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Study of changes in Photosyntheic rate, chlorophyll content, proline content and membrane stability in Indian wheat cultivars under drought stress

Megha Singh, Vaishali and Lalit Kumar

Abstract
The study reveals that photosynthetic rate, chlorophyll content, proline accumulation and membrane stability index of Indian wheat cultivars gets affected when grown under PEG-induced drought conditions. The seeds of 20 wheat cultivars were treated with 10%, 20% and 30% PEG solution for 48 hours and were sown in field. The physiological parameters i.e. photosynthetic rate and chlorophyll content were measured at anthesis stage and 15 days after anthesis along with this biochemical parameters i.e. proline content and membrane stability index were also measured. It was observed in the present study that osmotic stress significantly reduced the chlorophyll content and photosynthetic rate of flag leaf in wheat germplasm. Among all the tested genotypes HD2733 and K9107 performed better under both irrigated and drought conditions, whereas the DBW16 and DBW17 were seems to be the sensitive genotypes under drought environment. The germplasm HD2733 shows higher value of proline content, whereas wheat genotypes exhibited high variability for MSI values. After increasing the stress level the proline accumulation is recorded to be increases in these genotypes.

Keywords: Biochemical analysis, chlorophyll content, drought stress, membrane stability index physiological analysis, photosynthetic rate, proline content and wheat

Abbreviations: Cm – centimetre, G – gram, PEG – Polyethylene glycol, SPAD - Soil Plant Analysis Development, IRGA – Infra Red Gas Analyzer

Introduction
Researchers reported that climatic changes generate difficult conditions for producing needed quantity of the crop for fulfilling necessities of the population. Reduced plant production is the cause of environmental changes and alarming threats towards food security (Mickelbart *et al.* 2015) [36]. Abiotic stresses like drought, saltiness, heavy rainfall and extreme temperature shows adverse impacts on growth of plant, metabolism and physiology. Wheat is massively affected by shifts in climatic conditions, therefore, abiotic stress like drought brought major decline in the productivity of especially wheat crops (Shao *et al.* 2005; Kirigwi *et al.* 2007; Huseynova and Rustamova 2010) [50, 26, 21]. Continual drought occurrence that resulted due to change in weather condition will show reduced world's wheat productions which in turn do not provide justifiable agricultural production and supportable nutrition (Li *et al.* 2009; Mwadzingeni *et al.* 2016) [32, 37].
Cell division, cell enlargement and differentiation processes are directly related to genome, physiology, ecology, morphology, growth and development of the plant that are sensitized towards drought (Taiz and Zeiger, 2006) [51]. The harmful effects of drought are going to reach drastic increase, therefore, it is necessary to begin search for wheat genotypes which are tolerant to drought stress.

Physiological Characters and Drought
During moisture stress, photosynthesis is constrained due to which the carbohydrate in stem that are responsible for grain growth becomes limited (Ehdaie *et al.* 2006) [14]. In a study, spring and winter wheat both are found to have differences in concentration of water-soluble carbohydrates in stems around anthesis during water stress (Ruuska *et al.* 2006; Foulkes *et al.* 2007; Yang *et al.* 2007) [47, 58]. Photosynthesis is particularly sensitive to water deficit because the stomata close to conserve water as available soil water declines. Stomatal closure deprives the leaves of carbon dioxide and photosynthetic carbon assimilation is decreased in favor of photo-respiratory oxygen uptake (Pei *et al.*, 2000) [42]. In photorespiration, H_2O_2 is produced at very high rates by the glycollate oxidase reaction in the peroxisomes (Noctor *et al.*, 2002) [39]. Moreover, superoxide production by the photosynthetic electron transport chain (via the Mehler reaction) is exacerbated by drought (Noctor *et al.*, 2002) [39].

Arjenaki *et al*., (2012) [3] revealed that chlorophyll content is one of the major factors affecting photosynthetic capacity. Reduction or no-change in chlorophyll content of plant under drought stress has been observed in different plant species and its intensity depends on stress rate and duration. The chlorophyll content also decreases in drought stress. Low chlorophyll content is observed in harsh environment. Chlorophyll content might be considered as consistent indicator in screening plant germplasm for drought tolerance. Energetic status of the chloroplast increases as a consequence of the drought stress which has a direct relationship to that of increased amount of total chlorophyll and Chl*a* and Chl*b* among the stressed induced varieties (Ranjbarfordoei *et al*., 2000) [45]. Plant cells accumulate some compatible solutes such as proline, proteins, betain, etc. for osmotic adjustment to protect cell membrane (Delauney and Verma, 1993) [12]. Under osmotic stress environment these compatible solutes are overproduced aiming to facilitate osmotic adjustment. These compounds accumulated in high quantities generally in cytoplasm of stressed cells deprived of interfering with macromolecules and acted as osmoprotectant. Assessment of cell membrane stability as a mechanism of drought stress tolerant variety is also important. The degree of cell membrane injury induced by moisture stress may be easily estimated through measurement of electrolyte leakage from cells. The more the concentration of PEG increases more the electrolyte leakage will be observed in the different cultivars. The sensitive varieties will show higher MSI values as compared to drought tolerant varieties (Chakraborty and Pradhan 2012) [9].

