

The effects of drought stress on yield, relative water content, proline, soluble carbohydrates and chlorophyll of bread wheat cultivars

Shamsi Keyvan

Islamic Azad University, Kermanshab Branch, Iran.

Corresponding author email: keyvan@iauksh.ac.ir

Keywords

Bread wheat, Drought stress, Relative water content, Proline, Soluble carbohydrates, Chlorophyll

1 SUMMARY

The experiment carried out in 2007 and 2008 in the Dryland Agriculture Research sub – Institute Sararood, Kermanshah, Iran in order to study the effects of drought stress on yield, proline content, soluble carbohydrates content, relative water content and chlorophyll content of bread wheat cultivars under field conditions. The experiments were base on split plot in a randomized complete block design with three replications. The main plots included drought stress treatments at 4 levels: I1- drought stress at the start of stem elongation stage (31 Zadoks); I2 - drought stress at the start of boot stage (43 Zadoks); I3- drought stress at the start of grain filling stage (70 Zadoks) and I4- full irrigation. The Subplots included cultivars treatments at 3 levels: Chamran (C1), Marvdasht(C2), and Shahriar (C3). A significant difference ($p<0.01$) was observed between the drought stress treatments. The results showed that with an increase in the Intensity of drought stress on wheat cultivars, there was a decrease in relative water content , total chlorophyll content and increased proline content, but was not observed on trend relating to soluble carbohydrates content. The Chamran cultivar (C1) on drought stress treatment (I1) had the lowest decrease in relative water content and total chlorophyll content than with control treatment (I4). Also this cultivar had the highest drought tolerance and yield stability.

2 INTRODUCTION

Plants under natural and agricultural conditions are exposed to stress constantly. Drought limits plant growth and field crops production more than any other environmental stresses (zhu, 2002). Almost 32 percent of wheat culture face up to various types of drought stress during the growth season in developing Countries (Morris et al, 1991). Drought stress is the most important limiting factor of field crops in Iran. Most parts of Iran's cultivation land is placed in arid and semiarid regions and because of water deficiency, plant stress appears and wheat performance reduces severely in these regions. Drought stress is a decrease of soil water potential so plants reduce their osmotic

potential for water absorption by congestion of soluble carbohydrates and proline and in other words osmotic regulation is performed (Martin et al, 1993). Therefore osmotic regulation will help to cell development and plant growth in water stress (Pessarkli, 1999). It is defined that decrease of relative water content close stomata and also after blocking of stomata will reduce photosynthesis rate (Cornic, 2000). It is reported that high relative water content is a resistant mechanism to drought, and that high relative water content is the result of more osmotic regulation or less elasticity of tissue cell wall (Ritchie et al, 1990). The difference of relative water content (RWC) has been reported



as 18.6 and 21.8 percent for most resistant and most sensitive genotypes to drought stress in 2 years of experiment. (Merah, 2001). In studies that performed on 4 cultivars of bread wheat, RWC reduced to 43 percent (from 88% to 45%) by moisture stress (Siddique et al, 2000). (Mationn et al, (1989) represented a similar report as regards a drop in the amount of RWC in tolerant and sensitive cultivars of barley. Chlorophyll concentration has been known as an index for evaluation of source (Herzog, 1986), therefore decrease of this can be consideration as a nonstomata limiting factor in the drought stress conditions. There are reports about decrease of chlorophyll in the drought stress conditions (Majumdar et al, 1981; Mayoral et al, 1981; Kuroda et al, 1990) Also, it is reported that chlorophyll content of resistant and sensitive cultivars to drought and thermal stress reduced. But resistant cultivar to drought and thermal stress conditions had high chlorophyll content (Sairam et al, 1997). Other reports have represented that drought stress did not have effect on chlorophyll concentration (Kulshreshtha et al, 1987). (Pastori & Trippi,

(1992) expressed that resistant genotypes of wheat and corn had higher chlorophyll content than sensitive genotypes under the oxidative stress. Ashraf et al, (1994) also reported that drought stress will reduce concentration of chlorophyll b more than chlorophyll a. For the first time, accumulation of proline in plant tissues that have missed water was reported in 1954 (pessarkli , 1999). However it is reported that proline content in resistant wheat cultivars was more than in sensitive cultivar under the drought and salinity stress (kao, 1981). Also accumulation of proline has been reported under the drought stress in another various crops such as chick pea (*cicer arietinum*), (Ayerb & Tenori, 1998) corn (*zea mays*), (Serraj & Sinclair, 2002) and peanut (*Arachis hypogaea*) (Smith et al, 2002). Materials such as soluble carbohydrates have a role in osmotic regulations and conservation mechanism (Martin et al. 1993). The purpose of this research was to study drought stress effect on some biochemical processes of bread wheat cultivars, so that responses of these cultivars can be evaluated in resistance to drought stress.

