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GERMINATION, SEEDLING GROWTH AND PHYSIO-BIOCHEMICAL INDICES OF BREAD WHEAT (*Triticum aestivum* L.) GENOTYPES UNDER PEG INDUCED DROUGHT STRESS

SUMMARY

The establishment of seedlings at early growth stages of plants is one of the important determinants for higher yield potential under stress conditions. Therefore, high germination rate and vigorous seedling growth under water stress conditions is preferred to increase productivity. Considering this issue, an experiment was conducted to know the response of drought stress on the germination and seedling growth traits of available wheat cultivars. The study was carried out at the Laboratory of Horticulture in Agriculture Faculty, Cukurova University, Turkey. Seeds of five bread wheat genotypes viz. Wafia, Lucilla, Envoy, DZ19-2 and DZ19-1 were placed and grown in Petri-dishes under polyethylene glycol (PEG 6000) induced drought stresses of 0 (control=distilled water), -0.2, -0.4 and -0.6 MPa conditions for evaluation the properties of germination and seedling growth. Regarding the root length and dry weight, the genotypes Wafia, Lucilla and DZ19-1 were found superior and seemed to be relatively tolerant to drought stress over other genotypes. Regarding biochemical analysis, the proline content increased with increasing drought levels, where the maximum proline was observed in the DZ19-1 genotype. Due to drought stress, the shoot proline content increased in drought-tolerant wheat genotypes whereas the proline level decreased in the sensitive ones compared to the control. Considering the study, the genotype DZ19-1 can be treated as drought tolerant genotype, and further investigations are needed, however, to support our understanding of the drought stress effects during the whole life cycle of wheat.

Keywords: Water stress, growth indices, biochemicals, wheat genotypes, proline

INTRODUCTION

Crop production is usually restricted by abiotic stress factors such as drought, temperature, and salinity. During crop production, seed germination and seedling establishment are the most sensitive stages to abiotic stresses particularly

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to water deficit stress (Patade *et al.*, 2011; Kizilgeci *et al.*, 2020). The incidence of drought is increasing day by day owing to global warming which ultimately affecting the agriculture. Drought stress at germination stage reduced the seedling emergence and establishment (El Sabagh *et al.*, 2019; Salih and Tuncurk, 2020). Drought stress delays seed germination declines germination rate, and also significantly changes the physiology and biochemistry in seedling (Bateman *et al.*, 2016). Extreme water shortage causes a considerable reduction of morpho-physiological and metabolic activities in plants, and ultimately reduces yield and quality of crop (Aslam *et al.*, 2013). In some cases, plants exhibit adaptation to withstand water stress (Mickky and Aldesuquy, 2017). Under drought stress, the morphology, physiology, biochemistry in a plant would be adversely changed (Duan *et al.*, 2017). Germination, a very sensitive stage in plants life cycle (as it determines the degree of crop establishment), is severely affected by salinity (Vardar *et al.*, 2014).

Wheat is a major cereal crop in many parts of the world, and it is commonly known as king of cereals. Wheat grain yield is depressed by environmental stresses such as drought, heat, low temperatures, low fertility (especially nitrogen) and soil salinity (Barutcular *et al.*, 2017; Dončić *et al.*, 2019; Panfilova, and Mohylnytska, 2019; Guo *et al.*, 2020; Zaman *et al.*, 2020). Therefore, a great attention has been made to produce wheat under various environmental stresses (Mickky and Aldesuquy, 2017; Mubeen *et al.*, 2020 Popović *et al.*, 2020). Drought is a deleterious factor among environmental stresses that can reduce wheat yield (Abbasi *et al.*, 2015; Barutçular *et al.*, 2017). There are many factors which are greatly influenced the growth and productivity of crops by alleviating the drought stress effects, and selection of and cultivation drought tolerant cultivars is the most important one. Therefore, existing wheat varieties should be evaluated under drought stress conditions at seed germination and early seedling growth stages, and promising drought tolerant varieties should be recommended for crop cultivation (Alom *et al.*, 2016). Hence, screening of wheat genotypes should be made under naturally or artificially induced drought stress, and polyethylene glycol (PEG 6000) is used to induce artificial drought stress. PEG is a flexible, water-soluble polymer, and creates very high osmotic pressures. These properties make PEG one of the most useful molecules for applying osmotic pressure in biochemistry and bio-membranes experiments, in particular when using the osmotic stress technique (Partheeban *et al.*, 2017; Wikipedia, 2020). The present study was, therefore, undertaken to evaluate and select tolerant wheat genotypes for verifying their responses under different levels of PEG induced drought stress during the seed germination and seedling growth stages.

