

EFFECT OF PEG INDUCED WATER DEFICIT STRESS ON PHYSIO-BIOCHEMICAL CHARACTERISTICS OF DIFFERENT PEARL MILLET VARIETIES

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Abstract: The present study aimed to scrutinize six pearl millet varieties, differing in their drought sensitivity to evaluate drought tolerance through physio-biochemical parameters. The main purpose of this work was to screen the highly tolerant and susceptible genotypes under PEG-6000 induced water deficit stress (WDS). WDS was induced in seedling on 10th and 20 day of germination by exposing them to different stress levels *i.e.* T1 (Control); T2 (5% PEG) and T3 (10% PEG). Significant reductions in parameters viz. shoot length, root length, seedling vigour index I, seedling vigour index II and Membrane stability index was observed. The antioxidant enzyme activity (Catalase and Superoxide Dismutase) was assayed for these varieties under water stress. There was a profound decrease in the Catalase activity whereas the SOD activity was increased in the varieties selected for the study. The water stress induced by supplementing 5% PEG in soil was tolerable by the plants as compared to 10% PEG. The results obtained were useful in screening drought tolerant Pearl Millet genotype.

Keywords: WDS, PEG, Drought, Pearl millet, Enzyme activity

INTRODUCTION

Pearl millet (*Pennisetum glaucum* L.) is the most widely grown minor cereal crop world-wide among the millets. It is recognized as an important food and forage crop in many countries of Asia and Africa. As pearl millet can withstand drought and high temperature stress during either the vegetative or reproductive phases of its growth, hence is mostly preferred for arid and semiarid regions which experience frequent periods of dry weather. Currently, drought is one of the most important limiting factors for crop production and becoming an increasingly severe problem in many regions of the world (Aslamet *et al.*, 2006). Leaf rolling, stomata closure, deeper penetration of roots, higher relative water content and better osmotic adjustment are some of the mechanism that plants employ to overcome water stress.

Since physiological responses of plants to drought stress may vary at different developmental stages, it is considered that different indicators should be used for the phenotyping of drought tolerance (Tuberosa, 2012). Various methods have been employed from time to time to identify drought tolerant genotypes and efforts have been made in the past to screen tomato varieties which differed in drought tolerance (George *et al.*, 2013).

The most popular approach for induction of drought stress is to use high molecular weight osmotic substances, like polyethylene glycol (PEG). It have been used often as abiotic stress inducer in many studies to screen drought tolerant germplasm (Jatoi *et al.*, 2014). It is a polymer and considered as better

chemical to induce water stress artificially. PEG induced osmotic stress is inductee to decrease cell water potential. PEG is a non-penetrable and nontoxic osmotic substance which is used to lower the water potential and it has been used to simulate drought stress. It is commonly established plants that adapt PEG show high level of tolerance to drought stress as compared to cell lines that fall short under the induced stress condition.

Rajasthan is the major state with an area of 4.77 m. ha under pearl millet production. Since moisture loss from sandy soils of western Rajasthan is fast, sowing is completed within 2-3 days of the rainfall. Farmers of this region therefore tend to undertake risk in sowing even under sub-optimal conditions results in poor plant stand. Therefore developments of cultivars that germinate and produce vigorous stand under limited soil moisture are expected to contribute to successful pearl millet production in the arid regions. In past few decades, research for identification of drought tolerant genotypes has taken an impetus with special reference to stress management. But pearl millet, although an important crop of Rajasthan has not been exploited in this regard further, the aim of this study was to investigate the effects of osmotic stress generated by different levels of PEG-6000 on seedling stage of pearl millet genotypes. The primary objective of the present study was to screen out the most tolerant and most sensitive pearl millet genotypes under artificially induced PEG drought stress.

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MATERIALS AND METHODS

Experimental materials

Seeds of six pearl millet genotypes with different sensitivity to water-deficit stress i.e. (MH 1996, MH 1998, MH 1993, MH 2024, RHB 177 and KBH108.) were obtained from Pearl millet Research Station, RARI, Durgapura, Jaipur.

Experimental details

The experiment was laid out in randomized complete block design with two factors (genotypes and water stress level) and three replications. Fifty seeds of each genotype were grown in plastic pots filled with coco peat. To achieve a uniform emergence of seedlings, pots were filled with potting mixture. Seedling vigour index I at was measured by the formula given by Singh and Kakralya (1995).

