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

Response of Maize Hybrids to Peg - Induced Osmotic Stress at Pre Germination and Germination Phase

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Abstract: *Keeping in view the alarming situation of drought globally, the present study was initiated to analyze osmotic stress tolerance at germination and early seedling growth stages of maize* (Zea mays *L.). The effect was investigated in three maize hybrids, DHM-117, PMH-3 and HQPM-7 in four Polyethylene glycol-6000 (PEG) concentrations i.e., control, 10 %, 20%, 30% and 40% solutions. Results showed a decrease in germination from 100% in control to 5 % in 40% PEG concentration. Root traits* viz., *root length, number of lateral roots, fresh weight and proline content increased with increasing osmotic potential of PEG and peaked at 20% PEG, and then declined sharply. All the shoot traits showed a decreasing trend with increasing osmotic potential. The estimation of cumulative drought index indicated hybrid PMH-3 to be most responsive to the progressive water deficit conditions. This hybrid can be utilised as a source for drought tolerance in breeding programme.*

Keywords: *PEG-6000, Proline, Root traits, Imbibition, Cumulative drought index*

INTRODUCTION

Abiotic stress causes crop loss worldwide, reducing average yields in major crop plants by more than 50% (Boyer 1982). One of the most important abiotic factors limiting plant germination and early seedling- growth stages is water stress brought about by drought and salinity (Almansouri *et al.* 2001). Plant growth, yield, cell membrane integrity, pigment, osmotic adjustment and photosynthetic activity is influenced by drought (Anjum *et al.*, 2011; Prabha *et al.* 2009) The susceptibil-ity of plants to drought stress depends on degree of stress, plant species and their developmental stages (Demirevska *et al.* 2009).

Water stress acts by decreasing the percentage and rate of germination and seedling growth (Khayatnezhad *et al.* 2010). Germination of seeds, one of the most critical phases of plant life, is greatly influenced by drought. Once seed has germinated, root is the first organ to be exposed to water deficit. Therefore, under water deficit conditions, it is assumed that osmotic adjustment in the root occurs before that in the leaf, to enhance turgor pressure for continued root growth and absorption of water and nutrients. Thus, osmotic adjustment in the root is expected to delay the onset of water deficit in the shoots. Acclimation of plants to water deficit leads to morphological and physio-biochemical responses at cellular and whole-plant level. Accumulation of protective solutes, such as proline and soluble sugar is a unique response to environmental stresses, specifically to drought stress (Sakamoto and Murata 2002).

Breeding for drought - resistant cultivars is hampered by non-availability of suitable method for screening a large number of genotypes in uniform dry environments. Under natural field conditions, rainfall eliminates water deficits. Hence,

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in-vitro drought-screening methods are necessary to screen genotypes at different stages, facilitating our understanding of drought-resistance traits. Richards (1978) suggested evaluating germination of genotypes as a useful criterion in screening for water- stress tolerance. Osmotic solution induced drought is one of the methods for the evaluation of tolerance to drought during the germination phase. Khakwani *et al.* (2011) demonstrated that drought tolerance in six varieties of wheat evaluated by *in vitro* germination tests correlated with field drought-tolerance levels. Exposure to polyethylene glycol with large molecular weight (PEG-6000) is more appropriate than to smaller molecules such as PEG-4000, to create osmotic stress, because germination percentage of seed in polyethylene glycol 6000 and in soil with the same water potential is approximately equal (Emmerich and Hardgree 1990).

In India maize is mostly grown as a rain-fed crop during *kharif* season. However, *kharif* season crop is affected by less rain coupled with poorly distributed rainfall patterns leading to drought. To stabilize maize production, development of drought tolerant maize is a viable solution. Keeping this in view, the present study was conducted to evaluate the three tropical maize hybrids for rainfed cultivation across the country for early seedling traits in PEG 6000 - induced stress conditions.