MATERIAL AND METHODS

The study was carried out at the Horticulture Laboratory, Faculty of Agriculture, Cukurova University, Turkey. The form of experiment was factorial which was laid out in a completely randomized design (CRD) with thrice in replication. Five bread wheat genotypes (Wafia, Lucilla, Envoy, DZ19-1 and

DZ19-2) were placed to petri dishes under four levels of PEG induced drought stresses viz. control (distilled water), -0.2, -0.4 and -0.6 MPa under laboratory conditions to study the properties of germination and early seedling growth.

Seeds of different wheat genotypes were treated with a 5% solution of sodium hypochlorite for 10 min, and residual chlorine effect was removed by washing the seeds thoroughly with distilled water. The 20 seeds were placed on two layers of Whatman no. 2 filter paper with 12 cm diameter Petri-dishes. Day and night lengths were 18/6 h, with $24\pm 1^\circ\text{C}$. Drought conditions were created by using PEG-6000 according to the treatment specification.

The seedlings were grown up to 7 days and then harvested. The germination and seedling growth traits like seed vigor, shoot and root lengths, coleoptile length, fresh weight of shoot and root fresh weight were measured. Turgor weight, shoot and root dry weight were measured for each plant after drying the samples in an oven at 72°C temperature for 48 h, and the relative water content (RWC) was calculated by using the following formulae:

$$\text{RWC} = (\text{FW} - \text{DW}) \times 100 / (\text{TW} - \text{DW})$$

Where, FW: Fresh weight, DW: Dry weight and TW: Turgid weight.

The proline content was measured following the protocol of Bates *et al.* (1973). The optical density of the extracted sample was recorded at 528nm wavelength in spectrophotometer, and the proline was calculated using a calibration curve of L-proline concentration (0- $5\mu\text{g/ml}$).

The chlorophyll (a, b, and total), and carotenoid contents were measured following the method developed by Agarwal (1986) with a slight modification by Higazy *et al.* (1995). The absorbance reading was taken from spectrophotometer at 480, 649 and 665 nm wavelengths.

The data were analyzed by partitioning the total variance with the help of a computer using MSTAT-C program (Gomez and Gomez, 1984). Duncan's Multiple Range Test was used to compare the treatment means (Duncan, 1955).

RESULTS AND DISCUSSION

The results of the germination and various growth characteristics of wheat seedlings under different drought stresses are shown in Table 1. Data revealed that drought stress adversely affected the seed vigor, root, coleoptile and seedling lengths, fresh and dry weights of root and shoot, RWC, proline, chlorophyll, and carotenoid content. The result of the present findings corroborates with the results of Partheeban *et al.* (2017), Kizilgeci *et al.* (2017), Guo *et al.* (2020), where they also noted that water deficit stress suppressed the membrane integrity by stimulating the lipid peroxidation as significant increase of membrane leakage.

Germination under drought condition is very important because it reflects the seedling establishment and crop yield (Mwale *et al.*, 2003). Drought is a serious threat in agriculture among the abiotic stresses that limits the seed germination, seedling growth, plants growth and yield (Aslam *et al.*, 2013; El Sabagh *et al.*, 2019). The response of drought stress on the wheat cultivars varied

regarding the germination rate, and germination index, used as drought tolerance indices at germination stage (Öztürk *et al.*, 2016).

Table 1. Analysis of variance for seedling growth properties for genotypes and PEG concentrations

SOV	df	RL	CL	SL	RFW	SFW	RDW	SDW	RWC	SV	Proline	Chl-a	Chl-b	Cd	Total Chl
Genotypes	4	**	**	**	*	**	**	**	ns	**	**	**	**	**	**
Stresses	3	**	**	**	**	**	**	**	**	**	**	**	**	**	**
G×S	12	ns	ns	ns	**	*	ns	**	**	ns	**	**	**	**	**
CV (%)		13.71	8.02	14.25	25	13.2	18.18	9.09	18.75	10.57	24.4	9.61	17.39	12.18	9.26

