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Original Research Article

In-vitro Based Screening of Promising Wheat (*Triticum aestivum* L.) Genotypes for Osmotic Stress Imposed at Seedling Stage

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A B S T R A C T

The effects on yield of crops depend on severity of drought and the stage of plant growth during which it occurs. Seed germination is the stage of growth that is sensitive to water deficit. In order to study the effects of drought stress on germination indices in wheat genotypes, an experiment was carried out under laboratory conditions where seeds of eight genotypes (AKAW-4842, AKAW-4907, AKAW-4925, AKAW-4926, AKAW-5010, AKAW-5012, AKAW-5014, AKAW-5017) with two check variety [AKAW-3717 (Tolerant) and AKAW-3722 (Susceptible)] were raised in petri dishes and were either treated with distilled water (control) or different concentration (15%, 25%) of polyethylene glycol 6000 solution, it create artificial osmotic stress condition and observed its effect on germination percentage, shoot and root length, fresh and dry weight of shoot and root. The results of experiments with reference to genotypes revealed that, genotype AKAW-4842 shows highly similarity to the tolerant check variety. It gave maximum seed germination (93.04), shoot length (16.2cm), root length(18.2 cm), while the genotype AKAW-5017 shows maximum shoot fresh weight (0.28 g), root fresh weight (0.19 g). Among the genotypes tested AKAW-4842 and AKAW-5017 are tolerant genotypes had the potential to perform better under osmotic stress condition. Whereas AKAW-4926, AKAW 4907 and AKAW 5014 were shows moderate tolerant. Moreover, the genotypes AKAW-5012, AKAW-5010 and AKAW-4925 are the sensitive genotypes under drought environment. It is concluded from present in-vitro studies that osmotic stress significantly reduced the seed germination, shoot/root length, fresh and dry weight in all wheat genotypes. The maximum reduction was found at higher osmotic stress induced by PEG-6000 (25%) significantly

Keywords

In-vitro screening, Wheat genotypes, seed germination, early seedlings, PEG-6000, Osmotic stress

Introduction

Drought is one of the most common environmental stresses that affect growth and development of plants (Iqbal *et al.*, 1999; Yang *et al.*, 2004). It is assumed that by the year 2025, around 1.8 billion people will face absolute water shortage and 65% of the world's population will live under water-stressed environments. 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). 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 the most important cereal crop for the majority of world's populations. It is the most important staple food of about two billion people (36% of the world population) worldwide. It 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 (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). 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. Selection for dought tolerance at phase of seedlings is most usually practical using poly ethylene glycol (PEG 6000) in the medium (Rauf *et al.,* 2006)

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 conducted at the Biotechnology centre, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, India during the year 2017.

The experiment was laid out under complete randomized design (CRD) with three replication. In the present study seeds of eight wheat genotypes (AKAW-4842, AKAW-4907, AKAW-4925, AKAW-4926, AKAW-5010, AKAW-5012, AKAW-5014, AKAW-5017) with two check variety [AKAW-3717(Tolerant) and AKAW-3722(Susceptible)] were used.

Seeds of cultivars were obtained from wheat research unit Dr. PDKV, Akola. Seeds of ten genotypes were exposed to three stress level of PEG-6000 [T1, Control (distilled water), T2 (15 %) and T3 (25 %)]. PEG-6000 was prepared by dissolving the required amount of PEG in distilled water at room temperature. 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.

Twenty five seeds of individually wheat genotype were placed in a petri dish and then kept in an incubator for 10 days at 25/20^{o}c day/night temperature. Distilled water and PEG solutions were applied (5 ml each) on alternate days for each genotype. Seeds were considered germination when the developing radical stretched 2mm in length. Seed germination percentage was noted after 120 hrs of incubation. Growth attributes were studied in term of shoots and root length, fresh and dry weight.

Seed germination Percentage (%)

After 5 days (120 hours), seed germination ratio was considered by using the behind formula:

$$\text{Germination Percentage} = \frac{\text{Number of Seeds Germinated}}{\text{Total Number of Seeds Germinated}} \times 100$$

Shoot and Root length

The shoot and root length was measured in centimeter with ruler after one week of sowing at the time of experiment termination.

