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Morphological, physiological and biochemical analysis of wheat genotypes under drought stress

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Abstract

Drought is a common abiotic stress that seriously affects wheat (*Triticum aestivum* L.) production in many parts of the world, particularly in arid and semi-arid regions. Therefore, maintaining a sufficient yield under drought conditions has become a priority, particularly considering global environmental changes and increase in world population. In present study wheat genotypes were evaluated for different morphological, physiological and biochemical characters under drought stress. Drought conditions were imposed by holding water irrigation for 15 day before flowering stage. Morphological parameter like plant height, leaf area, and number of tillers recorded in the range of 56.8 - 69.3cm, 14.5 - 44.1 cm, 3.4 - 5.2 respectively. Physiological parameter like chlorophyll content, relative water content was recorded in the range of 44.8 to 58.9, 69.3% to 86.6% respectively. Under biochemical parameter, proline content was recorded in the range 0.11 to 0.86 umole/gmfw. All these parameters were greatly affected under imposed drought conditions. Almost all the parameters were showed decline trend under imposed drought conditions except proline content.

Keywords: Drought, Plant height, Chlorophyll, Proline.

Introduction

Wheat (*Triticum aestivum* L.) belongs to family Poaceae (Lorenz and Kulp, 1991) [12]. Abiotic stress is an adverse effect on organism by climatic factor likely various type drought, salinity, osmotic stress, high metal etc. Drought is a common abiotic stress that seriously affects wheat (*Triticum aestivum* L.) production in many parts of the world, particularly in arid and semi-arid regions. Therefore, maintaining a sufficient yield under drought conditions has become a priority, particularly considering global environmental changes and increase in world population (Huaqi *et al.*, 2002) [6]. Drought is the most common environmental stress affecting about 32% of 99 million hectares under wheat cultivation in developing countries and at least 60 million hectares under wheat cultivation in developed countries (Shamsi *et al.*, 2011) [16].

The percentage of drought affected land areas more than doubled from the 1970s to the early 2000s in the world (Isendahl and Schmidt, 2006) [7]. For the purpose of crop production, yield improvement, developing of drought tolerant varieties is the best option (Siddiqe *et al.*, 2000). Therefore, physiological and biochemical approaches have a great importance in order to understand the complex responses of plants to water deficiency and to develop new varieties. The productivity of crop plants could be increased through studying the nature of adaptation of cereal crops to water stress and by identifying the possible physiological markers for evolving the best-adapted and high yielding wheat varieties for areas affected by water stress (Mujtaba *et al.*, 2002) [13].

During water stress, there is considerable potential for increased accumulation of superoxide and hydrogen peroxide resulting from the increased rate of O₂ photo-reduction in chloroplasts (Robinson and Bunce, 2000). In fact it has been reported that much of the injury to plants caused by exposure to various stresses is associated with oxidative damage at the cellular level. Mechanisms of ROS (Reactive oxygen species) detoxification exist in a, plants (Mundree *et al.*, 2002) [14]. Changes of antioxidants reflect the impact of environmental stresses on plant metabolism (Herbinger *et al.*, 2002) [5]. The level of response depends on the species, the development and the metabolic status of the plant, as well as the duration and intensity of the stress.

Material and Method**Plant material and growth condition**

The present investigation was conducted during Rabi session conducted at SardarVallabhbai

Patel University of Agriculture and Technology, Meerut, U.P. India (North West Plains Zone, India, 28.990 N and 77.700 E), Different genotypes of wheat with diverse background were used. Different drought levels were produced at reproductive stage for to observe the effect of water scarcity on morphological, physiological and biochemical levels. Fifteen wheat genotypes HD-3086, PBW373, PBW226, DBW17, PBW590, DBW71, DBW16, K802, K9107, MP4010, HD2733, HD2864, K1256, K9423, and K6525 were procured from IIFSR Modipuram, Meerut, Uttar Pradesh. Different wheat genotypes selected on the basis of diverse backgrounds were grown in pots (12x14). The crop was exposed to partial drought (at different growth stages) with re-watering after produced a specific drought. The experiment was conducted in three replications having drought condition and another was raised under irrigated conditions. Irrigation water was supplied by sprinklers to provide two water regimes during plant growth. Control regime was watered at 15 day interval throughout the growing season. Drought was created in this rain free environment by with-holding irrigation after 15 days from sowing and giving supplementary irrigations every three weeks during 90 days post-sowing. Wheat genotypes were evaluated for morpho-physiological traits (irrigated conditions) and for drought tolerance (water stressed conditions) at different growth stages.