The objectives undertaken for the present investigation were physiological and biochemical evaluation of wheat genotypes for drought tolerance.

Material and Method

Collection of Plant Materials

Seeds of twenty wheat cultivars were obtained from Seed Production Unit, Department of Genetics and Plant Breeding, Sardar Vallabhbhai Patel University Of Agriculture and Technology, Meerut, were used in this study. The cultivars are: K-802, K-1256, K-607, K-9107, K-6525, K-9423, DBW-71, DBW-16, DBW-17, MP-4010, MP-3336, PBW-226, PBW-373, PBW-590, PBW-71, PBW-533, HD-2733, HD-3086, HD-3095 and HD-2864.

Methods

Field Trial

The experiment was conducted at field laboratory of Department of Agriculture Biotechnology, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, U.P., during *rabi* season of 2015-16 and 2016-17. Rabi is the winter season in Northern India, where crop is sown in October-November and harvested in March-April. The seeds of all genotypes were treated with three concentrations (10%, 20% and 30%) of Polyethylene glycol (PEG) for 48 hours in Petri plates in three replications along with control and allow them to germinate. After 48 hours, the seedlings were transferred to pots and field and maintained under optimum conditions till maturity. The Experiments was conducted under Randomized Block Design (RBD) throughout the study. In each block 25 seeds were spreaded randomly. Every replication was tagged with their genotype name and PEG concentration (10%, 20% and 30%). After transferring the seeds, the pots and field was only irrigated in excessively dry condition for maintaining the stress; otherwise it was depended on rain water.

The crop was observed for various physiological and biochemical parameters. The observations were recorded from each introgression lines in each replication at different growth stages.

Physiological Characterization

Photosynthetic Rate

Infra-Red Gas Analyzer (IRGA) was used to measure the photosynthetic rate of leaves. At seedling stage three readings were taken from different leaves of same plant in each treatment and their average was considered for determination of photosynthetic rate (μmol/m^2 sec).

Chlorophyll Content

Chlorophyll meter SPAD-502 was used to measure the relative chlorophyll content of the leaves. Three readings were taken from different leaves of same plant in each treatment and their average was considered for determination of chlorophyll content (μg/cm^2). The chlorophyll content was recorded at anthesis and 15 days after anthesis.

Biochemical Characterization

Estimation of Proline Content

***Sulphosalicylic acid* (3%):** 3g Sulphosalicylic acid dissolved in 80 mL of double distilled water and make up volume upto 100 ml.

***Ninhydrin*:** 2.5g ninhydrin dissolved in 40 ml of 6M H_3PO_4 acid and 60 ml glacial acetic acid.

***Toluene*:** 2 ml of toluene is used in reaction mixture according to protocol.

***H_3PO_4*: [**6M]- 43.6 ml H_3PO_4 taken and make up upto 100 ml with distilled water.

***Glacial acetic acid*:** 60 ml of glacial acetic acid used in preparing ninhydrin reagent.

For estimation of Proline content in wheat germplasm at their respective treatments, 100 mg of fresh leaf tissue was taken from plants. Leaf tissues were grinded in 1ml of aqueous Sulphosalicylic acid (3%). Tubes (1.5 ml) were centrifuged at 7000 rpm for 5 minutes in a centrifuge the supernatant was separated and mixed with equal volume of glacial acetic acid. Then add 0.5 ml of ninhydrin and incubated for 30 min at 100˚C in boiling water bath and then placed on ice bath for 5 min for cooling. Thereafter, 2 ml of toluene was mixed in reaction mixture and mixed properly. The aqueous phase was transferred in new tubes. The reaction mixture was warm at 25˚C and chromophore was measured at 520nm.

