



Science

## **IDENTIFICATION OF DROUGHT TOLERANT WHEAT GENOTYPES UNDER WATER DEFICIT CONDITIONS**

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### **ABSTRACT**

*Climate change is emerging phenomena and causing frequent drought which lead to scarcity of water, which ultimately negatively affecting wheat (*Triticum aestivum* L.) yield all around the world. The aim of this study was to explore the potential drought tolerant wheat genotypes for candidate genes exploration. This study was conducted during the year 2014-2015 at Plant Physiology Division, Nuclear Institute of Agriculture (NIA) Tandojam. The six wheat genotypes (cv. MT-1/13, MT-2/13, MT-3/13, MT-4/13 Chakwal-86 and Khirman) were investigated for their response at germination and seedling stage under different water stress treatments (0, -0.5, -0.75 and -1.0 MPa) in controlled conditions. The results of experiments with reference to genotypes revealed that genotype Chakwal-86 shows maximum seed germination (82.58 %), while the genotype Khirman shows maximum shoot length (7.23 cm), root length (15.1 cm), shoot fresh wt. (5.85 g 10-1shoots), root fresh wt. (3.45 g 10-1roots), shoot dry wt. (1.33 g 10-1shoots), root dry wt. (0.69 g 10-1roots). Among the genotypes tested Khirman and MT-4/13 are the tolerant genotypes had the potential to perform better under drought conditions, whereas MT-4/13 and Chakwal-86 were moderate tolerant under water stress conditions. Moreover, the genotypes i.e. MT-1/13 and MT-2/13 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 six wheat genotypes. The maximum reduction was found at higher osmotic stress induced by PEG-6000 (-1.0 MPa) significantly.*

### **Keywords:**

*wheat genotypes, seed germination, early seedlings, PEG-6000.*

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## 1. INTRODUCTION

Wheat (*Triticum aestivum* L.), one of the most significant staple food crop, it accounts for about 20 % of the human food supply and is cultivated on about 215 million hectares globally (WHEAT 2014). The lack of adequate available water is the most common constraint for wheat production in low rainfall and poorly irrigated areas. Water stress results in a significant reduction in the yield (Bano *et al.*, 2012). Water deficit is one of the further most main biologically issues that retard plant growth and productivity in arid regions (Kafi and Salehi, 2012). Plants usually experience water supply fluctuations during life cycle due to changing climatic factors (Tan *et al.*, 2006; Izabela *et al.*, 2013). Between all the abiotic stresses, drought probably has the most significant effect on growth and yield which plants may be encounter in both natural and agricultural systems (Bartels and Sunkar, 2005). Therefore, it is significant to study the mechanism of drought stand of plant nature in order to improve their agronomic characters to facilitate developing cultivar with increased resistance.

Selection for drought tolerance at phase of seedlings is most usually practical using poly ethylene glycol (PEG 6000) in the medium (Rauf *et al.*, 2006). PEG 6000 molecules are inert, nonionic and basically impermeable chains and have commonly been used to move water stress without causing any significant physiological damage to crop plants (Carpita *et al.*, 1979). PEG can be used to change the osmotic potential of nutrient clarification culture and can transfer plant water shortage in a comparison exact way, proper to untried proprieties (Lagerwerff *et al.*, 1961).

Earlier studies focalization on identification of the drought tolerant wheat genotypes using different concentrates of PEG 6000 have showed significant differences for different seedling particularities (Rauf *et al.*, 2006; Singh *et al.*, 2008). Important differences among the wheat genotypes have also been observed for cell membrane stability (CMS), number of tillers and 100-seed weight. A positive connection was observed between CMS and number of tillers in wheat (Shafeeq *et al.*, 2006). The seedling traits when pooled together could discriminate between drought tolerant and susceptible genotypes (Noorka and Khaliq, 2007).

Possible development of crops for drought tolerance may require a search of physiological attributes and the exploration of their genetic variation in germplasm (Farooq *et al.*, 2009; Jatoi *et al.*, 2012). The production of wheat can be increased by bringing more area under cultivation or by increasing it's per hectare yield (Ahmad, 2002). Currently, it is impossible to increase area due to other compare crops, limited supply of irrigation water and reduction in cropped area due to expanding cities and industries (Rafiq *et al.*, 2005).

