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National Academy of Agricultural Science (NAAS) Rating : 3. 03

Impact of Osmotic Stress on Seed Germination and Seedling Growth in Mungbean (*Vigna radiata L. Wilczek*)

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Abstract: *Screening of drought was undertaken investigate the effect of water stress on thirtytwo different accessions of mungbean (Vignaradiata L. Wilczek) and its physiological responses to drought tolerance at seedling stage. Water stress was simulated by non-ionic water soluble polymer polyethylene glycol of molecular weight 6000. After fourteen days, data were recorded for easily measurable seedling traits as shoot length,root length, fresh shoot weight, dry shoot weight, fresh root weight and dry root weight under control as well as water stress conditions. Significant differences were observed among the accessions, treatments and their interactions for evaluated plant traits suggesting a great amount of variability for drought tolerance in mungbean. Differential sensitivity of seedling traits was noted due to water stress created by PEG. However, shoot related traits were the most sensitive against the water stress.*

Keywords: *Vigna radiata L. Wilczek., Polyethylene glycol (PEG), water stress, seedling traits.*

INTRODUCTION

Mungbean [*Vigna radiata* (L.) Wilczek] is an important pulse crop of global economic importance and is the best of all pulses on nutritional point of view. Also it has a certain function of detoxification, increasing appetite, and lowering blood pressure, cancer and other health effects (Yin *et al.*, 2015; Bouchenak and Lamri-Senhadji, 2013). This legume is characterized by a relatively high content of proteins rich in leucine, phenylalanine, lysine, valine, isoleucine and certain vitamins.

Mungbean is reported to be more susceptible to water deficits than many other grain legumes (Sunil Kumar *et al.*, 2015). The successful breeding for drought tolerance is availability of reliable methods for screening of desirable genotypes (Feller 2006). Water potential studies enabled the identification of varieties suitable for growing under moisture stress situations. Varieties that are found to germinate under reduced water potential do not usually fail to germinate and establish into seedlings (Kumar, 2003).

Water stress affects various physiological processes associated with growth, development, and economic yield of a crop (Allahmoradi *et al.*, 2011). Water deficit disturbs normal turgor pressure, and the loss of cell turgidity may stop cell enlargement that causes reduced plant growth (Srivalli *et al.*, 2003). Water stress increases root shoot ratio, thickness of cell walls and amount of cutinization and lignifications (Srivalli *et al.*, 2003). Water is the most important and widelyoperative limiting factor for crop production. Responses of plants to water deficit condition havebeen employed to make a physiological evaluation of drought resistance. Plant abiotic stresses such as drought stress along with the growing world population stress and percapita food consumption threaten stable global foodavailability. Drought or any other abiotic stresses results in reduction of yield and plant growth. They limit the photo synthesis and consequently, limited availability of photo synthetic assimilates and energy to the plant. It is imperative for plants to use this limited supply of nutrients to their maximal advantage to survive understress.

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Apparently, under drought stress conditions, anurgent need for plants would be to increase the uptake of water, which is usually more available deep down in thes oil (Xiong *et al.* 2006). Identification and understandingthe mechanisms of drought tolerance in sorghum have been major goals of plant physiologists and breeders include prolific root system, ability to maintain stomatalopening at low levels of leaf water potential and highosmotic adjustment and various seedling parameters.Water stress affects almost everydevelopmental stage of the plant. However, damaging effects of this stress was more noted when it coincidedwith various growth stages such as germination; seedling shoot length, root length and flowering (Rauf, 2008; Khayatnezhad, *et al.* 2010).

Significant progress has been made in understanding plant growth under drought stress. Water deficit is sensed by the roots which begin to synthesize ABA within 1 hour of the onset of the water stress. ABA is transported via xylem from roots to leaves within minutes to hours. Root length is an important trait against drought stress in plant varieties; in general, variety with longer root growth has resistant ability for drought (Leishman and Westoby, 1994; Kaydan and Yagmur, 2008). Dhanda *et al.* (2004) reported that the osmotic membrane stability of the leaf segment was the most important trait, followed by root-to-shoot ratio and root length on the basis of their relationships with other traits for drought tolerance. These water sensitive stages maybe exploited to discriminate genotypes on the basis of their resistance to water stress.