Membrane Stability Index

Membrane stability index was determined for different genotypes and their respective treatment. 100mg fresh leaf tissues were taken in test tubes containing 10 mL of double distilled water in two sets. Test tubes in one set were kept at 40˚C in a water bath for 30 min and electrical conductivity of the water containing the sample was measured (C_1) using a conductivity bridge. Test tubes in the other set incubated at 100˚C in the boiling water bath for 15 min and their electrical conductivity was measured as above (C_2). MSI was calculated using the formulae given below:

MSI = [1- C_1/C_2] x 100

Results

Physiological Characters

Photosynthetic Rate

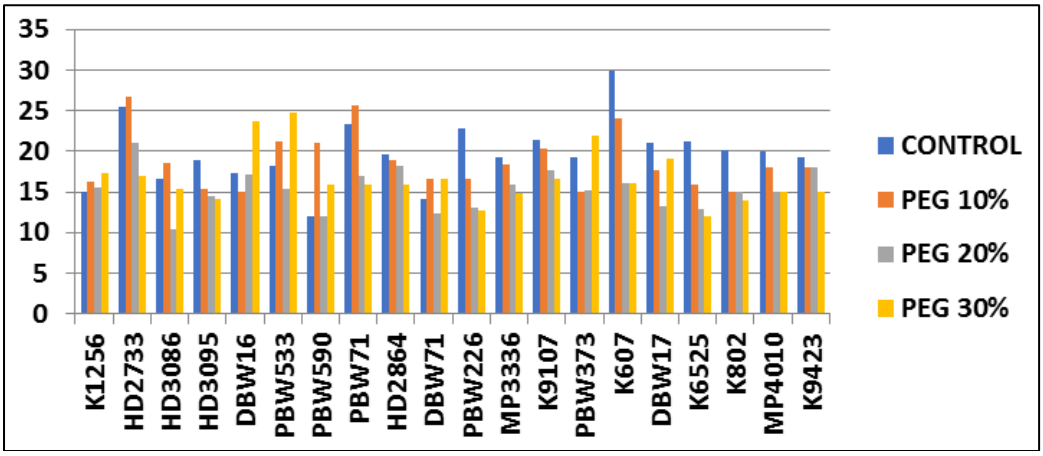
This parameter is an important among all other parameters as it directly affects the final yield of the crop. The photosynthetic rate of five randomly selected plants of each variety was recorded using Infra-Red Gas Analyzer at anthesis and after 15 days of anthesis. The photosynthetic rate at anthesis was ranged from 29.86µmol/m^2sec in genotype K607 to 12.05µmol/m^2sec in genotype PBW590 under control condition (Table 1; Fig 1.A, B). However the photosynthetic rate was found to be decreased with increasing concentration of polyethylene glycol from 0 to 30%. In stress condition at T1 and T2 level, the genotype HD2733 shows maximum photosynthetic rate i.e. 26.63µmol/m^2sec and 20.95µmol/m^2sec respectively. Whereas the genotype PBW533 shows maximum photosynthetic rate 24.67µmol/m^2sec at T3 level of PEG treatment at anthesis stage. On the other hand, the minimum photosynthetic rate was 14.97µmol/m^2sec in genotype PBW373 shows at T1, 10.36µmol/m^2sec in genotype HD3086 at T2 and12.01µmol/m^2sec in genotype K6525 minimum at T3 level of stress treatment.

After 15 days of anthesis, the photosynthetic rate observed was ranged from 10.77µmol/m^2 sec in genotype PBW590 to 26.96µmol/m^2sec in genotype K607 under control condition (Table 1; Fig 1). Under imposed stress condition at three levels, the genotype HD2733 shows maximum photosynthetic rate 24.06µmol/m^2sec at T1and 18.91µmol/m^2sec at T2 and PBW533 shows maximum photosynthetic rate22.28µmol/m^2sec at T3 level of PEG treatment. Whereas the genotype PBW373 shows minimum photosynthetic rate 13.53µmol/m^2sec at T1, HD3086 shows minimum Photosynthetic rate9.28µmol/m^2sec at T2 andK6525 minimum photosynthetic rate 10.83 µmol/m^2sec at T3 level of stress treatment, after 15 days of anthesis.

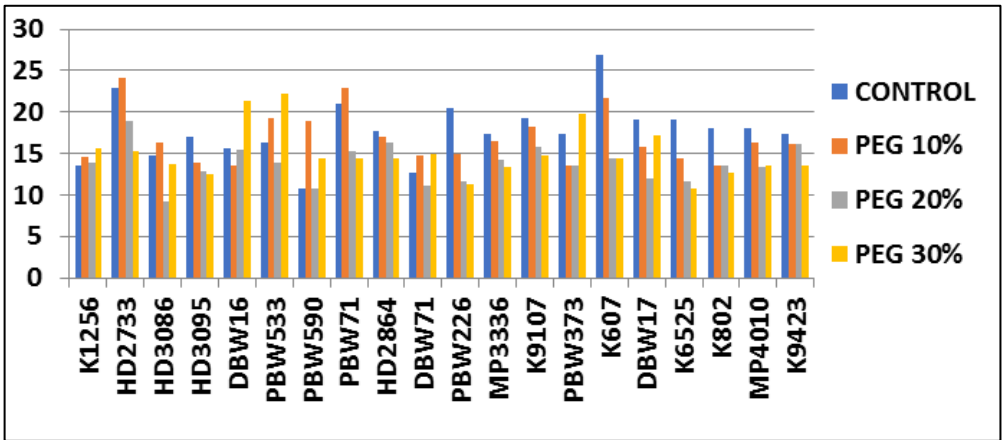
Table 1: Physiological characters of wheat genotypes.

