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RESEARCH PAPER

Standardization of optimum Polyethylene Glycol 6000 dose in groundnut (*Arachis hypogaea* L.) for *in vitro* screening against induced moisture stress

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Abstract: Groundnut (*Arachis hypogaea* L.) is an important oilseed crop in India and the second most important legume in the world, generally grown as a rainfed crop, where crop is frequently subjected to drought stress leading to 50-80 *per cent* yield loss. Therefore, identification of genotypes that have a better ability to use limited available water is critical to enhance crop productivity. Breeding for tolerance to drought is limited due to non-availability of rapid method of screening large number of genotypes. Hence, a fast-screening mechanism would be helpful in selecting valuable genotypes conferring drought tolerance. An attempt was made to standardize optimum Polyethylene glycol concentration to screen against induced osmotic stress under *in vitro* condition using 15 groundnut genotypes which included stabilized breeding lines and three released varieties as checks (Dh-256, TMV-2 and JL-24). The osmotic stress was induced using different levels of PEG 6000 (0, -3, -4, -5 and -6 bars) in the laboratory using two factorial complete randomized design with two replications during 2021-22. Significant differences were observed for germination per cent, lengths of root, shoot and seedlings, root and seedling length stress tolerance indices and seed vigour at different levels of PEG-6000 among the genotypes. There was also significant interaction between genotypes and PEG levels for all of the traits. The germination percentage and all the seedling traits exhibited greater reduction with the increase in the PEG concentration in comparison to control. The optimum concentration of PEG 6000 appeared to be -4 bar for screening groundnut genotypes for moisture stress tolerance under *in vitro* condition, since beyond -4 bars, germination and all the seedling traits have shown more than 50 per cent reduction compared to control (distilled water). Beyond -4 bars of PEG 6000 concentration seemed to have detrimental effect on seed germination and other seedling parameters.

Key words: PEG 6000, Osmotic stress, *In vitro*, Germination, Groundnut

Introduction

Groundnut (*Arachis hypogaea* L.) is an important oilseed crop in India and the second most important legume in the world, generally grown as a rainfed crop, ranking next to soybean in production. In tropical and subtropical regions of the world, groundnut is an important source of vegetable oil (36 –54%) which is composed of 80 *per cent* unsaturated fatty acids and protein (22 - 36%). It is also a good source of vitamins such as thiamine, riboflavin and niacin and minerals like phosphorous, iron and zinc.

India is the second largest producer of groundnut in the world next to China. In India, groundnut is grown in an area of 6.10 million hectares with a production of 9.95 million tonnes and productivity of 1631 kg ha^{-1} (FAO, 2020). The productivity of groundnut in India is low (1631 kg ha^{-1}) compared to Israel (5892 kg ha^{-1}), USA (4254 kg ha^{-1}), China (3906 kg ha^{-1}) and Argentina (3498 kg ha^{-1}) (FAO, 2020). Low rainfall and prolonged dry spells during the crop growth period are the main reasons that cripples the groundnut productivity in India.

Approximately 70 *per cent* of the global groundnut growing areas are located in semi-arid regions, where drought is a key environmental constraint limiting groundnut production. According to recent estimation, global groundnut productivity incurred an annual loss of approximately 6 million tons due to drought alone (Bhatnagar *et al*., 2014). Moreover, due to agro-ecological changes, the crop is facing high risk of moisture stress ever before. Further, drought is also known to predispose groundnut to aflatoxin contamination making them unfit for human consumption (Blankenship *et al*., 1984).

