materials, resulting in an accumulation of excess current photosynthetic materials, particularly in the leaves of the stem (Azhand et al. 2015). Researchers noted that the maximum weight of the barley stem was achieved between 7 and 20 days after flowering. Followed by a decline due to reduced current photosynthesis (from leaf senescence) and an increase in the transfer rate of stored materials from the stem to the seed (Ehdaie and Waines 2011).

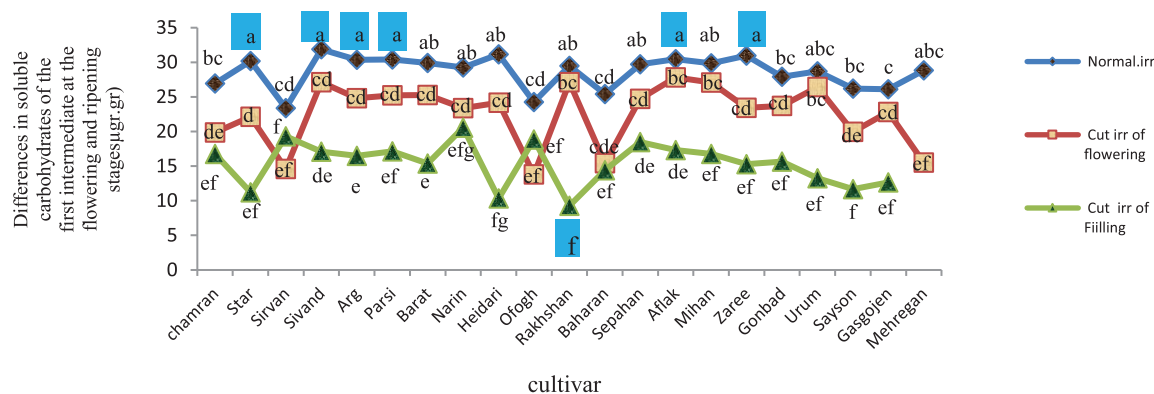


Figure 3. Interaction effects of cut irrigation and cultivars on differences in soluble carbohydrates of the first intermediate at the flowering and ripening stages.

Differences in soluble carbohydrates in the second internode at the flowering and ripening stages

The Comparison of mean interaction effects revealed that the highest difference in soluble carbohydrates of the second internode at the flowering and physiological ripening stages was observed in from the usual irrigation and Sivand cultivar treatment (60.134 mg.gFw⁻¹), which was not significantly different from the (V11*S0) and (V9*S0) treatments. The lowest difference in soluble carbohydrates of the second internode at the flowering and physiological ripening stage was found in the Ofogh variety either withholding irrigation at the seed filling stage treatment (12.894 mg.gFw⁻¹).

Storage and re-transmission were primarily associated related with the lower internodes under water and drought conditions, with the penultimate internodes and inflorescence tail following closely behind (Scofield et al. 2009). The penultimate internodes and inflorescence tail were found to have the highest carbohydrate storage in wheat (Kumar et al. 2006). Efficiency of conversion and ability to transfer storage materials to seeds depended on the Sink capacity, variety and environmental conditions (Ahmadi et al. 2009). The capacity of the sink was identified as a crucial factoring the distribution of photosynthetic materials in cereals (Li-ang et al. 2013). Researchers have noted that increasing the ratio of Sink to source; does not impact the amount of carbohydrates-transfer in wheat (Blum et al. 2011). The capacity of the Sink plays a significant role in materials redistribution (Ehdaie and Waines 2011). Blum (2005) suggested that the source-to- Sink ratio affecting retransmission with high and low ratios increasing and decreasing retransmission, respectively (Jubany-Mari et al. 2010).

Superoxide dismutase antioxidant enzyme (SOD)

The results of analysis of variance indicated that irrigation, cultivars and their interaction had a significant effect on the activity of superoxide dismutase enzyme at the 1% level ($P < 0.01$). Based on the results, the cessation of irrigation was accompanied by an increase in the activity of the superoxide dismutase enzyme compared to the control. The results of mean comparison showed that drought

stress increased the activity of superoxide dismutase enzyme in different cultivars. Withholding irrigation at the flowering stage had the greatest effect on the increase of this enzyme. The highest and lowest SOD enzyme levels were obtained from withholding irrigation at the flowering stage and Gosgogen cultivar (19.06 U.mg protein) and normal irrigation with the Sirvan variety (7.06 U.mg protein) treatment, respectively (Fig. 4).

