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ORIGINAL ARTICLE

Evaluation of Chlorophyll Fluorescence and Biochemical Traits of Lettuce under Drought Stress and Super Absorbent or Bentonite Application

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The effects of two superabsorbents (natural-bentonite) and (synthetic-A 200) on the chlorophyll fluorescence index, proline accumulation, phenolic compounds, antioxidant activity and total carbohydrate in lettuce (*Lactuca sativa* L.) was evaluated. For this purpose, a factorial experiment using completely randomized design with superabsorbents at 3 levels (0, 0.15, 0.30 w/w%), drought stress at 2 levels (60 and 100% of field capacity) and 4 replicates was conducted. Results showed that photosystem photochemical efficiency (Fv/Fm) II under drought stress (60% FC) as well as lower levels of bentonite superabsorbent polymer reduced. The minimum and maximum proline content were obtained in 0.3% bentonite, 100% FC and 0 benetonite, 60% FC, respectively. The lowest and highest phenolic compounds was corresponded to the highest levels in both super absorbents and control respectively, so that the super absorbent and bentonite, reduced phenolic compounds by 62.65 and 66.21% compared to control. 0 and 0.15 wt % bentonite in high drought stress (60% FC) showed the highest and 0.3 wt % bentonite and 100% FC attained the lowest level of antioxidant activity. Control bentonite treatment beds at 60% FC and beds containing 0.3 wt. % bentonite in 100% FC, showed the lowest and the highest total carbohydrate content respectively. Results of this study indicate that bentonite can reduce the negative effects of drought stress similar to artificial super absorbent.

Key words: water deficit stress, synthetic superabsorbent, natural superabsorbent, Chlorophyll fluorescence indicators

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Water forms the main factor concerning oriented agriculture in arid regions, thus the convergence of all agricultural operations must be used to optimize water utilization and to maximize efficiency per unit water (Koochaki and Alizadeh, 1986). Hence, the management of some of modifying additives such as super absorbent hydrogel in order to optimize the use of water in

agriculture to increase crop yield under drought conditions is of particular importance. Super absorbent polymer hydrogel are extremely hydrophilic and with high speed of water absorption and capacity, it provides water and nutrients for roots if required easily. These super absorbents which exist in natural and artificial forms are odorless and safe compounds for environment and plant tissue (Kabiri, 2005). Bentonite belongs to a group of super absorbent natural mineral 1:2 and is a mixture of clay minerals with high montmorillonite contents. It also shows high adhesion ability (Abedi-koupai and Sohrab, 2004). Due to the structure of the material, bentonite has the ability to absorb water and minerals, and also prevents the leaching of minerals from the soil, thus improves soil fertility (Stejskal. 1996). Further researches indicate that water limitation leads to photosynthesis decline and growth reduction which itself can perform in terms of lower yields (Cronic and Massacci, 1996). Photosynthesis reduction under drought stress in related to clutter biochemical pathways. Photosystem II is the most sensitive part to drought stress and oxygen receiving complex tension and recipient of the reaction center complexes in the system faces the greatest damage from drought (Giardi, 1996). Today, chlorophyll fluorescence is proposed as an index to measure the effects of environmental stresses, including water stress on crop species and determination of their drought resistance (Moffatt *et al*., 1990). In other words, chlorophyll fluorescence measurement proves the intact membranes of tylacoeids and shows the relative efficiency of electron transfer from the photosystem II to photosystem I. The lowest fluorescence of the system (Fo), appears when first Kinnon Primary quinone electron acceptor of photosystem II, is at fully oxidized state (open state photosystem reaction center II), and it increases gradually with reduction. This process will continue until full recovery of its molecules. In case of full reduction of the photosystem center, the highest fluorescence will show (Fm). Drought stress in fact by affecting carbon replication negatively reduces electron compliance and transport capacity and the system will reach (Fm) state faster resulting in varied fluorescence (Fv) decline. However, with increasing light intensity, the photosynthetic system loses the excess energy through non-photochemical blackouts via non-radiation process using a reducing energy regulation induced stimulation method. With this regulating mechanism, while protecting the reaction center, which causes the least damage to the center (Bhardway and Singhal, 1981). Hence photosystem II photochemical efficiency is expressed as the Fv/Fm ratio (the ratio of variable fluorescence to maximum fluorescence). Therefore, environmental stresses reduce this ratio by concerning photosystem II (Ma *et al*., 1995). One of the evaluation criteria for choosing varieties under drought stress is proline accumulation in various plant organs (Leinhose and Bergman, 1995). When plants are subjected to drought stress, increased protein breakdown and amino acid amides is accelerated. One of these amino acids is proline (Barker *et al*., 1993). Osmoregulation is a physiological process during which the accumulation of a series of plant material in cells, increases osmotic potential of the tissue under stress and the plant remains in a desirable state. Most of these compounds are amino acid and sugar. Proline and soluble sugar accumulation in plants as a defense mechanism are important in osmoregulation (Irigoyen *et al*, 1992). Increased proline and carbohydrates have been reported in

