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EVALUATION OF MORPHO-PHYSIOLOGICAL RESPONSES OF WHEAT GENOTYPES AGAINST DROUGHT STRESS IN PRESENCE OF A LEONARDITE DERIVED HUMIC FERTILIZER UNDER GREENHOUSE CONDITION

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ABSTRACT

The present study was carried out for evaluation response of 12 bread wheat genotypes to a liquid humic fertilizer based on Leonardite against terminal drought stress. Analysis of variance of data showed that there was considerable variability among genotypes in all of the studied traits except of maximum total fluorescence value (Fm), which demonstrate the presence of genetic diversity among wheat genotypes. Irrigation mean squares were also significant for all the studied traits except of Fv/Fm, total tillers, fertile tillers, plant height and spike length showing that the water stress has significant effect on traits. Irrigation and Leonardite interaction was significant for Fv/Fm, Fm, fertile tillers and plant height, non-significant irrigation and leonardite interaction for another traits it could be indicated that used potassium humate in this study has no effect on these traits in normal and drought stress conditions. As result, it concluded that in our experimental conditions, grain yield of wheat genotypes was reduced under drought stress condition. But fluorescence parameter increased in drought stress condition and humic fertilizer didn’t affect genotypes. Genotype MV17/zrn produced the highest biological yield, spike weight, spike length, number of grain per spike and grain yield. Therefore, Genotype MV17/zrn performed better than others.

Key words: Bread wheat, Potassium humate, Drought stress, Chlorophyll fluorescence.

INTRODUCTION

Wheat is the most important crop in the world and it is cultivating in about 228 million hectare around the world. Iranian farmers cultivate on an average 6.6 million hectares of wheat each year of which about 4.2 million hectares under rain fed (drought stressed) and the remaining of total wheat areas is irrigated or under irrigation (Rostaei, 2007; Shahryari and Mollasadeghi, 2011a). There is a need to increase wheat productivity worldwide, particularly in developing countries and to increase genetic potential of wheat, it is important for to understand the physiological and genetic basis of yield (Yang *et al.,* 2006; Shahryari *et al.,* 2008). Significant increase of wheat yield within the past 50 years has been owes breeding specialists’ sustained efforts, in order to produce improved varieties and quality and quantity increasing of product. Continuing these efforts to reform and introduction of high yielding varieties gives strength to the life expectancy of future generations. Hence, another factor that increases the quantity and quality of crops, particularly wheat, can be the application of humic materials in organic agriculture (Molasadeghi and Shahryari, 2011).

Humic substances (HS) play an important role from the agronomical point of view influencing significantly the quality and productivity of the soil. In addition to the improvement of the soils’ physical properties and moisture conditions, HS also show a high base exchange capacity, which is important for soil fertility (Zhang and He, 2004; Peoa-Mendez *et al.,* 2005). Currently, humic materials are used as additives in fertilizers (Madejun *et al.,* 2001; Albiach *et al.,* 2001, Arancon *et al.,* 2004). Presence of HS is important during all stages of plants’ development but particularly vital in the early stages. That is why the pre-planting treatment of seeds is very important. Even before germination begins, vital forces are awakened, and the immune system is stimulated. The use of humates guarantees high quality, vitamin-rich produce. Thus, humic preparations are the reliable protection for plants and crops against harmful admixtures from our environment (soil, subsoil waters, rain-water, and the atmosphere), which is more polluted each day. They also protect crops from unfavorable environmental factors (drought, ionizing radiation, etc.). HS determine the structure and the fertility of the soil. They are an effective measure in solving ecological problems, such as pollution of soil and subsoil waters by chemicals used in agriculture. Therefore, treating vegetating plants with humates ensures their continuous nutrition with vital macro- and micro-elements. Humates stimulate micro-organisms and therefore are conducive to humus restoration (Shahryari and Mollasadeghi, 2011b). Potassium humate as a humic fertilizer increases the quality of crops miraculously, and makes plant more tolerant against biotic and abiotic stresses (Molnal *et al.,* 2004; Shahryari *et al.,* 2011a). Gadimov *et al.* (2009) concluded that humates are miraculous natural substances for increasing quantity and quality of crop yields. They expressed that a practical- scientific perspective and programming need to application of this technology in the world; especially in the developing countries. Also, they expressed action rate of these materials are related to origin and quality of HS. Quality of commercial humates is related to procedure of extraction and percent of humic acids and fulvic acids. Shahryari *et al.* (2009) observed that potassium humate increases the wheat production from 2.4 to 3.61 ton/ha in well watered conditions. Yang *et al.* (2004) suggested that humic materials can affect physiological processes of plant growth directly or indirectly.