Among these criticalstages, water stress induced during seedling stage hasbeen exploited in various crop species to screen germplasm or breeders populations *i.e.* Wheat (Dhanda *et al.* 2004), sorghum (Gill *et al.* 2002; Bibi *et al,* 2010), maize (Mohammadkhani and Heidari, 2008; Farsiani and Ghobadi, 2009; Khayatnezhad, 2010) and sunflower (Rauf *et al.* 2008). Rauf (2008) narrated several benefits of screening genotypes at seedling stages such as low cost, ease of handling, less laborious and getting rid of susceptible genotypes at earliest. Furthermore seedling traits have also shown moderate to high heritability with additive type of genetic variance within and over environments (Rauf *et al.* 2008).

Field experiments related to water stress hasbeen difficult to handle due to significant environmental or drought interactions with other abiotic stresses (Rauf, 2008). An alternative approach is to induce water stress through polyethylene glycol (PEG) solutions forscreening of the germplasm (Kulkarni and Deshpande, 2007; Khodarahmpour, 2011; Rajendran *et al.* 2011). Polyethylene glycol glycols withmolecular mass of 6000 and above are non-ionic, watersoluble polymers which are not expected to penetrate intact plant tissues rapidly. This solution interferes withthe roots to absorb water due to reduction of osmotic potential (Dodd and Donovan, 1999; Sidari *et al.* 2008). An artificially created water-stress environment is used to provide the opportunity in selecting superiorgenotypes out of a large population. On the basis of thesegrounds, experiment was carried out to categorize mungbean germplasm against drought stress; to select suitable accessions for drought tolerance and also to determine the suitability of various seedling traits for selection of tolerant or susceptible genotypes to drought stress.

MATERIALS AND METHODS

For this study, thirty two genotypes of mungbean were collected from various ecological zones of India and were assessed for drought tolerance using PEG 6000. The genotypes were placed in the moistened germination paper according to Bayoumi *et al.* (2008) to provide appropriate moisture stress for seed germination. When seedlings were at stage of first true leaf initiation (12 days after treatment) data were recorded at four different moisture levels on germination percentage, root length, shoot length, root fresh weight, root dry weight, shoot fresh weight, shoot dry weight, relative water content, vigour index and tolerance index.

Action of PEG 6000

Polyethylene glycol molecules with a M. Wt $\geq$ 6000 (PEG 6000) are inert, non ionic and virtually impermeable chains that have frequently been used to induce water stress and maintain a uniform water potential throughout the experimental period (Hohl and Schopfer, 1991; Lu and Neumann, 1998). Molecules of PEG 6000 are small enough to influence the osmotic potential, but large enough to not be absorbed by plants (Carpita *et al.*, 1979; Saint-Clair, 1976). Because PEG does not enter the apoplast, water is withdrawn from the cell. Therefore, PEG solution mimic dry soil more closely than solutions of low molecular osmotica, which infiltrate the cell wall with solutes (Veslues *et al.*, 1998). Chemical mutagens namely Ethyl Methane Sulphonate (EMS) and colchicine were used for inducing mutation.

Drought stress at was imposed through three concentrations of osmotic potentials of 0 (as control), –0.3, –0.5, and –0.7 MPa using PEG-6000 (Polyethylene Glycol –6000 mw) following the method of Michel and Kaufmann (1973) before the start of the experiment.

S. No.	Concentration (MPa)	PEG 6000 in 1000 ml of distilled water (g)
1.	Control (0)	–
2.	–0.3	115
3.	–0.5	196
4.	–0.7	235

