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RESEARCH PAPER

In vitro response of groundnut (*Arachis hypogaea* L.) genotypes for tolerance to osmotic stress

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Abstract: The present work was carried out to assess the tolerance of groundnut genotypes to induced osmotic stress using twenty-six groundnut genotypes along with four checks. The osmotic stress was induced using polyethylene glycol (PEG-6000) and the genotypes were treated with three different levels of PEG (0, -3 and -6 bars) in the laboratory using two factorial complete randomized design with two replications during 2020-21. Significant differences were observed among genotypes and PEG levels for all the traits. There was significant interaction between genotypes and PEG levels for all the traits. Germination and seedling traits decreased with the increase in PEG concentration and the genotypes exhibited differential response to induced osmotic stress tolerance. The components of genetic variation for different traits under *in vitro* conditions revealed the existence of greater magnitude of variation for the all the traits and especially variation was greater at -6 bars of PEG concentration and there was significant differences among the genotypes. The PCV, GCV, heritability and GAM varied from low to high across the traits at different levels of PEG. The traits showing higher heritability coupled with high GAM indicated the possibility of improving these traits by selection. Seed germination was significantly affected by the osmotic potential induced by PEG in all the groundnut genotypes under the study. Seeds germinated more often and vigorously under mild stress (0, -3 bars) than heavy stress (-6 bars) of PEG concentration. The genotypes,GND14(7.45 cm), GND 18 (2.59 cm) and Dh 257 (1.93 cm) recorded highest shoot length at 0, -3 and -6bars of PEG levels, respectively. In the present work, the genotypes *viz.* K1812, ICGV15090, GND 6, GND 4 and GND 10 proved to be having osmotic stress tolerance.

Key words: Components of variation, Groundnut, *In vitro*, Osmotic stress, Seedling parameters

Introduction

Groundnut (*Arachis hypogaea* L.) is an important oilseed crop in India and the second most important legume in the world. It is generally grown as a rainfed crop, ranking next to soybean in production. Groundnut is one of the major sources of dietary protein, minerals and vitamins for vegetarians. Groundnut kernels contain 36-54 per cent of oil, which is composed of 80 per cent-unsaturated fatty acids (Arya *et al*., 2016).

It is grown in more than 100 countries covering an area of 27.49 million hectares with an annual production of 46.98 million tonnes and productivity of 1590 kg ha^{-1} (FAO, 2019). The productivity of groundnut in India is low (1554 kg ha^{-1}) compared to Israel (7389 kg ha^{-1}), USA (4397 kg ha^{-1}), China (3492 kg ha^{-1}) and Argentina (2848 kg ha^{-1}) (FAO, 2018). In India, low rainfall and prolonged dry spells during the crop growth period are the main reason that cripples the groundnut productivity.

Abiotic stresses are an integral part of 'climate change', which can change soil-plant-atmosphere continuum thereby influencing the productivity of crops. Approximately 70 per centof the global groundnuts growing areas are located in semi-aridregions,where drought is a key environmental constraint limiting groundnut production. According to recent estimation, global groundnut productivity in curred annual loss of approximately 6 million tonnes due to drought alone (Bhatnagar *et al*., 2014). Moreover, dueto agro-ecological changes, the crop is facing high risk of moisture stress ever before. Further,drought is also known to predispose peanut to aflatoxin contamination (Blankenship *et al*., 1984) making them unfit for human consumption. Yield losses due to drought are highly variable in nature depending on timing, intensity and duration, coupled with other location-specific environmental stress factors such as high irradiation and temperature.

The most economic/feasible and productive way of employing crops indrought-prone areas is by screening and selection of genotypes with substantial water stress tolerance amalgamated with appropriate crop management practices to reduce water loss. Information on the response of different genotypes to drought and exploitation of this variability is an important requirement for crop improvement in drought prone areas. Several workers have investigated effects of drought on groundnut at different stages. The flowering and pegging stages of the groundnut life cycle are considered to be more sensitive to water deficit leading to reduced yield (Suvarna *et al*., 2004).