Drought is an arising threat to world; most of the countries of the world are facing the problem of drought. The insufficiency of water is the principle environmental stress and to enter heavy damage in many part of the world for agricultural products (Nofouzi *et al.,* 2008; Khan *et al.,* 2010). Drought stress can reduce grain yield. Nouri-Ganbalani *et al.,* 2009 have estimated the average yield loss of 17 to 70% in grain yield due to drought stress. Therefore, drought stress is the most widespread environmental stress, which affect growing and productivity. It induces many physiological, biochemical and molecular responses on plants, which enable plants to develop tolerance mechanisms adapted to limited environmental conditions (Habibpor *et al.,* 2011). Response of plants to drought stress depends on time and place (Cattivelli *et al.,* 2008). Furthermore, responses to drought stress are extremely different according to the plant genetic background. Inter and intra-species variations in drought tolerance are almost well known (Rampino *et al.,* 2006). Morphological characters such as number of tillers, grains per spike , fertile tillers per plant, 1000-grain weight, peduncle length, awn length, plant height, spike length, kernel number per spike, grain weight per spike, etc. affect the wheat tolerance to the moisture shortage in the soil (Aminzadeh, 2010; Ahmadizadeh *et al.,* 2011a,b). Grain yield in wheat can be analyzed in terms of three yield components (number of spikes per square meter, number of kernels per spike, and kernel weight) that appear sequentially with later developing components under control of earlier-developing ones (Shahryari and Mollasadeghi, 2011b). Chlorophyll content is positively associated with photosynthetic rate which increases biomass production and grain yield. Significant relationships between chlorophyll content and yield and yield components facilitate selection of high yielding genotypes (Nori *et al.,* 2011).

The present study was carried out to assess the performance of different genotypes under application of a liquid humic fertilizer against terminal drought under greenhouse condition.

MATERIALS AND METHODS

Experiments were undertaken on 12 wheat (*Triticum aestivum L.*) genotypes planted under well watered, terminal drought, well watered with humic fertilizer (HF) and terminal drought with humic fertilizer conditions in a three replicated experiment on the basis of completely randomized block design. The experiment was conducted in the greenhouse conditions in Agricultural Research Station of Islamic Azad University, Ardabil branch, Iran (Northwest of Iran), during the 2009- 2010 years. Applied humic fertilizer was potassium humate derived from Leonardite. Treatments by HF were done at four stages: 1) preplanting on seeds, 2) tillering, 3) stem elongation, 4) after anthesis. Preplanting seed treatment was done on the basis of 220 ml HF in 10 liter water for 1 ton seeds. The plastic pots which 20cm diameter and 30cm height had filled with 10kg soil. Each plastic pot filled with a mix of cultivated soil, sand and manure with a ratio of 1:1:1 and four seeds planted in 3cm depth with equal spaces. At three leaves stage, in order to vernalize, the pots were moved out of the greenhouse from 21 December until 30 January for 40 days. After this period, the pots were moved to the greenhouse once again. All the pots were watered in three days period to reach the irrigation capacity. In flowering stage, drought stress was exerted through every day watering control pots and not watering stress pots until they reached to 80% soil moist evacuation via weight. The studied characters were plant height, number of tillers, peduncle length (cm), spike length (cm), grain per spike numbers, fertile tillers per plant, 1000-grain weight (g), awn length (cm), grains per spike, harvest index, grain yield (g), leaf chlorophyll content and chlorophyll fluorescence. Chlorophyll fluorescence parameters were measured using a pulse amplitude modulation chlorophyll fluorometer CCM (Opti_Scince- America). Minimal fluorescence, F0, was measured in 15 min dark-adapted leaves using weak modulated light of < 0.15 μmol m-2 s-1 and maximal fluorescence, Fm, was measured after 0.8 s saturating white light pulse (>5500 μmol m-2 s-1) in the same leaves. All measurements were from the middle part of the abaxial side of the leaves. Fo is the initial fluorescence emission by antenna Chl a molecules. Fm is the maximum total fluorescence value. The Fv/Fm ratio measures the efficiency of excitation energy capture by open PSII reaction centers representing the maximum capacity of light dependent charge separation. The chlorophyll contents of the leaves of flag were measured by the chlorophyll meter device (CCI-200) which was manufactured by the Opti-science company.

