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Screening of potential wheat genotypes for drought tolerance using polyethylene glycol

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Abstract

Screening for early drought tolerance by using three treatments of PEG-6000, control (T_1), 15% (T_2) and 25% (T_3) was employed in six wheat genotypes (DH 114, DH 40, DH 65, C 306, DH 52 and DH 5). The genotypes were evaluated for shoot length, coleoptile length, root length and root to shoot ratio. From the results, shoot, coleoptile and root length decreased with increased PEG concentrations. DH 40 expressed highest shoot length in T_2 and T_3 with least reduction and C 306 witnessed longer coleoptile with least reduction over control. DH 114 expressed highest root length in all treatments, while DH 40 had least reduction (3.31 and 7.96% in T_2 and T_3, respectively). C 306 in T_3 and DH 114 in T_2 were superior for root to shoot ratio. DH 40, DH 114 and C 306 possessed early drought tolerance behaviour and might be productive in wheat improvement programmes.

Key words: Coleoptile length, DH 40, drought tolerance, PEG.

Staple food crop production has a pivotal role in livelihood and economy of the farmers, while commercial production has major weightage for every country's economic status. Among major staple food crops of the world, cereals are the leading contributors, among which bread wheat (*Triticum aestivum* L.) is one with largest contribution. Bread wheat is the most important cereal crop of Himachal Pradesh with production of 564 thousand tonnes in 2019 covering an area of 360 thousand hectares (Anonymous, 2019). Himachal Pradesh being a hilly region covering various agro-climatic zones, wheat is cultivated in the *rabi* except in dry temperate regions of the state, where it is cultivated in summer (April-May to September-October). Bread wheat, being a *rabi* season crop is exposed to a number of environmental stresses namely, drought, cold and heat stress among which drought stress is the major contributor to crop losses. The state's 80% cultivable area is under rain-fed agriculture, making crop cultivation a challengeable task. The rain-fed wheat cultivation face serious problems of water stress which hinders the production and productivity of wheat.

Moisture or drought stress occurs at all stages irrespective of growth stages which largely depends on the local environmental conditions. Drought is a stress condition which changes with time and severity with spells ranging from long drought season with less rainfall to short season where seedlings completely depend on available soil moisture. Based on the severity, drought stress in wheat can be more threatening at the seedling stage, mid-season water stress, terminal stress or a combination of any two or three (Chachar *et al.* 2014). Selection and breeding for drought tolerance is a challenging aspect as wheat plant experience dramatic changes in the physiological, biochemical and morphological parameters which need to be measured and understood. Drought indices include biochemical, physiological and morphological parameters, among which comparison of yield loss under water stressed condition with normal condition have been used for screening drought-tolerant genotypes. Screening and identification of wheat genotypes that can tolerate water stress is important to boost the wheat production which can be achieved by exploring genetic potential from available germplasm of wheat. Knowledge of character association for seedling traits under water deficit conditions is also important for understanding yield limiting factors.

Polyethylene glycol (PEG-6000) is used as low molecular weight osmotica wherein it withdraws water not only from the cell but also from the cell wall of the plant. PEG induces reduction in growth of germinated seeds and hinders seedling growth thereby affecting

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development of shoot more than primary roots. Therefore, PEG mimics soil drying and induces drought stress in plants which complements the breeder in selection of suitable desirable drought tolerant trait at early seedling stages in *in vitro* conditions (Nepomuceno *et al*. 1998). The present investigation was executed to identify superior wheat genotypes under drought stress condition which elucidates the response through various early seedling traits.

Materials and Methods

The present investigation to evaluate the response of early drought stress, six wheat genotypes *viz*., DH 114 (released variety of CSK HPKV, Palampur), DH 40, DH 65, DH 52, DH 5 and C 306 were subjected to experimentation. The parentage and their source have been mentioned in the Table 1. The DH genotypes subjected to the study were developed by utilizing the wheat × maize (Laurie and Bennett 1988) and wheat × *Imperata cylindrica* (Chaudhary *et al.* 2005)- mediated chromosome elimination approach of doubled haploidy breeding in the Molecular Cytogenetics & Tissue Culture Lab (MCTL), Department of Genetics & Plant Breeding, CSK HPKV, Palampur. These genotypes were obtained from the Department of Genetics & Plant Breeding, CSK HPKV, Palampur (H.P.). C 306 is a national variety known for its extra ordinary drought tolerance attributes. The DH genotypes under study have desirable morpho-physiological and superior yield contributing traits and therefore needed experimentation for early drought tolerance behaviour.