*= 5% level of significance; ** = 1% level of significance; ns: not significant
 SOV: Source of variance, RL: Root length, CL: Coleoptile length, SL: Seedling length RFW: Root fresh weight SFW: Seedling fresh weight RDW: Root dry weight SDW: Seedling dry weight RWC: Relative water content SV: seedling vigour Chl-a : chlorophyll-a Chl-b: chlorophyll-b Cd: Carotenoid

Root length

Under drought stress condition, behaviour of roots is an important trait that triggers to other growth traits, and plants under drought stress enhance deeper roots to absorb available water in the soil which enables higher drought tolerance (Španić *et al.*, 2017). The ANOVA of root length of bread wheat genotypes during the germination period under different levels of PEG-6000 induced stresses is presented in Table 1, whereas its mean values are given in Table 2. The differences between genotypes and concentrations were found significant at 1% level. Statistically insignificant interaction between genotypes × levels of PEG-6000 induced stress was observed. In the present study, the root length decreased significantly with increasing drought stress among the genotypes (Table 2). However, the shortest root length (8.45 cm) was recorded at DZ19-2 genotype as compared to other genotypes as they showed statistically similar root length. The genotype DZ19-1 showed the longest root lengths numerically (10.65 cm). The longer root length provides an advantage of plant roots to go deep in soil for uptake of water and nutrients. In general, it can be argued that DZ19-1, Wafia and Lucilla genotypes tolerated drought at all levels of drought stress. Nature of root length under drought stress is considered as an important index to select drought tolerant genotypes (Turner, 1997), considering the root morphology and growth rate (Malik *et al.*, 2002). Remarkable decrease in root length with increasing PEG concentrations has been observed by Jajarmi *et al.* (2009). Under drought stress, plant could show the relative adaptability in terms of morphological, physiological, and biochemical changes in order to ensure the relatively stable growth of plant, for example, reduced leaf area and increased root activity (Mwadingeni *et al.*, 2016; Duan *et al.*, 2017). Root is the main organ of plant to absorb water and nutrient, which reacts against drought stress at first (Janiak *et al.*, 2015; Duan *et al.*, 2017).

Table 2. Response of PEG 6000 induced drought stress on the root length (cm) of wheat genotypes

Root length (cm)	Genotypes	Levels of stress (MPa)				
		0 (control)	-0.2	-0.4	-0.6	Mean
	DZ19-2	12.62	7.92	7.55	5.72	8.45 b
	Lucilla	12.86	12.02	9.03	6.24	10.04 a
	DZ19-1	14.29	11.49	9.81	7.03	10.65 a
	Envoy	12.80	11.53	9.31	6.23	9.97 a
	Wafia	14.83	11.61	8.30	5.70	10.11 a
	Mean	13.48 a	10.91 b	8.8 c	6.18 d	

Means within columns followed by different lowercase letters are significantly different

Table 3. Response of PEG 6000 induced drought stress on the coleoptile length (cm) of wheat genotypes

Coleoptile length (cm)	Genotypes	Levels of stress (MPa)				
		0 (control)	-0.2	-0.4	-0.6	Mean
	DZ19-2	2.65	2.74	3.17	2.81	2.84 a
	Lucilla	2.41	2.55	2.75	2.43	2.54 c
	DZ19-1	2.75	3.05	2.96	2.96	2.93 a
	Envoy	2.79	2.89	2.86	2.48	2.76 ab
	Wafia	2.49	2.73	2.82	2.52	2.64 bc
	Mean	2.62 c	2.79 ab	2.91 a	2.64 bc	

Means within columns followed by different lowercase letters are significantly different

Table 4. Response of PEG 6000 induced drought stress on the seedling length (cm) of wheat genotypes

Seedling length (cm)	Genotypes	Levels of stress (MPa)				
		0 (control)	-0.2	-0.4	-0.6	Mean
	DZ19-2	12.15	10.13	10.85	7.11	10.06 b
	Lucilla	14.73	12.19	10.69	7.43	11.26 ab
	DZ19-1	15.81	13.65	12.46	8.16	12.52 a
	Envoy	12.54	12.19	10.01	6.90	10.41 b
	Wafia	16.10	10.67	9.68	6.83	10.82 b
	Mean	14.27 a	11.77 b	10.74 b	7.28 c	

Means within columns followed by different lowercase letters are significantly different