To achieve sustainable groundnut production, it is crucial to identify cultivars that are resistant to drought. But the large scale rapid and accurate screening of genotypes is hindered by non-availability of land and resources. Hence, a fast-screening mechanism would be helpful in selecting valuable groundnut genotypes with defined growth strategies conferring drought tolerance suitable for breeding programs. Added to this, there are very scanty reports on the screening methodologies for moisture stress tolerance in groundnut crop and there is an urgent need to find suitable methodology for moisture stress tolerance screening in groundnut. However, screening for moisture stress tolerance by inducing artificial osmotic stress under *in vitro* conditions using Polyethylene glycol (PEG)-6000 was proved to be a reliable method in many crops (Ahmed *et al.,* 2009; Geetha *et al*., 2012; Yohannes *et al*, 2014; Harish Babu and Gobu, 2016). One of the important findings is that a positive correlation between drought tolerance of the genotypes in the field and in laboratory experiments was noted (Kosturkova *et al*., 2014). Hence, an attempt was made to optimize the concentration of PEG-6000 for moisture stress tolerance screening in groundnut.

Material and methods

The groundnut seeds of fifteen genotypes (GND 1, GND 2, GND 3, GND 4, GND 5, GND 6, GND 10, GND 11, GND 12, GND 13, GND 15, GND 16, Dh 256, TMV 2 and JL 24) used in the present investigation were obtained from All India Coordinated Research Project on Groundnut, MARS, University of Agricultural Sciences, Dharwad, Karnataka, India.

The experiment was laid out in a Factorial Complete Block Design (CRD) with two replications. The genotypes were subjected to osmotic stress at germination stage induced by Polyethylene Glycol-6000 (PEG-6000) of different levels 0 (normal), -3 bars (15.30 g of PEG in 100 ml of water),-4 bars (18.00 g of PEG in 100 ml of water), -5 bars (20.40 g of PEG in 100 ml of water) and -6 bars (22.60 g of PEG in 100 ml of water). For the control treatment, sterile distilled water was used instead of PEG-6000 for seed germination and seedling growth.

Ten seeds per genotype per replication were surface sterilized with 70 per cent ethanol for one minute. Then, the seeds were rinsed thoroughly with distilled water for three times and seeds were placed in petri-plates having wet germination paper. Seeds were moistened with distilled water for control petri-plates and with different concentrations of PEG-6000 solution for treatment petri-plates and were incubated for10 days at room temperature. At periodic interval, one ml of distilled water was added to control petri-plates and one ml of different concentrations of PEG-6000 solution for treatment petri-plates to manage the germination paper adequately moist during the period of incubation. Germination percentage (%), root length (cm), shoot length (cm) and seedling length (cm) were recorded at 10 days after incubation. Further, the seed vigour (Ashraf *et al*., 2006), root length stress tolerance index (RLSI) and seedling length stress tolerance index (SLSI) were calculated by the following formulae.

$$\text{Seed vigour} = \text{seedling length (cm)} \times \text{Germination percentage} = \text{Root length stress tolerance index} = \frac{\text{Root length of stressed seedlings (cm)}}{\text{Root length of stressed seedlings (cm)}} \times 100$$

$$\text{Seedling length stress tolerance index} = \frac{\text{Seedling length of stressed seedlings (cm)}}{\text{Seedling length of stressed seedlings (cm)}} \times 100$$

Results and discussion

The results of factorial ANOVA revealed that different traits *viz.,* germination percentage, root length, shoot length, seedling length, seed vigour, root length stress tolerance index and seedling length stress tolerance index recorded under PEG induced moisture stress showed highly significant differences among genotypes, different levels of PEG and interaction of different levels of PEG with genotypes was also significant (Table 1). These results suggested genotypic differences for these characters, effect of moistures stress induced by PEG on various traits and differential behaviour of genotypes to different levels of PEG induced moisture stress. The results indicate the presence of genetic variability and scope for identification of genotypes tolerant to induced drought.

