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Effect of Polyethylene Glycol Induced Water Stress on Seedling Development of Lentil (*Lens culinaris* Medik.) Genotypes

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Abstract The present study was carried out in order to study the effect of PEG-6000 induced water stress on early seedling growth traits of lentil in the laboratory of the Department of Genetics and Plant Breeding, BCKV, West Bengal. India. Five lentil genotypes viz., WBL-77, WBL-81, Ranjan. Asha and Hul-57 were tested for drought tolerance under nine different external water potential viz., 0, -2, -3, -4, -5, -6, -7, -8, -9 bar using PEG-6000 as osmoticum. The experiment was carrried out in three replication under asymmetrical factorial completely randomized design and data on various seedling growth traits like root length, shoot length, root fresh weight, shoot fresh weight, root dry weight and shoot dry weight was recorded after ten days. A noteworthy reduction was noticed in all the studied characters due to imposition of drought stress, however the reduction was in a progressive manner to that of the external water potential imposed by PEG-6000. A positive correlation was observed between shoot length and root length at all stress conditions.

Keywords Lentil, Drought, PEG, Seedling traits.

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Introduction

The available literature and observations clearly indicated that "climate change" has a negative impact on agriculture. Out of the many abiotic stress drought has been considered as the most devastating one affecting the plant growth. Taking into consideration global water scarcity and increases in demand for non-agricultural uses of water, expansion of the area under irrigation in developing countries does not appear to be a realistic scenario to address the challenge of food security. Therefore, food security in the twenty-first century will rely increasingly on the release of cultivars with improved potentiality to combat drought and with high yield stability [1].

Lentils are rich sources of protein, folic acid, dietary fiber, vitamin C, B vitamins, essential amino acids like lysine and arginine and trace minerals like iron, cobalt and iodine [2]. In the Indian subcontinent, lentil is primarily grown in the post-rainy winter season on receding soil moisture. The available soil water during post-rainy season is about 200-250 mm, which is bare minimum to meet the normal evapotranspiration. Thus, the crop invariable suffers from moisture stress of varying degrees during different stages of growth.

Identification of lentil genotypes that can withstand inadequate water condition is vital to increase the crop production and this can be accomplished only by exploring the drought tolerant gramplasm of lentil. Various methods have been employed from time to time to identify drought tolerant genotypes. According to Botwright et al. [3], early vigor of seedling with good development can be used as a trait of interest for the selection of tolerant germplasm [3]. A positive correlation between drought tolerance of the genotypes in the field and in laboratory experiments has been reported earlier [4]. Because of the potential of maintaining uniform water potential during the experimental period PEG has been used often as drought stress inducer in many studies to screen drought tolerant germplasm [5]. Current study was planned to understand the effect of different PEG-6000 concentrations on different seedling traits of lentil.

Materials and Methods

The seed materials of five lentil genotypes viz., WBL-77, WBL-81, Ranjan, Asha and Hul-57 used in the experiment were collected from AICRP on MULLaRP (Mung bean, Urd bean, Lentil, Lathyrus, Rajma and Peas), Kalyani center, BCKV. The experiment was carried out in three replicates under asymmetrical factorial completely randomized design. PEG was used for inducing the water stress that was experienced by roots. The solutions of different water potential were prepared by using PEG-6000 following the method described by Michel and Kaufmann [6]. The formula for osmotic potential (Ψs) given by them is as follows.

$$(\Psi s) = -(1.18 \times 10^{-2})\, C - (1.18 \times 10^{-4})\, C^2 + (2.67 \times 10^{-4})\, C\, T + (8.39 \times 10^{-7})\, C^2 T$$

Where,

Ψs = Osmotic potential (in bars),
C = Concentration of PEG-6000 (g kg^{-1} H_2O)
T = Temperature (°C)

Following the above equation desired solution of Ψs could be prepared . Table 1 presented below was used to prepare osmotic solutions having different Ψs.