Laboratory Experiment

Solution of PEG 6000 corresponding to the Control and the three concentrations was prepared in 1000 ml distilled water. Ten seeds were selected from each genotype which was uniform in size and shape. The selected seeds of the thirty two genotypes were rolled in germination paper for the various concentrations and rolled to form a paper towel according to Bayoumi *et al.*, (2008) to provide appropriate moisture stress for seed germination. The seeds in the rolled germination paper were tightly held by using rubber bands at the either end. The concentrations of control and the PEG 6000 (–0.3, –0.5, and –0.7 MPa) were prepared and poured into buckets upto 2 inches. Then the paper towels for the various concentrations were marked and placed in the respective PEG 6000 concentrations at room temperature (26 ± 2°C). When seedlings were at stage of first true leaf initiation (12 days after treatment) data were recorded at Control and three different moisture levels. Five plants of each accession from each replication and treatment were evaluated for shoot length (SL), root length (RL), fresh shoot weight (FSW), dry shoot weight (DSW), fresh root weight (FRW), and dry root weight (DRW), vigour index and tolerance index. Length based traits were measured with measuring tape after carefully uprooting the seedling and dissecting into roots and shoot. Fresh shoot or root weight was measured on digital analytical balance whiledry shoot and root weight was measured by puttingshoots and roots in kraft paper bags separately and dried in the oven at 70 °C for constant dry weight (Kaydan and Yagmur, 2008). The average dry shoot and root weight was then calculated.

RESULTS

The rate of germination varied in the genotypes. All genotypes recorded 80 to 100 per cent germination in the control. Water deficiency decreased the germination percentage considerably in all the genotypes (Figure 1). The decrease in water potential gradient between seeds and their

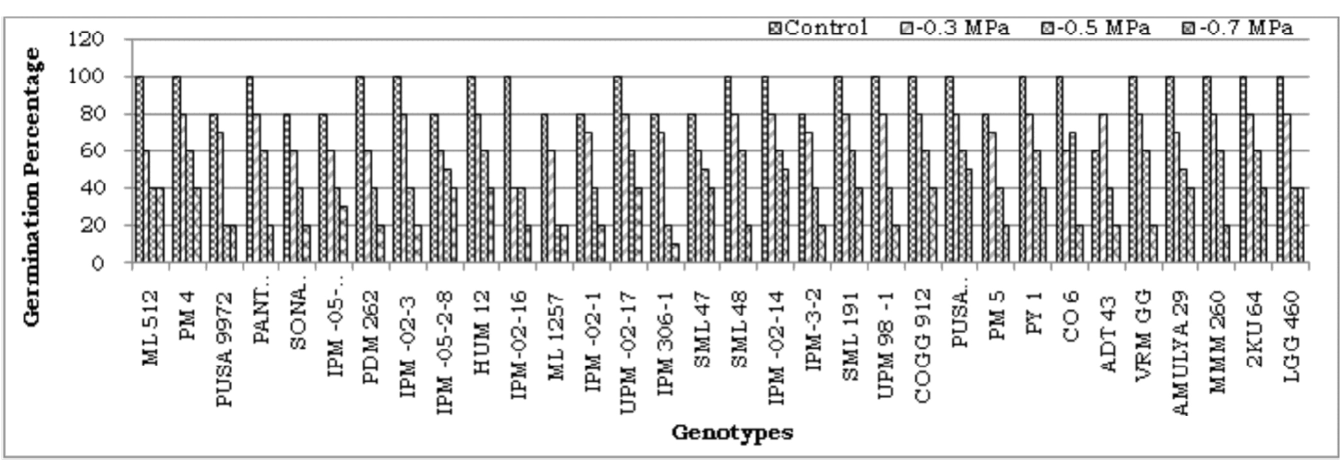

Figure 1: Germination percentage in mungbean exposed to different osmotic potentials induced by PEG 6000

surrounding media by the effects of PEG affects seed germination, due to limited water uptake by the seeds. A decline in germination percentage under increasing moisture stress has been reported in mungbean (Dutta and Bera 2009) and pea (Okcu *et al.* 2005). In the reduced water potential the average germination varied between 40 to 80 per cent in –0.3 MPa, 20 to 70 in –0.6 MPa and 10 to 50 in –0.7 MPa. The genotype IPM 306-1 showed the minimum germination percentage at water potential of –0.7 MPa of PEG, whereas genotypes IPM –02 – 14 and PUSA BOLD 2 recorded germination percentage of 50 percent, thus showing the physiological mean of tolerance to moisture stress.