MATERIALS AND METHODS

Plant Material

To study the response of genotypes to osmotic stress induced by PEG-6000, three popular maize hybrids suitable for different agro-ecology of country *viz.*, HQPM- 7, PMH- 3 and DHM-117 released by All India Coordinated Maize Programme were studied. Among the hybrids, HQPM-7 is a late- maturing Quality Protein Maize hybrid with parentage of HK1-193-1 x HKI-161; its parental inbred lines were derived from CIMMYT QPM germplasm. PMH-3 is late- maturing, flint hybrid with high response to inputs recommended for cultivation in North India (Delhi, Punjab, Haryana and Western Uttar Pradesh). In 2010, nutrient- responsive DHM-117 was released for Peninsular India; it is characterized by medium maturity, stay-green character and tolerance to lodging.

Stress treatment and experimental set-up

To induce osmotic stress, poly ethylene glycol (PEG) 6000 was used at concentrations of 0% (control), 10% (-0.15 MPa), 20% (-0.49 MPa), 30% (-1.03 MPa) and 40% (-1.76 MPa) (w/w). Water potential values were calculated according to Burlyn and Mirrill (1973) based on theoretical values of nutrient solution and experimental values of the PEG solution. Fully mature, homogenous seed lot of hybrids was surface sterilized with 1.0% sodium hypochlorite solution and then washed with distilled water three times. The experiment was set up as a two- factor, completely randomized design with two replications. First factor was hybrids and the second factor was PEG concentrations (control, 10, 20, 30 and 40%). In every replication, five Petri dishes of each hybrid per PEG treatment, along with control, were used. Two layers of filter paper were laid in each Petri dish and 10 seeds were placed in it.

Observations

***Imbibition or water uptake*:** For recording imbibition in response to various levels of water potential or treatments (0, 10, 20, 30, 40% PEG), seeds of each hybrid was initially weighed (W_1) and placed in Petri dish with filter paper layers. In each Petri dish, 10 ml solution of respective treatment was added and incubated at 25^0C. After 24 hours, seeds were reweighed (W_2) one by one. Water absorption was calculated using the formula

$$\text{Water absorption (\%)} = [(W_2 - W_1)/W1] \times 100$$

Imbibition (water uptake) in response to various levels of water potential or treatments (0, 10, 20, 30, 40% PEG) in each hybrid of maize was recorded.

***Germination assay and seedling-growth parameters*:** The observations regarding germination were made after every 24 hours and continued till the completion of germination. The emergence of radicle and plumule was taken as an indicator or measure of germination. After seven days the germination percentage was calculated as follows:

Germination percentage (GP)% = (Number of seeds germinated/Total seeds soaked) X 100

After germination, seedling parameters, *viz.*, axile root length (ARL), shoot length (SL), lateral root number (LR), root fresh weight (RFW) and shoot fresh weight (SFW) were measured.

Proline estimation: For Proline estimation, a 100mg plant sample was homogenized in 10 ml of 3% sulfosalicylic acid. This was centrifuged at 4000 rpm for 10 min and the supernatant was collected. The left over pellet was extracted with 10 ml of 3% sulfosalicylic acid at 4000 rpm for 10 min. After centrifugation, both the supernatants were mixed together. In 2 ml supernatant, 2 ml acetic acid and 2 ml ninhydrin solution were added. This was further kept in boiling water bath for 1 hour and then the reaction was terminated by placing in an ice box. After cooling, 4 ml toluene was added in each test tube, mixed and kept for sometime at room temperature. Chromatophore (only toluene phase) was sucked out with a pipette. Absorbance (O.D. value) was measured at 520 nm against reagent blank. The amount of proline content present in the extract was calculated using standard curve prepared from graded concentration of proline. Total proline was determined by use of the following formula:

Proline content (mg/g fresh weight) = (F X 1000 X Total weight) / 100mg x Vol. of replicate

where, O.D Factor (F) = Weight/ O.D value

Cumulative drought tolerance index

CDTI = $(\Sigma\ x_1+x_2+....+x_n / n) / ((\Sigma\ y_1+y_2+....+y_n / n)$

where, $x_1,x_2...x_n$ are traits studied at 30% PEG and y_1, y_2, ...y_n traits observed in control.

Data recorded were subjected to two-way analysis of variance (ANOVA) procedures. All data were presented as mean value ± standard error. Significant differences between means were determined by Fisher's least significant difference test (LSD) at P<0.05.