German chamomile in response of decreased soil moisture (Arazmjo *et al*., 2009). On the other hand, it seems that the phenolic compounds widely found in plants with high antioxidant activity are mainly gained from plant extracts (Bahramikia and Yazdanparast, 2008; Chatchawan *et al*., 2008; Karpinska *et al*., 2001; Candan *et al*., 2003; Senji and Yuuya, 2008; Yadegarinia *et al*., 2006; Muret *et al*. 2007). The key role of phenolic compounds as lubricants eliminating free radicals has been reported in several articles (Katalinic *et al*., 2006; Theriault *et al*., 2006; Aeschbach *et al*., 1994). It should be noted that phenolic compounds can effectively act as a hydrogen donor thus effectively act as an antioxidant (Golluce *et al*., 2007). Starch and sucrose metabolic products as mediators of glycolysis fatty acids for the production of carbon skeletons for construction and nucleotides go to work (Dennis *et al*., 2000; Kafi *et al*., 2009). Researchers found a high correlation between the accumulation of soluble sugars (sucrose, glucose and fructose) and drought tolerance in plants (Hoekstra and Buitink, 2001; Crowe *et al*., 1990; Ritchie *et al*., 1990). Chlorophyll fluorescence, proline content, phenolic compounds, antioxidant capacity and total carbohydrates, are among factors showed changes under drought stress and plant growth and yield can by affected by them. So to evaluate growing response of lettuceunder drought stress using two kinds of super absorbent natural and artificial, an experiment was conducted.

MATERIALS AND METHODS

To evaluate the effect of drought stress, super absorbent and bentonite on chlorophyll fluorescence index, shoot proline accumulation, phenolic compounds, antioxidant activity, and total carbohydrate of lettuce (*Lactuca sativa* L.), a factorial trial based on a completely randomized design with 4 replicates was conducted in Ferdowsi University of Mashhad during 2012-2013 in greenhouse conditions. Pots with 25 cm height and 30 cm width were used and after weighing each pot, they were filled with a mixture of surface soil and sand (2:1). Soil test analysis results indicated a sandy loam type and moisture content at field capacity and permanent wilting point were determined 24.4 and 12.5%, respectively (Table 1). A 200 superabsorbent at 3 levels (S0(0), S1(0.15), S2(0.3) w/w% equal to 0, 15, 30 g/10 kg soil per pot) and bentonite (B0(0), B1(0.15), B2(0.3) w/w % equal to 0, 15, 30 g/10 kg soil per pot) was mixed with the soil. Characteristics of the bentonite used are shown in Table 2. *Lactuca sativa* L. *cv* Siaho seeds were used for seedling production in this experiment and were transplanted to trial pots at 4-leaf stage. Full irrigation (100% FC) was performed up to 6-8 leaf stage to ensure establishment and deployment of transplants, by then drought stress treatments in two levels 60% (DS1) and 100% (DS2) was applied using field capacity method via daily weighing. Finally chlorophyll fluorescence, shoot proline content, phenolic compounds, antioxidant activity and total carbohydrate were measured.