The analysis of variance (ANOVA) for each character was performed and mean comparisons followed by the Duncan's new multiple range tests (Steel *et al.*, 1997). The data were statistically analyzed by MSTAT-C and SPSS software's.

RESULTS AND DISCUSSION

Analysis of variance of recorded data showed that there was considerable variability among genotypes for all of the studied traits except of Fm, which demonstrate the presence of genetic diversity among wheat genotypes. Garcia Del-Moral *et al.* (2003) also reported significant differences between genotypes for grain yield, number of grains per spike and grain weight. Also Ahmadizadeh *et al.* (2011b) studying genetic diversity of durum wheat landraces from Iran and Azerbaijan reported highly significant differences among the genotypes in all of the morphological traits. Mollasadeghi *et al.* (2011), Molasadeghi and Shahryari, 2011 and Ahmadizadeh *et al.* (2011a) also reported similar results. Irrigation mean squares were also significant for all the studied traits except for Fv/Fm, total tillers, fertile tillers, plant height and spike length showing that the water stress has significant effect on these traits. Irrigation × Leonardite effect was significant for Fv/Fm, Fm, fertile tillers and plant height (Table 1); non-significant effect of I × L on other traits indicated that potassium humate used in this study in normal and drought stress conditions had no effect on these traits.

Genotype × irrigation (G × I) effect was significant for Fv/Fm, Fo, Fm, CCI and grain yield (Table 1), showing variation of genotypes over environments. Nori *et al.* (2011) evaluated physiological responses of durum wheat landraces to terminal drought stress and reported significant differences between genotype and irrigation interaction for Fv/Fm, Fo, Fm and CCI. Ahmadizadeh *et al.* (2011b) also reported significant differences due to genotype × irrigation interaction for grain yield in 37 durum wheat genotypes. This could provide a wide scope for breeding of under study traits, along with yield and its components, under drought stress conditions. On the other hand, Mollasadeghi *et al.* (2011) showed non-significant Genotype × Irrigation effect for agro-morphological traits that was similar to result of this study. In most studies related to the physiological basis of genetic improvement of yield increase, it was concluded that wheat breeding has not been a significant change during long history (Shahryari *et al.,* 2011b). Interactions between irrigation and humate with genotypes (G x I x L) were not significant for measured traits except plant height. This finding is in line with that of Shahryari and Shamsi (2009).

Comparison of genotypic means showed that Sardari and Saratovskaya-29 had the highest fertile tillers. The highest number of total tillers was determined in genotype Sardari (Table 2). Also with attention to results of Table 3 between genotypes there were significant differences for total tillers and fertile tillers in four conditions (Table 3). Drought stress caused yield reduction spike yield and grain yield, it happens due to fertile spikes reduction and the number of grains per spike (Ahmadizadeh *et al.,* 2011a). Genotypes Sabalan and MV17/zrn had the maximum spike length. Genotype Saratovskaya-29 had the maximum plant height (Table 2). There were significant differences between genotypes for spike length and plant height in four conditions (Table 3). Average plant height of all genotypes was 62.59 and 60.88 cm under normal + humic fertilizer and stress condition, respectively (Figure 1). Due to the capacity of tall wheat genotypes for extracting water from soil and the effective role of stored materials in the stem of these genotypes in grain yield under end seasonal drought, produced more performance compared to short genotypes (Innes *et al.,* 1985).

