ISSN 2226-3063 e-ISSN 2227-9555
Modern Phytomorphology 12: 124–130, 2018

https://doi.org/10.5281/zenodo.1812103

RESEARCH ARTICLE

Physiological, biochemical and yield responses of wheat cultivars to deficient water stress

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Received : 02.10.2018 | **Accepted :** 14.11.2018 | **Published :** 16.11.2018

Abstract

Two similar and concurrent experiments were carried out in the Agricultural Research Center of Islamic Azad University, Kermanshah Branch and Agricultural Research Center of Islamabad during 2016-2017. The experiments were performed in a split-plot format in a randomized complete-block design based on 3 replications. The main plots were assigned to 4 different regimes of irrigation: (I1) full irrigation during the growth period followed by 50% of the soil moisture depletion; (I2) water deficit stress from the start of the flowering stage (Z61) to the milking stage (Z77) associated with irrigation after 80% of the soil moisture depletion; (I3) water deficit stress from the beginning of the flowering stage (Z61) to the ripening stage (Z93) followed by irrigation after 80% of the soil moisture depletion; and (I4) water deficit stress from the start of the milking stage (Z77) to the ripening stage (Z93) followed by irrigation after 80% of the soil moisture depletion. The 3 cultivars of C1 (Sirvan), C2 (Pishtaz), and C3 (Marv dasht) were treated using the allocated sub-plots. The results revealed that by increasing the drought stress intensities on the wheat cultivars, Grain Yield (GY), Relative Water Content (RWC), and Total Chlorophyll Content (TCC) were decreased except for Proline Content (PC). Sirvan cultivar subjected to the treatment of drought stress (I3) led to the highest reduction in RWC and TCC compared to the control treatment (I1). The results of this study indicated that the wheat cultivars of Islamabad Agricultural Research Center had higher GY (5129 $kg.h^{-1}$), TCC (58 mg. g^{-1} fr.wt.), and RWC (92%) values than those of the Agricultural Research Center of Islamic Azad University, Kermanshah branch.

Keywords: Grain yield, Proline content, Relative water content, Water deficit, Wheat

Introduction

Among all the abiotic stresses (drought, coldness, high salinity, etc.), drought is the most severe limiting factor in plant growth and crop production (Seki et al. 2001). Drought is the condition, in which the amount of available water in the environment does not meet the requirements of a plant due to its high transpiration rates (Tuberosa 2012). Wheat is one of the most important crops in Iran and worldwide. In all the areas where it is grown in Iran, drought is a major limiting factor influencing its yield. Plant growth, GY, and quality are severely affected by drought, which causes it to undergo molecular, biochemical, physiological, and morphological changes (Zarafshar et al. 2014). Wheat yield under drought stress suffers serious moisture deficit throughout its growth period from seedling to full maturity (Bilal et al. 2015). Several studies have been conducted on the spring and winter wheat to evaluate the effects of water deficit stress on the crop production. Yield is mostly lowered when drought stress occurs during the heading or flowering and soft dough stages. GY is a critical and direct parameter for measuring drought damage. It has been well established that drought during the flowering and grain-filling stages can severely reduce wheat GY (Farooq et al. 2014). As stated by Pandey & Shukla (2015), many aspects of plant physiology, including net photosynthesis, RWC, TCC, and photosystem-II activity, are influenced by drought. It has been reported that RWC can be a direct indicator of water content for plants undergoing drought stress (Lugojan & Ciulca 2011).

It showed higher RWCs to be maintained by resistant compared to susceptible cultivars. Chlorophyll content has a positive relationship with photosynthesis rate (Gutierrez-Rodriguez et al. 2004) and maintaining its content at a high level for a long period of time can be one of the strategies for augmenting GY at water deficit conditions (Guo et al. 2008). On the other hand, Manivannan et al. (2007) stated that plant photosynthesis may be limited by drought stress, which can consequently lead to large reductions in chlorophyll content, chl*b* content, and finally TCC in crops. Moreover, Ashraf et al. (1994) reported that drought stress reduced the concentration of chlorophyll *b* (chl*b*) more than chlorophyll *a* (chl*a*). Plants can partly protect themselves against moderate drought stresses by accumulating osmolytes. However, Verbruggen & Hermans (2008) studied proline metabolism in plants mainly in response to osmotic stress. Therefore, the main purpose of this investigation was to analyze the physiological, biochemical and yield responses of wheat cultivars to drought stress.