Chlorophyll fluorescence indices: Chlorophyll fluorescence index (Fo, Fm, Fv, Fv/Fm) was measured using a strain gauge (Plant stress meter). Leaves darkness adaptation for 30 min, illumination device 400 microeinstein per square meter per second and irradiation time was set at 2 seconds.

Proline content: The proline content was estimated by the method of Bates *et al*. (1973). The plant material was homogenized in 3% aqueous sulfosalicylic acid and the homogenate was centrifuged at 10,000 rpm. The supernatant was used for the estimation of the proline content. The reaction mixture consisted of 2 ml of acid ninhydrin

and 2 ml of glacial acetic acid, which was boiled at 100 ◦C for 1 h. After termination of reaction in ice bath, the reaction mixture was extracted with 6 ml of toluene, and absorbance was read at 520 nm.

Total phenolic compounds: The amount of total phenolic compounds in plant extract samples was measured by the method of Folin Sykaltv (McDonald *et al*., 2001). According to this method, the test tube to 0.1 ml of ethanol extract (at a concentration of 1 mg/mL) or gallic acid standard ethanol solution (concentration from 25-300 µg) 5 ml Folin Sykaltv reagent (diluted with distilled water at 1:10) and 4ml sodium bicarbonate 7.5% was added and mixed. After 30 minutes at ambient temperature of the laboratory, spectrophotometer (Farmasya K L model of SPECT II Nova of England) absorption at 765 nm was recorded. Amounts of total phenol extract samples were determined using the standard curve in terms of mg gallic acid per gram of extract.

Measurement of antioxidant activity by DPPH: Antioxidant activity was estimated by the method of Abe *et al*. (1998). 100 mg of fresh plant material was extracted by methanol 99% (v/v %). Extract were then centrifuged at 3500 rpm for 5 minutes and DPPH solution was added. After an incubation period of 30 min at 25^0C, the absorbance at 517 nm was recorded. The free radical-scavenging activity of each solution was then calculated as percent inhibition according to the following equation:

% inhibition= (A blank – A sample)/A blank × 100

Total carbohydrates: 500 mg of fresh leaf samples were weighted and extracted with 10 ml methanol 99% (v/v %). Extracts were then centrifuged for at 3500 rpm for 15 minutes. 3 ml of Anthron indicator was added to the prepared samples at this stage and then they were boiled at 100 ^{0}C for 10 minutes. The absorbance read was 630 nm (Hedge and Hofreiter, 1962).

Statistical analysis: This experiment was conducted as a factorial experiment based on completely randomized design. Data were analyzed as factorial ANOVAs using SAS Version 9.1. Where significant (P≤0.05) treatment effects were determined by ANOVA, data means were separated by the LSD test.

RESULTS

Chlorophyll fluorescence indices

Results of variance analysis showed that water restrictions have a significant effect on all traits of chlorophyll fluorescence parameters and their interactions (P≤0.01) (Table 3). The difference between Fo and Fm (Fv) declines in drought stress conditions because of increased Fo and Fm reduction. In addition, combined treatments of super absorbent and bentonite with increased restrictions of water, Fv/Fm decreased and lowest value is observed in non-treated retaining moisture. Combination treatment of 0.3% bentonite and super absorbent obtained maximum photosystem photochemical II which represents a higher photosynthesis (Table 5). Results of chlorophyll fluorescence parameters showed that drought stress (60% FC) and reduction of bentonite and super absorbent levels reduced photochemical II efficiency (Fv/Fm) due to water limitation (Table 6).

Proline content

Results of shoot proline content in all of bentonite, drought stress and super absorbent except super absorbent and bentonite interaction were observed significant (P≤0.01) (Table 3). Shoot proline content increased by 15.62% from 100 to 60% FC. According to the results of mean comparison of the effects of drought and bentonite, the lowest proline content to the 0.3% w/w

bentonite and 100% FC (0.45), and the maximum value obtained without bentonite and 60% FC, (0.71 mg/g fresh weight) (Table 4). Combining of 0.3% w/w of bentonite and 0.3% w/w of super absorbent A 200 at 100% of field capacity reduced the proline 60.24%, compared to Control (Table 6).Combination of 0.3% w/w of bentonite and 0.3% w/w of Super Polymer with an average minimum Proline 0/39 mg per g fresh weight showed the best combination of media used to reduce injuries to drought stress (Table 5).

