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Full Length Research Paper

Effect of end-season drought stress on chlorophyll fluorescence and content of antioxidant enzyme superoxide dismutase enzyme (SOD) in susceptible and tolerant genotypes of durum wheat

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This experiment was conducted with 12 genotypes of durum wheat originating from Iran and Azerbaijan Republic in both stressed and non-stressed conditions in Agricultural Research Station, Islamic Azad University of Ardabil in a randomized complete blocks design with 4 replications and in two years, 2008 to 2009 and 2009 to 2010 agricultural years. In this experiment, in addition to physiological traits, traits like leaf chlorophyll content, initial fluorescence (F0), maximum fluorescence (FM), variable fluorescence (FV), efficiency potential (FV/FM) and the amount of superoxide dismutase enzyme (SOD) had been measured. The results showed that stress tolerant varieties had higher chlorophyll content and it is increased by stress operations of amount of superoxide dismutase enzyme in varieties to overwhelming stress. In this study, genotypes 8, 10 and 11 had stress tolerance, chlorophyll fluorescence levels as desirable, appropriate chlorophyll amount and ultimately optimized yield in stressed conditions. Also, the higher amount of superoxide dismutase enzyme (SOD) in these varieties, this represents these varieties can cope desirably with drought stress conditions. It is probably that genotypes 10 and 11 are consistent with regional conditions. Also high and meaningful correlation between chlorophyll content and yield (r = 0.56*) showed that by increasing the amount of chlorophyll, the yield rate will be increased. Finally, it was found stress tolerant and high-yield varieties had higher superoxide dismutase, as well as high amount of chlorophyll.

Key words: Chlorophyll fluorescence, drought stress, durum wheat, superoxide dismutase enzyme (SOD).

INTRODUCTION

Drought stress is one of the environmental factors limiting photosynthesis of plants (Malakouti et al., 2005). Two photic systems II (PS II) is very sensitive to inhibitory environmental factors and drought stress results in damage to PS II reaction centers. Using chlorophyll fluorescence techniques, it can be observed that there is an imbalance between metabolism and the production processes (Malakouti et al., 2005). Study of Chlorophyll fluorescence parameters is a simple and non-destructive technique and can be quickly measured. In F_0, the potential to applying stimulated energy photochemically is highest, and therefore the photochemical reduction of fluorescence is also highest. When light intensity is sufficient, fluorescence will increase from F_0 to its value, F_M. It represents the gradual increase in fluorescence yield while reducing acceleration of photochemical reactions.

Chlorophyll fluorescent measuring is a relatively new technology that in recent years to study the effects of different stresses including drought, salinity and temperature on photosynthetic efficiency (or yield) of leaves in the farm (or field) and greenhouse conditions convention is used (Zobayed et al., 2005). Climate

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Table 1. Genotypes name and regions.

No.	Genotype	Region	No	Genotype	Region
1	Leucurum(Tabriz)	Iran	7	(Omrabi15)	Iran
2	Melanopus(Cheiltoxm)	Iran	8	Leucurum(Kermanshah)	Azerbaijan
3	Leucurum(Germi)	Azerbaijan	9	Apulicum(xanlar)	Iran
4	Reichenbachi(11077)	Iran	10	Melanopus(Ahar)	Azerbaijan
5	Saiymareh	Iran	11	Hordeiforme(Maraghe)	Azerbaijan
6	Hordeiforme(shamxi)	Iran	12	Leucurum(Sarab)	Azerbaijan

changes in recent decades, leading to a decrease in the rainfall amount and distribution of it in the arid and semi arid regions of the world including the Middle East. So it seems according to the patterns of occurrence of drought changing, changing the appropriate strategies for reducing the difference between actual yield and yield potential of crops in these areas is necessary (Ort, 2002).

Factors that affect the amount of chlorophyll are: a) the light intensity in the amount of leaf chlorophyll and even different chloroplasts array has an effect within the cell. Chlorophyll of the shadow-friendly plants is more of the amount of light-friendly plants chlorophyll; b) The temperature is involved in the chlorophyll efficiency or its yield; so that in the plants which have the C_4 at a temperature from 30 to 45°C and in the plants with C_3 at a temperatures from 10 to 25°C, the chlorophyll has the best yield; c) The age of leaf with its chlorophyll content is directly related. Since the beginning of leaf emergence until its full growth, photosynthetic growth rate is increased and then gradually decreases. Yellow and old leaves due to loss of chlorophyll lose their photosynthetic power.

The water in synthesis of chlorophyll is very important. After a heavy rain the amount of chlorophyll is increased, but in the arid time its value decreases. On the other hand, if the soil is water saturated, leaves chlorophyll content decreases. Leaf water content to maintain the maximum amount of chlorophyll should be high (Bohrani and Habibi, 1992). In the green plants chlorophyll tissue under environmental stress in leaves of susceptible cultivar is decreased, but increased in the resistant cultivar and resistant cultivars leaves are to the susceptible cultivar has a darker green color. Rapid loss of chlorophyll in cold-sensitive cultivars is caused to decrease photosynthetic activity. Several environmental factors on plants cause chlorosis or yellowing.

