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In vitro screening of sunflower genotypes for stress tolerance using PEG-6000

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Abstract
Productivity of sunflower under moisture stress is primarily determined by seed germination and the seedling vigour for appropriate crop stand. Screening of genotypes for moisture stress under field has uncertainties due to the uncontrolled conditions, interaction of biotic and abiotic stresses and variability in environmental factors. Hence, in the present investigation, response of seventeen sunflower genotypes to moisture stress at germination and seedling stage was recorded by using polyethylene glycol (PEG-6000) as drought stimulator under laboratory conditions. Four levels of osmotic stress (-0.3 MPa, -0.6 MPa, - 0.9 MPa, -1.2 MPa) were created and performances were monitored against the control. All genotypes recorded reduced seedling growth parameters with increasing osmotic stress. However, five genotypes (RSLP-41, RSLP-14, CMS-104 B, RSLP-24 and RSLP-33) showed seed germination and root length even at -0.9 MPa where all other genotypes did not germination at this higher concentration of PEG-6000 indicating moisture tolerance. However, at -1.2 MPa concentration none of the genotypes survived this indicates the lethality of PEG dose.

Keywords: Sunflower, moisture stress, PEG-6000, seedling traits

1. Introduction

Sunflower is crop of tropical and subtropical regions with semi-arid to arid climate and frequently grown in dry lands or on supplementary irrigation. Since it is a photo insensitive crop, cultivated primarily as a rainfed crop both in *Kharif* and with irrigation in residual soil moisture *rabi* seasons and hence the crop is influenced by several weather factors both biotic stress and abiotic stress such as heat, drought and other abiotic stresses (Robert *et al.*, 2016) [8]. Among abiotic stress drought is the one of the most limiting of all abiotic stress as it causes more than 70 percent reduction in biomass and seed yield in sunflower (Ravi Shanker, 1991) [7]. In India, the major area is under rainfed cultivation. The crop experiences severe moisture stress during flowering and terminal growth stages. In order to overcome moisture stress at such critical stages of crop, along with water management technologies there is need identify the moisture stress tolerant genotypes at germination stage itself as it decides the optimum plant population which intern helps to harvest better yields. However, uncontrolled conditions, heterogeneity in soil, huge amount of plant material, time and labour experiments in the field, makes the screening experiments more difficult. Hence laboratory experiments are more reliable and easier to conduct as compared to field trials. Germination stage is an important stage to maintain adequate population for obtaining prominent yield. Germinating the seeds in media with different water potentials is a convenient method. Under laboratory Polyethylene glycol (PEG-6000) was used to model the *in vitro* osmotic stress effects and; is usually used as drought simulator as it is considered as non-penetrable, harmless and best way to create osmotic stress without causing any toxicity to the plant cells (Praveen *et al.*, 2021) [6]. The PEG, with draws water from the cell without entering into the apoplast and therefore, PEG stress mimics the dry soil. Hence, different PEG concentrations can be effectively used for screening genotypes to drought tolerance under laboratory condition.

The present investigation was mainly to identify genotypes which can grow under moisture stress conditions through germination test under laboratory using moisture stress inducing agent PEG-6000.

2. Materials and Methods

In vitro screening of genotypes is an alternate and easy method to screen large number of genotypes with limited space and time. A total of 17 sunflower genotypes which includes four maintainer lines, four restorer lines, five inbred and four hybrids were screened by using chemical *i.e.*, Poly ethylene glycol (PEG-6000). Many studies indicated that PEG-6000 is water stress inducing agent it is the best to screen genotypes for drought stress tolerance under laboratory conditions (Geetha *et al.*, 2012) [4]. Polyethylene glycol with a molecular weight of 6000 (PEG-6000) was used as a drought simulator and four levels of osmotic stress are -0.3 MPa, -0.6 MPa, -0.9 MPa, and -1.2 MPa were developed by dissolving 12.24 g, 17.90 g, 22.40 g and 26.19g of PEG per 80 ml distilled water respectively are against the control and two replications were maintained for each osmotic stress in two factorial-CRD fashion. Germination paper was completely soaked in respective PEG solutions and placed in the petri plate. Then uniform sized dry seeds (10 No's) were placed in the petri plate and kept at room temperature for seven days. PEG solutions were applied to every plate once in three days after draining out the previously applied solution. On the 7th 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 of all genotypes of sunflower seedlings.