Materials and Method

This research was conducted in the Agricultural Research Centre of Islamic Azad University, Kermanshah branch, Iran (47°8' E, 34°23' N; elevation 1351 m) and Islamabad Agricultural Research Centre, Kermanshah, Iran (47°26' E, 34°8' N; elevation 1346 m) during 2016-17 as shown in Tab. 1.

The experiments were carried out in a split-plot format based on a randomized complete block design in 3 replications. The main plots were allocated to 4 different irrigation regimes: (I1) full irrigation during the growth period followed by 50% of the soil moisture depletion; (I2) water deficit stress from the start of the flowering stage (Z61) to the milking stage (Z77) associated with irrigation after 80% of the soil moisture depletion; (I3) water deficit stress from the beginning of the flowering stage (Z61) to the ripening stage (Z93) followed by irrigation after 80% of the soil moisture depletion; and (I4) water deficit stress from the start of the milking stage (Z77) to the ripening stage (Z93) followed by irrigation after 80% of the soil moisture depletion. The sub-plots were allocated to the treatments of the three cultivars of C1 (Sirvan), C2 (Pishtaz), and C3 (Marvdasht). Based on De-marton's climate classification method, the climate of the Agricultural Research Center of Islamic Azad University, Kermanshah is cold and semi-arid with a mean annual rainfall of 435 mm and mean annual temperature of 13.5°C. Also, the climate of Islamabad Agricultural Research Center is cold and temperate with a mean annual rainfall of 538 mm and a mean annual temperature of 10.5°C. In the Agricultural Research Center of Islamic Azad University of Kermanshah, the silty clay soil texture with a pH value of 7.3 consisted of the Total Organic Matter (TOM) of 1.8%, Electrical Conductivity (EC) of 1.3 ds m^{-1}, Total Nitrogen (TN) of 8.13, available phosphorus of 0.04 $mg.kg^{-1}$, available potassium of 330 $mg.kg^{-1}$, and zinc, iron, and manganese contents of 1, 5.3, and 3.3 $mg.kg^{-1}$, respectively. The loamy clay soil texture examined in Islamabad Agricultural Research Center had a pH value of 7.5, TOM of 1.6%, EC of 1.1 ds m^{-1}, TN of 9.15, available phosphorus of 0.04 $mg.kg^{-1}$, available potassium of 220 $mg.kg^{-1}$, and zinc, iron, and manganese contents of 1.2, 5, and 4.2 $mg.kg^{-1}$, respectively. At the beginning of the season, the experimental area was prepared with Moldboard plow/Disking (MD). Each plot included 8 rows of 5 m length separated by a space of 20 cm, while the plots and blocks were kept apart within

Table 1 Meteorological condition in 2016-2017.

	Agricultural Research Centre of Islamic Azad University, Kermanshah Branch		Agricultural Research Centre of Islamabad	
	Air temperature °C	Precipitation mm	Air temperature °C	Precipitation mm
Months	Monthly Mean	Monthly Mean	Monthly Mean	Monthly Mean
10	18.7	0	16.9	0
11	10.6	115.9	8.7	131
12	3.1	0.5	2.9	0.8
1	4.4	4.9	2.2	10.4
2	3	76.7	1.5	68.2
3	4.4	23.3	2.6	34.3
4	11.8	24.4	13.3	45.7
5	18.4	15.6	17	17.9
6	24.9	0	22.5	0
7	28.1	0	27.5	0
8	29.8	0	27.4	0
9	25.9	0	23.2	0

the distances of 1 and 2 m, respectively. 400 seeds per square meter were considered for the wheat density. The planting procedure was arranged during the 2nd week of November. The 1st step of irrigation was immediately taken after seed planting. Water quantity for each irrigation cycle was determined based on the areas of the test plots and continuous measurements of their moisture contents using a wet HH2 device. Drought stress was imposed on the treatments by stopping irrigation during the targeted growth stages. Irrigation of all the plots through an installed pipeline system was performed by controlling the water input volume for each plot using an adjustable counter. Hand weeding was carried out to keep the crops free from weeds throughout the growth period. The GY was measured by harvesting the plants of the 4th and 5th rows of 3 m length from each plot center during the maturity stage. TCC, as well as the contents of chl*a* and chl*b*, was determined by extracting 0.5 g of leaf tissue using acetone 80% (v/v). The pigments were spectrophotometrically estimated at 645, 663 and 480 nm based on Arnon's method (1949). 80% acetone was applied as the blank. The relevant equations can be expressed as follows:

TCC (mg. mL^{-1}) = 0.0202 × A645 + 0.00802 × A663

Chl*a* (mg/mL^{-1}) = 0.0127 × A663 – 0.0027 × A645

Chl*b* (mg/mL^{-1}) = 0.0229 × A64 5– 0.0046 × A663

Leaf RWC was determined through the following formula:

RWC = (Mf-Md)/ (Mt-Md) × 100

Where Mf, Mt, and Md are leaf fresh mass, turgid mass, and dry mass, respectively (Boyer et al. 2008). Estimation of soluble carbohydrates was based on anthrone reagent application (Yemm et al. 1954). Free PC quantification of the wheat flag leaves was based on the proposed procedure by Bates et al. (1973). The two software systems of MSTATC and SPSS were utilized to conduct the obtained data analysis. The targeted traits underwent the analysis of variance. Duncan's Multiple Range Test (MRT) was employed for comparing the means ($p= 0.05$) and Pearson's correlation analysis was applied for assessing the correlations between the parameters. Finally, Microsoft Excel was used for constructing the diagrams.

Results and Discussion

Grain yield (GY)

As depicted in Tab. 2, water deficit stress and cultivar have a significant effect on GY ($p<0.01$). The highest and lowest GY value was observed to be related to Sirvan (C1) and Marvdasht (C3) cultivars, respectively (Tab. 2). Additionally, the highest and lowest GY value was found to belong to the treatments of I1 and I3, respectively. As reported by Samarah et al. (2009) and Alqudah et al. (2011), GY could be drastically alleviated by water deficit stress during the grain-filling period. Bahrani et al. (2009) declared declined GY of wheat to be caused by water deficit stress at the post-anthesis phase. Similar results were obtained by Moussavi-NIK et al. (2007), which discovered decreased wheat GY as a result of no irrigation treatment after pollination. All these findings are congruent with those of our experiments. In this regard, Nouri-Ganbalani et al. (2009) came to the same conclusions.

Relative water content (RWC)

Highly significant results were achieved based on the analysis of variance representing changes in leaf RWC under the impacts of water deficit stress and cultivar from the statistical viewpoint ($p<0.01$). Furthermore, the interaction between cultivar and water deficit stress indicated a significant effect on leaf RWC ($p<0.01$). The results are illustrated in Tab. 2. During our experiments, the leaf RWCs of all the cultivars were observed to significantly decline based on the levels of water deficit stress (Tab. 2). Accordingly, varied leaf hydration statuses and water deficit conditions, as well as physiological water levels, were evidenced in the different cultivars under study. Similar leaf RWC results were reported by Siddiqui et al. (2015) for faba bean cultivars under drought conditions. Among the studied cultivars, Sirvan (C1) and Marvdasht (C3) cultivars respectively revealed the highest and lowest RWCs under mild (I4), moderate (I2), and severe (I3) water deficit stress conditions (Fig. 1a). These findings indicated that Marvdasht (C3) and Pishtaz (C2) leaves would lose water quicker than those of Sirvan cultivar (C1) during water deficit stress conditions. The physiological analysis of RWC demonstrated the greater tolerance of Sirvan cultivar (C1) to water deficit stress as compared to the other studied wheat cultivars. The author suggests that the differences in the RWCs of the cultivars under water deficit stress conditions might be due to their different abilities to absorb water from soil or stomata abilities to alleviate water loss. This finding is in a good line with those of the other studies demonstrating the higher leaf RWCs of drought-resistant compared to drought-sensitive cultivars (Matin 1989; Turkan et al. 2005).

Chlorophyll content

As depicted in Tab. 2, water deficit stress and cultivar have significant effects on the contents of chl*a* and chl*b*, chl *a*-to-*b* ratio, and TCC ($p<0.01$). Also, the interaction between cultivar and water deficit stress has significant impacts on TCC ($p<0.01$) and ($p<0.05$) chl*a* content. The results were indicative of a significant decline in the TCC due to the decreases in the contents of chl*a* and chl*b* in all the studied wheat cultivars. As shown in Tab. 2, the highest and lowest reductions of chl*a* and chl*b* contents

Table 2. Effect of cultivar and water deficit stress on yield, physiological and biochemical characteristics in wheat (data derived mean two location).