Chlorophyll is one of the basic pigments of plants that a reduction of concentration causes chlorosis, growth reduction and the yield (Khoshkhoghm and Ando, 1995). Plants cope with oxidative stress with induced high-efficiency defense systems which are able to destroy or neutralize free radicals. This defense system includes superoxide dismutase (SOD), catalysis (CAT), Ascorbate peroxidase (APX) and glutathione reductase (GR) and non-enzymatic systems including ascorbate, tocopherol, carotenoids, and miscellaneous compounds (that is, flavonoids, mannitols and polyphenols) (Baker, 2004). Multiplicity and plurality of defense systems is due to producing reactive oxygen sorts (http://tse-co.blogfa.com) in cells and different under-cell sectors, and they are also different for features like diffusion capability, solubility and tendency to react with various biological molecules. Therefore, a series of interconnected molecules to acting in defense of both organic phase and in all parts of the cell membrane to disable radical is as fast as they are formed, are needed.

Drought stress lead to damage in chlorophyll and change in rate of chlorophyll fluroscence and finally decreas in yield. Varieties with high chlorophyll content are more resistant to drought stress condition.

This study was conducted to evaluate effect of drought stress on physiological indices, fluorescence index, superoxide dismutase enzyme and the relationship between chlorophyll content and drought stress tolerance in durum wheat genotypes in Ardabil region.

MATERIALS AND METHODS

The present work was carried out at the agriculture research center, Islamic Azad university of Ardabil branch, Iran, in two agricultural years (2008 to 2009 and 2009 to 2010), using 12 Iran and Azerbaijan oriented durum wheat cultivars (Table 1), arranged as randomized complete block design (RCBD) under irrigated and rain fed conditions with four replications. Randomization was done for three replications by MSTATC software. This experiment was done by use of 50 varieties in previous years. Selected varieties for study on chlorophyll had uniformity in maturation. Irrigation was performed according to local custom and wheat need for both conditions to flowering stage, and stress treatment was exposed to stress after flowering.

Stress treatments included:

1. Whole irrigated (100% used water based on the plant demand at various growing stages).
2. Limited irrigation (water supply until a thesis and after wards drought employment as water is withheld until the end of growing stage). Nylon covers were used for control of water under stress treatments.

To determine physical and chemical properties of soil tests, soil sampling before land preparation operations were performed. Samples 0 to 30 cm and 30 to 60 cm depths were selected after laboratory analysis of soil and water in the Islamic Azad University of Ardebil; the results in Table 2 are shown, and the results of rainfall for 2 years are in Figure 1.

Each genotype was planted on five rows placed 150 cm apart

Table 2. Soil analysis results.

Soil type	Soil texture			Absorbent Potassium (ppm)	Absorbent Phosphorus (ppm)	Total nitrogen (%)	Organic carbon (%)	Neutral-reacting material (%)	Electrical conductivity		Saturation	Depth (cm)
	Sand	Silt	Clay						(PH)	(ds /m)		
Clay loam	31	41	28	460	8-Apr	0/103	0/97	8-Apr	8-Jul	Feb-66	48	0-30
Clay	40	36	24	290	2	0/056	0/47	7	2-Aug	4-Feb	45	30-60

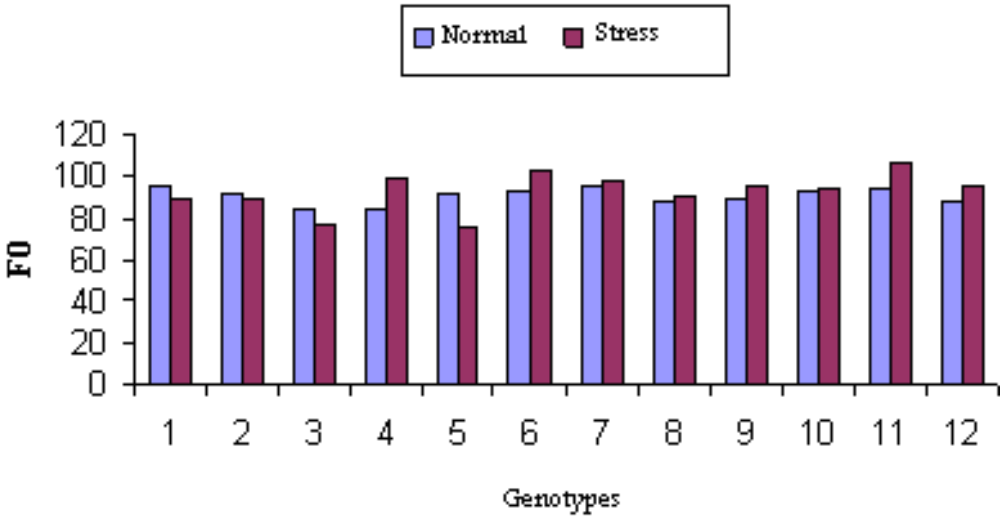

Figure 1. The amount of initial fluorescence (F0) for under-study varieties.