When plant are exposed to stress, a large amount of reactive oxygen species is produced. In many plants, the enzyme system is activated to destroy these radicals (Khan and Ashraf 2008). There is a direct relationship between plants tolerance to drought stress with activity levels of antioxidant enzymes (Ferrarese et al. 2003). Antioxidant enzymes neutralize Free radicals, which can cause the inactivation of photosynthetic enzymes (Lum et al. 2014). Plants re-pond by increasing the activity of enzymes such as Catalase, Superoxide Dismutase and Glutathione Peroxides which increase with active oxygen species in plant tissue under stress conditions (Koyro et al. 2012). The enzyme superoxide dismutase (SOD) serves the first line of defense against reactive oxygen radicals in the cell and converts superoxide radical into hydrogen peroxide (Sunkar 2010).

In this study, withholding irrigation during the flowering stage caused the most damage to DNA. To deal with this damage, the amount of antioxidants increased. In very sensitive varieties, the amount of antioxidants increased to combat cell death factors. The reason can be attributed to stress-tolerant varieties which with the increase of macromolecules under stress conditions prevented the production of degradation biomarkers. Of course, antioxidants in the cell, and assimilation products, it was spent on the production and filling of the seed. However, in sensitive and semi-tolerant cultivars, the plant tried to maintain its life with this mechanism and prevent cell death.

Catalase antioxidant (CAT)

The results of analysis of variance showed that the simple effects of irrigation and variety as well as the mutual effects of the treatments on catalase enzyme activity (CAT) were significant at the one percent level ($P < 0.01$). The amount of catalase enzyme activity increased with the withholding

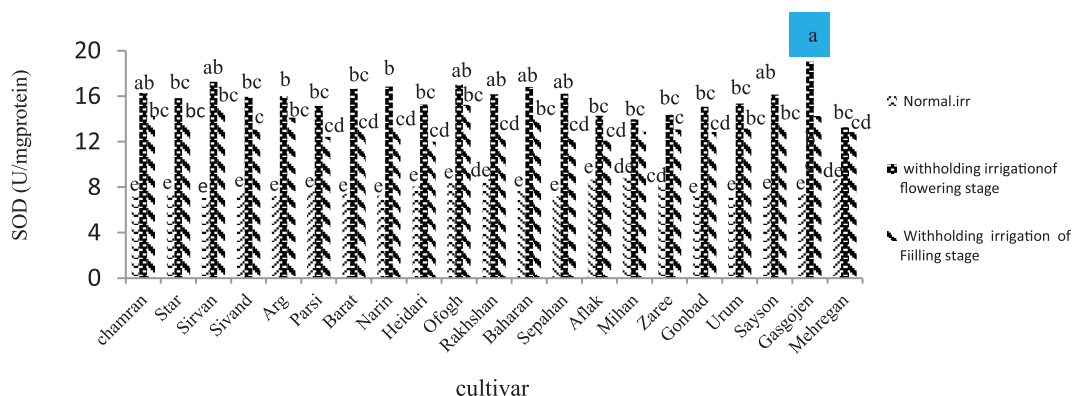


Figure 4. Interaction effects of cut irrigation and cultivars on the amount of SoD.

of irrigation. The treatments involving the interruption of irrigation during flowering and seed filling were in statistical group A. The results indicated that the interruption of irrigation increased the activity of Catalase enzyme (CAT) in different cultivars. The highest and lowest activity of Catalase enzyme were observed in the treatments involving withholding irrigation during the flowering stage for the Saison cultivar (52.91 U.mg protein) and normal irrigation for the Sirvan cultivar (11.81 U.mg protein), respectively.

