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Morpho-physiological character association studies of seedling traits in ten wheat (*Triticum aestivum* L.) genotypes under osmotic stress conditions

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Abstract
In the past, many environmental stress especially water stress, negatively influenced the productivity and production of wheat (*Triticum aestivum* L.), one of the most important cereal crop in the world. Breeding current high yielding water stress tolerant wheat genotypes is a research priority specifically for these regions where climate change is predicted to result in more water stress conditions. A strategy that evaluates genotypes for physiological responses to water stress at earlier growth stages may be more targeted to water stress. Therefore, present investigation, ten wheat genotypes were screened following a completely randomized design under controlled condition. Different concentrations of PEG-6000 were used to induce osmotic stress at the germination growth stage. The analysis of variance indicated that significant differences among treatments for all seedling traits. All genotypes also differed significantly in germination percentage, shoot and root length, fresh and dry weight of shoot and root. Significant treatment interaction revealed that genotypes responded variably to osmotic stress treatments; hence provided better opportunity to select a water stress tolerant genotype at seedling growth stages. Data revealed a decreasing trend in values for all traits with the increasing concentration of PEG-6000. The relative minimum decrease over average due to osmotic stress was 9.04 percent in seed germination, 20.58 percent in shoot length, 9.01 percent in root length, 10.04 percent in shoot fresh weight, 3.30 percent in shoot dry weight, 11.57 percent in root fresh weight and 11.01 percent in root dry weight. However, relative decrease of individual genotype for various seedling traits might be more meaningful which indicated that genotype AKAW-5017 showed minimum reduction in root length, fresh root weight, dry root weight, fresh shoot weight and dry shoot weight while the minimum decline of seed germination and shoot length observed in AKAW- 3717. However, AKAW-4842, AKAW- 4907, and AKAW-4926 gave almost equally lower reduction for seed germination, root length, and shoot length and were consider as water stress tolerant. Correlation coefficient studies revealed considerable and positive correlation among seedling traits. The result indicated that increase in one trait may cause a simultaneous an increase in other traits; hence, selection for any of these seedling attributes will lead to developing water stress tolerant wheat genotypes.

Keywords: *Triticum aestivum*, water stress, genotypes, seedlings traits, PEG-6000

1. Introduction
In past year, globally wheat (*Triticum aestivum* L) is second ranked most important cereal crop for staple food. Many environmental factors especially drought stress is considered to be one of the major constraints in worldwide which have negatively influenced the productivity and production (Ahmed *et al*., 2017) [7]. The effect of water stress on crop yield depends on stage of plant growth during which it occur. The most threatening problem in wheat production is the shortage of water at the seedling stage, mid-season water stress, terminal stress or a combination of any two or three. Seed germination and seedling growth characters are very important factors in determining yield (Rauf *et al*., 2006) [53]. Dhanda *et al*. (2004) [14] indicated that seed vigor index and shoot length are among the most sensitive to drought stress, followed by root length and coleoptiles length. The rate of seed germination and the final germination percentage as well as the amount of water absorbed by the seeds were considerably lowered with the rise of osmotic stress level (Heikal *et al*., 1981) [19].

There are many studies such as the selecting plant species or the seed treatments that are helpful for alleviating the negative effect of drought stress on plant (Ashraf *et al*., 1992; Almansouri *et al*., 2001; Okcu *et al*., 2005; Kaya *et al*., 2006; Iqbal and Ashraf, 2007) [2, 3, 49, 31].

Identifying the water stress tolerant wheat genotypes at germination and early seedling growth stage under low osmotic pressure is practiced as a reliable physiological indicator by the researchers (Chachar *et al*., 2016) [12]. Hence, the investigation of water stress based on the germination of different genotypes is a forward step to identify the most tolerant genotypes under low osmotic potential. Selection for water stress tolerance at germination and the early seedling stage is frequently accomplished using simulated water stress induced by chemicals like polyethylene glycol (PEG 6000). It imposes water stress under *in-vitro* conditions that maintains uniform water potential throughout an experimental period, whereby a large set of genotypes can be screen accurately (Manoj and Uday, 2007) [44]. The advantage of using polyethylene glycol (PEG) compared to others osmotic solutions is that due to the high molecular weight (6000-8000) PEG cannot enter the plant cells, instead, the water is withdrawn from the cell and cell wall without affecting or hurting the cell structure (Van Berg and Zeng, 2006) [58]. While other osmotic solutions of low molecular weight could be toxic to plant as they are easily be taken by the plant (Hamza, 2012) [24]. Polyethylene glycol molecules are known to be inert, no-ionic, and virtually impermeable to cell membranes and can induce uniform water stress without causing direct physiological damage (Kulkarni and Deshpande, 2005) [32]. It can be used to modify the osmotic potential of nutrient clarification culture and thus pant water deficit in a relatively controlled manner (Lagerwerff *et al*., 1961; Money, 1989; Zhu *et al*., 1997; Lu and Neumann, 1998; Rauf *et al*., 2006; Van *et al*., 2006; Hamza, 2012) [38, 43, 59, 39, 53, 58, 24]. PEG as a water stress causing factor can reduce water potential, resulting in the growth reduction of germinated seeds and seedling (Zhu *et al*., 2006) [60]. It is envisaged from the above findings that PEG solutions can be used in the laboratory for screening water stress resistant genotypes. Therefore, present experiment was designed to treat seeds of 10 wheat genotypes with different concentration (0%, 15% and 25%) of PEG solution for screening of water stress tolerance wheat genotypes under laboratory condition. The results showed that all genotypes of wheat have different responses to water stress, indicating that it is feasible to choose the best genotype for resisting water stress in controlled conditions. However, seeds treated with 25% PEG did not germinate at all. The reason of this failure might be the high concentration of the chemical (PEG) which desiccated endosperm of the seeds rapidly, eventually they failed to grow (Khakwani *et al*., 2011; Baloch *et al*., 2012) [36, 9]. Selection of physiological traits associated with the drought tolerance is essentially enhanced to increase the efficiency of selection (Ciuca *et al*., 2010) [10]. Screening for water stress tolerance based on the field trials is costly and time-consuming, in addition to the typical condition required to express their effective genes responsible for the studied characteristics. Therefore, preliminary screening methods are commanded for the field criteria (Kim *et al*., 2001) [29]. Early screening as a physiological dissection of drought tolerance is one of the approaches to assist plant breeder in rapid detection of suitable genotype to be involved in the next breeding program.

