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Physiological responses associated with drought stress during grain filling period in Wheat (*Triticum aestivum* L.)

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Abstract
Drought stress induced plant physiological responses were studied in two wheat varieties *viz.* WH 1105 and WH 1025 because drought is a major threat for crop productivity. Plant physiological parameters such as relative water content (RWC), osmotic potential, chlorophyll ratio and SPAD-chlorophyll contents were determined in leaf samples during grain filling period under drought stress. Relative water content and osmotic potential were decreased in both wheat varieties, however more decrease was observed in WH 1105 than WH 1025. Osmotic potentials of 0.75 - 1.00 Mpa and 0.82 - 1.20 Mpa were observed in WH 1025 and WH 1105 respectively under drought condition. Chlorophyll concentration is an index for evaluation of source sink relationship. The maximum chlorophyll *a/b* ratio of 4.61 was obtained in WH 1025 at 7th DAA and decreased from 7th to 28th DAA in both wheat varieties. Reduction in the ratio was more in WH 1105 than WH 1025 under drought stress. Reduction in chlorophyll content (SPAD units) was noticed at 28th days after anthesis (DAA) in both varieties.

Keywords: Physiological, Wheat, *Triticum aestivum*

Introduction
Wheat is the one of most important cereal crop in India and its production has considerably increased from past few decades. Indeed the crop productivity is low in many parts of the country compared to other countries due to climatic, edaphic and agronomic factors. Changed precipitation patterns are common occurrence across the country with frequent onset of severe drought episodes. However wheat cultivation under irrigated areas is also affected by different levels of moisture stress (Najafian G. *et al.*, 2010) [23]. All stages of crop growth are critically affected by drought and impede crop performance. Drought rapidly reduces expansion of leaves and stomatal conductance and may eventually impact primary events in photosynthesis (Passioura 2007) [25]. Water deficit conditions affect photosynthetic process and results reduced yields. The principal reasons for reduced yields are low photosynthetic efficiency owing to disruption and operation of metabolic events at low pace due to inadequacy in supply of photosynthetic assimilates. The severity and duration of stress determine the extent of yield loss. Drought occurrence particularly at grain filling period hampers starch deposition in grains owing to the metabolic limitations of starch synthesizing enzymes ultimately causing low yields. Studies on physiological changes associated drought stress disclose few intricating processes to understand adverse oxidative stress affects. Drought stress drastically affects chlorophyll content, osmotic potential and relative water content of plants. The link between various physiological responses of crop plants to drought and their tolerant mechanisms are still elusive. Relative water content, osmotic potential and chlorophyll content are among the main physiological criteria that influence plant-water relations and have been used for assessing drought tolerance (Kocheva *et al.* 2009; Ezat-Ollah *et al.* 2012 [10]; Usha & Bhumika, 2012) [18, 32]. The first response in virtually all plants to acute water shortage is the closure of stomata to prevent transpirational water loss, which may result in response to decrease in leaf turgor and water potential besides increasing the canopy temperature. Stomatal closure may lead to increased susceptibility to photo damage. The major process involved in protection against photo damage is probably the increase in non-photochemical quenching energy dissipation. In this mechanism zeaxanthin dissipates the excess energy in chloroplast *via* non radiative processes which alleviates excitation pressure on photo system II centres by diverting light energy into heat.

Materials and Methods

Seeds of two varieties of wheat *viz.* WH 1105 (Drought sensitive) and WH 1025 (Drought tolerant) were obtained from Wheat and Barley Section, Department of Genetics and Plant Breeding, College of Agriculture, CCSHAU, Hisar. Seeds of uniform size were sown in micro plots by keeping recommended spacing at research field area of department of crop physiology in the university farm under randomized block design (RBD), with pre- sown irrigation only for drought condition and recommended irrigation schedules for control. Plants were allowed to grow up to maturity.