A great variation is observed in seedling growthbehavior in terms of length and accumulation of fresh and dry weight in tested genotypes under normal condition. In the present study, decrease in the external osmoticpotential caused reduction in seedling growth in all genotypes (Figures 2 to 7). Reduction in shoot length was comparatively more than the root length. Shoot length varied between 6.5 (ML 512) to 13.3 (IPM 306-1) at –0.7 MPa of water potential. All the genotypes recorded reduced shoot growth when compared to the control.

The maximum root length was recorded in IPM -02-14 (12.5 cm) followed by VRM GG (11.8 cm) at –0.7 MPa and the minimum root length was recorded in HUM 12 (6.3 cm) followed by SML 1151. The seedlings of tolerant genotypes had maintained longer root length as compared to susceptible genotype under conditions of water deficit. Maintenance of root growth during water deficit is anobvious benefit to maintain an adequate water supply (O'Toole and Bland 1987). Decreasing water

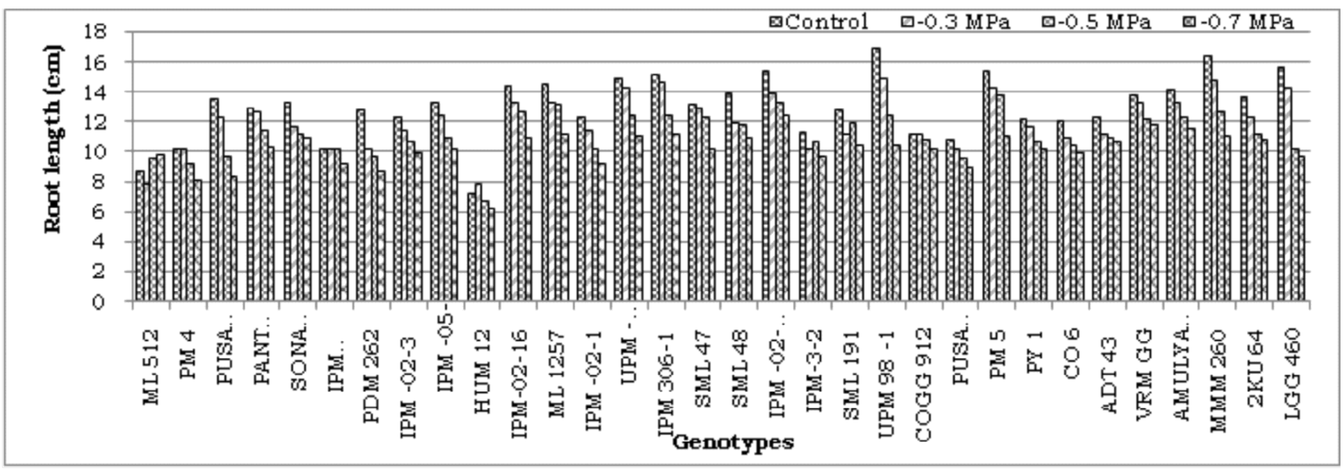

Figure 2: Root length in mungbean exposed to different osmotic potentials induced by PEG 6000