The prime objective of the experiment was to study the early drought tolerance behaviour of six bread wheat genotypes by imposing drought stress through PEG (polyethylene glycol)-6000. Treatment of plants with PEG has been widely used to induce abiotic stress to screen for drought tolerance (Jatoi *et al*. 2014). The seeds of the genotypes were surface sterilised with 4% sodium hypochlorite solution followed by washing with distilled water to remove the adhered sodium hypochlorite solution. The experiment was conducted in completely randomized design (CRD) with three replications each in *in vitro* condition. Ten seeds of each genotype were sown in a petriplate with three treatments *viz*., control-0% (T_1), 15% (T_2) and 25% (T_3) PEG-6000 placed on a germination paper. Petriplates were covered with the lid to avoid moisture loss and were incubated at temperature 25±2℃ with 60-70% humidity. The responses of six genotypes in different treatments were evaluated after 7 days of incubation. The responses of genotypes were recorded based on following parameters: shoot length (cm), root length (cm), coleoptile length (cm) and root shoot ratio. Per cent change in the parameters in T_2 and T_3 over control was also analysed. These are the important morphological attributes that categorize the wheat genotypes for early drought tolerance behaviour. For statistical analysis, ANOVA and Duncan's Multiple Range Test (DMRT) (Duncan, 1955) was performed.

Results and Discussion

Shoot length (cm)

Early drought stress imposes severe impact on seedling emergence and crop stand (Noorka and Khaliq 2007). Drought conditions slow down the mechanism of seedling growth by lowering the water potential. Increased shoot length with high vigour under moisture stress reduces the chance of crop failure at early seedling growth stages. Therefore, shoot length is one of the parameters that clearly differentiates genotypes exhibiting variable responses under early moisture stress. The analysis of variance revealed that mean sum of square due to genotypes, PEG concentration and their interaction was significant for shoot length (Table 2).

Table 1. Parentage and source of genotypes used in the present study

Sr. no	Genotypes	Parentage	Source
1.	DH 114	VWFW 452 × WW 24	Department of Genetics & Plant Breeding, CSK HPKV, Palampur
2.	DH 40	Saptdhara × HW 3024	--do--
3.	DH 65	WW 24 × PW 552	--do--
4.	DH 52	W 10 × HW 3024	--do--
5.	DH 5	Saptdhara × HPW 184	--do--
6.	C 306	RGN/CSK3//2*C5 91/3/C217/N14 //C281	--do--

The experiment revealed variable results in control (T_1), 15% (T_2) and 25% (T_3) PEG treatment (Table 3). The shoot length varied from 8.2 cm to 11.0 cm in control treatment wherein DH 40 (11.0 cm) manifested maximum shoot length followed by DH 5 (10.9 cm), while, minimum shoot length was exhibited by DH 52 (8.2 cm). In treatment T_2, the shoot length was reduced exponentially. The genotype DH 40 expressed highest shoot length (8.2 cm) and DH 5 witnessed least shoot length (3.6 cm) accounting 24.97 and 67.14% reduction over control, respectively. Treatment T_3 further decreased the shoot length over treatment T_1 which ranged from 3.0 cm (DH 5) to 6.6 cm (DH 40) with the reduction of 72.51 and 40.11% reduction over control (T_1), respectively. Minimum reduction in shoot length was recorded in DH 114 in T_2 (23.26%) and T_3 (32.10%) over control (T_1), whereas DH 5 exhibited maximum reduction of 67.15 and 72.51% in T_2 and T_3, respectively over control (T_1) (Figure 1a). From the results obtained in the study, the shoot length decreased with increase in PEG concentration. Genotypes DH 40 and DH 114 as compared to other genotypes were less affected at low and high levels of moisture stress. PEG induces artificial moisture deficit condition which decreases turgor pressure and reduces cell division resulting in poor shoot growth (Lagerwerff *et al.* 1961). Previous studies of Khakwani *et al.* (2011) have also reported significant reduction in shoot length of wheat varieties at the seedling stage under PEG stress.