Phenolic compounds

Based on the results collected, it was revealed that the effects of water stress, bentonite, super absorbent and their interaction in phenolic compounds was greatly significant (Table 3) (P≤0.01). Greater amounts of phenolic compounds were observed in water restriction conditions (60% FC) and (control) so that compared to 100% FC and Combining of 0.3% w/w of bentonite and 0.3% w/w of super absorbent A 200 it was about 95/72% higher. Lowest and highest amounts of phenolic compounds, corresponds to the highest level of both super absorbent at 100% FC and control at 60% FC respectively (Table 6). The interactions of bentonite and super absorbent, difference between all beds were found in terms of phenolic compounds. Beds containing 0.3% w/w of bentonite and 0.3% w/w of superabsorbent polymer (8.98%) and the substrate with no superabsorbent (58/13%) showed the highest levels of phenols (Table 5).

Antioxidant activity

Based on the results of ANOVA table, drought stress, super absorbent and bentonite had a significant effect on antioxidant activity (Table 3) (P≤0.01). Water stress (60% FC) as well as lower levels and lacking both super absorbent increased the antioxidant activity (Table 6). 0 and 0.15 bentonite in 60% F.C stress, showed the maximum antioxidant activities (66.75 and 67.41 percent) and 0.3 bentonite in 100% F.C, was observed the lowest (26.24%) antioxidant activity recorded (Figure 1). Mean comparisons associated with increased levels of functionality superabsorbent and bentonite to decreased antioxidant activity in beds. The highest antioxidant activity (67%) was observed in control substrates (Table 5).

Total carbohydrates

Results (Table 3) showed a significant effect on all levels at (P≤0.01) and only the interaction effect of drought stress and super absorbent for total carbohydrates was significant at (P≤0.05). Changes of total carbohydrates are affected by stress and utilization of both artificial and natural super absorbent was significant, therefore an increase can be notified along with elevated humidity and increasing super absorbent levels (Table 6). No bentonite bed at 60% FC and beds containing 0.3% bentonite in 100% FC, showed the lowest and highest total carbohydrates respectively, (Table 4). Total carbohydrates in combination treatments of 0.3% w/w bentonite and 0.3% w/w of super absorbent polymer was maximum and 0.15% bentonite and no superabsorbent and control was the lowest among treatments and no difference significant was observed (Figure 2).

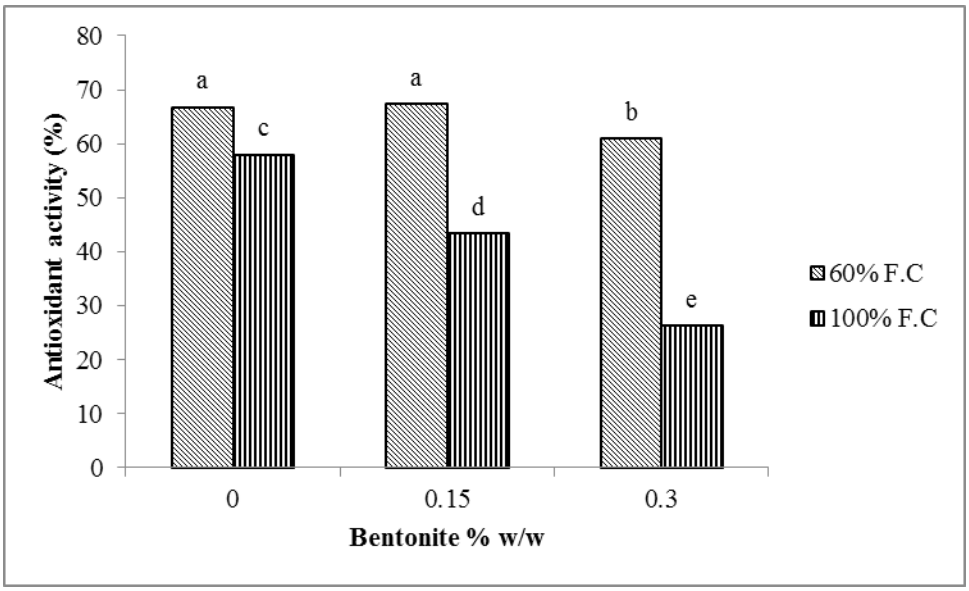

Figure 1. Interaction effects of drought stress and bentonite on Antioxidant activity