The germination phase is of prime importance in the growth cycle of plants as it determines the crop stand and yield of the crop. Factors adversely affecting seed germination may include drought stress and salinity stress. Polyethylene glycol-6000 is known to induce osmotic stress which affects *per cent* germination in many crop plants at varying concentrations (Khodarahmpour, 2011; Harish Babu and Gobu, 2016). PEG induced moisture stress significantly reduced the seed germination in all the groundnut genotypes under investigation and the greater reduction was observed at -6 bars PEG concentration (96.33%) . The mean germination percentage was maximum in control (0 bars) (99.33%) followed by -3 bars (81.67 %), - 4 bars (69.67%), -5 bars (6.33%) and -6 bars (3.67%) reflecting reduction in germination percentage with increased PEG concentration. The range was observed to be wider at -3 bars (55% – 100%) followed by - 4 bars (50% – 90%), -5 bars (0 % – 40%) and -6 bars (0% - 25%) (Table 2). The results are similar to the findings reported by Rekha and Usha (2019) and Abdul *et al* (2022) who reported that germination percentage of groundnut markedly decreased with increase in PEG concentration and highest reduction was observed at -6 bars of PEG treatment.

The mean germination percentage at - 4 bars of PEG was 69.67 which is also the minimum germination percentage required in groundnut, proving that this concentration is a threshold level for the *in vitro* screening of groundnut seeds. Beyond - 4 bars of PEG concentration seemed to have detrimental effect on seed germination. The results of the present work clearly indicate that the - 4 bars of PEG concentration can be used as selection criterion in groundnut for screening the genotypes under induced osmotic stress. Shankar *et al*. (2016) reported - 2.95 bars of PEG as ideal concentration for screening of groundnut genotypes. On the contrary the work of Rekha and Usha (2019) reported that -12 bars of PEG concentration is a threshold value for the good germination of groundnut seeds.

Germination was significantly affected by the osmotic potential. Germination percentage of all the groundnut

Table 1. Factorial ANOVA for different traits under PEG induced moisture stress in groundnut

Source of variation	df	G%	SL	RL	Se L	SV	RLSI	SLSI
Levels of PEG	4	9652.33**	38.61**	603.68**	941.54**	10126332.88**	9533.96**	0698.64**
Genotypes	14	1352.21**	1.39**	14.45**	20.19**	203590.06**	292.54**	273.83**
Levels of PEG x Genotypes	56	118.76**	0.46**	5.92**	7.49**	79349.42**	102.94**	85.50**
Error	75	17.66	0.002	0.01	0.01	360.20	0.67	0.55
Total	149	11140.96	40.462	624.06	969.23	10409632.6	9930.11	11058.52

Table 2. Mean and range for different traits under PEG induced moisture stress in groundnut

Traits	Mean					Range			
	0 bars	-3 bars	-4 bars	-5 bars	-6 bars	0 bars	-3 bars	-4 bars	-5 bars	-6 bars
G (%)	99.33	81.67	69.67	6.33	3.67	90.00-100.00	55.00 -100.00	50.00 - 90.00	0 -40.00	0 -25.00
RL (cm)	11.64	4.48	3.07	0.27	0.10	4.85 - 16.83	2.80 - 6.77	1.50 - 5.03	0 –1.90	0 - 0.80
SL (cm)	3.19	1.92	1.02	0.05	0.00	1.78 - 5.85	1.23 - 2.25	0.32 - 1.65	0 - 0.40	0 .00
Se L(cm)	14.85	6.41	4.10	0.31	0.10	6.88 - 21.35	4.03 - 8.40	1.82 - 6.41	0 –2.23	0 - 0.80
SV	1479.81	531.71	290.52	11.14	1.95	618.75 -2134	241.5-839.5	90.75 -438.50	0 -78.05	0 -16.00
RLSI (%)	-	42.08	28.29	2.68	1.06	-	23.20 - 67.11	9.50 - 44.94	0 -24.05	0 -10.13
SLSI (%)	-	46.36	28.80	2.46	0.81	-	27.40 - 65.26	12.00 - 44.76	0 -20.88	0 –7.49

