

Seed Germination under Simulated Water Stress as Drought Tolerance Test in Maize

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ABSTRACT Six inbreds possessing various degrees of drought tolerance along with their 15 F_1 s were used to study seed germination and early seedling growth under simulated drought using polyethylene glycol (PEG) and mannitol solution at 0.5 MPa (-5.0 bars). Germination percentage decreased greatly under osmotic stress. The results obtained in PEG and mannitol were parallel. However, tolerant parents and hybrids showed less decrease in germination percentage. Reduction in root and shoot length and increase in root-shoot ratio were some other effects of osmotic stress on early seedling growth. Osmotic stress caused greater decrease in shoot length. However, tolerant genotypes showed lower decrease in root length and increased root-shoot ratio. Almost similar trend was noticed in F_1 , F_2 and backcrosses. This suggested the utility of laboratory screening by taking per cent decrease in germination and increased root-shoot ratio as criteria for preliminary screening of genotypes for drought tolerance. The germination percentage in osmotic stress showed positive correlation with root-shoot ratio and grain yield under rainfed and limited irrigation conditions.

Key words: Drought tolerance, osmotic stress, germination, PEG, mannitol

Moisture stress is one of the most important factors limiting the crop productivity. The breeding for drought tolerance has been a challenge to the plant breeders. Breeding for drought tolerance has been accomplished by yield. Empirical screening for drought tolerance based on grain yield requires full season and is not an efficient approach for screening large number of germplasm. Also, under field conditions maintaining satisfactory control of soil moisture is difficult [1]. An alternative and quick approach is to screen germplasm under laboratory or green house conditions. Studying seed germination and seedling growth under high osmotic stress simulating drought is one such methods. Different crop species and osmotic substrates have been used in studying the effects of simulated water stress on seed germination. According to Manohar and Heydecker [2], polyethylene glycol of high molecular weight (4000 or more) possess osmotic properties with no evidence of toxicity. Thimman [3] found mannitol suitable for limiting water uptake in plants without

affecting metabolic activity. Present investigation utilized both PEG and mannitol for simulating moisture stress (drought) during seed germination and early seedling growth tests. The salient findings of the study are presented.

MATERIALS AND METHODS

The base material for the present investigation consisted of six inbreds, three of which possessed tolerance (Ib3, Ib5 and Ib6) and the remaining three possessed susceptibility to drought (Ib1, Ib2 and Ib4). These inbreds were mated in a diallel mating system excluding reciprocals. Six inbred lines as parents (three tolerant and three susceptible), 15 F_1 hybrids and four standard varieties as checks viz., PC-3, PC-4, PEHM-I and PEHM-II were evaluated under both Delhi and Hyderabad conditions for recording yield data. Thus experimental material was subjected to screening in the field as well as in the laboratory utilizing polyethylene glycol and mannitol as osmotica to simulate drought during germination.

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In Delhi, the experiment was conducted under irrigated (IR-D) and rainfed conditions (RF), whereas, in Hyderabad, it was carried out under irrigated (IR-H) and limited irrigation (LI) conditions. The irrigated trial was provided with normal number of irrigations (five irrigations), limited irrigation trial was given only three irrigations such that it is subjected to stress at flowering and post-flowering stages. The rainfed experiment was grown exclusively as rainfed and no irrigation was given. The trials were separated by a four meters wide bund to avoid the seepage effects of irrigation water on rainfed trial in Delhi and on limited irrigation experiment in Hyderabad. The water requirement of the maize during kharif is about 500 mm rainfall and rainfall between 100-200 mm around flowering period indicate that area is marginal for maize production [4]. In the present study the total precipitation received during kharif 2000 in Delhi was about 340 mm and around flowering it was about 155 mm. Therefore, it can be considered as a rain deficient season. In Hyderabad conditions, experiment was conducted in rabi season (2001-2002) and hence moisture stress treatment was given by with-holding the irrigation during flowering and post-flowering stages (limited irrigation). The spacing followed was 75 cm x 20 cm and each row consisted of 20 plants. Five plants were randomly selected for recording observations on anthesis-silking interval (ASI-the difference between days to 50 per cent silking and days to 50 per cent tasseling) and yield per plant (g).