2.1 Observations were recorded for the following parameters by using the formulae.

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

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

Seed vigor = [Seedling length (cm)× Seed germination (%)]

$$\text{Root length stress tolerance index} = \frac{\text{Root length of stressed plants}}{\text{Root length of control plants}} \times 100$$

$$\text{Seedling length stress tolerance index} = \frac{\text{Seedling length of stressed plant}}{\text{Seedling length of control plant}} \times 100$$

3. Results and Discussion

Analysis of variance revealed that the mean sum of squares due to treatments, genotypes and genotypes × treatment interactions were highly significant for all the traits studied, reflecting the presence of substantial variability indicating genotypes involved in study and different treatments imposed are quite diverse to test set of genotypes. The interaction effects of the genotypes used were screened by using different concentrations PEG at -0.3 MPa, -0.6 MPa, -0.9 MPa and -1.2 MPa respectively along with the control. Seven traits studied showed significant differences at different concentration of PEG. However, none of the genotypes did survive at -1.2 MPa concentration, indicating the lethality of PEG dose.

Seed germination is a critical phenological step in plant life. If germination occurs under inappropriate environmental conditions, it may not permit the development of a fertile plant and in turn, the survival of the species. This suggests that the tolerance to water stress at the germination stage is conferred by the maternal genetic environment during seed development and that it is conserved across the different life stages of the plant. On a practical point of view, this also suggests that germination assays involving seed and seedling traits in osmotic conditions could be used as a proxy for evaluating plant drought tolerance in breeding programmes. Higher water retention was observed in roots than in leaves (Vassilevska-Ivanova *et al.*, 2016) [13]. Since roots are the first part of the plant exposed to stress conditions, their reaction is associated with tolerance to abiotic stress. Osmotic stress also decreased root and shoots length. The water content in plant roots and leaves has been shown to be associated with drought tolerance in cultivated sunflower. The effect of PEG-6000 at different levels of concentrations has marked impact on the some of the traits like seed germination, root length and shoot length which can correlated and validated with drought tolerance in plants.

3.1 Seed germination

Effect of PEG-6000 concentrations induced stress on seed germination in sunflower genotypes presented in Table 1. In the control, all the genotypes showed more than 80 percent germination. As the concentration of PEG increased the seed germination percentage decreased across all genotypes. At control and -0.3 MPa all the genotypes have recorded significant seed germination percentage. At -0.6 MPa of PEG -6000, out of 17 genotypes only six genotypes *viz.*, RSLP-41 (85.00%), CMS -104B (80.00%), RSLP-14 (80.00%), RSLP-24 (77.50%), RSLP-33 (75.00%) and R-630 (75.12%) recorded highest seed germination percentage. Whereas, as the concentration of PEG increased from -0.6 Mpa to -0.9 Mpa, the seeds of genotype R-630 did not germinate, however still five genotypes showed good levels of seed germination percentage *i.e.*, CMS-104B (10.00%), RSLP-14 (10.00%), RSLP-33 (15.00%), RSLP-24 (20.00%) and RSLP-41 (20.00%). However, none of the genotypes did survive at -1.2 MPa concentration, indicating the lethality of PEG dose. The majority of the genotypes showed decreased the germination percent with increased concentrations of PEG-6000 compared to control. This may be due to reduction in imbibition, oxygen uptake and hydrolysis of food reserves (Toosi *et al*., 2014) [12]. Similar results are observed with reports of Vassilevska-Ivanova *et al*. (2016) [13], Praveen *et al*. (2021) [6], Santhosh *et al.* (2018) [10] germination percentage decreased with increase in osmotic stress.

3.2 Root length and Shoot length

The root length and shoot length varied among the genotypes ranged from 0 to 11.75 cm and 0 to 7.01cm respectively. However, at -0.6 MPa concentration only seven genotypes exhibited significant differences for root length and shoot length *viz.*, KBSH-78, RSLP-33, RSLP-14, PM-81, R-630, RSLP-41 and RSLP-24. Substantial decrease in root length and shoot length was resulted from the PEG induced water deficit in all the genotypes when compared to control. This reduced root length and shoot length is due to prominent effect of water stress was shown to be on shoot growth as compared to the root growth, reduced cell division and enlargement in the root and shoot growing region and ultimately reduced root growth during seed germination under stress condition (Muscolo *et al*. (2013) [5].