3 MATERIALS AND METHODS

This research was done, in 2007-2008 on the Dryland Agriculture Research sub- Institute Sararood (47°, 20' E; 34°, 20' N), 1361 meter elevated from sea level. Based on dumarten's climate classification method, this area is a part of the cold semiarid regions. Sararood test soil had clay – loamy texture with EC= 1.3 ds.m-2 and PH=7.3. Main plots consisted of four drought stress treatments, i.e.: impose drought stress at initiation of stem elongation stage (31 of the Zadoks) (I1), at booting stage (43 of the Zadoks) (I2), at initiation of grain filling stage (70 of the Zadoks) (I3), and full irrigation (I4). Subplots included three cultivars, i.e.: Chamran (C1), Marvdasht (C2), and Shahriar (C3). Seeding operations were carried out on 23 November 2007. Based on soil analysis, required fertilizers were used as follows: 100 kg P2O5/ha-1 and 60 kg N/ ha-1 prior to planting and 60kg N/ ha-1 were used as topdressing in tillering stage. Each plot included 8 rows 20 cm

apart, 4 meter long, 1 and 2 meter distances were taken between test plots and replicates, respectively. Density was taken at 400 seeds per square meter.

3.1 Chlorophyll determination:

Chlorophyll content was determined by froze and Archioze method (Ferus & Arkosiva , 2001).

3.2 Relative water content: Relative water content was determined according to Schonfeld et al. (1999).

3.3 Proline determination: Proline was determined in flag leaves according to Pesci and Beffagna (1984).

3.4 Total soluble carbohydrate content: Total soluble carbohydrates were extracted according to a modified procedure described by Wardlaw and Willenbrink (1994).total amounts of wsc (mg wsc/100 mg dry weight) were determined as fructose equivalents using the anthrone colorimetric assay (Yemm and willis,



1954) at 620 nm on an IG-721 spectrophotometer.

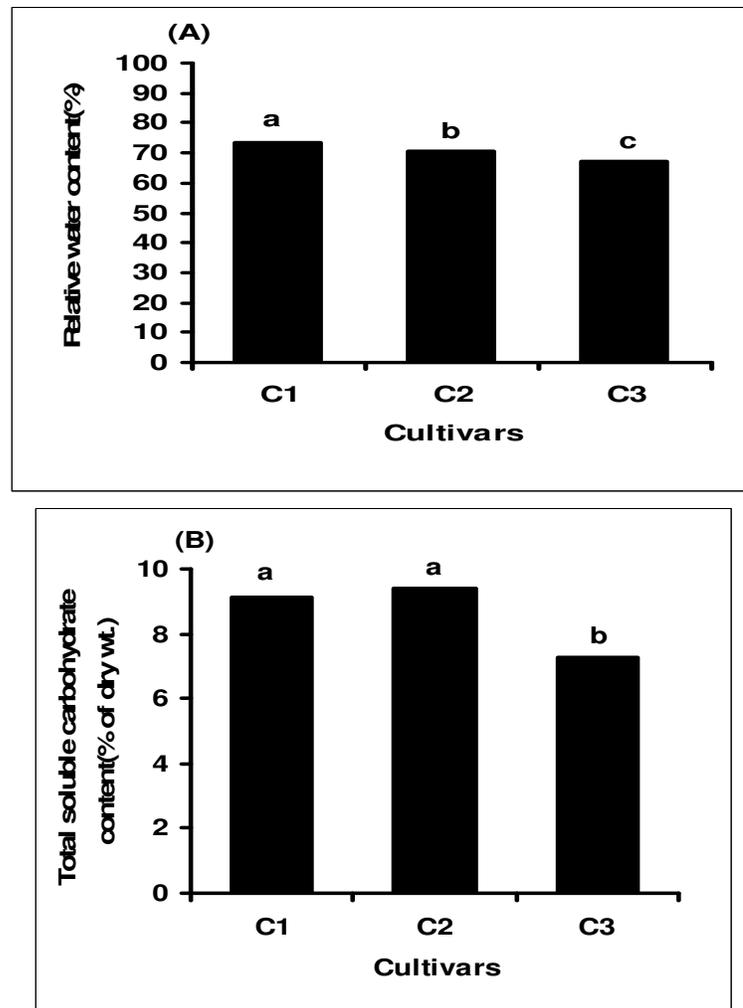
3.5 Grain yield: At the end of growth period, plants from rows 4 and 5 of each plot, 3 meter long, were harvested from each plot center, and grain yield were determined.

