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# In-vitro screening technique for drought tolerance of wheat (*Triticum aestivium* L.) genotypes at early seedling stage

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Drought significantly reduces yield of many crop plants including wheat in the world. Identification of wheat genotypes that can thrive on limited water is vital to boost the wheat production of rainfed areas. Six wheat genotypes were tested for drought tolerance using 0, -0.5, -0.75 MPa Polyethylene Glycol 6000 solutions at Department of Crop Physiology, Faculty of Crop Production, Sindh Agriculture University, Tandojam, Pakistan during 2012-13. Data were recorded on various seedling parameters like germination percentage, Shoot, root length, fresh and dry weight. The seedling traits showed a decreasing trend in response to increased concentrations of PEG 6000. Wheat genotype DH-3/48 and Chakwal-86 were found the best for germination percentage (77.31 and 80%) under high water stress (-0.75 MPa). Whereas, genotypes Khirman showed the maximum shoot and root length shoot fresh weight, with regards to maximum shoot dry weight value whereas, genotype DH-3/48 showed the maximum values (0.11 g/10 shoots), while the maximum root dry weight was recorded in genotype Chakwal-86 (0.14 g/10 roots) at the high water stress respectively. The genotypes Khirman and DH-3/48 found superior and might be productive in further breeding programmes for drought tolerance. Selection can be made on the basis of these characters at early growth stage to screen a large population for drought stress.

**Keywords:** In-vitro screeinig, Drought stress, Wheat genotypes

## Introduction

The availability of adequate water resources throughout a crop season is a burning issue worldwide as well as in the especially arid regions of Pakistan, Groundwater has been depleted, surface water is shrinking,

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irrigation water is rationalized. Fish industry may suffer when farmers use more water for irrigation. There is a need to save every drop of water to keep the groundwater from being depleted, rivers, lakes and wetlands from drying out and to save endangered species from extinction.

One of the important challenges facing crop physiologists and agronomists is understanding and overcoming the major abiotic stresses in agriculture which reduces crop productivity and yield. One of these stresses particularly predominant in arid and semi-arid regions is drought stress, which decreases plant growth and development and also crop yield ( Moayedi *et al.,* 2009). Drought, one of the environmental stresses, is the most significant factor that restricts plant growth and crop productivity in the majority of agricultural fields of the world (Abedi and Pakniyat, 2010). Thus, drought indices which provide a measure of drought based on yield loss under drought conditions in comparison to normal conditions have been used for screening drought-tolerant genotypes (Talebi *et al.,* 2009).

Wheat (*Triticum. aestivum* L.) is responsible for feed of one-third of the world population with more than half of their calories and nearly half of their protein. The forecasted global demand for wheat in year 2025 may rise up to 750 million tons (Mujeeb-Kazi and Rajaram, 2002). 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. Various factors affect the yield of a crop like seed germination, seedling vigor, growth rate, and mean emergence time and desiccation tolerance (Crosbie *et al*., 1980; Noorka *et al*., 2007).

Seed germination and seedling growth characters are very important factors in determining yield (Rauf *et al.,* 2007). Dhanda *et al.* (2004) 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). 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). Selection of drought tolerance at early seedling stage is frequently accomplished using simulated drought induced by chemicals like polyethylene glycol (PEG6000).

Polyethylene glycol (PEG6000) can be used to modify the osmotic potential of nutrient solution culture and thus induce plant water deficit in relatively controlled manner (Carpita *et al.*, 1979; Money, 1989; Zhu *et al.*, 1997). Lu and Neumann (1998); Kulkarni and Deshpande (2007) showed that Polyethylene glycol molecules are inert, no-ionic, virtually impermeable to cell membranes and can induce uniform water stress without causing direct physiological damage. PEG as a factor causing drought stress by reducing water potential results in reducing growth in seed germinated and stopping seedling growth so that this effect has been observed more in the shoot than primary roots (Khaheh *et al.*, 2000; Zhu, 2006). Dodd and Donovan (1999) also suggested that PEG prevent water absorption by seeds, but penetrable ions by reducing potential inside cell results in water absorption and starting to germinated.