(Because, seeds were obtained from a single plant and were very low), with four replications. Distances between irrigated and drought blocks were 1 m but were 2 m between the two irrigated or drought blocks. Upon the planting, irrigated was performed for whole blocks to moisten soil profile in the photosphere of all cultivars to facilitate germination. Irrigation was done as flooding at the harvest time, to prevent border effect, 50 cm of each row from both sides were eliminated to harvest and following traits were measured: plant height, total number of tillers, fertile tillers, peduncle length, main spike length, main spike weight, total plant dry weight, number of seeds per spike, number of seed seeds per main spike and seed weight per main spike. Also, seed yield of each block was measured (All the farms were sampled). Drought stress can produce ROS. At the first stage, ROS is causing damage on chlorophyll. Excessive ROS production can cause oxidative stress, which damages plants by oxidizing photosynthetic pigments, membrane lipids, proteins and nucleic acids (Yordanov et al., 2000). Because of this, an experiment was done in the stress condition.

Chlorophyll content of the flag leaves was detected with a chlorophyll meter device CCI-200 which was manufactured by Opti-science company. This device is measures the chlorophyll content index of leaves.

In order to measuring chlorophyll fluorescence rate, one month after anthesis, we apply from plant stress meter (Bio Monitor SCI AB) device which is portable. To do this, first, two special clamps are attached two complete third and fourth leaves above the plant after ensuring their valves are closed, so that were leaves placed in darkness and light reaction of photosynthesis is stopped, and for this purpose leaves are placed in darkness for 40 min and

Table 3. Mean comparison of meaningful traits for under-study genotypes.

Genotypes	Leaf chlorophyll	Plant height	Main spike length	Grain weight per main spike	Spikes weight	Yield
1	33/ 71^{BC}	120/2^{BC}	4/89^{D}	1/43^{A}	4/07^{D}	81/65^{AB}
2	33^{BCD}	102/4^{D}	6/46^{AB}	1/25^{A}	4/64^{CD}	89/70^{AB}
3	19/83^{FG}	113/3^{CD}	6/26^{BC}	1/84^{A}	6/55^{ABC}	77/63^{AB}
4	23/86^{EF}	120/5^{BC}	5/15^{BCD}	1/69^{A}	6/19^{ABC}	84/92^{AB}
5	15/13^{G}	134/3^{AB}	5/72^{BCD}	1/43^{A}	5/83^{ABCD}	79/47^{AB}
6	25/20^{DEF}	102/5^{D}	5/29^{CD}	1/53^{A}	5/01^{BCD}	96/63^{A}
7	94/19^{A}	117/9^{C}	7/40^{A}	1/41^{A}	5/02^{BCD}	60/72^{B}
8	30/56^{BCDE}	122/7^{BC}	5/14^{CD}	1/38^{A}	7/21^{A}	92/35^{A}
9	27/11^{CDEF}	108/3^{CD}	5/72^{BCD}	1/42^{A}	6/84^{AB}	78/20^{AB}
10	38/38^{AB}	140/6^{A}	6/19^{BC}	1/42^{A}	5/69^{ABCD}	74/68^{AB}
11	42/91^{A}	81/07^{E}	5/71^{BCD}	1/73^{A}	6/18^{ABC}	89/25^{AB}
12	13/27^{G}	110/5^{CD}	5/44^{BCD}	1/34^{A}	5/55^{ABCD}	84/43^{AB}

afterwards the clamps attached to the fiber optic of device and the clamp valves are opened, and as we turn on the device, the modulated 695 nm light shining to leaf through the fiber optic and fluorescence parameters like initial fluorescence (F0), maximum fluorescence (FM), variable fluorescence (FV) and the yield potential (FV / FM) which are appeared on device are noted. Fourth and fifth leaves from the top of the plant were chosen for measurements. Because they had the modest condition in making light and shadow set. To increase the test accuracy, three devices applied for simultaneously measurements at 11:00 am.

To extract the superoxide dismutase enzymes (SOD) and catalysis (CAT), 0.5 g leaf, along with 5 ml cold buffer is cooled already in a chinaware tub and then pulverized in a container of ice. The buffer includes potassium phosphate 0.1 mol with PH = 7.5 which has 0.5 m/M EDTA. Blending process had been passed by means of cotton (Mel Mel) and the resulted extract was placed on centrifuge at 15 000 round (model Eppendorf) for 15 min. High transparent material can be seen as enzyme extract (Sairam and Saxena, 2000).