4 RESULTS AND DISCUSSION

In this research increase of drought stress caused a significant ($p < 0.01$) increase in proline amount. The mean comparison of flag leaf proline amount in the different wheat cultivars

3.6 Statistical Analysis: MSTATC and SPSS software were used to analyze obtained data. Analysis of variance was performed on targeted traits. Duncan's multiple range tests was used to compare means, and Excel software was used to construct diagrams.

represented shows that Chamran cultivar (c1) had the most amount of proline in flagleaf and Shahryar cultivar (c3) had the lowest amount (Figure1).



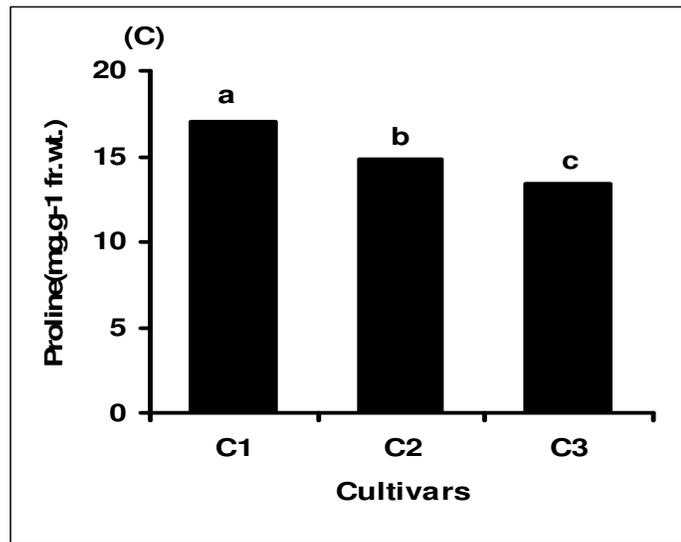
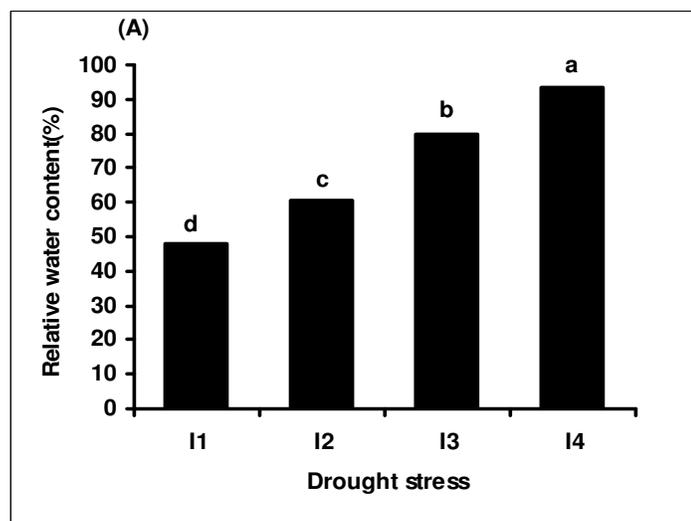


Figure 1: Effects of cultivar treatment on relative water content(A), total soluble carbohydrate content(B), proline (C) in the flag leaf of wheat. Letters on bars indicate results of Duncan's multiple range test. Different letters on the histograms indicate that the means differ significantly ($P < 0.05$)

Also, the mean comparison of flagleaf proline amount in various treatments of drought stress represented that the treatment of drought stress (I1) had the most amount that from a statistical

point of view had a significant difference ($P < 0.01$) with another levels (Figure 2).