Identification of wheat genotypes that can tolerate limited water condition is vital to boost the wheat production which can be achieved only by exploring maximum 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. The present study was planned to identify wheat genotypes which could tolerate well under water stress conditions.

## Materials and methods

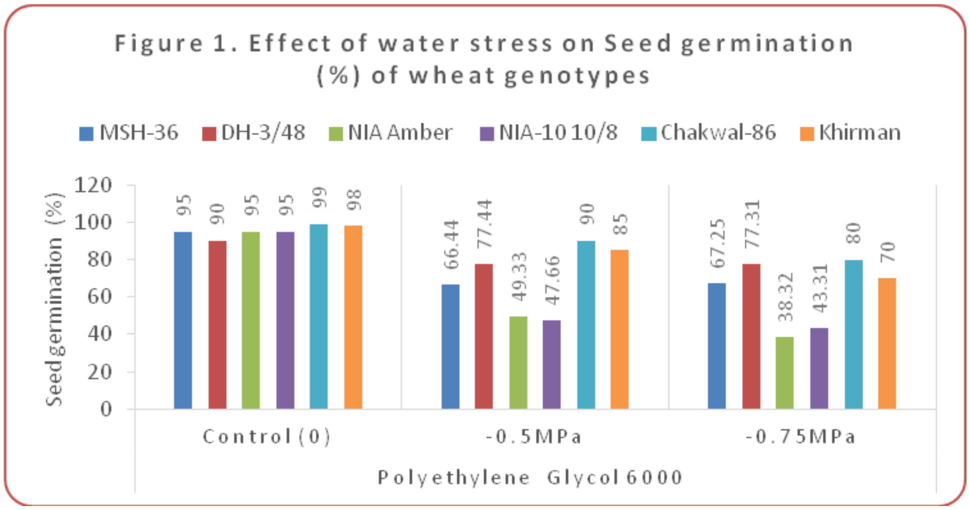
In order to study the effects of water stress, using polyethylene glycol, on germination indices and seedling growth parameters in wheat, an experiment was conducted in Department of Crop Physiology, Faculty of Crop Production, Sindh Agriculture University, Tandojam, Pakistan, during the year 2012-13. The form of experiment was factorial, using a completely randomized design (CRD) with three replications. In the present study seeds of six wheat cultivars (MSH-36, DH-3/48, NIA Amber, NIA-10 10/8, Chakwal and Khirman) were used. Seeds of cultivars were obtained from Nuclear Institute of Agriculture (NIA) Tandojam. Seeds of six cultivars were exposed to three stress level of PEG-6000 (0.0, -0.50, and -0.75MPa). PEG-6000 was prepared by dissolving the required amount of PEG in distilled water at 30$^{\circ}$C. Wheat seeds were surface sterilized with 5% sodium hypochlorite solution for 15 minutes. After the treatment the seeds were washed three times with distilled water. 30 seeds were grown in each replicate on plastic bowls at different treatment of PEG-6000. The bowls were covered with transparent plastic sheet to prevent the loss of moisture by evaporation under laboratory condition (24±2 °C) for 12 days. Seeds were considered germinated when they exhibited radicle extension of > 3 mm.

The experiment was terminated by harvesting seedlings 12 days after grains soaking and traits including seed germination, shoot, and root length, fresh and dry weight. All the data collected were subjected to analysis of variance to discriminate the superiority of treatment means and LSD test was applied by the method of Gomez and Gomez (1984) to compare the means.

