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IN VITRO SCREENING OF SUNFLOWER PRE-BREEDING LINES USING PEG-6000

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ABSTRACT

Seed germination is considered as the first and foremost fundamental life stages of a plant, which influences the final crop stand, growth and yield, especially under rainfed conditions. In the present study 40 pre-breeding lines of sunflower derived from *H. argophyllus* along with two checks *i.e* RSLP-14 and R-127-1 were screened for seedling traits such as germination percentage, root length, shoot length, seedling length and seedling vigour index for moisture stress under laboratory conditions using 4 levels of osmotic stress (-0.3 MPa, -0.6 MPa, -0.9 MPa, -1.2 MPa) with PEG-6000. Analysis of variance revealed highly significant differences for all five traits studied. Increase in PEG concentration reduced all traits however at the highest stress level (-1.2 MPa) few genotypes performed better than at lower concentrations such as PBM-8 sustained a high germination percentage (93%) while PBM-4 (4.22 cm) and PBM-6 (6.16 cm) recorded superior root length. In shoot length, PBM-1 (0.43 cm) and PBM-2 (0.45 cm) showed better performance, whereas PBM-1 (1.77 cm), PBM-4 (5.16 cm) and PBM-6 (7.16 cm) exhibited higher seedling length. Similarly, PBM-1 (124.06), PBM-6 (676.92) and PBM-18 (487.41) maintained higher seedling vigour index. Based on rank sum analysis PBM-35, PBM-8, PBM-23, PBM-6 and PBM-13 were identified as moisture stress tolerant genotypes in sunflower.

***Key words*:** Sunflower, pre-breeding lines, moisture stress, PEG-6000, seedling traits

Introduction

Drought is one of the most critical abiotic stresses limiting crop productivity, particularly in arid and semi-arid regions. Sunflower, a major oilseed crop contributing nearly 87% to global vegetable oil production (Razzaq *et al.*, 2015) is highly vulnerable to water scarcity. Uneven rainfall distribution and water shortage during its growth period cause severe reductions in achene and oil yield (Tahir *et al.*, 2002). Among the different growth stages, germination, seedling and flowering are most affected by drought, with germination being the most sensitive stage. Water deficit during this stage hampers seedling establishment, leading to poor crop performance. Identifying drought-tolerant accessions from existing germplasm istherefore, a crucial step towards developing tolerant varieties or hybrids. Although several screening methods have been employed to evaluate drought tolerance, field-based experiments are often constrained by variable environmental conditions, soil heterogeneity and the requirement for extensive time, labour and plant material. In contrast, laboratory-based screening offers a simpler and more controlled alternative. Polyethylene glycol (PEG-6000) is commonly used *in vitro* as a drought simulator, as it induces osmotic stress without penetrating plant tissues and effectively mimics drought conditions (Kaya *et al.*, 2006). The present study aimed to identify sunflower genotypes capable of withstanding moisture stress through laboratory-based germination tests using PEG-6000 as the stress-inducing agent.

Materials and Methods

In vitro screening provides a convenient and efficient approach to evaluate a large number of genotypes within limited space and time.PEG 6000 has been widely used to induce drought stress in plants, especially when it comes to early stages of their life cycle. A total of 40 pre-breeding lines of sunflower derived from *H. argophyllus* along with two checks *i.e.*, RSLP-14 and R-127-1 were screened by using chemical *i.e.*, Poly ethylene glycol (PEG-6000). The experiment was laid out in a factorial CRD with three replications, each containing 10 seeds. Four levels of osmotic stress -0.3 MPa, -0.6 MPa, -0.9 MPa and -1.2 MPa along with control were developed by dissolving 12.24g, 17.90g, 22.40g, 26.19g and 0g of PEG per 100ml of distilled water, respectively. Germination paper was completely soaked in respective PEG solutions and placed in Petri plates. Then uniform sized dry seeds are placed in petri plate and kept at room temperature for seven days. On the seventh day of treatment, the percentage of germination and morphology of the root and shoot was noted down from each replication of absolute control and PEG treated seeds for all the lines. For the statistical analyses, the OPSTAT statistical software package was employed as the analytical tool.