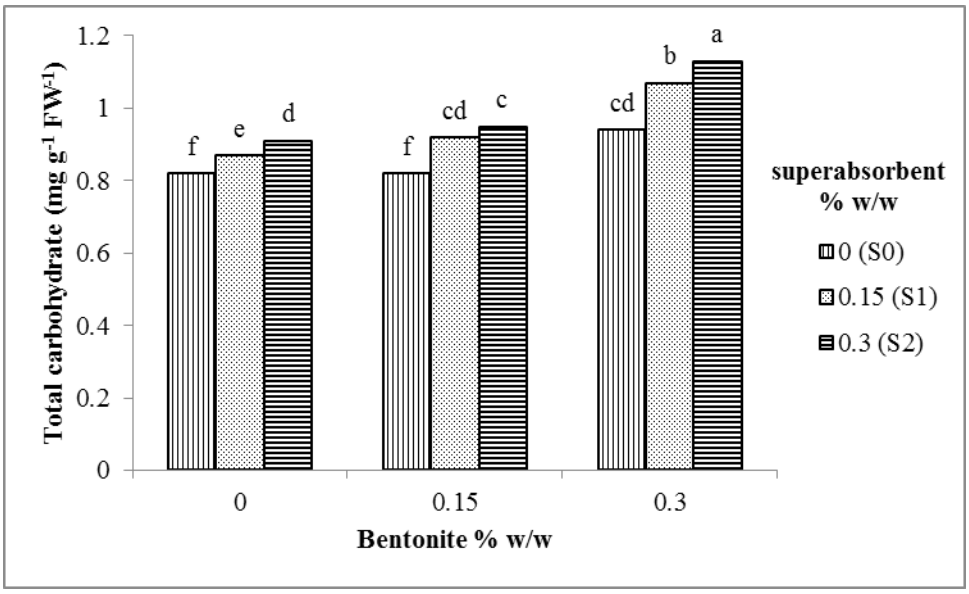

Figure 2. Interaction effects of bentonite and superabsorbent on Total carbohydrate

Table 1 : Physical and chemical characteristics of soil

Permanent Wilting Point (PWP) (%)	Field Capacity (F.C) (%)	Organic Compounds (%)	EC ($dS.m^{-1}$)	pH	K (ppm)	P (ppm)	N (ppm)	Texture, sandy loam (%) Sandy	loam	clay
12.5	24.4	0.281	1.20	8.12	25.01	1.22	8.25	70	18	12

Table 2 : Wt% bentonite constituents tested (%)

SiO_2	61.5-71.7
Al_2O_3	11.5-15.1
Fe_2O_3	2.4-3.2
MgO	1.1-3.4
Na_2O	0.5-3.5
K_2O	0.4-1.1
CaO	1.6-2.7
TiO_2	0.3-0.6

Table 3 : Mean square of drought stress , bentonite, superabsorbent levels and their interaction on Biochemical traits and Chlorophyll Index Fluorescence