G- Germination percentage, RL- Root length, SL- Shoot length, Se L- Seedling length, SV- Seed vigour, RLSI- Root length stress tolerance index, SLSI- Seedling length stress tolerance index

genotypes markedly decreased with increase in PEG concentration (Fig. 1). Seeds of different genotypes germinated more often and vigorously under mild stress (0 bars, -3 bars and -4 bars of PEG concentration) than heavy stress (-5 and-6 bars of PEG concentration) (Plate 1). Most of the genotypes failed to germinate at the higher stress levels of -5 and - 6 bars of PEG concentration, with the exception of three genotypes (GND 1, GND 3 and Dh 256) which germinated at all the levels of PEG treatment (Plate 1). The *per cent* germination of these genotypes at - 6 bars of PEG was 20% (GND 1), 25% (GND 3) and 10% (Dh 256) (Table 3).The *per cent* germination of GND 1 and GND 3 was significantly higher than the drought tolerant check Dh 256 (Fig 1), indicating that these genotypes are tolerant to induced moisture stress. The three genotypes *viz.*, GND 2, GND 12 and JL 24 recorded highest *per cent* reduction in germination at -3 bars (GND 2 - 33.33% ; GND 12– 45% and JL24 – 45%) and - 4 bars (GND 2 – 44.44% ; GND 12 – 50% and JL24–50%) of PEG treatment and at -5 bars and -6 bars completely failed to germinate indicating that these are drought susceptible genotypes (Plate 1). Similar response of GND 2 and JL 24 was revealed by Abdul *et al.*(2022). Maximum reduction in *per cent* germination (61%) was recorded at 30% PEG - 6000 concentration when compared to control (Shankar *et al*.,2016).The seedling length is a combination of shoot length and root length and considered as important trait to screen the genotypes for osmotic stress. The effect of PEG induced moisture stress showed decreased seedling length with increased concentration (Fig 2). The average mean of genotypes for seedling length was higher at 0 bars of PEG (14.85 cm) followed by -3 bars (6.41 cm), - 4 bars (4.10 cm), -5 bars (0.31 cm) and - 6 bars (0.10 cm). At - 4 bars PEG, the seedling length significantly decreased (4.10 cm) and showed greater variation (1.82 cm – 6.41 cm) as compared to -6 bars of PEG concentration (Table 2), proving that this concentration is a threshold value for *in vitro* screening of groundnut. Beyond -4 bars of PEG concentration seemed to have detrimental effect on seedling development. The genotypes GND 1, GND 4 and GND 10 had high mean seedling length with minimal *per cent* reduction of 55.64 *per cent*, 63.18 *per cent* and 65.95 per cent respectively in comparison to tolerant check Dh 256 (73.38%) at – 4 bars of PEG, reflecting their superiority for the induced osmotic stress. Further the GND 1 and GND 3 coupled with Dh 256 check showed seedling length at -5 and -6 bars of PEG and remaining all other genotypes failed to exhibit the seedling length (Fig 2). The genotype, GND 12 (87.89 %), GND 13 (83.47 %) and GND16 (79.03%) recorded maximum *per cent* reduction

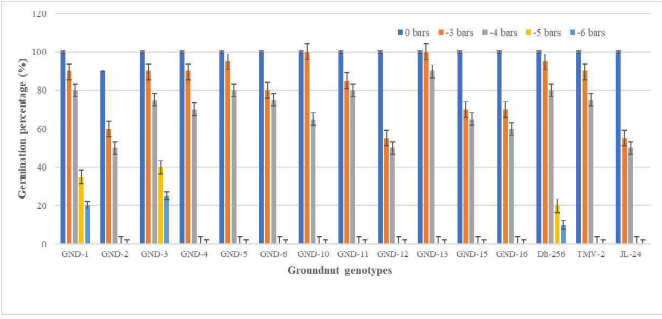

Fig.1 Effect of PEG-6000 induced moisture stress on germination percentage of groundnut genotypes. Bars indicate the mean values ± S.E.M.