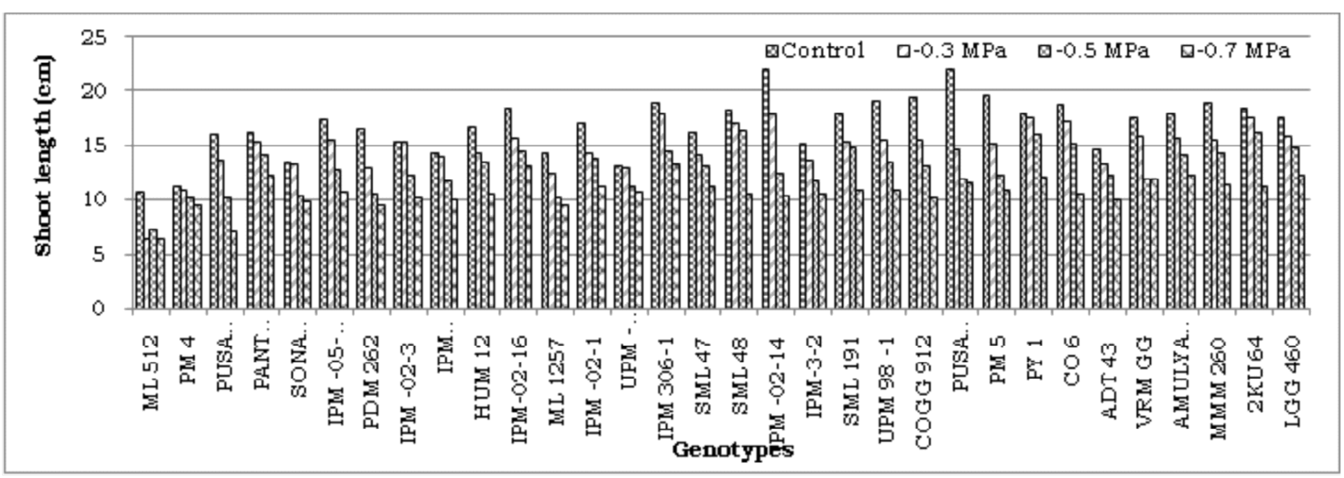

Figure 3: Shoot length in mungbean exposed to different osmotic potentials induced by PEG 6000

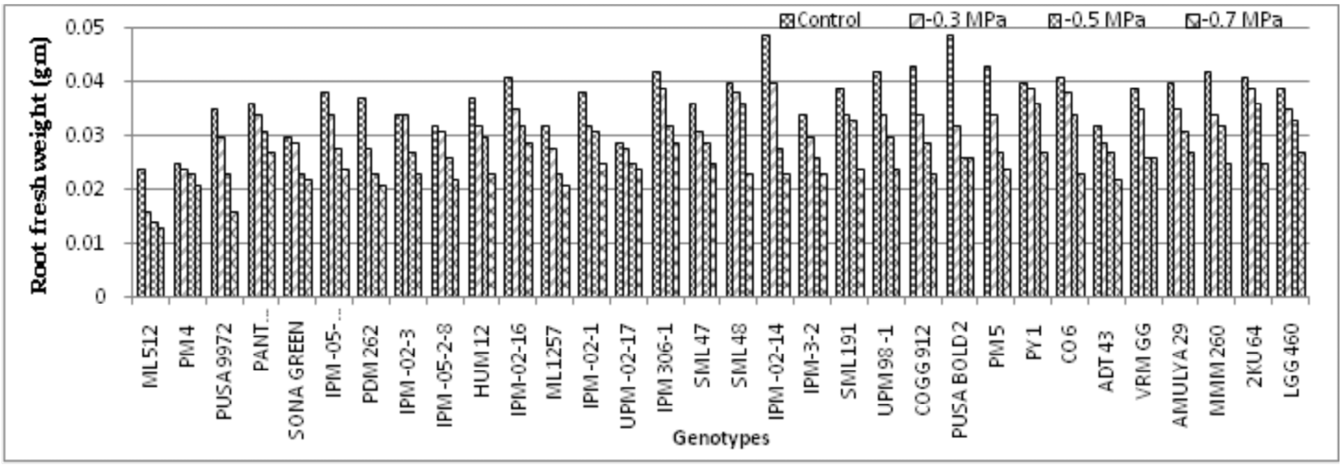

Figure 4: Root fresh weight in mungbean exposed to different osmotic potentials induced by PEG 6000