		Chlorophyll Index Fluorescence				Biochemical traits			
Source of Variance	Df	Fo	Fm	Fv	Fv/Fm	Total carbohydrate (mg g^{-1} FW^{-1})	Antioxidant activity (%)	Total phenolics (mg g^{-1} FW^{-1})	Proline (mg g^{-1} FW^{-1})
DS(Drought Stress)	1	0.1289**	1.311**	1.112**	0.250**	0.069**	9114.97**	4274.42**	0.185**
B(Bentonite)	2	0.2175**	0.235**	0.186**	0.025**	0.231**	2165.80**	5556.49**	0.132**
S(Superabsorbent)	2	0.1768**	1.208**	0.777**	0.140**	0.126**	2555.90**	4524.57**	0.331**
DS.B	2	0.0168**	0.032**	0.008**	0.019**	0.049**	1019.46**	676.28**	0.007**
DS. S	2	0.0131**	0.087**	0.001**	0.033**	0.000 ns	876.28**	186.29**	0.005**
S.B	4	0.0149**	0.072**	0.039**	0.011**	0.006**	333.71**	313.75**	0.002*
S.DS.B	4	0.0084**	0.007**	0.004**	0.008**	0.002 ns	277.07**	39.72**	0.003**
Error	54	0.00019	0.00162	0.00023	0.00034	0.011	1.97	1.033	0.00094

ns, * and ** are Non-significant, significant at the 5% and 1% probability levels, respectively.

Table 4 : Means comparisons interaction of drought stress and bentonite levels on Biochemical traits and Chlorophyll Index Fluorescence

		Chlorophyll Index Fluorescence				Biochemical traits		
	Treatment	Fo	Fm	Fv	Fv/Fm	Total carbohydrate (mg g^{-1} FW^{-1})	Total phenolics (mg g^{-1} FW^{-1})	Proline (mg g^{-1} FW^{-1})
Drought Stress	Bentonite							
60%	0	0.47a	0.86f	0.36e	0.40d	0.85c	60.39a	0.71a
	0.15	0.25d	0.94e	0.36e	0.37e	0.90b	31.15c	0.63b
	0.30	0.28c	1.09d	0.54d	0.47c	0.91b	21.45d	0.59c
100%	0	0.33b	1.22b	0.61c	0.49b	0.88b	32.72b	0.63b
	0.15	0.21e	1.15c	0.65b	0.55a	0.89b	22.17d	0.54d
	0.30	0.21e	1.34a	0.75a	0.55a	1.13a	11.87e	0.45e

*In each column means followed by similar letter not significantly different (LSD: 0.05).

Table 5 : Means comparisons interaction of bentonite and superabsorbent levels on Biochemical traits and Chlorophyll Index Fluorescence

		Chlorophyll Index Fluorescence				Biochemical traits		
Treatment		Fo	Fm	Fv	Fv/Fm	Antioxidant activity (%)	Total phenolics (mg g^{-1} FW^{-1})	Proline (mg g^{-1} FW^{-1})
Bentonite	Superabsorbent							
0	0	0.52a	0.84g	0.34g	0.38f	67.00a	58.13a	0.78a
	0.15	0.37b	1.10d	0.51e	0.45e	61.75c	53.39b	0.67bc
	0.30	0.31c	1.18c	0.60d	0.50d	58.37d	28.14e	0.56fg
0.15	0	0.26d	0.89f	0.35fg	0.38f	65.37b	44.02c	0.70d
	0.15	0.26d	1.02e	0.51e	0.48d	57.25d	23.95f	0.57b
	0.30	0.17f	1.24b	0.66c	0.52c	43.50e	12.01g	0.49d
0.30	0	0.36b	0.89f	0.36f	0.39f	61.75c	29.34d	0.66e
	0.15	0.21e	1.21bc	0.73b	0.60a	38.25f	11.67g	0.51e
	0.30	0.16f	1.54a	0.85a	0.54b	30.73g	8.98h	0.39f

*In each column means followed by similar letter not significantly different (LSD: 0.05).

Table 6 : Means comparisons interaction of Drought stress,bentonite and superabsorbent levels on Biochemical traits and Chlorophyll Index Fluorescence