Healthy, viable seeds of each lentil genotype were surface sterilized by immersing the seeds in 70% ethanol for 2 minutes followed by thorough washing with distilled water. Twelve seeds of a genotype were arranged in a row with even space over a glass plate (20 cm × 30cm) wrapped with a blotting paper. To prevent the seeds from sliding down when the set was kept in a slant position in a stand, another glass strip (20 cm × 2 cm) was placed over the seeds with the help of a piece of thermocol at the two ends and guarder in such a way that the seeds remained in their position and the seedlings grew without any hindrance. The whole set was then placed in a transparent polythene bag. There were 27 such sets for each genotypes representing three replications for each individual water potential. In each plate, PEG solution of desired water potential was used as germinating medium. The seeds were then allowed to germinate and grow for 10 days under indoor laboratory condition under sufficient light, 70—80% relative humidity and at a temperature range of 20—25°C. Data from six randomly selected competitive seedlings on the different growth parameters viz., root length, shoot length, root fresh weight, shoot fresh weight, root dry weight and shoot dry weight were recorded from 10 days old seedling from each plate.

Results and Discussion

Root length

Roots are the primarily affected plant part under drought conditions than any other parts [7]. The effect of osmotic stress induced by the PEG on the root length of five lentil genotypes is presented in Fig. 1. A strong negative correlation was noted in between PEG concentration and root length. The cor-

Table 1. Osmotic potential of polyetylene glycol (PEG) 6000 solutions at 25 °C.

Osmotic potential (bars)	0	-1.0	-2.0	-3.0	-4.0	-5.0	-6.0	-7.0	-8.0	-9.0
Concentratation of PEG 6000 (g/kg H_2O)	0	78	119	151	178	202	224	243	262	279

relation coefficient values calculated for Hul-57 (*r*=0.898), Ranjan (*r*=-0.912), WBL-77 (*r*=-0.950), WBL-81 (*r*=-0.959) and Asha (*r*=-0.905). A gradual reduction in root length with an increasing concentration of PEG was the common tendency observed among all genotypes which is in concordance with