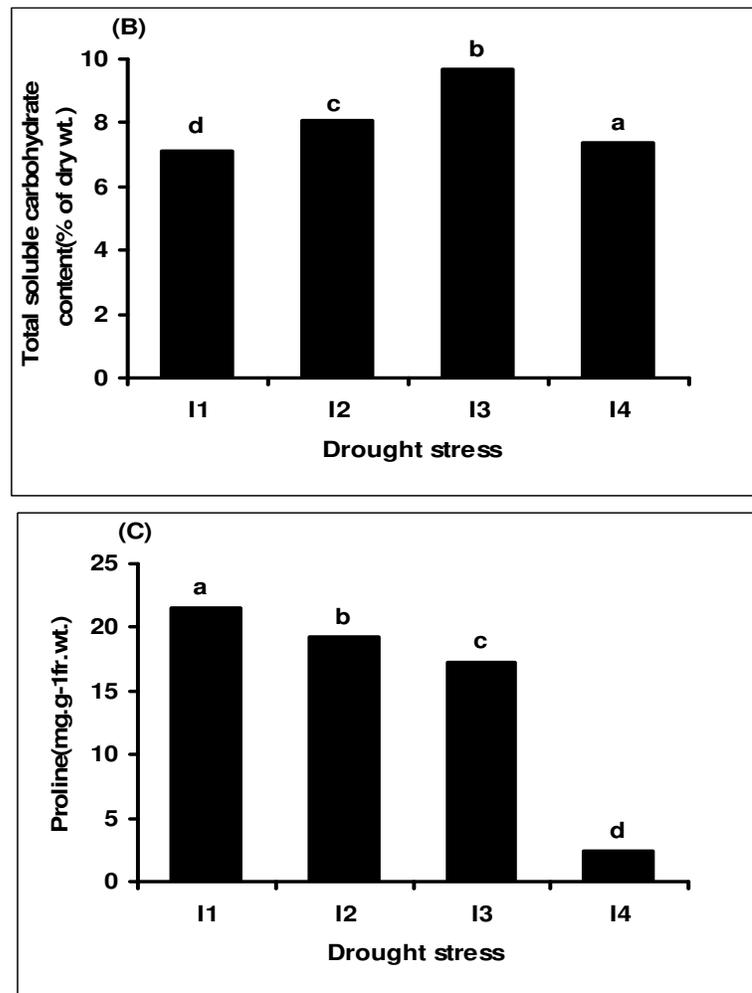


Figure 2: Effects of drought stress treatments on relative water content (A), total soluble carbohydrate content (B), proline (C) in the flag leaf of wheat. Letters on bars indicate results of Duncan's multiple range test different letters on the histograms indicate that the means differ significantly ($P < 0.05$).

Laik and Doftling, (1985) the proline amount measured in 2 cultivars amount was significant and was more in resistant cultivar. Ten & Hollaran ; (1982) expressed that the crops under the stress, accumulate the most amount of free proline in their leaves tissue by studying of changes and correlation of proline accumulation in spring wheat cultivars and comparison of performed experiments by another researchers . Increasing of proline amount due to drought stress has reported in another researches (Heuer, 1994; Safarnejad et al, 1996; Mattioni, 1997 ; Sharpe & Verslues, 1999; ; Staden et al, 1999; Serraj & Sinclair, 2002; Safarnejad, 2004). The results of variance

analysis represented that changes of flagleaf soluble carbohydrates amount under the effect of cultivar and drought stress and from a statistical point of view were so significant ($p < 0.01$) . The mean comparison of flag leaf soluble carbohydrates amount in different wheat cultivars represented that Chamran cultivar (c1) and Marvdasht cultivar (c2) had the most amount of soluble carbohydrates and Shahryar cultivar (c3) had the lowest amount. Also, the mean comparison of flagleaf soluble carbohydrates amount in varison treatments of drought stress represented that the treatment of drought stress (I3) had the most amount. According to wheat cultivars, concentration of



leaf soluble carbohydrates is variable between 1.1 to 10.4 percent on 14 to 20 days after flowering and according to cultivar, drought stress may be reduce or increase the concentration of these combinations in the leaf (Hossain et al, 1990). The difference of results of this study to results of some previous experiments on significant increase of soluble carbohydrates concentration that are under drought stress may be due to the test method. (Morgan, 1992). Drought stress had a significant effect on the content of chlorophyll a and b, and on the total of chlorophyll ($p < 0.01$). It had a significant effect on the rate chlorophyll a to b ($p < 0.05$). Content of chlorophyll a decreased because of drought stress and decrease of chlorophyll a, was lowest

in Chamran cultivar (c1) and highest in Shahryar cultivar (c3). Various cultivars were different significantly in different levels of drought stress so that the lowest decrease of chlorophyll a concern to Chanran cultivar (c1) and control treatment (I4) and the highest decrease related to Shahryar cultivar (c3) and treatment (I1). As the same of chlorophyll a, content of chlorophyll b decreased under effect of drought stress. In the light of chlorophyll b rate, various cultivars had a different reaction to drought stress, so that, the highest decrease of chlorophyll b was in Shahryar cultivar (c3) and the lowest decrease of chlorophyll b was in Chamran cultivar (c1) (Table 1).