			Chlorophyll Index Fluorescence				Biochemical traits			
	Treatment		Fo	Fm	Fv	Fv/Fm	Total carbohydrate (mg g^{-1} FW^{-1})	Antioxidant activity (%)	Total phenolics (mg g^{-1} FW^{-1})	Proline (mg g^{-1} FW^{-1})
Drought stress	Bentonite	Superabsorbent								
60%	0	0	0.60 a	0.71 j	0.21 l	0.29 h	0.81 i	71.00 a	70.34 a	0.83 a
		0.15	0.43 b	0.91 g	0.41 i	0.44 f	0.85 hi	66.25 b	66.95 b	0.73 b
		0.30	0.38 c	0.98 f	0.47 h	0.48 e	0.89 gh	63.00 cd	43.87 d	0.57 de
	0.15	0	0.22 fg	0.83 h	0.23 kl	0.27 h	0.83 i	69.75 a	43.72 d	0.75 b
		0.15	0.31 d	0.95 fg	0.34 j	0.35 g	0.92 efg	69.25 a	33.24 f	0.60 cd
		0.30	0.21 g	1.05 e	0.51 g	0.48 e	0.95 ef	63.25 c	16.50 j	0.54 e
	0.30	0	0.36 c	0.83 h	0.25 k	0.29 h	0.83 i	66.50 b	31.10 g	0.77 b
		0.15	0.27 e	1.12 d	0.65 e	0.57 b	1.01 d	62.75 cd	18.32 i	0.55 e
		0.30	0.21 g	1.33 c	0.72 c	0.54 cd	1.06 c	53.50 f	14.95 k	0.46 f
100%	0	0	0.43 b	0.97 fg	0.47 h	0.48 e	0.82 i	63.00 cd	45.93 c	0.73 b
		0.15	0.31 d	1.29 c	0.62 f	0.48 e	0.89 fgh	57.25 e	39.83 e	0.62 c
		0.30	0.24 f	1.39 b	0.73 c	0.52 d	0.93efg	53.75 f	12.40 l	0.55 e
	0.15	0	0.30 d	0.95 fg	0.46 h	0.48 e	0.80 i	61.00 d	44.32 d	0.65 c
		0.15	0.20 g	1.08 de	0.67 d	0.62 a	0.91 efg	45.25 g	14.66 k	0.54 e
		0.30	0.12 i	1.43 b	0.81 b	0.56 bc	0.96 e	23.75 h	7.53 m	0.44 f
	0.30	0	0.36 c	0.96 fg	0.47 h	0.48 e	1.04 cd	57.00 e	27.59 h	0.56 de
		0.15	0.16 h	1.31 c	0.82 b	0.62 a	1.14 b	13.75 i	5.02 n	0.47 f
		0.30	0.11 i	1.75 a	0.97 a	0.55 bcd	1.21 a	7.97 j	3.01 o	0.33 g

*In each column means followed by similar letter not significantly different (LSD: 0.05).

DISCUSSION

Chlorophyll fluorescence indices

One of the attributes that have been affected by drought stress is the photosystem II yield quantum. Light redundancy is characterized by reduced photosystem II performance. This event occurs in two conditions; first, when the leaves are suddenly exposed to intense light which damages photosystem II center and second when exposed to water restriction. In this case, reducing the surge in non-radiation excitation energy results in energy release as heat (Mohammad *et al*., 1996). Ashraf *et al*. (2007) also reported low photosystem II quantum yield in maize and, Mehrjerdi *et al*. (2012), on pea and Mamnoei and Sharifi (2010) on barley too. Similar results have additionally been reported in a variety of studies (Fardad and Shirdeli, 1996; Mohammad *et al*., 1996; Legg *et al*., 2000).

Proline content

Proline is one of the membrane and protein protectants in stress conditions. Reduction in the rate of proline oxidation led to increased proline accumulation, which plays an important role in alleviating negetive effects of drought stress (Paleg *et al*., 1985; Santoro *et al*., 1992; Kiyosue *et al*., 1996). Different studies showed that the accumulation of proline is associated with drought tolerance of plants (Van Rensburg *et al*., 1993). Biological synthesis of proline starts with glutamic acid and reduction take place in following. Electrons from NADPH and NADH is provided for this process to carboxylase D-proline acid 5 (DP5C) so that in the final stage proline is formed by turning of P5C (Pyrronline-5-carboxylic acid) and enzyme-labeled DP5C. In fact, the synthesis of proline is derived from glutamate (drought stress) and Rtynyn (salt stress) which is due to the induction of enzyme activity (P5CS and P5CR) and inhibition of oxidizing enzymes such as PRODH and P5COH (Hare *et al*., 1999). Proline content increased along with drought stress severity. Proline molecules consist of both hydrophilic and hydrophobic segments. Soluble proline can affect other protein solubility and prevents abnormal albumin denaturation. This proline is characteristic is because the interaction between proline and other hydrophobic proteins are set due to the increased hydrophilic protein levels, which in turn increased the stability and prevented denaturation. Although proline content was not significantly affected by irrigation levels in German chamomile (Pirzad, 2007), but our results were in agreement with (Sanchez *et al*., 1998) on pea and (Zaifnejad *et al*., 1997) in sorghum. Proline accumulation in plants helps a brief period of drought survival and subsequent recovery therefore it will have a positive effect on yield performance. But this situation will not be true in long-term stress and proline accumulation will even have a negative effect on plant yield, because photosynthetic sources are derived to lateral functions other than seed formation (Sanchez *et al*., 1998).