Coleoptile length

The differences between genotypes and stress levels were determined at 1% level of significance but the interaction between genotypes × PEG-6000 stress was found statistically insignificant (Table 1). The coleoptile length of wheat cultivars was significantly influenced by the imposition of drought stress, and it decreased considerably with the increasing concentration of PEG6000 induced stresses (Table 3). However, the coleoptile lengths were seen to increase

up to -0.4 MPa stress, and thereafter decreased at -0.6 MPa stress, but the values were higher over control condition. This shows that wheat genotypes tend to increase coleoptile lengths in response to drought stress. Wheat genotypes showed differential responses due to magnitude of drought stresses. The genotype DZ19-1 produced the highest coleoptile length which was statistically similar to Envoy and DZ19-2 under drought stress, while the lowest was recorded for Lucilla. Drought stress suppressed the seed germination which retarded the growth of young seedling, reduced the length of plumules and radicles, fresh weight of seedlings and relative water content of wheat genotypes (Mickky and Aldesuquy, 2017).

Seedling length

The variations among the genotypes as well as stress levels were found to be significant at 1% level, but the interaction between genotypes \times PEG-6000 concentrations was found to be statistically insignificant (Table 1). Wafia genotype had the longest shoot lengths (16.10 cm) under control conditions followed by DZ19-1 (15.81 cm). As drought stress increased, there was a noticeable decline in seedling length. The shortest shoot length (6.83 cm) was observed in Wafia genotype under severe stress condition, which had the highest shoot length under control conditions demonstrating its sensitivity to drought conditions (Table 4). The genotype DZ19-1 had the longest shoot lengths of 8.16 and 12.52 cm under control condition and drought stress of -0.6 MPa, respectively indicating that DZ19-1 is resistant to drought stress. Similarly, in their research, Kizilgeci *et al.* (2017) reported that PEG induced stress of -1.2 MPa caused a drastic reduction of seedling length in comparison to -0.3 MPa stress. Drought stress severely reduced the seedling growth of wheat genotypes (Španić *et al.*, 2017).

Root fresh weight

The root fresh weight of all wheat genotypes was markedly decreased with the increasing PEG induced stress over control. The mean root fresh weights of genotypes decreased with increasing the drought stress concentration (Table 5). The genotype Wafia had the highest root fresh weight under the control condition which was followed by DZ19-2 and Lucilla, while the highest root fresh weight (50.0 mg) was observed in DZ19-1, and the lowest (16.8 mg) was in Lucilla genotype at -0.6 MPa stress condition. DZ19-1 was observed to maintain the maximum root fresh weight compared to other genotypes under increased drought stress, although it maintained the minimum root fresh weight (46 mg) at the control condition and can be treated as drought stress resistant genotype.

Seedling fresh weight

The Seedling fresh weight was significantly affected by the PEG induced drought stress, genotypes, and their interaction (Table 1). The differences among the genotypes and stress levels were highly significant (1% level), and their interaction effect was also significant (5% level). There was no statistical difference between control and -0.2 MPa induced stress on the seedling fresh

weight, while the average seedling fresh weight decreased as the stress level increased. Nonetheless, DZ19-1 genotype obtained the highest seedling fresh weight (42.9 mg) under severe drought stress, indicating its tolerance to drought stress (Table 6). These results were adhering to the results of Sayar *et al.* (2010) and Španić *et al.* (2017), who reported that the seedling fresh weight of wheat genotypes decreased with increasing the osmotic stress.

Root dry weight

The root dry weight was influenced significantly (1% level) by different genotypes, and stress levels whereas no remarkable variation regarding the root dry weight was observed by the interaction effect of genotypes and stress levels (Table 1)

Table 5. Response of PEG 6000 induced drought stress on the root fresh weight (mg) of wheat genotypes

Root fresh weight (mg)	Genotypes	Levels of stress (MPa)				Mean
		0 (control)	-0.2	-0.4	-0.6	
	DZ19-2	68.2a	44.0ef	50.7cf	21.6gh	46.1bc
	Lucilla	68.4ab	53.1c-f	45.5def	16.8h	45.9bc
	DZ19-1	46.0def	36.1fg	37.8fg	50.0c-f	42.5c
	Envoy	61.8a-d	47.7c-f	58.1a-e	43.0ef	52.7ab
	Wafia	73.8a	64.2abc	48.9c-f	38.0fg	56.2a
	Mean	63.7a	49.0b	48.2b	33.9c	

Means within columns followed by different lowercase letters are significantly different

Table 6. Response of PEG 6000 induced drought stress on the seedling fresh weight (mg) of wheat genotypes

