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Optimization of osmotic stress induced by polyethylene glycol-6000 fordrought tolerance screening in Groundnut (*Arachis hypogaea L.,*)

: Agriculutural Science

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ABSTRACT

Groundnut is a premier oilseed crop of India. Drought is the chief abiotic stress causing up to 50-80% crop loss in groundnut. An attempt was made to optimize the osmotic stress induced by different concentrations of PEG-6000 for moisture stress tolerance screening in groundnut cv. TMV-2. Six different concentrations of PEG-6000 (5, 10, 15, 20, 25 and 30 percent) along with a control (sterile distilled water) were used. Significant differences were observed for germination per cent, lengths of root, shoot and seedlings, seed vigour and root/shoot ratio at different concentrations of PEG-6000 ($p < 0.05$). The PEG-6000 concentration above 15% has reduced per cent germination and seed vigor almost by 50%. However, at 15% PEG-6000, a significant increase in root/shoot ratio was recorded. Hence, 15% PEG-6000 (equivalent to -2.95 bars) appears to be an ideal concentration for moisture stress tolerance screening of groundnut genotypes.

INTRODUCTION

Groundnut (Arachis hypogaea L.) is the fourth most important oilseed crop and thirteenth most important food crop in the world (Coulibaly et al., 2013). Groundnut is also considered as "poor man's Almond" because it is a good source of protein (25-28%), vitamins and minerals comparable to other dried nuts. India is the second largest producer of groundnut in the world next to China. In India, groundnut occupies an area of 47.6 lakh ha with a production of 46.9 lakh tones with an average productivity of 985 kg/ha (2012-13). More than 85% production of groundnut comes from 5 States namely-Gujarat (38%), Andhra Pradesh (16%), Tamil Nadu (14%), Rajasthan (9%) and Karnataka (8%).

One of the chief production constraints in groundnut in Karnataka particularly in rain-fed crop is the drought during vegetative and reproductive phases. The identification of drought tolerant cultivars in groundnut is very important in order to realize sustainable production of groundnut in the state. 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 drought 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 [Geetha et al., 2012; Yohannes, 2014; Harish Babu and Gobu, 2016;]. Hence, an attempt was made to optimize the concentration of PEG-6000 for moisture stress tolerance screening in groundnut.

MATERIALS AND METHODS

The optimum concentration of polyethylene glycol-6000 (PEG-6000) for moisture stress tolerance screening of groundnut under in vitro conditions was standardized using a popular cultivated variety of the region viz., TMV-2 obtained from Zonal Agricultural and Horticultural Research Station, Babbur farm, Hiriyur. The kernels of the groundnut were subjected to osmotic stress at germination stage induced by PEG-6000 at different concentrations viz., 5, 10, 15, 20, 25 and 30 per cent in 4 replications. For control, sterile distilled water was applied instead of PEG-6000 solution.

For each of the concentrations of PEG-6000 solution, 20 seeds per replication were surface sterilized with 0.1% HgCl2 for 2 minutes followed by washing with sterile distilled water three times. Later, the groundnut seeds were kept in 200 mm diameter petri-plates having a moisturized germination paper. Seeds were moisturized with 5, 10, 15, 20, 25 and 30% PEG-6000 solution for treatment plates and with sterile distilled water for control plates and kept for incubation for 10 days (Thill, 1979; Geetha et al., 2012). Using a sterile pipette, a small quantity of PEG-6000 solution/sterile distilled water was added to petri-plates regularly to maintain the uniform level of osmotic stress as induced by different concentrations of PEG-6000 throughout the incubation period. Germination was recorded on daily basis. The observations on germination per cent, root length (cm), shoot length (cm), seedling length (cm) were recorded 10 days after incubation. Further, the seed vigour and root: shoot ratios were computed (Geetha et al., 2012; Harish Babu and Gobu, 2016). The seed vigor was calculated by the following formula given by Maisuria and Patel (2009): Seed vigour = Seedling length (cm) × Germination percentage. The statistical analysis of the data on the individual characters was carried out using WINDOSTAT software package (Version 9.2)

RESULTS AND DISCUSSION

The success of a drought tolerance screening method depends on identifying a critical level of stress induced by a particular concentration of an agent capable of inducing moisture stress (Yohannes et al., 2014; Harish Babu and Gobu, 2016). In the present study, we have used various concentrations of polyethylene glycol-6000 (PEG-6000) for inducing variable degrees of osmotic stress to identify a 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 short time.

Analysis of variance revealed significant differences for different traits in groundnut cv. TMV-2 at various concentrations of PEG-6000. Significant differences were observed for percent germination, root length and seedling length ($p < 0.01$). However, shoot length, seed vigor and root/shoot ratio exhibited significant differences at 5% level of probability (Table 1) at different concentrations of PEG-6000. The mean performance of groundnut for various traits at different concentrations of PEG-6000 is presented in the Table 2.