Therefore the aim this study was to exploration of genetic diversity among selected wheat genotypes that can tolerate limited water condition. Knowledge of traits associated is also important for understanding yield limiting factors. The present study was planned to select the drought tolerant wheat genotypes below osmotic stress (PEG 6000) at germination and seedling stage.

## 2. MATERIALS AND METHODS

The current studies were conducted at plant physiology Division of Nuclear Institute of Agriculture (NIA), Tandojam as collaborated research between SAU and NIA, Tandojam during the year 2014-15. Polyethylene glycol (PEG) was used in different concentration, 0.0, 0.5, 0.75, and 1.0 MPa to create artificial stress. Preliminary laboratory experiment was conducted to screen out of 6 genotypes/line collected from Plant Breeding division, NIA Tandojam. Good healthy wheat seed were manually selected and upper treated with 5% sodium hypochlorite (NaOCl) solution for 10 minutes, washed with refined water several times, and briefly blotted into fine quality filter paper. Seed were germinated in protected clean petri dishes covering germination paper moisturized with 10ml of changed concentration of PEG-600 separately. Twenty seeds of individually wheat genotype will be placed in a petri dish covered with black muslin cloth and then kept in an incubator for 8 days at 25/20°C day/night temperature. Seeds were considered germination when the developing radicle stretched 2mm in length. Seed germination percentage was noted after 192 hrs of incubation.

To study growth attributes in response to osmotic stress expansion was conducted in glass bowls (15 and 10cm in depth). Twenty imbibed seeds of 6 wheat genotypes were sown over plastic screen and placed in glass bowels containing PEG solution of four different grades including control (D.H<sub>2</sub>O), -0.5 MPa, -0.75 MPa, and -1.0Mpa. The bowls were placed in a programmed growth cabinet under a 10 h photoperiod (4.96 μmol m<sup>-2</sup> s<sup>-1</sup>). The seedlings were harvested after 20 days. Growth attributes were studied in term of shoot and root length, fresh and dry weight.

### Seed germination Percentage (%)

After 3 days (72 hours), seed germination ratio was considered by using the behind formula:

$$\text{Seed germination (Percentage)} = \frac{\text{Germinated seeds} \times 100}{\text{Total Seeds}}$$

### 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.

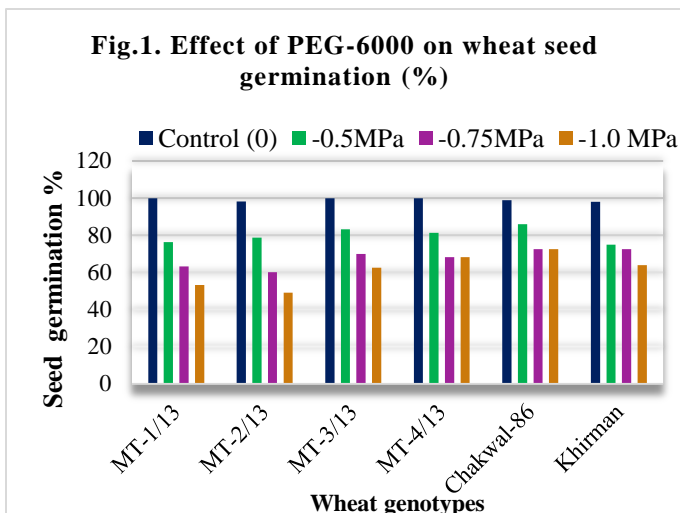
### Shoot/Root fresh and dry weight

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°C for 72 hours and weighed again for dry weighed.