Measurement of plant height, Number of Tillers, Spike length, Number of spikelet's per spike, Chlorophyll content, Related Water Content (RWC) and Proline concentration (PC)

Observations on morpho- physiological traits were recorded at different growth stages. In each pot, five random plants were tagged to record these observations. Average of treatment was taken. The traits studied and techniques adopted to record the observations are given below. The plant height was measured from bottom of the plant i.e. from soil level to the base of the spike and represented in centimeter. Wheat plants of selected genotypes in their early stages of growth exhibit marked difference in their growth habit. Number of tillers recorded at heading stages to avoid any ambiguity in the counting. The average spike length of five plants on the main Culm from the base of the spike to the top of the last spikelet excluding awns was recorded in centimeter. Total numbers of spikelet's on main spike of all five plants were counted at the time of maturity and average was recorded. The chlorophyll content in the flag leaf was determined using a SPAD meter. Five flag leaves of each genotype grown in rainfed condition were measured after anthesis stage. Three measurements in the middle of the flag leaf were made randomly for each plant and the average sample was used for analysis.

Relative water content (RWC) was determined by the method described by (Barrs and Weatherly, 1962) [2]. Leaf material (100 mg) taken and kept in double distilled water in a petridish for two hours to make the leaf tissue turgid. The turgid weights of the leaf materials were taken after carefully soaking the tissues between the two filter papers. Subsequently this leaf material was kept in a butter paper bag and dried in oven at 65°C for 24 hours and their dry weights were recorded. The RWC was calculated by using the formula. The PC was determined according to the method of (Bates *et al.*, 1973) [3]. Plant material (0.5 g) taken at anthesis stage was grinded with 10 ml of 3% sulfosalicylic acid. The

homogenate was filtered, and 1 ml of glacial acetic acid and 1 ml of acid ninhydrin reagent were added to a 1 ml of filtrate. Then the mixture was shaken by hand and incubated in boiling water bath for 1 h. After that, it was transferred to ice bath and warmed to room temperature. Toluene (2 ml) was added to the mixture and the upper toluene layer was measured at 520 nm using UV spectrophotometer.

Result and Discussion

The studied wheat genotypes were evaluated for drought tolerance by observing various morphological, physiological and biochemical characters. The drought condition was simulated by holding the water irrigation after 75 days of sowing (approx. 15 days before the flowering stage). All the morphological, physiological and biochemical parameters were studied after 90 days of sowing (at flowering stage).

Morphological observations

The morphological traits were studied *viz* plant height, leaf area, no of tiller three parameters studied and data were recorded as follows:

Plant height observed under irrigated and drought condition

Morphological characters are the important attributes for a plant to determine their stress level. Presently, five plants were randomly selected from each variety and the data was recorded. Plant height was recorded from control and treated plants. Plant height was measured (in cm) from base of the plant to the tip of the auxiliary shoot and presented in Table 1.1. The result showed that the plant height under control condition varied from a lower value of 56.8cm in genotype HD-2733 higher value of 69.3cm in MP-4010. Under simulated drought condition the plant height was reduced significantly and varied for 44.7 cm in genotype DBW-17 to 65.7 cm in genotype MP-4010. The average difference in plant height under control and irrigated condition was 2.45. The maximum difference was observed in PDW-373(4.1) and minimum difference was observed in PBW-226 (1.3). The results suggested that the genotype PDW-373 showed maximum difference may be rare sensitive toward drought condition while the genotype PBW-226 showed minimum difference may be more resistant toward drought conditions in reference to plant height. Similar results were reported by Jatoi *et al.*, 2011 [8] in wheat, therefore we are in confirmation of our findings with this trait and varieties used in the present study.