$SV I = \text{Germination \%} \times (\text{mean of seedling length (root + shoot)}/100)$

Seedling vigour index was measured by the formula given by Singh and Kakralya (1995).

$SV II = \text{germination \%} \times \text{Seedling dry weight}$

Membrane Stability Index

The pearl millet membrane stability index (MSI) was determined according to the method of Premchand *et al.*, (1990) as modified by Sairam (1994). Shoot portion (0.1 g) of different treatments and control were thoroughly washed in running tap water and double distilled water and thereafter placed in 10 ml of double distilled water at 40°C for 30 minutes. After the end of this period their electrical conductivity was recorded by EC meter (C1). Subsequently the same samples were placed on boiling water bath (100° C) for 10 min and their electrical conductivity was recorded as above (C2). The membrane stability index (MSI) was calculated as:

$$MSI = [1 - (C1/C2)] \times 100$$

Catalase (CAT) enzyme activity:

It was assayed by measuring the disappearance of H₂O₂ according to Teranishiet *al.*, (1974). 1gm fresh plant material was taken and homogenized it in 50mM chilled phosphate buffer (pH 7.0) and then it was centrifuged. For CAT activity, the reaction mixture will contain 2.7 ml 50mM phosphate buffer (pH7.0), 0.1ml enzyme extract, 0.2 ml 200mM H₂O₂ solution. Decrease in absorbance will be recorded at 410 nm for 3 minutes.

leaving the top 5 cm empty, sowing and then covered with 2 cm of potting mixture. Plants were allowed to germinate and irrigated regularly. There were three treatments comprising T1: (control i.e. no PEG); T2 (5% PEG); T3: (10% PEG). The germination was calculated by incubating 10 seeds of each variety under three treatments T1, T2 and T3. The shoot and root length was measured with the help of meter scale and thread. Various physio-biochemical indices have been monitored at two stages of early growth, i.e. at 10 days after sowing and 20 days after sowing. The growth parameters- shoot length, root length, seedling vigour index I and II, membrane stability index, catalase and superoxide dismutase enzyme activity were recorded.

Seedling Vigour Index

Superoxide dismutase (SOD) enzyme activity:

1gm of plant material was homogenized in 50mM phosphate buffer+ 0.25 ml triton+ 1% PVPP and then centrifuged at 10000 rpm. For SOD activity the reaction mixture contain 1.5 ml reaction buffer, 0.2 ml methionine, 0.1 ml enzyme extract, 0.1 ml Na₂CO₃, 0.1 ml NBT , 0.1 ml EDTA and 0.1 ml Riboflavin. Three test tubes were taken. One contain all things and was kept in dark (Blank A), second test tube contain all things except enzyme extract and was kept in light (Blank B), third test tube contain all things and was kept in light (Blank C). Absorbance was recorded at 560 nm. One unit of SOD activity was defined as amount of enzyme which causes 50% inhibition of photochemical reaction of NBT.

Statistical Analysis

Data were subjected to analysis of variance (ANOVA) using SAS 9.1 software. The least significant differences (LSD at p = 0.05%) test was used to define significant differences among treatment means.

RESULTS

Effect of PEG on Germination

The various concentrations of PEG had a significant effect on the germination of the seeds. Germination was significantly affected by the osmotic potential, by cultivars and their interaction (Table 1). An increase in PEG stress markedly decreased the germination percentage of all cultivars compared to their relative controls. Genotype KBH108 and MH2024 showed 100% germination in 5% PEG induced water stress while MH1996 showed least germination in both 5% and 10% PEG induced stress.