The highest number of grain per spike was determined in genotype Mv17/zrn (Table 2). Also with attention to results of Table 3 between genotypes there were significant differences for number of grains per spike in four conditions (Table 3). Number of grains per spike was decreased in stress condition (Figure 2). Genotype MV17/zrn had the most grain yield. There were significant differences between genotypes for grain yield under normal and normal + humic fertilizer condition at 1% probability level, also there were significant differences between genotypes for grain yield under stress + humic fertilizer condition at 5% probability level but there were no significant differences between genotypes for grain yield under stress condition (Table 3). The grain yield decreased under stress conditions (Figure 3). Foulkes *et al.* (2002) and Austin (1987) reported that the yield in stress condition in anthesis stage and after that has significant reduction relative to non-stressed condition. Seyedbagheri (2008) evaluated commercial humic acid products derived from lignite and leonardite in different cropping systems from 1990 to 2008. The results of those evaluations differed as a result of the source, concentration, processing, quality, types of soils and cropping systems. Under that research, crop yield increased from a minimum 9.4 to a maximum 35.8%.

The highest 1000-grain weight was determined in genotype Sardari (Table 2). Also with attention to results of Table 3 between genotypes there was a significant difference for 1000 grain weight in four conditions (Table 3). 1000-grain weight decreased in stress condition (Figure 4). Guttrieri *et al.* (2001) reported that selection of drought tolerant genotypes leads to reconnaissance genotypes with high 1000-grain weight. The results were in accordance with the findings of Sinha (1987), Takami *et al.* (1990) and Wardella and Willenbrink (1994), they reported that drought stress

Table 1. ANOVA for measured traits in wheat genotypes affected by humic fertilizers under drought stress and normal irrigation in greenhouse condition.

		Mean squares												
S.O.V	**D.F**	**Fv/Fm**	**Fo**	**Fm**	**CCI**	**Total tillers/plant**	**Fertile tillers/plant**	**Biologi-cal yield**	**Spike weight**	**Plant height**	**Spike length**	**Grains/ spike**	**1000-grain weight**	**Grain yield**
Irrigation (I)	1	5.5 x 10^{-4ns}	2101.9**	19748**	266.48**	2.564 ^{ns}	0.002 ^{ns}	10.76**	4.369**	22.11 ^{ns}	0.04 ^{ns}	474.4**	1888**	4.97**
Leonardite (L)	1	0.0013^{ns}	39.58 ^{ns}	444.5 ^{ns}	13.467 ^{ns}	0.016 ^{ns}	0.002 ^{ns}	0.361 ^{ns}	0.336^{ns}	30.48 ^{ns}	1.8x10^{-4} ^{ns}	18.40^{ns}	25.61 ^{ns}	0.055 ^{ns}
I x L	1	0.0056*	171.5 ^{ns}	12191*	18.3 ^{ns}	2.263 ^{ns}	1.074**	0.307^{ns}	0.014^{ns}	198.1**	2.2 x10^{-4} ^{ns}	27.60^{ns}	73.76 ^{ns}	0.001 ^{ns}
Ea	8	0.008	358.8	12429	80.843	2.783	0.512	0.56	0.239	62.87	0.613	163.7	10.66	0.205
Genotype (G)	11	0.0013 ^{ns}	145.86*	3072.8 ^{ns}	573.36**	21.90**	2.978**	7.98**	3.112**	1205**	19.02**	912.9**	467.0**	1.29**
G x I	11	0.0046**	133.92*	10369*	67.936**	0.719 ^{ns}	0.29 ^{ns}	0.529 ^{ns}	0.284^{ns}	17.55 ^{ns}	0.275 ^{ns}	40.95^{ns}	29.81 ^{ns}	0.164*
G x L	11	5.8 × 10^{-4} ^{ns}	44.495 ^{ns}	1991.8 ^{ns}	67.413*	0.919 ^{ns}	0.162 ^{ns}	0.121 ^{ns}	0.081^{ns}	4.411 ^{ns}	0.329 ^{ns}	19.96^{ns}	31.38 ^{ns}	0.036 ^{ns}
G x I x L	11	9.8 × 10^{-4} ^{ns}	113.8 ^{ns}	2960.8 ^{ns}	31.652 ^{ns}	0.636 ^{ns}	0.306 ^{ns}	0.356 ^{ns}	0.226^{ns}	30.56*	0.269 ^{ns}	25.40^{ns}	17.00 ^{ns}	0.069 ^{ns}
Eb	88	0.0014	69.55	2113.3	30.723	1.069	0.210	0.291	0.164	15.96	0.544	34.69	28.39	0.075

ns: non significant differences; *: significant at $p<0.05$; **: significant at $p<0.01$

Table 2. Mean values of morpho-physiological parameters, measured from 12 wheat genotypes affected by humic fertilizers under drought stress and normal irrigation in greenhouse condition.