The selection of genotypes for drought tolerance should be based on various physiological, biochemical and morphological traits that impart tolerance there by increasing the yield under moisture stress. 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. Seed germination percentage and early seedling growth are critical stages for crop establishment and

are extra sensitive to drought stress during the seedling stages. Therefore,evaluating plant response to drought at early seedling stage was commonly attained using chemical desiccators such as polyethylene glycol (PEG).Several work shave shown that *in vitro* screening technique using PEG is one of the reliable approaches for the selection of suitable genotypes to study in detail on water scarcity on plant germination indices (Ahmad *et al.,*2013).

In the present study, various concentrations of polyethylene glycol 6000(PEG-6000) were used for inducing variable degrees of osmotic stress to identify an ideal concentration of PEG-6000 capable of identifying moisture stress tolerance in groundnut genotypes so that such a level of osmotic stress can be used to screen a large germplasm for moisture stress tolerance under *in vitro* conditions in a very shorttime.

Material and methods

A total of thirty genotypes (including four checks) were evaluated in a complete randomized block design with three levels of PEG concentration in the laboratory during 2021. The genotypes were subjected too smotic stress at germination stage induced by Polyethylene Glycol-6000 (PEG-6000) at different levels (0-normal, - 3 bars and -6 bars) (Shankar *et al.,* 2019). For control, 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 put up in sterilized petri-plates having wet germination paper. Seeds were moistened with distilled water (25 ml) for control petri-plates and with different concentrations of PEG-6000 solution of 25 ml for treatment petri-plates and were incubated for 10 days at room temperature. At periodic interval, one ml of distilled water/PEG solution was added to petri-plates to keep the germination paper adequately moist during the period of incubation. Harvesting of seedlings was done on tenthday. The germination was recorded on day-to-day basis. The germination percentage was calculated on the basis of normal seedlings obtained in the final count. The root length and shoot length data was recorded on five randomly selected seedlings. Further, seed vigour, seedling length stress tolerance index (SLSI) and root length stress tolerance index (RLSI) were estimated to have a greater understanding on the drought tolerance potentiality of different genotypes. Seed vigour was determined using the following formula (ISTA, 1985). Seed vigour = Seedling length (cm) × Germination percentage

Root length stress tolerance index (RLSI) and seedling length stress tolerance index (SLSI) were calculated as given by Ashraf *et al.* (2006) using the following formulas.

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

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

The statistical analysis of the data on the individual characters was carried out on the mean values of five random seedlings and analyzed by using OPSTAT software package (version 9.2). The analysis of variance for each character was computed by adopting two Factorial Completely Randomized Design. The coefficient of variation both at phenotypic and genotypic levels for all the characters were computed by applying the formula as suggested by Burton and Devane (1953). PCV and GCV were classified into low (0 – 10 %), moderate (11 – 20 %) and high (21 % &above) as suggested by Sivasubramanian and Menon (1973). Heritability in broad sense for all the characters was computed by using the formula suggested by Hanson *et al*. (1956). Heritability was classified into low (0 – 30 %), moderate (31 – 60 %) and high (61 % &above) as suggested by Robinson *et al*. (1949). The predicted genetic advance was estimated according to the formula given by Johnson *et al*. (1955). The genetic advance as per cent of mean was categorized into low (0 – 10 %), moderate (11 – 20 %) and high (>21 & above) as suggested by Johnson *et al*. (1955). Further, the per cent change over the control was calculated for different levels of PEG for all the traits in order to know the response of different groundnut genotypes to induced osmotic stress.

Results and discussion

The success of a osmotic stress tolerance screening methods depends on identifying a critical level of stress induced by a particular concentration of an agent capable of inducing moisture stress (Babu and Gobu, 2016). In the present work, various concentrations of polyethylene glycol 6000 (PEG-6000) were used for inducing variable degrees of osmotic stress to identify an ideal concentration of PEG-6000 and to know the genotypic responses for various seedling parameters.