## Result and discussions

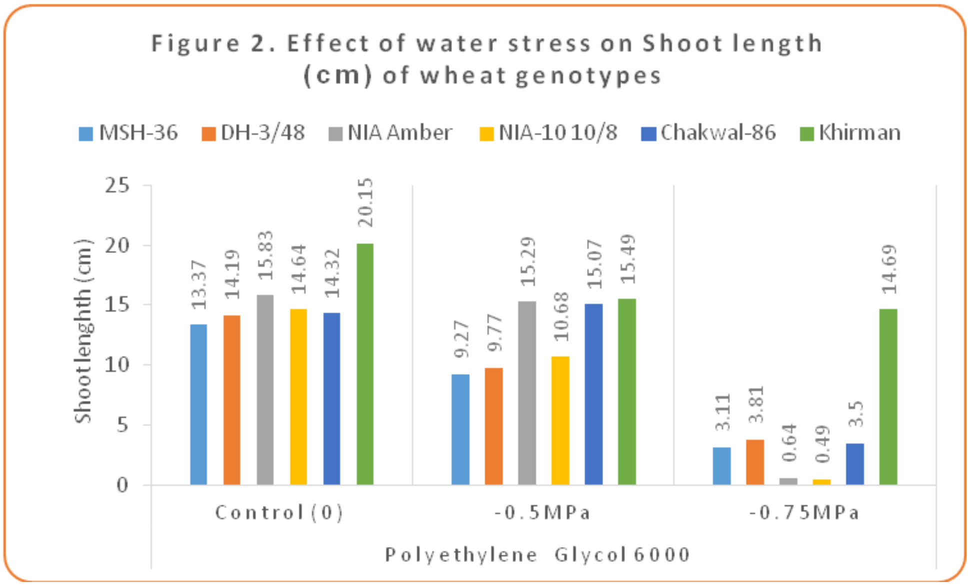
### *Seed germination (%)*

As screening procedure, the survival ability of the six wheat genotypes to tolerate chemical desiccation by PEG during germination stage is exhibited in Figure (1) In all cultivars, the germination percentage was highest at control treatment and started to decrease as the water stress level increasing using PEG. The cultivars differences in response to water stress for germination were greatly significant. The culivars Chakwal-86 and Khirman had higher germination percentage than the other cultivars regardless of water stress. However, the cultivar NIA Amber generally had the lowest germination percentage regarding of water stress. Hegarty (1977) indicated that water stress at germination stage can result in delayed and reduced germination or may prevent germination completely. Also, once a seed attains a critical level of hydration it will precede without cessation toward full germination. However, physiological changes do occur at hydration levels below this critical level that can cause an inhibition of germination. Dodd and Donavon (1999) reported that reduction in germination percentage can result from PEG treatments that decrease the water potential gradient between seeds and their surrounding media. Different cultivar response to these osmotic stress treatments suggests a great deal of genetic variation among cultivars that could be utilized to develop new wheat cultivars adapted to arid and semiarid regions.