Shoot/Root fresh and dry weight

Shoots/roots were separated and weighed in grams (g) with an electronic digital balance. Shoots and roots were dried in hot air oven at 65^{o}C for 72 hours and weighed again for dry weighed.

Results and Discussion

Seed germination (%)

In the present study, ability of the eight genotypes with two check variety of wheat under chemical desiccation, induced by PEG (6000) during early seedling stage was assesed under in-vitro conditions. Data relevant the effect of osmotic stress induced by PEG on seed germination percentage (%) as presented in fig.1. Plate no.1. At control level seed germination percentage was highest and started to decrease as the osmotic stress level was increased by using PEG-6000 in all wheat genotypes. Under the control level, maximum seed germination were recorded in check tolerant variety AKAW-3717 and AKAW-4842, AKAW-5017 genotypes (99.67 and 99.51, 99.25%) while the minimum seed germination was recorded in AKAW-5014 (97.7%). Similarly under higher osmotic stress level (25%) the maximum seed germination was recorded in AKAW-3717 and AKAW-4842 i.e. (61.33, 63%) whereas no seed germination found in the check susceptible variety AKAW-3722 and AKAW-5012, AKAW-4925 genotype (0 %), respectively.

Osmotic stress decreases water potential gradient between seeds and their surrounding environment hence Dodd and Donavon (1999) reported that it can be a cause of reduction in seed germination. 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.

Shoot length (cm)

Seedling development under laboratory conditions have been accepted and 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 as depicted in fig.2., Plate no.1

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 AKAW-3717 (17.5 cm), while AKAW-3722 cultivar recorded lowest value (13.5 cm) followed by AKAW-4925 (14.2 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 AKAW-3717 followed by AKAW-5017genotypes, while the minimum shoot length was recorded in AKAW-5012 respectively. The decreasing trend in shoot length under increase the osmotic stress was reported by Chachar *et al.,* (2014a, 2014b).

Root length (cm)

For root length parameter, genotype AKAW-3717 had the maximum root length (10.5) followed by AKAW-4842 under control treatment. While the genotype AKAW-3722 and AKAW-5015 (4.5 and 3.5 cm) genotypes showed the minimum root length associated with 25% PEG-6000 treatment as depicted in fig.3., Plate no.1.

The decreasing trend in root length was also reported by many other scientists (Chachar *et al.,* 2016; Chachar *et al.,* 2014a, 2014b), who found that water stress had a significant effect on root length. 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 fresh weight (g 10^{-1}shoots)

The shoot fresh weight values were decreased with increasing water stress in all wheat genotypes (fig.4). Maximum shoot fresh weight was observed in AKAW-3717(0.295 g 10^{-1}shoots). Whereas, the minimum shoot fresh weight values were observed in AKAW-3722 and AKAW-5012(0.265 and 0.261 g 10^{-1}shoots). Results revealed that significant decrease with increasing water stress. High water stress condition (25%PEG-6000) there was comparatively higher reduction in plant biomass with increasing water stress of the growing media.

Fig.1 Germination (%)

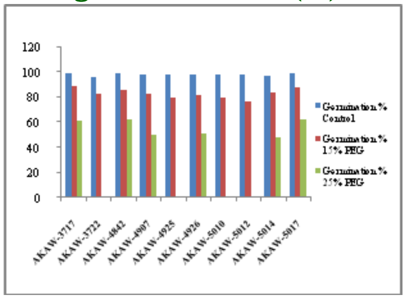


Fig.2 Shoot length (g)

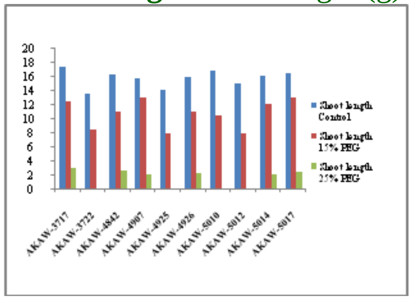


Fig.3 Root length (g)