Seedling fresh weight (mg)	Genotypes	Levels of stress (MPa)				Mean
		0 (control)	-0.2	-0.4	-0.6	
	DZ19-2	67.2a	67.8ab	59.4bcd	33.7h	57.0a
	Lucilla	41.4fgh	54.2cde	49.6d-g	35.6h	45.2b
	DZ19-1	72.1a	63.5abc	56.1bcd	42.9e-h	58.7a
	Envoy	74.4a	56.8bcd	52.9c-f	37.4gh	55.4a
	Wafia	64.4abc	62.7abc	49.5d-g	37.7gh	53.6a
	Mean	63.9a	61.0a	53.5b	37.5c	

Means within columns followed by different lowercase letters are significantly different

The root dry weight decreased with the increasing drought stress levels. The genotype Envoy maintained the highest and lowest root dry weight (15.7 and 11.1 mg) both at control and -0.6 MPa PEG conditions, respectively (Table 7). The minimum root dry weight was recorded in Wafia genotype at severe water stress (-0.6 MPa) which reduced root dry weight by 42.85% over control, whereas the reduction was only by 31.25% in Envoy genotype at same condition. In root development, Envoy, DZ19-1 and Wafia genotypes showed better results in stress

conditions compared to other genotypes when examined in length and age. The decreasing trends of the shoot and root dry weight with increasing drought stress was reported previously by Chachar *et al.* (2014) and Molla *et al.*, (2019). Our results are line with the results of Jatoi *et al.* (2014), who concluded that the root dry weight of wheat genotypes was reduced gradually with the increasing PEG concentrations.

Seedling dry weight

Seedling dry weight is the consequence of plant physiological and biological activities. Seedling dry weight values were decreased with the increase of PEG6000 concentrations in all wheat genotypes. Differences among the genotypes, stress levels, and their interaction were found to be significant at 1 % level. According to the results, the shoot dry weight ranged from 8.2 mg to 16.8 mg owing to the interaction between stress levels and genotypes (Table 8). When the differences among water stress levels were analysed, the seedling dry weight decreased statistically with the imposing of moderate and severe (-0.6 MPa) drought stresses. The Wafia genotype, which had the highest seedling dry weight under control conditions, was found to reduce drastically (52.94%) at -0.6 MPa compare to other genotypes. Islam *et al.* (2018) depicted that the seedlings dry weight decreased with the increasing PEG6000 induced stress levels in rice genotypes. Rana *et al.* (2017) found a wide difference among wheat genotypes to stress tolerance based on their tolerance index for seedling dry weight.

Relative water content

Relative water content (RWC) was used as a measure of drought tolerance indicator. PEG-induced drought stress considerably reduced the RWC in the shoot over control (Table 9). The differences among the stress levels were significant regarding the RWC at the level of 5%, and the interaction between PEG-6000 induced stress and genotypes was found significant at the level of 1%. However, the differences among the genotypes were found statistically insignificant. From the study, it is shown that the RWC decreased with the increment of drought stresses. Among all, the genotype Lucilla showed the highest RWC at severe stress conditions. Many researchers (Yassin *et al.*, 2019; Monsur *et al.*, 2020) confirmed that proline is accumulated in crops under stress environment. Drought stress induced by PEG decreased the water status in shoot which is consistent with the study of Guoxiong *et al.* (2002), who reported that PEG induced drought stress significantly decreased the RWC. It is reported earlier that larger seeds produced longer root lengths that help to uptake more water resulting in higher RWC in shoot (Kaydan and Yagmur, 2008)

Seed vigour

Vigour test does not only measure the percentage of viable seeds in a sample, it also reflects the ability of those seeds to produce normal seedlings under adverse growing conditions alike to field conditions. The differences among the genotypes and water deficit stresses regarding the seed vigour were found significant at 1% level, but their interactions were found statistically insignificant (Table 1).

