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VARIETAL DIFFERENCES AGAINST PEG INDUCED DROUGHT STRESS IN COWPEA

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Abstract: The effect of PEG-4000 induced water stress on germination parameters was studied. Total 5 cowpea varieties (BL-1, BL-2, EC-4216, Kohinoor and Local) were tested for drought tolerance using 0, -0.075, 0.1 and -0.2 MPa osmotic stress and the data was recorded on various seedling parameters like germination energy, mean daily germination, seedling vigor index, seedling distribution and seedling density. The experiment was carried out in three replicates under Complete Randomized Design. Reduction in germination parameters was observed in all varieties at highest level (-0.2 MPa) of PEG solution. All germination parameters of seedlings which were study in present investigation were more negatively affected in case of Kohinoor where as less negatively affected in case of BL-2 when compared with control. Based on the observations it was concluded that verity BL-2 had shown better tolerances under limiting water conditions among the varieties studied.
Keyword: Cowpea, Germination parameters, PEG-4000, Water stress.
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INTRODUCTION

Abiotic stresses play a major role in determining crop and forage productivity (Rao, 2013) and also affects the differential distribution of the plant species across different types of environments (Chaves *et al.,* 2003). Climate change exacerbates abiotic stress on a global scale, with increased irregularity and unpredictability and as a result, adaptation strategies need to be developed to target crops to specific environments (Beebe *et al.,* 2011). Drought is one of the major limiting factors in crop production; it can affect various morphological and physiological traits associated with plant growth and development (Dulai *et al.,* 2006). Furthermore, it has reported that the availability of water for agriculture purpose reduced day by day; and under present scenario it became limited in various agriculture zone of world (Carter, 1989). Legumes (*Leguminosae* or *Fabaceae*) belong to the second most important plant family in agriculture after the *Poaceae* or grass family. Cowpea (*Vigna unguiclata* (L.) Wap) is an legumes crops which widely grown in all the arid and semi-arid regions of the world because it has ability to grow under serve water stress conditions and maintained a fix amount of water inside the cell (Kumar *et al*., 2008). They provide the largest single source of vegetable protein in human diets and livestock feed (forages) and contribute to agriculture, the environment and human health (reviewed in Dita *et al*., 2006). In the balance water in plant will be happen when is absorption water from roots equal the water loss from stomata and water potential is negative (Stikic and Davies, 2000). Various methods have been employed from time to time to identify drought tolerant varieties and efforts have been made in the past to screen cowpea varieties which differed in drought tolerance (George *et al.,* 2013). PEG is a polymer and considered as better chemical than others to induce water stress artificially (Kaur *et al.,* 1998). Many studied Polyethylene glycol (PEG) compounds used to induce artificial water stress in petri dish (*in vitro*) for plants to maintain uniform water potential and screen drought tolerance varieties (Ahmad *et al.,* 2013; Jatoi *et al.,* 2014). The upsurge in concentration of PEG caused a decrease in germination percentage, seedling vigour in certain crop plants (Khodarahmpour, 2011). Several reports have shown that in vitro screening technique using PEG is one of the dependable approaches for the selection of desirable genotypes to study in detail on water scarcity on plant germination indices (Kocheva *et al.*, 2003). The aim of this study was simple and quick screening of cowpea varieties that have higher tolerance to drought. To achieve this we intend to understand the effect of different PEG-4000 concentrations on germination energy, seedling vigor index, mean daily germination, seedling distribution and seedling density of selective cowpea varieties.

EXPERIMENTAL

The screening experiments were planned with completely randomized design (CRD) with three replications. The varieties used in the present study were obtained from Indian Grassland and Forage Research Institute (IGFRI), Jhansi, UP, India. The names of the varieties are BL-1, Bl-2, EC-4216, Kohinoor and Local. Seeds were disinfected with 0.1% $HgCl_2$ for 3-5 minutes with gentle shaking at room temperature. After the surface sterilization the seeds were washed with autoclaved distilled water or until traces of sodium hypochlorite got removed. Ten seeds from each variety were germinated on two layers of filter paper in 9-cm Petri dishes with respective PEG treatment. Seeds of five varieties were incubated at 25±2ºC at different stress level of PEG-4000 *viz.* 0 (control), -0.075, -0.1 and -0.2 MPa. The Petri plates were covered to avoid the loss of moisture by evaporation under laboratory condition (25±2°C) for 10 days. The experiment was terminated by harvesting seedlings after 10 days and germination energy, mean daily germination, seedling vigor index, seedling distribution and seedling density was calculated by using the following formulae:

Germination energy (GE) = n/N x 100 (Guo *et al.,* 2013).
Where n= Number of germination of seed in 4 days and N= Total number of seeds
Mean Daily germination (MDG) = Total Geminated Seeds/Days of Total Germination of Seeds (Kafi and Goldani, 2001).
Seedling Vigor Index (SVI) = Seedling Length x Geminated Seeds Percentage/ 100 (Abdul-Baki and Anderson, 1970).
Seedling distribution = Fresh weight/ Length
Seedling density = Dry weight/Length (Arduini *et al.*, 1994)
The Statistical analysis was carried out using ANOVA was carried out to test the variation at 0.05 significance.