Leaf samples were collected at four stages starting from 7th day after anthesis (7, 14, 21 and 28 days). Plants samples were brought to the laboratory by keeping them in ice box after cutting from plant. Leaf material was cut into small bits and used for determination of leaf relative water content (RWC), Osmotic potential and chlorophyll content. The SPAD chlorophyll content was measured by method described by Hui *et al.* (2007 [14] by using chlorophyll meter.

The standard statistical tools were used for analysis of data recorded in different experiments by using software "OPSTAT", developed at the Computer Center, College of Basic Science and Humanities, CCS Haryana Agriculture University Hisar.

Relative water content

Relative water content (RWC) was measured by the method of Barrs & Weatherley (1962). Leaf samples were excised, weighed immediately and placed them in petriplates containing about 20 ml of distilled water at constant temperature in diffused light for 6 hours. When leaves became fully turgid, the samples were taken out from petriplates, adhered water was blotted off with rough filter paper and reweighed for turgid weight. Then samples were kept for oven dry and dry weight was recorded.

RWC was calculated by the following formula:

$$\text{RWC (\%)} = \frac{(\text{Fresh weight - Dry weight})}{(\text{Turgid weight - Dry weight})} \times 100$$

Osmotic Potential

The osmotic potential of leaf was estimated by the method of Morgan (1980) [19] with psychrometric technique using a model VAPRO 5520 vapour pressure osmometer (Wescor INC., Lorganan, Utah, USA). The leaf was excised, sealed in an ependorff tube individually and quickly frozen at -20 ºC. Before measuring the osmotic potential, the sample was thawed for 60 min. at 25 ºC. The sample was then transferred into 2 ml capacity syringe and collected the sap by press to squeeze out. The filter paper disc was placed on the sample holder of the instrument, applied one drop of the extracted leaf sap, pushed the holder inside the chamber and locked it. About 80 seconds later the osmotic potential reading so displayed on the digital screen was recorded. The osmometer was calibrated by using standard solutions of NaCl and the calculations were done as following:

40 m osmo = -1 bar

-10 bar = 1 Mpa

Chlorophyll content

Chlorophyll content was estimated by the method of Hiscox & Isrealstam (1979) [12], where leaf material (100 mg) was washed, blotted to dry and submerged in 10 ml of dimethyl sulphoxide (DMSO) and allowed for overnight. During the course of incubation at room temperature the chlorophyll was extracted in DMSO whose absorbance was read at 663 nm and 645 nm for chlorophyll 'a' and chlorophyll 'b' estimations respectively and amount was calculated from the following formula:

$$\text{Chl 'a' (mg/g)} = \frac{(12.3 \times A_{663} - 0.86 \times A_{645})V}{1000 \times \text{W}} \times 100$$

$$\text{Chl 'b' (mg/g)} = \frac{(19.3 \times A_{645} - 3.6 \times A_{663})V}{1000 \times \text{W}} \times 100$$

Where

V = Volume of DMSO

W = Weight of the sample

Chlorophyll content-SPAD

The SPAD chlorophyll content was measured by following method of Hui *et al.* (2007) [14], where the chlorophyll meter, (model no. Minolta SPAD-502 Plus) was used to measure the greenness or the relative chlorophyll content of leaves. The meter makes instantaneous and non-destructive readings on a plant, based on the quantification of light intensity (peak wavelength: approximately 650 nm: red LED) absorbed by the tissue sample. A second peak (peak wavelength: approximately 940 nm: infrared LED) was emitted simultaneously with red LED to compensate the thickness of leaf.

Results

Relative Water Content

The data for relative water content obtained under irrigated and drought stress conditions is given in Table 1. Relative water content of wheat leaves was measured between 7th to 28th days after anthesis. The progressive decrease in relative water content was observed under drought stress condition. The maximum relative water content was measured at 7th day after anthesis and minimum at 28th day after anthesis.

The relative water content under irrigated condition ranged from 70.16 to 89.00 per cent and 58.00 to 78.00 per cent under drought condition from 7th to 28th days after anthesis. The percent reduction at 7th, 14th, 21st and 28th days after anthesis was 15.24, 16.28, 21.90 and 22.19 in WH 1105 while in WH 1025 the per cent reduction was 11.38, 12.73, 14.69 and 14.65 respectively. WH1025 showed minimum per cent reduction of 11.38 while WH 1105 showed minimum per cent reduction of 15.24.