2. Materials and Methods

2.1 Plant material

The experimental material of the present investigation comprised of ten genotypes of wheat (*Triticum aestivum*) procured from Wheat Research Unit Dr. Panjabrao Deshmukh Krishi Vidyapeeth Akola.

2.2 Osmotic stress experiment

The laboratory experiment was laid out in completely randomized design (CRD) with two factors: genotypes and water stresses. This study was conducted in a growth room of the Department of Botany (Biotechnology centre), Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, India. Ten wheat genotypes were studied to see the effect of osmotic stress imposed by using polyethylene glycol (PEG) 6000. Three osmotic solutions (including distilled water) were applied during the germination period on the wheat genotypes with three replicates, using Poly ethylene glycol (PEG) of molecular weight 6000. Solutions were prepared according to weight by volume i.e. T1, (control with distilled water), T2 (15% PEG solution) and T3 (25% PEG solution) by dissolving separately calculated amounts of PEG 6000 in distilled water (0, 15.0, 25.0g PEG 6000/100 ml), respectively, at 25°C. Seeds were surface sterilized with 5 percent sodium hypochlorite solution for 15 minutes. Residual sodium hypochlorite solution was removed by thorough washing with sterilized distilled water. Whatman filter paper was used and moistened with 5 ml PEG solution or distilled water. Twenty five seeds from each genotype with three replicates were placed in Petri dish (90 mm diameter) for all treatments and were placed in growth room at 25 ± 2°C with 65 percent relative humidity for 10 days. Two ml of designated treatment solution was applied alternate days into each Petri dish after thorough washing and draining the previous left solution.

The germinated seeds were counted daily for the experiment duration, started from the second day after sowing. Seeds were considered germinated when they exhibited radicle extension more than 2 mm. The following characteristics were measured:

- **Germination Percentage (%):** Counted after 10th days of treatment by using following formula:
  Germination% = (The number of germinated seeds /total number of seeds) × 100
- **Shoot Length (cm):** Shoot length was measured with the help of scale by selecting ten seedlings randomly on the 10th day of germination. Average length was used for statistical analysis.
- **Root Length (cm):** Average root length of ten randomly selected plants was recorded with the help of scale on the 10th day of germination.
- **Seedling vigor index:** The seedling vigor of seed was assessed by the method suggested by Abdul baki and Anderson (1972) [1] by using formula,
  Seedling vigor = Average percentage germination × Seedling length on the day of finalcount (root + shoot) (cm)
- **Shoot fresh/dry and Root fresh/dry weight (g plant^{-1}):** Ten days old plumules and radicles emerged under control and stresses were harvested. Immediately fresh weights were weighed and dried in hot air oven at 70°C till stable weight following which dry weights were measured.

2.3 Statistical analysis

Data was subjected to analysis of variance (ANOVA), using OPSTAT software at both 1% and 5% level of probability. Cluster analysis based on squared Euclidean distance was also performed to classify the genotypes using UPGMA (Un-weighted Pair Group Method for Arithmetic mean) for cluster analysis and dendrogram was generated as per the procedure given by (Nei and Li, 1979). These computations were performed using the program XLSTAT software (www.xlstat.com).

3. Result and Discussions

3.1 Seed germination (%)

Seedling emergence is one of the most sensitive growth stages that are resistance to water deficit. Therefore, seed germination is prerequisites for successful stand establishment of crop plants (Baloch *et al*., 2012) [9]. Under semiarid region, low moisture is frequently limiting factor during germination (Khakwani *et al*., 2011) [36]. Because of germination is on the most valuable trait for early seedling stage of crop plant, it seems that water stress condition had more sensitive than other genotypes (Homayoun *et al*., 2011) [23]. Earlier studies focalization, germination in solution with high osmotic potential is one of the most important laboratory methods suggested for screening water stress tolerance of crop plants (Rauf *et al*., 2006) [53]. Therefore, in the present investigation, ability of the ten wheat genotypes under chemical desiccation, induced by PEG (6000) during early seedling stage was assessed under in-vitro conditions. Data relevant the effect of osmotic stress induced by PEG on seed germination percentage (%) as depicted in (Figure A, Plate 1). Data indicated that increase in osmotic stress caused a significant decrease in germination percentage in all genotypes under study (Table 1). Out of 10 genotypes tested, three genotypes (AKAW-3717, AKAW-4926 and AKAW-5017) showed maximum of 85% seed germination (Table 1). The average of germination percentage was 98.54% and 83.21% in unstressed and 15% osmotic stress treatment. AKAW-4907 exhibited better germination percentage (99.93%) followed by AKAW-4925 (99.67%) under unstressed condition whereas overall minimum germination percentage (97.93%) recorded in AKAW-5010. Maximum germination percentage (89.67%) was recorded in AKAW-3717 followed by AKAW-5017 (87%) under 15% osmotic stress. AKAW-5012 exhibited minimum germination percentage (77.33%) under osmotic stress condition. The genotype AKAW-3717 showed minimum reduction in germination percentage (9.4%) followed by AKAW-5017 (12.34%) under stress condition.