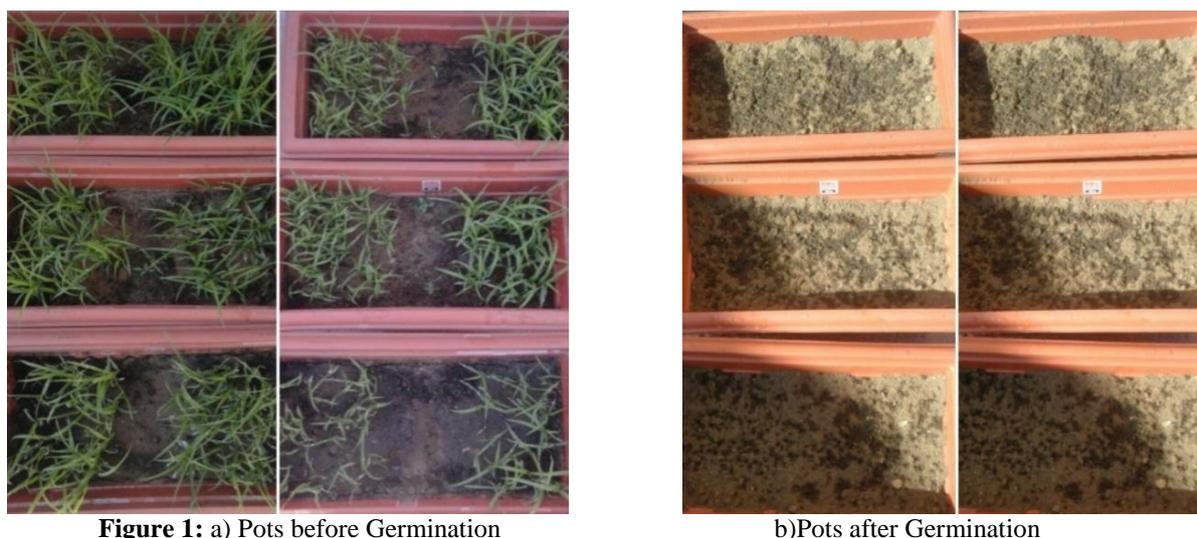


Table 1. Effect of PEG treatments on germination of pearl millet hybrids:

S. No	Hybrids	T1	T2	T3
1	MH1996	80	70	70
2	MH1998	90	90	80
3	MH1993	100	80	80
4	MH2024	100	100	90
5	RHB177	100	90	70
6	KBH108	100	100	90

Effect of PEG on shoot and root length

For all genotypes, the shoot lengths of seedlings decreased with an increase in water stress (Table 2). 5% PEG treatment decreases the length of the coleoptile by 11.16 to 16.50% while 10%PEG reduces this dimension by 11.31 to 16.53% in all genotypes.GenotypeKBH 108 produced the longest shoot (17.4 cm) at control condition while showed

highest reduction 16.50% and 16.53% at 5% and 10% PEG treatment respectively. While MH1996 showed lowest reduction 11.16% and 11.31% at 5 % and 10% PEG treatment respectively. PEG treatments decrease the root length of all the genotypes (Table2). In contrast, 10% PEG treatment increase the root length in all genotypes compared to 5% PEG.

Table 2. Effect of treatments on root length and shoot lengthof pearl millet hybrids:

Hybrids	Shoot length			Root length		
	T1	T2	T3	T1	T2	T3
MH1996	12.1	11.4	9.5	10.5	9.3	8.8
MH1998	15.7	15.2	14.4	11.6	10.8	9.9
MH1993	13.1	11.8	11.2	11.2	10.9	9.9
MH2024	13.3	12.9	11.9	14.9	11.4	10.9
RHB177	16.9	15	13.8	12.7	11.9	10.9
KBH108	17.4	15.7	15.2	14.6	13.1	12

Effect of PEG on Seedling vigor index I and Seedling vigor index II

Among the hybrids, MH 1996 was affected the least by drought stress because it gave the lowest reduction rate for seed vigour (Table 3). Seed vigour decreased with increase in concentration of PEG

solution (Table 3). There were significant differences among hybrids for seed vigour in all drought levels. Among six pearl milletgenotypeKBH 108 produced the highest seed vigour and there were significant differences among hybrids for seed vigour in 5% and 10% PEG treatment.

Table 3. Effect on PEG on Seedling vigor index I and Seedling vigor index II of pearl millet hybrids

Hybrid	Seedling vigour index I			Seedling vigour index II		
	T1	T2	T3	T1	T2	T3
MH1996	18.08	16.56	12.81	2.78	2.48	1.61
MH1998	22.77	23.4	19.44	3.87	2.79	2.15
MH1993	24.3	22.7	18.99	3.81	2.36	1.44
MH2024	28.2	24.3	20.62	5.07	4.09	3.28
RHB177	29.6	24.21	17.29	4.74	4.14	2.29
KBH108	32	25.92	24.48	5.86	4.9	4



Figure 2: Effect of PEG induced water stress on Seedling length of Pearl millet hybrid

Effect of PEG on Membrane Stability Index:

As shown in table 4, the values for stability of cellular membranes in the leaf tissues studied revealed that there was a significant decline in MSI of stressed plants in all hybrids at both treatment level. A major impact of plant environmental stress is cellular membrane modification, which results in its perturbed function or total dysfunction. The cellular

membrane dysfunction due to stress is well expressed in increased permeability and leakage of ions out, which can be readily measured by the efflux of electrolytes. The variety which shows maximum membrane stability index is KBH108 and minimum membrane stability index was observed in MH1996 (Table 4).