Catalase enzyme facilitates the conversion of hydrogen peroxide to water and oxygen without the need for an auxiliary substrate (Jiang and Zhang 2011). This enzyme aids in the survival of the plant by eliminating various of reactive oxygen species and preventing cell membrane (Mittler 2009). The decreased activity of Catalase enzyme (CAT) in different genotypes is likely due to an imbalance in the implementation of defense mechanisms. Consequently with the accumulation of active forms of oxygen, attacking key metabolic and defense mechanisms of the cell, these genotypes become more sensitive to drought stress conditions compared to Heydari, Sivand and Rakhshan genotypes. This result in productive assimilates being used to sustain the plant until the end of the growth stage.

Glutathione peroxidase antioxidant enzyme (GPX)

Withholding irrigation increased the activity of the glutathione peroxidase enzyme (GPX). The results of the average comparison indicated that interrupting irrigation increased the activity of the glutathione peroxidase enzyme in different cultivars. The highest and lowest amounts of GPX were obtained from the treatments of withholding irrigation at the flowering stage for the Aflak variety (21.38 U.mg protein) and normal irrigation for the Gasgogen variety (9.34 U.mg protein), respectively.

GPX is one of the most important water soluble antioxidants present in most cell organelles, especially chloroplasts. This antioxidant has the ability to directly react with active oxygen radicals such as superoxide and hydroxyl and neutralize them (Boguszewska et al. 2010). It appears that the amount and activity of this group of enzymes in plants are related to their drought tolerance, which aligns with the opinions of researchers (Sunkar 2010).

Malondialdehyde degradation biomarker (MDA)

The results of the analysis of variance was effective in determining the effects of irrigation levels and variety and as well as the interaction effects of treatments at one and five percent probability levels on MDA (Table 2). MDA levels increased with the withholding of irrigation. The highest amount of MDA was observed in the treatment where irrigation was withheld at the flowering stage treatment (37.5 $\eta\text{mol.mg}^{-1}$ protein). The highest MDA levels at 28.61 ($\eta\text{mol.mg}^{-1}$ protein) was belonged to found in the Ofogh and Gasgogen cultivars, while the Sirvan, Narin and Sepahan cultivars were grouped statistically in group A. The maximum MDA was recorded in the treatment with withholding irrigation at the flowering stage and the Gosgogen cultivar (40.68 $\eta\text{mol.mg}^{-1}$ protein). Conversely, the lowest MDA levels were obtained from in the treatment with normal irrigation and the Sivand cultivar (11.45 $\eta\text{mol.mg}^{-1}$ protein) (Fig. 5).

Lipids peroxidation occurs, leading to the destruction of biological membranes, indicating oxidative stress that is created under various stresses including drought. MDA is used as an indicator to determine the severity of oxidative damage to lipids (Jin et al. 2006). An increase in the amount of free radicals causes damage to the cell membrane, with the most obvious result being the oxidation of fatty acids in the membrane. An increase in MDA levels signifies is a sign of cell membrane damage (Mo et al. 2008), which is consistent with the results of this study.

D-tyrosine degradation biomarker (DT)

Based on the results of analysis of variance, the simple effects of irrigation levels and variety on DT were significant at the one percent level ($P < 0.01$). However, the interaction effects of the treatments were not statistically significant on DT. The results showed that the maximum DT was obtained from withholding irrigation at the flowering stage treatment (19.13 $\eta\text{mol.mg}^{-1}$ protein), which was increased by 87.37% to compare to normal irrigation. The highest amount of DT was observed in the Sirvan (17.33 $\eta\text{mol.mg}^{-1}$ protein) and Ofog (17.12 $\eta\text{mol.mg}^{-1}$ protein), cultivars. Arg, Baharan, Gonbad, Gasgogen and Mehran cultivars were in statistical group A.