The results of factorial ANOVA revealed that different traits *viz.,* germination per centage, shoot length, root length and seed vigour studied under PEG induced moisture stress showed highly significant differences ($p<0.01$) for genotypes, different

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

Source of Variation	df	G(%)	SL(cm)	RL(cm)	SeL(cm)	SV	RLSI(%)	SLSI(%)
Levels of PEG	2	7251.67**	251.41**	153.19**	793.63**	8357795.00**	37395.78**	21044.18**
Genotypes	29	1198.40**	2.43**	2.37**	6.99**	86862.00**	932.32**	277.37**
Levels of PEG×Genotypes	58	335.58**	1.17**	1.57**	2.43**	18922.91**	467.48**	345.02**
Error	90	14.44	0.06	0.03	0.10	1038.22	27.85	6.79
Total	179	8800.08	255.07	157.17	803.15	8464618.00	38823.44	21673.36

**and* Significant at 1%and 5% Probability level respectively

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

Note: The replication means were non-significant for all the traits

Table 2. Mean,range, components of genetic variation for different traits under PEG induced moisture stress

Characters	Mean			Range			PCV(%)			GCV(%)			h^2(%)			GAM(%)		
	0bar	-3bar	-6bar	0bar	-3bar	-6bar	0bar	-3bar	-6bar	0bar	-3bar	-6bar	0bar	-3bar	-6 bar	0bar	-3bar	-6bar
Germination(%)	99.17	90.50	77.33	85- 100	50- 100	20- 100	3.39	17.82	33.86	2.51	16.91	33.52	54.82	90.08	98.02	3.83	33.07	68.37
Shoot length(cm)	5.01	2.01	1.09	3.00–7.45	1.18–2.60	0.00–2.12	27.16	17.24	65.15	25.97	15.76	64.56	91.46	83.52	98.19	51.17	29.66	131.79
Rootlength(cm)	3.78	1.76	0.63	1.92–8.94	0.65–3.0	70.00–2.00	39.25	33.47	80.35	38.56	33.01	78.33	96.52	97.30	95.03	78.04	67.09	157.29
Seedling length(cm)	8.79	3.77	1.72	5.45–14.02	1.87-5.45	0.00-4.05	22.65	22.62	68.39	21.90	22.14	67.94	93.49	95.83	98.68	43.63	44.65	139.03
Seedlingvigor	872.96	340.65	153.63	545-1402	93.50-545.00	0.00-364.5	23.49	26.50	76.30	22.85	25.33	75.74	94.67	91.36	98.55	45.80	49.86	154.90
Root length stress tolerance index (%)	—	52.14	16.83	—	17.94-94.72	0.00-52.46	—	44.46	82.13	—	42.47	79.92	—	91.26	94.69	—	83.59	160.21
Seedling lengthstress toleranceindex (%)	—	44.69	18.20	—	25.75-71.67	0.00-37.77	—	30.27	63.85	—	29.45	62.92	—	94.64	97.11	—	59.01	127.73

levels of PEG and interaction of different levels of PEG with genotypes (Table 1). These results suggest the presence of genetic variability among the genotypes for these characters; indicate the effect of osmotic stress induced by PEG on various traits and differential response of genotypes to different levels of PEG. Similarly differential responses of groundnut genotypes to different levels of PEG for germination percentage and different seedling parameters was reported by Shankar *et al.* (2019).

Genetic components of variation

The components of genetic variation for different traits under *in vitro* conditions revealed the presence of greater magnitude of variation among the genotypes for all the traits and especially variation was greater at -6 bars of PEG concentration and there was significant differences among the genotypes (Table 2).

Germination percentage

The germination phase is of prime importance in the growth cycle of plants as it determines the standard establishment and final yield of the crop. Factors adversely affecting seed germination may include drought stress and salinity stress. 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. Polyethylene glycol-6000 is known to induce osmotic stress which affects per cent germination in many crop plants at varying concentrations (Khodarahmpour, 2011; Babu and Gobu, 2016)

The mean germination percentage was maximum in control (0 bars) (99.17%) followed by -3 bars (90.50 %) and -6 bars (77.33 %) reflecting reduction in germination percentage with increased PEG concentration. The range was observed to be wider at -6 bars (20-100%) followedby-3 bars (50-100%) and 0 bars(85-100%). The results are similar to the findings reported by Rekha and Usha (2019) who reported that germination percentage of groundnut markedly decreased with increase in PEG concentration.