RESULTS

In ANOVA, there was significant differences among treatments for axile root length, shoot length, number of lateral roots and root proline (Table 1 & 2), while there was no significant difference among hybrids for any of the traits studied. Analysis of variance revealed significant hybrid-treatment interaction for root length.

Effect of PEG-6000 induced osmotic stress imbibition and germination

Results showed the gradual decrease in imbibition (water uptake) in all hybrids (Fig. 1) with increase in PEG concentration from 0 to 40%. PMH-3 showed highest water uptake of 46.94% in control followed by DHM-117 and HQPM-7. The overall mean of imbibition percentage was 38.1 among hybrids. In hybrids, HQPM-7 and PMH-3 showed decreasing trend in germination among treatments from control to 40% PEG (Fig 1). Complete germination (100%) was observed in DHM-117 in control, 10% and 20% PEG concentrations. At extreme 40% PEG, germination was highest in PMH-3 followed by HQPM-7 and DHM-117. PMH-3 had an overall mean percentage of 66% followed by HQPM-7 (58%) and DHM-117 (52%) across treatments. Hence, hybrids exhibited germination and imbibition at different stress levels at varying percentage.

Effects of PEG-6000 induced osmotic stress on seedling traits

Hybrids, HQPM-7 and DHM-117 exhibited declining axile root length from control to 40% PEG

Table 1
ANOVA for traits investigated for the three hybrids of maize in response to drought

	ARL (cm)	*SL (cm)*	*RFW (g)*	*SFW (g)*	*LR*	*RP (mg/g)*	*GP (%)*	*IP (%)*
Treatment	96.94*	79.238*	0.19	0.360	18.63*	3.24*	0.950487	0.078794
Hybrid	2.28	1.47	0.01	0.003	3.70	0.26	0.019229	0.079642
Interaction	14.82*	0.89	0.04	0.003	1.53	0.09	0.11742	0.025412

* significance at P<0.05; ARL-Axile Root length; SL- Shoot length; RFW- Root fresh weight; SFW-Shoot fresh weight; LR-Number of lateral roots; Root proline-RP

Table 2
Effects of osmotic stress , genotypes and their interaction; mean values + standard error for axile root length (ARL), shoot length (SL), root fresh weight (RFW), shoot fresh weight (SFW) , number of lateral roots (LR) and root proline content (Pro)

Factor	*ARL(cm)*	*SL(cm)*	*RFW(g)*	*SFW(g)*	*NLR*	*RP(mg/g)*
Genotypes						
DHM-117	5.12+1.86	3.52+1.55	0.24+0.09	0.18+0.09	3.41+1.03	0.67+0.77
PMH-3	5.88+2.15	2.86+1.41	0.28+0.11	0.19+0.10	3.40+0.93	0.96+ 0.76
HQPM-7	6.04+2.12	3.60+1.92	0.20+0.08	0.22+0.13	2.40+0.68	0.92+ 0.61
PEG-6000 (%)						
Control	9.23 + 1.86	8.67 + 0.73	0.39 + 0.08	0.57 + 0.03	4.33 + 0.33	0.54+0.49
10%	8.23 + 0.54	5.33 + 0.44	0.38 + 0.03	0.33 + 0.03	3.67 + 0.67	1.20+0.14
20%	8.00 + 2.53	2.0 + 0.29	0.34 + 0.14	0.08 + 0.01	4.67 + 0.89	1.50+0.21
30%	2.90 + 0.35	0.63 + 0.24	0.09 + 0.01	0.02 + 0.01	2.67 + 0.33	0.97+0.16
40%	0	0	0	0	0	0

