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Physiological and Biochemical Responses in Five Wheat Cultivars to Supplemental Irrigation

L. Moradi[1], A. Siosemardeh[1*], Y. Sohrabi[1], B. Bahramnejad[1], and F. Hosseinpanahi[1]

ABSTRACT

The objective of this study was to assess changes in proline, Glycine Betaine (GB), photosynthetic pigment concentration, Membrane Stability Index (MSI), and grain yield of wheat cultivars induced by Supplemental Irrigation (SI) in the terminal growth stages. Two field experiments with four irrigation levels (rainfed, SI at booting stage, SI at booting and anthesis stages, SI at booting, anthesis, and grain filling stages) and five cultivars (Rejaw, Sardari, Homa, Azar2, and Sirwan) were conducted during the 2015-2017 growing seasons. Results indicated that, overall, SI increased cultivars' chlorophyll concentration, MSI, and grain yield. Proline and GB highly accumulated under drought stress, but rapidly decreased after SI; the severity of the decrease in proline concentration was more remarkable. Sardari and Azar2 cultivars exhibited greater MSI, proline, and GB accumulation during drought stress and more rapid recovery from drought. Our results suggest that the ability of the wheat cultivars to maintain functions during drought and recover after SI during the terminal growth stage is essential for determining final grain yield.

Keywords: Chlorophyll, Drought stress, Grain filling, Grain yield, Proline.

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INTRODUCTION

Due to unbalance rainfall distribution in the Mediterranean region, drought stress occurs mainly during the terminal stages of rainfed wheat growth (Oweis and Hachum, 2009). Under such conditions, Supplemental Irrigation (SI) is a highly efficient practice to reduce the adverse effects of drought stress and improve grain yield. SI uses limited water resources in critical crop growth stages (irrigation limited to one or several stages of growth) in rainfed conditions (Oweis and Hachum, 2009; Sahar *et al.,* 2019).

Assessment of Membrane Stability Index (MSI) is widely used as a physiological indicator and a method for measuring drought tolerance. Drought stress inhibits the development of cell membranes and increases electrolyte leakage from the cell. Due to the injury of the cell membrane, the contents inside the cell leak out; but drought tolerant cultivars have less electrolyte leakage (Kapoor *et al.,* 2020). Plant leaf chlorophyll concentration is an essential factor in photosynthetic capacity and dry matter production in the plant (Li *et al.,* 2018). The study and measurement of photosynthetic green pigments can provide important information about the physiological responses of plants to various environmental factors such as drought stress (Li *et al.,* 2018; Ma *et al.,* 2020). When the chlorophyll content of the leaves decreases by 50% compared to the natural green leaves, the most obvious sign of senescence, which is the yellowing of the leaves, appears. Therefore, by measuring the chlorophyll content of the leaf, leaf senescence can be examined (Cha *et al.,* 2002). Drought stress at the terminal stages of growth causes damage to cell membranes, decreases leaf chlorophyll concentration by photo-oxidation and chlorophyll degradation

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[1] Department of Agronomy and Plant Breeding, Faculty of Agriculture, University of Kurdistan, Sanandaj, Islamic Republic of Iran.
[*]Corresponding author; e-mail: a33@uok.ac.ir

and chlorosis, and ultimately leads to early leaf senescence and reduces grain yield (Abid *et al.,* 2018; Ma *et al.,* 2020).

Under drought stress, the plants reduce inner water potential to evade dehydration, uphold potential water balance, and maintain cellular functions by synthesizing and accumulating compatible osmolytes such as proline and Glycine Betaine (GB) (Farooq *et al.,* 2014). Osmotic adjustment, maintaining turgor pressure, and cell volume are critical to preserving metabolic activity under low water potential conditions. In addition, osmotic adjustment facilitates the recovery of metabolic activities in the post-stress period and re-irrigation (Abid *et al.,* 2018). Accumulation of proline helps the plant to recover its normal growth shortly after removing stress and, therefore, in some cases, can have a positive effect on grain yield. However, in the long run, it can negatively affect grain yield because the photosynthetic sources of the plant lead to processes other than grain filling (Kao *et al*., 1981; Ashraf and Foolad, 2007). Proline and glycine betaine protect plants from stress through different courses, including contribution to removing Reactive Oxygen Species (ROS), protection of macromolecules from denaturation, maintaining cell membrane integrity, and regulating cellular pH (Ashraf and Foolad, 2007; Amini *et al.,* 2015; Annunziata *et al.,* 2019). Proline and GB also serve as nitrogen and carbon source for plants under severe stress (Zhang *et al*., 2014; Amini *et al.,* 2015; Tian *et al.,* 2017).

Although the adverse effects of drought stress on the physiological activities of many plant species are well documented, research on SI and its effects on plant physiological responses and recovery period after irrigation is relatively limited. Under drought stress conditions, availability of water, even a tiny amount of rain, can significantly affect physiological and biochemical responses in the plant. Therefore, it can be essential to investigate the mechanisms involved in drought tolerance and the effect of SI on drought stress recovery. The specific objectives of the current study were to: (a) Evaluate chlorophyll concentration at booting, anthesis, and grain filling stage, (b) Investigate MSI at booting, anthesis, and grain filling stage, (c) Analyze proline and GB concentration during drought stress and three days after SI (recovery from drought stress), (d) Measure the effect of end-of-season drought stress on grain yield of studied wheat cultivars, and (e) Examine relationships between chlorophyll, proline, GB concentration, and MSI with grain yield.