Treatments	GY (kg.h⁻¹)	RWC (%)	TSCC (mg. TSC100. dry.wt⁻¹)	PC (mg. g⁻¹fr. wt.)	TCC (mg. g⁻¹fr. wt.)	Chla (mg. g⁻¹fr. wt.)	Chlb (mg. g⁻¹fr. wt.)	Chla/Chlb (mg. g⁻¹fr.wt.)
Water deficit stress								
I1	6935a	93.24a	7.54a	4.74d	66.53a	44.68a	17.67a	2.76b
I2	4359c	61.88c	9.23c	20.33b	37.74c	29.47c	3.97d	9.24c
I3	3590d	49.65d	6.62d	25.63a	30.63d	22.45d	6.89c	3.36b
I4	3556b	78.34b	8.40b	17.23c	57.80b	37.99b	12.98b	3.75a
Cultivar								
C1	5954a	79.58a	9.06a	18.76a	51.56a	38.54a	12.56a	3.11a
C2	5873a	71.33b	9.21a	15.54b	47.23b	33.85b	11.67b	3.33a
C3	5429b	64.93c	7.76b	13.53c	46.75b	30.66c	11.13b	2.36b
Location								
Azad	4751b	81.43b	9.43a	20.66a	56.12b	27.63b	8.93b	2.79b
Islamabad	5129a	92.35a	7.60b	14.53b	58.47a	36.57a	9.84a	3.54a
Water deficit stress	**	**	**	**	**	**	**	**
Cultivar	**	**	**	**	**	**	*	ns
Water deficit stress ×								
Cultivar	ns	**	ns	**	**	*	ns	ns
Location	*	**	*	**	*	ns	*	*
CV%	4.84	5.62	12.34	7.84	11.17	4.84	11.63	18.28

GY-Grain yield, RWC-Relative water content, TSCC-Total soluble carbohydrates content, PC- Proline content, TCC-Total chlorophyll content, Chla/Chlb-Chlorophyll a/b, Chlb-Chlorophyll b, Chla-Chlorophyll a. Within treatment means followed by the same letter are not significantly at p<0.05 according to Duncan's multiple range test. * -P<0.05, ** -p<0.01, ns-Non signification

have occurred to Marvdasht (C3) and Sirvan (C1) cultivars, respectively. Losses in the chlorophyll contents of such plants as, sunflower indicate the negative impacts of water deficit stress (Manivannan et al. 2007). On the other hand, Rong-Hua et al. (2006) reported that the drought-tolerant genotypes of barley possessed significantly higher chlorophyll contents compared to the drought-sensitive genotypes when subjected to water deficit stress. The highest decreases in the TCC and contents of chl*a* and chl*b* belonged to Treatment I3 as compared to the control treatment (I1) (Tab. 2). The maximum and minimum TCC and chl*a* content were found in the treatments of I1C1 and I2C3, respectively (Fig. 1b and 1c). This result is in good agreement with those of the other studies showing reduced or unchanged chlorophyll levels in other species during drought stress periods depending on drought duration and severity (Kpyoarissis et al. 1995). Wheat chlorophyll reduction under water deficit stress mainly results from the inhibited chain of photosynthetic electron transport and chlorophyll biosynthetic enzymes (Tavakkoli et al. 2010).

Proline content (PC)

As exhibited in Tab. 2, water deficit stress and cultivar have significant effects on PC (p<0.01). Also, there is a significant interaction between cultivar and water deficit stress (p<0.01). Proline is known to include the osmolytes produced by plants under drought stress. Accumulations of proline and soluble carbohydrates by plant tissues occur through an adaptive reaction when involved in the conditions of water deficit stress (Sheela Devi & Sujatha 2014). On the other hand, an increase in proline concentration caused by water deficit stress has been reported in other research studies (Bowne et al. 2012; Qayyum et al. 2013). Hayat et al. (2012) stated that proline overproduction in the plants undergoing varied environmental stresses induces a stress tolerance status via cell turgor maintenance, membrane stabilization, and regulation of Reactive Oxygen Species (ROS) concentrations. In our experiments, proline was found to significantly accumulate in the stressed (I2, I3, and I4) compared to the control (I1) plants (Tab. 2). This study demonstrated that the drought-resistant cultivar of Sirvan (C1) had a higher PC than Marvdasht drought-sensitive cultivar (C3) (Tab. 2). The maximum and minimum PCs were observed in the treatments of I1C1 and I2C3, respectively (Fig. 1d). An enhancement of proline concentration triggered by water deficit stress has been reported in other research studies (Serraj & Sinclair 2002). We deduced that proline accumulation might have had a role in wheat tolerance against water deficit stress. These results are in line with the data previously reported for wheat, in which higher proline amounts have been discovered to be accumulated in the drought-tolerant compared to the drought-sensitive genotypes (Safarnejad 2004).