Table 1: Effect of drought stress on relative water content (%) in wheat flag leaf during grain development

S. No.	DAA	WH 1105			WH 1025		
		Irrigated	Drought	% Reduction	Irrigated	Drought	% Reduction
1	7	89.00	75.43	15.24	88.02	78.00	11.38
2	14	83.01	69.49	16.28	88.98	77.65	12.73
3	21	83.03	64.84	21.90	79.05	67.44	14.69
4	28	74.54	58.00	22.19	70.16	59.88	14.65
CD at 5%		E= 4.23, DAA = 1.53, E X DAA = 5.11			E = 2.79, DAA = 1.12, E X DAA = 3.59		

Osmotic Potential

Table 2 shows the osmotic potential (-MPa) in leaves of wheat under irrigated and drought stress conditions at different days after anthesis. Reduction in osmotic potential was observed under drought stress condition at different days after anthesis. WH 1025 showed less reduction (23.20, 25.36, 26.03 and 26.58 per cent) while WH 1105 showed more reduction (29.73, 32.63, 37.33 and 35.79 per cent) at 7th, 14th, 21st and 28th days after anthesis respectively under drought stress condition.

The maximum per cent reduction (37.33) in osmotic potential was observed at 21st day after anthesis in WH 1105 where the measured osmotic potential was 0.63, 0.63, 0.76 and 0.88 under irrigated condition and 0.82, 0.84, 1.05 and 1.20 under drought stress condition at 7th, 14th, 21st and 28th days after anthesis respectively. Similarly, maximum per cent reduction (26.58) in osmotic potential was observed at 28th day after anthesis in WH 1025 where the measured osmotic potential was 0.61, 0.66, 0.73 and 0.79 under irrigated condition and 0.75, 0.83, 0.92 and 1.00 under drought stress condition at 7th, 14th, 21st and 28th days after anthesis respectively.

Table 2: Effect of drought stress on osmotic potential (-Mpa) in flag leaf of wheat during grain development

S. No.	DAA	WH 1105			WH 1025		
		Irrigated	Drought	% Reduction	Irrigated	Drought	% Reduction
1	7	0.63	0.82	29.73	0.61	0.75	23.20
2	14	0.63	0.84	32.63	0.66	0.83	25.36
3	21	0.76	1.05	37.33	0.73	0.92	26.03
4	28	0.88	1.20	35.79	0.79	1.00	26.58
CD at 5%		E = 0.10, DAA = 0.14, E X DAA = NS			E = 0.14, DAA = 0.09, E X DAA = 0.22		

Chlorophyll 'a'/'b' ratio

Data in Table 3 exemplifies the chlorophyll 'a'/'b' ratio of flag leaf of two wheat varieties. The chlorophyll 'a'/'b' ratio in WH 1105 under irrigated condition was 4.10, 4.05, 3.87 and 3.40 while under drought stress condition it was 3.03, 2.99, 2.94 and 2.67 at 7th, 14th, 21st and 28th days after anthesis respectively. The per cent reduction in ratio of chlorophyll 'a'/'b' varied from 26.09 to 21.47 from 7th to 28th days after anthesis.

In WH 1025, the chlorophyll 'a'/'b' ratio was 4.61, 4.45, 4.33 and 4.34 under irrigated condition and 4.19, 4.02, 3.90 and 3.86 under drought condition. The per cent reduction in ratio of chlorophyll 'a'/'b' varied from 9.11 to 11.05 from 7th to 28th days after anthesis. The chlorophyll 'a'/'b' ratio decreased under drought stress, however decrease was more in WH 1105 compared to WH 1025.