MATERIALS AND METHODS

A two-year field study was performed in the University of Kurdistan Research Farm, located in Dehgolan Plain (35˚ 19′ 10″ N, 47˚ 18′ 55″ E, 1,864 m above sea level). The soil texture was loam and other physical soil parameters are shown in Table 1. The monthly climatic conditions of the experimental site during the wheat growing season are shown in Table 2.

This experiment was conducted in split plots in a randomized complete block design with three replications in two growing seasons (2015-2016 and 2016-2017). Four irrigation levels (I0, I1, I2, and I3) were randomized on the main plot, and five wheat cultivars (Sardari, Azar 2, Homa, Rejaw, and Sirwan) were randomized on a sub-plot. Irrigation levels included I0: no-irrigation (rainfed condition), I1: SI at the booting/Z41 stage, I2: SI at the booting/Z41 and anthesis/Z61 stages, and I3: SI at the booting/Z41, anthesis/Z61, and grain filling/Z79 stages (Zadoks *et al.,* 1974). The size of the main plot was 18.5×9 m^2 and the size of a sub-plot was 2.9×9 m^2. The distance between the main plots was 2 m, and the distance between the sub-plots was 1.5 m. The amounts of SIs are shown in Table 3. The target relative water content in the 0–50 cm soil layer after SI was 75% Field Capacity (FC). Soil samples were collected using a soil corer at 50 cm in all experimental plots. The Soil Water Content

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Table 1. Soil nutrient status at 0– 30 cm depth of experimental field before sowing.

Growing	Organic C (%)	Total N (g	Available P (mg	Available K (mg
2015-2016	0.87	1.2	13.3	327.1
2016-2017	0.92	0.9	8.0	349.10

Table 2. A synopsis of weather conditions in 2015-2017 growing seasons.

Month	2015-2016			2016-2017		
	Precipitation	T_{max} (˚C)	T_{min}	Precipitation	T_{max} (˚C)	T_{min}
Oct.	89.20	13.98	7.40	1.63	19.69	8.40
Nov	35.36	10.92	1.40	8.50	12.86	0.50
Dec	34.07	-3.54	-7.61	64.12	7.50	-2.40
Jan.	49.45	6.37	-2.53	11.35	5.98	-4.16
Feb.	30.66	2.91	-1.62	30.45	3.42	-6.72
Mar	45.12	8.09	-1.62	86.55	10.37	1.21
Apr.	55.93	12.33	2.50	45.52	18.10	6.68
Ma	14.6	16.85	5.75	26.66	23.94	11.02
Jun.	0.51	22.86	10.44	0	30.23	15.62
Tot	354.9	-	-	274.78	-	-

Table 3. The amount of each Supplemental Irrigation (SI) in four treatments consists of (I0) no-irrigation, (I1) SI at booting stage, (I2) SI at booting and anthesis stages, and (I3) SI at booting, anthesis, and grain filling stages.

	2015-2016				2016-2017			
	I0	I1	I2	I3	I0	I1	I2	I3
	(mm)				(mm)			
Booting	-	29.93	29.	29.93	-	38.6	38.6	38.6
Anthesis	-	-	40.	40.95	-	-	45.4	45.4
Grain filling	-	-	-	49.16	-	-	-	54.8
Total irrigation (mm)	-	29.93	70.	120.04	-	38.6	84.0	138.

(SWC) was measured using oven drying. The amount of SI was calculated by the equation described by Ekren *et al.* (2012), as follows:

$I= 10\times\rho b\times D_h\times(\theta_t-\theta_n)$,

Where, I (mm) is the amount of SI, and ρb (g cm^3) is the soil bulk density, D_h is the thickness of the soil profile measured for SWC pre-irrigation, θ_t (%) is the target SWC on a weight basis after SI, and θ_n (%) is the SWC on a weight basis pre-irrigation. θ_t was calculated as follow: $\theta_t= (\theta_{max}\times\theta_{tar})$ where, θ_{max}(%) is the FC and θ_{tar}(%) is the SWC (in this study, 75%). SWC in FC were 282.9 and 285.6 (mg water g^{-1} dry soil) in 2015/2016 and 2016/2017, respectively. The soil bulk density was 1.56 and 1.6 (g cm^{-3}) in 2015/2016 and 2016/2017, respectively. Irrigation was done using drip tape and installing a meter with an accuracy of 1 liter.

Crop Management

All plots were supplied with 150 kg ha^{-1} of urea and 200 kg ha^{-1} of triple superphosphate at the time of seeding. Wheat seeds were sown at a density of 350 plants.m^{-2} on October 27, 2015, and October 23, 2016. The wheat was harvested on July 8 and July 6, in 2015 and 2017, respectively. The other management practices, such as tilling, planting, weed control, and pest

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control, were similar to conventional wheat practices.

Measurements

Flag leaves sampling to measure chlorophyll concentration was performed (randomly collected) three days after SI at booting, anthesis, and grain filling stages. Samples were frozen by liquid nitrogen and stored at −40◦C until chlorophyll concentration assays were performed. Concentrations of chlorophyll were estimated according to Arnon (1967). Eventually, the amount of leaf chlorophyll per gram fresh weight was converted to the reference of dry weight (DW) to deduce the influence of water content.