Coleoptile length

Coleoptile is the cylindrical organ that ensheath the first leaf and shoot apex in cereal and grass seedlings. Coleoptile protects the wheat seedlings from soil desiccation and it contributes largely to seedling emergence, therefore coleoptile length is considered as drought resistant attribute in wheat seedlings (Song-ping *et al.* 2007). The analysis of variance revealed that mean sum of square due to genotypes, PEG concentration and their interaction was significant for coleoptile length (Table 2). The results of the experiment revealed that in treatment control (T_1),C 306 exhibited maximum coleoptile length (3.3 cm) followed by DH 40 (3.2 cm), while DH 114 manifested minimum length (2.5 cm). In T_2, the highest response for coleoptile length was shown by C 306 (3.1 cm) followed by DH 40 (2.9 cm) with only 7.42 and 10.50% reduction over T_1, respectively. In T3, DH 40 and C 306 showed same coleoptile length (2.7 cm) (Table 3). Per cent reduction was lowest in DH 40 with only 15.84% while C 306 exhibited 17.45% reduction. Maximum reduction for coleoptile length was observed in DH 52 in T_2 and T_3 treatments (29.50 and 36.73%, respectively) (Figure 1b). Longer coleoptile length generally favour the high vigour in wheat seedlings than the smaller coleoptile length under soil desiccated or moisture stress condition (Rebetzke *et al*. 2005). From the present study, it is evident that coleoptile length decreases with increase in moisture stress. Similar results have also been reported by Bayoumi *et al.* (2008). In the study, variety C 306 followed by DH 114 and DH 40 recorded longer coleoptile length favouring high seedling emergence during moisture stress in field condition. The superiority of C 306 for longer coleoptile length under moisture stress was also reported by Kumari *et al.* (2014). These genotypes were less affected by both PEG concentrations indicating drought tolerance attributes at both level of stresses.

Root length (cm)

Root components play a crucial role in elevating the positive plant responses to drought stress. Profuse root system is a mechanism of desiccation avoidance in natural vegetation. The analysis of variance revealed that mean sum of square due to genotypes, PEG concentration and their interaction was significant for

Table 2. Analysis of variance for different traits in six wheat genotypes

Sr.No.	Traits	Source	Mean sum of square			
			Genotype (Factor A)	PEG (Factor B)	Interaction (A × B)	Error
		df	5	2	10	36
1.	Shoot length		15.40*	126.44*	3.48*	0.48
2.	Coleoptile length		1.273*	2.156*	0.054*	0.031
3.	Root length		19.603*	14.219*	14.219*	0.084
4.	Root to shoot ratio		0.159*	0.690*	0.041*	0.009

root length (Table 2). From the study, the genotypes expressed variable root lengths in control (T_1), T_2 and T_3 treatments (Table 3). In control (T_1), the root length of DH 65 was limited to 4.2 cm, whereas in treatment T_2 and T_3, root length of DH 5 was limited to 3.5 and 2.8 cm, respectively. In control, T_2 and T_3, DH 114 manifested maximum length (9.0, 7.3 and 6.6 cm, respectively). Per cent reduction of root length after treatment T_2 over control (T_1) revealed that DH 40 followed by DH 65 were least affected (3.31 and 4.20, respectively). Treatment T_3 recorded least reduction of root length in DH 40 and DH 65 (7.96 and 12.51%, respectively).