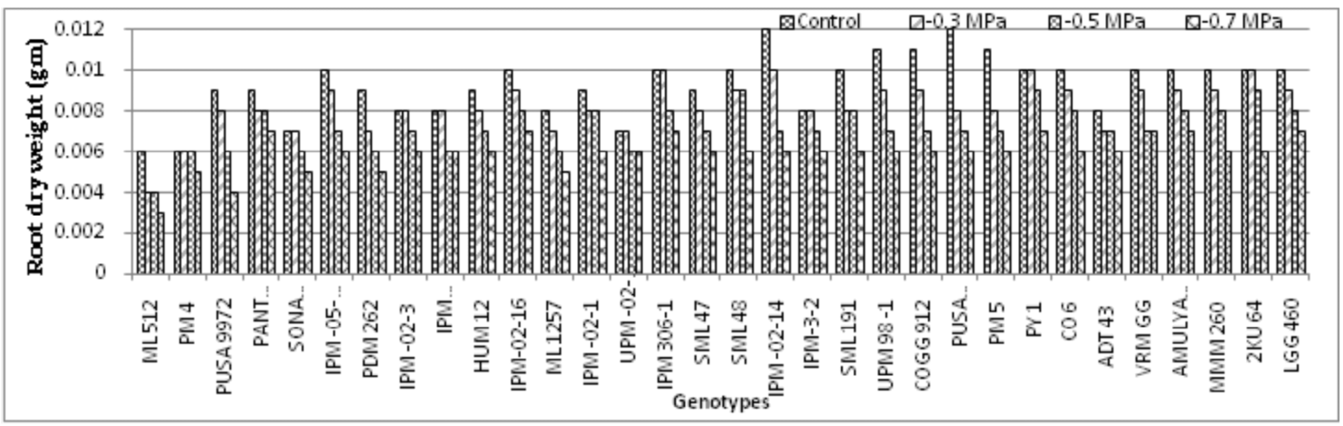

Figure 5: Root dry weight in mungbean exposed to different osmotic potentials induced by PEG 6000

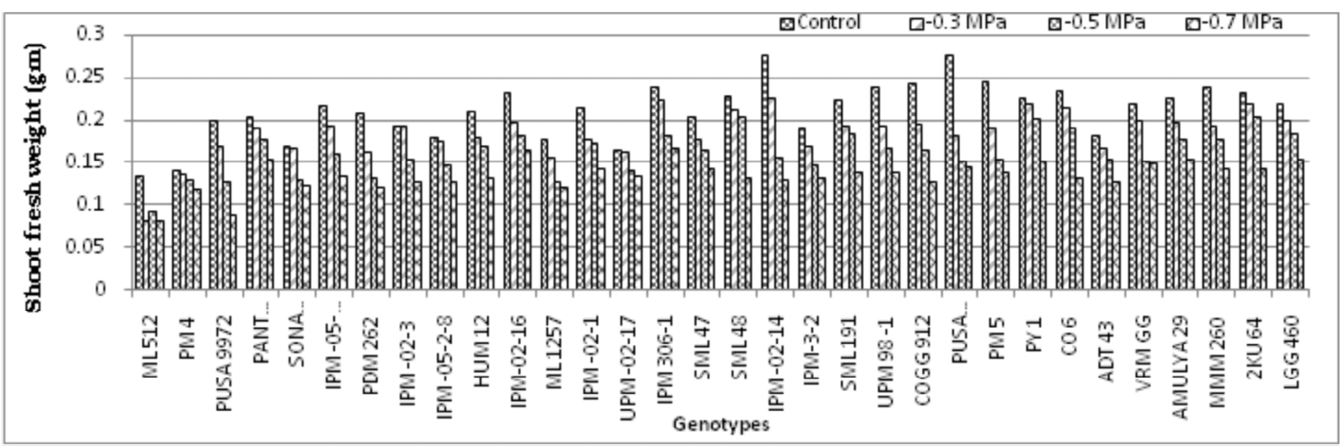

Figure 6: Shoot fresh weight in mungbean exposed to different osmotic potentials induced by PEG 6000

potential by PEG caused are markable reduction in fresh and dry weight of shoot and root (Figures 4 to 7). Significant reduction in seedling growth interms of length, fresh and dry weight of shoot and root among the genotypes might be attributed to their differential response in term of tolerance level to moisture stress. The results are in accordance of Meo, 2000, Bibi *et al.*, 2010; Ali *et al.*, 2011a). Bibi *et al.*, 2010 observed that most of the morphological and physiological characters at seedling stage are affected by water stress in mungbean. Drought stress suppressed shoot growth more than root growth and in certain cases root growth increased (Salih *et al.*, 1999; Younis *et al.*, 2000; Okçu *et al.*, 2005;