Table 7. Effect of PEG 6000 induced drought stress on the root dry weight (mg) of wheat genotypes

Root dry weight (mg)	Genotypes	Levels of stress (MPa)				
		0 (control)	-0.2	-0.4	-0.6	Mean
	DZ19-2	11.3cde	8.3e	8.2e	7.6e	8.8d
	Lucilla	15.2ab	16.7a	15.6ab	9.1de	14.1a
	DZ19-1	13.2abc	10.9cde	12.4bcd	10.2cde	11.7bc
	Envoy	15.7ab	13.8abc	13.2abc	11.1cde	13.4ab
	Wafia	14.0abc	13.0abc	10.3cde	8.1e	11.3c
	Mean	13.9a	12.5ab	11.9b	9.2c	

Means within columns followed by different lowercase letters are significantly different

Table 8. Response of PEG 6000 induced drought stress on the seedling dry weight (mg) of wheat genotypes

Seedling dry weight (mg)	Genotypes	Levels of stress (MPa)				
		0 (control)	-0.2	-0.4	-0.6	Mean
	DZ19-2	14.8abc	16.4ab	12.3c-f	7.8j	12.8a
	Lucilla	12.1d-g	11.1e-h	9.6g-j	8.2ij	10.2c
	DZ19-1	12.7cde	11.3d-h	12.3c-f	8.2ij	11.1bc
	Envoy	13.9bcd	9.9f-j	9.4hij	8.9hij	10.5bc
	Wafia	16.8a	10.6e-i	11.0e-h	8.3ij	11.7ab
	Mean	14.1a	11.9b	10.9b	8.3c	

Means within columns followed by different lowercase letters are significantly different

Table 9. Effect of PEG 6000 induced drought stress on the relative water content of wheat genotypes

Relative water content (%)	Genotypes	Levels of stress (MPa)				
		0 (control)	-0.2	-0.4	-0.6	Mean
	DZ19-2	0.86 ab	0.72 b-e	0.55 e-h	0.51 f-h	0.66
	Lucilla	0.97 a	0.70 b-g	0.67 b-h	0.55 e-h	0.72
	DZ19-1	0.76 b-d	0.62 c-h	0.57 d-h	0.52 f-h	0.62
	Envoy	0.82 a-c	0.73 b-e	0.48 h	0.47 h	0.62
	Wafia	0.71 b-f	0.71 b-f	0.51 f-h	0.50gh	0.60
	Mean	0.82 a	0.70 ab	0.56 b	0.51 b	

Means within columns followed by different lowercase letters are significantly different

The average seed vigour decreased statistically with the increasing levels of drought stress. When the average seed vigour among genotypes was examined, the highest germination was observed in Envoy genotype, and the lowest was in

DZ19-1, which was statistically similar to DZ19-2, Wafia, and Lucilla genotypes (Table 10). The size of seed is positively correlated with the seed vigor, and larger sized seeds have a tendency to produce more vigorous seedlings (Ries and Everson, 1973). It was pointed out that bolder seeds produced vigorous seedlings, taller plants with greater tillering, and higher dry matter as compared to smaller seeds under drought stress (Westoby *et al.*, 1996).

Table 10. Response of PEG 6000 induced drought stress on the seed vigour (%) of wheat genotypes

Seed vigour (%)	Genotypes	Levels of stress (MPa)				Mean
		0 (control)	-0.2	-0.4	-0.6	
	DZ19-2	75.00	83.33	83.33	58.33	75.00 b
	Lucilla	91.67	85.00	75.00	73.33	81.25 b
	DZ19-1	88.33	90.00	80.00	61.67	80.00 b
	Envoy	98.33	93.33	85.00	80.00	89.17 a
	Wafia	98.33	88.33	78.33	58.33	80.83 b
	Mean	90.33 a	88.00 a	80.33 b	66.23 c	

Means within columns followed by different lowercase letters are significantly different

Proline

Proline is one of the most important metabolites which acts as cytosolute different types of environmental stresses like drought, high temperature, nutrient deficiency, exposure of heavy metals, and high acidity induced to accumulate proline for adaptation of plants (Oncel *et al.*, 2000; Chen and Murata, 2002; Yasar *et al.*, 2006; Wang, 2012).

The increased proline content under drought helps for osmotic adjustment. Genotypes, PEG-6000 induced stresses, and their interaction were statistically significant at 1% level (Tables 1 & 11). At increased PEG concentrations, the amount of proline increased compared to the control condition. However, the DZ19-1 genotype accumulated the highest amount of proline (2.19 micromol/g) under control condition, and maintained the similar trends at all stress levels, indicating it as a drought tolerant genotype. The genotype Envoy produced the lowest proline content under stress conditions (Table 11). Plants under drought produce compatible solutes to maintain cell turgor, which results in reduced osmotic stress in plants (Silva *et al.*, 2010). One of the important compatible solutes is proline, which acts as a protectant to defend cell membrane and also as an ROS scavenger that could provide energy for growth and survivability under stress condition (Sankar *et al.*, 2007; Rezayian *et al.*, 2018).