Observations were recorded for the following parameters

$$\text{Seed germination (\%)} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds kept for germination}} \times 100$$

Seedling length (cm) = Total length of shoot (cm) + Total length of root (cm)

Seedling Vigour Index-I = [Seedling length (cm) × Germination (%)]

Results and Discussion

The analysis of variance revealed highly significant differences among the 42 sunflower genotypes for all the five traits studied indicating the presence of substantial genetic variability under PEG-6000 induced osmotic stress. Significant genotype × treatment interactions for all traits indicated that the relative performance of genotypes varied across moisture stress levels, highlighting the importance of multi environment screening for reliable selection (Table 1).

Laboratory screening with polyethylene glycol (PEG) has been widely recognized as a reliable approach for imposing controlled moisture stress conditions (Geetha *et al.*, 2012). PEG acts solely as an external osmotic agent, it creates a consistent water-deficit environment that closely simulates drought without causing toxic effects on plant growth and development under controlled concentrations (Ivanova *et al.*, 2014). Increased PEG concentrations progressively lowers the water potential of the germination medium. The reduction in external water availability limits seed imbibition, slows activation of metabolic processes and consequently leads to a marked decline in germination percentage. Similarly, restricted water uptake reduces turgor pressure and inhibits cell division and elongation, resulting in shorter root and shoot growth, while the combined suppression of these traits substantially lowers the seedling vigour index. Genotypes that maintain comparatively higher germination, greater root and shoot elongation and higher vigour index under severe osmotic stress exhibits stronger osmotic adjustment and early-stage dehydration tolerance, making PEG-based screening an effective and rapid method for identifying drought-tolerant sunflower genotypes.

The mean performance of 42 sunflower genotypes for five different characters at different concentration of PEG -6000 is presented in Table 2.

Seed germination among the 42 sunflower genotypes varied from 100% under control conditions to 0% at -1.2 MPa. The mean germination percentage declined progressively from 93% in the control to 89%, 87%, 76% and 61% at -0.3, -0.6, -0.9 and -1.2 MPa respectively, indicating a strong inhibitory effect of osmotic stress on seed imbibition and metabolic activity. Under non-stress conditions, 28 genotypes showed significantly higher germination, which decreased to 24 at -0.3 MPa and 20 at -0.6 MPa. With increasing stress intensity (-0.9 MPa) only nine genotypes maintained significantly higher

Table 1: ANOVA for five different characters in 42 sunflower genotypes under laboratory evaluation using PEG-6000.

		Mean sum of squares				
Source of variation	**d.f**	**Germination percentage**	**Root length (cm)**	**Shoot length (cm)**	**Seedling length (cm)**	**Seedling vigour index-I**
Genotype	41	2174.71 **	55.21 **	9.25 **	98.84**	1124912.52 **
Treatment	4	21765.64 **	1459.48 **	1356.41 **	5451.35**	51324074.96 **
T × G	164	308.89**	15.19**	3.14 **	26.96**	257331.84 **
Error	420	21.43	6.83	1.15	12.31	104018

**Significant at 1 % probability

Table 2: Genotype × Treatment interaction effects for five traits by using different PEG-6000 concentrations.