The laboratory screening was done at Central Seed Testing Laboratory, IARI, New Delhi. Polyethylene glycol and mannitol were used to simulate drought stress during seed germination. The six parents (three drought tolerant and three drought susceptible) were used to standardize the appropriate osmotic pressure of PEG, and mannitol solution to be used for screening. The germination in water was used as control. The study was conducted with three replications following paper towel method for seed germination, PEG and mannitol [1]. To prepare 0.5 MPa solutions, 202 g of PEG 6000 or 36.99 g of mannitol were dissolved in one litre of luke warm distilled water. The paper towels were completely wetted with water, PEG or mannitol solutions and seeds were kept in between

two paper towels. Twenty seeds from each parent and F_1 cross; 50 seeds from F_2 and back cross population for each replication were used. The paper towels were then carefully rolled in a butter paper. They were kept in germinator at 25° C for 12 days in dark and the following observations were recorded viz., germination percentage, root length (in cm was recorded from base to the tip of main root), shoot length (in cm was recorded from base to the tip of the leaf blade), root to shoot ratio (the ratio of root length to the shoot length) and plant height stress index (PHS I), which was calculated as the ratio of the shoot length under osmoticum to the shoot length under water as follows:

$$PHSI = \frac{\text{Shoot length of stressed seedlings}}{\text{Shoot length of control seedlings}} \times 100$$

The correlation coefficients were calculated to determine the degree of association of characters with yield and also among themselves. Phenotypic and genotypic correlations were computed by using the formulae given by Weber and Moorthy [5].

RESULTS AND DISCUSSION

The osmotic pressure (OP) of 0.5 MPa was found to be useful among 0, 0.25, 0.5, 0.75 and 1.0 MPa levels as this OP level was clearly differentiating tolerant and susceptible genotypes and abnormal seed germination was less. Under higher OP levels, abnormal germination of seeds was high. The germination percentage was above 95 per cent in all genotypes except PC-3 and PC-4 when germinated in water (Table 1). When germinated in PEG and mannitol, there was a decrease in germination percentage in all genotypes. The germination percentage among parents ranged from 95.0 (Ib5) to 98.3 per cent (Ib1) in water, from 60.6 (Ib1) to 85.6 per cent (Ib3) in PEG and from 63.6 (Ib1) to 87.3 per cent (Ib3) in mannitol. Among hybrids, it varied from 94.0 (Ib1 x Ib5) to 99.3 per cent (Ib2 x Ib6) in water, from 65.6 (Ib1 x Ib4) to 91.6 per cent (Ib3 x Ib5) in PEG and from 72.6 (Ib1 x Ib4) to 92.3 per cent (Ib3 x Ib5) in mannitol. Thus, the percentage of germination in PEG and mannitol decrease compared to that in water. Appreciable decrease was observed in susceptible inbred lines (Ib1, Ib2 and Ib4) and tolerant inbreds

(Ib3, Ib5 and Ib6) had higher germination under osmotic stress. Williams et al. [6] studied germination percentage in mannitol solution and found the usefulness of this method to classify genotypes as tolerant and susceptible ones. Genotypes with high germination percentage can be considered as tolerant [7-9]. Similar reports were available in wheat [10] and soybean [11]. Among hybrids Ib3 x Ib5, Ib3 x Ib6 and Ib5 x Ib6 and other crosses involving Ib3 recorded higher germination (Table 1). These hybrids, under moisture stress in field conditions, showed lower decrease in yield and short PHSI, which are established criteria for screening for drought tolerance [4]. When germinated in water, genotypes did not show much difference compared to that under osmotic stress.