3.3 Seedling length and Seed vigor

The seedling length varied among 17 genotypes which are ranged from 0 cm to 17.41 cm. whereas seed vigor ranged from 0 to 1741 among the genotypes tested. At -0.6 MPa concentration six genotypes have shown significant difference for this trait *viz.*, KBSH -78, RSLP-33, RSLP-14, PM-81, RSLP-41 and RSLP-24. The reduction in seedling length and seed vigour under increased osmotic stress could be due to fall in mobilization of reserves to plumule thus preventing seedling growth. Whereas the seed vigour which might be due to the result of membrane rupture, which might have resulted in loss in seedling vigor. (Singh and Singh, 1983) [11].

3.4 Root length stress tolerance index (RLSTI) and Seedling length stress tolerance index (SLSTI)

The root length stress tolerance index and seedling length stress tolerance index varied from among the genotypes ranged from 0 to 98.50 and 0 to 87.90 respectively. At -0.6 MPa of PEG-6000 only eleven genotypes showed significant difference for RLSTI and SLSTI *viz.*, RSLP- 14, RSLP-33, KBSH -78, PM-81, RSLP-24, PM-66, RSLP-41, CMS-38B, RSFH-700, R-630 and RSFH-1887 (Fig-2). The root length stress tolerance index and seedling length stress tolerance index decreased with increased PEG-6000 concentrations, these results accordance with reports of Ahmad *et al.* (2009) [1] and Saense *et al.* (2012) [9].

Table 1: Interaction effects of Genotype and Treatment for seven seedling traits by using different concentrations of PEG-6000

Genotype	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
01. CMS-104B	100.00*	95.00*	80.00*	10.00	0.00	57.00	5.06*	3.12*	0.73	0.10	0.00	1.8
02. CMS-105B	97.50*	94.00*	5.00	0.00	0.00	39.30	4.07*	3.24*	0.03	0.00	0.00	1.47
03. CMS-107B	99.00*	96.50*	40.00	0.00	0.00	47.10	5.41*	3.23*	0.74	0.00	0.00	1.88
04. CMS-38B	100.00*	96.50*	30.00	0.00	0.00	45.30	4.93*	3.60*	1.43	0.00	0.00	1.99
05. R-630	100.00*	80.00*	75.00*	0.00	0.00	50.00	6.22*	4.21*	2.43*	0.00	0.00	2.77
06. R-127-1	100.00*	15.00	10.00	0.00	0.00	25.00	2.86*	0.67	0.25	0.00	0.00	0.76
07. PM-81	100.00*	85.00*	45.00	0.00	0.00	46.00	6.99*	5.59*	3.74*	0.00	0.00	3.26
08. RGM-49	95.00*	90.00*	25.00	0.00	0.00	42.00	6.15*	5.06*	0.44	0.00	0.00	2.33
09. PM-66	92.50*	90.00*	35.00	0.00	0.00	43.50	4.63*	4.06*	1.56	0.00	0.00	2.05
10. RSLP-14	95.00*	85.00*	80.00*	10.00	0.00	54.00	6.56*	5.65*	5.14*	0.15	0.00	3.5
11. RSLP-24	100.00*	90.00*	80.00*	20.00	0.00	57.50	10.34*	5.01*	3.43*	0.28	0.00	3.61
12. RSLP-33	100.00*	85.00*	77.50*	15.00	0.00	57.50	6.58*	5.68*	6.15*	0.24	0.00	3.73
13. RSLP-41	100.00*	95.00*	85.00*	20.00	0.00	60.00	11.75*	7.54*	3.49*	0.43	0.00	4.64
14. RSFH-1887	100.00*	77.50*	15.00	0.00	0.00	38.50	4.58*	4.42*	1.08	0.00	0.00	2.02
15. KBSH-44	100.00*	82.50*	0.00	0.00	0.00	36.50	4.66*	2.96*	0.00	0.00	0.00	1.52
16. RSFH-700	100.00*	82.50*	30.00	0.00	0.00	42.50	5.80*	1.84*	1.13	0.00	0.00	1.75
17. KBSH-78	100.00*	77.50*	55.00	0.00	0.00	46.50	11.48*	10.03*	6.61*	0.00	0.00	5.62
Mean	98.77	83.35	45.15	4.41	0.00	46.34	6.35	4.46	22.58	0.07	0.00	2.63
G X T	46.34						2.63					
S. Em ±	1.81						0.25					
C.D	11.43						0.71					

*Indicates the genotypes have shown significance difference for the particular traits