Table 1.1: Mean value of plant height in drought and irrigated condition of wheat genotype in centimeter (cm)

S.No.	Genotypes	Plant height		Difference b/w irrigated and drought
		Irrigated	Drought	
1	HD-3086	57.9	56.5	1.4
2	PDW-373	68.2	64.1	4.1
3	PBW-226	61.3	60.0	1.3
4	DBW-17	58.5	54.7	3.8
5	PBW-590	61.5	59.6	1.9
6	DBW-71	61.6	59.1	2.5
7	DBW-16	59.8	57.7	2.1
8	K-802	64.7	62.3	1.7
9	K-9107	60.2	58.4	1.8
10	MP-4010	69.3	65.7	3.6
11	HD-2733	56.8	54.8	2.0
12	HD-2864	62.7	59.8	2.9
13	K-1256	65.9	62.9	3.0
14	K-9423	66.4	64.0	2.4
15	K-6525	58.6	56.3	2.3
Mean				2.45

Flag Leaf area observed under irrigated and drought condition

Leaf area of flag leaf is directly related to higher photosynthesis and high chlorophyll content and therefore is a crucial factor for understanding the proper growth and development of plant. For this randomly selected seven plants of each variety were taken and seven leaves from each plant were taken for calculation of leaf area. The average of them was considered for explanation. It was measured by multiplying the leaf length and width with multiplying factor (multiplication factor was $F=0.76$). The calculated leaf area of control and treated plants are being represented in Table 1.2. Result showed that the leaf area under control condition varied from a lower value of 14.5cm^2 in genotype PDW-373 to a higher value of 44.1cm^2 in HD-2733. Under simulated drought condition the plant height was reduced significantly and varied from 10.2cm^2 in genotype PDW-373 to 35.1cm^2 in genotype HD-2733. The average difference in leaf area under irrigated and drought condition was 5.44. The maximum difference was observed in HD-2733 (9.0) and minimum difference was observed in PBW-226 (2.6). In contrast, PDW-373 had the shortest leaf (14.5cm^2) which was identical to K-802 (19.4cm^2) and decreased percentage control to treatment highest K-6525 (35.68%) and lowest PBW-226 (9.15%) under drought condition. Similar results were also reported by (Khakwani *et al.*, 2011) [9]. They observed higher leaf area in range 12.48 to 41.49cm^2 . In general all genotypes showed reduced leaf area under drought condition; many researchers considered that it may be due to decrease in the photosynthetic activity of plant leaves due to water stress. In many research, it was observed that due to water shortage cell sap of leaves decreases so volume of cell and parenchymatous cell size decreased.

Table 1.2: Mean value of leaf area in drought and irrigated condition of wheat genotype in centimeter (cm)

S. No.	Genotypes	Leaf area		Difference b/w irrigated and drought
		Irrigated	Drought	
1	HD-3086	25.6	20.6	5.0
2	PDW-373	14.5	10.2	4.3
3	PBW-226	28.4	25.8	2.6
4	DBW-17	23.0	18.4	4.6
5	PBW-590	24.6	18.8	5.8
6	DBW-71	22.8	17.2	5.6
7	DBW-16	24.6	18.9	5.7
8	K-802	19.4	14.9	4.5
9	K-9107	20.2	14.5	5.7
10	MP-4010	33.1	27.7	5.4
11	HD-2733	44.1	35.1	9.0
12	HD-2864	25.4	20.9	4.5
13	K-1256	24.6	18.3	6.3
14	K-9423	25.1	20.1	5.0
15	K-6525	21.3	13.7	7.6
Mean				5.44