The root length among parents varied from 14.93 (Ib3) to 19.53 cm (Ib6) when germinated in water, from 7.70 (Ib2) to 11.97 cm (Ib6) in PEG and from 11.15 (Ib3) to 16.10 cm (Ib6) in mannitol. Among crosses it varied from 17.73 (Ib1 x Ib2) to 24.80 cm (Ib3 x Ib4) when germinated in water, from 10.97 (Ib1 x Ib4) to 14.03 cm (Ib3 x Ib5) in PEG and from 11.60 (Ib2 x Ib3) to 15.70 cm (Ib3 x Ib5) in mannitol. Most of the crosses having Ib3 as a parent had more root length, when germinated in osmotic stress. The shoot length among parents ranged from 14.95 (Ib3) to 17.0 cm (Ib6), when germinated in water, from 3.40 (Ib3) to 4.90 (Ib4) in PEG and from 3.50 (Ib3) to 5.10 (Ib1) in mannitol. Among hybrids, it varied from 17.50 (Ib1 x Ib3) to 22.05 cm (Ib3 x Ib4), when germinated in water, from 4.25 (Ib1 x Ib3) to 6.25 cm (Ib1 x Ib5) in PEG and from 3.95 (Ib1 x Ib3) to 5.90 cm (Ib1 x Ib5) in mannitol. Reduction in root length and shoot length was observed in PEG and mannitol germinated seedlings. Reduction in shoot length was more than that in root length. Besides, reduction was more in some genotypes like Ib2, Ib4, Ib3 x Ib4 and Ib4 x Ib5, whereas, Ib3 and Ib3 x Ib5 had least reduction for root length. Natural corollary of this pattern of reduction in root and shoot length is increase in root-shoot ratio in PEG and mannitol germinated seedlings. Such reports were available in wheat [10], soybean [11] and maize [9]. The likely explanation is that moisture stress affects growth of shoot more than it does for

photosynthesis so that the amount of assimilates available for root growth is increased.

The root-shoot ratio oscillated around 1.0 when germinated in water but it was more than 2.0 in most of the genotypes under osmotic stress. When germinated in PEG, it varied from 1.62 (Ib2) to 2.98 (Ib3) and in mannitol, it varied from 2.80 (Ib1) to 4.03 (Ib6) among parents. Among hybrids when germinated in PEG, it varied from 2.09 (Ib1 x Ib2) to 3.12 (Ib3 x Ib5) and in mannitol, it ranged from 2.64 (Ib1 x Ib5) to 4.19 (Ib3 x Ib5). The plant height stress index varied from 0.23 (Ib3) to 0.32 (Ib4), when germinated in PEG and from 0.23 (Ib3) to 0.33 (Ib1) in mannitol among parents. Among hybrids, PHSI ranged from 0.21 (Ib3 x Ib4) to 0.35 (Ib1 x Ib5) in PEG and from 0.21 (Ib3 x Ib5) to 0.33 (Ib1 x Ib5) in mannitol. The PHSI values were well below 1.0 in all genotypes when germinated under both PEG and mannitol (Table 1). Thus, genotypes with higher germination percentage in osmotic stress had lower PHSI and higher root-shoot ratio, as they had less shoot length. This indicated that drought tolerant types had lower PHSI and higher root-shoot ratio compared to susceptible ones. Similar results were earlier reported by Subba Rao [9].

The correlation coefficients showed that genotypic correlation coefficients were more than phenotypic correlation coefficients for majority of the characters (Tables 2&3). The table 2 represents genotypic and phenotypic correlation coefficients among germination and seedling characters, when germinated in water and yield under irrigated conditions. The phenotypic correlation coefficients are given above the diagonal and genotypic correlation coefficients are given below the diagonal of matrix (Table 2). The data showed that the phenotypic correlations were positive and significant for root length with shoot length (0.718), root-shoot ratio (0.517) and yields in Delhi (IR-D 0.420) and in Hyderabad (IR-H 0.552). Shoot length had positive and significant correlations with irrigated yield in Delhi and Hyderabad. The yield in Delhi-irrigated conditions was having significant correlation with yield in Hyderabad irrigation (0.870). Same trend was observed even in genotypic correlations, except that germination percentage and root length had negative and significant correlation (-0.382).