Wheat Genotype	Photosynthesis Rate(µmol/m^2sec) At Anthesis				Photosynthesis Rate(µmol/m^2sec) 15DAAnth			
	C	T1	T2	T3	C	T1	T2	T3
K1256	15.02	16.31	15.47	17.30	13.51	14.63	13.89	15.54
HD2733	25.45	26.63	20.95	16.97	22.86	24.06	18.91	15.25
HD3086	16.56	18.47	10.36	15.35	14.84	16.28	9.28	13.77
HD3095	18.96	15.38	14.41	14.09	17.06	13.85	12.87	12.58
DBW16	17.29	15.06	17.16	23.64	15.56	13.51	15.42	21.34
PBW533	18.16	21.25	15.43	24.67	16.37	19.18	13.83	22.28
PBW590	12.05	21.06	12.01	15.90	10.77	18.94	10.84	14.44
PBW71	23.27	25.58	16.98	15.85	21.05	22.92	15.23	14.36
HD2864	19.66	18.88	18.12	15.94	17.66	16.93	16.28	14.34
DBW71	14.09	16.52	12.33	16.56	12.76	14.82	11.04	14.93
PBW226	22.77	16.64	13.05	12.620	20.55	14.90	11.66	11.33
MP3336	19.28	18.34	15.91	14.90	17.310	16.57	14.26	13.39
K9107	21.40	20.26	17.67	16.53	19.34	18.28	15.83	14.83
PBW373	19.20	14.97	15.14	21.98	17.28	13.53	13.61	19.75
K607	29.86	24.03	16.05	16.04	26.96	21.65	14.49	14.36
DBW17	21.05	17.58	13.32	19.11	19.05	15.79	12.04	17.22
K6525	21.15	15.98	12.87	12.01	19.02	14.35	11.56	10.83
K802	20.08	15.03	15.02	14.02	18.05	13.54	13.46	12.61
MP4010	19.96	18.06	14.98	15.06	18.01	16.24	13.45	13.53
K9423	19.34	18.10	18.02	15.05	17.38	16.18	16.22	13.47
Gen. Mean	19.73	18.71	15.266	16.68	17.77	16.81	13.711	15.01
C.V.	0.267	0.233	0.239	0.233	0.197	1.134	0.301	0.300
F Prob.	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
S.E.M.	0.021	0.018	0.015	0.016	0.014	0.078	0.017	0.018
C.D. 5%	0.060	0.050	0.042	0.045	0.040	0.218	0.047	0.052
C.D. 1%	0.080	0.066	0.055	0.059	0.053	0.289	0.063	0.068

C-Control, T1-Treatment with PEG 10%, T2- Treatment with PEG 20%, T3- Treatment with PEG 30%



(A)



(B)

Fig 1: (A) Graphical representation of Photosynthesis Rate of 20 wheat genotype at the time of anthesis, (B) Graphical representation of Photosynthesis Rate of 20 wheat genotype after 15 day of anthesis.