Superoxide dismutase activity was evaluated in accordance with Sayram and Srivastava (2000). A 3 ml reaction mixture included 13 m/mol Mitionin, 25 m/mol nitro bluetetrasolium (NBT), 0.1 m/M EDTA, 50 m/M buffer phosphate (pH = 7.8), 50 m/mol bicarbonate sodium and 0.1 ml enzyme extract. Adding 2 m/mol riboflavin and placing pipes under 15 watt fluorescent bulbs light at a distance of 30 cm and for 15 min, SOD enzyme reaction would be start. After 15 min, the tubes covered with aluminum foil and the lamps were turned off. Control tube lacking the enzyme created the most color. Numbers of optic absorption of samples were recorded in the wavelength 560 nm. It is noteworthy that one unit of enzyme activity as an enzyme content results in decreasing absorption amount of samples to 50% at the afore-mentioned wavelength as compared with control sample. Catalysis activity was evaluated according to Liu and Huang (2000). A 3 ml reaction mixture contained 50 m/M buffer phosphate (pH = 7), 15 m/mol hydrogen peroxide (H_2O_2) and 0.1 ml is enzyme extract. CAT enzyme reaction would be started by adding enzyme extract. Optical absorption changes of samples was recorded every 20 s and performed for 5 min. Liu and Huang (2000) reported that each 0.01 change in the absorption of samples in one minute, is recognized as a unit of catalysis enzyme activity (Liu and Huang, 2000).

To determine the susceptibility and tolerance rates of the genotypes, stress tolerance index (Fernandez, 1992) was used:

Stress tolerance index (STI):

STI= (Ypi*Ysi)/Yp2 (Fernandez, 1992)

Where, Ysi= yield of cultivar in stress condition; Ypi= yield of cultivar in normal condition; Yp= total yield mean in normal condition

Traits were measured based on instruction of randomized complete blocks design in variance analysis and treatments mean were compared by LCD method. Also, in order to analyze experiment data, we used softwares PATH2, SPSSI6, Excel. Causality analysis had been performed about yield on the basis of residual traits from multivariable regression by stepwise.

RESULTS AND DISCUSSION

The data was analyzed with mean of two years: Mean comparison of traits (Table 3 and 4) showed that for leaf chlorophyll, genotypes 7 and 11 had the highest value. Presence of leaf chlorophyll to increase photosynthesis in plant is essential and is also one of the effective factors to increasing yield. So it likely seems that these genotypes have a higher yield than other genotypes. Yield comparison of genotypes was nearly consistent with this theory and yield level of genotype 10 was higher than the other genotypes, whereas genotype 7 has the lowest yield. But genotype 7 had the most main spike length and was promising in main spike for grain weight. Spike-related traits that are involved in the yield levels can also be very important, genotype 8 having the highest ears weight, was ranked as second between genotypes for yield. So focus on these traits can be useful on the yield increase. Existence of significant interactions in ANOVA table led to non-significant main effects.

Evaluation interactions between genotypes and under-study conditions (Table 5) showed that genotype 5 has the highest grain yield in irrigation conditions, genotype 7 has a maximum plant height in irrigation conditions, genotype 2 has the highest amount of total weight of the plant in irrigation conditions, genotype 4 has the highest

Table 4. Mean comparison of genotype interactions in the environment.

Interaction of G×C	Plant height	Total plant weight	Spikes weight	Yield	Harvest index
1×N	127/7^{ABCD}	7/63^{ABCDE}	3/35^{G}	93/55^{ABCD}	25/43^{BCDEFG}
2× N	106/6^{EFGH}	9/7^{A}	7/41^{DEFG}	75/25^{ABCD}	19/69^{EFG}
3× N	126/7^{ABCDE}	6/2^{CDE}	7/02^{ABCD}	89/45^{ABCD}	20/54^{DEFG}
4× N	124/6^{ABCDE}	8A^{BCDE}	8/67^{A}	91/2^{ABCD}	23/12^{BCDEFG}
5× N	134/3^{ABCD}	8/8^{ABCD}	6/6^{ABCDEF}	108/4^{A}	25/59^{BCDEFG}
6× N	115/2^{DEF}	6DE	4/01^{EFG}	102/4^{ABC}	25/45^{BCDEFG}
7× N	143/1^{A}	9/13^{ABC}	4/12^{EFG}	85/55^{CD}	17/36^{FG}
8× N	124/6^{ABCDE}	7/35^{ABCDE}	8/25^{AB}	103/7 ^{AB}	24/8^{BCDEFG}
9× N	124/8^{ABCDE}	7/8^{ABCDE}	7/63^{ABC}	80/25^{ABCD}	22/4^{CDEFG}
10× N	142/8^{A}	7/5^{ABCDE}	6/47^{ABCDEF}	85/65^{ABCD}	19/42^{EFG}
11× N	89/43^{HI}	7/5^{ABCDE}	6/65^{ABCDE}	89/75^{ABCD}	26/03^{BCDEFG}
12× N	132/4^{ABCD}	6/65^{BCDE}	6/15^{ABCDEFG}	98/05^{ABC}	24/2^{BCDEFG}
1×S	112/8^{DEFG}	8/28^{ABCDE}	4/79^{CDEFG}	69/75^{ABCD}	27/12^{BCDEFG}
2× S	98/30^{FGH}	5/52^{E}	4/57^{DEFG}	104/2^{AB}	46/68^{A}
3× S	99/89^{FGH}	9/1^{ABC}	4/08^{ABCDEFG}	65/8^{ABCD}	31/65^{BCDE}
4× S	116/4^{CDEF}	7/8^{ABCDE}	3/72^{FG}	78/65^{ABCD}	28/9^{BCDEFG}
5× S	136/3^{ABC}	6/2^{CDE}	4/87^{CDEFG}	50/5^{D}	15/07^{G}
6× S	89/75^{HI}	8/75^{ABCD}	6/01^{ABCDEFG}	90/8^{ABCD}	36/9^{AB}
7× S	92/74^{GHI}	8/68^{ABCD}	5/92^{ABCDEFG}	62/9^{BCD}	36/33^{ABC}
8× S	120/8^{BCDE}	7/65^{ABCDE}	6/17^{ABCDEFG}	81/05^{ABCD}	26/5^{BCDEFG}
9× S	91/85^{HI}	9/2^{AB}	6/05A^{BCDEFG}	76/15^{ABCD}	28/1^{BCDEFG}
10× S	138/4^{AB}	6/65^{BCDE}	4/92^{CDEFG}	63/7^{BCD}	19/97^{EFG}
11× S	72/71^{I}	8/49^{ABCDE}	5/72^{BCDEFG}	88/75^{ABCD}	34/47^{ABCD}
12× S	88/64^{HI}	8/85^{ABCD}	4/95^{CDEFG}	70/8^{ABCD}	31/09B^{CDEF}