Germination is considered as one of the first and most fundamental life stage of a plant which contribute significantly in growth and yield production of wheat varieties. The resistance against water stress during the germination makes a plant stable, vigorous and healthy. Water stress results in late and reduced germination or might inhibit germination absolutely. Once a seed reaches a critical level of hydration it will head toward full germination. But it's also identified that physiological variations will arise under partial hydration when germination is prevented (Hunter and Erickson, 1952) [18]. The reduction in germination percentage was due to the effect of PEG 6000 that decreases the water potential gradient among seeds and their adjacent media and badly affects seed germination (Dodd and Donovan, 1999) [13]. Diverse genetic differences were found among the cultivars with respect to germination and there was a substantial decline in germination in all wheat cultivars. Genotypes considered being water stress tolerant as their germination percentage did not reduce significantly with the increased moisture stress during germination, because some plants can develop their biochemical and physiological function to tolerate the water deficient condition (Chachar *et al*., 2016) [12]. These findings are in agreement with the outcomes of Harshvardhan *et al*. (2014) [25] and Alaei *et al*. (2010) [4] who reported that, with the increase in water stress, germination percentage is decreases. Good germination under water stress is an important parameter for screening different wheat varieties for drought resistance. So such varieties having good germination percentage and rate should be selected for good crop yield.

3.2 Shoot length (cm)

Shoot length continuously decreases by exposure to different osmotic stress levels. Water stress tolerance in genotype is described by slight decrease of shoot growth in water stress environments. Measurements of shoot length of seedling subjected to osmotic stress have been suggested for water stress tolerance (Ahmadizadeh *et al*., 2011) [5]. Shoot lengths were recorded after 10 days of seedling growth. All wheat genotypes showed variation in shoot length in response to the PEG induced stress (Table 1). In present study, data relevant the effect of osmotic stress induced by PEG on shoot length (cm) as illustrated in (Figure B, Plate 1). Increase in osmotic stress caused a substantial decrease in shoot length in wheat genotypes. Mean shoot lengths were 16.23 and 10.91 cm in unstressed and 15% osmotic stress respectively which clearly shows decrease over unstressed condition. Highest shoot length (17.83 cm) recorded in AKAW-3717 followed by AKAW-5017, AKAW-4842 (16.8 cm) and AKAW-5014 (16.7 cm) while lowest shoot length (14.5 cm) recorded in AKAW-3722. Shoot length is inversely proportional to water stress. AKAW-3717 gave better shoot length (14.16 cm) followed by AKAW-5017 (13.3cm) and AKAW-4842, AKAW-4926 (12.2 cm) under 15% osmotic stress condition. Minimum values of shoot length (7.9 cm) recorded in AKAW-4925 and AKAW-5012 at 15% osmotic potential. The genotype AKAW-3717 showed minimum reduction in shoot length (20.58%) followed by AKAW-5017 (20.83%) under stress condition.

In wheat, seedling growth in laboratory environments has been recognized as appropriate growth phase for testing the drought tolerance. It could be speculated that presence of increased concentrations of PEG during the growth of seedling prevents the developmental traits and survival of wheat seedling. In the present study, larger decline in shoot length was recorded under stress conditions (at osmotic potential of 25%). Similarly finding, presence of increased different concentration of PEG 6000 during the seedling growth stage had certainly reduced the shoot development. The water stress tolerant is qualified by a small reduction of shoot growth under water stressed condition (Moushachi *et al*., 2012) [46]. Also many researchers viz., Othmani *et al*., 2019; Soni *et al*., 2014; Perez *et al*., 2007; Okcu *et al*., 2005) [50, 54, 51,49] documented that shoot growth reduced under water stressed conditions. All these findings are in accordance with the statement of Hsiao and Xu (2000) [21] ; Munns and Sharp (1993) [42] conveyed that shoot growth was often more reduced than root growth under stress conditions, a phenomenon commonly found in dry soils.

3.3 Root length (cm)

Root length in wheat crop considered as the most powerful trait among the seed growth parameters in water stress tolerance selection program (Hassan *et al*., 2016) [26]. Root growth is an important parameter for plant tolerance to drought stress as roots are the main engine for meeting transpirational demand and play an important role in making water available to plants (Huang and Gao, 1999; Liu and Huang, 2000) [20, 40]. A deeper and more extended root system allow the seedlings to extract more water in surrounded soil. Decrease in the root length might be resulted from the diminish relative turgidity and protoplasm dehydration, that brings down cell expansion and delaying the cell division (Mujtaba *et al*., 2016) [47]. Root lengths were recorded after 10 days of seedling growth (Table 1). In present study, data indicated that increase in osmotic stress caused a significant decrease in root length as presented in (Figure C, Plate 1). Maximum root length (19.20 cm) recorded in AKAW-5012 followed by AKAW-4907 (19.12 cm) and AKAW-5017 (19.03 cm) whereas minimum root length (18.13 cm) recorded in AKAW-4842. Root length decreased rapidly with increase in osmotic potential in wheat cultivars under study. AKAW-5017 gave better root length (17.27 cm) followed by AKAW-4926 (16.83 cm) and AKAW-3717 (16.13 cm) at 15% osmotic potential. Minimum values of root length (13.54 cm) recorded in AKAW-3722 at 15% osmotic potential. The genotype AKAW-4926 showed minimum reduction in root length (9.1%) followed by AKAW-5017 (9.28%) under stress condition.