Chlorophyll Content

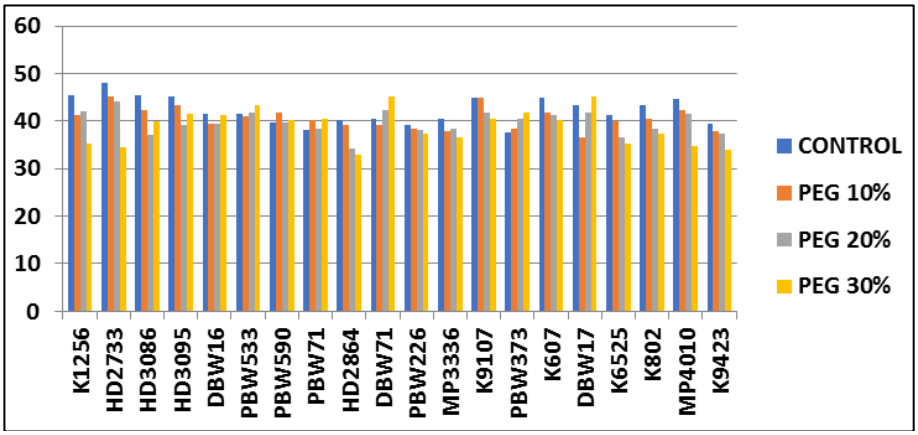
The chlorophyll content is one of the essential parameter among all of the observed parameter responsible for the development of the plant and response under stress conditions. Data on chlorophyll content of different wheat genotypes were recorded at anthesis and after 15 days of anthesis (Table 2; Fig 2). At the time of anthesis, the chlorophyll content was ranged from minimum 37.63µg/cm^{2}in genotype PBW373 to maximum 48.16µg/cm^{2}in genotype HD2733 under control condition. However the chlorophyll content was found to be decreased with increasing concentration of polyethylene glycol from 0 to 30%. Under imposed drought stress at three level, the genotype HD2733 shows highest 45.08µg/cm^2, 42.38 µg/cm^2 at T1 level (10%) and T2 level (20%)of PEG treatment respectively, whereas the genotype DBW71shows highest 45.22µg/cm^2 chlorophyll content at T3 level(30%) of PEG treatment. On the other hand the genotype DBW17 shows lowest chlorophyll content 36.58 µg/cm^2 at T1, genotype HD2864 show lowest chlorophyll content 34.22µg/cm^2 and 32.83µg/cm^{2}at T2 and T3 respectively.

After15 days of anthesis, the chlorophyll content was ranged from37.76µg/cm^2 in genotype PBW373 to 49.26µg/cm^{2}in genotype HD2733under control condition. Under the imposed stress condition at three levels, the maximum chlorophyll content was 45.15µg/cm^2 in genotype HD2733 at T2, 44.49 µg/cm^2 in genotype DBW71at T3 level of PEG treatment Whereas the minimum chlorophyll content was 43.87µg/cm^2 in genotype DBW17 at T1, 34.36µg/cm^2 in genotype HD2864at T2 and 32.88µg/cm^{2}in genotype HD2864 at T3level of stress treatment recorded after 15 days of anthesis.

Table 2: Physiological characters of wheat genotypes.

Wheat Genotype	Chlorophyll Content(µg/cm^2) at Anthesis				Chlorophyll Content(µg/cm^2) at 15DAAnth			
	C	T1	T2	T3	C	T1	T2	T3
K1256	45.56	41.18	42.17	35.21	46.45	45.10	42.08	35.21
HD2733	48.16	45.08	44.21	34.56	49.26	42.23	44.32	40.26
HD3086	45.55	42.29	37.03	40.04	47.64	40.99	37.30	34.68
HD3095	45.13	43.46	39.06	41.64	48.74	43.60	39.28	41.60
DBW16	41.46	39.46	39.56	41.31	40.18	39.59	39.89	41.53
PBW533	41.45	41.05	41.87	43.40	41.99	41.24	41.92	43.65
PBW590	39.63	41.85	39.66	40.22	40.14	42.01	39.72	40.52
PBW71	38.23	40.19	38.43	40.48	38.41	40.48	38.47	40.75
HD2864	40.25	39.11	34.25	32.83	40.43	39.25	34.36	32.88
DBW71	40.57	39.30	42.38	45.18	45.64	39.36	42.81	41.36
PBW226	39.12	38.38	38.22	37.36	39.78	38.45	38.23	37.47
MP3336	40.61	37.86	38.34	36.46	40.82	37.62	38.36	36.63
K9107	44.92	44.85	41.77	40.53	45.05	45.15	41.87	40.77
PBW373	37.63	38.51	40.44	41.89	37.76	38.81	40.58	42.08
K607	44.93	41.92	41.35	40.21	45.10	42.24	41.56	40.32
DBW17	43.36	36.58	41.67	45.22	45.60	43.87	41.87	36.49
K6525	41.29	39.95	36.56	35.40	41.75	40.22	36.48	35.68
K802	43.43	40.48	38.47	37.45	43.36	40.73	38.68	37.83
MP4010	44.59	42.34	41.48	34.64	44.47	42.32	41.84	34.84
K9423	39.47	37.97	37.33	33.89	40.09	38.14	37.46	34.26
Gen. Mean	42.27	40.59	39.72	38.89	42.79	40.72	39.86	39.09
C.V.	0.340	0.094	0.244	0.165	0.168	0.349	0.140	0.135
F Prob.	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
S.E.M.	0.059	0.016	0.040	0.026	0.029	0.058	0.023	0.022
C.D. 5%	0.165	0.044	0.111	0.073	0.082	0.163	0.064	0.060
C.D. 1%	0.218	0.058	0.147	0.097	0.109	0.216	0.085	0.080