Geno-types	Seed Germination (%)						Root length (cm)					
	Control	-0.3 MPa	-0.6 MPa	-0.9 MPa	-1.2 MPa	Mean	Control	-0.3 MPa	-0.6 MPa	-0.9 MPa	-1.2 MPa	Mean
PBM-1	97*	80	90	77	70	83	7.96	2.62	2.55	0.87	1.34	3.07
PBM-2	83	60	57	43	47	58	12.06*	1.73	1.08	0.87	0.51	3.25
PBM-3	83	97*	70	73	60	77	10.97	6.63	5.85	1.51	1.66	5.32
PBM-4	77	90	93*	80	67	81	6.68	21.58*	5.50	3.33	4.22	8.26
PBM-5	100*	97*	97*	93*	83	94	8.17	9.75	3.10	4.23	0.98	5.25
PBM-6	90	97*	87	90	93*	91	11.50*	10.68	6.20	5.12	6.16	7.93
PBM-7	97*	77	70	60	43	69	9.49	7.88	2.90	2.22	1.13	4.72
PBM-8	93*	93*	100*	87	93*	93	11.42*	18.90*	12.01*	6.70	1.09	10.02
PBM-9	90	93*	93*	97*	73	89	9.05	9.62	6.40	3.64	1.64	6.07
PBM-10	93*	87	87	87	63	83	9.20	7.71	6.38	4.02	1.57	5.78
PBM-11	97*	100*	80	83	47	81	14.65*	15.03*	10.73	4.24	0.50	9.03
PBM-12	87	93*	93*	77	60	82	8.90	7.82	3.93	2.99	1.23	4.97
PBM-13	100*	97*	97*	97*	83	95	11.50*	9.45	7.11	5.14	3.15	7.27
PBM-14	90	97*	87	90	83	89	6.27	3.89	3.22	2.42	2.79	3.72
PBM-15	83	73	50	57	37	60	6.95	6.61	3.75	2.83	0.00	4.03
PBM-16	97*	93*	100*	77	50	83	11.04	11.69*	8.09	6.04	2.06	7.78
PBM-17	93*	77	80	70	50	74	12.95*	5.29	1.94	1.71	1.53	4.68
PBM-18	97*	100*	93*	93*	93*	95	11.39*	9.01	10.47	3.93	4.69	7.90
PBM-19	90	83	83	43	40	68	12.51*	5.86	5.08	1.62	0.00	5.01
PBM-20	90	90	83	70	63	79	10.60	12.98*	5.10	2.49	4.06	7.05
PBM-21	97*	97*	93*	73	53	83	14.27*	5.51	2.23	0.87	1.07	4.79
PBM-22	100*	100*	93*	80	60	87	9.27	5.70	3.87	1.24	0.97	4.21
PBM-23	100*	100*	97*	90	97*	97	13.75*	14.14*	7.26	3.60	3.57	8.46
PBM-24	87	80	67	53	30	63	8.37	3.86	2.66	1.87	0.00	3.35
PBM-25	97*	80	83	50	53	73	5.77	2.11	1.75	0.97	0.76	2.27
PBM-26	97*	57	80	57	43	67	8.85	3.68	3.51	1.57	0.52	3.62
PBM-27	97*	100*	100*	93*	60	90	10.88	8.53	5.91	2.61	2.48	6.08
PBM-28	100*	100*	97*	80	63	88	9.35	7.11	6.14	6.79	3.39	6.56
PBM-29	97*	97*	100*	87	67	89	9.98	4.50	8.22	3.10	1.20	5.40
PBM-30	93*	83	80	70	43	74	7.65	2.38	3.10	2.12	0.57	3.16
PBM-31	97*	97*	83	77	47	80	13.35*	7.35	5.36	5.22	1.47	6.55
PBM-32	97*	93*	93*	83	43	82	13.42*	5.42	5.86	5.78	0.72	6.24
PBM-33	97*	87	100*	90	83	91	14.86*	3.73	5.45	4.50	1.33	5.97
PBM-34	100*	100*	100*	100*	93*	99	9.45	9.12	7.11	4.11	3.53	6.67
PBM-35	100*	100*	100*	93*	80	95	15.43*	12.55*	9.40	4.81	1.68	8.78
PBM-36	83	80	73	53	47	67	10.02	6.16	3.74	2.32	0.47	4.54
PBM-37	97*	97*	90	93*	60	87	9.89	8.92	3.78	3.68	1.30	5.51
PBM-38	100*	100*	100*	100*	87	97	9.14	9.36	10.41	6.44	2.24	7.52
PBM-39	80	73	83	70	70	75	8.46	8.51	7.07	4.23	2.65	6.18
PBM-40	97*	100*	97*	83	77	91	11.92*	15.14*	3.87	5.42	3.30	7.93
RSLP-14	100*	80	77	43	0	60	6.07	5.17	3.47	4.74	0.00	3.89
R-127-1	90	70	60	37	0	51	4.28	3.71	2.82	2.87	0.00	2.73
Mean	93	89	87	76	61	81	10.18	8.03	5.34	3.45	1.75	5.75