In order to measure the MSI, flag leaves samplings were randomly collected three days after each irrigation at booting, anthesis, and grain filling stages. MSI was evaluated by measuring electrolyte leakage according to Liu *et al*. (2005).

Flag leaves samplings to determine the proline and GB concentration were randomly collected before and after irrigation (three days after irrigation) at booting, anthesis, and grain filling stages. Samples were frozen by liquid nitrogen and stored at −40 ◦C until proline concentration assays were performed. Proline and GB concentration were assessed using the method of Bates *et al.* (1973) and Grieve and Grattan (1983), respectively.

At the maturity stage, to determine the grain yield, 3 m^2 area cut in the center of each plot and was expressed at a 12% moisture content (Zhang *et al.,* 2019).

Statistical Analysis

In order to analyze the combined variance of the data, after Bartlett's test and ensuring homogeneity of variance, the split-plot combine analysis model based on a randomized complete block design was used. Analysis Of Variance (ANOVA) was performed using SAS 9.4 software. The means were compared using Duncan's multiple range test at P≤ 0.05 (Duncan, 1955). Pearson correlation coefficients amongst the measured variable were determined using Proc corr.

RESULTS

In the grain filling stage, compared to the anthesis stage, Chl a concentration in I0, I1, I2, and I3 treatments decreased by 34.49, 36.72, 28.23, and 26.58%, respectively. Also, the rate of decrease in Chl b concentration in the grain filling stage and I0, I1, I2, and I3 treatments compared to the anthesis stage was 39.26, 40.74, 29.75, and 27.90%, respectively [Figure 1 (A-C)].

Three days after the last irrigation in the grain filling stage, a combined ANOVA for photosynthetic pigments concentration revealed significant effect of year, irrigation, and cultivar on these traits. The significant year×cultivar interaction detected for these traits was also significant (Table 4). The Chl a, Chl b, and Chl T concentrations in the 2015-2016 season was more than the 2016-2017 season. Overall, SI had a positive effect on Chl concentration. The Chl a, Chl b, and Chl T concentrations decreased in the order I3> I2> I1> I0 (Table 4). The chlorophyll concentration decreased in all cultivars during the 2016-2017 growing season compared to 2015-2016. However, the incline of concentration reduction in Homa and Sirwan cultivars was higher than in other cultivars [Figure 2 (A-C)].

MSI was 80.90, 83.36, 82.65, and 82.35%, respectively, in I0, I1, I2, and I3 treatments at booting stage, while at grain filling stage declined to 45.74, 48.13, 58.72, and 61.50%, respectively, in I0, I1, I2, and I3 treatments. The Sirwan cultivar had the smallest MSI value compared to other cultivars during three growth stages (Figure 2-D).

The results of the combined ANOVA for MSI three days after the last irrigation in the grain filling stage showed that MSI was affected by year, irrigation, and cultivar.

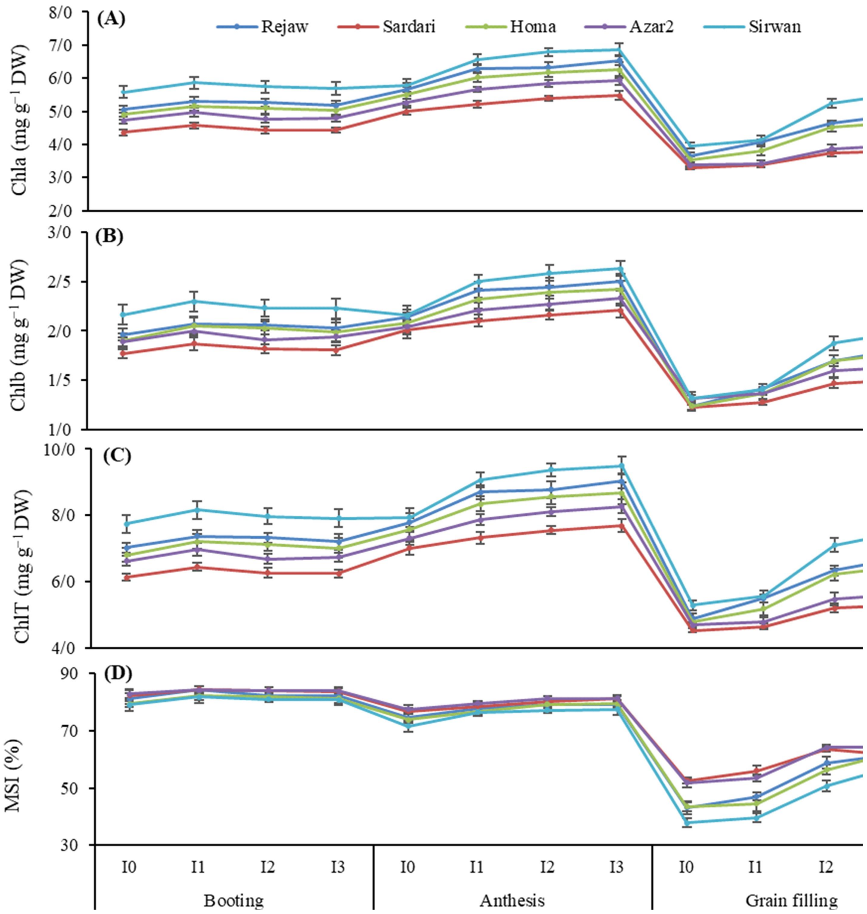

Figure 1. Effect of Supplemental Irrigation (SI) on chlorophyll concentration (A-C) and Membrane Stability Index (MSI, D) in wheat cultivars during 2015-2017 growing seasons. Each mean is accompanied by a standard error (n= 6). I0= No-irrigation, I1= SI at booting stage, I2= SI at booting and anthesis stages, and I3= SI at booting, anthesis, and grain filling stages.