Table 3: Effect of drought stress on chlorophyll 'a'/'b' ratio in flag leaf of wheat during grain development

S. No.	DAA	WH 1105			WH 1025		
		Irrigated	Drought	% Reduction	Irrigated	Drought	% Reduction
1	7	4.10	3.03	26.09	4.61	4.19	9.11
2	14	4.05	2.99	26.17	4.45	4.02	9.66
3	21	3.87	2.94	24.03	4.33	3.90	9.93
4	28	3.40	2.67	21.47	4.34	3.86	11.05

Chlorophyll content

Data in Table 4 demonstrate the chlorophyll content (SPAD units) of flag leaf of two wheat varieties. Chlorophyll content decreased under drought stress. The chlorophyll content in WH 1105 under irrigated condition was 49.82, 47.66, 46.14 and 44.22 SPAD units while under drought stress condition the value was 39.00, 38.00, 37.68 and 37.96 SPAD units at 7th, 14th, 21st and 28th days after anthesis respectively. The per cent reduction decreased from 21.72 to 14.16 from 7th to 28th days after anthesis. In WH 1025, the chlorophyll content under irrigated condition was 51.44, 49.58, 45.40 and 43.28 SPAD units while under drought stress condition 41.86, 41.12, 40.34 and 39.48 SPAD units with a per cent reduction decreased from 18.62 to 8.78 from 7th to 28th days after anthesis. The per cent reduction was higher in WH 1105.

The data also revealed that more chlorophyll content was noted in WH 1025 and less in WH 1105 at all stages of anthesis. The maximum chlorophyll content was 51.44 observed in WH 1025 at 7th day after anthesis under irrigated condition, after that decreased gradually up to 28th day after anthesis. Similar trend was also observed in WH 1105.

Table 4: Effect of drought stress on chlorophyll content (SPAD units) in flag leaf of wheat during grain development

S. No.	DAA	WH 1105			WH 1025		
		Irrigated	Drought	% Reduction	Irrigated	Drought	% Reduction
1	7	49.82	39.00	21.72	51.44	41.86	18.62
2	14	47.66	38.00	20.27	49.58	41.12	17.06
3	21	46.14	37.68	18.34	45.40	40.34	11.15
4	28	44.22	37.96	14.16	43.28	39.48	8.78
CD at 5%		E = 1.43, DAA = 0.91, E X DAA = 2.86			E= 2.34, DAA = 1.48, E X DAA = 4.08		

Discussion

Relative water content (RWC) is regarded as a measure of plant water status, reflects the metabolic activity of the plant organization and is used as an index of dehydration in most plants (Anjum *et al.* 2011; Huang *et al.* 2013) [2]. It is closely related to physiological functions of plants and is often used to show the relationship between plant leaves and water content. RWC also indicates the ability to sustain water content and wilting degree of leaves. Table 1 depicts that RWC of wheat leaves was higher at initial stage of grain development and declined as the dry matter accumulates and finally reached to maturity. The RWC content decreased under drought stress condition over irrigated condition with more decrease in WH 1105. Our results are in accordance with the work of Sairam *et al.* (1998) [26]; Kocheva *et al.* (2009) [18]; Chunmei *et al.* (2011) [8]; Svetlana *et al.* (2011) [31]; Ezat-Ollah *et al.* (2012) [10] who showed reduction in RWC under drought stress. The present study is also supported by Agnes *et al.* (2009) [1] who found that drought stress significantly decreased RWC values even at anthesis in two sensitive wheat cultivars. Mingyang *et al.* (2015) [21] showed that with increased duration of drought stress, the RWC of leaves of four wheat cultivars seedlings decreased significantly.