Root length of all cultivars used in the present study was significantly reduced by osmotic stress. Similar outcomes were experienced by Huang and Fu (2000) [22]. Differences in root length were also highly significant among cultivars. Root length is less affected by water stress as compared with shoot length. The halt in cell division and cell elongation resulted in tuberization ultimately causing decrease in root and shoot length. This results in adjustments in the root system which allows the plant to enter a static mode till the environment becomes favorable again (Fraser *et al*., 1990) [16]. When those varieties having elongated root length under water stress conditions reflected an adaptive reaction involving an increase in root length to reach deeper water in the soil suggested by Soni *et al*. (2014) [54]. Though root length was affected due to water stress, considerable reduction due to PEG at higher concentration (Othmani *et al*., 2019) [50]. The outcomes are in accordance with the findings of Perez *et al*. (2007) [51] stated that water stress reduces the root growth.

3.4 Shoot fresh weight (g 10^{-1}shoots)

Osmotic stress caused a significantly decrease in shoot fresh weight of all genotypes under the present study (Table 2). Data relevant the effect of osmotic stress induced by PEG on shoot fresh weight (g) as illustrated in (Figure D, Plate 1). AKAW-3717 exhibited maximum SFW (3.11 g) followed by AKAW-4842, AKAW-4926 and AKAW-5017 (3.00 g) whereas minimum SFW (2.95 g) recorded in AKAW-4907. Shoot fresh weight decreased, with increase in osmotic stress. AKAW-3717 exhibited better SFW (2.77 g) followed by AKAW-5017 (2.69 g) at 15% osmotic potential. Least values of SFW (2.18 g) recorded in AKAW-4925 at 15% osmotic potential. Water stress depresses the wheat shoot growth rather than root development. Moreover, distinct genetic differences were found among the genotypes with respect to shoot growth subjected PEG. Shoot fresh weight was more affected than root fresh weight. Shoot fresh weight showed that genotypes AKAW-3717 and AKAW-5017 were less affected as compared to other genotypes. The genotype AKAW-5017 showed minimum reduction in shoot fresh weight (10.4%) followed by AKAW-3717 (11.12%) while genotype AKAW- 4925 showed maximum reduction in shoot fresh weight under stress condition. The decrease in shoot biomass was attributed to lesser number and development of reduced leaves with increased concentration of PEG in the growing medium. Similarly findings by Kaydan and Yagmur (2008) [33]; Kamran *et al*. (2009) [34] and Khan *et al*. (2013) [37] revealed that shoot fresh weight is adversely affected under the stress environment.

3.5 Shoot dry weight (g 10^{-1}roots)

Data relevant the effect of osmotic stress induced by PEG on shoot fresh weight (g) as illustrated in (Table 2). The effect of osmotic stress on shoot fresh weight (SDW) of wheat genotypes presented in (Figure E, Plate 1). AKAW-3717 gave maximum SDW (2.56 g) followed by AKAW-5017 (2.50 g) whereas minimum SDW (2.03 g) recorded in AKAW-4926. Shoot dry weight decreased, with increase in osmotic stress. AKAW-3717 exhibited better SDW (2.41 g) followed by AKAW-5017 (2.35 g) at 15% osmotic potential respectively. AKAW-4925 gave least values of SDW (1.56 g) at 15% osmotic potential. Water stress adversely affects the shoot growth of wheat genotypes. The genotype AKAW-4926 showed minimum reduction in shoot fresh weight (2.80%) followed by AKAW-4907 (3.30%) while genotype AKDW-5012 (31.4) showed maximum reduction in shoot fresh weight under stress condition. Genetic variations were recorded among the genotype with respect to shoot growth subjected to osmotic stress conditions. Shoot dry weight is more affected than root dry weight. Shoot dry weight indicated that genotypes AKAW-4926, AKAW-5017 were less affected as compared to other genotypes. The decline in shoot biomass was due to water stress. The outcomes are in agreement with the results of Khan *et al*. (2010) [35] reported that shoot dry weight is affected due to osmotic stress