N: Normal condition; and S: stress conditions.

Table 5. Yield means in stressed and irrigation conditions and stress tolerance index of Fernandez.

Genotypes	Yield in normal conditions	Yield in stress conditions	STI Index
1	55/93	75/69	73/0
2	25/75	15/79	67/0
3	45/89	Aug-65	66/0
4	Feb-91	65/88	91/0
5	45/113	May-50	64/0
6	45/137	Aug-95	48/1
7	55/58	Sep-62	41/0
8	65/118	May-81	1-Aug
9	25/80	15/76	69/0
10	65/85	Jul-63	61/0
11	75/98	75/88	Sep-00
12	May-98	Aug-70	78/0

ears weight in irrigation conditions and unlike these results, genotype 2 has the highest harvest index under stressed conditions. According to achieved results, genotypes which have the most ears weight, have the highest harvest index. In summary, results of this study showed that the irrigation is better effective than the stress conditions on genotypes, which this result was not unexpected. Khayatnezhad et al. (2010) also reported similar results.

Among the most striking responses of plants to stressful environmental factors is photosynthesis loss due to dysfunction in photo-system II activity. When the light is normal, most part of it would be consumed in photochemical activities and photosynthetic process, and

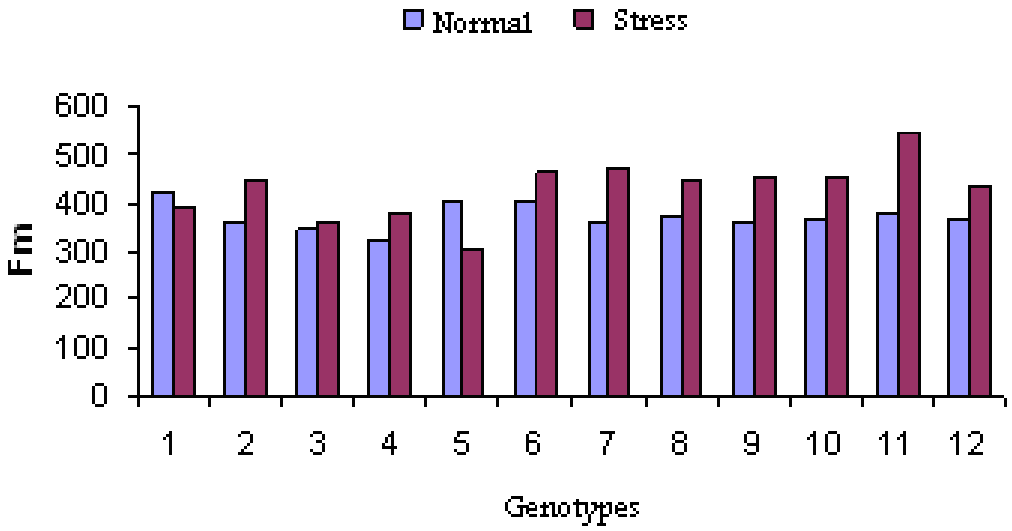

Figure 2. The amount of maximum fluorescence (FM) for under-study genotypes.