No	**Genotype**	**Fo**	**CCI**	**Total tillers/plant**	**Fertile tillers/plant**	**Biological yield**	**Spike weight (g)**	**Plant height (cm)**	**Spike length (cm)**	**Grains/ spike**	**1000-grain weight (g)**	**Grain yield**
1	Gascogne	103.3 A	46.35 AB	4.43 B-D	1.31 F	3.26 CD	1.91 BC	52.65 G	9.12 EF	34.96 DE	41.64 B	1.46 B
2	Sabalan	92.13 B	34.35 D	2.59 F	1.44 EF	3.86 B	2.22 B	62.75 CD	12.08 A	40.81 BC	38.43 BC	1.58 B
3	4057	93.80 B	46.27 AB	4.54 BC	1.67 D-F	2.64 E	1.62 CD	53.85 FG	8.22 GH	36.34 CD	29.95 E	1.12 CD
4	Ruzi-84	94.93 B	40.95 C	2.66 F	1.45 EF	3.54 BC	2.09 B	68.13 B	9.39 D-F	40.45 BC	39.73 BC	1.59 B
5	Gobustan	95.69 B	41.23 C	4.79 B	1.91 CD	2.88 DE	1.64 C	66.14 BC	9.99 B-D	30.68 EF	38.12 B-D	1.17 CD
6	Saratovskaya-29	96.86 AB	41.66 BC	3.60 D-E	2.47 AB	2.91 DE	1.22 E	83.78 A	9.73 C-E	27.94 F	33.41 DE	0.93 DE
7	Mv17/zrn	93.13 B	38.35 CD	2.61 F	1.29 F	4.67 A	2.78 A	64.14 CD	11.60 A	52.21 A	37.06 B-D	1.92 A
8	Sardari	91.86 B	24.80 E	7.12 A	2.83 A	1.64 G	0.98 E	57.83 E	8.78 FG	16.47 G	53.72 A	0.85 E
9	4061	93.94 B	41.45 C	2.40 F	1.55 D-F	3.74 B	2.21 B	61.43 D	10.33 BC	41.97 B	36.43 CD	1.55 B
10	4041	95.75 B	33.87 D	3.82 C-E	1.77 DE	2.72 E	1.70 C	55.97 E-F	9.17 EF	32.81 D-F	37.64 B-D	1.23 C
11	Sissons	93.83 B	48.55 A	4.39 B-D	2.27 BC	2.19 F	1.29 DE	42.90 H	7.89 H	32.37 D-F	29.61 E	0.96 DE
12	Toos	88.86 B	48.07 A	3.00 EF	1.40 EF	3.62 BC	2.13 B	57.18 EF	10.53 B	37.21 B-D	40.19 BC	1.54 B

Values with the same superscript letters are no significantly different at $P < 0.05$.

Table 3. Supplementary analysis of interaction effects

S.O.V	**Fv/Fm**	**Fo**	**Fm**	**CCI**	**Total tillers/plant**	**Fertile tillers/plant**	**Biologic al yield**	**Spike weight (g)**	**Plant height (cm)**	**Spike length (cm)**	**Grains/ spike**	**1000-grain weight (g)**	**Grain yield**
Normal (N)	0.003^{NS}	88.02*	4501.2^{NS}	180.56**	5.84**	0.878*	3.579**	1.357**	286.36**	5.072**	295.34**	103.10**	0.59**
N + Leonardite	0.002^{NS}	112.08**	4298.6**	151.33**	7.66**	0.852**	2.295**	1.033**	334.73**	4.873**	297.11**	183.9**	0.54**
Stress (S)	0.0013*	135.28^{NS}	4940.6^{NS}	111.03**	3.78**	0.823**	1.354**	0.408**	403.67**	4.879**	192.01**	107.4*	0.24^{NS}
S + Leonardite	0.0014^{NS}	102.6*	4654.5**	297.42**	6.88**	1.183**	1.758**	0.905*	233.24**	5.074**	214.75**	150.7**	0.18*

ns, * and **:nonsignificant, significant at 5% and 1% probability levels, respectively.