Also, the interaction of irrigation×cultivar for MSI was significant (Table 4). In 2016-2017 (54.98%), the average MSI was higher than 2015-2016 (52.08%). The MSI decreased in the order I3> I2> I1> I0. MSI of all cultivars (ranging from 37.8 to 64.2) was enhanced with SI frequency (Figure 2-D).

SI reduced proline concentration in all three stages and all cultivars. At booting, anthesis, and grain filling stages, comparison of proline concentration before and three days after recovery (in the applied treatments) decreased the average concentration of this osmolyte by 32.85, 36.03, 31.17%, respectively. The smallest and greatest changes in proline concentration before and after SI in the grain filling stage were related to Rejaw (25.20%) and Sardari (35.25%), respectively (Figure 3-A).

The results of the combined ANOVA for proline concentration three days after recovery in the grain filling stage showed that year, irrigation, cultivar, and the interaction of year×irrigation,

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Table 4. Mean comparisons of chlorophyll (Chl a, Chl b, and Chl T), proline, and Glycine Betaine (GB) concentration, Membrane Stability Index (MSI), and grain yield affected by the growing season, Supplemental Irrigation (SI) and cultivars.[a]

Mean comparisons	Three days after SI at grain filling stage						Grain yield (kg ha^{-1})
	Chl a	Ch (mg g^{-1} DW)	Chl	MSI (%)	Proline (µg g^{-1} FW)	GB (mg g^{-1} DW)	
Year	*	*	*	*	**	*	**
2015-	4.18^{a}	1.5	5.74^{a}	54.98^{a}	643.62^{b}	7.64^{b}	3945.2^{a}
2016-	3.98^{b}	1.4	5.43^{b}	52.06^{b}	724.41^{a}	8.18^{a}	3641.6^{b}
Irrigation	**	**	**	**	**	**	**
I0	3.57^{c}	1.2	4.83^{c}	45.74^{d}	923.22^{a}	9.09^{a}	3172.1^{d}
I1	3.77^{b}	1.3	5.13^{b}	48.13^{c}	915.90^{a}	9.10^{a}	3659.9^{c}
I2	4.40^{a}	1.6	6.06^{a}	58.72^{b}	553.13^{b}	7.28^{b}	4053.2^{b}
I3	4.58^{a}	1.7	6.33^{a}	61.50^{a}	343.81^{c}	6.17^{c}	4290.3^{a}
Cultivar	**	**	**	**			**
Rejaw	4.32^{b}	1.5	5.86^{b}	52.68^{b}	685.76^{c}	7.55^{c}	4074.4^{a}
Sardari	3.55^{d}	1.3	4.92^{e}	58.33^{a}	811.32^{a}	9.15^{a}	3316.6^{c}
Homa	4.14^{c}	1.5	5.66^{c}	51.74^{b}	610.12^{d}	7.59^{c}	3909.8^{a}
Azar2	3.66^{d}	1.4	5.14^{d}	58.41^{a}	749.95^{b}	8.47^{b}	3677.1^{b}
Sirwan	4.72^{a}	1.6	6.36^{a}	46.44^{c}	562.92^{e}	6.79^{d}	3990.5^{a}
Y×I	ns	ns	ns	ns	**	ns	ns
I×C	**	**	**	**	**	**	**
Y×C	ns	ns	ns	ns	**	*	**
Y×I×C	ns	ns	ns	ns	ns	ns	ns

[a] Values within a group in a column bearing followed by the same letter are not significantly different at P≤ 0.05 as determined by Duncan's test. I0= No-irrigation. I1, I2 and I3 as defined previously.

*: P≤ 0.05; **: P≤ 0.01, ns: Not significant error within-group variance.

irrigation×cultivar, and year×cultivar had significant effects on this trait. The proline concentration in all water treatments during 2016-17 was higher than in the 2015-2016 growing season (Figure 4). Sardari cultivar had a higher proline concentration than other cultivars in different irrigation treatments. The greatest proline concentration was observed in this cultivar under one SI condition (1,117 µg g^{-1} FW) (Figure 5-A). Proline concentration ranged from 533.2 to 833 µg g^{-1} FW across the cultivars and growing season. It was more or less increased during 2016-2017 compared to the 2015-2016 growing season (Figure 6-A).

In I0, I1, I2, and I3 treatments, the GB concentration in the latest determination stage compared to the first measurement stage was higher by, respectively, 87.94, 83.865, 48.65, and 48.72%. At booting, anthesis, and grain filling stages, the comparison of GB concentration before and three days after recovery decreased the average concentration of this osmolyte by 12.25, 8.54, and 7.64%, respectively.

The combined analysis for GB concentration three days after the last irrigation at the grain filling stage indicated that the effect of year, irrigation, cultivar, and interaction of irrigation×cultivar and year×cultivar were significant (Table 4). GB concentration of all cultivars in 2016-2017 compared to the 2015-2016 growing season was increased, and the most variation of 11.99% was recorded in Sirwan cultivar (Table 4 and Figure 5-B). The highest concentration of GB was observed in the Sardari cultivar and under I1 (10.58 mg g DW) and I0 condition (10.51mg g DW) (Figure 6-B).