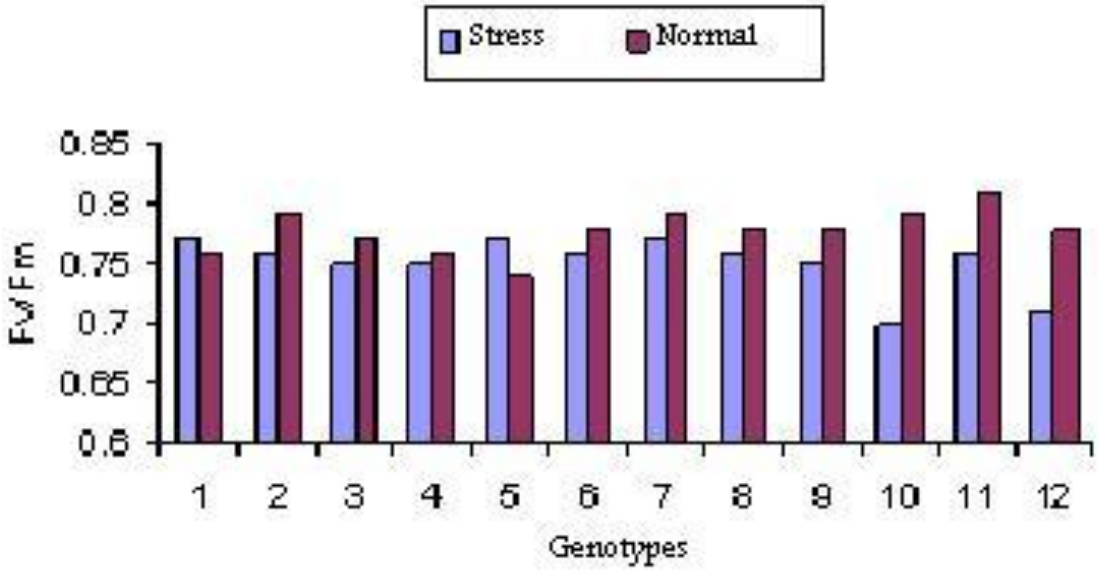

Figure 3. Quantum yield rate for under-study varieties in drought stress and irrigation conditions.

eventually a few section of light energy is emitted as fluorescence that named potential fluorescence (F0). The results showed that the greatest value of F0 was obtained in stressed conditions which represent destruction of PSII reaction centers in drought stress conditions. Havaux et al. (1998), stated that drought stress do not alone creates meaningful changes in F0, and usually heat stress alone or in combination with drought stress can cause the destruction or damage to PSII reaction centers and thus resulting in F0 rise. In this experiment, the amount of F0 in genotypes 1, 2 and 3 was more under irrigation conditions than stress conditions which represent maintaining of PSII centers and thus they are resistant to degradation of these centers in stressed conditions (Figure 2).

When the leaves are exposed to light saturation pulse, all molecules called quinine are at least temporarily restored in the state and because of stability of photochemical reactions of photo-system II, the fluorescence had been highly increased which is called max fluorescence (FM) (Briggs et al., 1972). In current experiment, genotypes 1 and 5 had more max fluorescence in drought conditions than in irrigation conditions and in the rest genotypes; conditions were contrary with these two genotypes (Figure 3).

Amount of FV/FM represents the maximum quantum

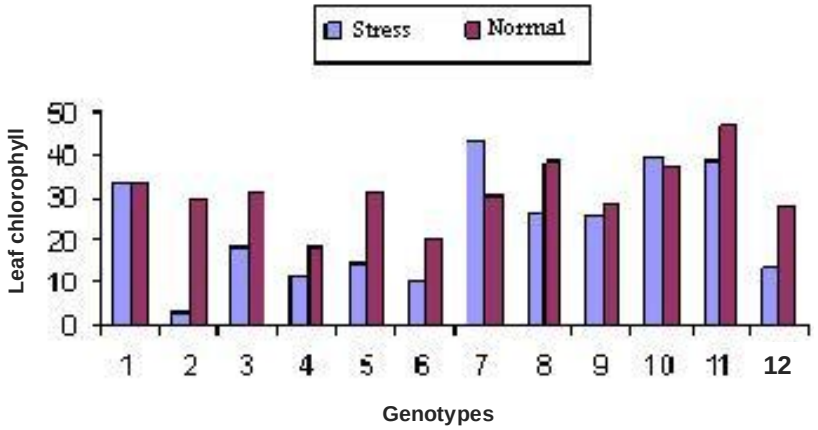

Figure 4. Amount of leaf chlorophyll for under-study varieties in drought stress and irrigation conditions.

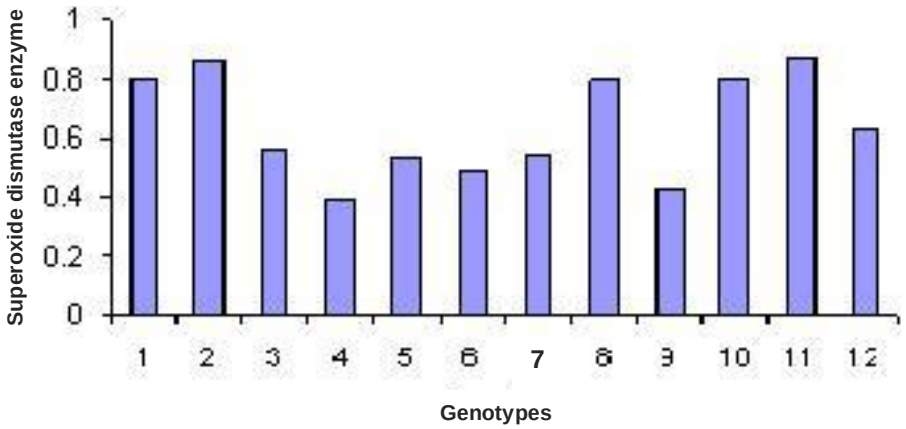

Figure 5. The average superoxide dismutase enzyme for under-study varieties.

efficiency of photo-system II and a criterion of plant photosynthesis performance, so that the value of this parameter for the most common plant species in normal environmental conditions is about 0.83 (Fracheboud, 2006). According to Paknejad et al. (2007), drought stress will reduce the quantum yield.