	S. E. m	C.D @ 1%	S. E. m	C.D @ 1 %
G	1.195	4.374	0.675	2.470
T	0.412	1.509	0.233	0.852
G×T	2.673	9.780	1.509	5.523

MPa- Mega pascal; *Significant at 1 % probability

Continue ...2

Geno-types	Shoot length (cm)						Seedling length (cm)					
	Control	-0.3 MPa	-0.6 MPa	-0.9 MPa	-1.2 MPa	Mean	Control	-0.3 MPa	-0.6 MPa	-0.9 MPa	-1.2 MPa	Mean
PBM-1	7.83*	2.43	1.39	0.37	0.43	2.49	15.79	5.05	3.94	1.24	1.77	5.56
PBM-2	9.06*	1.15	0.59	0.19	0.45	2.29	21.11*	2.87	1.67	1.06	0.95	5.54
PBM-3	8.69*	3.30	1.83	0.60	0.25	2.93	19.66*	9.93	7.67	2.11	1.90	8.26
PBM-4	4.44	6.35*	1.93	1.24	0.94	2.98	11.12	27.93*	7.43	4.56	5.16	11.24
PBM-5	8.40*	3.71	1.26	2.57	0.50	3.29	16.57*	13.46	4.36	6.80	1.48	8.53
PBM-6	10.43*	5.48*	3.08	1.40	1.00	4.28	21.93*	16.16	9.28	6.52	7.16	12.21
PBM-7	9.23*	3.35	1.18	0.57	0.28	2.92	18.73*	11.23	4.08	2.79	1.42	7.65
PBM-8	11.46*	7.39*	4.02	1.43	0.37	4.93	22.87*	26.30*	16.03	8.13	1.45	14.96
PBM-9	9.75*	4.40	1.91	1.20	0.28	3.50	18.79*	14.02	8.30	4.83	1.91	9.57
PBM-10	9.93*	4.22	1.96	1.03	0.32	3.49	19.13*	11.93	8.34	5.05	1.89	9.27
PBM-11	6.70*	4.35	2.25	0.64	0.07	2.80	21.34*	19.39*	12.99	4.88	0.58	11.83
PBM-12	8.21*	2.65	0.88	0.39	0.21	2.47	17.11*	10.47	4.81	3.37	1.44	7.44
PBM-13	11.64*	4.61	2.45	0.71	0.38	3.96	23.14*	14.06	9.56	5.85	3.53	11.23
PBM-14	8.29*	3.06	1.29	0.66	0.44	2.75	14.56	6.95	4.51	3.08	3.23	6.46
PBM-15	5.23	3.19	1.25	0.48	0.00	2.03	12.18	9.80	5.00	3.31	0.00	6.06
PBM-16	11.33*	6.89*	2.80	0.92	0.12	4.41	22.37*	18.58*	10.89	6.96	2.18	12.20
PBM-17	9.20*	2.53	0.62	0.18	0.17	2.54	22.15*	7.82	2.55	1.88	1.69	7.22
PBM-18	7.93*	4.93	3.05	0.71	0.46	3.42	19.32*	13.95	13.52	4.64	5.15	11.32
PBM-19	11.33*	2.78	1.32	0.16	0.00	3.12	23.84*	8.64	6.40	1.79	0.00	8.13