3.6 Root fresh weight (g 10^{-1}shoots)

Data relevant the effect of osmotic stress induced by PEG on root fresh weight (g) as depicted in (Figure F, Plate 1). Osmotic stress produced a significant decrease in RFW of all wheat genotypes presented in Table 3. AKAW-5017 exhibited maximum RFW (2.81 g) followed by AKAW-4842 (2.78 g) and AKAW-3717 (2.75 g) whereas minimum RFW (2.49 g) recorded in AKAW-5012. Increases in osmotic stress caused a remarkable decrease in RFW. AKAW-3717 gave better RFW (2.42 g) followed by AKAW-5017 (2.39 g) and AKAW-5014 (2.35 g) at 15% osmotic potential of respectively. AKAW-5012 and AKAW-4925 gave least values of RFW (1.70 g and 1.78 g) at 15% osmotic potential respectively. The genotype AKAW-5017 showed minimum reduction in shoot fresh weight (11.7%) followed by AKAW-5017 (12.04%) while genotype AKDW- 5012 (31.05) showed maximum reduction in shoot fresh weight under stress condition. Water stress drastically reduces the root fresh weight in wheat seedling. Seedling survival in drought prone environments may depend upon the species ability to compensate for the negative effect of low water potentials in the soil and atmosphere by adjusting root and shoot morphological and physiological patterns (Kramer, 1983; Morgan, 1984) [28, 41]. The results obtained in this study reveal that RFW is more affected than that of dry weight and are in agreement with the results obtained by Murillo-Amador *et al*. (2002) [45] in cowpea. These results correlates with finding with Sayar *et al*. (2010) [35] who found that osmotic stress caused a significant decrease in root fresh weight in all genotypes.

3.7 Root dry weight (g 10^{-1}roots)
The effect of osmotic stress on root dry weight (RFW) of wheat genotypes significantly decreased as illustrated in Table 3. Genotypes respond differently to various levels of osmotic stress for RDW as presented in (Figure G, Plate 1). AKAW-3717 gave maximum RDW (2.04 g) followed by AKAW-4907, AKAW-4926 (1.99 g) and AKAW-5017 (1.90 g) whereas minimum RDW (1.74 g) recorded in AKAW-4925. Root dry weight deceased, with increase in osmotic stress. AKAW-4842 exhibited better RDW (1.80 g) followed by AKAW-3717 (1.78 g) at 15% osmotic potential respectively. AKAW-4925 gave least values of RDW (1.19 g) at 15% osmotic potential. Seedling dry weight indicated that cultivar AKAW-3717 was less affected by drought stress as compared to other cultivars. The genotype AKAW-4842 showed minimum reduction in shoot fresh weight (11.1%) followed by AKAW-5017 (12.04%) while genotype AKDW-5017 (32.72) showed maximum reduction in shoot fresh weight under stress condition. Water stress adversely affects the root dry weight. Moreover, diverse genetic differences were found among the cultivars with respect to seedling growth subjected PEG. Root dry weight is less affected than fresh weight. The outcomes obtained in this study revealed that dry mass was less affected than fresh weight and results are in agreement with the findings of Bayoumi *et al*. (2008) [8]; Ahmad *et al*. (2017) [7] who reported that osmotic stress caused significant effects on root dry weight in all genotypes.

3.8 Correlation coefficient among seedling traits
Correlation analysis is one the most important statistical parameters for selection and crop improvement. Correlation analysis revealed that shoot length exhibited significant and positive correlation with root length (0.71), fresh root weight (0.86), fresh root weight (0.93), dry shoot weight (0.94), dry root weight (0.86) and germination percentage (0.80) (Table 4) which indicated that by increasing these attributes, shoot length will increase. Correlation coefficients illustrated that root length showed highly positive and significant association with fresh shoot weight (0.50), fresh root weight (0.74), dry shoot weight (0.65), dry root weight (0.62) and germination percentage (0.51). Fresh shoot weight showed significant correlation with fresh root weight (0.75), dry shoot weight (0.89), dry root weight (0.83) and germination percentage (0.73).
Fresh root weight showed significant correlation with dry shoot weight (0.92), dry root weight (0.90) and germination percentage (0.77). Dry shoot weight exhibited significant and positive correlation with dry root weight (0.91) and germination percentage (0.81). Positive and highly significant correlation was observed between shoot length and dry shoot weight (0.94). These finding led to the conclusion that desirable traits possessed by different genotypes can be used for the development of water stress tolerant wheat genotypes. Khan *et al*. (2002) [30] and Khan *et al*. (2013) [37] reported that germination percentage exhibited positive and significant correlation with root length, fresh shoot weight, dry shoot weight, and dry root weight. Many researchers (Rakesh *et al*.,1998; Noorka *et al*., 2007 and Ahmad *et al*., 2013) [52, 48, 6] reported that genotypes showed variable response for water stress related traits under different stress level.

3.9 Clustering of genotypes based on physiological parameters from laboratory screening using xlstat software (Gower coefficient)
The differences in the responses to water stress by wheat genotypes was measured by employing xlstat software. Based on the physiological screening data, all the genotypes were grouped into four clusters, signaling the difference response of wheat genotypes to osmotic stress. Cluster IV had the maximum number of genotypes (4) followed by cluster II (3) and cluster I (2). The cluster III is monogenotypic. The ten genotypes grouping into four clusters are illustrated in Table 5. Clustering of wheat genotypes based on the physiological screening under laboratory condition using PEG-6000 is presented in Figure 1. The tolerant genotypes were grouped in cluster I while moderate tolerant and susceptible genotypes grouped in cluster II, III and IV. Gower method was found to be a useful tool in assessing the relative contribution of various physiological parameters in wheat genotypes under water stress and it may be used to select genetically superior genotype for breeding water stress tolerant lines. The contribution of each character towards the variation in response to water stress in wheat genotypes is depicted in Table 6. The seed germination (83.20%) contributed highest. Thus, seed germination may be given high emphasis while screening large number of genotypes for their water stress tolerant ability. This is contradictory with Ashraf *et al*. (1992) [2] reported that under water stress condition, seed germination is not the standard for predicting plants showed water stress tolerance. Other characters like root length (15.68%), shoot length (10.90%), fresh shoot weight (2.45%), fresh root weight (2.11%), dry shoot weight (1.96%) and dry root weight (1.51%) contributed very little. The response of genotypes may be varying to different factors, which could be reflected in their respective seed performances.
Cluster analysis has been utilized to elucidate the disparity and assemble the genotypes based on water stress tolerance indices (El-Mohsen *et al*., 2015) [15]. Similarly, finding Ahmad *et al*. (2017) [7] and Guoth *et al*. (2009) [17] stated that the germination test is found to be useful for identifying the genotypes capable of quickly establishing themselves under low soil moisture conditions which shows correlation with water stress tolerance. The present investigation for screening water stress tolerance ability of wheat genotypes was found to be relevant in choosing the best accession for tolerant water stress. PEG-6000 is considered as the best macromolecular compound to stimulate water stress and it is widely used in water stress physiology and genetic mechanism for plants (Christmann *et al*., 2005; Verslues and Bray 2004; 2006) [11, 56, 57].