Number of tillers observed under irrigated and drought condition

Number of tiller were counted from first three plant and averaging them for all genotypes in each pot at flowering stage. The calculated number of tiller of control and treated plants was presented in Table 1.3. The result showed that the number of tiller under control condition varied from a lower value of 3.4 in genotype K-6525 to a higher value of 5.2 in PBW-226. Under simulated drought condition the number of tiller was reduced significantly and varied from 2.1 in genotype

K-6525 to 4.3 in genotype PBW-226. The average difference in number of tiller under control and treatment condition was 1.14. The maximum difference was observed in HD-2733(1.5) and minimum difference was observed in PBW-226 (0.9). The highest no of tiller was recorded in PBW-226 (5.2). In contrast, K-6525 had the shortest tiller in plant (3.4) which was identical to K-1256 and PBW-373 (3.4). And decreased percentage control to treatment highest K-6525 (35.68%) and lowest PBW-226 (9.15%) under drought condition. Similar result was reported by (Khakwani *et al.*, 2011) [9]. They observed higher number of tillers in range 2.67 to 4.83.

Table 1.3: Mean value of number of tillers in drought and irrigated condition of wheat genotype in centimeter (cm)

S.No.	Genotypes	No. of Tillers		Difference b/w irrigated and drought
		Irrigated	drought	
1	HD-3086	4.0	3.1	0.9
2	PDW-373	3.4	2.5	0.9
3	PBW-226	5.2	4.3	0.9
4	DBW-17	4.8	3.6	1.2
5	PBW-590	3.6	2.7	0.9
6	DBW-71	4.2	3.1	1.1
7	DBW-16	4.6	3.3	1.3
8	K-802	5.2	4.0	1.2
9	K-9107	3.6	2.6	1.0
10	MP-4010	4.0	2.9	1.1
11	HD-2733	5.2	3.7	1.5
12	HD-2864	3.8	2.6	1.2
13	K-1256	3.4	2.1	1.3
14	K-9423	4.0	2.7	1.3
15	K-6525	3.4	2.1	1.3
Mean				1.14

Physiological parameters

Chlorophyll content observed under irrigated and drought condition

Chlorophyll content is an important aspect among all parameters which directly linked to the yield of the plant. During the flowering stage, the plant require highest amount of chlorophyll for proper development of grains. Chlorophyll content was measured using a portable Minolta chlorophyll meter SPAD 502. The average of triplicate reading was recorded at each two upper expanded leaflet. The calculated chlorophyll content of control and a treated plant was presented in Table 2.0. The result showed that the chlorophyll content under control condition varied from a lower value of $40.8\mu\text{g}/\text{cm}^2$ in genotype DBW-71 to a higher value $58.9\mu\text{g}/\text{cm}^2$ in PBW-226. Under simulated drought condition the chlorophyll content was reduced significantly and varied from $30.8\mu\text{g}/\text{cm}^2$ in genotype DBW-71 to $54.3\mu\text{g}/\text{cm}^2$ in genotype PBW-226. The average difference in chlorophyll content under control and treatment condition was 8.32. The maximum reduction was observed in MP-4010 (16.0) and minimum difference was observed in PBW-226 (4.6). The highest chlorophyll content was recorded in PBW-226 (58.9) under control conditions. In contrast, DBW-71 had the higher range (SPAD reading) (40.8) which were identical to DBW-16 (42.00) under drought while chlorophyll content decreased under control condition in genotypes MP-4010 (31.0%) and PBW-226 (7.8%). Similar results were reported by (Sibomana *et al.*, 2013) [17].

The lower chlorophyll content was recorded under drought condition means a genotype may be a drought tolerant in nature. Our results are in agreement with the study of (Nyachiro *et al.*, 2001) [15], who described a significant

decrease of chlorophyll *a* and *b* caused by water deficit in *Triticum aestivum* genotypes. It is generally considered that chlorophyll degraded with drought conditions as photo-oxidation increased due to stress.