PBM-20	7.18*	5.22	1.75	0.38	0.35	2.98	17.78*	18.21*	6.85	2.87	4.40	10.02
PBM-21	8.04*	3.31	0.96	0.35	0.09	2.55	22.31*	8.82	3.19	1.22	1.15	7.34
PBM-22	6.80*	3.08	1.13	0.43	0.12	2.31	16.07	8.79	5.00	1.68	1.09	6.52
PBM-23	10.35*	5.13	2.77	1.21	0.54	4.00	24.10*	19.27*	10.02	4.81	4.11	12.46
PBM-24	6.68*	2.29	0.99	0.43	0.00	2.08	15.05	6.15	3.65	2.29	0.00	5.43
PBM-25	6.45*	1.36	0.86	0.30	0.18	1.83	12.22	3.47	2.61	1.27	0.94	4.10
PBM-26	7.66*	2.07	1.61	0.49	0.11	2.39	16.51*	5.75	5.13	2.06	0.63	6.01
PBM-27	9.72*	5.06	1.94	0.72	0.35	3.56	20.60*	13.59	7.85	3.33	2.83	9.64
PBM-28	9.28*	4.40	2.19	1.05	0.47	3.48	18.63*	11.52	8.33	7.84	3.87	10.04
PBM-29	8.86*	2.93	2.59	0.69	0.33	3.08	18.84*	7.43	10.80	3.78	1.53	8.48
PBM-30	6.47*	1.75	1.16	0.42	0.16	1.99	14.12	4.13	4.27	2.54	0.73	5.16
PBM-31	7.09*	2.99	1.40	1.09	0.33	2.58	20.44*	10.34	6.75	6.31	1.80	9.13
PBM-32	6.68*	1.99	1.61	0.92	0.12	2.26	20.11*	7.42	7.47	6.70	0.83	8.50
PBM-33	12.36*	4.42	2.79	1.32	0.42	4.26	27.22*	8.14	8.24	5.81	1.75	10.23
PBM-34	11.87*	5.63*	2.87	1.17	0.61	4.43	21.32*	14.75	9.99	5.28	4.14	11.10
PBM-35	10.50*	5.90*	3.21	1.10	0.38	4.22	25.93*	18.45*	12.62	5.91	2.06	12.99
PBM-36	7.40*	2.86	1.01	0.32	0.16	2.35	17.42*	9.02	4.75	2.64	0.63	6.89
PBM-37	8.06*	3.99	1.98	1.20	0.27	3.10	17.95*	12.90	5.76	4.88	1.57	8.61
PBM-38	7.09*	5.02	3.48	1.30	0.45	3.47	16.23	14.38	13.89	7.75	2.69	10.99
PBM-39	8.14*	4.77	2.77	0.83	0.46	3.39	16.60*	13.28	9.84	5.06	3.10	9.58
PBM-40	7.31*	5.86*	1.37	0.93	0.46	3.19	19.23*	21.00*	5.24	6.35	3.76	11.12
RSLP-14	4.86	3.12	1.85	1.86	0.00	2.34	10.93	7.70	5.94	8.57	0.00	6.63
R-127-1	4.65	2.71	1.70	0.65	0.00	1.94	8.93	6.08	5.14	3.27	0.00	4.68
Mean	8.39	3.87	1.88	0.82	0.31	3.06	18.58	11.88	7.25	4.31	2.06	8.82