Table 1. Mean values for germination percentage and seedling characters in maize under laboratory conditions along with grain yield under field conditions

Parents	Germination (%)		Root length (cm)		Shoot length (cm)		Root-shoot ratio		PHSI		Yield per plant (g)		Anthesis silking interval										
	Water	PEG	Water	PEG	Water	PEG	Water	PEG	Water	PEG	IR-D	RF-D	IR-H	LI-H									
Ib ₁	98.33	60.67	63.67	15.07	10.07	14.30	15.40	4.40	5.10	0.98	2.29	2.80	0.29	0.33	105.8	70.4	85.3	57.3	3.33	7.00	3.00	7.33	
Ib ₂	97.00	65.67	69.00	16.97	7.70	13.70	16.60	4.75	4.80	1.02	1.62	2.86	0.29	0.29	99.5	68.8	72.4	53.8	3.33	6.00	2.67	6.00	
Ib ₃	97.67	85.67	87.33	14.93	10.10	11.15	14.95	3.40	3.50	1.00	2.98	3.19	0.23	0.23	126.2	105.0	104.9	90.0	2.00	2.67	2.00	2.67	
Ib ₄	95.00	69.00	64.67	19.27	9.27	14.60	15.45	4.90	4.95	1.25	1.89	2.95	0.32	0.32	106.5	79.9	75.6	60.1	3.67	5.33	2.33	5.67	
Ib ₅	97.33	79.67	85.33	18.03	11.83	13.05	15.90	4.45	3.95	1.13	2.67	3.30	0.28	0.25	110.5	91.5	93.4	76.5	2.67	4.67	2.67	4.00	
Ib ₆	97.67	76.67	82.00	19.53	11.97	16.10	17.00	4.05	4.00	1.15	2.95	4.03	0.24	0.24	111.4	91.6	93.6	76.6	3.67	4.33	2.00	4.33	
F ₁ ^S																							
Ib ₁ ×Ib ₂	97.67	70.67	72.67	17.73	10.97	13.90	17.65	5.25	5.20	1.01	2.09	2.68	0.30	0.29	145.8	90.8	111.7	75.8	3.67	6.33	3.33	6.33	
Ib ₁ ×Ib ₃	96.67	86.00	84.33	19.03	12.27	14.90	17.50	4.25	3.95	1.09	2.89	3.79	0.24	0.23	144.5	115.2	119.8	100.2	4.00	4.00	3.00	4.00	
Ib ₁ ×Ib ₄	96.33	65.67	72.67	20.30	12.33	14.50	18.25	5.30	5.05	1.11	2.34	2.88	0.29	0.28	144.6	95.0	121.8	80.0	3.33	6.33	2.00	6.33	
Ib ₁ ×Ib ₅	99.33	79.00	77.00	19.03	13.33	15.55	17.75	6.25	5.90	1.07	2.14	2.64	0.35	0.33	134.6	111.5	111.7	91.6	3.67	4.33	2.67	4.33	
Ib ₁ ×Ib ₆	98.67	77.67	79.67	18.30	10.97	14.20	18.70	5.20	5.15	0.98	2.11	2.76	0.28	0.28	137.6	106.9	112.5	91.9	3.33	5.33	3.33	5.33	
Ib ₂ ×Ib ₃	97.67	81.67	82.33	19.10	13.03	11.60	18.70	4.60	4.50	1.02	2.83	2.59	0.25	0.24	155.6	115.0	128.9	100.0	3.33	4.33	4.33	4.33	