Total soluble carbohydrate content

As represented in Tab. 2, water deficit stress and cultivar have significant effects on the total carbohydrate content (p<0.01). Among the studied cultivars, Sirvan (C1) and Marvdasht (C3) cultivars respectively had the highest and lowest carbohydrate contents under mild (I4), moderate (I2), and severe (I3) water deficit stress conditions

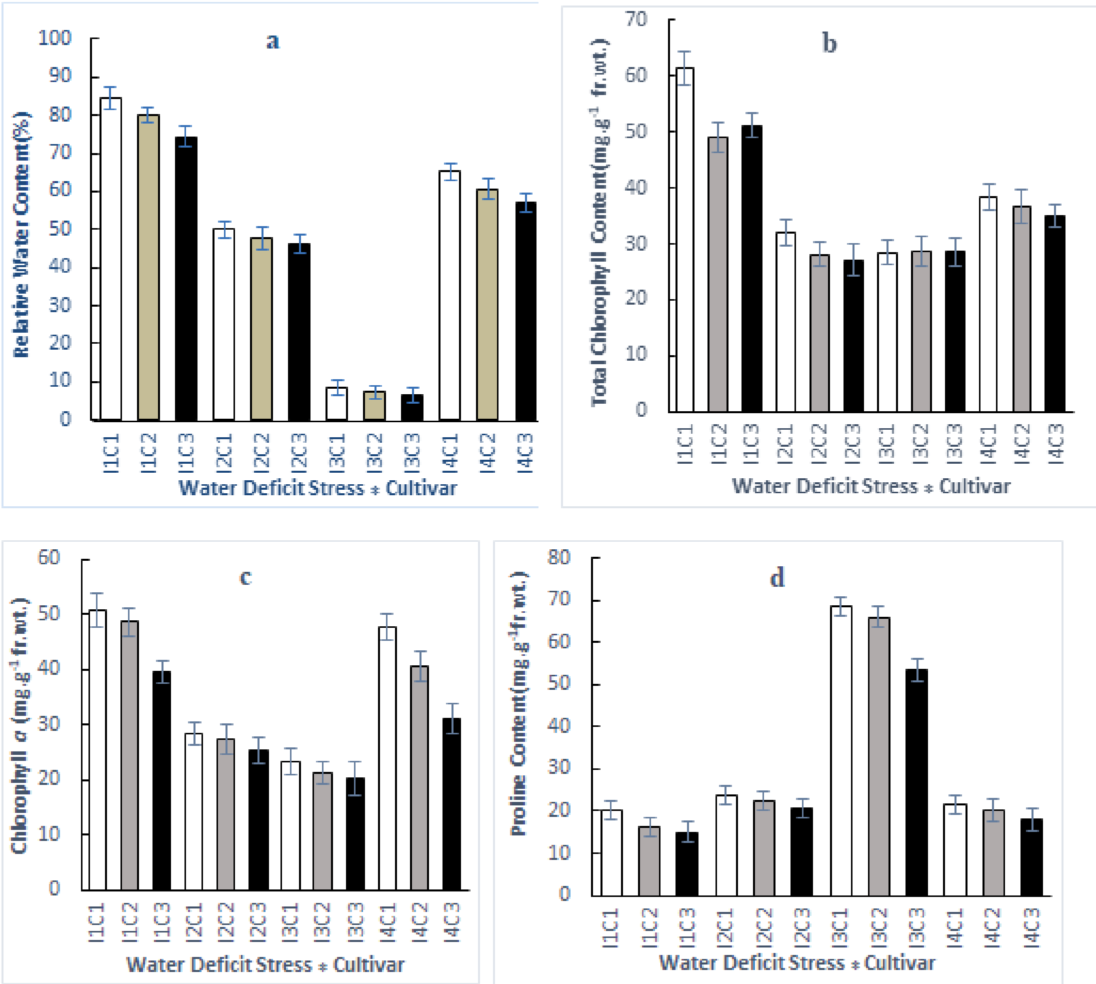

Figure 1. Effect of cultivar and water deficit stress on **a**-Relative water content, **b**-Total chlorophyll content, **c**-Chlorophyll a, **d**-Proline content. (I1) full irrigation during the growth period followed by 50% of the soil moisture depletion, (I2) water deficit stress from the start of the flowering stage) Z61(to the milking stage (Z77) associated with irrigation after 80% of the soil moisture depletion, (I3) water deficit stress from the beginning of the flowering stage (Z61) to the ripening stage (Z93) followed by irrigation after 80% of the soil moisture depletion, and (I4) water deficit stress from the start of the milking stage (Z77) to the ripening stage (Z93) followed by irrigation after 80% of the soil moisture depletion.. C1-Sirvan cultivar, C2- Pishtaz cultivar, C3- Marvdasht cultivar.