Evaluation of quantum yield (FV/FM) for under-study genotypes (Figure 4) showed that genotype 11 in both water stress and drought conditions have the greatest value. While genotype 10 in irrigation condition for chlorophyll fluorescence has the lowest value. Zhao et al. (2007) in evaluation of effect of salinity on chlorophyll fluorescence of oats leaf reported that increased levels of salinity increases the amount of chlorophyll fluorescence and reduce leaf chlorophyll content. So it likely seems that drought stress and salinity caused inhibition of chlorophyll synthesis and increased chlorophyll analysis. This represents reducing chlorophyll efficiency in performing photosynthesis at stress occurred condition. Zhao et al. (2007) stated that chlorophyll stops synthesis in severe water shortages. Under dehydration conditions, activities of the most enzymes (such as nitrate reducing enzyme) are reduced.

According to earlier researchers and also crucial role of chlorophyll in photosynthesis process and plant growth, it is likely that increasing chlorophyll results increases the efficiency of photosynthesis and eventually increases the yield. The results also indicate that the genotypes have significant decrease of chlorophyll levels in 0.01% and in both stressed and non-stressed conditions. Only genotype 7 had the greater amounts of chlorophyll in stressed conditions which it can be because of its genetic characteristics (Figure 5). There are evidences that water stress reduces the chlorophyll content of leaf (Ashraf et al., 2001). While in other studies it is not observed such reduction in chlorophyll in stress conditions (Ahmadi and Biker, 2001). Ahmadi and Beigar (2001) also reported that short-term water stress that caused typical wilt and

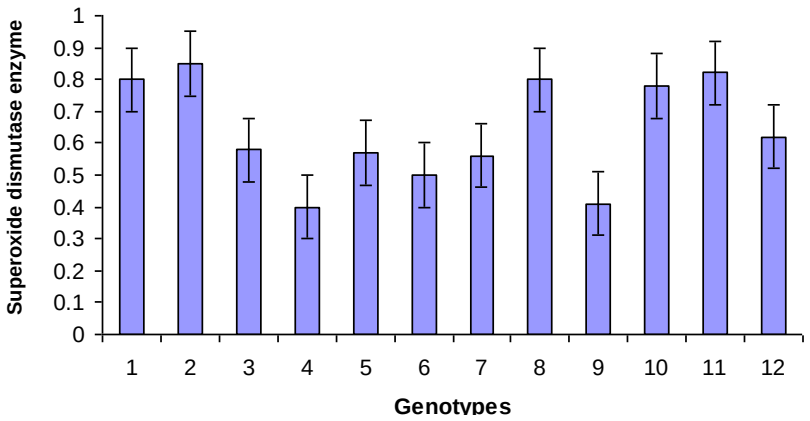

Figure 6. The average superoxide dismutase enzyme for under-study varieties.

complete halt of pure photosynthesis (Pn) in wheat, do not affect leaf chlorophyll, but it increased ratio of chlorophyll a/b (Ahmadi and Biker, 2001). No reduction in chlorophyll content in wheat and sunflower plants and also increasing ratio of chlorophyll a/b had been reported in the other studies. Antolin et al. (1995) found that by increasing drought stress leaf chlorophyll content will be decreased, but the ratio of chlorophyll a/b will be increased (Ahmadi and Biker, 2001). It is noteworthy that some researchers believed that increasing of ratio of a/b chlorophyll causes the darkness of leaves and also increased chlorophyll meter yield (Salehi et al., 2004). Salehi et al. (2004) reported that indices in wheat such as amount of nitrogen and chlorophyll content are increased in response to drought stress and this response is particularly significant in flag leaves (Salehi et al., 2004). They also expressed that the drought stress results in increasing chlorophyll content in fall rosy (safflower) varieties. He pointed out to a strong positive relationship between nitrogen, chlorophyll and SPAD, and added that increasing chlorophyll meter yield indicated increasing chlorophyll content per unit leaf area. Also, Chapman and Barreto (1997) have stated that the chlorophyll meter number affected by thickness of plant leaves will change. They noted that leaf thickness may change with regard to product type, growth stage, cultivar and environmental conditions. So it can perhaps link the difference between wheat varieties for chlorophyll meter number in control conditions (no drought stress) to available differences among varieties for leaf thickness (Chapman and Barreto, 1997). Increasing SPAD number in stressed conditions is also probably due to reduced leaf area and chlorophyll concentration in low levels of leaves. Machado and Paolsan (2001) have been introduced rapid physiological changes such as tubing leaves, reduced leaf area and an increase in stomatal resistance as mechanisms avoiding drought stress (Machado and Paolsan (2001).