Table 1: Effect of cultivar and Drought stress on chlorophyll (total, a, b and a/b ratio) and grain yield.

Traits	chl a ($mg \cdot g^{-1} fr.wt.$)	chl b ($mg \cdot g^{-1} fr.wt.$)	a/b ($mg \cdot g^{-1} fr.wt.$)	a+b ($mg \cdot g^{-1} fr.wt.$)	GY ($kg \cdot ha^{-1}$)
Drought stress					
I ₁	21.65D	7.707 D	3.037 B	29.07 D	3663 C
I ₂	27.06 C	2.940B	9.520 C	36.74 C	4312 B
I ₃	39.81 B	11.90 B	3.468 A	53.90 B	4725 B
I ₄	46.35 A	16.66 A	2.857 B	62.60 A	6793 A
Cultivar					
C ₁	37.55 A	12.49 A	3.122 AB	50.04 A	5118 A
C ₂	34.55 B	10.97 B	3.248 A	44.98 B	4967 A
C ₃	29.09 C	10.88 B	2.819 B	40.72 B	4536 B

With in treatments followed by the same letters are not significant at $p < 0.05$ according to Duncan's multiple range test .chl a chlorophyll a, chl b chlorophyll b, a/b chlorophyll a/b, a+b: total chlorophyll, GY: grain yield

Drought stress decreased the total rate of chlorophyll (the sum of chlorophyll a and b), too. The highest decrease of the total of chlorophyll, ratio to control treatment (I4), belonged to treatment (I1). In this case, various cultivars had different reactions, so that the highest rate of decrease of the total of chlorophyll (the sum of chlorophyll a and b) was in Shahryar cultivar (c3) and the lowest rate of decrease belonged to Chamran cultivar (c1) (Table 1). Cultivar and drought stress made an effect on the changes of flag leaf RWC and it was very significant statistically ($p < 0.01$).

In various wheat cultivars, the mean comparison mean of flag leaf RWC showed Chamran cultivar (c1) with 73.58 percent has percent has highest flag leaf RWC and Shahryar cultivar (c3) with 67.33 percent had lowest (Figure1). Also, in different treatments of stress, the mean comparison of flag leaf RWC showed drought stress treatment (I1) with 47.89 percent had highest decrease of RWC than control treatment (I4) with 93.56 percent (Figure 2). In fact, RWC, in drought stress decreased in all under testing cultivars and the same results of this test have reported in beans



(korir et al, 2006). On the other hand, difference in RWC of cultivars that are under drought stress may be for this reason that the ability of more absorption of water from soil or ability of stomata to reduce the loss of water is different. Schonfeld et al, (1988) expressed with increase of drought stress of wheat, RWC decrease and usually but not always, in drought stress conditions, the cultivars that are resistant to drought have more RWC. These results are the same as reports of Sinclair & Ludlow, (1985) and generally are agreement with result of this study. Cultivars and drought stress had a very significant effect on the grain yield ($p < 0.01$).

In various cultivars the mean comparison of grain yield showed Chamran cultivar (c1) had highest grain yield (5118 kg.h⁻¹) and Shahryar cultivar had lowest (4536 kg.h⁻¹). Also, mean comparison of grain yield in different treatment of drought stress showed drought stress treatment (I1) with 3663 kg.h⁻¹ had highest decrease of grain yield than control treatment (I4) with 6793 kg.h⁻¹ (Table 1).

Generally, following the effect of drought stress on chlorophyll content (the sum of chlorophyll a and b) that is means, production potential and storage capacity reduced too. This storage, in wheat, plays a main role in neutralizing the effect of drought on grain filling (Blum, 1998).