Osmotic adjustment is considered a crucial process in plant adaptation to stress as it is triggered by production of osmotic compounds like proline (Hamilton & Heckathorn, 2001; Mutlu & Buzcuk, 2007; Bakht *et al.* 2011) [11, 22, 3] and methylated quaternary ammonium compounds e.g. glycine betaine and alanine betaine (Rathinsabapthi *et al.* 2001; Sakamoto & Murata, 2002) [26, 30]. In addition to decreasing cell osmotic potential, these solutes may protect the cell membrane under dehydration. Thus osmotic adjustment is a major mechanism of drought resistance in crop plants (Constantina *et al.* 2008) [7]. The present study demonstrates that osmotic potential reduced under drought stress condition, where a more reduction in osmotic potential was observed in WH 1025 as compared to WH 1105 (Table 2). The results of present investigation corroborate the previous reports of Zhang *et al.* (1995) [33]; Karin *et al.* (2002) [16]; Moud & Yamagishi (2005) [20]. The decrease in osmotic potential could be due to accumulation of osmo-protectant solutes such as glycine-beatine, proline and soluble sugars. The results are also in close proximity with that of Chunmei *et al.* (2011) [8] who showed that the leaf water potential declined in both transgenic and wild type wheat plants. After drought stress for three days or more, the leaf water potential of transgenic lines L2 and L4 were much more negative than in wild type plants and the differences were stastically significant.

Chlorophyll concentration has been known as an index for evaluation of source-sink relationship, therefore its decrease can be considered as a non-stomata limiting factor in drought stress condition (Shamsi, 2010) [29]. Leaf chlorophyll content varies within wide limits. According to the majority of investigators, the ratio between chlorophyll 'a' and 'b' is 3:1. These values vary as a function of plant growth and development, plant cultivar and a number of environmental factors. It should also be stressed that color of the leaves of certain cultivars and varieties is not always directly correlated with chlorophyll concentration (Biljana & Stojanovic, 2005) [5]. In the present study, maximum chlorophyll *a/b* ratio of 4.61 was obtained in WH 1025 at 7th DAA and decreased from 7th to 28th DAA in both the wheat varieties. The reduction in chlorophyll ratio was more in WH 1105 than WH 1025 indicating that WH1025 had maintained higher amounts of chlorophyll during drought stress period (Table 3). Minor changes in chlorophyll content and chlorophyll a/b ratio indicated that the pigment apparatus is comparatively resistant to dehydration in WH 1025. Ezat-Ollah *et al.* (2012) [10] also suggested that as a consequence of drought stress chlorophyll *a/b* ratio decreased significantly. Nikolaeva *et al.* (2010) [24] also reported that drought stress had differential influence on chlorophyll content in wheat leaves and showed decrease in chlorophyll content by 13-15 per cent after 7 day drought period. Clement *et al.* (2010) [9] reported canopy chlorophyll contents ranged between 38 and 475 mg m^{-2}. The results are at par with previous results of Baisak *et al.* (1994) [4] who also reported reduction in leaf chlorophyll concentration in wheat under drought stress. Khakwani *et al.* (2012) [17] also reported that chlorophyll content was significantly and positively correlated with all the traits expect stomatal density and specific leaf area traits where a significant but negative trend was observed. Sairam & Srivasthava (2002) [28] showed that salinity tolerant wheat *cv*. Kharchia 65 showed lesser decline in chlorophyll than salt sensitive HD 2687.

The increased Soil Plant Analysis Development (SPAD) values in heat stressed wheat plants after salicylic acid application reflect higher chlorophyll synthesis. Its values have a significant association with photosynthesis and leaf nitrogen content (Iqbal *et al.* 2013) [15]. The results presented in Table 4 showed that chlorophyll SPAD units decreased under drought stress at different developmental stages of grain and higher reduction in SPAD units were observed in WH 1105 than WH 1025 indicating that WH 1025 had maintained chlorophyll contents considerably during the period of drought stress. The reduction in chlorophyll SPAD units was much more pronounced in WH 1105 however, a lesser reduction was observed in WH 1025. The results are supported by the previous work of Hui *et al.* (2007) [14] who reported that high temperature stress significantly reduced SPAD values in flag leaves of wheat during grain filling period.

Conclusions

Relative water content, osmotic potential and leaf membrane stability decreased in both wheat varieties under drought stress, however more reduction for these parameters was observed in WH 1105. Reduction in relative water content (RWC) was maximum in WH 1105 (22.19 per cent) and minimum in WH 1025 (11.38 per cent) during different stages of grain development.