High chlorophyll content is a desirable characteristic because it indicates a low degree of photo inhibition of photosynthetic apparatus, therefore reducing carbohydrate losses for grain growth (Farquhar *et al.*, 1989) [4]. Growth and photosynthesis are two of the most important processes abolished, partially or completely by water stress (Kramer and Boyer, 1995) [10], and both of them are major cause of decreased crop yield.

Table 2.0: Mean value of chlorophyll content in drought and irrigated condition of wheat genotype in $\mu\text{g}/\text{cm}^2$

S. No.	Genotypes	Chlorophyll content ($\mu\text{g}/\text{cm}^2$)		Difference b/w irrigated and drought
		Irrigated	Drought	
1	HD-3086	47.7	41.6	6.1
2	PDW-373	56.0	48.9	7.1
3	PBW-226	58.9	54.3	4.6
4	DBW-17	51.3	42.6	8.7
5	PBW-590	46.7	40.6	6.1
6	DBW-71	40.8	30.8	10.0
7	DBW-16	42.0	33.3	8.7
8	K-802	52.1	42.0	10.1
9	K-9107	42.3	35.5	6.8
10	MP-4010	51.6	35.6	16.0
11	HD-2733	51.8	44.3	7.5
12	HD-2864	46.3	37.6	8.7
13	K-1256	51.3	42.6	8.7
14	K-9423	43.6	36.6	7.0
15	K-6525	46.6	37.8	8.8
Mean				8.32

Relative Water Content (RWC) observed under irrigated and drought condition

Relative water content was measured following the standard method of data from three leaves per four plants in each germplasm was recorded and averaging them. The calculated Relative water content of control and a treated plant was presented in Table 2.1. The result showed that the Relative Water Content (RWC) under control condition varied from a lower value of 69.3% in genotype HD-2864 to a higher value of 86.6% in PBW-226. Under simulated drought condition relative water content was reduced significantly and varied from 60.9% in genotype HD-2864 to 80.7% in genotype PBW-226. The average difference in Relative water content under control and treatment condition was 8.47. The maximum difference was observed in MP-4010 (1.3) and minimum difference was observed in PBW-226 (5.9). The maximum relative water content was recorded in PBW-226 (86.6%) under control (irrigated condition). In contrast, HD-2864 had the lowest relative water content (69.3%) which was identical to K-9107 (71.1%) and genotype MP-4010 showed high amount of chlorophyll (13.0%) under control while lowest showed by the genotype PBW-226 (6.81%) under drought condition. Similar result was reported by (Bajji *et al.*, 2001., Jatoi *et al.*, 2011 and Khakwani *et al.*, 2011) [1, 8, 9] in wheat. They observed higher relative water content in range 69.3% to 81.1%, 75.67% to 90% and 52.44% to 96.87%, respectively.

RWC was found higher in the genotypes with are having

higher resistance against drought in comparison to other genotypes which do not have drought resistance. In other words, plant having higher yields under drought stress should have high RWC. So, based on these results, mentioned genotypes which are classified as high and medium yielding genotypes in condition of drought stress showed high level of RWC. Decrease in RWC in plants under drought stress may depend on the reduction of plant vigor and observed in many genotypes which studied by other research groups (Liu *et al.*, 2002) [11].

Table 2.1: Mean value of RWC (Relative Water Content) in drought and irrigated condition of wheat genotype in percentage (%).

S.No.	Genotypes	Relative water content (%)		Difference b/w irrigated and drought
		Irrigated	Drought	
1	HD-3086	77.1	69.4	7.7
2	PDW-373	75.5	67.6	7.9
3	PBW-226	76.6	70.7	5.9
4	DBW-17	79.0	68.9	10.1
5	PBW-590	81.1	70.8	10.3
6	DBW-71	77.7	68.7	9.0
7	DBW-16	75.7	67.0	8.7
8	K-802	78.2	70.2	8.0
9	K-9107	71.1	62.2	8.9
10	MP-4010	79.2	68.9	10.3
11	HD-2733	80.4	71.5	8.9
12	HD-2864	69.3	60.9	8.4
13	K-1256	77.8	70.1	7.7
14	K-9423	80.1	72.8	7.3
15	K-6525	78.8	70.8	8.0
Mean				8.47