Results indicated that grain yield was affected by year, irrigation, and cultivars. A

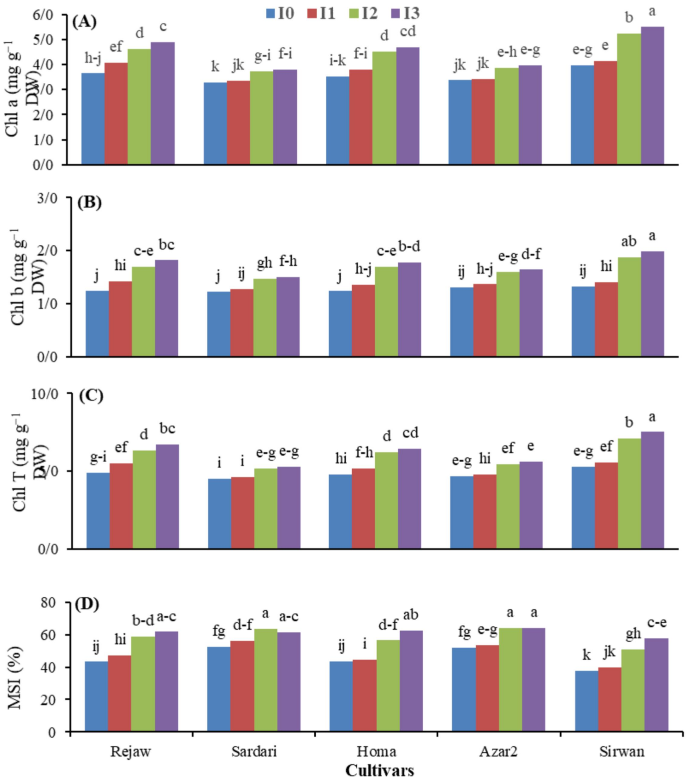

Figure 2. Mean comparisons of interaction effects of irrigation×cultivars on chlorophyll concentration [Chl a (A), Chl b (B), and Chl T (C)] and Membrane Stability Index (MSI, D) three days after SI at grain filling stage. Columns designated by the same letter are not significantly different at the P≤ 0.05 level as determined by Duncan's test. I0, I1, I2, and I3 as defined previously.

significant irrigation×cultivar and year×cultivar treatment interaction was detected for grain yield (Table 4). Three SI (I3) increased the grain yield of Sirwan, Homa, Rejaw, Azar2, and Sardari by 49.25%, 42.57, 38.27, 29.94, and 14.55% compared to the no SI treatment, respectively (Table 4 and Figure 5-C). On average, in both seasons, Sardari had significantly lower grain yield than other cultivars (Table 4 and Figure 6-C).

The data in Table 5 reveal a significant positive relationship between grain yield with Chl a (r= 0.78, P≤ 0.01), Chl b (r= 0.77, P≤0.01), Chl T (r= 0.79, P≤ 0.01), MSI (r= 0.43, P≤ 0.01). The relationship between grain yield with proline (r= -0.70, P≤ 0.01) and GB (r= -0.73, P≤ 0.01) concentration was negatively significant.

DISCUSSION

In the Mediterranean region, an uneven precipitation pattern decreases moisture available to the plant, particularly in the terminal stages of growth. In rainfed agriculture, this pattern usually causes drought stress, reduced growth, and wheat grain yield. SI at critical growth stages can alleviate the adverse effects of drought stress on growth and improves grain yield (Oweis and Hachum, 2009; Sahar *et al.,* 2019; Zhang *et al.,* 2019).

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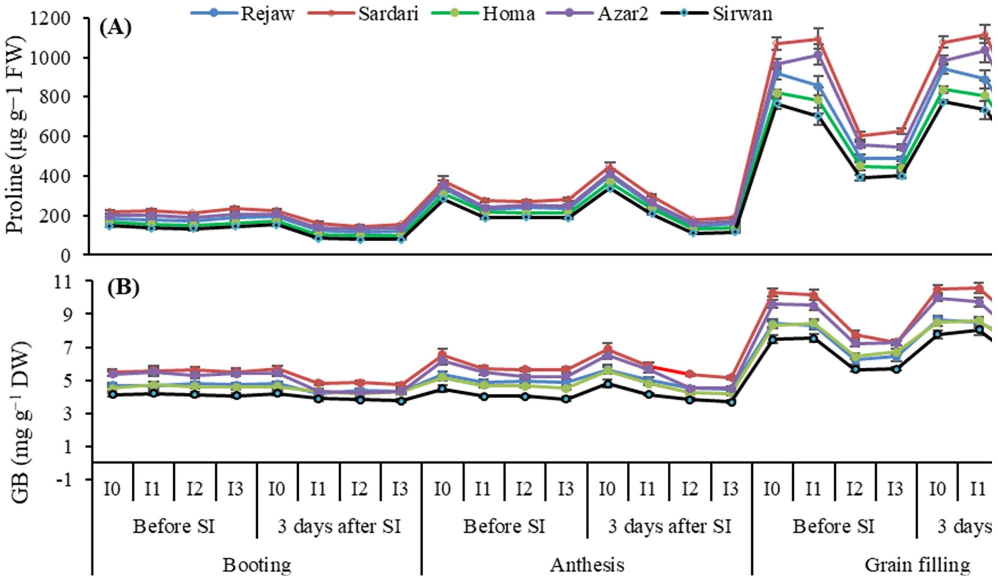

Figure 3. Effect of Supplemental Irrigation (SI) on proline (A) and Glycine Betaine (GB, B) in wheat cultivars. During 2015-2017 growing seasons. Each mean is accompanied by a standard error (n= 6). I0, I1, I2, and I3 as define previously.