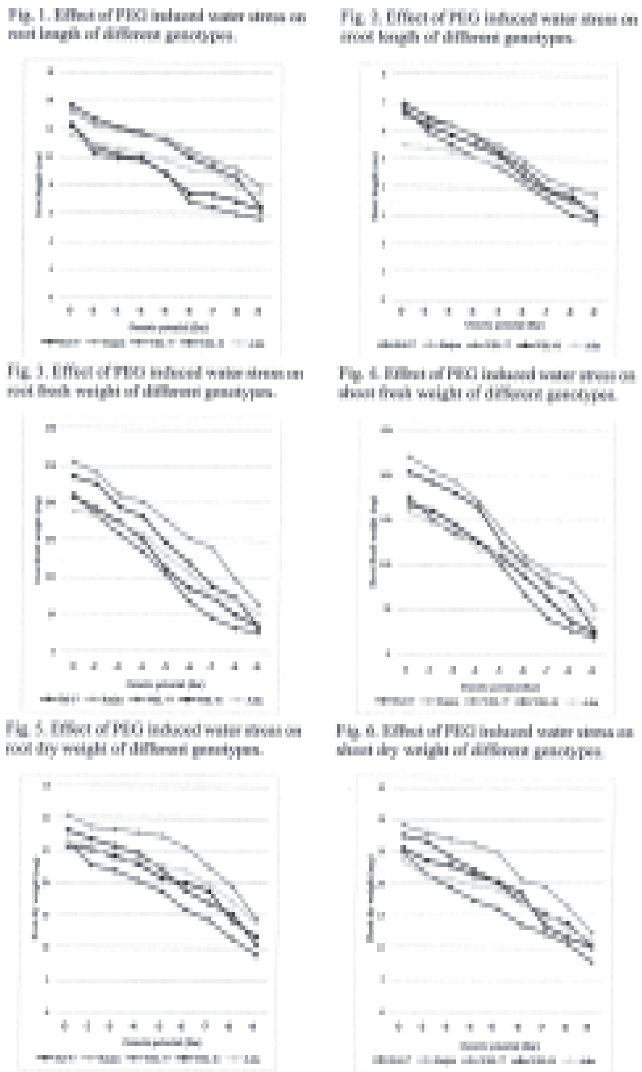

Fig. 1. Effct of PEG induced water stress on root length of different genotypes. **Fig. 2.** Effect of PEG induced water stress on root length of different genotypes. **Fig. 3.** Effect of PEG induced water stress on root fresh weight of different genotypes. **Fig. 4.** Effect of PEG induced water stress on shoot fresh weight of different genotypes. **Fig. 5.** Effect of PEG induced water stress on root dry weight of different genotypes. **Fig. 6**. Effect of PEG induced water stress on shoot dry weight of different genotypes.

the earlier report [8]. However the reduction rate in root is different in the genotypes investigated, which may be due to their differential genetic make-up. Considering the critical difference (CD) value, significant difference was observed in root length among all genotypes except between WBL-77 and WBL-81. Among all the lentil genotypes examined at -2 bar osmotic stress, significant difference has been observed among all genotypes except WBL-81 and Asha. At -3, -4 and -5 bar osmotic stress significant difference was observed between all varieties except between Hul-57 and Ranjan and WBL-77 and WBL-81. At -6, -7, -8 bar water potential all genotypes revealed significantly difference among them. Similarly at -9 bar water potential all genotypes revealed significantly difference among them except Hul-57 and WBL-81.

Kulkarni and Deshpande [9] reported that early rapid elongation of roots is a key trait of drought tolerance. According to Abdel-Raheem et al. [10] root system with the ability of better growth under stress conditions can be considered as tolerant germplasm . In the current study the genotype Ranjan exhibited higher root length than other four genotypes at stress conditions and also revealed lower rate of reduction than other, so it may be taken as tolerant one. In the other hand the genotypes WBL-77 revealed lower root length in most of the stressed conditions as well as higher rate of reduction, so this may be taken as drought susceptible one.

Shoot length

The effect of osmotic stress induced by the PEG on the shoot length of five lentil genotypes is presented in Fig. 2. All the genotypes revealed strong negative correlation between shoot length and PEG concentration. The correlation coefficient values calculated for Hul-57 (*r*=-0.917), Ranjan (*r*=0.888), WBL-77 (*r*=0.960), WBL-81 (*r*=0.925) and Asha (*r*=0.863). A positive correlation between shoot length and root length has been identified and it clearly indicated that increase in root length helps to increase in shoot length. All the varieties witnessed a reduction in shoot length with increasing concentration of PEG, which was previously reported in several studies [9, 10,11]. The variable reduction rate of shoot in different genotypes in response to induced osmatic stress is a commonly observed phenomenon which may be due to the different tolerant capacity of the plant.

Comparison was carried out between varieties at different stress conditions. In control condition, a significant difference has been found between HUL-57 and Ranjan and Asha and other four genotypes. At -2 bar water potential a significant difference was found between HUL-57 and WBL-77, Ranjan and WBL-77 and Asha and other four genotypes. Both WBL-77 and Asha exhibited significant difference from other 3 genotypes at-3, -4 and -5 bar water potential. At -6 bar water potential significant difference was observed between WBL-77 and HUL-57, WBL-77 and Ranjan, Asha and HUL-57, Asha and Ranjan and WBL-81 and Ranjan. At -7 bar water potential Ranjan was found to be significantly different from other 4 genotypes. At-8 and -9 bar water potential, WBL-77 revealed a very low mean value and it was also significantly different from other 4 genotypes. In almost all stress conditions Ranjan exhibited higher mean value, while WBL-77 witnessed the lower mean value. Interestingly similar observation was also reported in case of root length.