Ib ₂ ×Ib ₄	93.67	68.00	75.00	19.37	12.07	13.85	20.85	5.65	5.20	0.93	2.14	2.67	0.27	0.25	145.0	86.5	108.6	76.5	3.33	5.00	3.00	5.00	
Ib ₂ ×Ib ₅	97.67	80.33	83.67	18.93	12.53	14.80	19.25	6.00	5.60	0.98	2.09	2.64	0.31	0.29	147.5	106.0	105.3	84.6	3.67	4.00	2.00	4.00	
Ib ₂ ×Ib ₆	94.00	79.33	80.33	21.97	12.13	14.90	19.65	5.70	5.15	1.12	2.13	2.91	0.29	0.26	155.8	109.0	118.7	94.0	3.33	4.67	2.67	4.67	
Ib ₃ ×Ib ₄	97.67	84.33	81.67	24.80	13.40	14.45	22.05	4.60	4.75	1.12	2.92	3.07	0.21	0.22	141.2	113.8	112.3	92.3	3.67	3.67	2.67	3.67	
Ib ₃ ×Ib ₅	96.00	91.67	92.33	18.97	14.03	15.70	19.90	4.50	3.75	0.95	3.12	4.19	0.23	0.19	150.9	125.4	118.3	98.8	2.33	2.33	2.00	2.67	
Ib ₃ ×Ib ₆	98.33	87.00	86.00	21.63	13.67	14.20	19.90	4.40	4.10	1.09	3.11	3.46	0.22	0.21	139.8	119.4	137.2	107.0	3.33	3.33	2.67	3.67	
Ib ₄ ×Ib ₅	95.00	80.00	81.33	22.93	12.63	13.65	18.70	5.25	4.80	1.23	2.41	2.85	0.28	0.26	151.3	101.7	111.0	86.7	3.67	4.33	3.00	4.33	
Ib ₄ ×Ib ₆	98.33	77.67	77.67	19.20	12.07	14.15	19.35	5.45	4.90	0.99	2.22	2.89	0.28	0.25	144.7	96.7	125.1	81.7	4.67	5.33	2.67	5.33	
Ib ₅ ×Ib ₆	96.33	86.00	88.00	20.10	13.67	15.35	20.05	6.05	4.15	1.00	2.26	3.71	0.30	0.21	160.0	128.3	130.0	106.7	2.67	3.33	3.33	3.33	
Checks																							
PC-3	93.67	74.33	73.67	18.57	12.90	14.55	19.85	6.35	5.00	0.94	2.03	2.91	0.32	0.25	114.6	76.8	92.5	71.5	4.33	4.67	2.67	4.67	
PC-4	93.67	85.00	81.33	16.27	12.03	14.20	19.05	5.65	4.35	0.85	2.13	3.27	0.30	0.23	120.9	87.1	102.8	78.1	4.67	5.00	3.00	5.00	
PEHM-I	97.33	82.67	84.67	14.97	10.30	14.15	18.35	5.60	5.20	0.82	1.84	2.73	0.31	0.28	125.2	100.6	105.2	85.3	3.00	3.33	2.33	3.33	
PEHM-II	97.67	79.00	81.67	17.73	11.27	13.60	19.10	4.70	5.25	0.93	2.40	2.59	0.25	0.27	117.4	78.1	98.5	70.1	3.67	4.33	3.33	4.33	
Semi ±	1.01	2.32	2.82	0.50	0.51	0.19	0.29	0.22	0.17	0.03	0.13	0.12	0.014	0.009	8.89	5.93	11.11	6.49	0.55	0.66	0.58	0.65	