Flag leaf osmotic potential ranged from 0.75 to 1.00 –Mpa in WH 1025 and 0.82 to 1.20 –Mpa in WH 1105 under drought condition. Chlorophyll content (SPAD units) and chlorophyll fluorescence declined under drought stress and WH 1105 showed higher reduction than WH 1025. Reduction in chlorophyll was 21.72 to 14.16 per cent and 18.62 to 8.78 per cent in WH 1105 and WH 1025 respectively from 7th to 28th days after anthesis

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References

1. Agnes G, Jolan C, Maria S, Adrienn G, Laszlo C, Irma T *et al*. Glutathione transferase activity and expression patterns during grain filling in flag leaves of wheat genotypes differing in drought tolerance: Response to water deficit. Journal of Plant Physiology 2009;166:1878-1891.
2. Anjum SA, Xie XY, Wang LC, Saleem MF, Chen M, Wang L. Morphological, physiological and biochemical responses of plants to drought stress. African Journal of Agricultural Research 2011;6(9):2026-2032.
3. Bakht J, Jamal Y, Shafi M, Sher H. Response of maize (*Zea mays* L.) to seed priming with NaCl and salinity stress. Spanish Journal of Agriculture 2011;9:252-261.
4. Baisak R, Rana D, Acharya PBB, Kar M. Alterations in the activities of active oxygen scavenging enzymes of wheat leaves subjected to water stress. Physiologia Plantatrum 1994;35:489-495.
5. Biljana B, Stojanovic J. Chlorophyll and carotenoid content in wheat cultivars as a function of mineral nutrition. Archives of Biological Science Belgrade 2005;57(4);283-290.
6. Barrs HD, Weatherley PE. A re-examination of the relative turgidity technique for estimating water deficits in leaves. Australian Journal of Biological Sciences 1962;15:413-428.
7. Constantina B, Elena P, Aurel G, Nicolae NS. Relationship between genetic differences in the capacity of osmotic adjustment and other physiological measures of drought resistance in winter wheat *(Triticum aestivum* L.). Romanian Agricultural Research 2008;25:7-11.
8. Chunmei H, Weiwei Z, Qiang G. Enhancement of drought resistance and biomass by increasing the amount of glycine betaine in wheat seedlings. Eupytica 2011;177:151-167.
9. Clement A, Martine G, Frederic B, Willy W. Comparartive analysis of three chemometric techniques for the spectroradiometric assessment of canopy chlorophyll content in winter wheat. Computers and Electronics in Agriculture 2010;73:165-173.
10. Ezat-Ollah F, Mohsen F, Shokuh D. Comparison between