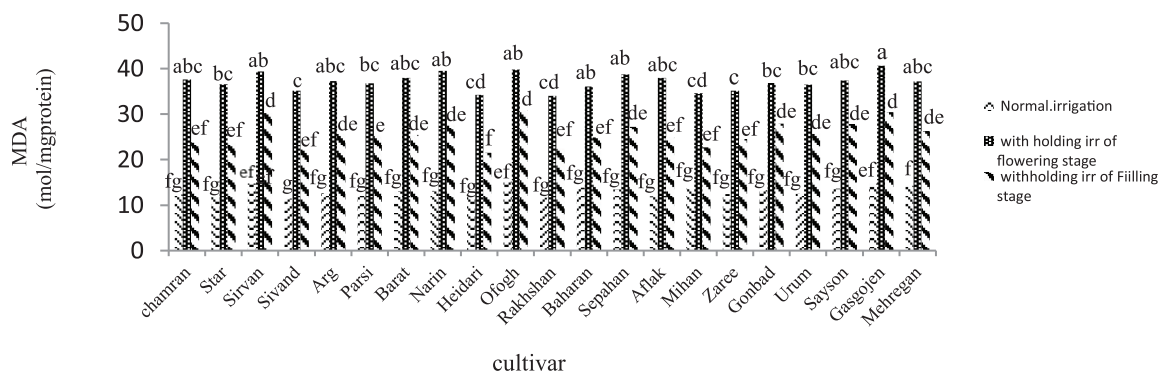


Figure 5. Interaction effects of cut irrigation and cultivars on the biomarker of degradation MDA

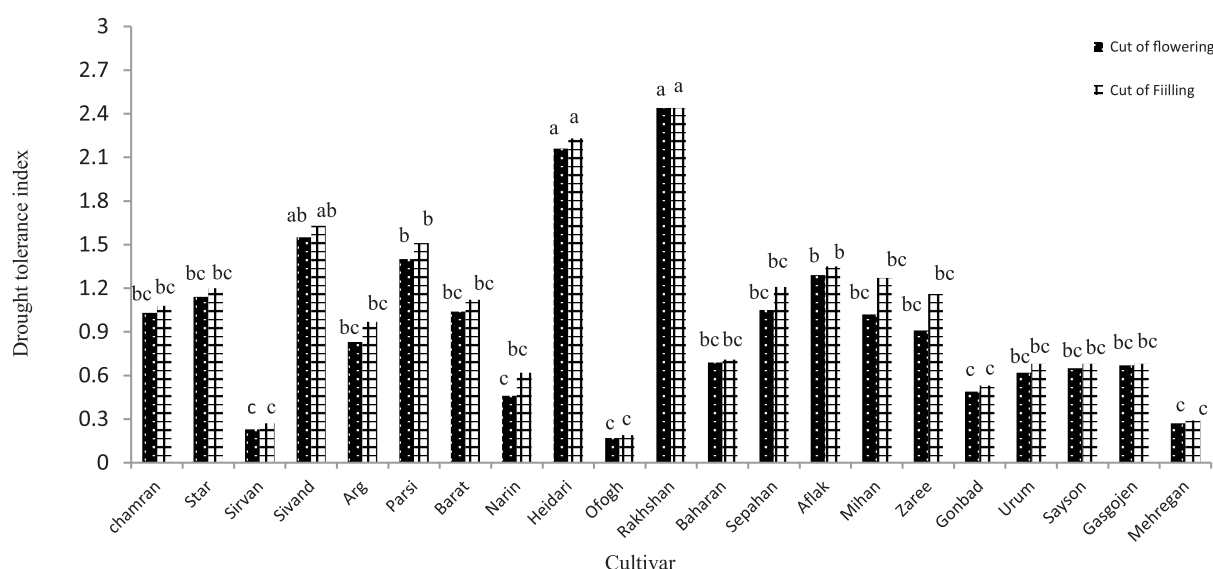


Figure 6. Drought tolerance index cultivars.

Table 4. Mean comparison of oxidative stress and biochemical traits evaluation of wheat at irrigation in Shahriar Region.

Drought Stress	Grain yield (kg.ha)	Proline (mg.g ⁻¹ FW)	S.O.D (unit. mg ⁻¹ pro)	CAT (unit. mg ⁻¹ pro)	GPX (unit. mg ⁻¹ pro)	M.D.A (nmol. mg ⁻¹ pro)	Di-Ty (nmol. mg ⁻¹ pro)	8-OH-dg (nmol. mg ⁻¹ pro)
Normal irrigation	6656.8 ^a	0.52 ^c	7.71 ^c	15.36 ^b	11.62 ^c	13.03 ^c	10.21 ^c	7.45 ^b
Withholding irrigation at the flowering stage	3919.1 ^b	0.86 ^a	15.02 ^a	44.78 ^a	17.41 ^a	37.15 ^a	19.13 ^a	8.54 ^a
Withholding irrigation at the grain filling	4315.4 ^b	0.76 ^b	13.34 ^b	39.03 ^a	15.32 ^b	25.76 ^b	15.32 ^b	7.89 ^{ab}

Means which have at least one common letter are not significantly different at the 5% level using DMRT.