Table 1: Effect of PEG on seed germination percentage (%), shoot length and root length (cm)

Genotypes	Seed germination (%)			Shoot length (cm)			Root length (cm)		
	US (0%)	S (15%)	% DOS	US (0%)	S (15%)	% DOS	US (0%)	S (15%)	% DOS
AKAW-3717	99.07	89.67	9.4	17.83	14.16	20.58	19.10	16.17	13.36
AKAW-3722	96.66	81.44	15.74	14.5	8.17	43.66	19.03	13.54	31.90
AKAW-4842	99.52	80.17	12.42	16.8	12.3	26.79	18.13	16.13	11.02
AKAW-4907	99.93	84.15	16.78	15.9	10.3	35.22	19.12	15.14	20.91
AKAW-4925	99.67	80.73	18.99	14.8	7.9	46.62	18.63	15.80	15.20
AKAW-4926	98.13	85.33	10.13	16.3	12.3	24.54	18.5	16.83	9.01
AKAW-5010	97.93	82.13	16.12	17	10.5	38.24	18.33	14.50	24.90
AKDW-5012	98.7	77.33	21.64	15.5	7.9	49.03	19.20	15.13	21.18
AKAW-5014	96.63	84.14	12.61	16.7	12.2	26.95	18.43	16.3	11.57
AKAW-5017	99.25	87	12.34	16.8	13.3	20.83	19.03	17.27	9.28
Average	98.54	83.21	13.62	16.23	10.91	33.25	18.75	15.68	16.83
SE(m) Factor A Factor B Factor(A×B)	 0.23 0.52 0.73		 - - -	 0.12 0.26 0.36		 - - -	 0.01 0.03 0.04		 - - -
CD Factor A Factor B Factor(A×B)	 0.08 0.18 0.25		 - - -	 0.33 0.74 1.05		 - - -	 0.04 0.08 0.12		 - - -
CV	1.45		-	0.16		-	0.12		-

Note: Average, CD and SE (m) were calculated using replicated data in the observations, Significant at 0.01 probability level: DOS (Decreased over stressed); US- Unstressed; S- Stressed

Table 2: Effect of PEG on shoot fresh and dry weight (g)

Genotypes	Shoot fresh weight (g)			Shoot dry weight (g)		
	Unstressed (0%)	Stressed (15%)	% DOS	Unstressed (0%)	Stressed (15%)	% DOS
AKAW-3717	3.11	2.77	11.12	2.56	2.41	5.90
AKAW-3722	2.95	2.35	20.41	2.27	1.68	26.2
AKAW-4842	3.0	2.57	15.40	2.35	2.11	5.47
AKAW-4907	2.83	2.41	14.77	2.12	2.04	3.30
AKAW-4925	2.89	2.18	24.61	2.22	1.56	29.5
AKAW-4926	3.0	2.51	12.55	2.03	1.98	2.80
AKAW-5010	2.97	2.41	18.51	2.3	1.76	23.3
AKDW-5012	2.92	2.32	20.74	2.37	1.63	31.4
AKAW-5014	3.0	2.34	22.09	2.32	2.08	6.20
AKAW-5017	3.0	2.69	10.4	2.50	2.35	5.90
Average	2.90	2.30	18.36	2.31	1.98	14.01
SE(m) Factor A Factor B Factor(A×B)	 0.02 0.04 0.06		 - - -	 0.06 0.05 0.20		 - - -
CD Factor A Factor B Factor(A×B)	 0.05 0.12 0.17		 - - -	 0.02 0.05 0.07		 - - -
CV	0.05		-	4.4		-

Note: Average, CD and SE (m) were calculated using replicated data in the observations, Significant at 0.01 probability level: DOS (Decreased over stressed)

Table 3: Effect of PEG on root fresh and dry weight (g)

Genotypes	Root fresh weight (g)			Root dry weight (g)		
	Unstressed (0%)	Stressed (15%)	% DOS	Unstressed (0%)	Stressed (15%)	% DOS
AKAW-3717	2.75	2.42	12.04	2.04	1.78	12.93
AKAW-3722	2.51	1.84	26.61	1.78	1.29	27.52
AKAW-4842	2.78	2.31	12.53	2.0	1.80	11.01
AKAW-4907	2.68	2.19	16.59	1.99	1.67	16.20
AKAW-4925	2.49	1.78	27.98	1.74	1.27	27.41
AKAW-4926	2.71	2.38	12.32	1.99	1.61	19.21
AKAW-5010	2.57	1.82	30.19	1.82	1.32	27.58
AKDW-5012	2.49	1.70	31.05	1.77	1.19	32.72
AKAW-5014	2.62	2.35	12.38	1.89	1.46	22.79
AKAW-5017	2.81	2.39	11.57	1.97	1.73	12.84