Table 4. Effect of treatments on MSI of pearl millet hybrids

Hybrid	T1	T2	T3
MH 1996	67.33	60	53.67
MH1998	76	71	64.33
MH1993	77.67	74	65
MH2024	76.67	71	64.67
RHB177	74.67	68	62.33
KBH108	79.65	75	67.33

Effect of PEG on Catalase and Superoxide dismutase activity

In the present investigation as shown in figure 2, there was observed decrease in the CAT activity with the increase in the concentration of PEG creating water stress. In the present study, the CAT activity was found decreasing with the increasing PEG

concentration as well as duration of drought induction. The variety which shows maximum antioxidant catalase activity is MH1996 and minimum catalase activity was observed in KBH 108.

In the present investigation as shown in figure 3, there was observed increase in the SOD activity with

the increase in the concentration of PEG creating water stress. SOD is a major scavenger of O₂ and its enzymatic action results in the formation of H₂O₂ and O₂. The SOD activity of leaves increases at both mild and severe stress condition as compare to the control plant leaves. The enhancement of SOD activity under

stress condition shows a well organised defence system against ROS under stress condition. The variety which shows maximum SOD activity is KBH 108 and minimum SOD activity was observed in MH 1996. The readings wastaken in Umg⁻¹ prot⁻¹.

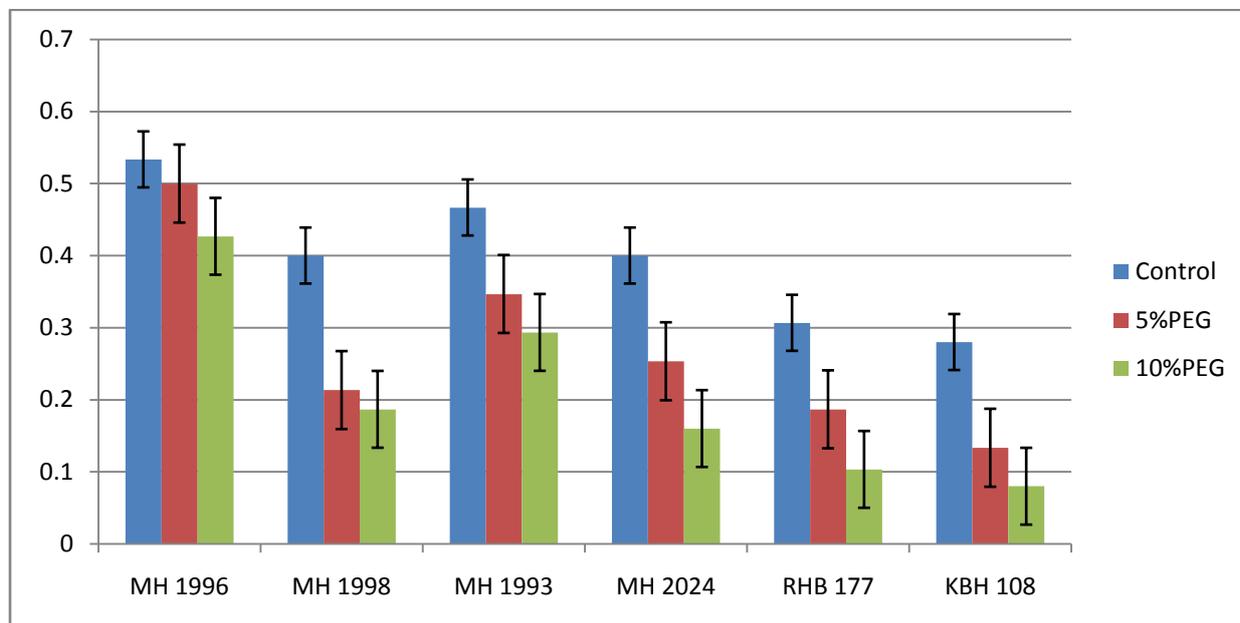


Figure3: Effect of PEG on Catalase activity of pearl millet hybrids. The bars indicates standard error (\pm SE) of mean (n = 3). All means are significantly different at $p \leq 0.05$.