The results of this research specified that these cultivars when under effect of drought stress have a reduction of their chlorophyll content, RWC, proline and carbohydrates. Their yield is more to stability. It has reported under drought stress rate of chlorophyll a to b has increased on wheat (Ashraf et. al, 1994). Decrease of chlorophyll content and water potential of soil has represented in plants such as sunflower (Synerri et al, 1993) and Tobacco (Pastori & Trippi, 1992) that these results and reports correspond with results of this research. Correlation table between traits under studying represented that there is a positive correlation between rate of proline and soluble carbohydrates ($r = 0.310^*$). There is a positive and very significant correlation between RWC and chlorophyll a ($r = 0.944^{**}$), chlorophyll b ($r = 0.900^{**}$) and total chlorophyll (the sum of chlorophyll a and b) ($r = 0.947^{**}$), that is similar to results of work (Sigien & Leszczynska, 2004). On the other hand, there is a high and significant correlation between grain yield and RWC ($r = 0.880^{**}$), proline ($r = 0.860^{**}$) whole of chlorophyll (the sum of chlorophyll a and b) ($r = 0.843^{**}$), chlorophyll a ($r = 0.840^{**}$) and chlorophyll b ($r = 0.877^{**}$) that these results are similar to works of (Bennet et al, 1987; Schonfeld et al 1988; Bayoumi et al, 2008) (Table2).

Table 2: Correlation coefficient between studied traits

Traits	Chl a	Chl b	a+b	P	TSC	RWC	GY
Chl a	1						
Chl b	0.860**	1					
a+b	0.945**	0.926**	1				
P	- 0.695**	- 0.826**	- 0.728**	1			
TSC	0.231**	0.250	0.187	0.310*	1		
RWC	0.944**	0.900**	0.947**	0.805**	0.117	1	
GY	0.840**	0.877**	0.843**	0.866**	0.087	0.880	1

$P < 0.05$; $P^{**} < 0.01$; ns: Non - significant. Chl a: Chlorophyll a; chl b: Chlorophyll b; a+b: Chlorophyll a+b; P: proline content, TSC: Total soluble carbohydrate, RWC: Relative water content, GY: Grain yield

5 CONCLUSION

According to results, it can be concluded that plants in drought stress time, make changes in some of their physiological and biochemical features. One of these responses is proline

accumulation; the results showed its accumulation due to drought conditions (Girousse et al, 1996; Ingram & Bartles, 1996; Sharpe & Verslues, 1999; Safarnejad, 2004).

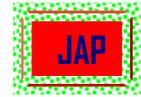


Also accumulations of soluble carbohydrates increase the resistance to drought in plant (Kameil & Losel, 1993). Also, the results of this investigation represented, in drought stress

conditions that the cultivars that have more relative water content (RWC) and chlorophyll content are more resistant to drought stress and their yield is stability.

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Proline content and soluble carbohydrate were increased in all of the three cultivars with enhance of NaCl concentration. By increasing of salinity stress accumulation of proline and soluble sugar content in leaves of Nosrat cultivar was more than other cultivars. As saltiness increases resistance natural responses in this plant gets better considering less decrease in chlorophyll amount and strategy of more production about praline and sugar solution compared to two other items.Â and measurement of proline, soluble sugar and chlorophyll content (a, b, a+b), (2) to evaluate abilities of these cultivars under salinity, (3) to compare and to introduce the most constant of cultivars to agricultural society. Materials and methods. Drought stress is one of the major abiotic stresses in agriculture worldwide. This study was carried out to investigate the effect of drought stress on proline content, chlorophyll content, photosynthesis and transpiration, stomatal conductance and yield characteristics in three varieties of chickpea (drought tolerant Bivaniej and ILC482 and drought sensitive Pirouz). A field experiment with four irrigation regimes was carried out in a randomized complete block design with three replications. Treatments included control (no drought), drought stress imposed during the vegetative phase, drought stress imposed during anthesis and drought stress during the vegetative phase and during anthesis. All physiological parameters were affected by drought stress. Water drought stress on soluble carbohydrates, chlorophyll deficit effects on solute contribution to osmotic and proline in four wheat cultivars with different adjustment as a function of leaf ageing in three climatic conditions of Iran, Iranian Journal of durum wheat (*Triticum durum* Desf.) cultivars Agricultural Sciences.Â Mohanty, N., 2003. Photosynthetic characteristics of "- amino acids in sugar beet plants in response to and enzymatic antioxidant capacity of flag leaf and osmotic, water and salt strees. Environmental and the grain yield in two cultivars of *Triticum aestivum* experimental botany, 36(1): 29-38. L.exposed to warmer growth conditions. J. Plant Physiol., 160: 71-74. 1192. Related Papers.