Biochemical parameters

Proline content observed under irrigated and drought conditions

Proline is known to be produced in higher amount under stress conditions as it helps the plants to withstand resistant against stress conditions. The data from four plants in each germplasm was recorded and average them. The calculated proline content of control and a treated plant was presented in Table 3.0. The result showed that the proline content under control condition varied from a lower value of 0.108 $\mu\text{mole}/\text{gmfw}$ in genotype DBW-16 to a higher value of 0.866 $\mu\text{mole}/\text{gmfw}$ in PBW-590. Under simulated drought condition the proline content was increment significantly and varied from 0.123 $\mu\text{mole}/\text{gmfw}$ in genotypes DBW-16 to 0.922 $\mu\text{mole}/\text{gmfw}$ genotype PBW-590. The average difference in proline content under control and treatment condition was 0.074. The maximum difference was observed in PBW-226 (0.164) and minimum difference was observed in PDW-373 (0.040).

In contrast, DBW-16 had the low value of proline content (0.11 $\mu\text{mole}/\text{gmfw}$) which was identical to PBW-373 (0.13 $\mu\text{mole}/\text{gmfw}$). Increased percentage of proline in control treatment was found higher in the genotype MP-4010 (13.0%) and lowest in genotype PBW-226 (6.81%) under drought condition. Similar results were reported by (Singh *et al.*, 2014). They observed higher proline content in the range 1.06 $\mu\text{mole}/\text{gmfw}$ to 1.37 $\mu\text{mole}/\text{gmfw}$ and 0.22 $\mu\text{mole}/\text{gmfw}$ to 1.64 $\mu\text{mole}/\text{gmfw}$.

Table 3.0: Mean value of Proline content in drought and irrigated condition of wheat genotype in $\mu\text{mole/gmfw}$

S. No.	Genotypes	Proline content ($\mu\text{mole/gmfw}$)		Difference b/w irrigated and drought
		irrigated	Drought	
1	HD-3086	0.216	0.276	0.060
2	PDW-373	0.125	0.165	0.040
3	PBW-226	0.488	0.652	0.164
4	DBW-17	0.313	0.354	0.041
5	PBW-590	0.866	0.922	0.056
6	DBW-71	0.310	0.401	0.091
7	DBW-16	0.108	0.123	0.015
8	K-802	0.389	0.458	0.069
9	K-9107	0.263	0.323	0.060
10	MP-4010	0.454	0.605	0.151
11	HD-2733	0.580	0.738	0.158
12	HD-2864	0.455	0.522	0.067
13	K-1256	0.488	0.536	0.048
14	K-9423	0.163	0.203	0.040
15	K-6525	0.667	0.722	0.055
Mean				0.074

Conclusion

Drought induces significant alterations in plant physiology and biochemistry of plant cells. Some plants have a set of physiological adaptations that allow them to tolerate water stress conditions. The degree of adaptations to the decrease of water potential caused by drought may vary considerably among species. Researchers linked various physiological responses of plant to drought with their tolerance mechanisms, such as: pigment content and stability and high relative water content. Understanding of physiological mechanisms that enable plants to adapt to water deficit and maintain growth and productivity during stress period could help in screening and selection of tolerant genotypes and these traits can be utilized in breeding programs. To investigate suitable stress resistance indices for screening of genotypes under drought condition, grain yield of cultivars under both non-stress and stress conditions were measured for calculating different sensitivity and tolerance indices. Drought causes accumulation of proline, betaine, sugars and active osmotic substances in many crops including wheat. These substances increase osmotic potential of cell sap and so plants becomes compatible to drought. Thus tolerant genotype showed higher content of ABA, proline, glycine betaine, total sugars, reducing sugars and have higher water content in compare to susceptible genotypes, which contained more of glycine, betaine and potassium but had lower ABA and water content.

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