	S. E. m	C.D @ 1%	S. E. m	C.D @ 1 %
G	0.277	1.012	0.906	3.31
T	0.095	0.349	0.313	1.14
G×T	0.619	2.264	2.026	7.41

MPa- Mega pascal; *Significant at 1 % probability

Continue ...2

Genotypes	Seedling vigour Index-I					
	Control	-0.3 MPa	-0.6 MPa	-0.9 MPa	-1.2 MPa	Mean
PBM-1	1539.46*	403.63	354.37	96.98	124.06	503.70
PBM-2	1762.79*	172.40	100.40	50.20	42.95	425.75
PBM-3	1635.09*	985.46	536.95	148.36	114.10	683.99
PBM-4	852.86	2513.69*	683.64	365.07	336.38	950.33
PBM-5	1656.67*	1288.60	427.19	643.84	124.17	828.09
PBM-6	1973.61*	1581.11*	811.26	587.01	676.92	1125.98
PBM-7	1813.24*	877.47	285.26	167.10	66.16	641.85
PBM-8	2127.83*	2480.50*	1603.33*	686.50	116.27	1402.89
PBM-9	1691.24*	1307.68	774.00	468.38	143.88	877.04
PBM-10	1773.91*	1038.40	733.15	430.61	115.49	818.31
PBM-11	2074.07*	1938.57*	1038.97	414.02	28.27	1098.78
PBM-12	1481.31*	959.78	450.61	262.35	86.40	648.09
PBM-13	2314.29*	1372.60	927.45	557.55	291.93	1092.76
PBM-14	1309.93	677.84	388.11	276.84	266.77	583.90
PBM-15	1011.94	727.10	250.05	186.48	0.00	435.12
PBM-16	2157.31*	1731.13*	1089.05	536.19	109.08	1124.55
PBM-17	2060.29*	623.08	204.34	131.78	84.65	620.83
PBM-18	1865.66*	1394.76	1270.68	441.44	487.41	1091.99
PBM-19	2145.11*	707.32	543.13	75.46	0.00	694.20
PBM-20	1599.97*	1638.55*	588.36	201.02	273.25	860.23
PBM-21	2151.55*	853.01	293.65	87.63	66.75	690.52
PBM-22	1607.14*	878.57	470.39	134.00	65.45	631.11
PBM-23	2410.00*	1927.14*	967.27	432.44	411.43	1229.66
PBM-24	1276.28	491.95	244.00	131.13	0.00	428.67
PBM-25	1169.24	277.39	219.42	63.33	50.40	355.96
PBM-26	1607.21*	345.02	410.03	108.92	26.70	499.58
PBM-27	2001.02*	1359.05	785.24	310.23	169.70	925.05
PBM-28	1863.33*	1151.90	812.70	627.33	245.97	940.25
PBM-29	1830.62*	732.83	1080.48	334.41	106.23	816.91
PBM-30	1322.07	356.22	341.34	177.62	34.67	446.38
PBM-31	1972.20*	997.99	560.18	473.54	85.70	817.92
PBM-32	1967.42*	678.46	685.50	550.11	41.67	784.63
PBM-33	2625.02*	709.11	823.81	522.89	146.96	965.56
PBM-34	2132.38*	1475.24	998.57	528.10	388.99	1104.65
PBM-35	2592.86*	1845.24*	1261.90	548.82	164.99	1282.76
PBM-36	1439.54	721.92	334.99	154.67	28.03	535.83
PBM-37	1749.81*	1265.71	517.94	456.85	94.00	816.86
PBM-38	1622.86*	1437.62	1388.57	774.76	237.42	1092.25
PBM-39	1327.91	995.52	826.42	354.17	217.28	744.26
PBM-40	1872.25*	2099.52*	515.68	528.11	294.77	1062.07
RSLP-14	1092.86	615.68	467.99	373.48	0.00	510.00
R-127-1	803.25	425.89	308.40	130.67	0.00	333.64
Mean	1744.84	1096.68	651.78	345.96	151.55	798.16
	S. E. m			C.D @ 1%		
G	83.274			304.732		
T	28.732			105.142		
G×T	186.206			681.401		

MPa- Mega pascal; *Significant at 1 % probability germination. Even at the most severe stress level (-1.2 MPa), five genotypes namely PBM-23 (97%), PBM-6, PBM-8, PBM-18 and PBM-34 (93%) exhibited superior germination, indicating their inherent tolerance to moisture stress during early seedling establishment (Plate 1).

Root length across the 42 genotypes declined sharply from a mean of 10.18cm under control to 8.03, 5.34, 3.45 and 1.75cm at -0.3, -0.6, -0.9 and -1.2MPa, respectively. Although 15 genotypes exhibited significantly higher root length under control, only PBM-8 maintained a significantly superior root length (12.01 cm) at -0.6 MPa, and no genotype performed well at higher stress levels. Shoot length showed an even stronger reduction, ranging from 12.36 cm in control to 0 cm at -1.2 MPa, while 38 genotypes performed well under control, only seven genotypes retained higher shoot length at -0.3 MPa, and none showed superiority beyond -0.6 MPa. These reductions emphasize the pronounced inhibitory effect of osmotic stress on elongation growth, attributed to restricted cell division and expansion in both roots and shoots, with shoots being more sensitive.

Seedling length among 42 sunflower genotypes declined progressively with increased PEG concentration, ranging from 27.93cm at -0.3 MPa to 0cm at -1.2 MPa. Mean seedling length decreased from 18.58cm (control) to 2.06cm (-1.2 MPa). Under control conditions 31 genotypes exhibited significantly higher seedling length whereas at -0.3 MPa, eight genotypes maintained significantly higher values with PBM-4 (27.93cm), PBM-8 (26.3cm), PBM-40 (21cm), PBM-11 (19.39cm) and PBM-23 (19.27cm) being the highest (Plate 2).