reduces one-thousand grain weight and irrigation increases it. In fact, irrigation during grain filling stage helps to increase synthesis of photosynthates and their translocation to grain resulting increased grain weight. In contrast, the lack of sufficient moisture at this critical period may result decrease of 1000-grain weight noticeably. Plaut *et al.* (2004) also concluded that water shortage at the anthesis stage reduced significantly grain formation and its fertility, and at grain filling stage, it reduced photosynthetic mobilization capacity to seeds significantly, causing the shrinkage of grain and reduction of one-thousand grain weight.

Kobota *et al.* (1992) declared that weight reduction of grain wheat was subsequent to water access reduction due to reduction in further transformation process of into spike. Genotype Mv17/zrn had the most biological yield (Table 2). However, in few studies, it was seen meaningful increasing in biological yield during under-study years (Perry and Antuono, 1989; Siddique *et al,.* 1989). It was also found that less than 20% increase in grain yield was due to increased biological yield (Perry and Antuono, 1989). The only exception is a report presented by Hucl and Baker (1987) in Canada. They achieved not only a positive and meaningful increasing for the biological yield during the under-study years, but also stated that the major increase in grain yield is due to increased biological yield (Rahimyan and Banayan, 1997). Genotype Mv17/zrn had the most spike weight (Table 2). Evaluation of CCI distribution showed that, the variation in the CCI was well, ranging from 24.8 to 48.55. It seems that greater changes in the CCI in genotypes are induced by four conditions (Figure 5). There were significant differences at 1% probability level between genotypes for CCI in four conditions. With regard to the significant interaction between genotype and conditions, the highest average CCI values were observed in Sissons and Toos, and lowest average CCI values were observed in Sardari (Table 2). There were significant differences between genotypes for Fo in all conditions except stress condition. Also there were significant differences between genotypes for Fm in stress + humic fertilizer, and normal + humic fertilizer (Table 3). There were no significant differences between genotypes for Fv/Fm in all conditions except stress condition (Table 3). Genotype Mv17/zrn was placed in the superior group from most traits points of views, it had been an indicator of high potential of the genotype from agronomy and morphological points of views. Therefore, considering the results of mean comparison of the traits, the genotype can be introduced as the superior genotype. Tas and Tas (2007) pointed out that chlorophyll content decrease with ripeness of seed in stress condition, which confirms the results of this experiment. Kulikova *et al.* (2005) expressed that in spite of numerous studies on the biological effects of humic substances, the mechanism of their action remains unclear.

Results of this study showed that CCI, spike weight, number of grains per spike, 1000-grain weight and grain yield decreased in drought stress conditions. Nouri-Ganbalani *et al.* (2009) demonstrated that drought stress results in reduced pollination and reduces the number of grains per spike. Also other researchers expressed that the average of some mopho-physiological traits were decreased under drought stress (Khayatnejad *et al.,* 2010; Ahmadizadeh *et al.,* 2011a,b).

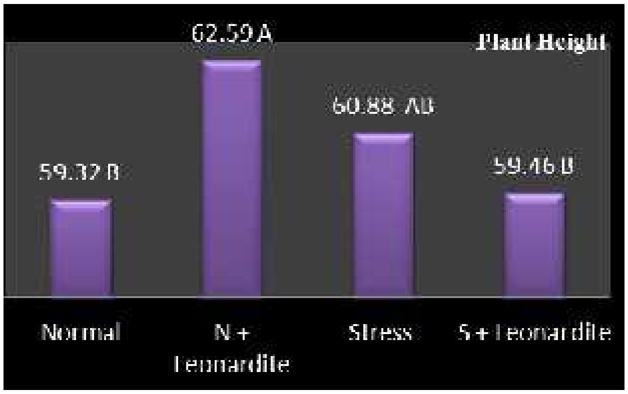

Figure 1. Plant height of bread wheat genotypes under different conditions.