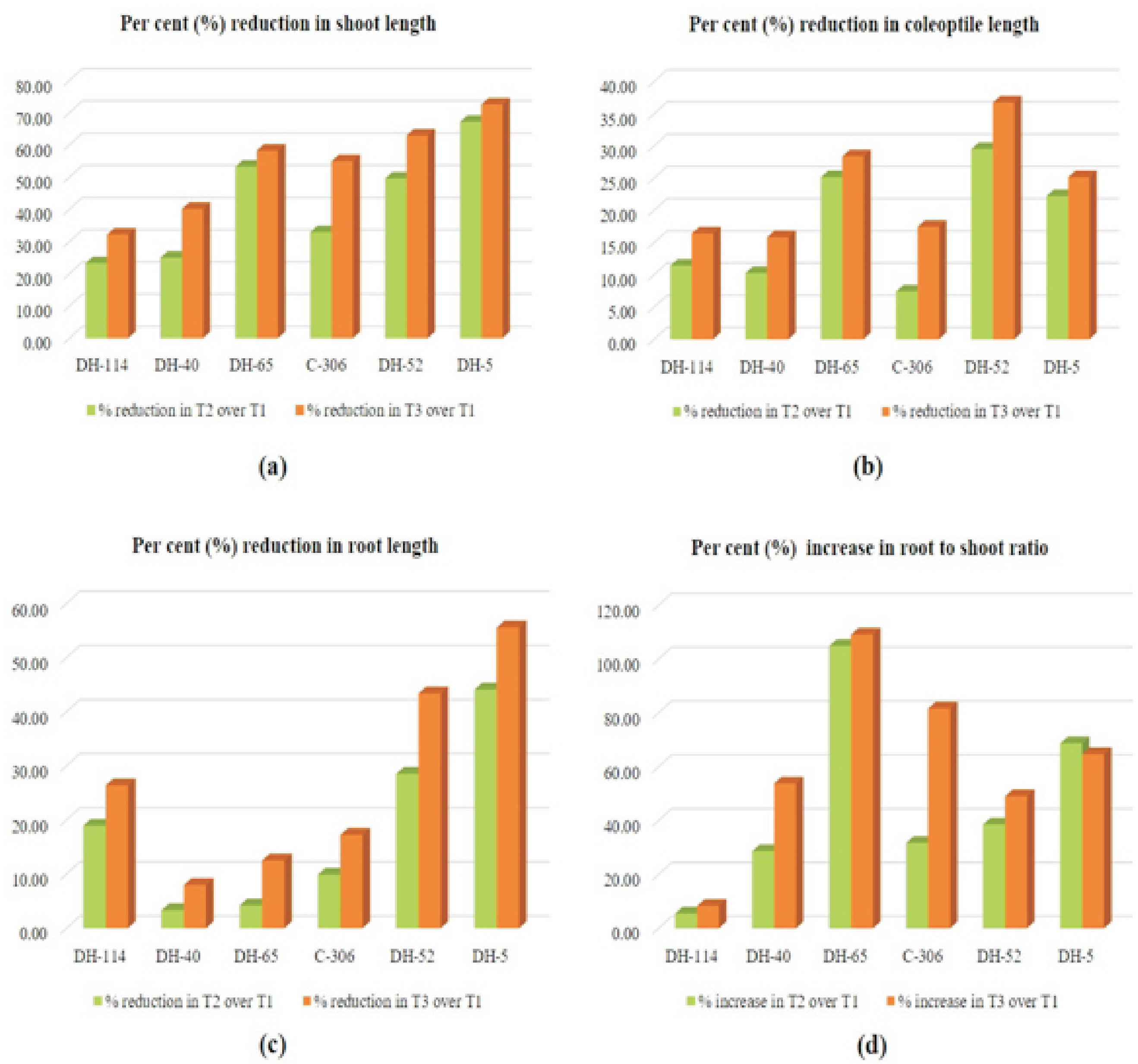

Figure 1. Per cent change in parameters in T2 and T3 over control (a) shoot length (b) coleoptile length (c) root length and (d) root to shoot ratio

Table 3. Effect of various PEG treatments on shoot length, coleoptile length, root length and root to shoot ratio in wheat genotypes