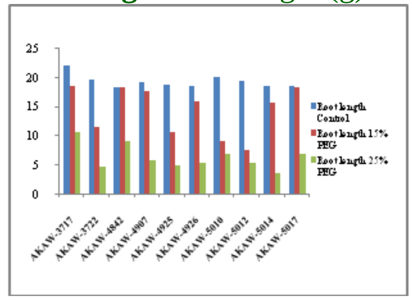


Fig.4 Fresh Shoot weight (g)

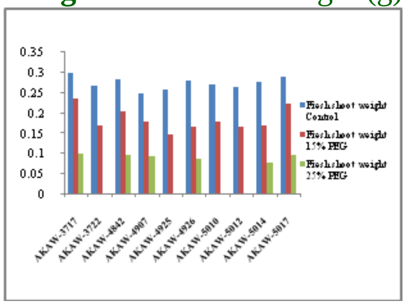


Fig.5 Fresh Root weight (g)

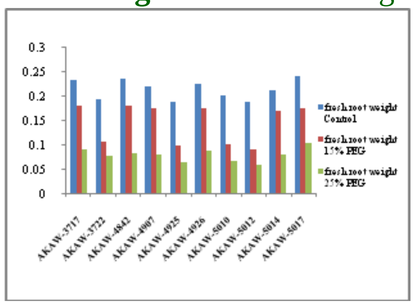


Fig.6 Dry Shoot weight

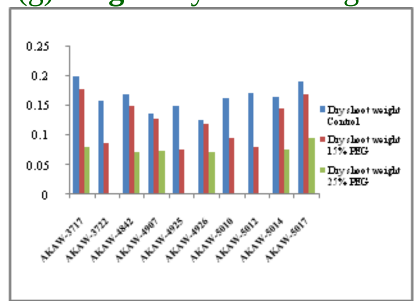
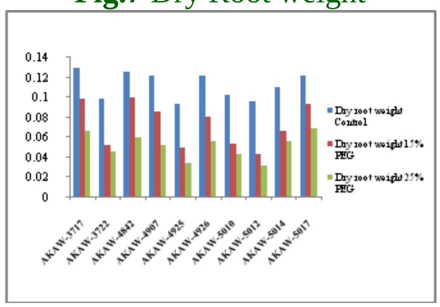


Fig.7 Dry Root weight



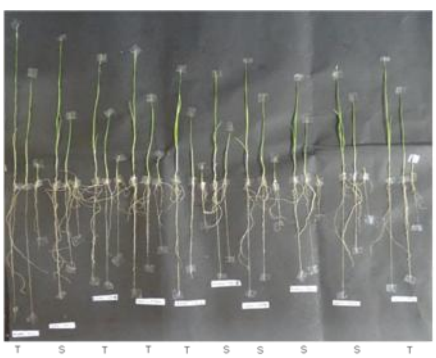


Fig.2- Wheat genotypes arranged with different concentration of PEG treatment

Plate no.1

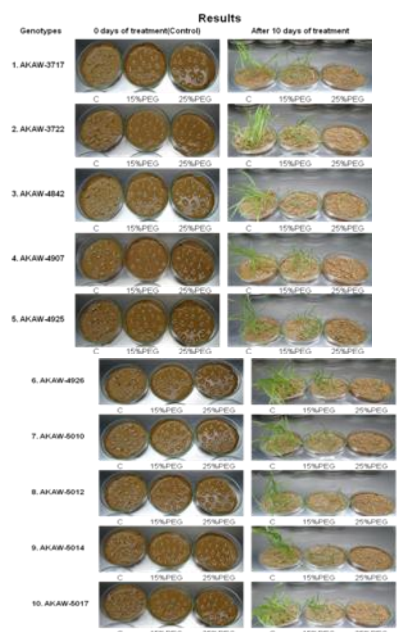


Fig.1- Screening of Wheat genotypes with different concentration of PEG-6000 at early seedling stage

Here again genotypes AKAW-3717 and AKAW-5017 showed maximum shoot fresh weight (0.098 and 0.095 g 10^{-1}shoots), followed by AKAW-4842, AKAW-4907 and AKAW-4926 (0.095, 0.0910 and 086 g 10^{-1}shoots) and AKAW-5014, however no shoot development in the genotypes AKAW-3722, AKAW-4925, AKAW-5010 and AKAW-5012 was observed under treated condition supplemented with 25%PEG.