Root fresh weight

The effect of osmotic stress induced by the PEG on the root fresh weight of five lentil genotypes is presented in Fig. 3. A negative correlation was observed root fresh weight and PEG concentration for all five genotypes. The correlation coefficient values calculated for Hul-57 (*r*=-0.907), Ranjan (*r*=0.902), WBL-77 (*r*=-0.945), WBL-77 (*r*=-0.945), WBL-81 (*r*=-0.925) and Asha (*r*=-0.886), Under 0, -2, -3, -4, -5, -6 and -8 bar water potential all five genotypes were found to be significantly different from each other. However at -7 bar a significant different was observed between all genotypes except between WBL-81 and Asha. Similarly at -9 bar water potential a significant difference was observed between all genotypes except between HUL-57 and WBL-81. Out of the five genotypes, Ranjan performed better while WBL-77 exhibited lower mean value under most of the conditions. Decline in root fresh weight due to imposition of water stress is a common phenomenon observed in all genotype, which has been earlier reported [12].

Shoot fresh weight

Fig. 4 represents the effect of osmotic stress induced by the PEG on the shoot fresh weight of five lentil genotypes. In all the five genotypes a negative correlation was found between shoot fresh weight and PEG concentration. The correlation coefficient values calculated for Hul-57 (*r*=0.896), Ranjan (*r*=-0.915), WBL-77 (*r*=-0.927), WBL-81 (*r*=-0.898) and Asha (*r*=-0.895). A significant difference was observed between all the genotypes at 0, -2, -3, -5, -6, -7, -8, -9 bar water potential, while at -4 bar water potential all genotypes revealed significant difference between them except WBL-77 and WBL-81. The same two genotypes viz., Ranjan and WBL-77 exhibiting higher and lower mean value respectively for root fresh weight also revealed the same result for shoot fresh weight.

Root dry weight

The effect of osmotic stress induced by the PEG on the root dry weight of five lentil genotypes is presented in Fig.5. In all the five genotypes a negetive correlation was found between root dry weight and PEG concentration. The correlation coefficient values calculated for Hul-57 (r=-0.878), Ranjan (r=-0.809), WBL-77 (r=0.933), WBL-81 (r=-0.849) and Asha (r=-0.0844). Decrease in root dry weight because of water stress has been reported earlier [13]. At control condition significant difference was noted among all varieties except between WBL-77 and Asha. At -2 bar osmotic stress significant difference was observed among all genotypes except between HUL-57 and Asha. In the rest of solutions viz., -3, -4, -5, -6, -7, -8, -9 bar water potential all the genotypes revealed significant difference among themselves. Here also the genotype Ranjan performed better while the performance of the genotypes WBL-77 was poorer under different stress conditions.

Shoot dry weight

The effect of osmotic stress induced by the PEG on the root fresh weight of five lentil genotypes is presented in Fig.6. In all the five genotypes a negative correlation was found between root dry weight and PEG concentration. These results were in accordance with those reported earlier [14]. The correlation coefficient values calculated for Hul-57 (*r*=-0.912), Ranjan (*r*=-0.854), WBL-77 (*r*=-0.961), WBL-81 (*r*=-0.877) and Asha (*r*=-0.900). Significant difference was observed among all genotypes at 0, -2,-3,-4,-5, -7 and -8 bar water potential. In case of -6 and -9 bar water potential significant difference was observed among all genotypes except between HUL-57 and Asha and HUL-57 and WBL-81 respectively. Out of the five genotypes Ranjan performed better while WBL-77 performed poorer under most of the stress condition.

Conclusion

Water stress has been considered as one of the most devastating abiotic stress affecting the plant growth and productivity. Development of new varieties is one of the ultimate methods to overcome the problem associated with the drought stress. The development of new varieties could be assisted by screening of germplasm for higher drought tolerance. The present study was planned to standardize the screening procedure as well as to identify the tolerant genotypes that can be used for future breeding program. All the five genotypes revealed strong negative correlation between PEG concentration and all the studied characters. The genotype Ranjan showed better growth than others stress condition, so this may be taken as the tolerant one, while the genotype WBL-77 had a very poor performance under different water stress condition, so this may be taken as the susceptible one. The results highlight the importance of the PEG as an artifical stress inducer for quick screening in the laboratory conditions for identification of drought tolerant germplasm for breeding programs in lentil.

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