The per cent germination in groundnut varied from 39 to 100 at various concentrations of PEG-6000 and it was 100 per cent in case of control (distilled water or 0% PEG-6000). Maximum reduction in per cent germination (61%) was recorded at 30% PEG-6000 concentration when compared to control as 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). Further, the PEG-6000 has affected root and shoot elongation at various concentrations. The root length in groundnut varied from 1.83 (30% PEG-6000) to 4.11 cm (15% PEG-6000) at different concentrations of PEG-6000. However, the root elongation was 2.87 cm under control where distilled water was used instead of polyethylene glycol-6000. The shoot length in groundnut was in the range of 1.36 (30% PEG-6000) to 2.08 cm (10% PEG-6000) at variable concentrations of PEG-6000. However, the shoot length at control was 3.00 cm. The total

seedling length varied from 3.19 to 6.07 cm at different concentrations of PEG-6000 and it was 5.87 cm in case of control. Among different concentration regimes of PEG-6000, maximum seedling length was recorded at 15% while it was minimum at 30% concentration.

The seed vigor exhibited a wide range of variation from 123.93 to 468.55 at variable concentrations of PEG-6000 and it was 587 in case of control (distilled water). The highest value of seed vigor i.e., 468.55 was recorded at 10% PEG-6000 while it was least (123.93) at 30% PEG-6000. Since seed vigor is the product of total seedling length and per cent germination, it is influenced by parameters like root length, shoot length and germination per cent (Harish Babu and Gobu, 2016). Further, the root/shoot ratio, an important parameter to judge the moisture stress tolerance ability of a crop or genotype was computed and it also showed a considerable variation ranging from 1.34 to 2.11 at different concentration levels of PEG-6000. However, the root/shoot ratio in case of control was 0.95. Generally, under moisture stress conditions, plants tend to elongate their roots much faster than their shoots in search of moisture for their survival which invariably results in higher values for root/shoot ratio as evident in many crops (Khodarahmpour, 2011; Yohannes et al., 2014; Harish Babu and Gobu, 2016). In the present investigation on groundnut, the root/shoot ratio was maximum at 15% PEG-6000 wherein the groundnut has put in a maximum elongation of roots compared to shoots indicative of a critical value of osmotic stress in groundnut. However, at 30% PEG-6000, the root/shoot ratio declined indicating that the osmotic stress at this level is injurious to groundnut as the root and shoot elongation were severely affected (Verslues et al.,1998). From the foregoing results of this investigation, it can be summarized that, the optimum concentration of PEG-6000 at 15% appears to be ideal for screening groundnut genotypes for moisture stress tolerance under in vitro conditions because the germination percent, seed vigour and root/shoot ratio have shown more than 50% reduction compared to control (distilled water) beyond 15% PEG-6000 and have detrimental effect on the both seed germination and seed vigour. Similar findings were reported in corn, sorghum, sunflower and other crops (Ahmed et al., 2009; Khodarahmpour, 2011; Geetha et al., 2012; Yohannes et al., 2014; Harish Babu and Gobu, 2016). Based on the findings of the present investigation, the polyethylene glycol-6000 at 15% concentration (equivalent to -2.95 bars) can be used for screening a large number of germplasm collections of groundnut in a short time under in vitro conditions.

Table 1: Analysis of variance for different traits in groundnut at various concentrations of polyethylene glycol-6000

Source	Degrees of freedom	Germination (%)	Root length (cm)	Shoot length (cm)	Seedling length (cm)	Seed vigor	Root/Shoot ratio
Treatments	6	2889.74**	2.30**	0.29*	3.84**	76117.00*	0.33*
Replications	3	12.81	0.06	0.05	0.18	2235.00	0.03
Error	18	10.05	0.04	0.02	0.08	599.70	0.02

*Significance at 5% **Significance at 1%

Table 2: Mean performance of groundnut cv. TMV-2 for various traits at different concentrations of Polyethylene glycol-6000

Sl. No.	Concentration of polyethylene glycol-6000	Germination (%)	Root length (cm)	Shoot length (cm)	Seedling length (cm)	Seed vigor	Root/ Shoot ratio
1	5%	100.00	2.60	1.72	4.32	431.50	1.52
2	10%	94.75	2.86	2.08	4.94	468.55	1.38
3	15%	56.5	4.11	1.96	6.07	342.58	2.11
4	20%	47.25	3.28	1.87	5.14	243.75	1.77
5	25%	43.75	2.68	1.53	4.20	183.93	1.76
6	30%	39.00	1.83	1.36	3.19	123.93	1.34
7	0% [Distilled Water]-Control	100	2.87	3.00	5.87	587	0.95
	Mean	63.54	2.89	1.75	4.64	299.04	1.65
	Range	39.00-100.00	1.83-4.11	1.36-3.00	3.19-6.07	123.93-587.00	0.95-2.11
	CV	4.99	7.30	8.66	6.38	8.19	9.09
	CD (5%)	4.78	0.32	0.23	0.45	36.91	0.23
	CD (1%)	6.61	0.44	0.31	0.62	51.03	0.31

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