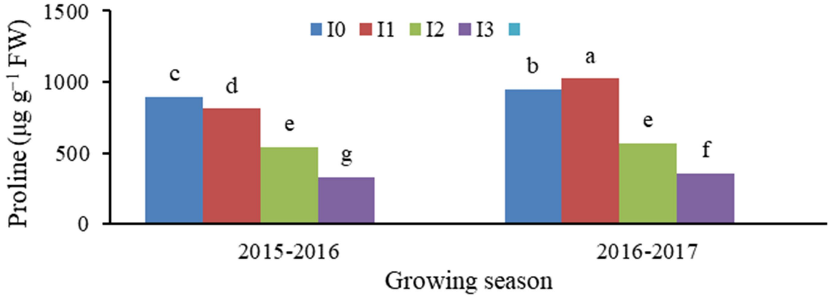

Figure 4. Mean comparisons of interaction effects of year×irrigation on proline concentration three days after Supplemental Irrigation (SI) at grain filling stage. Columns designated by the same letter are not significantly different at the P≤ 0.05 level as determined by Duncan's test. I0, I1, I2, and I3 as define previously.

Drought stress reduces the chlorophyll concentration through increased degradation of pigments, decreased synthesis, and disorders of enzyme activity involved in the synthesis of photosynthetic pigments (Saeidi and Abdoli, 2015). In the present study, concentrations of the photosynthetic pigments in the anthesis stage were higher than in the booting and grain filling stages. Since sampling of flag leaf was done, and the flag leaf was not completely developed at the booting stage, concentration of pigments increased at the anthesis stage. In the grain filling stage in SI treatments, photosynthetic pigment concentration was significantly higher than in rainfed conditions [Table 4 and Figures 1 (A-C) and 2 (A-C)]. The overlap of senescence and grain filling period and the synchronization of these two processes are most important in determining grain yield. Except for grain weight, other yield components were

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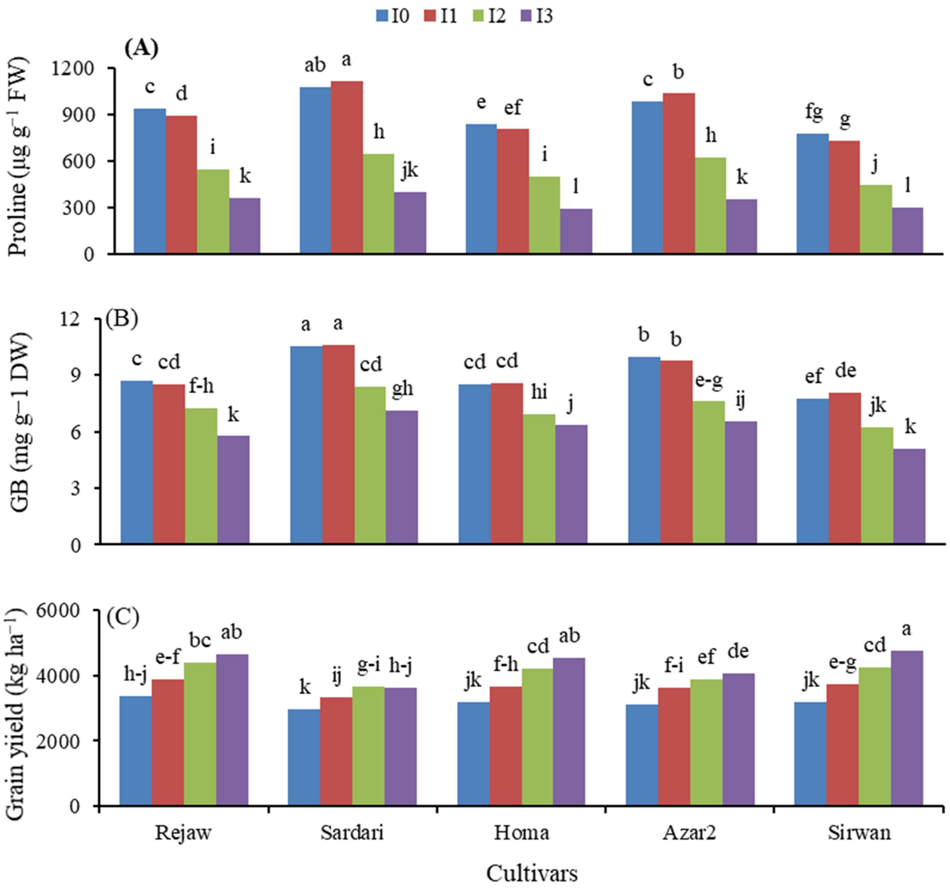

Figure 5. Mean comparisons of interaction effects of irrigation×cultivars on proline (A) and Glycine Betaine (GB, B) concentration, and grain yield (C) three days after Supplemental Irrigation (SI) at grain filling stage. Columns designated by the same letter are not significantly different at the P≤ 0.05 level as determined by Duncan's test. I0, I1, I2, and I3 as define previously.

determined before the onset of terminal senescence. Hence, in cultivars that stay-green longer, the grain yield increases due to continued photosynthesis and an increase in grain weight (Chen *et al.,* 2010). SI delays leaf senescence and maintains photosynthetic activity, and ultimately increases grain yield. In our study, a significant and positive relation between grain yield and Chl concentration supports this conclusion (Table 5).