## 3. RESULT AND DISCUSSIONS

### *Seed germination (Percentage)*

In the present study, ability of the six 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 presented in fig.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 MT-1/13 and Chakwal-86 (100 and 99%) while the minimum seed germination was recorded in Khirman (98%). Similarly under higher



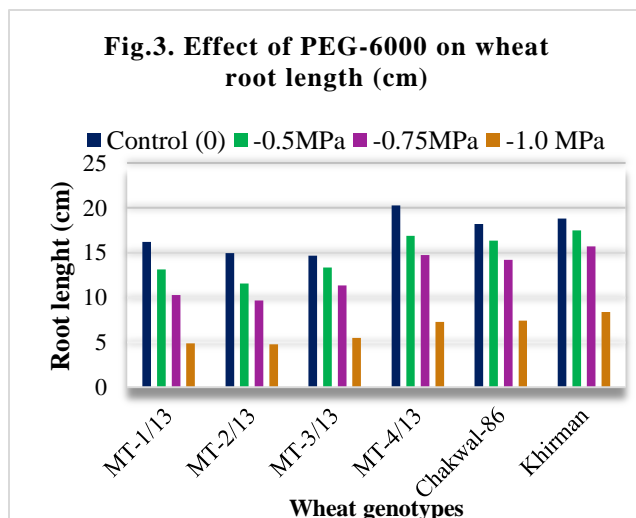
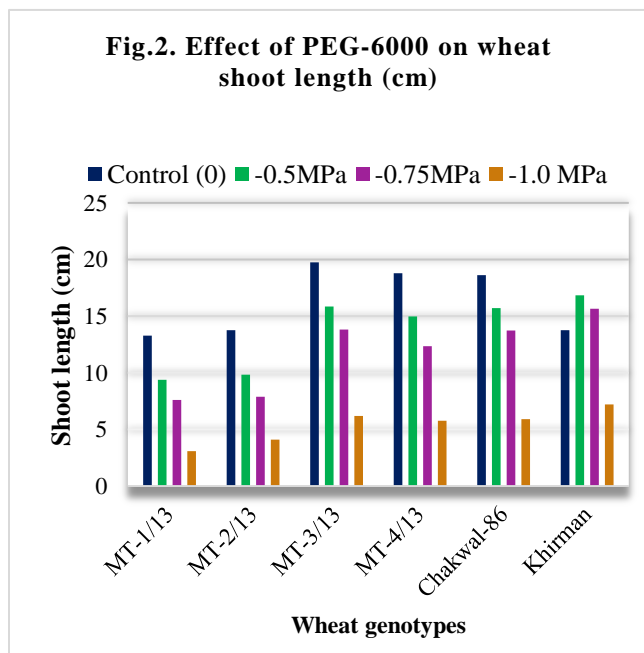
osmotic stress level (-1.0MPa) the maximum seed germination was recorded in Chakwal-86 i.e. (72.66%) whereas the minimum seed germination under high osmotic stress was recorded in the genotype MT-2/13 which was (49.11%), respectively. Delayed and reduced germination can be resulted from water stress at germination stage or it may retard germination completely. However once a seed gets at sufficient level of hydration it will prevented toward full germination (Hegarty, 1977). Dodd and Donavon (1999) concluded that osmotic stresses reduce seed

germination and seedling growth under osmotic stress conditions. 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. Exploration of genetic variation among the genotypes that could be useful to develop new genotypes that can be adopted in arid and semiarid regions was suggested by Alaei *et al.* (2010) and Jajarmi (2009).

**Shoot and root length (cm)**

In the present study wheat genotypes were tested under control (0), -0.5, -0.75 and -1.0 MPa showed significant decrease in shoot and root growth. Maximum shoot length was recorded in Khirman followed by, Chakwal-86 (16.17 and 14.29 cm) as depicted in fig.2. Whereas, the Fig.3 revealed that maximum root length was recorded in Khirman and MT-4/13 (15.1 and 14.80 cm), moreover, minimum root length was recorded in MT-1/13 (6.39 cm). Increasing concentration of osmotic stress reduced shoot and root length of all the wheat genotypes. Maximum reduction was recorded at the highest water stress level (i.e.-1.0 MPa). Under control conditions, the genotype MT-4/13, Khirman and Chakwal-86 showed the maximum root length