Phenolic compounds

Plants have the ability to produce antioxidant compounds such as phenolic compounds under drought stress to protect cells against active radicals. Many phenolic compounds are known to act as antioxidants and can effectively remove hydroxyl and Proxyl radicals and prevent fat oxidation (Boscaiu *et al*., 2010). Changes in total phenols in pea leaves showed a significant reduction in elevated drought stress levels (Mehrjerdi *et al*., 2012). Phenolic compound decrease was also reported in sweet potatoes

under water stress (Lin *et al*., 2006). In triticale, water stress increased phenolic compound production in susceptible cultivars, but resistance cultivars change were not insignificant (Hura *et al*., 2007). This decrease could result from degradation of these compounds in response to oxidative stress conditions.

Antioxidant activity

Pootesmaeil *et al*. (2006) observed an increase in antioxidant enzymes such as catalase; superoxide dismutase and glutathione peroxidase activity in red bean and by reaching super absorbent polymer used up to 7%, enzyme activity reduced due to drought stress alleviation. It also stated that the increased enzyme activities above mentioned represent the stress condition and their effect on reducing oxidative stress alleviation and free radical scavenging role. Sheikh (2004) in an experiment on pea stated that water stress significantly increased catalase activity and super acid dismutase. Saie *et al*. (2005) showed that catalase activity was significantly affected by drought stress. They considered antioxidant enzyme activity as a determinant for drought tolerance in forage sorghum. Shokravi (2004) stated that glutathione peroxidase activity in sunflower plants will significantly increase under drought stress compared to control. Drought stress increased the activity of antioxidant enzymes (Oman *et al*., 2005). Hence the ability of these enzymes can be used as a marker for selection of drought-tolerant varieties of sunflower nuts. Jensen *et al*., (1996) stated that elevated drought stress, enhanced superoxide dismutase activity after 24 hours. Beds lacking super absorbent and drought stress conditions, highest antioxidant activity were observed in the current study, which are consistent with Pootesmaeil *et al*. (2006), Rahmani, (2007) and Jin *et al* (2006).

Total carbohydrates

Soluble sugars are among compatible osmolyts which are accumulated in stress situations and may act as osmotic protectant or osmotic guardians (Irigoyen *et al*., 1992). Various reports showed an increase in soluble sugars on pea (Sanchez *et al*., 1998) and alfalfa (Irigoyen *et al*., 1992), in response to drought stress. German chamomile showed no increase in soluble sugar even with the most severe levels of drought stress (Pirzad and Razban, 2011), whereas trigonella enhanced carbohydrate content in terms of water shortages (Shokhmgar *et al*., 2009). Carbohydrate enhancement as an osmotic regulator in drought stress (Sanchez *et al*., 1998) and protection of proteins against free radical oxidative damage is important. Reduction of carbohydrates in response to drought stress mentioned in some investigations has met their participation in osmoregulation with skepticism (Thakur and Rai 1980). On the other hand noticeable amounts of carbon that could have been used for plants growth are utilized for osmotic compounds (sugars) production and osmotic adjustment which led to growth decline (De Herralde et. al., 1998). No increase in soluble sugar of German chamomile, occurred even with the most severe drought (Pirzad, 2007).

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