Generation and accumulation of ROS under drought stress causes injury to many cellular compounds such as lipids, proteins, carbohydrates, and nucleic acids. ROS induce lipid peroxidation and damage proteins, leading to membrane destruction, membrane permeability, electrolyte leakage from the membrane, and a decline in MSI (Jiang and Huang, 2001; Chen *et al.,* 2010). Bewley *et al.* (1979) reported the maintenance of cell membrane stability during drought stress as the main factor of plant tolerance to drought stress. In this study, due to the decrease in precipitation and increase in air temperature, the amount of damage to the cell membrane increased from the booting stage onwards. The highest

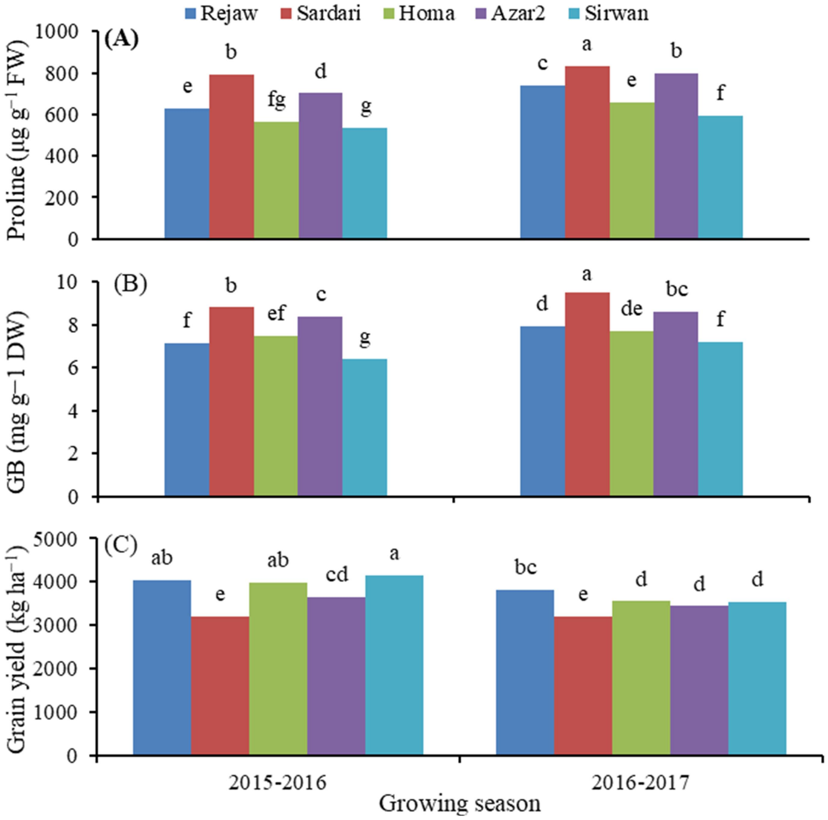

Figure 6. Mean comparisons of interaction effects of year×cultivars on (A) proline and (B) Glycine Betaine (GB) concentration, and (C) grain yield three days after SI at grain filling stage. Columns designated by the same letter are not significantly different at the P≤ 0.05 level as determined by Duncan's test.

Table 5. Pearson's correlation coefficients amongst five cultivars between chlorophyll (Chl a, Chl b, and Chl T), proline, and Glycine Betaine (GB) concentration, Membrane Stability Index (MSI) three days after SI at grain filling stage, and grain yield under four irrigation treatments during 2015-2017 growing seasons.

	Chl a	Chl b	Chl T	MSI	Proline	GB	Grain
Chl a	1						
Chl b	0.92^{**}	1					
Chl T	0.99^{**}	0.96^{**}	1				
MSI	0.24^{**}	0.50^{**}	0.32^{**}	1			
Proline	-0.80^{**}	-0.78^{**}	-0.76^{**}	-0.49^{**}	1		
GB	-0.74^{**}	-0.77^{**}	-0.81^{**}	-0.33^{*}	0.91^{**}	1	
Grain	0.78^{**}	0.77^{**}	0.79^{**}	0.43^{**}	-0.70^{**}	-	1

* P ≤0.05; ** P≤ 0.01, ns: Non-significant.

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MSI was related to the booting stage. There was a significant difference between cultivars in terms of MSI under different irrigation treatments. In general, the average MSI in Sirwan in all stages, especially the flowering and grain filling stages, was lower than other cultivars. Abid *et al.* (2018) revealed that the percentage of membrane damage in different wheat cultivars due to drought stress differed in corroboration with our findings. Examination of the changes in MSI at different growth stages showed that SI had positive effects on maintaining cell membrane integrity (Figures 1-D and 2-D). Considering a significant positive correlation between MSI and leaf chlorophyll concentration, the reduction in MSI was accompanied by a decline in photosynthetic pigment concentration (Table 5). A drastic decrease in the MSI during the grain filling stage can be due to the start of the senescence process (Khan *et al.,* 2015).