The phenotypic and genotypic coefficient of variation for germination percentage was low at 0 bars of PEG (3.39 % & 2.51 %), moderate at -3 bars (17.82 % & 16.91 %) and high at–6 bars (33.86% & 33.52%) indicating that the genotypes responded very differently to-6 bars PEG treatment and this can be deployed in identifying the genotypes tolerance to osmotic stress.

The PCV and GCV were low at 0 bars, moderate at – 3 bars and high at –6 bars of PEG treatment. The high PCV and GCV at -6 bars clearly indicate that there existed genotypic differences which was expressed by the genotypes when they were exposed to higher concentration of PEG as compared to the 0 bars and lower PEG concentration (-3 bars). The trait showed high heritability at all the levels of PEG treatments coupled with low GAM at 0 bars and high at -3 and -6 bars (Table 2). The results clearly indicate that the germination percentage can be used as selection criterion in groundnut for screening the genotypes under induced osmotic stress. These results are in line with that of Kaya *et al.* (2006), Ahmed *et al.* (2009), Gobu *et al.* (2014) and Shankar *et al.*(2017and 2019) as for the usage of PEG-6000 for screening genotypes in different crop plants for induced moisture stress tolerance.

Shootlength (cm)

Apart from seed germination, early seedling growth parameter like shoot length is also considered as important trait to screen genotypes against moisture stress. In the present work, the shoot length decreased with the increase in PEG-6000 concentrations (Table 2) and had a low mean of 1.09 cm as against control (5.01cm). These findings are in accordance with the results reported by Abdul(2019), Rekha and Usha (2019) and Shankar *et al.* (2019) in groundnut. The shoot length had a very low range value in -6 bars PEG treatment (0.0 - 2.12 cm) as against control having greater variation (3.00 - 7.45cm). Higher phenotypic and genotypic coefficient of variation coupled with high heritability and genetic advance over mean was observed in all the PEG treatments except moderate PCV and GCV in -3 bars of PEG. High estimates of genetic parameters indicate the role of additive gene action in the expression of the trait. Hence, selection based on the phenotype would be beneficial

in improvement of this trait.The findings are in accordance with Shankar *et al.*(2019).

Root length (cm)

In the present work, the root length decreased with the increase in PEG concentration. The root length varied from 0.00 to 2.00 cm (-6bars) and greater variation observed in control (1.92–8.94 cm). The phenotypic coefficient of variation were high at all the levels of PEG concentrations and recorded 39.25 per cent (0 bars), 33.47 per cent (-3 bars) and 80.35 per cent (-6 bars). High GCV of 38.56 per cent, 33.01 per cent and 78.33 per cent were noticed at 0, -3 and -6 bars PEG, respectively. The trait showed high heritability coupled with high GAM at all the levels of PEG treatment. Shankar *et al.* (2019) also reported similar findings. The results of present work and earlier reports clearly indicate that there exists a chance of this trait being under the influence of additive gene action which offers a better scope for selection of genotypes for osmotic tolerance based on increased root length under induced stress.

Seedling length (cm)

The seedling length is a combination of shoot length and root length and considered as important trait to screen the genotypes for osmotic stress. It has a mean of as low as 1.72 cm compared to higher mean in control (8.79 cm). The shoot length ranged from 0.00 to 4.05 cm at -6 bar of PEG, clearly indicate that seeds though germinated but could not grow further as a result some of the genotypes did not put forth plumule and radicle growth resulting in zero shoot length. All the genetic parameters recorded were high. These findings are in accordance with the results reported by Abdul (2019), Rekha and Usha (2019) and Shankar *et al.* (2019) in groundnut. In general, genotypes with longer seedling length especially longer root length are tolerant to osmotic stress (Leishman and Westoby, 1994).