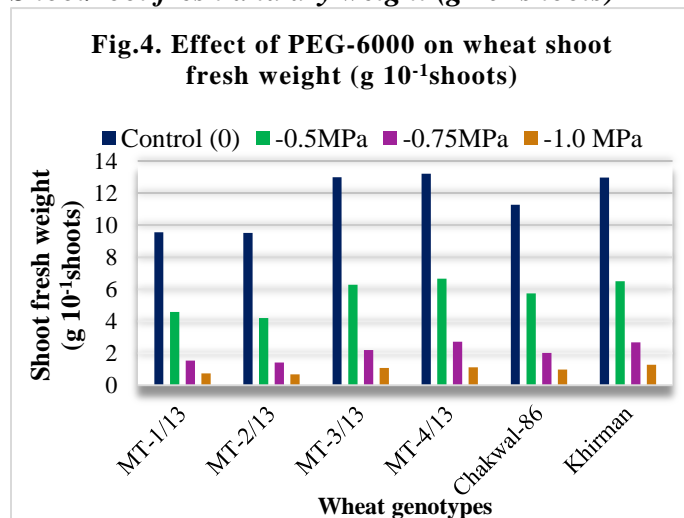
(i.e. 20.28, 18.81and 18.21 cm) followed by genotypes MT-1/13 (16.09 cm), MT-2/13(14.95 cm), while minimum root length i.e. (14.68 cm) was observed in genotypes



MT-3/13, respectively.

Under highest water stress conditions the genotype Khirman also showed maximum root length (8.39 cm), followed by Chakwal-86 (7.43 cm), MT-4/13(7.29 cm), and MT-3/13 (5.49 cm), while minimum root length was observed in genotypes MT-1/13 and MT-2/13 (i.e. 4.89 and 4.79 cm), respectively. Reduction in the shoot and root length under water stress environment result of an inhibition of cell division and elongation reported by Fraser et al. (1990). The decreasing trend in shoot and root growth was also reported by Kamran et al. (2009) and Chachar et al. (2014a 2014b) under water stress.

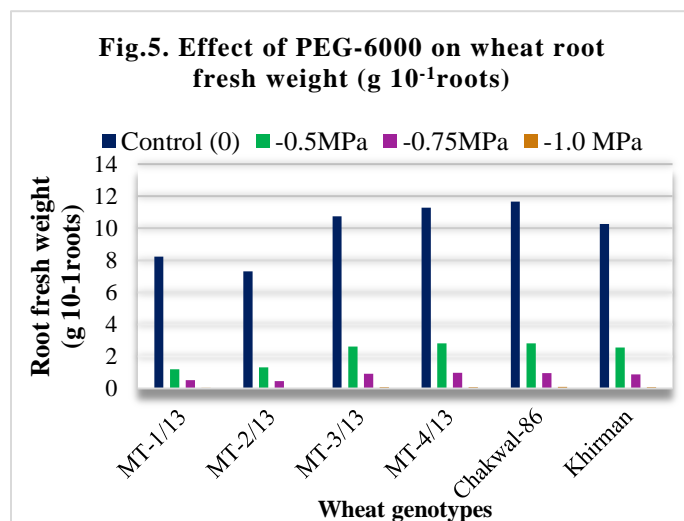
### Shoot/root fresh and dry weight ( $g 10^{-1}$ shoots)



The shoot and root fresh weight values were decreased with increasing water stress in all wheat genotypes (fig.4 and fig5). Maximum root and shoot fresh weight was observed in Khirman. Whereas, the minimum root and shoot fresh weight values were observed in MT.4/13 and MT-3/13 (5.93 and 5.63  $g 10^{-1}$ shoots). The results for shoot and root dry weight in the presented in fig.6 and fig.7. Results revealed that significant decrease with increasing water stress. High water stress condition (-1.0 MPa) there was comparatively higher reduction in plant biomass with increasing water

stress of the growing media. Here again genotypes Khirman and MT-4/13 showed maximum shoot fresh weight (1.29 and 1.13  $g 10^{-1}$ shoots), followed by MT-3/13 (1.09  $g 10^{-1}$ shoots) and Chakwal-86 (0.98  $g 10^{-1}$ shoots). While minimum shoot fresh weight (0.75 and 0.69  $g 10^{-1}$ shoots) was observed in genotype MT-1/13 and MT-2/13, respectively.