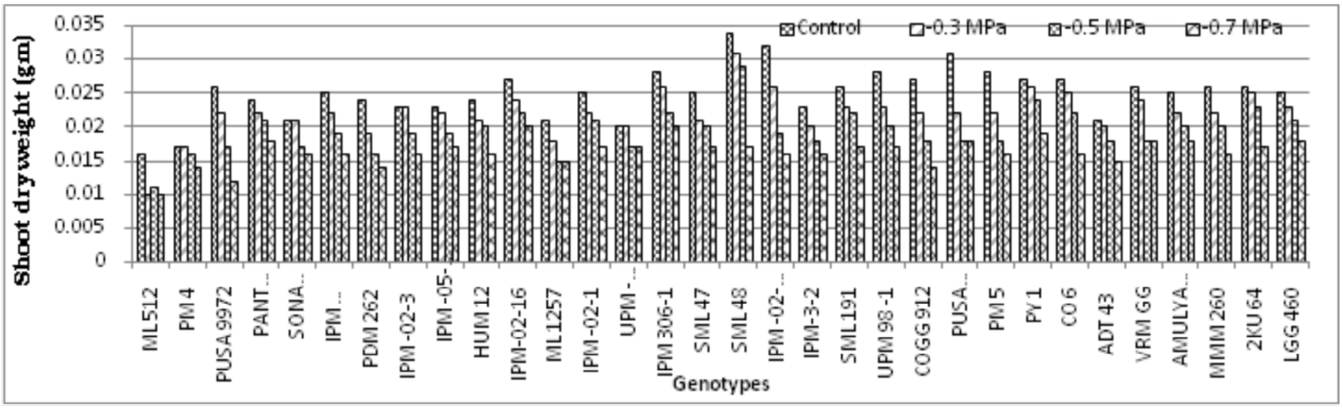

Figure 7: Shoot dry weight in mungbean exposed to different osmotic potentials induced by PEG 6000

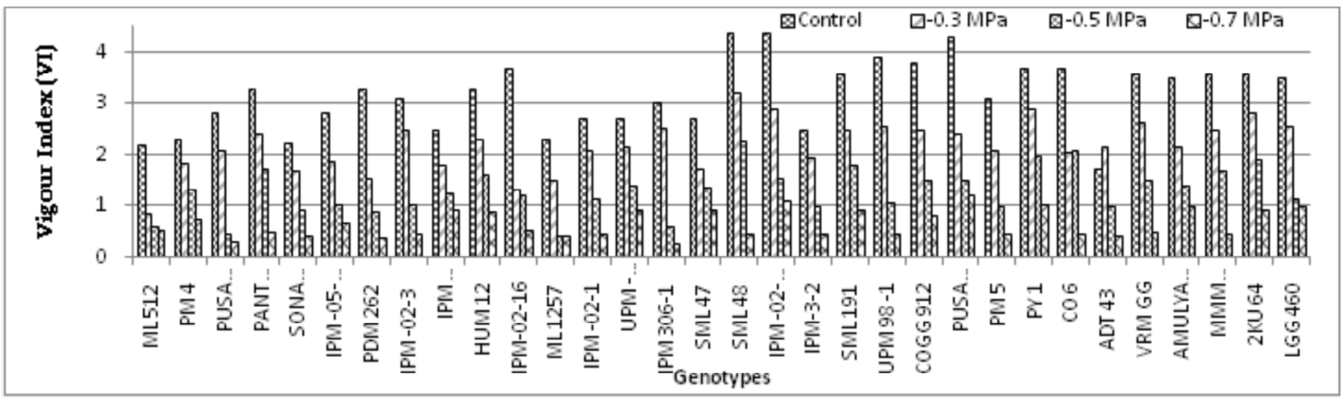

Figure 8: Vigour index in mungbean exposed to different osmotic potentials induced by PEG 6000