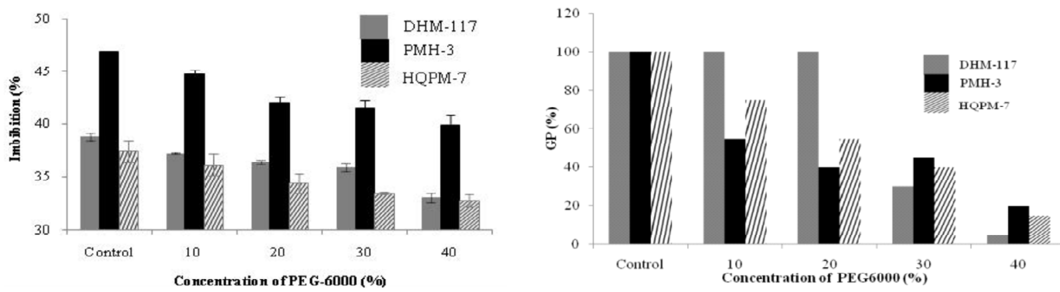

Figure 1: Effect of osmotic stress on imbibition percentage and germination percentage of three tropical maize hybrids

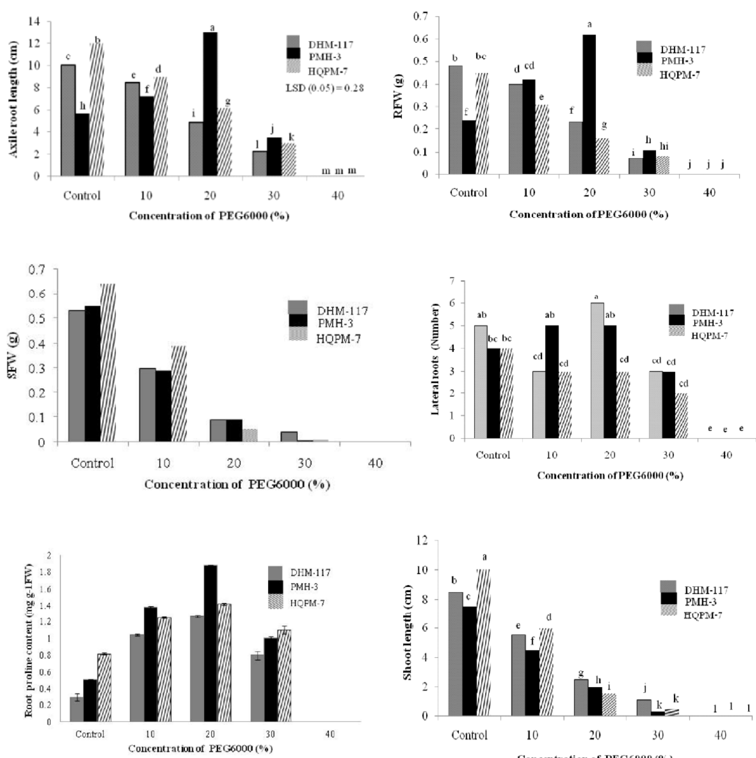

Figure 2: Comparison of mean effects of three tropical maize hybrids and osmotic stress levels on axile root length (ARL), shoot length (SL), root fresh weight (RFW), shoot fresh weight (SFW), number of lateral roots (LR) and root proline content

levels. PMH-3 showed highest (13cm) root length at 20% PEG, while failed to produce more shoot growth at the same level and water stress depressed the shoot growth over the root growth (Fig. 2). Increase in PEG concentration induced lateral roots (five roots) in PMH-3 at 10% and 20% PEG and decreased to three roots in 30% PEG. DHM-117 root penology was bushy compared to other hybrids with highest number of lateral roots. In DHM-117, 10% PEG concentration decreased the number of lateral roots (three roots) in comparison to control. While it increased to six roots in 20% PEG and later decreased to three roots at 30% PEG.

In PMH-3 root fresh weight reached a peak at 20% (0.62g) and then decreased in 30% treatment, whereas root fresh weight decreased with increasing PEG levels in other two hybrids. PEG decreased shoot fresh weight of hybrids at 10 and 20% relative to control (Fig. 2). At 40% PEG-6000 concentration all seedling parameters were completely inhibited after seven treatment days. Progressive osmotic stress from 10 to 40% induced an accumulation of proline in water stressed hybrid seedlings (Fig 2). The proline content increased as the drought stress progressed and reached a peak at 20% PEG stress level, and then decreased thereafter.