C-Control, T1-Treatment with PEG 10%, T2- Treatment with PEG 20%, T3- Treatment with PEG 30%



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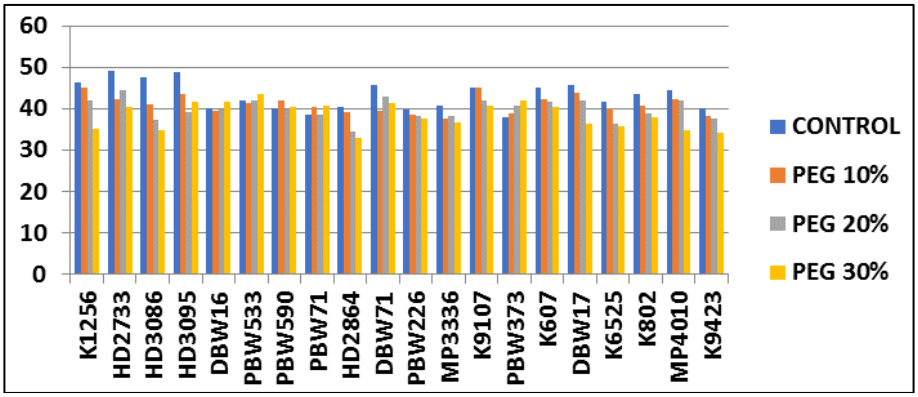


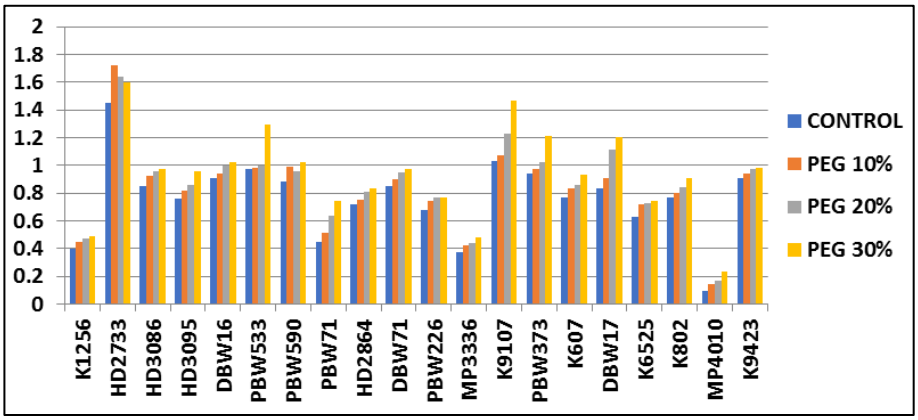
(B)

Fig 2: (A) Graphical representation of Chlorophyll Content of 20 wheat genotype at the time of anthesis. (B) Graphical representation of Chlorophyll Content of 20 wheat genotype after 15 day of of anthesis.

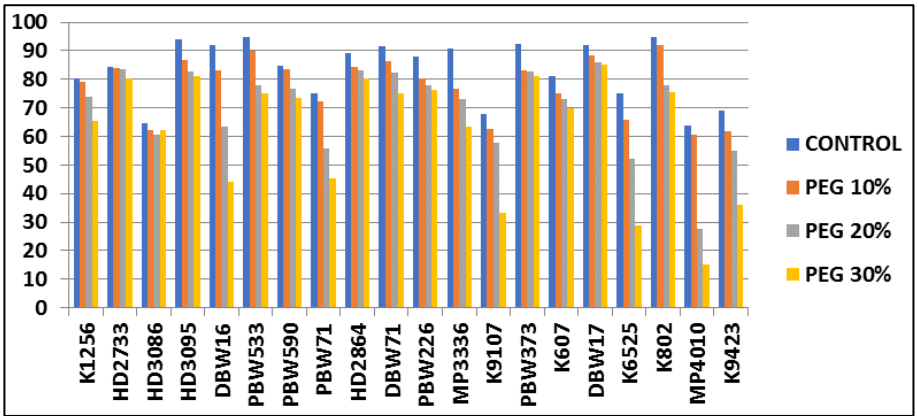
Table 3: Biochemical characters of wheat genotypes.