The amount of free radicals of oxygen increased under drought stress. This increment was caused by the destruction of proteins, nucleic acids and fatty acids of the membrane. Therefore, the amount of Di-tyrosine (a product of protein degradation) was increased in this experiment. It had been reported that decreasing amounts of DT result from damage to membranes and per oxidation of membrane proteins (Tao et al. 2010), which was consistent with the results of researchers (Wang et al. 2019).

Dihydroxyguanosine degradation biomarker (D-OH-dG)

The results of variance analysis show that the simple effects of irrigation and cultivar were significant on D-OH-dG at the probability level of one percent ($P < 0.01$). However, the mutual effects of irrigation and variety treatments had no did not have a significant impact on D-OH-dG and there were no significant differences, among treatments placing them all in the same statistical group. The highest amount of D-OH-dG was found in plants that had irrigation with held at the flowering stage (8.54 $\mu\text{mol.mg}^{-1}$ protein). The maximum D-OH-dG levels were observed in the Ofogh and Gosgogen cultivars.

In this study, the levels of D-OH-dG increased as a result of withholding irrigation, which can lead to

changes and mutations in plants. This is why the highest levels of this biomarker were seen in plants where irrigation was withheld during the flowering stage. In these cases, DNA was less damaged and D-OH-dG was predicted in smaller amount. It appears that DNA had a stronger and more effective defense system compared to lipids and proteins. Leading to less destruction by free radicals, it is also possible that antioxidant enzymes played a role in reducing the rate of destruction; which is consistent with previous research finding (Tao et al. 2010; Wang et al. 2019).

Fernandez drought tolerance coefficient

The results showed that the Rakhshan cultivar exhibited the highest tolerance to drought when irrigation was withheld at the flowering and seed filling stages, with an average of 2.44, placing it in statistical class A. The Ofogh variety had the lowest tolerance, with average of 0.17 and 0.19 when irrigation was stopped at those stages.

Additionally, the results revealed that the genotypes Sivand, Heidari and Rakhshan had the highest Fernandez drought tolerance index values. Indicating that these genotypes were more drought tolerant than others. The genotypes were classified into three groups based on this index Genotype Ofogh was placed in group D (less

STI - lower yield in both non-stress and stress environments), while genotypes Rakhshan, Heidariand and Siv-and in group A (more STI - higher yield in both environments), The remaining and genotypes were placed in group B (less STI - higher yield in both environments) with no genotypes falling in to group C (more STI - lower yield).

Conclusion

The results of this research showed that drought stress caused morphological and biochemical changes in various wheat cultivars, with the antioxidant defense system responding to this stress. Based on the results obtained, drought stress was linked to a decrease in

Table 5. Mean comparison of functional and biochemical traits of different wheat cultivars in Shahriar region.