Seedling Vigour Index-I across the genotypes ranged from 2625.02 (PBM-33) under control to 0 at -1.2 MPa. In control, 32 genotypes exhibited significantly higher values whereas at -0.3MPa, nine genotypes retained higher vigour index. At -0.6MPa, only PBM-8 (1603.33) maintained significantly higher vigour index, whereas at -0.9 MPa and -1.2 MPa, none of the genotypes showed a significant difference for this trait. Singh and Singh (1983) reported that decline in seedling vigour was due to fall in mobilization of reserves to plumule thus preventing their growth under stress.

Although a general reduction in germination and seedling traits was observed with increasing PEG concentration, a few genotypes exhibited comparatively superior performance under higher osmotic stress levels than at lower concentrations. Notably, PBM-6 (93%), PBM-8 (93%) and PBM-23 (97%) maintained higher germination percentages at the highest PEG level. Similarly, PBM-1 (1.34 cm), PBM-4 (4.22 cm), PBM-6 (6.16 cm) and PBM-20 (4.06 cm) recorded greater root length, while PBM-1 (1.77 cm), PBM-4 (5.16 cm), PBM-6 (7.16 cm) and PBM-18 (5.15 cm) exhibited higher seedling length under severe stress conditions. In terms of seedling vigour index, PBM-1 (124.06), PBM-6 (676.92), PBM-18 (487.41) and PBM-20 (273.25) showed relatively higher values. Such genotype-specific responses under severe osmotic stress may be attributed to stress-induced activation of adaptive mechanisms, including enhanced mobilization of seed reserves, improved osmotic adjustment and sustained root growth.

The experimental results obtained through *in vitro* screening of sunflower pre-breeding lines are in parallel with Ahmad *et al*., (2009), Saensee *et al*., (2012), Geetha *et al*., (2012), Santhosh *et al*., (2014), Vassilevska-Ivanova *et al*., (2014), Razzaq *et al*., (2017), Sheshaiah *et al*., (2017), Clapco *et al*., (2018), Praveen *et al*., (2021) and Vikas *et al*., (2023) indicating the effectiveness of PEG-6000 in screening sunflower genotypes for moisture stress. These findings confirm the effectiveness of PEG-6000 as a rapid and reliable laboratory-based screening tool for identifying drought-tolerant sunflower genotypes at the early seedling stage. PEG-based *in vitro* screening allows large numbers of genotypes to be evaluated quickly, economically and within limited space, making it a more efficient and reliable preliminary screening method than field testing.

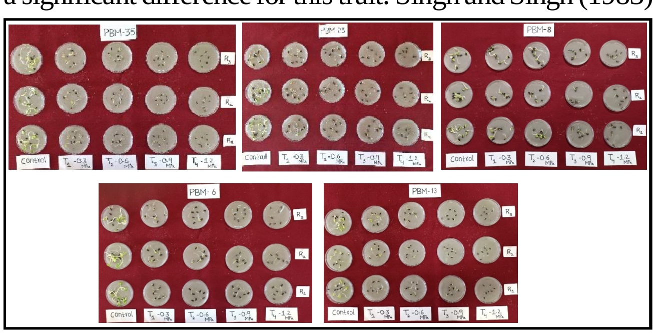

Plate 1: Performance of promising sunflower genotypes based on germination per cent under different PEG-6000 concentrations.

Plate 2: Performance of promising sunflower genotypes based on seedling characters under different PEG-6000 concentrations.

Conclusion

Generally, germination percent, root length, shoot length, seedling length and seedling Vigour Index-I reduced with increase in osmotic stress, however few genotypes performed better at higher stress levels. PBM-8 maintained significant root length and seedling Vigour Index-I even at -0.9 MPa. Based on rank sum analysis PBM-35, PBM-8, PBM-23, PBM-6 and PBM-13 were identified as drought tolerant at the seedling stage, reflecting strong osmotic stress resilience.

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