Wheat Genotype	Proline (µg/gfw)				MSI(%)			
	C	T1	T2	T3	C	T1	T2	T3
K1256	0.40	0.45	0.47	0.49	80.17	78.99	73.90	65.39
HD2733	1.45	1.72	1.64	1.60	84.26	84.15	83.41	80.47
HD3086	0.85	0.92	0.96	0.97	64.79	62.34	60.57	62.04
HD3095	0.76	0.82	0.86	0.96	94.06	86.66	82.90	81.26
DBW16	0.91	0.94	1.00	1.02	92.16	83.04	63.47	44.01
PBW533	0.97	0.98	1.00	1.29	94.64	90.09	77.79	75.29
PBW590	0.88	0.99	0.96	1.02	84.90	83.64	76.61	73.42
PBW71	0.45	0.51	0.64	0.74	75.05	72.47	55.60	45.47
HD2864	0.72	0.75	0.81	0.83	89.26	84.31	83.31	80.47
DBW71	0.85	0.90	0.95	0.97	91.52	86.52	82.37	75.17
PBW226	0.68	0.74	0.77	0.77	87.87	80.26	77.75	76.46
MP3336	0.37	0.42	0.44	0.48	90.99	76.67	72.99	63.23
K9107	1.03	1.07	1.23	1.47	67.79	62.77	57.82	33.25
PBW373	0.94	0.97	1.02	1.21	92.21	82.99	82.93	81.05
K607	0.77	0.83	0.86	0.93	81.01	74.97	72.98	69.86
DBW17	0.83	0.91	1.11	1.20	91.86	88.57	86.15	85.29
K6525	0.63	0.72	0.73	0.74	74.98	65.94	52.05	28.65
K802	0.77	0.80	0.84	0.91	94.77	91.95	77.82	75.57
MP4010	0.09	0.14	0.17	0.23	63.68	60.56	27.45	15.26
K9423	0.91	0.94	0.97	0.98	68.88	61.79	54.97	36.24
Gen. Mean	0.77	0.83	0.88	0.94	74.48	76.29	72.80	70.31
C.V.	7.797	5.997	7.791	1.195	0.029	0.070	0.066	2.929
F Prob.	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
S.E.M.	0.024	0.020	0.028	0.005	0.009	0.022	0.020	0.841
C.D. 5%	0.068	0.057	0.078	0.013	0.024	0.061	0.055	2.361
C.D. 1%	0.091	0.075	0.103	0.017	0.032	0.081	0.073	3.126

C-Control, T1-Treatment with PEG 10%, T2- Treatment with PEG 20%, T3- Treatment with PEG 30%



(A)



(B)

Fig 3: (A) Graphical representation of Proline of 20 wheat genotype, (B) Graphical representation of MSI of 20 wheat genotype.

Biochemical Characterization

Proline Content

Proline content accumulation is one of the most remarkable characters studied under stress condition. The accumulation of this compound is thought to represent an important adaptive response to stress. In the present study, the proline content under control condition range from 0.09µg/gfw in genotype MP4010 to 1.45µg/gfw in K802 (Table 2.; Fig 2). However the proline concentration is found to be increases as the polyethylene glycol concentration was increases upto 30%. Under stress treatment at three levels, the maximum proline content is recorded to be increased in genotype HD2733 from1.60 µg/gfw at T1, 1.64 µg/gfw at T2 and 1.72 µg/gfw at T3 level of tress treatment. Whereas genotype MP4010 shows the lowest 0.14µg/gfw, 0.17 µg/gfw and 0.23µg/gfw of proline content respectively at T1, T2, and T3 level of stress treatment.

Membrane Stability Index

Data pertaining to membrane stability index as affected by different genotypes of wheat and polyethylene glycol concentrations have been genotypes shows significant variation in membrane stability index in wheat genotype. The membrane stability index was ranged from 63.68% in genotype MP4010 to 94. 77% in genotype K802 under control condition. The minimum membrane stability index in genotype MP4010 was further reduced to 60.56% at T1, 27.45% at T2 and 15.26% at T3 level of stress treatment. However the maximum membrane stability index was 91.95% in genotype K802 at T1, 83.41% in genotype HD2733 at T2 and 85.29% in genotype DBW17 at T3 level of stress treatment. (Table 3.; Fig 3).

Discussion

In present study, poly ethylene glycol (PEG) has been used as drought stress inducer to screen drought tolerant germplasm among the 20 wheat genotypes. PEG has been used effectively to induce water stress for selecting desirable genotypes to study in detail on water scarcity on plant growth stages. (Sakthivelu *et al.*, 2008; Landjeva *et al.*, 2008; Almaghrabi, 2012; Ahmad *et al.*, 2013; Jatoi *et al.*, 2014) [48, 29, 2, 1, 22]. PEG is a polymer and considered as better chemical than others to induce water stress environment artificially by decreasing cell water potential (Larher *et al.*,1993; Kaur *et al.*,1998; Govindaraj *et al.*, 2010) [30, 25. 18].