Seed vigour

The seed vigour was higher in control (872.96) and least in -6 bars of PEG (153.63). Seed vigour exhibited a wide range of variation from 0.00 to 1402 at variable concentrations of PEG-6000. Since seed vigour is the results of total seedling length and per cent germination, it is influenced by parameters like root length, shoot length and germination per cent (Babu and Gobu, 2016). The upsurge in concentration of PEG caused decrease in germination per centage and seed vigour in certain cropplants (Khodarahmpour, 2011). The trait exhibited high PCV and GCV at different levels of PEG concentration. The trait showed high heritability at 0 (94.67 %), -3 (91.36 %) and -6 bars (98.55 %). High GAM was observed at 0, -3 and -6 bars of PEG with a value of 45.80 per cent, 49.86 per cent and154.90 per cent, respectively.This trait seems to be less influenced by environmental factors as indicated by high heritability and high genetic advance overmean (Shankar *et al.,* 2019).

Root length stress tolerance index (RLSI) and seedling length stress tolerance index (SLSI)

The present work on the effect of osmotic stress created by PEG-6000 indicated that root length stress tolerance index (RLSI) and seedling length stress tolerance index (SLSI) decreased with the increase in PEG concentration (Table 2).Ahmed *et al*. (2009) evaluated six sunflower hybrids and concluded that variation among sunflower hybrids for RLSI could be used as a reliable indicator of drought tolerance.The mean of genotypes for root length stress tolerance index (RLSI) was greater at -3 bars of PEG (52.14 cm) compared to-6 bars (16.83 cm). The-3 bars of PEG treatment exhibited greater magnitude of variation as noted from wide range values (17.94 -94.72 cm) and low at -6bars (0.00-52.46 cm). The over all mean of genotypes for seedling length stress tolerance index (SLSI) was 44.69cm (-3bars) and 18.20 cm (-6 bars). The range values were found to vary from 25.75-71.67 cm (-3 bars) and 0.00-37.77 cm (-6 bars). The components of variation were high for both RSLI and SLSI. These characters can be used effectively for selection of groundnut genotypes with better moisture stress tolerance capacity. The findings are in agreement with Shankar *et al.* (2019) and Ahmed *et al.* (2009). The present study strongly supports that, RLSI and SLSI can be exploited to screen groundnut genotypes for induced osmotic stress tolerance.

The significant deviation in mean performance of groundnut genotypes determined by the seedling growth parameters as germination percentage, shoot length, root length and seedling length, is an indication that seedling growth parameters are dependable and efficient method for screening groundnut genotypes for osmotic stress tolerance. In addition to this, 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).

Mean performance of genotypes under PEG induced moisture stress

The mean performance of genotypes and per cent change over the control is presented in Table 3. Germination percentage of groundnut markedly decreased with increase in PEG concentration. Seeds germinated more often and vigorously under mild stress (0 and -3 bars PEG concentrations) than heavy stress (-6 bars). At -6 bars PEG concentration, a drastic reduction in germination rate was observed. Rekha and Usha (2019) reported a significant decline in the germination percentage at -12 bars PEG, indicating that-12 bars PEG concentration is a threshold value for screening the groundnut genotypes under *in vitro* condition. The highest percentage reduction was noticed in GND 2 (78.95 %) followed by GND 18 (64.71%) at the highest level of PEG induced stress (-6 bars). Maximum reduction in per cent germination (61%) was recorded at 30 % PEG-6000 concentration when compared to control (Shankar *et al*., 2017).

All the genotypes showed decreased shoot and root length with the increased PEG concentration. The shoot length and root length reduced drastically at 6 bars of PEG concentration. The mean shoot length was 1.09 cm at -6 bars as against 5.01 cm at 0bars (control) of PEG concentration. At -6 bars among the genotypes, Dh 257 (57.32 %) and ICGV 15090 (58.02 %) exhibited minimum reduction. The mean root length at -6 bars of PEG was 0.63 cm as against control (0 bar) 3.78 cm

Table 3. Mean performance and percentage change (%C) over control for different traits under PEG induced moisture stress