Proline and GB, two compatible osmolytes, accumulate in many plants due to osmotic stresses such as drought stress (Ashraf and Foolad, 2007; He *et al.,* 2011; Chun *et al.,* 2018). Tolerant cultivars usually accumulate more proline and GB than sensitive cultivars (Ashraf and Foolad, 2007; Chun *et al*., 2018). Proline and GB accumulation in the plant rises clearly under drought stress conditions, but its concentration declines posthaste after re-irrigation (Dien e*t al.,* 2019). Therefore, we monitored the proline and GB levels in flag leaves of wheat cultivars during drought and recovery periods at booting, anthesis, and grain filling stages. Our results indicated that drought stress enhanced proline and GB concentration in wheat cultivars, agreeing with previous reports of proline and GB accumulation during drought (He *et al.,* 2011; Chachar *et al.,* 2016). Evaluation of the trend of changes in proline and GB concentration in different stages demonstrates that these osmolytes' accumulation increased in the order booting<anthesis<grain filling (Figure 3A). The increase in proline and GB accumulation after the booting stage is due to the decrease in soil moisture and increase in air temperature in the terminal stages of growth. The results of previous investigations showed that the reduction in proline concentration in plants after removal of drought stress and re-irrigation had a steeper slope compared to GB (He *et al.,* 2011, Abid *et al.,* 2018). In the present study, although SI at the booting, anthesis, and grain filling stages reduced the concentration of both osmolytes compared to before irrigation, proline and GB concentrations three days after the recovery period showed that the severity of the decrease in proline concentration was more remarkable. Sardari and Azar2 exhibited greater accumulation of proline and GB during drought stress and more rapid recovery following drought (after SI) (Figure 3-A and Table 4). There was a significant negative correlation between the concentration of photosynthetic pigments and the concentration of proline and GB (Table 5). The decrease in the concentration of photosynthetic pigments under stress conditions might be due to osmotic regulators such as proline and GB, which are high in nitrogen in their structure (Sun *et al.,* 2009).

The results of this investigation revealed that, overall, the concentration of photosynthetic pigments, MSI, and grain yield in 2015-2016 was higher than the 2016-2017 growing season. In contrast, the proline and GB concentration was higher in the second growing season, which could be due to lower precipitation in the growing season (Table 2) and more severe drought stress during this growing season.

CONCLUSIONS

Supplemental Irrigation (SI) is a practice with high efficiency in mitigating the adverse effects of drought stress on rainfed wheat growth and grain yield in the Mediterranean region. In the present study, we determined the effect of SI at booting, anthesis, and grain filling stages on chlorophyll (Chl a, Chl b, and Chl T), proline, and GB, concentration, MSI changes, and grain yield in five wheat cultivars during two growing seasons. Our results indicated that SI treatments enhanced grain yield significantly through increased photosynthetic pigments, delay senescence, and MSI. Variation was observed among the cultivars in terms of the studied traits. Proline and GB accumulation and MSI in Sardari and Azar2 cultivars were higher than in other cultivars. In addition, these cultivars recovered more rapidly than other cultivars after SI at different stages. More studies are required to assess the effect of SI at different growth stages, especially emergence and jointing stages, on physiological and biochemical changes in various wheat cultivars.

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ارزیابی پاسخ‌های فیزیولوژیکی و بیوشیمیایی پنج رقم گندم به آبیاری تکمیلی

ل. مرادی، ع. سی‌وسه مرده، ی. سهرابی، ب. بهرام‌نژاد، و ف. حسین پناهی

چکیده

این آزمایش به منظور بررسی تاثیر آبیاری تکمیلی بر عملکرد دانه و روند تغییرات غلظت کلروفیل a، کلروفیل b، کلروفیل کل، پرولین و گلیسین بتائین و شاخص پایداری غشا پنج رقم گندم به صورت کرت‌های خرد شده در قالب طرح بلوک‌های کامل تصادفی با سه تکرار و در دو سال زراعی (۱۳۹۵-۱۳۹۴ و ۱۳۹۶-۱۳۹۵) طراحی و به اجرا در آمد. سطوح آبیاری (دیم، یکبار، دو بار و سه بار آبیاری در بهار) به عنوان فاکتور اصلی و پنج رقم گندم (سرداری، آذر ۲، هما، ریژاو و سیروان) به عنوان فاکتور فرعی در نظر گرفته شد. به طور کلی آبیاری تکمیلی باعث باعث افزایش غلظت کلروفیل a، کلروفیل b، کلروفیل کل و عملکرد دانه در ارقام مورد بررسی شد. تجمع پرولین و گلیسین بتائین که تحت شرایط تنش به شدت افزایش یافت بعد از اعمال آبیاری تکمیلی سریعا کاهش پیدا کرد، شدت کاهش غلظت پرولین بیشتر از گلیسین بتائین بود. میزان تجمع پرولین و گلیسین بتائین و همچنین میزان پایداری غشای سلول در ارقام سرداری و آذر ۲ بیشتر از سایر ارقام مورد بررسی بود. علاوه بر این ریکاوری از تنش خشکی این ارقام نسبت به سایر ارقام بیشتر بود. نتایج این مطالعه نشان داد که حفظ کارکرد ارقام در طول دوره تنش و همچنین ریکاوری آنها بعد از اعمال آبیاری تکمیلی نقش مهم و تعیین کننده‌ای در عملکرد دانه دارد.

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