(Tab. 2). Accumulation of soluble carbohydrates serves as a vital mechanism for plant adaptation to drought stress (Zhou & Yu 2010). Our findings were indicative of the positive influence of water deficit stress on total carbohydrate content (Tab. 2). In all the cultivars, the total contents of carbohydrates were seen to augment by enhancing the levels of water deficit stress. These data are congruent with those achieved by Ali et al. (2016), who reported the elevated contents of soluble sugar in the leaves of the plants exposed to water deficit stress. Therefore, enhancement of total soluble carbohydrate accumulation is proposed as an efficient condition in response to water deficit stress in wheat, while providing an osmotic adjustment in this species.

Correlation between yield and physio-biochemical traits

The correlation coefficients between yield and physiological and biochemical traits suggested that RWC was positively and significantly correlated with chl*a* (0.72**), chl*b* (0.94**), TCC (0.91**), and proline (0.67**). Similarly, yield was significantly correlated with chl*a*, chl*b*, TCC, and proline, except for the total soluble carbohydrate and RWC, which indicated insignificant correlations (Tab. 3). Similar results were achieved by Bayoumi et al. (2008).

Table 3. Coefficients of correlation between yield, physiological and biochemical traits in wheat (data derived mean two locations).

Treatments	Chla	Chlb	TCC	PC	TSCC	RWC	GY
Chl*a*	1						
Chl*b*	0.89**	1					
TCC	0.97**	0.87**	1				
PC	-0.53**	-0.56*	-0.65**	1			
TSCC	0.45*	0.34 ns	0.23 ns	0.43*	1		
RWC	0.72**	0.94**	0.91**	0.67**	0.23 ns	1	
GY	0.76**	0.75**	0.73*	0.56**	0.14ns	0.78ns	1

Chla-Chlorophyll *a*, Chlb-Chlorophyll *b*, TCC-Total chlorophyll content, PC-Proline content, TSCC-Total soluble carbohydrates content, RWC-Relative water content, GY-Grain yield. * -P<0.05, ** -p<0.01, ns-Non signification

Physiological, biochemical and yield traits

The results of the analysis of variance revealed significant differences between the GYs, chl*a* and chl*b* contents, TCCs, and total soluble carbohydrate contents (p=0.05) of the different cultivars of the two studied regions, while highly significant differences (p=0.01) were evidenced between their RWCs, and PCs (Tab. 2). GY, chl*a* and chl*b* contents, chl *a*-to-*b* ratio, TCC, and RWC were higher in all the experimental treatments dealt with in Islamabad Agricultural Research Center compared to the Agricultural Research Center of Islamic Azad University of Kermanshah (Tab. 2). This was due to the higher distribution patterns of precipitation during the winter and spring in Islamabad region in contrast to the higher mean temperatures during March and April in the region of Islamic Azad University of Kermanshah in the year of the experiment. Moreover, the mean temperatures of the latter region were higher during the terminated wheat growth periods of May and June, thus resulting in the shortened reproductive period of wheat and its reduced yield (Tab. 1).

Conclusion

The results of this investigation revealed reduced wheat yield in all the studied cultivars under water deficit stress conditions. The different abilities of drought tolerance in the wheat cultivars could be reflected by their differential responses to the varied imposed water deficit stresses. The results of this research demonstrated that the studied cultivars experienced declined RWCs and chlorophyll contents, but augmented PCs and carbohydrates when facing water deficit stresses. Nonetheless, a high stability and potential for yield were documented for 'Sirvan' cultivar under drought stress conditions. Our correlation studies revealed a significant association between such agro-physio-biochemical traits as GY, RWC, total soluble carbohydrate, PC, and the contents of chl*a*, chl*b*, and chl *a+b* from among the other yield components. Hence, these relationships can serve as the selection criteria for screening the cultivars of potentially high drought resistance and GYs when having to deal with water deficit stress conditions. These findings further indicated the possibility of concurrently improving wheat yields and its biochemical and physiological traits at the conditions of water deficit stress.

Acknowledgment

This work was supported by a grant from the Research Council of Islamic Azad University, Kermanshah branch, Iran.

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