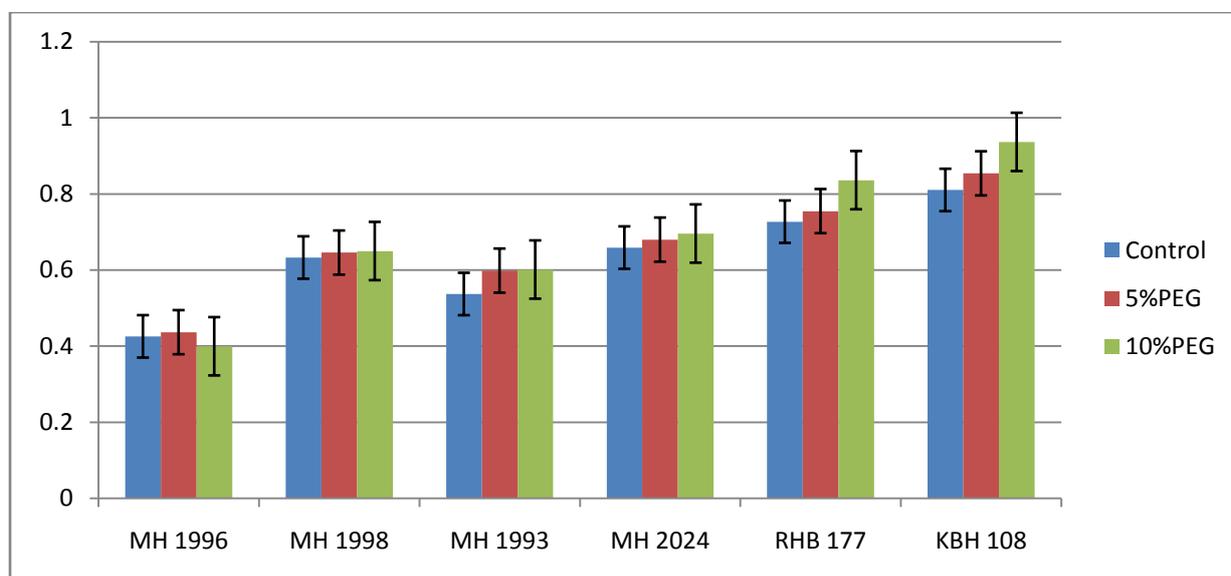


Figure4: Effect of PEG on Superoxide Dismutase activity of pearl millet hybrids. The bars indicates standard error (\pm SE) of mean (n = 3). All means are significantly different at $p \leq 0.05$.

DISCUSSION

Water stress due to drought is one of the most significant abiotic factors that limit the seed germination, seedling growth, plants growth and yield (Hartmann *et al.*, 2005, Van den Berg and Zeng, 2006). Several methods have been developed to screen drought tolerant germplasm in plant species.

Based on the literature available, PEG is considered as a superior chemical to induce water stress (Kaur *et al.*, 1998). Polyethylene glycol (PEG) molecules are inert, non-ionic, virtually impermeable chains and have been used frequently to induce water stress in crop plants (Carpita *et al.*, 1979; Landjeva *et al.*, 2008). Among investigated germplasm, KBH108 and MH2024 showed 100% germination in 5% PEG

induced water stress while MH1996 showed least germination in both 5% and 10% PEG induced stress than the other genotypes. The higher germination rates of the genotype may be due to their capability to absorb water even under PEG induced water stress. Hearty, (1997) and Turk *et al.*, (2004) reported that water stress at germination stage delayed or reduced or hinder germination completely. However, once the grain attains a critical level of hydration it will lead to full seed germination. If, the physiological changes happen below the critical level it lead to complete inhibition of seed germination. Dodd and Donavon, (1999) stated that PEG induced reduction in germination percentage was because of reduction in the water potential gradient between seeds and their surroundings. Several reports on wheat varieties suggest that germination rate was affected by various abiotic stresses (Bayoumi *et al.*, 2008; Jajarmi, 2009; Alaei *et al.*, 2010). A higher level of germination under stress condition was observed in *Vigna conitifolia*, however this finding may not be applicable to all cases and it depends on the germplasm used in screening (Soni *et al.*, 2011). Similar results like reduction in germination rate with the increase PEG were noted in chick pea also (Kaur *et al.*, 1998).