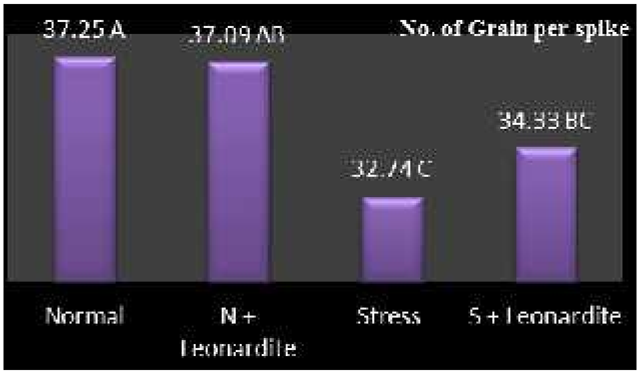

Figure 2. Number of grains per spike of bread wheat genotypes under different conditions.

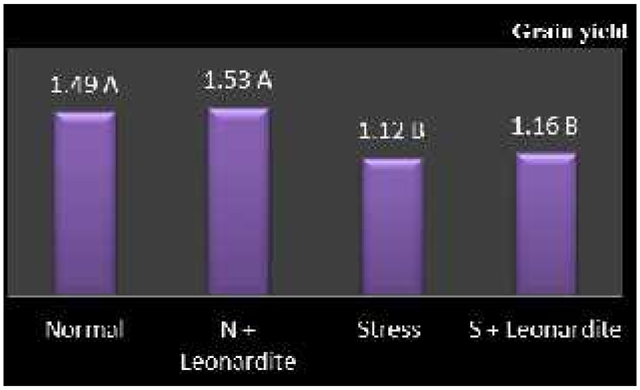

Figure 3. Grin yield of bread wheat genotypes under different conditions.

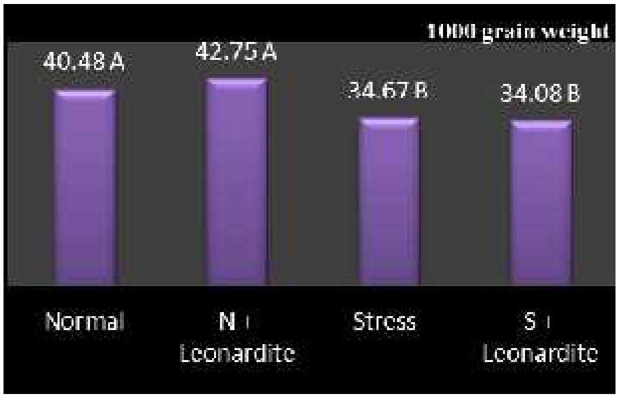

Figure 4. 1000-grain weight of bread wheat genotypes under different conditions.

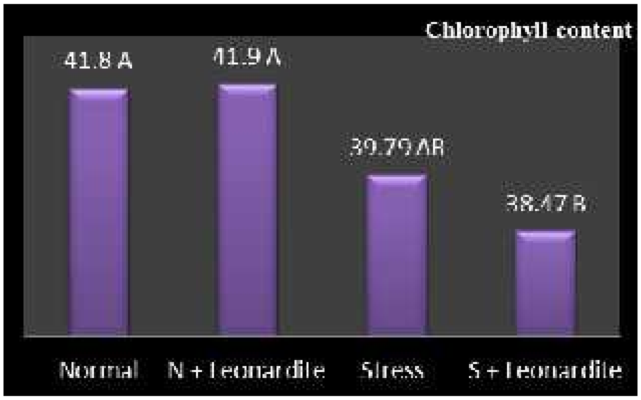

Figure 5. Chlorophyll content of bread wheat genotypes under different conditions.

Conclusion: Drought affected the morphological behavior of wheat genotypes. From present results, it can be concluded that grain yield of wheat genotypes was reduced under drought stress condition. But fluorescence parameter increased in drought stress condition and applied humic fertilizer had no effect on genotypes. Genotype MV17/zrn produced the highest biological yield, spike weight, spike length, number of grains per spike and grain yield. Therefore, Genotype MV17/zrn performed better than others. Many reports indicated that these traits can be utilized as screening criteria for stress tolerance. In the present study the findings are very similar to the former case. This study strongly supports the assertion that morphological traits can be utilized to screen wheat genotypes for drought tolerance.

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