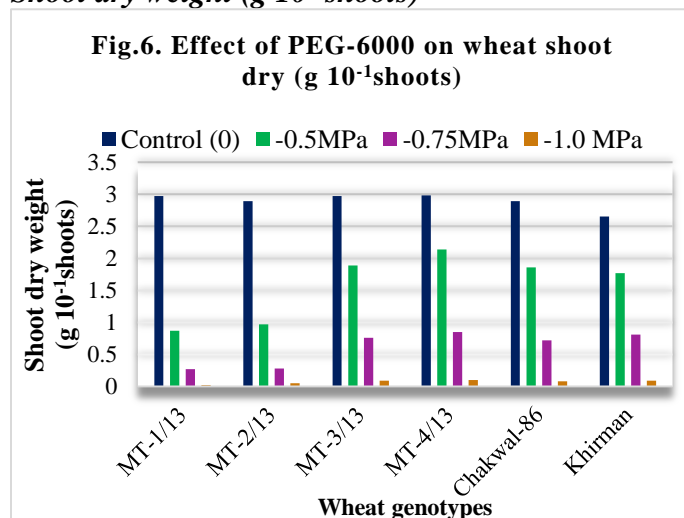
### Root fresh weight ( $g 10^{-1}$ roots)



There was decrease in root fresh weight with the increasing in water stress in all wheat genotypes. The decrease was more in -1.0 MPa as compared to control. Mean values for root fresh weigh in three treatments were recorded as 9.91, 2.22, 0.79 and 0.07  $g 10^{-1}$ roots under control, -0.5, -0.75 and -1.0 MPa respectively. Under control treatment the genotype Chakwal-86 showed maximum root fresh weight (11.66  $g 10^{-1}$ shoot), followed by MT-4/13 (11.29), MT-3/13 (10.75), Khirman (10.26), and MT-1/13 (8.24  $g 10^{-1}$ shoot) was recorded, whereas minimum root fresh weight (7.31  $g 10^{-1}$

<sup>1</sup>roots) was observed in genotypes MT-2/13, respectively. Root fresh weight at the highest water stress was observed as maximum in genotype Chakwal-86 (i.e. 0.10 g 10<sup>-1</sup>roots), followed by Khirman (0.09), MT-3/13 (0.09 g 10<sup>-1</sup>roots), and MT-4/13 (0.08). While, the genotypes MT-1/13 and MT-2/13 showed minimum (0.05 and 0.03 g 10<sup>-1</sup>shoots) values for root fresh weight at highest osmotic stress, respectively.

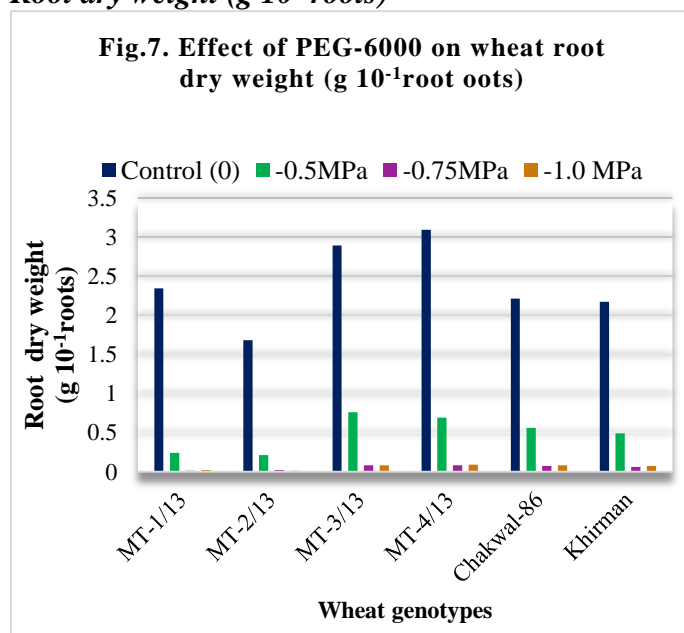
### Shoot dry weight (g 10<sup>-1</sup>shoots)