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).

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

There was decrease in root fresh weight with the increasing in water stress in all wheat genotypes. The decrease was more in 25% PEG as compared to control (fig.5). Under control treatment the genotype AKAW-5017 showed maximum root fresh weight (0.24g 10^{-1}shoot), followed by AKAW-4842, AKAW-3717, AKAW-4926 and AKAW-5014 (0.23, 0.23, 0.22 and 0.21 g 10^{-1}shoot) was recorded, whereas minimum root fresh weight (0.18 g 10^{-1}roots) was observed in genotypes AKAW-5012, respectively. Root fresh weight at the highest water stress was observed as maximum in genotype AKAW-5017 (i.e. 0.10 g 10^{-1}roots), followed by AKAW-3717 (0.09), AKAW-4842 (0.08g 10^{-1}roots), and AKAW-4926 (0.08g 10^{-1}roots). While, the genotypes AKAW-5012 and AKAW-4925 showed minimum (0.05 and 0.06 g 10-1shoots) values for root fresh weight at highest osmotic stress, respectively. Root morphology and biomass are very important traits while selecting drought tolerant genotypes (Steven *et al.*, 2016).

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

In (fig.6) under control condition shoot dry weight of the genotype AKAW-3717 showed maximum dry weight (0.20g 10^{-1}shoot), followed by AKAW-5017, AKAW-4842, and AKAW-5012 (0.19, 0.16, and 0.17 g 10^{-1}shoot) was recorded, whereas minimum root fresh weight (0.12 g 10^{-1}roots) was observed in genotypes AKAW-4926, respectively. Root fresh weight at the highest water stress was observed as maximum in genotype AKAW-5017 (i.e. 0.09 g 10^{-1}roots), followed by AKAW-3717 (0.08), AKAW-4842 (0.07g 10^{-1}roots), and AKAW-4926 (0.78g 10^{-1}roots). However no shoot development in the genotypes AKAW-3722, AKAW-4925, AKAW-5010 and AKAW-5012 was observed under treated condition supplemented with 25% PEG.

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

There was decrease in root fresh weight with the increasing in water stress in all wheat genotypes (fig.7). The decrease was more in 25% PEG as compared to control. Under control treatment the genotype AKAW-3717 showed maximum root dry weight (0.12 g 10^{-1}shoot), followed by AKAW-4842, AKAW-4907, AKAW-4926 and AKAW-5017 (0.12g 10^{-1}shoot) was recorded, whereas minimum root fresh weight (0.09 g 10^{-1}roots) was observed in genotypes AKAW-5012, respectively. Root fresh weight at the highest water stress was observed as maximum in genotype AKAW-5017 (i.e. 0.06 g 10^{-1}roots), followed by AKAW-3717 (0.06g 10^{-1}roots), AKAW-4842 (0.05g 10^{-1}roots), and AKAW-4926 (0.05g 10^{-1}roots). While, the genotypes

AKAW-5012 and AKAW-4925 showed minimum (0.03g 10^{-1}shoots) values for root fresh weight at highest osmotic stress, respectively.

Plants have developed biochemical and physiological approaches to tolerate in water deficits environments. 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 AKAW-3717, AKAW-5017 and AKAW-4842 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. It is concluded from present studies that osmotic stress significantly reduced the seed germination shoot and root length, fresh and dry weight. Among the genotypes tested AKAW-3717, AKAW-4842 and AKAW-5017 are the tolerant genotypes had the potential to perform better under drought conditions, whereas AKAW-4907,AKAW-4926 and AKAW-5014 was moderate tolerant under water stress conditions. Furthermore the genotypes i.e. AKAW-3722, AKAW-5010, AKAW-4925 and AKAW-5012 are the sensitive genotypes under drought environment. Furthermore, significant and positive correlation among various seedling traits revealed that by improving dry shoot weight will improve the overall performance of the crop. The genotypes with improved traits may be used as parents in wheat breeding for moisture stress conditions.

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