Chlorophyll a

The chlorophyll a (*Chl a*) content was significantly influenced by the genotypes and drought stress, and their interaction between drought stress and genotype. The values of *Chl a* decreased with the increase of drought stresses, and the lowest values were recorded at the severe drought stress in all the genotypes. Nevertheless, the genotypes DZ19-2 and DZ19-1 showed the highest

amount of *Chl a* (0.90 and 0.83 mg/g, respectively) under control conditions well as under severe drought condition (Table 12). *Chl* content is known as an index of photosynthates for the evaluation of source (Zobayed *et al.*, 2007), therefore decrease of *Chl a* can be considered as a non-stoma limiting factor in the drought stress conditions (Khayatnezhad and Gholamin, 2012). Stress can cause a change in chlorophyll content of plant leaves, and thus a change of photosynthetic function (Qian *et al.*, 2003). Manivannan *et al.* (2007) concluded that *Chl a*, *Chl b*, and the total chlorophyll contents in sunflower varieties are considerable reduced due to drought stress.

Table 11. Response of PEG 6000-induced drought stress on the proline content of wheat genotypes

	Genotypes	Levels of stress (MPa)				
		0 (control)	-0.2	-0.4	-0.6	Mean
Proline (micromol/g)	DZ19-2	0.81 e	1.34 de	2.40 ab	2.31 ab	1.72 b
	Lucilla	1.36 de	1.20 de	1.21 de	1.60 cd	1.35 cd
	DZ19-1	2.19 bc	2.67 ab	2.95 a	2.44 ab	2.56 a
	Envoy	1.00 de	0.95 de	1.27 de	1.16 de	1.10 d
	Wafia	1.01 de	1.26 de	0.96 de	2.72 ab	1.49 bc
	Mean	1.27 c	1.49 bc	1.76 ab	2.05 a	

Means within columns followed by different lowercase letters are significantly different

Chlorophyll-b

Drought stress had remarkable differential influence on the chlorophyll b (*Chl b*) content in the shoots. The *Chl b* was also significantly (1%) influenced by the interaction between PEG-6000 and genotypes (Table 1 & 13). DZ19-2 genotype accumulated the highest amount of *Chl b* in control condition (0.47 mg/g), and the lowest in drought stress of -0.6 MPa (0.21 mg/g). However, the DZ19-1 genotype showed the minimum resistance as against the drought stress of both -0.4 and -0.6 MPa. The minor changes in chlorophyll content and the stability of chlorophyll a/b ratio allow to conclude that the pigment apparatus is comparatively resistant to drought in wheat cultivars (Nikolaeva *et al.*, 2010). Decreased or unchanged chlorophyll level during drought stress has been reported in many species, depending on the duration and severity of drought stress (Hu *et al.*, 2007; Xu *et al.*, 2020).

Total chlorophyll

Chlorophyll fluorescent is used as a stress tolerant index to study the effects of different kinds of stresses (Zobayed *et al.*, 2007). Total chlorophyll content was remarkably influenced by the wheat genotypes; PEG-6000 induced drought stress, and their interaction (Table 1 & 14). In this study, the DZ19-2 genotype had the highest amount of chlorophyll under control. Although, the genotype Envoy protect its chlorophyll content as against the increased drought stress levels (like DZ19-2), and it was also statistically similar to DZ19-2 and

Envoy. The decrease in chlorophyll content under drought stress has been considered a typical symptom of oxidative stress, and Pigment photo-oxidation and chlorophyll degradation are responsible for diminishing the chlorophyll content in drought stress, and is considered as a typical symptom of oxidative stress (Sharif and Mohammadkhani, 2016). A similar dependence of chlorophyll content in dehydrated leaves was previously observed in barley seedlings by Pshibytko *et al.* (2004). The chlorophyll content in the non-stress treatment is higher than that of the drought stress treatments (Xu *et al.*, 2020).