In the present investigation, photosynthetic rate was recorded at anthesis and 15 days after anthesis. At both the stages photosynthetic rate was found reducing in the treatments when compared to controlled plants. The reduction percentage recorded from control to T1 was 5.17% &5.41%, T2 22.65% &22.85% and T3 15.46% &15.43%, respectively. The results are in accordance with the other reports where the photosynthetic activity is reducing with the increasing drought stress level (Krause *et al.*, 1991; Xu and Zhou, 2006; Liu *et al.*, 2007 and Ashraf, 2010) [27, 56, 33, 4]. Lawlor and Tezara (2009) [31] examined leaf cells under water deficient conditions and found reduced photosynthetic rate with decreased metabolic capacity. Wang *et al.*, (2016) [55] also studied alleviation in the inhibition of photosynthesis process in crops under different stress episodes due to decrease in stomatal conductance in wheat varieties. The perception of drought stress at plant level is indicated by decline in photosynthetic rate and plant growth (Cornic and Massacci, 1996) [11]. It is well documented that drought stress condition inhibits photosynthetic CO_2 fixation (Berry and Bjorkman, 1980) [6]. This reduces the expression of small and large

subunits particularly at anthesis and post anthesis stages which mainly results in significant reduction in photosynthetic rate under drought stress.

Chlorophyll content might be considered as consistent indicator in screening plant germplasm for drought tolerance (Rong-Hua *et al*., 2006) [46]. Tas and Tas (2007) [52] reported that significant reductions were observed in chlorophyll content and other physiological parameters under water stress and results of the present study are in agreement with the finding. The reduction percentage recorded from control to T1 was 3.97% & 4.84%, T2 6.03% & 6.85% and T3 8.11% & 8.65%, at both the stages, respectively. Other studies also documented the decline of chlorophyll contents in response to drought stress environments (Majumdar *et al*., 1991; Mayoral *et al*., 1981 and Kuroda and Imagawa, 1990) [34, 35, 28]. Chachar *et al.,* (2016) [8] examined 6 wheat varieties under drought stress and concluded with decreased chlorophyll content with increased stress. It is also reported that chlorophyll content of resistant and sensitive cultivars reduced under water stress conditions (Kaoau *et al*., 2007; Zaharieva *et al*., 2001) [24].

Osmotic adjustment is achieved by plant cells by accumulation of compatible solutes such as proline, proteins, polyols and betaine to protect membranes (Delauney and Verma, 1993) [12]. These compounds accumulated in high quantities generally in cytoplasm of stressed cells deprived of interfering with macromolecules and acted as osmoprotectants (Yancey, 1994) [57]. Proline has an important part in stabilizing cellular proteins and tissues in existence of high concentrations of osmoticum (Yancey, 1994 and Errabii *et al*., 2006) [57, 16]. In the present study, the proline content under control condition range from 0.09 µg/gfw in genotype MP4010 to 1.45 µg/gfw in K802. Under stress treatment at three levels, the proline content is observed to be increase slightly. The increase was recorded 7.79% in T1, 14.29% in T2 and 22.08% in T3 when compared to control plants. These results are in accordance to the findings of Tatar and Gevrek (2008) and Kameli and Losel (1996) [53, 23]. They also noticed that proline content increased under drought stress in wheat. The results of several studies also support the findings of the present study (Charest and Phan, 1990; Nayyar and Walia, 2003; Vendruscolo *et al*., 2007; Poustini *et al*., 2007; Tian and Lei, 2007; Patel and Vora 1985) [10, 38, 43, 54, 41].

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 using these traits in breeding programs. The cell membrane stability has been exclusively used as selection criterion for different abiotic stresses including drought and high temperature in wheat (Blum *et al.*, 2001 and Rahman *et al.*, 2006) [7, 44]. The cell membrane stability index is one of the sub-traits that has been used to study drought and heat stress and subsequently select tolerant genotypes (Ozturk *et al.*,2016; Elbasyoni *et al*., 2017) [40, 15]. Several associations were established between MSI and different agronomic traits under PEG treatment (Dhanda *et al.*, 2004) [13]. In the present work, membrane stability index under control condition was observed in range from 63.68% in genotype MP4010 to 94.77% in genotype K802. Under drought stress at three levels, the membrane stability index was observed to be reduced with increasing stress level. The reduction percentage recorded from control to T1 was 2.43%, T2 2.26% and T3 5.61%. Saneoka *et al.,* (2004) [49] and Azizi *et al.,* (2009) [5] also assessed the MSI under stress and non-stress condition and found that the plasma membrane stability in genotypes under stress was significantly lower that genotypes under non stress conditions. The results of Habibpor *et al.,* (2011) [20] and Gupta *et al*., (2012) [19] of their study on wheat under drought also support the results of the present study.

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