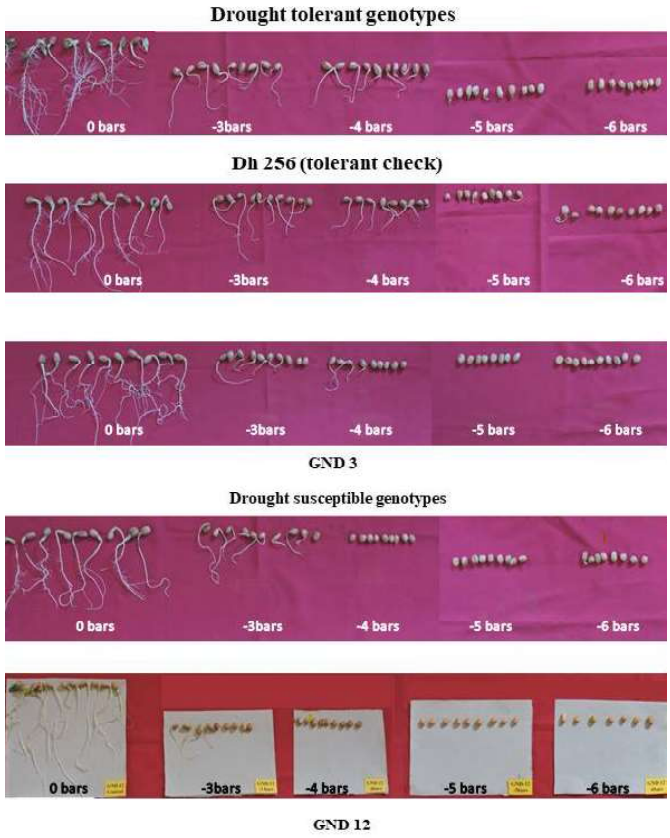

Plate 1. Response of **Groundnut genotypes to different levels of PEG-6000 induced moisture stress**

Table 3. Mean performance of groundnut genotypes and percent change (%C) over control for PEG induced moisture stress

Genotypes	Germination (%)									Seedling length (cm)								
	0	-3 bars	% C	-4 bars	% C	-5 bars	% C	-6 bars	% C	0	-3 bars	% C	-4 bars	% C	-5 bars	% C	-6 bars	% C
GND-1	100	90.00	-10.00	80.00	-20.00	35.00	-65.00	20.00	-80.00	10.68	6.87	-35.69	4.74	-55.6	4 2.23	-79.12	0.80	-92.51
GND-2	90.00	60.00	-33.33	50.00	-44.44	0.00	-100.0	00.00	-100.00	6.88	4.03	-41.45	1.82	-73.60	0.00	-100.00	0.00	-100.00
GND-3	100.00	90.00	-10.00	75.00	-25.00	40.00	-60.00	25.00	-75.00	15.78	5.87	-62.79	4.52	-71.38	1.95	-87.64	0.40	-97.47
GND-4	100.00	90.00	-10.00	70.00	-30.00	0.00	-100.00	0.00	-100.00	16.18	6.28	-61.17	5.96	-63.18	0.00	-100.00	0.00	-100.00
GND-5	100.00	95.00	-5.00	80.00	-20.00	0.00	-100.00	0.00	-100.00	16.29	6.51	-60.06	4.60	-71.75	0.00	-100.00	0.00	-100.00
GND-6	100.00	80.00	-20.00	75.00	-25.00	0.00	-100.00	0.00	-100.00	19.90	6.30	-68.33	5.85	-70.60	0.00	-100.00	0.00	-100.00
GND-10	100.00	100.0	00.00	65.00	-35.00	0.00	-100.00	0.00	-100.00	18.83	8.40	-55.41	6.41	-65.95	0.00	-100.00	0.00	-100.00
GND-11	100.00	85.00	-15.00	80.00	-20.00	0.00	-100.00	0.00	-100.00	7.88	5.12	-34.98	3.16	-59.94	0.00	-100.00	0.00	-100.00
GND-12	100.00	55.00	-45.00	50.00	-50.00	0.00	-100.00	0.00	-100.00	18.21	6.06	-66.75	2.21	-87.89	0.00	-100.00	0.00	-100.00
GND-13	100.00	100.0	00.00	90.00	-10.00	0.00	-100.00	0.00	-100.00	18.55	6.98	-62.39	3.07	-83.47	0.00	-100.00	0.00	-100.00
GND-15	100.00	70.00	-30.00	65.00	-35.00	0.00	-100.00	0.00	-100.00	10.36	5.62	-45.73	3.17	-69.44	0.00	-100.00	0.00	-100.00
GND-16	100.00	70.00	-30.00	60.00	-40.00	0.00	-100.00	0.00	-100.00	21.35	5.95	-72.12	4.48	-79.03	0.00	-100.00	0.00	-100.00
Dh-256	100.00	95.00	-5.00	80.00	-20.00	20.00	-80.00	10.00	-90.00	14.56	8.18	-43.83	3.88	-73.38	0.55	-96.22	0.32	-97.80
TMV-2	100.00	90.00	-10.00	75.00	-25.00	0.00	-100.00	0.00	-100.00	16.24	6.94	-57.25	4.17	-74.35	0.00	-100.00	0.00	-100.00
JL-24	100.00	55.00	-45.00	50.00	-50.00	0.00	-100.00	0.00	-100.00	11.04	7.00	-36.57	3.46	-68.69	0.00	-100.00	0.00	-100.00
Mean	99.33	81.67	-17.89	69.67	-29.96	6.33	-89.67	3.67	-96.33	14.85	6.41	-53.63	4.10	-71.22	0.31	-97.53	0.10	-99.19
C.D. (5%)	11.73	7.36	-	11.65	-	2.74	-	2.48	-	2.44	0.84	-	0.30	-	0.15	-	0.11	-
C.V. (%)	5.52	4.15	-	7.84	-	9.09	-	21.72	-	7.67	6.11	-	3.33	-	8.95	-	17.20	-