Table 12. Response of PEG 6000 induced drought stress on the chlorophyll a of wheat genotypes

	Genotypes	Levels of stress (MPa)				
		0 (control)	-0.2	-0.4	-0.6	Mean
Chlorophyll a (mg/g)	DZ19-2	0.90 a	0.59 b-d	0.52 d	0.51 d-f	0.63 a
	Lucilla	0.55 cd	0.41 g	0.39 g	0.25 h	0.40 d
	DZ19-1	0.83 a	0.63 bc	0.30 h	0.42 fg	0.55 b
	Envoy	0.68 b	0.51 de	0.67 b	0.43 e-g	0.57 b
	Wafia	0.65 b	0.41 g	0.42 fg	0.40 g	0.47 c
	Mean	0.72 a	0.51 b	0.46 c	0.40 d	0.52

Means within columns followed by different lowercase letters are significantly different

Table 13. Response of PEG 6000 induced drought stress on the chlorophyll b of wheat genotypes

	Genotypes	Levels of stress (MPa)				
		0 (control)	-0.2	-0.4	-0.6	Mean
Chlorophyll b (mg/g)	DZ19-2	0.47 a	0.28 c-e	0.22 e-1	0.21 e-1	0.29 a
	Lucilla	0.24 d-h	0.19 g-1	0.20 f-1	0.20 g-1	0.21 b
	DZ19-1	0.33 bc	0.25 d-g	0.16 1	0.17 1	0.23 b
	Envoy	0.36 b	0.30 b-d	0.27 c-f	0.17 1	0.27 a
	Wafia	0.21 e-1	0.17 h1	0.20 e-1	0.19 g-1	0.20 b
	Mean	0.32 a	0.24 a	0.21 bc	0.19 c	

Means within columns followed by different lowercase letters are significantly different

Carotenoid

Genotypes, PEG-6000 induced drought stresses, and their interaction were found significant at 1% level on the carotenoid content. Differential responses among the genotypes regarding carotenoid content were recorded under control and PEG induced drought stress conditions. The carotenoid content gradually decreased with increasing drought stress from 0 (Control) to -0.6 MPa. However, at the highest level of drought stress (-0.6 MPa), the genotype DZ19-2 recorded the highest carotenoid content (0.17 mg/g), which was statistically identical with Envoy, DZ19-1 and Wafia genotypes. In previous reports, Shmat'ko and Shvedova (1977) revealed that drought resistant wheat varieties exhibited a minor or no change in the pigment content compared to drought susceptible varieties.

The result differs from those of Wang (2003), who reported that the carotenoid content in leaves of winter wheat increased under drought stress.

Table 14. Response of PEG 6000 induced drought stress on the total chlorophyll of wheat genotypes

	Genotypes	Levels of stress (MPa)				
		0 (control)	-0.2	-0.4	-0.6	Mean
Total chlorophyll(mg/g)	DZ19-2	1.72 a	1.13 de	0.93 f	0.89 fg	1.17 a
	Lucilla	1.01 ef	0.74 gh	0.74 h	0.54 j	0.76 d
	DZ19-1	1.50 b	1.13 de	0.58 ij	0.73 h	0.99 b
	Envoy	1.30 c	1.38 bc	1.23 cd	0.75 gh	1.16 a
	Wafia	1.10 de	0.73 hi	0.78 gh	0.73 h	0.83 c
	Mean	1.33 a	1.02 b	0.85 c	0.73 d	0.98

Means within columns followed by different lowercase letters are significantly different

Table 15. Response of PEG 6000 induced drought stress on the carotenoid content of wheat genotypes

	Genotypes	Levels of stress (MPa)				
		0 (control)	-0.2	-0.4	-0.6	Mean
Carotenoid (mg/g)	DZ19-2	0.36 a	0.26 cd	0.19 fg	0.17 f-h	0.25 a
	Lucilla	0.21 ef	0.15 hi	0.14 hi	0.10 j	0.15 c
	DZ19-1	0.33 ab	0.25 cd	0.11 ij	0.14 hi	0.21 b
	Envoy	0.25 cd	0.16 gh	0.29 bc	0.15 g-i	0.21 b
	Wafia	0.24 de	0.14 hi	0.16 gh	0.14 hi	0.17 c
	Mean	0.28 a	0.19 b	0.18 b	0.14 c	

Means within columns followed by different lowercase letters are significantly different

CONCLUSIONS

Water stress significantly reduced the germination, seedling growth and physiological properties in all wheat genotypes, and the genotype DZ19-1 showed the best performance against the drought stress. The proline level was increased due to drought stress, and the highest proline levels were observed in the DZ19-1 genotype. Among the tested genotypes, DZ19-1 can be treated as a drought tolerant genotype due to performing most excellent growth during seedling stage under drought conditions.

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