cultivars	Grain yield (kg/ha)	Proline (mg.g ⁻¹ FW)	S.O.D (unit.mg ⁻¹ pro)	CAT (unit.mg ⁻¹ pro)	GPX (unit.mg ⁻¹ pro)	M.D.A (nmol.mg ⁻¹ pro)	Di-Ty (nmol.mg ⁻¹ pro)	8-OH-dg (nmol.mg ⁻¹ pro)
Chamran	5230.4 ^{bc}	0.8 ^{ab}	12.57 ^{abc}	36.27 ^b	14.25 ^{bc}	24.62 ^{bc}	14.65 ^{bc}	7.82 ^{abc}
Star	5499.4 ^{bc}	0.77 ^{ab}	12.22 ^{abc}	33.32 ^{bc}	15.19 ^{abc}	23.99 ^{cd}	14.34 ^{bc}	7.74 ^{abc}
Sirvan	2575.6 ^d	0.82 ^{ab}	13.17 ^{ab}	29.05 ^d	12.57 ^{cd}	28.15 ^{ab}	17.12 ^a	8.74 ^{ab}
Sivand	6059.5 ^{bc}	0.69 ^{bc}	12.19 ^{abc}	39.04 ^a	16.88 ^{ab}	22.92 ^{cd}	13.14 ^d	7.44 ^{bc}
Arg	4560.3 ^c	0.81 ^{ab}	12.44 ^{abc}	34.19 ^{bc}	14.13 ^{bc}	25.17 ^{bc}	16.01 ^{ab}	7.82 ^{abc}
Parsi	5716.4 ^{bc}	0.75 ^b	11.73 ^{bc}	33.67 ^{bc}	14.22 ^{bc}	24.81 ^{bc}	14.59 ^{bc}	7.62 ^{abc}
Barat	4741.7 ^c	0.74 ^{bc}	11.38 ^c	32.53 ^{bc}	15.22 ^{abc}	25.51 ^{bc}	14.61 ^{bc}	8.12 ^{ab}
Narin	2646.4 ^d	0.73 ^{bc}	12.46 ^{abc}	32.07 ^{cd}	14.34 ^{bc}	26.79 ^{ab}	14.89 ^{bcd}	8.13 ^{ab}
Heidari	8704.7 ^a	0.66 ^c	11.78 ^{bc}	38.22 ^{ab}	17.14 ^a	22.54 ^d	13.13 ^d	7.37 ^c
Ofogh	2295.7 ^d	0.82 ^a	13.53 ^a	28.24 ^d	12.37 ^d	28.61 ^a	17.33 ^a	8.82 ^a
Rakhshan	8416.5 ^a	0.59 ^d	12.52 ^{abc}	36.99 ^{ab}	15.41 ^{abc}	23.51 ^{cd}	12.81 ^d	7.37 ^c
Baharan	4881.2 ^c	0.73 ^{bc}	12.83 ^{abc}	31.49 ^{cd}	12.66 ^{cd}	24.92 ^{bc}	16.21 ^{ab}	8.27 ^{ab}
Sepahan	5131.8 ^c	0.79 ^{bc}	11.94 ^{bc}	33.56 ^{bc}	14.34 ^{bc}	26.47 ^{ab}	14.53 ^{bc}	7.69 ^{abc}
Aflak	5488.6 ^{bc}	0.76 ^{bc}	11.67 ^{bc}	32.76 ^{cd}	17.12 ^a	24.98 ^{bc}	13.46 ^{cd}	7.61 ^{abc}
Mihan	6407.1 ^b	0.62 ^{cd}	11.89 ^{bc}	36.27 ^b	16.38 ^{ab}	23.66 ^{bc}	14.19 ^{bc}	7.73 ^{abc}
Zare	5241.6 ^{bc}	0.59 ^d	11.91 ^{bc}	33.28 ^c	14.78 ^{bc}	24.05 ^c	14.09 ^{bc}	7.95 ^{abc}
Gonbad	4523.1 ^c	0.6 ^{cd}	11.72 ^{bc}	30.32 ^{cd}	13.85 ^c	26.03 ^{bc}	15.17 ^{abc}	8.22 ^{ab}
Oroum	5122.4 ^c	0.6 ^{cd}	11.87 ^{bc}	34.96 ^{bc}	16.06 ^{ab}	24.88 ^{bc}	14.91 ^{bcd}	7.94 ^{abc}
Sayson	5294.1 ^{bc}	0.63 ^{cd}	12.38 ^{abc}	37.45 ^{ab}	14.54 ^{bc}	26.31 ^b	14.79 ^{bc}	7.72 ^{abc}
Gasgogen	4791.2 ^c	0.69 ^{bc}	13.57 ^a	29.64 ^d	12.99 ^{cd}	28.61 ^a	16.65 ^{ab}	8.82 ^a
Mehregan	2506.1 ^d	0.75 ^b	11.61 ^{bc}	34.51 ^{bc}	15.82 ^{abc}	25.92 ^{bc}	16.02 ^{ab}	8.41 ^{ab}

Means which have at least one common letter are not significantly different at the 5% level using DMRT.