Genotypes	Shoot length (cm)	Coleoptile length (cm)	Root length (cm)	Root to shoot ratio
DH 114 (Control)	8.3[c]	2.5[ef]	9.0[a]	1.08[bcd]
DH 114 (T_2)	6.4[d]	2.2[fg]	7.3[b]	1.14[bc]
DH 114 (T3)	5.7[d]	2.1[gh]	6.6[cd]	1.17[ab]
DH 40 (Control)	11.0[a]	3.2[ab]	6.4[cde]	0.59[fgh]
DH 40 (T_2)	8.2[c]	2.9[bcd]	6.2[def]	0.76[ef]
DH 40 (T3)	6.6[d]	2.7[de]	5.9[efg]	0.9[de]
DH 65 (Control)	8.3[c]	2.9[bcd]	4.2[h]	0.51[h]
DH 65 (T_2)	3.9[e]	2.2[fg]	4.0[hi]	1.04[bcd]
DH 65 (T3)	3.5[e]	2.1[gh]	3.7[hij]	1.06[bcd]
C 306 (Control)	9.6[b]	3.3[a]	6.8[c]	0.73[fg]
C 306 (T_2)	6.5[d]	3.1[abc]	6.2[def]	0.96[cd]
C 306 (T3)	4.3[e]	2.7[de]	5.7[fg]	1.32[a]
DH 52(Control)	8.2[c]	2.8[cde]	5.6[g]	0.71[fg]
DH 52 (T_2)	4.1[e]	2.0[gh]	4.0[hi]	0.98[cd]
DH 52 (T3)	3.0[e]	1.8[h]	3.2[jk]	1.05[bcd]
DH 5 (Control)	10.9[a]	2.7[de]	6.3[cde]	0.58[gh]
DH 5 (T_2)	3.6[e]	2.1[gh]	3.5[ij]	0.98[cd]
DH 5 (T3)	3.0[e]	2.0[gh]	2.8[k]	0.96[cd]

Induction of osmotic stress affects the water uptake and reduces turgor pressure that eventually causes reduction of root length. Therefore, DH 5 is considered less responsive to water stress which expressed highest reduction in root length in both T_2 and T_3 over control (44.09 and 55.54%, respectively) (Figure 1c). The significant reduction of root length over PEG treatment is aligning with the reports of Khakwani *et al.* (2011). Leishman and Westoby (1994) reported that genotypes with longer root growth under moisture stressed condition were tolerant. Accordingly, it could be stated that DH 5 with least root length under moisture stress condition is susceptible and DH 40 with longest root length is tolerant to drought.

Root to shoot ratio

Root to shoot ratio tend to increase with increasing PEG concentration. The analysis of variance revealed that mean sum of square due to genotypes, PEG concentration and their interaction was significant for root to shoot ratio (Table 2). From the responses of shoot and root lengths in the present study, root to shoot ratio was calculated which varied accordingly with the treatment (Table 3). Among all treatments, root to shoot ratio was found to be highest in treatment T_3. In control (T_1), the ratio varied from 0.58 in DH 5 to 1.08 in DH 114, whereas after treatment T_2, genotypes showed an increase in root to shoot ratio and it varied from 0.76 (DH 40) to 1.14 (DH 114). After treatment T_3, C 306 with ratio 1.32 exhibited an increase of 81.67% over control. Overall, the root to shoot ratio was highest in DH 114 after T_2 and C 306 after T_3. The per cent increase in the ratio was found to be highest in DH 65 after both T_2 and T_3 over control (104.87 and 108.15%, respectively), but least increment was observed for DH 114 (5.45 and 8.29%) after T_2 and T_3 over control (Figure 1d). Genotypes with more of drought tolerance attributes have higher root to shoot ratio (Cui *et al.* 2008). The highest increase in root-shoot ratio under PEG-induced moisture stress indicated that PEG stress positively influenced root growth compared to shoot growth. The increase in the ratio is a result of greater reduction in shoot growth than an increase in root biomass, while reduced ratio is due to higher shoot growth. The findings of Khakwani *et al.* (2011) support the result of root to shoot ratio from the present investigation.

The genotypes under study revealed valuable results under control (T_1), 15% PEG (T_2) and 25% PEG (T_1). To conclude from the experiment, based on ranking for drought tolerant parameters at early seedling growth, DH 40, DH 114 and C 306could be classified as drought tolerant genotypes based on early seedling study on shoot length, root length, coleoptile length and root shoot ratio. To be precise, DH 40 is ranked above all genotypes with longer shoot length, coleoptile length and root length exhibiting lesser per cent reduction over control in PEG treatments. Genotypes DH 5 and DH 52 could be stated as susceptible to drought stress because of higher per cent reduction in all the four parameters in the study. While, DH 65 was moderate for all the parameters. Screening for drought tolerance in early stages of seedling growth is a successful tool in wheat breeding programme. These genotypes should be tested in field trials for their correlation between laboratory and *in vivo* (field) conditions. Furthermore, these genotypes can be utilised in future breeding programmes for development of drought tolerant cultivars.

Conflict of interest: There is no conflict of interest among the authors.

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