Genotype	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
01. CMS-104B	3.26*	1.51*	0.62	0.00	0.00	1.08	8.32*	4.63*	1.35	0.10	0.00	2.88
02. CMS-105B	3.16*	1.68*	0.00	0.00	0.00	0.97	7.23*	4.92*	0.03	0.00	0.00	2.44
03. CMS-107B	2.88*	1.67*	0.57	0.00	0.00	1.02	8.29*	4.90*	1.31	0.00	0.00	2.90
04. CMS-38B	2.79*	1.75*	0.19	0.00	0.00	0.95	7.72*	5.35*	1.62	0.00	0.00	2.94
05. R-630	2.70*	2.95*	0.67	0.00	0.00	1.33	8.92*	7.16*	3.10	0.00	0.00	4.10
06. R-127-1	3.07*	0.64	0.15	0.00	0.00	0.77	5.93*	1.31	0.40	0.00	0.00	1.53
07. PM-81	4.63*	3.45*	2.13*	0.00	0.00	2.04	11.62*	9.04*	5.70*	0.00	0.00	5.27
08. RGM-49	7.01*	2.83*	0.35	0.00	0.00	2.04	13.16*	7.89*	0.79	0.00	0.00	4.37
09. PM-66	5.61*	3.07*	0.99	0.00	0.00	1.93	10.24*	7.13*	2.55	0.00	0.00	3.98
10. RSLP-14	5.44*	2.08*	1.85	0.05	0.00	1.88	12.00*	7.73*	6.99*	0.20	0.00	5.38
11. RSLP-24	5.08*	1.79*	0.99	0.16	0.00	1.54	15.42*	6.80*	4.42*	0.44	0.00	5.15
12. RSLP-33	5.74*	2.78*	1.48*	0.12	0.00	2.02	12.32*	8.46*	7.63*	0.36	0.00	5.75
13. RSLP-41	4.09*	1.93*	1.99*	0.15	0.00	1.63	15.84*	9.47*	5.48*	0.58	0.00	6.27
14. RSFH-1887	5.36*	4.24*	0.55	0.00	0.00	2.03	9.94*	8.66*	1.63	0.00	0.00	4.05
15. KBSH-44	4.09*	2.91*	0.00	0.00	0.00	1.40	8.75*	5.87*	0.00	0.00	0.00	2.92
16. RSFH-700	5.17*	2.56*	0.00	0.00	0.00	1.55	10.97*	4.40*	1.13	0.00	0.00	3.30
17. KBSH-78	5.93*	3.65*	1.03*	0.00	0.00	2.12	17.41*	13.68*	7.64*	0.00	0.00	7.75
Mean	4.47	2.44	0.80	0.03	0.00	1.55	10.82	6.90	3.04	0.09	0.00	4.17
G X T	1.55						4.17					
S. Em ±	0.15						0.34					
C.D	0.42						0.98					

*Indicates the genotypes have shown significance difference for the particular traits

Genotype	Seed Vigour						Root Stress Tolerance Index (%)					
	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
01.CMS-104B	832.00*	441.20*	108.00	1.00	0.00	276.44	100	63.00*	15.80	1.87	0.00	16.14
02.CMS-105B	703.60*	462.42*	0.30	0.00	0.00	233.26	100	80.00*	0.73	0.00	0.00	16.14
03.CMS-107B	821.12*	473.24*	57.90	0.00	0.00	270.45	100	60.00*	10.30	0.00	0.00	14.07
04.CMS-38B	772.00*	517.01*	55.00	0.00	0.00	268.80	100	73.50*	28.18*	0.00	0.00	20.33
05. R-630	892.00*	572.80*	240.15	0.00	0.00	363.68	100	69.00*	26.65*	0.00	0.00	23.55
06. R-127-1	593.00*	21.60	4.00	0.00	0.00	123.72	100	27.00*	9.10	0.00	0.00	7.22
07. PM-81	1162.00*	759.70*	264.30	0.00	0.00	437.20	100	82.00*	55.65*	0.00	0.00	27.53
08. RGM-49	1242.00*	710.10*	19.00	0.00	0.00	394.22	100	74.50*	7.05	0.00	0.00	16.31
09. PM-66	946.20*	641.70*	114.60	0.00	0.00	340.50	100	89.50*	36.99*	0.00	0.00	25.29
10. RSLP-14	1200.00*	657.40*	561.05*	2.00	0.00	484.09	100	92.00*	84.94*	2.35	0.00	35.85
11. RSLP-24	1542.00*	612.80*	353.60*	8.80	0.00	480.75	100	49.00*	48.77*	2.60	0.00	15.65
12. RSLP-33	1232.00*	719.90*	572.80*	5.90	0.00	506.12	100	95.99*	87.50*	3.40	0.00	37.38
13. RSLP-41	1584.00*	900.10*	466.80*	14.80	0.00	593.14	100	66.00*	33.49*	3.55	0.00	20.6
14. RSFH-1887	994.00*	671.30*	25.00	0.00	0.00	338.06	100	98.50*	23.92*	0.00	0.00	24.48
15. KBSH-44	875.00*	484.35*	0.00	0.00	0.00	271.87	100	63.50*	0.00	0.00	0.00	12.70
16. RSFH-700	1097.00*	362.80*	37.80	0.00	0.00	299.52	100	31.50*	27.39*	0.00	0.00	11.77
17. KBSH-78	1741.20*	1060.85*	421.00*	0.00	0.00	644.61	100	88.00*	58.93*	0.00	0.00	29.38
Mean	1,072.30	592.31	194.19	1.91	0.00	372.14	100	70.76	32.68	0.81	0.00	26.06
G X T	372.14						20.85					
S. Em ±	31.39						3.17					
C.D	88.43						8.95					