Average	2.64	2.17	18.69	1.90	1.51	21.02
SE(m) Factor A Factor B Factor(A×B)	 0.03 0.07 0.09		 - - -	 0.01 0.02 0.03		 - - -
CD Factor A Factor B Factor(A×B)	 0.01 0.02 0.03		 - - -	 0.03 0.06 0.09		 - - -
CV	0.05		-	3.30		-

Note: Average, CD and SE (m) were calculated using replicated data in the observations, Significant at 0.01 probability level: DOS (Decreased over stressed)

Table 4: Correlation coefficients among various seedling traits of wheat genotypes treated with polyethylene glycol (PEG-6000) under *in vitro* condition.

	SL	**RL**	**FSW**	**FRW**	**DSW**	**DRW**
RL	0.71**					
FSW	0.86**	0.50**				
FRW	0.93**	0.74**	0.75**			
DSW	0.94**	0.65**	0.89**	0.92**		
DRW	0.86**	0.62**	0.83**	0.90**	0.91**	
GP	0.80**	0.51**	0.73**	0.77**	0.82**	0.67**

SL- shoot length; RL- root length; FSW- fresh shoot weight; FRW- fresh root weight; DRW- dry shoot weight; DRW- dry root weight; GP- Germination percentage; ** indicate significance at the 0.01 probability levels.

Table 5: Clustering of genotypes based on physiological parameters from laboratory screening

SN	Cluster	Name of genotypes
1	Cluster I	AKAW-3717, AKAW-5017
2	Cluster II	AKAW-3722, AKAW-4925, AKAW-5012
3	Cluster III	AKAW-4842
4	Cluster IV	AKAW-4907, AKAW-4926, AKAW-5010, AKAW-5014

Table 6: Contribution of different parameters from physiological screening using PEG-6000 in wheat genotypes under laboratory condition

SN	Source	Contribution
1	Seed germination percentage	83.21%
2	Shoot Length	10.90%
3	Root Length	15.68%
4	Fresh shoot weight	02.46%
5	Fresh root weight	02.19%
6	Dry shoot weight	01.96%
7	Dry root weight	01.51%