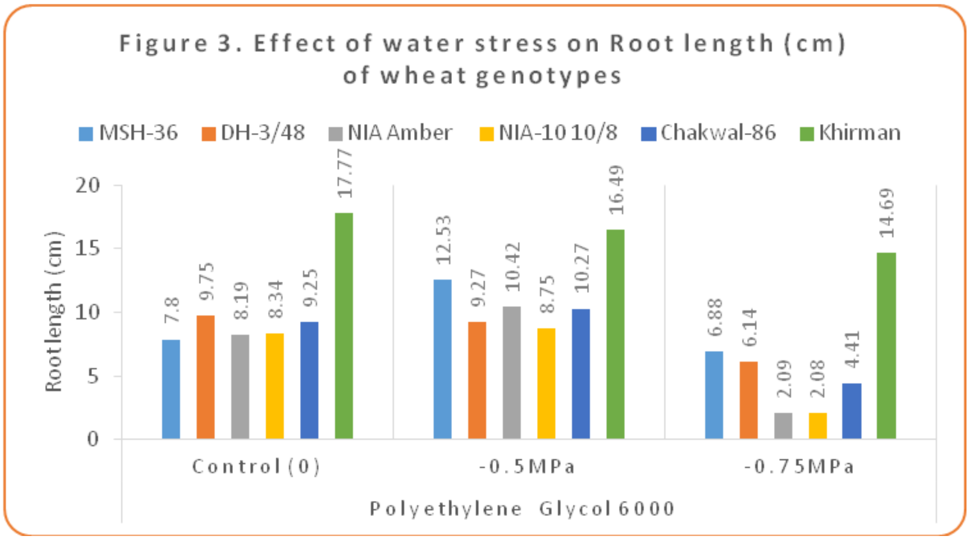

### *Shoot length*

Seedling development under laboratory conditions have been accepted an suitable growth stage for testing the drought tolerance in wheat it could be speculated that the presence of increased concentrations of PEG during the growth of seedling inhibits the developmental traits and survival of wheat seedling (Figure-2). The shoot length of different cultivars differed under different osmotic potential of PEG. In normal condition (control) the maximum value of shoot length was recorded for Khirman (20.15 cm), while MSH-36 cultivar recorded lowest value (13.37 cm) followed by DH-3/48 (14.19 cm). With increasing concentration of the PEG decline in shoot length was recorded. Under high PEG treatment (-0.75MPa), maximum shoot length was recorded in Khirman genotypes, while the minimum shoot length was recorded in NIA-10 10/8 respectively.