Strong negative correlation coefficient was noted between root length and PEG concentration. Roots are the primarily effected plant part under drought conditions than any other parts (Ghafoor, 2013). Root trait of all varieties provided useful information against different levels of PEG and this is very important attribute to study the drought stress. The germplasm which has better growth under stressed environment may have drought tolerance mechanism in it and these plants may have capability of holding a homeostasis under stressed conditions (Saxena and Toole, 2002). With few exceptions, the response of tomato varieties for root length was more or less similar against the different levels of PEG. A gradual reduction in root length with an increasing concentration of PEG was the common tendency observed among all varieties. The reduction rate in root length is different in the varieties investigated. The root length at control varied in between 10.5 to 14.6 cm in the pearl millet genotype with mean root length 12.58 cm. At the highest concentration of PEG (10%) a drastic reduction in root length in all pearl millet varieties was noted. It is well known fact that root architecture influences the yield and other agronomic traits, particularly under stress conditions (Ludlow and Muchow, 1990; Dorlodot *et al.*, 2007). Remarkable decrease in root length has been observed with increasing PEG concentrations was reported by Jajarmi *et al.*, (2009) and similar results like reduction in root length with increasing osmotic stress was identified in pea plants (Whalley *et al.*, 1998). Kulkarni and Deshpande, (2007) reported that early and rapid elongation of roots is a key trait of drought tolerance. A strong negative correlation

between shoot length and PEG concentration has been observed and a positive correlation between shoot length and root length was identified and it clearly indicated that increase in root length helps in increase of shoot length. All the varieties showed common trend i.e. reduction rate in shoot length with increasing concentration of PEG. The decline in shoot length traits in response to induced osmotic stress is a commonly observed phenomenon which is depends on the tolerance capacity of the plant. Decreasing in growth rate with increasing osmotic stress was reported in several studies (Waseem *et al.*, 2006; Abdel- Raheem *et al.*, 2007; Aazami *et al.*, 2010). Higher MSI can be in generally considered as drought tolerant. Our results are in agreement with the findings of Sairam and Shrivastava, (2001) who reported that during stress there was a decrease in MSI irrespective of the genotypes. Geravandi *et al.*, (2011) demonstrated that drought tolerant genotypes contained higher MSI as compared to drought sensitive genotypes. The germplasm which is showing better performance can be considered as drought tolerant. Hence, germplasm with the capability of early vigour under stress conditions may be beneficial by increasing seedling competitiveness against weeds (Lemerle *et al.*, 2001). The early vigour of seedling with good development can be used as a trait of interest for the selection of tolerant germplasm (Richards, 2000; Botwright *et al.*, 2002). Root system with the ability of better growth under (Abdel-Raheem *et al.*, 2007). Siddique *et al.*, (1990) explained that plants with better early vigour can increase the crop water use efficiency. Contradictory results have been reported for activities of antioxidant enzymes in number of different plant species. These variations in antioxidant enzymes induced by stress not only depend on severity and duration of the stress treatment and also depend on species and age of the plant (Carvalho, 2008). In our report, we observed that the activities of Catalase decreased by increasing the PEG concentration, whereas increase in SOD activity were observed in both progressive stresses induced by PEG as compare to the control. An increase in SOD activity and decrease in CAT activity was also reported during drought stress in Liquorice (Pan *et al.*, 2006). It was reported that SOD as well as CAT activities increases in response to PEG induced drought stress in gerbera and Sesame (Lai *et al.*, 2007; Fazeli *et al.*, 2007). Decreased activity of SOD and CAT was reported in wheat subjected to long term field drought as well as PEG induced water deficit in wheat (Simova-Stoilova, *et al.*, 2007, Abdul *et al.*, 2017). Plants are well endowed with antioxidant molecules and scavenging systems which establish a link between tolerance to water stress and rise in antioxidant enzyme concentration in photosynthetic plants.

CONCLUSION

Drought is a foremost stress which decreases the production of crops worldwide (Iqbal *et al.*, 1999; Yang *et al.*, 2004). The problem is particularly very serious in arid and semi-arid regions (Ashraf *et al.*, 1995), where many developing and under developed countries are located. Pearl millet is the fourth most important cereal crop in India, after rice, wheat and sorghum, where it is widely grown in the states of Rajasthan, Maharashtra, Gujarat and Haryana where the food security of the poorest population depends vastly on pearl millet production. As in Rajasthan drought condition are very prevalent, so by our studies on “Effect of PEG induced water stress on different varieties of Pearl millet” we find out that which variety can grow best in such an drought condition. KBH 108 is found to be most stable crop that can withstand drought condition and will be best suited to be grown in Rajasthan followed by RHB 177 and MH 2024.

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