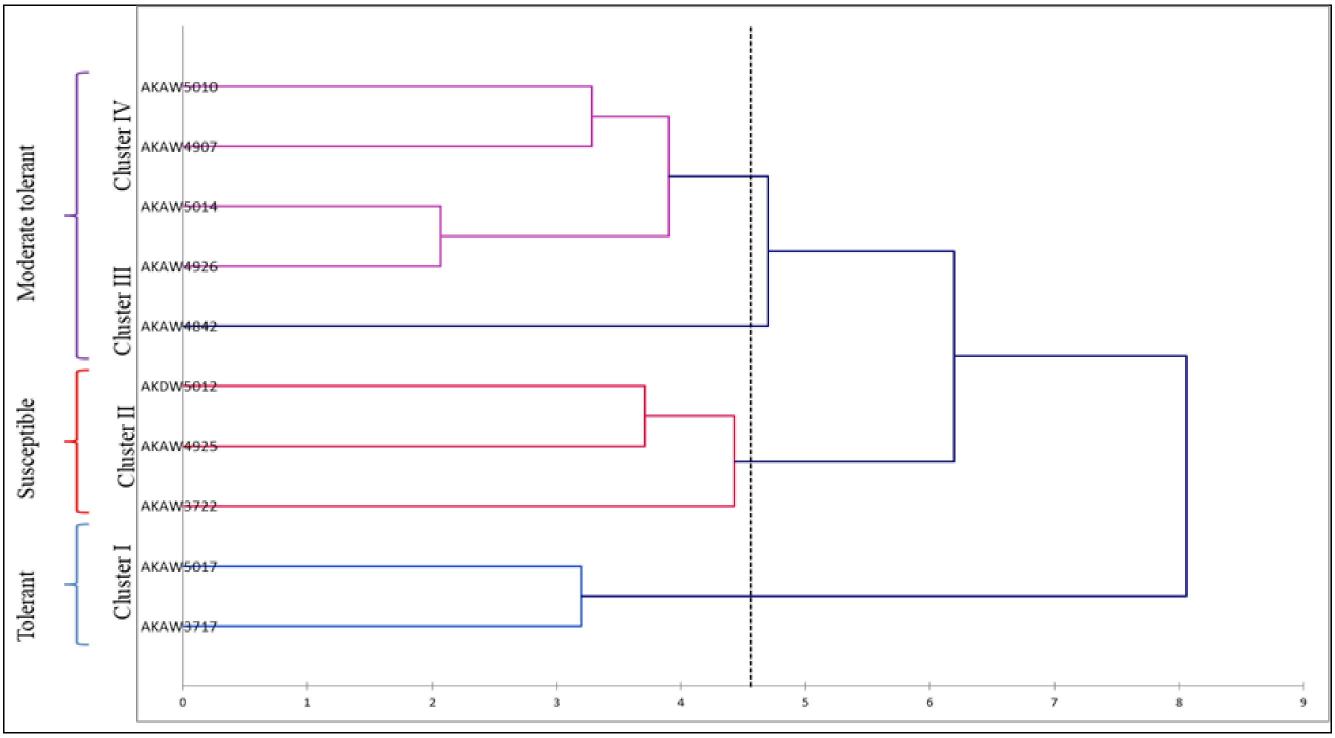

Fig 1: Cluster analysis of seedling traits under osmotic stress condition

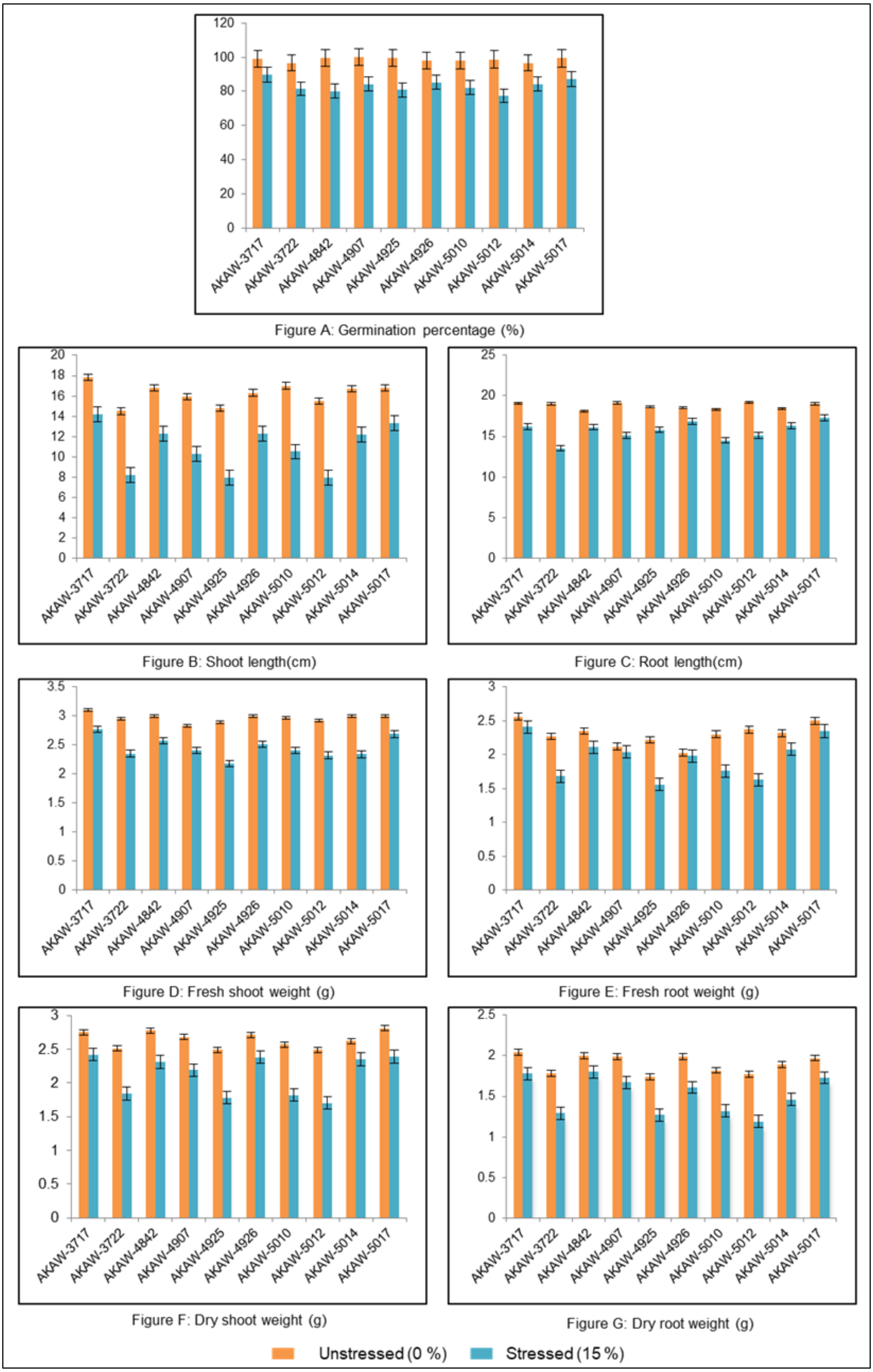

Plate 1: Physiological analysis of whet genotypes in response to stress using PEG-6000

4. Conclusion

The present work was conducted to evaluate the genetic potential of six wheat genotypes through artificially created osmotic stress by PEG-6000 in laboratory conditions followed by selection of genotypes based on easily measurable and inherited seedling traits contributing to water stress tolerance indices. In this study osmotic stress caused significant effect on all the seedling traits.

However, the effect on individual genotype was more meaningful which that genotype AKAW-3717 showed very less reduction in all seedling traits while minimum reduction was recorded in AKAW-5017. Similarly, AKAW-4926 and AKAW-4842 expressed minimum reduction in shoot/root fresh/dry weight suggesting their higher stress tolerant, so these seedling traits may be used as good indicator for water stress tolerance. Furthermore, significant and positive correlation among seedling traits depicted that by improving dry weight will improve the overall performance of the crop. Cluster analysis discriminate the genotypes at different level osmotic stress through the studied characteristics. Selection can be made on the basis of seedling traits to screen a large population for water stress. It would be cost effective, less time consuming and less laborious to select the germplasm at early stage. So is suggested that the findings may be helpful for selection of water stress tolerance wheat genotype under the deliberated traits. The genotypes with improved traits may be used as parents in wheat breeding for moisture stress conditions.

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