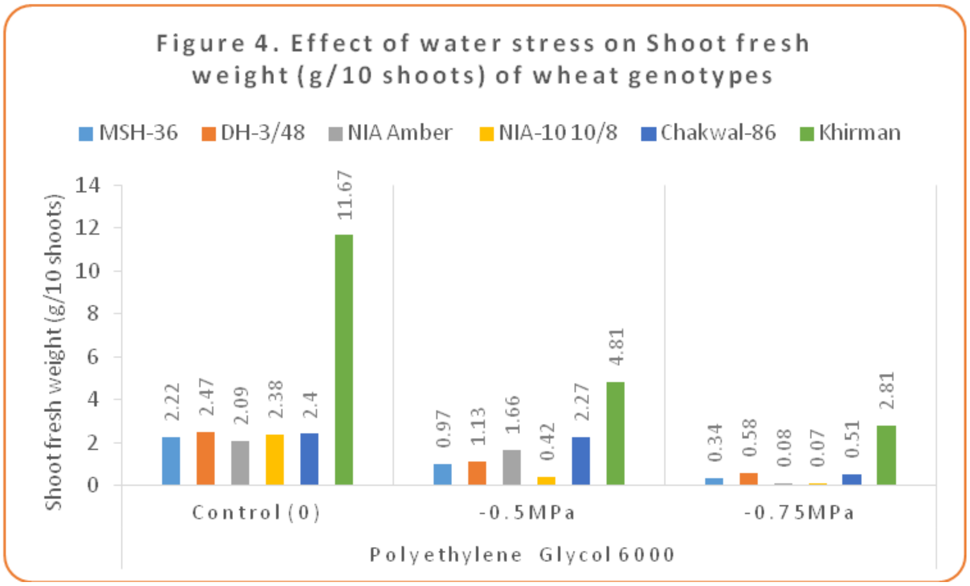

***Root length***

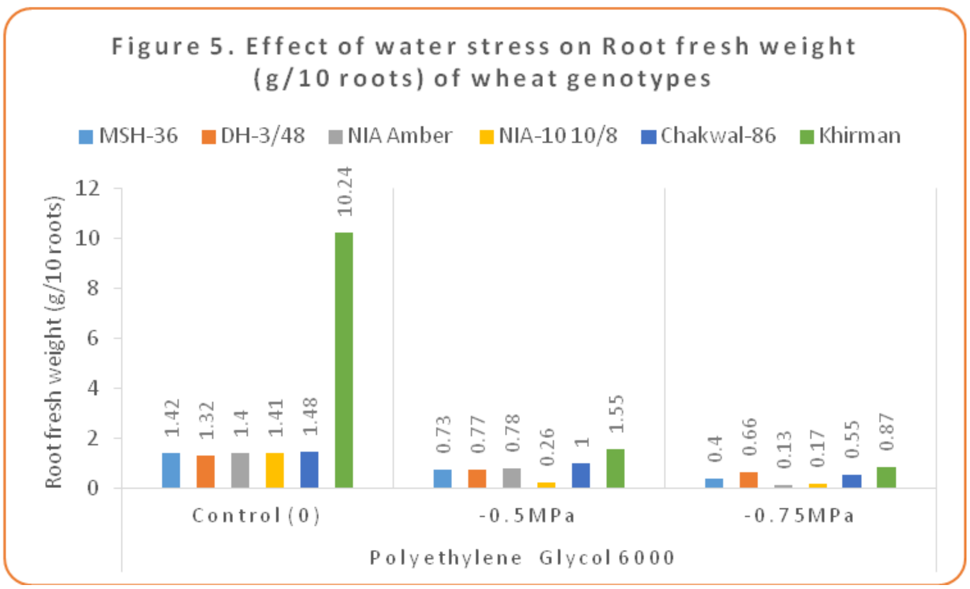
For root length parameter, genotype Khirman had the maximum root length (14.69) while the genotype NIA Amber and NIA-10 10/8 (2.09 and 2.08 cm) genotypes showed the minimum root length associated with -0.75MPa PEG-6000 treatment (Figure-3). Fraser *et al.*, (1990) reported that the reduction in the root length under drought stress may due to an impediment of cell division and elongation leading to Kind tuberization. This tuberization and the lignifications of the root system allow the conditions to become favorable again.




### *Shoot and root fresh weight*

The PEG prompted a decrease in the shoot and root fresh weight which were the greatest (11.6 shoots 7 and 10.24 g/10 roots) under control treatment in genotype Khirman, respectively. While under -0.75 MPa 2.81 g/10 shoots and 0.87 g/10 roots fresh weight were recorded. Greatest shoot and root fresh weight were recorded in in genotype Khirman (figure- 4 and 5), although the minimum value for shoot fresh weight was recorded in NIA-10 10/8 (0.07 g/10 shoots), whereas the minimum root fresh weight was recorded in genotype NIA Amber (0.17 g/10 roots) respectively. The reduction in shoot fresh weight was attributed to lower number and development of smaller leaves with increased PEG concentration of the growth media. It is important that drought resistance is categorized by small reduction of shoot growth under drought stressed condition (Ming *et al.*, 2012; Moucheshi *et al.*, 2012; Saghafikhadem 2012).