Genotypes	Germination(%)					Shootlength(cm)					Rootlength(cm)				
	0	-3 bar	%C	-6 bar	%C	0	-3 bar	%C	-6 bar	%C	0	-3 bar	%C	-6 bar	%C
GND1	100.00	90.00	-10.00	80.00	-20.00	4.22	2.12	-49.82	0.97	-76.99	2.75	2.60	-5.45	0.55	-80.00
GND2	95.00	50.00	-47.37	20.00	-78.95	3.27	1.22	-62.69	0.00	-100.00	2.62	0.65	-75.19	0.00	-100.0
GND3	100.00	90.00	-10.00	50.00	-50.00	3.00	1.73	-42.33	0.00	-100.00	2.45	1.13	-53.88	0.00	-100.0
GND4	100.00	100.00	0.00	100.00	0.00	6.25	2.12	-66.08	1.51	-75.84	3.82	2.46	-35.60	1.00	-73.82
JCG4801	100.00	70.00	-30.00	50.00	-50.00	4.72	2.15	-54.45	1.00	-78.81	3.07	2.30	-25.08	0.38	-87.62
ICGV 15090	100.00	100.00	0.00	95.00	-5.00	5.05	2.38	-52.87	2.12	-58.02	4.42	3.07	-30.54	1.33	-69.91
GND5	100.00	100.00	0.00	95.00	-5.00	5.62	1.79	-68.15	1.40	-75.09	4.57	1.38	-69.80	0.92	-79.87
GND6	100.00	100.00	0.00	100.00	0.00	5.38	2.24	-58.36	1.73	-67.84	6.30	1.54	-75.56	0.98	-84.44
GND7	100.00	95.00	-5.00	50.00	-50.00	3.32	1.18	-64.46	0.00	-100.00	4.18	0.75	-82.06	0.00	-100.0
GND8	95.00	70.00	-26.32	50.00	-47.37	3.30	2.60	-21.21	0.00	-100.00	4.12	1.87	-54.56	0.00	-100.0
GND9	100.00	90.00	-10.00	50.00	-50.00	3.15	2.53	-19.84	0.00	-100.00	3.35	1.54	-54.03	0.00	-100.0
GND10	100.00	100.00	0.00	90.00	-10.00	5.38	1.64	-69.61	1.27	-76.49	3.45	1.98	-42.61	0.52	-84.93
GND11	100.00	85.00	-15.00	70.00	-30.00	3.45	2.04	-41.01	0.98	-71.59	3.32	1.37	-58.89	0.45	-86.45
Higholeic 107	100.00	100.00	0.00	95.00	-5.00	5.94	1.97	-66.84	1.54	-74.07	5.29	1.50	-71.64	1.19	-77.50
GND12	100.00	60.00	-40.00	25.00	-75.00	6.57	1.82	-72.35	0.85	-87.05	2.08	1.54	-26.20	0.36	-82.93
GND13	100.00	100.00	0.00	100.00	0.00	4.56	2.13	-53.29	0.91	-80.04	2.83	1.82	-35.69	0.88	-69.08
GND14	100.00	100.00	0.00	100.00	0.00	7.45	1.93	-74.09	1.89	-74.63	2.22	1.95	-12.16	1.10	-50.45
K 1812	100.00	100.00	0.00	90.00	-10.00	6.91	2.18	-68.52	2.05	-70.33	3.81	2.50	-34.38	2.00	-47.51
GND15	100.00	70.00	-30.00	50.00	-50.00	3.92	2.39	-39.03	1.33	-66.07	3.40	2.85	-16.18	0.88	-74.12
GND16	100.00	100.00	0.00	100.00	0.00	4.03	1.91	-52.61	1.44	-64.39	5.38	1.32	-75.46	0.54	-89.96
GND17	100.00	100.00	0.00	95.00	-5.00	5.08	2.01	-60.43	1.90	-62.60	8.94	1.69	-81.10	0.89	-90.04
DBG4	100.00	100.00	0.00	100.00	0.00	7.13	1.87	-73.77	1.48	-79.24	3.52	1.25	-64.49	0.49	-86.08
GND18	85.00	50.00	-41.18	30.00	-64.71	3.77	2.59	-31.43	0.00	-100.00	3.60	2.47	-31.39	0.00	-100.0
GND19	100.00	100.00	0.00	100.00	0.00	4.91	2.11	-57.03	1.55	-68.43	4.96	1.88	-62.10	1.27	-74.40
GND20	100.00	100.00	0.00	100.00	0.00	6.28	1.87	-70.22	1.46	-76.75	4.35	1.58	-63.68	0.84	-80.69
DBG3	100.00	100.00	0.00	90.00	-10.00	7.44	2.15	-71.10	1.71	-77.02	2.98	1.82	-38.93	0.91	-69.63
Dh256(C)	100.00	100.00	0.00	65.00	-35.00	4.62	1.92	-58.44	0.77	-83.33	2.44	1.98	-18.69	0.25	-89.73
Dh257(C)	100.00	95.00	-5.00	95.00	-5.00	4.51	2.18	-51.77	1.93	-57.32	5.17	1.36	-73.69	0.85	-83.56
JL24(C)	100.00	100.00	0.00	100.00	0.00	5.63	2.02	-64.21	0.00	-100.00	2.20	1.47	-33.18	0.00	-100.0
TMV2(C)	100.00	100.00	0.00	85.00	-15.00	5.35	1.68	-68.60	0.95	-82.24	1.92	1.13	-41.15	0.33	-82.81
Mean	99.17	90.50	—	77.33	—	5.01	2.01	—	1.09	—	3.78	1.76	—	0.63	—
C.D.(5%)	4.63	10.39	—	7.53	—	0.81	0.29	—	0.20	—	0.57	0.20	—	0.23	—
C.V.(%)	2.28	5.61	—	4.76	—	7.93	7.00	—	8.76	—	7.32	5.49	—	14.97	—