Amount of superoxide dismutase enzyme had meaningful difference between the under-study genotypes, so that genotypes 2, 8 and 11 had the highest amount of the enzyme (Figure 6). The results showed that amount of this enzyme will be increased as increasing drought stress. This could be due to destruction of structures producing SOD. When the plant is exposed to stress, as increasing stress, plant antioxidant system would be active and while increasing activity of superoxide dismutase enzyme as a first defense barrier against the attack of oxygen radicals resist against damages due to stress (Sairam and Saxena, 2000) and this process continues until the plant can inhibit produced superoxide content in plant.

Results on other plants by researchers also showed that SOD activity in resistant varieties to drought and salinity is higher than the susceptible varieties and increased the amount of stress and aging plant enzyme shows a significant increase (Sgherri et al., 2000).

Zaeifizadeh and Goliov (2009) reported that resistant varieties are also having more chlorophyll. In their study of relationship between genotype and environmental conditions (drought and normal) on the amount of chlorophyll contents and the amount of superoxide dismutase reported that in drought resistant varieties, superoxide dismutase will be increased while increasing drought stress if the same process in susceptible varieties showed unmeaningful increasing or even decreasing chlorophyll of SOD super oxide dismutase.

It is reported that superoxide dismutase (SOD) is a potent antioxidant that destroy first produced material from one-capacity restoration of oxygen, that is, radical superoxide, so SOD recognized as early defense against free radicals of oxygen (Liu and Huang, 2000).

Results obtained from the stress tolerance index of Fernandez (STI) are in Table 5. The higher the STI this is indicated higher drought tolerance of that specific genotype, which this results in increasing potential yield of that genotype. Accordingly, genotypes 8 and 6 had the highest value of this index and were selected as the most tolerant genotypes. Also, genotypes 7 and 10 were most critical genotypes.

Table 6. Simple correlation of related traits to leaf with grain yield.

Trait	F0	Fm	Fv/Fm	Leaf chlorophyll	SOD	Yield
F0	1	0/35	0/397	0/333	0/197	0/143
Fm		1	0/76**	0/49	0/51	0/23
Fv/Fm			1	0/334	0/14	00-tcO
Leaf chlorophyll				1	0/52	*56/0
SOD					1	0/82*
Yield						1

Results of simple correlation (Table 6) between chlorophyll fluorescence and grain yield traits showed that there was positive and meaningful correlation between Fv/Fm and Fm, in other words, by increasing the amount of Fv/Fm, Fm also is increasing. Also positive and meaningful correlation between chlorophyll content and plant yield showed that by increasing the amount of chlorophyll, the yield will be increased.

Finally, it was found that genotypes 8, 10 and 11 had higher stress tolerance and higher rate of chlorophyll fluorescence, optimal chlorophyll content and ultimately optimal yield in stressed conditions. Also, the higher SOD enzyme levels in these varieties, this indicated these varieties can optimally cope with the drought stress conditions. Considering the likely genotypes 10 and 11 are consistent with regional conditions over time, so more and more useful studies should be carried out on genotype 8 that almost had more ideal conditions than other genotypes in current study.

Conclusion

According to obtained results it can be expressed that drought stress results in tangible reduction of yield components and ultimately yield itself through impact on the photosynthetic system. It seems chlorophyll fluorescence parameters can be useful as a device to evaluate affectability rate of optic responses of wheat photosynthesis under drought stress conditions. Also the high correlation between chlorophyll and plant yield showed that this trait can also be good criterion to identify higher yield under drought stress conditions. The higher amount of chlorophyll content and the more stable conditions of cell membranes, chlorophyll conditions to transfer electrons from photo-system II would be better and thus lead to higher pure photosynthetic quantum yield (Paknejad et al., 2007). In this case the construction of NADPH and ATP levels during the light reactions of photosynthesis had been increased, which ultimately leads to higher yield in the plant. So it seems higher chlorophyll meter content and its maintenance under drought stress means increasing the intensity of affect on plant and more reducing the leaf area. In fact, plant reduced transpiration levels by decreasing leaf area under drought stress to prevent wasting water and therefore, despite reducing the total amount of chlorophyll in leaves, chlorophyll content per unit leaf area increases (Salehi et al., 2004). Obtained results by researches has shown (Sairam and Srivastava, 2001) drought resistant wheat genotypes which had higher carotenoid and chlorophyll amounts under stressed conditions, are also more active antioxidant enzymes compared with susceptible varieties under stressed conditions. The results of this experiment are also consistent with the above mentioned researchers.

Finally, to select drought resistant varieties to stress, this process by chlorophyll content was more useful and also selection of varieties by SOD enzyme may be helpful in stressed conditions and therefore it is suggested that the increased activity of antioxidant enzymes could be an important factor in raising resistant rate of plants to moisture stress.

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