Mean values for shoot dry weight in different treatments were recorded as 2.89, 1.58, 0.61 and 0.07 g 10<sup>-1</sup>shoots in control, -0.5, -0.75 and -1.0 MPa, respectively. Under control condition, shoot dry weight of genotype MT-4/13 was maximum i.e. (2.98 g 10<sup>-1</sup>shoots), followed by MT-1/13, MT-3/13 (2.97 g 10<sup>-1</sup>shoots) and Chakwal-86 (2.89 g 10<sup>-1</sup>shoots). While, minimum shoot dry weight was observed in genotypes MT-2/13 and Khirman (2.89 and 2.65 g 10<sup>-1</sup>shoots), respectively. The maximum shoot dry weight was recorded in genotype MT-4/13 (0.10 g 10<sup>-1</sup>shoots),

followed by MT-3/13 and Khirman as (0.09 g 10<sup>-1</sup>shoots of each), at -1.0 MPa. The other genotype were also showing better SDW, was Chakwal-86 (0.08 g 10<sup>-1</sup>shoots) respectively. Minimum values for SDW (0.02 and 0.05 g 10<sup>-1</sup>shoots) were recorded in genotypes MT-1/13 and MT-2/13, respectively.

### Root dry weight (g 10<sup>-1</sup>roots)



Mean values for root dry weight in different treatments were recorded as 2.39, 0.49, 0.05 and 0.58 g 10<sup>-1</sup>roots in control, -0.5, -0.75 and -1.0 MPa, respectively. Under control condition, root dry weight of genotype MT-4/13 was maximum i.e. (3.09 g 10<sup>-1</sup>roots), followed by MT-3/13 (2.89 g 10<sup>-1</sup>roots) and MT-1/13 (2.34 g 10<sup>-1</sup>roots), whereas Chakwal-86 (2.21 g 10<sup>-1</sup>roots) and Khirman (2.17 g 10<sup>-1</sup>roots). While, minimum shoot dry weight was observed in genotype MT-2/13 (1.68 g 10<sup>-1</sup>roots), respectively. Under highest osmotic stress (-1.0 MPa) condition, root dry weight of genotype MT-4/13 was maximum i.e. (0.09 g 10<sup>-1</sup>roots), followed by MT-3/13 and Chakwal-86 (0.08 g 10<sup>-1</sup>

<sup>1</sup>roots each), whereas Khirman (0.07 g 10<sup>-1</sup>roots) and MT-1/13 (0.02 g 10<sup>-1</sup>roots). While minimum shoot dry weight was observed in genotype MT-2/13 (0.01 g 10<sup>-1</sup>roots), respectively.

The decreasing trend in shoot and root dry weight was also reported by many other scientists (Kamran *et al.*, 2009; Ahmad *et al.*, 2013; Chachar *et al.* 2014a, 2014b), who found that water stress had a significant effect on shoot and root dry weight. The decline in shoot/root fresh and dry weight was attributed due to lower number and development of smaller leaves with increased PEG (6000) level in growing media. Many scientist resported that drought resistance is considered by small reduction of shoot growth under water stress environment (Ming *et al.*, 2012; Mouchesh, *et al.* 2012 and Saghafikhadem 2012; Sassi *et al.*, 2012). Root morphology and biomass are very imporant traits while selecting drought tolerant genotypes (Steven *et al.* 2016). The decreasing trend in root and shoot dry weight was also reported by other researchers (Kamran *et al.*, 2009; Ahmad *et al.*, 2013; Izabela *et al.*, 2013) who found that water stress had a significant effect on root and shoot dry matter production.

#### 4. CONCLUSION

Plants have developed biochemical and physiological approaches to tolerate in water deficits environments. It is concluded from present studies that osmotic stress significantly reduced the seed germination shoot/root length fresh and dry weight. Among the genotypes tested Chakwal-86 and MT-4/13 are the tolerant genotypes had the potential to perform better under drought conditions, whereas MT-3/13 and Khirman was moderate tolerant under water stress conditions. Furthermore the genotypes i.e. MT-1/13 and MT-2/13 are the sensitive genotypes under drought environment. Furthermore it is strongly recommended that tested genotypes should be included in future breeding programes for development of drought tolerant cultivars.

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