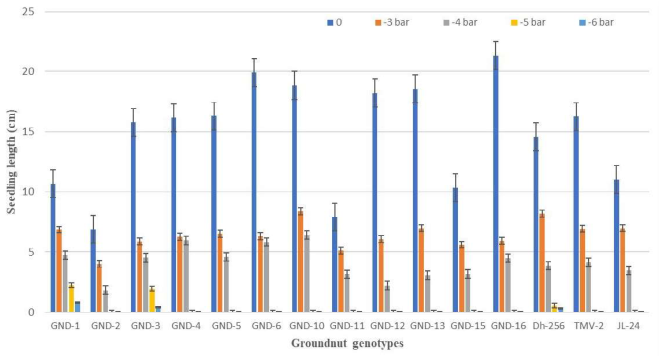

Fig.2 Effect of PEG-6000 induced moisture stress on seedling length of groundnut genotypes. Bars indicate the mean values ± S.E.M.

when compared to susceptible check JL 24 (68.69%) at -4 bars of PEG concentration, indicating these as drought susceptible genotypes. The differential response of genotypes to *in vitro* stress induced by PEG is also evident from the works of Rekha and Usha (2019) who reported that all the genotypes showed reduced shoot length with increasing PEG concentrations with exception at -2 bars PEG. There are significant genotypic variations in response to drought and their tolerance levels in groundnut as reported by Azevedo *et al.* (2010) and Vaidya *et al.* (2015).

The present work on the effect of moisture stress induced by PEG-6000 indicated that root length stress tolerance index (RLSI) and seedling length stress tolerance index (SLSI) decreased with the increase in PEG 6000 concentration (Table 4). Similar results were reported in six sunflower hybrid varieties studied by Ahmed *et al.* (2009). They also reported the presence of variation among sunflower hybrids for RLSI and it can be used as a reliable indicator of drought tolerance in sunflower. The genotypic means for RLSI and SLSI were higher at -3 bars (42.08% and 46.36%) than at - 4 bars (28.29% and 28.80%), - 5 bars (2.68% and 2.46%) and - 6 bars (1.06% and 0.81%) of PEG, respectively. The RLSI of GND 1 (10.13) at - 6 bars was higher in comparison to check Dh 256 (2.92). Similarly, the SLSI of GND 1 and GND 3 at - 6 bars was 7.49 and 2.53, repsectively and was higher than check (2.20). These results clearly indicate that GND 1 and GND 3 are tolerant to induced drought. These parameters can be used effectively for selection of groundnut genotypes with better moisture stress tolerance capacity at - 4 bars of PEG concentration. The results concur with those of Shankar *et al.* (2016) and Ahmed *et al.* (2009).