Genotype	Seedling Length Stress Tolerance Index (%)					
	Control	-0.3 MPa	-0.6 MPa	-0.9 MPa	-1.2 MPa	Mean
01. CMS-104B	100	56.22*	16.77*	1.07	0.00	14.81
02. CMS-105B	100	68.56*	0.43	0.00	0.00	13.79
03. CMS-107B	100	59.84*	12.23*	0.00	0.00	14.41
04. CMS-38B	100	70.57*	19.34*	0.00	0.00	17.98
05. R-630	100	80.97*	20.36*	0.00	0.00	26.17
06. R-127-1	100	23.82*	7.83	0.00	0.00	6.33
07. PM-81	100	78.00*	51.87*	0.00	0.00	25.97
08. RGM-49	100	61.61*	5.45	0.00	0.00	13.40
09. PM-66	100	70.73*	24.80*	0.00	0.00	19.10
10. RSLP-14	100	64.99*	58.70*	1.66	0.00	25.07
11. RSLP-24	100	44.45*	49.88*	2.60	0.00	13.48
12. RSLP-33	100	69.33*	62.35*	2.67	0.00	26.87
13. RSLP-41	100	62.09*	35.82*	3.85	0.00	20.35
14. RSFH-1887	100	87.90*	16.68*	0.00	0.00	20.91
15. KBSH-44	100	67.34*	0.00	0.00	0.00	13.46
16. RSFH-700	100	40.37*	10.00*	0.00	0.00	10.08
17. KBSH-78	100	80.02*	44.81*	0.00	0.00	24.96
Mean	100	63.93	25.73	0.68	0.00	18.07
G X T	18.07					
S. Em ±	2.88					
C.D	8.12					

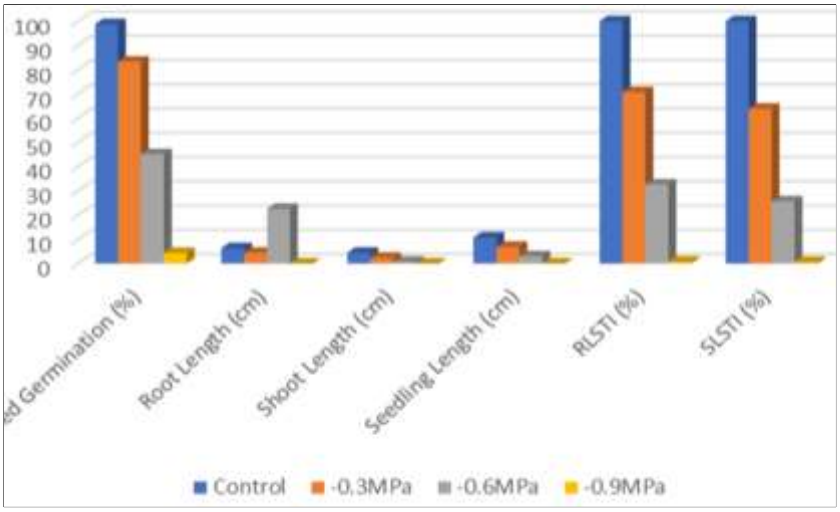

Fig 1: Seed germination percentage and seedling traits of sunflower seedlings incubated in different concentration of PEG-6000