Table 6. Mean comparison of CAT, GPX and M.D.A evaluation of different wheat cultivars undercut irrigation in Shahriar region.

cultivars	CAT (unit.mg ⁻¹ pro)			GPX (unit.mg ⁻¹ pro)		
	Normal irrigation	Non irrigation at the flowering stage	Non irrigation at the grain filling	Normal irrigation	Non irrigation at the flowering stage	Non irrigation at the grain filling
Chamran	11.37 ^{gh}	16.28 ^{cde}	15.12 ^{de}	14.87 ^{hi}	45.29 ^{cd}	39.65 ^{ef}
Star	12.46 ^{fg}	15.87 ^{de}	17.24 ^{cd}	15.04 ^{hi}	43.69 ^{cd}	41.22 ^{de}
Sirvan	10.23 ^{gh}	13.32 ^{fgh}	14.18 ^{ef}	11.81 ⁱ	42.67 ^{de}	32.68 ^g
Sivand	13.07 ^{efg}	19.46 ^{abc}	18.11 ^{bc}	18.97 ^g	51.86 ^{ab}	46.31 ^{cd}
Arg	11.44 ^{gh}	16.87 ^{cde}	14.09 ^{ef}	15.37 ^{gh}	46.92 ^c	40.28 ^e
Parsi	12.18 ^{fg}	16.21 ^{cde}	14.27 ^{ef}	17.92 ^{gh}	44.31 ^{cd}	38.79 ^{ef}
Barat	12.32 ^{fg}	17.04 ^{cd}	16.32 ^{cde}	15.38 ^{gh}	43.16 ^d	39.07 ^{ef}
Narin	11.67 ^{gh}	16.49 ^{cde}	14.86 ^{ef}	13.61 ^{hi}	45.89 ^{cd}	36.73 ^f
Heidari	12.89 ^{fg}	20.31 ^{ab}	18.23 ^{bc}	18.64 ^{gh}	50.41 ^{ab}	45.62 ^{cd}
Ofogh	10.34 ^{gh}	14.68 ^{ef}	12.09 ^{fg}	11.68 ^g	41.08 ^{de}	31.97 ^f
Rakhshan	13.48 ^{efg}	17.27 ^{cd}	15.49 ^{de}	17.82 ^{gh}	49.36 ^b	43.81 ^{cd}
Baharan	11.08 ^{gh}	14.82 ^e	13.13 ^{efg}	12.39 ^{hi}	43.86 ^{cd}	38.24 ^{ef}
Sepahan	12.17 ^g	16.03 ^{cde}	14.81 ^e	15.27 ^h	45.12 ^{cd}	40.31 ^e
Aflak	12.68 ^{fg}	21.38 ^a	17.42 ^{cd}	16.32 ^{gh}	42.73 ^{de}	39.24 ^{ef}
Mihan	11.92 ^{gh}	20.65 ^{ab}	16.58 ^{cde}	14.65 ^{hi}	51.48 ^{ab}	42.69 ^{de}
Zare	10.68 ^{gh}	18.31 ^{bc}	15.25 ^{de}	13.89 ^{hi}	47.31 ^{bc}	38.65 ^{ef}
Gonbad	11.16 ^{gh}	16.27 ^{cde}	14.12 ^{ef}	15.09 ^{hi}	41.82 ^{de}	34.07 ^f
Oroum	11.48 ^{gh}	20.89 ^{ab}	15.82 ^{de}	17.44 ^{gh}	51.68 ^{ab}	35.76 ^f
Sayson	11.67 ^{gh}	18.14 ^{bc}	13.52 ^{efg}	17.84 ^{gh}	52.91 ^a	41.61 ^{de}
Gasgogen	9.34 ^h	16.57 ^{cde}	13.07 ^{efg}	13.41 ^{gh}	42.69 ^{de}	32.83 ^f
Mehregan	10.29 ^{gh}	19.28 ^{abc}	17.88 ^c	15.27 ^h	48.36 ^{bc}	39.87 ^{ef}

Means which have at least one common letter are not significantly different at the 5% level using DMRT.

yield and yield components as well as an increase in antioxidant defense, proline and degradation biomarkers. Cutting irrigation at the flowering stage had a more detrimental effect on the plant compared to cutting

irrigation at the seed filling stage. Additionally, it was discovered that the Rakhshan variety had the highest seed yield, indicating that Rakhshan and Sivand cultivars were drought tolerant.

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