Genotypes	Seedling length(cm)					Seedling vigour				
	0	-3 bar	%C	-6 bar	%C	0	-3 bar	%C	-6 bar	%C
GND1	6.97	4.72	-32.30	1.52	-78.18	696.50	424.35	-39.07	121.60	-82.54
GND2	5.89	1.87	-68.25	0.00	-100.00	559.70	93.50	-83.29	0.00	-100.00
GND3	5.45	2.86	-47.52	0.00	-100.00	545.00	257.40	-52.77	0.00	-100.00
GND4	10.07	4.58	-54.52	2.51	-75.07	1007.00	458.00	-54.52	251.00	-75.07
JCG4801	7.79	4.45	-42.88	1.38	-82.28	779.00	311.50	-60.01	69.00	-91.14
ICGV 15090	9.47	5.45	-42.45	3.45	-63.57	947.00	545.00	-42.45	327.20	-65.45
GND5	10.19	3.17	-68.89	2.32	-77.23	1019.00	317.00	-68.89	220.50	-78.36
GND6	11.68	3.78	-67.64	2.71	-76.80	1168.00	378.00	-67.64	271.00	-76.80
GND7	7.50	1.93	-74.26	0.00	-100.00	749.67	183.35	-75.54	0.00	-100.00
GND8	7.42	4.47	-39.73	0.00	-100.00	705.50	312.90	-55.65	0.00	-100.00
GND9	6.50	4.07	-37.44	0.00	-100.00	650.00	366.00	-43.69	0.00	-100.00
GND10	8.83	3.61	-59.08	1.79	-79.77	883.00	361.33	-59.08	160.80	-81.79
GND11	6.77	3.40	-49.78	1.43	-78.88	677.00	290.50	-57.09	100.10	-85.21
Higholeic 107	11.23	3.47	-69.10	2.73	-75.69	1123.00	347.00	-69.10	258.80	-76.95
GND12	8.65	3.35	-61.26	1.21	-86.06	864.67	201.00	-76.75	30.25	-96.50
GND13	7.39	3.95	-46.55	1.79	-75.85	739.00	395.00	-46.55	178.50	-75.85
GND14	9.67	3.88	-59.88	2.99	-69.08	967.00	388.00	-59.88	299.00	-69.08
K 1812	10.72	4.68	-56.39	4.05	-62.22	1072.00	467.50	-56.39	364.50	-66.00
GND15	7.32	5.24	-28.39	2.21	-69.81	732.00	366.92	-49.87	110.50	-84.90
GND16	9.41	3.23	-65.67	1.98	-79.01	941.00	323.00	-65.67	197.50	-79.01
GND17	14.02	3.70	-73.61	2.79	-80.10	1402.00	370.00	-73.61	264.70	-81.12
DBG4	10.65	3.12	-70.70	1.97	-81.50	1065.00	312.00	-70.70	197.00	-81.50
GND18	7.37	5.05	-31.43	0.00	-100.00	621.30	254.50	-59.04	0.00	-100.00