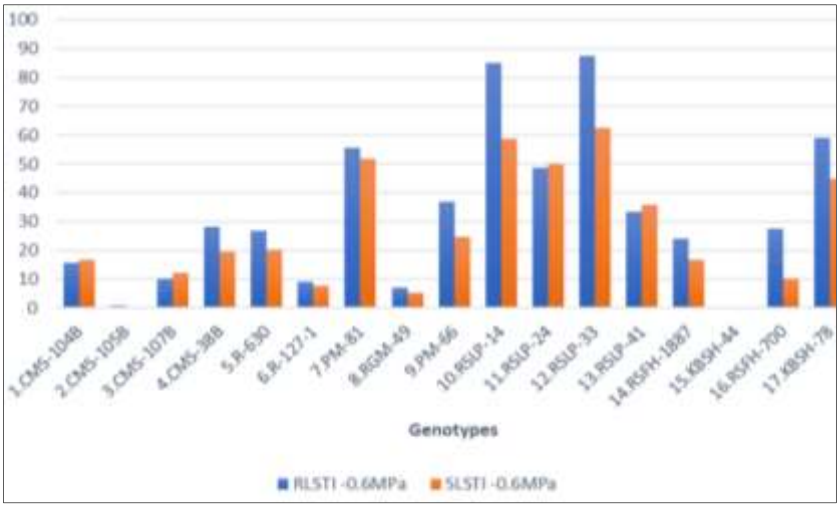

Fig 2: Differential response of sunflower genotypes for stress tolerance indices (root-RLSTI and seedling-SLSTI) at stress condition of -0.6 MPa of PEG-6000

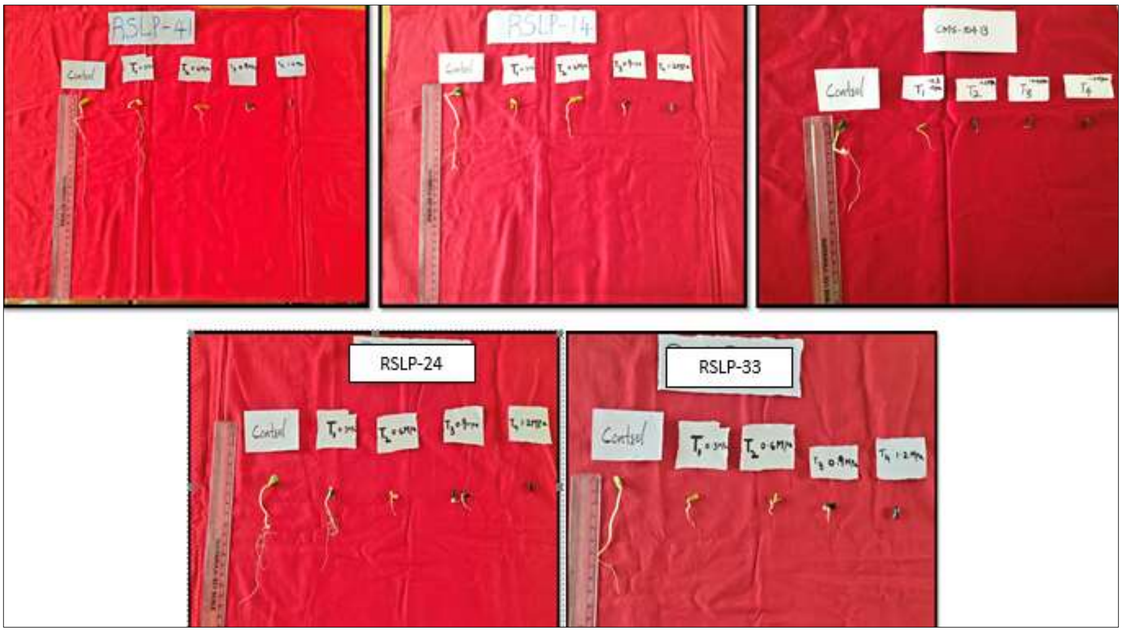

Plate 1: Performance of promising sunflower genotypes based on seedling characters under different PEG-6000 concentrations

4. Conclusion

Generally, seed germination, root length, shoot length and seedling length decreased with increase in PEG-6000 concentration among all sunflower genotypes. However, based on seed germination, seedling length and seed vigour studies in different PEG-6000 concentration five genotypes *viz*., RSLP-41, RSLP-14, CMS-104B, RSLP-24 and RSLP-33 were identified as moisture stress tolerant genotypes under lab conditions (plate 1). Further, these promising genotypes would be utilized in the crop improvement programme as donor parents for the seedling vigour related studies to enhance the yield and quality of sunflower seeds under moisture stress conditions at whole plant level (Field conditions).

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