RESULTS AND DISCUSSION

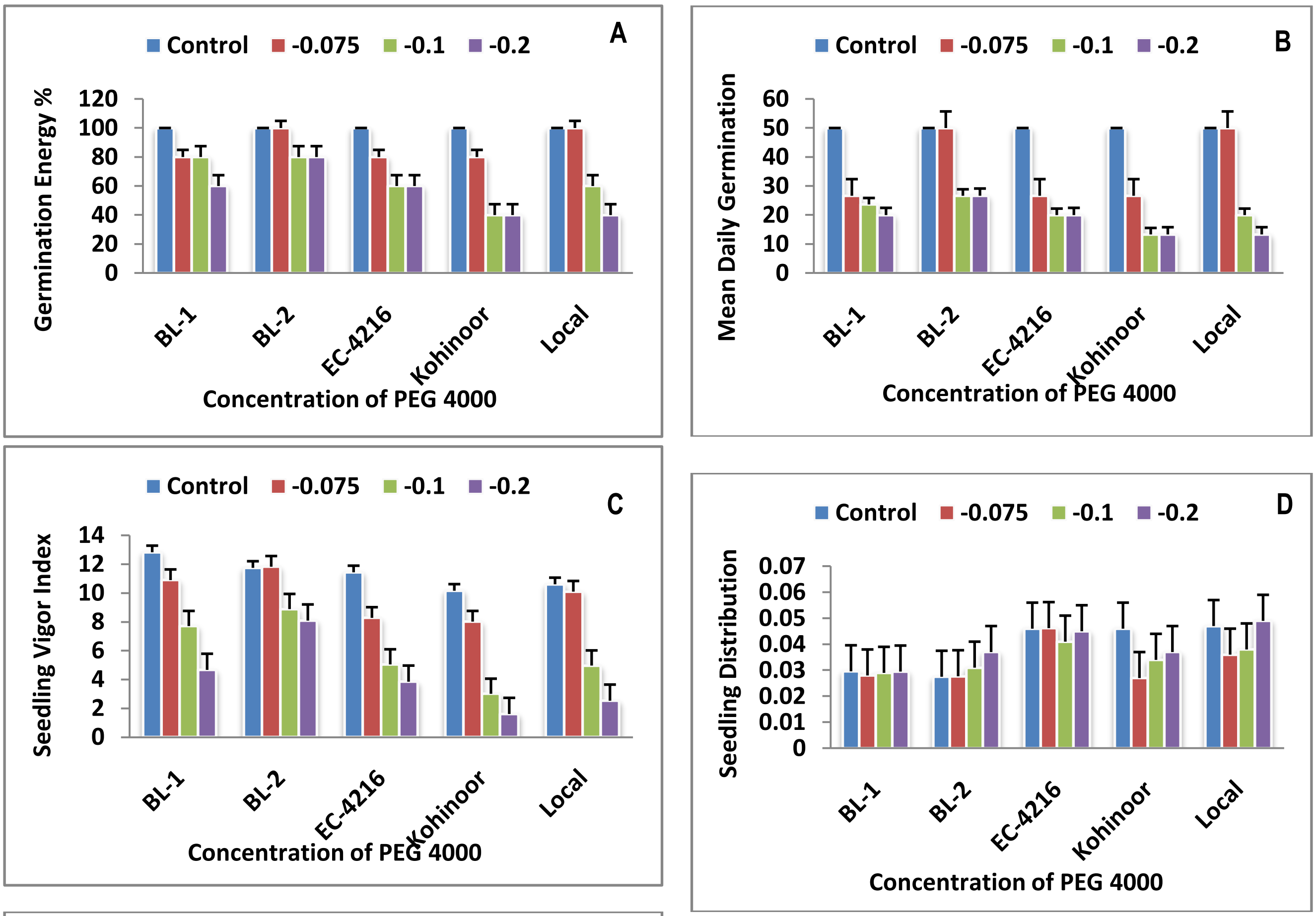
Germination Energy (GE): The effect of osmotic stress induced by the Poly Ethylene Glycol (PEG) on the germination energy of BL-1, BL-2, EC-4216, Kohinoor and Local varieties is presented in Figure 1. Seed germination and seedling development in lab conditions have been recognized as testing procedure in cowpea and it is well known that with the increase in PEG concentrations, the germination energy got decreased (Figure 1). The highest germination energy 100 % was recorded at control. The lowest negative impact on GE (80%) was recorded in BL-2 at maximum stress level *i.e*. -0.2 MPa. Further, Kohinoor and Local suffered maximum decreased in GE (40%) at this level (Figure 1a).