Conclusion

From the results of this investigation, it can be concluded that, the -4 bars of osmotic potential induced by PEG 6000 (18.00 g of PEG in 100 ml of water) appears to be ideal for screening groundnut genotypes for induced moisture stress tolerance under *in vitro* conditions since the germination percentage and seedling length had shown more than 50 *per cent* reduction compared to control (distilled water) beyond this PEG level. Beyond -4 bars of PEG concentration seemed to have detrimental effect on the both seed germination and seedling length. On the basis of germination percentage,

Table 4. Drought tolerance indices for PEG induced moisture stress in groundnut

Genotypes	Root length Stress tolerance index (RLSI)					Seedling length stress tolerance index (SLSI)				
	0	RLSI @ -3 bars(%)	-4 bars	-5 bars	-6 bars	0	-3 bars	-4 bars	-5 bars	-6 bars
GND-1	7.90	60.13	44.94	24.05	10.13	10.68	64.33	44.76	20.88	7.49
GND-2	4.85	57.73	30.93	0.00	0.00	6.88	58.58	26.45	0.00	0.00
GND-3	14.00	29.64	24.29	11.07	2.86	15.78	37.20	28.64	12.36	2.53
GND-4	12.50	33.20	36.32	0.00	0.00	16.18	38.81	36.84	0.00	0.00
GND-5	13.55	34.91	24.94	0.00	0.00	16.29	39.96	28.24	0.00	0.00
GND-6	14.05	28.83	29.96	0.00	0.00	19.90	31.66	29.40	0.00	0.00
GND-10	16.30	41.53	30.86	0.00	0.00	18.83	44.61	34.04	0.00	0.00
GND-11	5.75	53.91	39.13	0.00	0.00	7.88	65.26	40.10	0.00	0.00
GND-12	15.63	27.06	9.50	0.00	0.00	18.21	33.28	12.00	0.00	0.00
GND-13	14.65	33.04	15.36	0.00	0.00	18.55	37.63	16.55	0.00	0.00
GND-15	8.00	40.38	23.13	0.00	0.00	10.36	54.25	30.60	0.00	0.00
GND-16	16.83	23.20	18.12	0.00	0.00	21.35	27.40	20.98	0.00	0.00
Dh-256	10.95	57.08	29.22	5.02	2.92	14.56	56.18	26.65	3.78	2.20
TMV-2	12.22	43.13	28.23	0.00	0.00	11.04	63.41	31.34	0.00	0.00
JL-24	7.50	67.11	26.66	0.00	0.00	16.24	42.73	25.68	0.00	0.00
Mean	11.64	42.08	28.29	2.68	1.06	14.85	46.36	28.80	2.46	0.81
C.D. (5%)	2.27	-	-	-	-	2.44	-	-	-	-
C.V. (%)	9.09	-	-	-	-	7.67	-	-	-	-

seedling length, RLSI and SLSI parameters under drought stress imposed by PEG-6000, the promising genotypes were GND 1 and GND 3 coupled with tolerant check Dh 256. The - 4 bars of PEG concentration can be used for quick screening of large number of groundnut genotypes for induced moisture stress. In addition to seedling traits, RLSI and SLSI can be used for selection under induced moisture stress. Further investigations on physiological and biochemical studies will be helpful in selecting drought tolerant genotypes before utilizing them in the breeding programmes.

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