Table 3. Contd…..

	Seedlinglength(cm)					Seedlingvigour				
Genotypes	0	-3 bar	%C	-6 bar	%C	0	-3 bar	%C	-6 bar	%C
GND19	9.87	3.99	-59.57	2.82	-71.43	987.00d	399.00	-59.57	282.00	-71.43
GND20	10.63	3.45	-67.54	2.3	-78.36	1063.00	345.00	-67.54	230.00	-78.36
DBG3	10.42	3.97	-61.90	2.62	-74.90	1042.00	397.00	-61.90	235.35	-77.41
Dh256(C)	7.06	3.90	-44.72	1.02	-85.54	705.50	390.00	-44.72	66.25	-90.61
Dh257(C)	9.68	3.54	-63.48	2.78	-71.33	968.00	334.60	-65.43	264.25	-72.70
JL24(C)	7.83	3.49	-55.47	0.00	-100.00	783.00	348.67	-55.47	0.00	-100.00
TMV2(C)	7.27	2.81	-61.30	1.28	-82.39	727.00	281.33	-61.30	109.00	-85.01
Mean	8.80	3.75	—	1.77	—	5.01	294.57	—	140.96	—
C.D. (5%)	1.70	0.75	—	0.35	—	0.81	38.77	—	29.37	—
C.V. (%)	9.44	9.81	—	9.59	—	7.93	6.42	—	10.20	—

and the least reduction was shown by K1812 (47.51 %) followed by GND 14 (50.45 %). Among the genotypes, GND 2, GND 3,GND 7, GND 8, GND 9, JL 24 and GND 18 showed cent percent reduction for shoot and root length (Table 3). Rekha and Usha (2019), similarly reported that in groundnut the mean root length of all the genotypes was almost rudiment and differences were not found at -12 bar. All the genotypes showed reduced shoot length with increasing PEG concentrations with exception at -2 bars PEG. Shoot growth was completely inhibited in all the genotypes at -14 bars PEG concentration.

All the genotypes exhibited drastic reduction for seedling length and seed vigor with the increase in PEG concentration. The mean seedling length was1.77 cm as against control of 8.80 cm with ICGV 15090 recording the least reduction for shoot length (63.57 %) at -6 bars of PEG. Among the genotypes ICGV 15090 showed least reduction for seed vigour (65.45 %) at -6 bars of PEG treatment. Since seed vigour is the product of total seedling length and per cent germination, it is influenced by parameters like root length, shoot length and germination percent. The seed vigour exhibited a wide range of variation from 123.93 to 468.55 at variable concentrations of PEG and it was 587 in case of control (Shankar *et al*., 2017).

The optimum concentration of PEG appears to be -6 bars ideal for screening groundnut genotypes for moisture stress tolerance under *in vitro* conditions because the germination per cent, shoot length, root length, seedling length and seed vigour have shown more than 50 per cent reduction compared to control (distilled water), beyond -6 bars of PEG concentration seems to have detrimental effect on seed germination and other seedling parameters. Similar findings were reported in corn, sorghum, sunflower and other crops (Ahmed *et al*., 2009; Khodarahmpour, 2011; Geetha *et al*., 2012; Babu and Gobu, 2016). Based on the findings of the present investigation, genotypes *viz.* K1812, ICGV 15090, GND 6, GND 4 and GND 10 proved to be having osmotic stress tolerance and -6 bars concentration of PEG can be used for screening a large number of germplasm collections of groundnuts in a short time under *in vitro* conditions.

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