DISCUSSION

Water moves from high potential to low potential due to differences in the free energy content reducing water uptake. The gradient of water potential between dry seeds and pure water (0.0 bars) decreases rapidly with the addition of any soluble substance in water. In an experiment the induced (PEG 6000) osmotic stress expressed 38.1 percent of imbibition in the hybrids. According to Mohammadkhani and Heidari (2008) the decrease in water potential gradient between seed and media prevents the seed to absorb the desired amount of water. Elevated drought stress reduces osmotic potential and decreases water uptake by seeds there by inhibiting imbibition, germination (Almaghrabi and Abdelomoneim 2012) and seedling growth (Ashraf *et al.* 2002). Under reduced osmotic potentials, seed metabolism activation is delayed and seed germination takes place later (Kader and Jutz 2002; Swapna and Rajendrudu 2015).

Water availability and movement into the seeds is very important to promote germination, initial root and shoot elongation (Bewley and Black 1994).The significant interaction in root length indicated differences in osmotic stress tolerance among the three hybrids, as root traits determine drought tolerance in genotypes to a large extent (Khodarahmpour 2011). Aboveground part of seedling is more sensitive to increased PEG concentrations than the radicle (Radiæ et al. 2007). All hybrids in experiment invariably showed reduction in shoot length with increasing concentration in PEG. Decline in root and shoot length in response to drought might be due to either decrease in cell elongation resulting from the inhibitory effect of water shortage on growth promoting hormones, decrease cell turgor, cell volume and eventually cell growth (Banon et al. 2006), and/or due to blocking up of xylem and phloem vessels to hinder translocation (Lavisolo and Schuber 1998). HQPM-7 showed reduction in lateral roots with increasing PEG levels. Drought tolerant genotypes show profuse proliferation of root hairs and thicker roots with broader xylem vessels. The role of lateral roots in cereals like maize for water and nutrient uptake has been recognised.

Results of our study showed reduction of shoot and root fresh weight of hybrids in various treatments. Such reduction in fresh and dry biomass production of chosen maize lines could be expected since the early seedling stage is the most sensitive to water deficit (Zhao *et al.* 2006). Quick response of plants to stress elucidates the signalling of inbuilt protective mechanism to combat stress which include morphological, physiological and biochemical changes (Marcinska *et al.* 2013). The increase of proline content ranged from 0.3 mg/g fresh weight in DHM-117 (control) to 1.9 mg/g fresh weight in PMH-3 (20% PEG) in root. Proline accumulation is the first response of plants exposed to water deficit in order to reduce cell injury (Ghorbanli *et al.* 2012; Moharramnejad *et al.* 2015). Proline maintains water content, stores carbon and nitrogen after water stress recovery and stabilizes macromolecule, proteins and cell membranes in plant tissues (Pirzad *et al.* 2011).Drought tolerance in cereals is based on the iden-tification of drought traits and the selection of promising genotypes for varietal development. A calculated cumulative drought index indicated that hybrid PMH-3 (42.7%) could be ranked first in drought tolerance followed by DHM-117 and HQPM-7 (Fig 3). Hence, hybrid PMH-3 can adapt to the progressive water deficit conditions efficiently. The varying response to osmotic stress by maize hybrids confers that it has a genetic background to combat water shortage. Further studies need to be carried out to assess whether the genotypes marked as drought tolerant at initial stages maintain their degree of tolerance in later growth stages too.

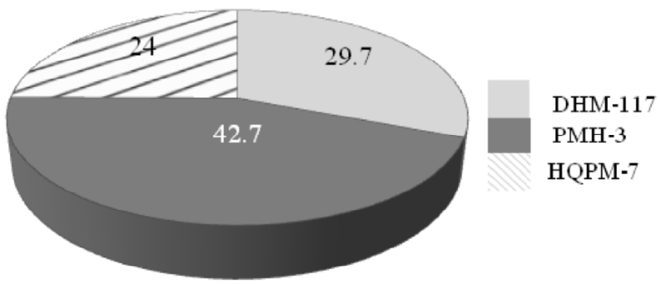

Figure 3: Cumulative drought tolerance index of hybrids

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