effective selection criteria ofdrought tolerance in bread wheat landraces of Iran. Annals of Biological Research 2012;3(7):3381-3389.
11. Hamilton EW, Heckathorn SA. Mitochondrail adaptation to NaCl, complex I is protected by anti-oxidants and small heat shock proteins, whereas complex II is protected by proline and beatine. Plant Physiology 2001;126:1266-1274.
12. Hiscox JD, Isrealstam GF. A rapid method for extraction of chlorophyll from leaf tissue without maceration. Canadian Journal of Botany 1979;57:1332-1334.
13. Huang CJ, Zhao SY, Wang LC, Shakeel AA, Chen M, Zhou HF. Alteration in chlorophyll fluorescence, lipid peroxidation and antioxidant enzymes activities in hybrid ramie (*Boehmeria nivera* L.) under drought stress. Australian Journal of Crop Science 2013;7(5):594-599.
14. Hui Z, Tingbo D, Qi J, Dong, J. Leaf senescence and grain filling affected by post-anthesis high temperatures in two different wheat cultivars. Plant Growth Regulation 2007;51:149-158.
15. Iqbal RKM, Noushina I, Asim M, Tasir SP, Nafees AK. Salicylic acid alleviates adverse effects of heat stress on photosynthesis through changes in proline production and ethylene formation. Plant Signalling and Behavior 2013;8(11):e26374-1-e26374-10.
16. Karin H, Michael T, Astrid W, Gerhard S, Alexander S, Dieter G. Complex interactive effects of drought and ozone stress on the antioxidant defence systems of two wheat cultivars. Plant Physiology and Biochemistry 2002;40:691-696.
17. Khakwani AA, Dennett MD, Munir M, Baloch M. Wheat yield response to physiologicallimitations under water stress condition. The Journal of Animal and Plant Science 2012;22(3):773-780.
18. Kocheva K, Kartseva T, Landjeva S, Georgiev G. Parameters of cell membrane stability and levels of oxidative stress in leaves of wheat seedlings treated with PEG 6000. General and Applied Plant Physiology 2009;35(3-4):127-133.
19. Morgan JM. Osmotic adjustment in the spikelets and leaves of wheat. Journal of Experimental Botany 1980;31(2):655-665.
20. Moud AAM, Yamagishi T. Application of projected pollen area response to drought stress to determine osmoregulation capability of different wheat (*Triticum aestivum* L.) cultivars. International Journal of Agriculture and Biology 2005;7(4):604-605.
21. Mingyang W, Lirong C, Fenling L, Min Z, Lun S, Shushen Y. Effect of drought stress on antioxidant enzymes in seedlings of different wheat genotypes. Pakistan Journal of Botany 2015;47(1):49-56.
22. Mutlu F, Buzcuk S. Salinity induced changes of free and bound polyamine levels in sunflower (*Helianthus annus* L.) roots differing in salt tolerance. Pakistan Journal of Botany 2007;39:1097-1102.
23. Najafian G, Kaffashi AK, Jafar-Nezhad A. Analysis of grain yield stability in hexaploid wheat genotypes grown in temperate regions of Iran using additive main effects and multiplicative interaction. Journal of agricultural Science and Technology 2010;12:213-222.
24. Nikolaeva MK, Maevskaya SN, Shugaev AG, Bukhov NG. Effect of drought on chlorophyll content and antioxidant enzyme activities in leaves of three wheat cultivars varying in productivity. Russian Journal of Plant Physiology 2010;57(1):87-95.
25. Passioura JB. The drought environment: physical, biological and agricultural perspectives. Journal of Experimental Botany 2007;58:113-117.
26. Rathinsabapthi B, Fouad WM, Sigua CA. β-alanine betaine synthesis in the Plumbaginaceae: Purification and charecterization of a tri functional S-adnosyl-methionine –dependent *N*-methyltransferase from *Limonium latifolium* leaves, Plant Physiology 2001;126:1241.
27. Sairam RK, Deshmukh PS, Sexana DC. Role of antioxidant system in wheat genotypes tolerance to water stress. Biolgia Plantarum 1998;41:387-394.
28. Sairam RK, Srivasthava GC. Changes in antioxidant activity in sub-cellular fractions of tolerant and susceptible wheat genotypes in response to long term salt stress. Plant Science 2002;162:897-904.
29. Shamsi K. The effect of drought stress on yield, relative water content, proline, soluble carbohydrates and chlorophyll of bread wheat cultivars. Journal of Animal and Plant Science 2010;8(3):1051-1060.
30. Sakamoto A, Murata N. The role of glycine betaine in the protection of plants from stress: clues from transgenic plants. Plant cell and environment 2002;25:163.
31. Svetlana VO, Aleksey VP, Marina DP, Tatyana AP, Andreas B. leaf dehydroascorbate reductase and catalase activity is associated with soil drought tolerance in bread wheat. Acta Physiologia Plantarum 2011;33:2169-2177.
32. Usha C, Bhumika P. Oxidative stress in five wheat vrieties *(Triticum aestivum* L.) exposed to water stress and study of their antioxidant enzyme defence system, water stress responsive metabolites and H_2O_2 accumulation. Brazalian Journal of Plant Physiology 2012;24(2):117-130.
33. Zhang CS, Lu Q, Verma DPS. Removal of feedback inhibition of Δ^1-pyrroline-5-carboxylate synthetase, a bifunctional enzyme catalysing the first two steps of proline biosynthesis in plants. The Journal of Biological Chemistry 1995;270:20491-20496.