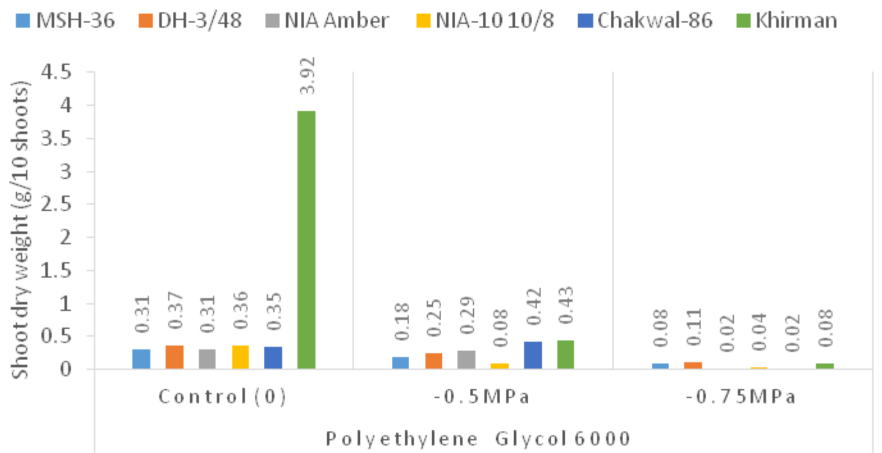




***Shoot and root dry weight***

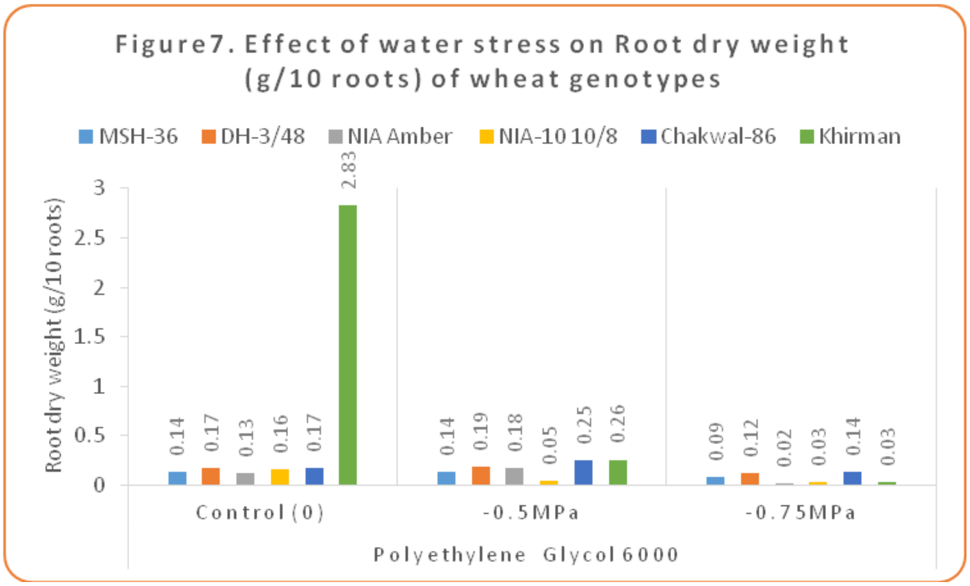
PEG caused a greater reduction in dry weight of shoot and root at higher concentrations compared to control condition (figure-6). However, maximum shoot dry matter value was recorded in DH-3/48 (0.11 g/10 shoots), while the minimum shoot dry weight value was recorded in NIA Amber and Chakwal-86 (0.02 g/10 shoots) under high concentration of PEG (-0.75

MPa), respectively.

Figure 6. Effect of water stress on Shoot dry weight (g/10 shoots) of wheat genotypes




On the other hand, there was a progressive decrease in root dry weight with increased osmotic stress. Higher value of root dry weight was found under control treatment for genotype Khirman (2.83 g/10 roots). While the dry matter accumulation at high water stress (-0.75 MPa) the genotype Chakwal-86 showed the maximum root dry weight values as 0.14 g/10 roots as compare to genotype NIA Amber (0.02 g/10 roots) respectively.




The tested cultivars varied significantly in their reaction to PEG for all seedling growth parameters. These results are in consonance with those of Sayar *et al.* (2008) who reported that seed germination under stress conditions could not distinguish among varieties. So it can be regarded as an inappropriate test for drought tolerance screening. Similar to present results, Roza *et al.* (2010) noted significant decrease in radicle and plumule length and dry matter. Longer coleoptile length helps seedlings to emerge from the soil even at low soil moistures levels, hence can play an important role in the establishment of plant stand.

## Conclusion

The present research work was conducted to evaluate the genetic potential of six wheat genotypes through artificially created water stress by PEG of molecular weight 6000 in laboratory conditions followed by selection of genotypes based on easily measurable and inherited seedling traits contributing to drought tolerance. The genotypes Khirman and DH-3/48 found superior and might be productive in further breeding programmes for drought tolerance. Selection can be made on the basis of these characters at early growth stage to screen a large population for drought 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 and fruitful for selection of drought stress in wheat under the deliberated traits.

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