PEG - Polyethylene glycol; PHSI - Plant height stress index; IR - Irrigated; RF - Rainfed; LI - Limited irrigation; D - Delhi; H - Hyderabad

Table 2. Genotypic (below diagonal) and phenotypic (above diagonal) correlation coefficients among germination and seedling characters in water and yield under irrigated conditions

Character	G	RL	SL	R/S	Yield	
					Delhi	Hyd
Germination (G)		-0.23	-0.17	-0.15	-0.26	0.06
Root length (RL)	-0.38*		0.72**	0.52**	0.42**	0.37*
Shoot length (SL)	-0.24	0.75**		-0.22	0.67**	0.55**
Root:shoot ratio (R/S)	-0.29	0.49**	-0.21		-0.23	-0.17
Yield IR-Del	-0.19	0.55**	0.79**	-0.24		0.87**
Yield IR-Hyd	0.06	0.50**	0.74**	-0.24	0.75**	

*&**Significant at 5% and 1% level, respectively; IR - Irrigated; Del - Delhi; Hyd - Hyderabad

Table 3 shows correlation coefficients among germination and seedling characters under osmotic stress (PEG and mannitol) and grain yield under moisture stress conditions (under RF and LI). The results showed that germination percentage had

positive and significant phenotypic correlation with roots-shoot ratio, grain yield under RF and yield under LI, when germinated in both PEG and mannitol. The phenotypic correlation was negative and significant between germination percentage

Table 3. Genotypic (below diagonal) and phenotypic (above diagonal) correlation coefficients among germination and seedling characters in PEG and mannitol, and yield under rainfed and limited irrigation conditions

Character	Osmoticum	Yield						
		G	RL	SL	R/S	PHSI	RF-Delhi	LI-Hyd
Germination (G)	PEG		0.609**	-0.153	0.633***	-0.459**	0.809**	0.764**
	Mannitol		-0.030	-0.519**	0.502**	-0.725**	0.756**	0.715**
Root length (RL)	PEG	0.692**		0.272	0.546**	-0.179	0.732**	0.704**
	Mannitol	-0.002		0.211	0.407**	0.041	0.137	0.047
Shoot length (SL)	PEG	-0.157	0.272		-0.650**	0.760**	0.108	0.053
	Mannitol	-0.654**	0.229		-0.802**	0.768**	-0.263	-0.293
Root:shoot ratio (R/S)	PEG	0.702**	0.547**	-0.654**		-0.797**	0.496**	0.521**
	Mannitol	0.631**	0.417**	-0.788**		-0.694**	0.355*	0.321*
PHSI	PEG	-0.511**	-0.286	0.732**	-0.852**		-0.287	-0.333*
	Mannitol	-0.877**	0.042	0.760**	-0.684**		-0.585**	-0.597**
Yield RF-Del	PEG	0.944**	0.874**	0.133	0.588**	-0.352*		0.970**
	Mannitol	0.87**	0.154	-0.337*	0.431**	-0.702**		0.950**
Yield LI-Hyd	PEG	0.933**	0.859**	0.080	0.622**	-0.392*	0.780**	
	Mannitol	0.890**	0.062	-0.359*	0.390*	-0.712**	0.780**	

*&**Significant at 5% and 1% level, respectively; Del - Delhi; Hyd - Hyderabad; PHSI - Plant height stress index; RF - Rainfed; LI - Limited irrigation; PEG - Polyethylene glycol

and PHSI. The correlation of PHSI with yield under moisture stress was negative and significant. Grain yield under RF had positive correlation with the yield under LI. The same trend was observed even in genotypic correlation coefficients. Thus, germination percentage, root-shoot ratio and PHSI are important characters under moisture stress, could be used to screen the germplasm in laboratory for drought tolerance.

The results of the present study indicate the usefulness of germination under osmotic stress in screening of germplasm for drought tolerance in maize. These results are in agreement with the reports of earlier researchers in different crops [6-9, 11]. However, the results of Ashraf and Abu-Shakra [12] and Winter et al. [13] in wheat were in variance with our results and they concluded that the technique might not be suitable for the cultivars used in the study meaning there might be genotype specific response. The present investigation involved limited number of drought tolerant and drought susceptible genotypes and hence, in future, large number of resistant and tolerant, genotypes drawn from diverse sources should be utilized to evaluate the efficacy of this technique.

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