Mean Daily Germination (MDG): All the verities subjected to stress under gone consistent decreased in mean daily germination (MDG) as level of osmotic stress progressed. MDG was 50 under control. Reduction in MDG 13.33 was observed in Kohinoor and local varieties, least reduction in BL-2 (26.66) at highest osmotic level (Figure 1b).

Seedling Vigor Index (SVI): Seedling vigor index decreased significantly with increasing osmotic stress. The highest seedling vigor index under PEG (-0.2 MPa) was related to cultivars BL-2 with 10.1, and lowest value was related to Kohinoor with 4 (Figure 1c).

Seedling Distribution: The seedling distribution of BL-2 and local varieties were increased and Bl-1, Kohinoor and EC-4216 were decreased with increasing PEG concentration, It was calculated that the seedling distribution maximum increased 34.54 percent in BL-2 and the maximum decreased Kohinoor *i.e.* 19.56 percent respect to control at highest level (-0.2 MPa) stress (Figure 1d).

Seedling Density: Seedling density was increased in all varieties but in Kohinoor seedling density was decreased with increasing PEG concentration. Seedling density in BL-2 *i.e.* 0.0064, 36.17 percent maximum increased and in Kohinoor i.e., 0.0034, 10.52 percent maximum reduction respect to control (Figure 1e).



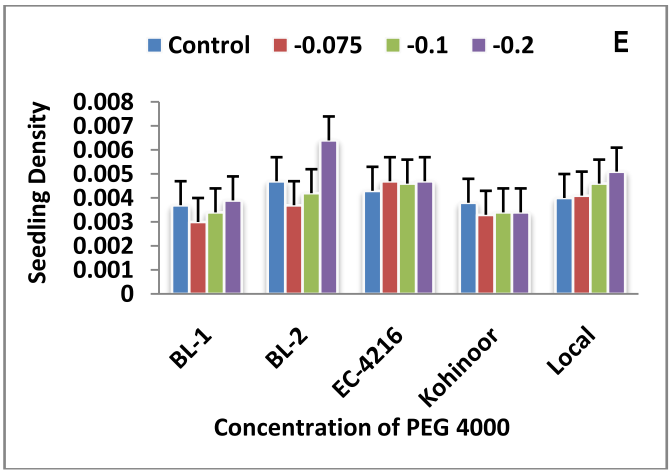


Figure 1: a) Germination energy; b) Mean daily germination; c) Seedling vigor index; d) Seedling distribution; e) Seedling density at different levels of PEG stress.
Bars represent standard (± S.E) error of mean.

Abiotic pressures like drought stress can impose limitations on crop productivity thus highlighting a greater need for understanding how plants respond to adverse conditions with the hope of improving tolerance of plants to environmental stress (Joseph *et al.,* 2010). Availability of water is one of the most important factors, which determine geographical distribution and productivity of plants (Bartels, 2001). Water stress is perceived as water deficit and can occur with different severity (Ramanjulu and Bartels, 2002). Hence, water stress is a very important limiting factor at the initial phase of plant growth and establishment (Shao *et al.,* 2008). In order to define drought stress tolerance or sensitivity of five varieties, growth parameters like germination energy, mean daily germination, seedling vigor index, seedling distribution and seedling density were tested under the effect of -0.075, -0.1 and -0.2 MPa osmotic stress. The current results showed that drought stress in cowpea plants generally caused a noticeable reduction in almost all growth criteria of cowpea cultivars Kohinoor (drought sensitive) and BL-2 (drought tolerant). Dhandas *et al.* (2004) indicated that seed vigor index is among the other germination traits are most sensitive to drought stress. So this has high sensitivity, the greatest decrease in comparison with other traits during stress. Ries and Everson (1973) reported that seed size is positively correlated with seed vigor. There was significant reduction in germination energy with an increase in PEG concentration. These results are consistent with the work of Sani *et al.,* 2014 and Sharon and Mehta, 2015 who described that high dosages of PEG delay germination processes. The results indicated that, PEG induced water stress caused noticeable decreases in seedling density and seedling distribution of cultivars. Since seedling density relates dry mass production to the unit seedling length and seedling distribution represents the fresh mass accumulated per the unit seedling length, the reduction in both density and distribution of cowpea seedling may reflect the effect of drought on decreasing fresh and dry masses. In this respect, Chopart *et al.* (2008) stated that evaluation of seedling density and distribution could be considered as a key factor for water and nutrient uptake by a plant in soil.

CONCLUSION

The development of new varieties could be assisted by screening of verity for higher drought tolerance. The present study was planned to standardize the screening procedure as well as to identify the better varieties that can be useful to scientific community. All the varieties showed strong negative correlation between PEG induced water stress. It was concluded from this work that the plant growth is significantly affected by water stress in cowpea cultivars in comparison to non-drought conditions. According to these results, Kohinoor would be classified as a susceptible and BL-2 tolerance to drought stress, in comparison to rest of the varieties. The results highlight the importance of the PEG as an artificial stress inducer for quick screening in the laboratory conditions for identification of drought tolerant varieties for breeding programs in cowpea.

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