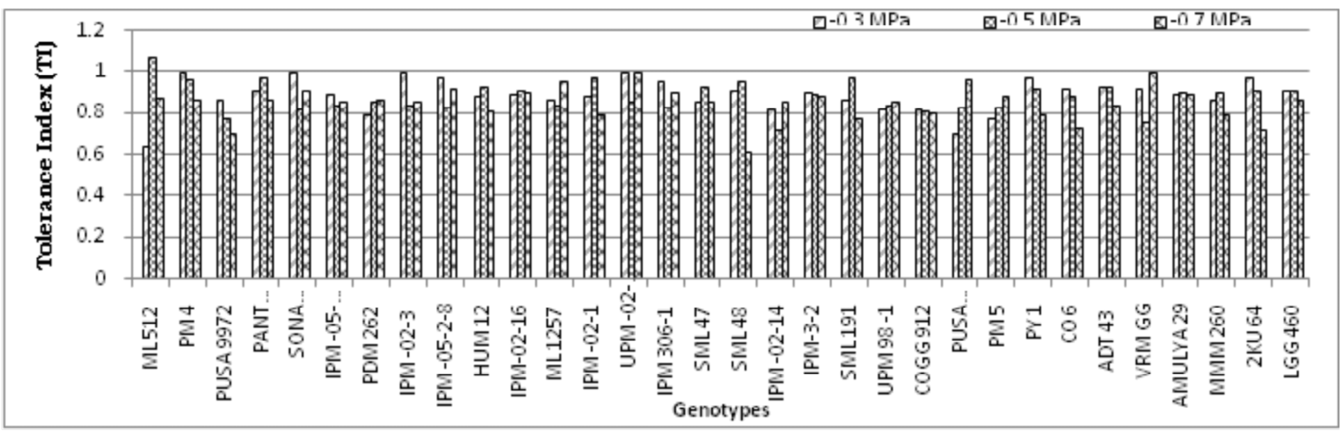

Figure 9: Tolerance index in mungbean exposed to different osmotic potentials induced by PEG 6000

Bibi *et al.*, 2010). Reduction in seedling growth is the result of restricted cell division and enlargement, as drought stress directly reduces growth by decreasing cell division and elongation (Kramer, 1983).

Roots are the place where plants first encounter water stress, it is likely that roots may be able to sense and respond to the stress condition (Xiong *et al.*, 2006; Khodarahmpour, 2011). It plays an important role in water stress tolerance by reduction in leaf expansion and promotion of root growth. Root length at seedling stage provides a fair estimate about the root growth in field (Ali *et al.*, 2011a, b; Rajendran *et al.*, 2011). Reduction in shoot length perhaps due to less water absorption and decrease in external osmotic potential created by PEG (Kaydan and Yagmur, 2008) but higher root: shoot ratios than the susceptible lines, which may have been responsible for their higher leaf water-potentials in the stressed environments. It appears that vigorous shoot growth corresponds to vigorous root growth under water stress.

Fresh and dry root weight was also decreased due to water stress in mungbean. Similar results were reported by Shiralipour and West (1984). Dry and fresh weights of roots were decreased during the drought period as their leaf size remained small to minimize transpiration, ultimately plant dry weight also reduced. Dry root weight (DRW) has been utilized as a selection criterion for drought tolerance by many plant breeders. Water uptake by the root is a complex parameter that depends on root structure, root anatomy, and the pattern by which different parts of the root contribute to overall water transport (Cruz *et al.* 1992).

The tendency of highly reduced water potential either to inhibit germination or suppresses the growth and development of seedlings was also noticed for vigour index. The genotype varied significantly in tolerance index under water stress conditions. The vigour index reduced gradually with the increase in the osmotic potential among the various genotypes studied. The tolerance index was found maximum in SML 1168. According to Dutta and Bera (2009) tolerance index (TI) which is based on the dry matter of a plant at a particular stage is universally considered as a more stable character than other morphological parameters.

Out of thirty two mungbean genotypes screened SML 48, PUSA 9972,CO 6 and 2KU 64 showed minimum value of tolerance index, while VRM GG followed by IPM-02-14 and PUSA BOLD 2 showed maximum value of tolerance index so they confirming that these genotypes are more tolerant to water stress condition and ‘SML 48, PUSA 9972, CO 6 and 2KU 64 are the most sensitive genotypes. The genotypes which performed better under osmotic stress in terms of lesser reduction in various aspects of growth might be related to their water stress tolerance 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 screen the germplasm at early stage. So is suggested that the findings may be helpful and fruitful for selection of drought stress in sorghum under the discussed traits.

ACKNOWLEDGEMENT

The author is highly acknowledged to University Grants Commission (UGC), Delhi for providing all the funds required for this research work.

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