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Screening for water stress induced by polyethylene glycol- 6000 in green gram (*Vigna radiata* (L.) R. Wilczek)

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Abstract
Twenty five mung bean genotypes were screened utilizing PEG-6000 at different water potentials (-0.3, -0.6 and -0.9 MPa) for drought tolerance. The analysis of variance indicated presence of significant difference for all the traits under different concentration of PEG (-0.3 MPa, -0.6 MPa and -0.9 MPa) besides control. With increase in concentration of PEG, significant reduction in germination percentage, root length and shoot length was noted as compared to control which ultimately leads to reduction in vigor index. Under stress condition, there is always decrease in root and shoot length but genotypes showing less relative reduction are considered as water stress / drought tolerant. In the present investigation, genotypes like Samrat, KL 4, Banka Local, LGG 524, MH 921, HUM 16 and Pusa Vishal depicted higher values for all the mentioned trait under reduced water potential hence can be utilised as parents for hybridization for drought tolerance breeding programme.

Keywords: Germination percentage, mung bean, osmoticum, vigour index, Water potential

Introduction
Mung bean (*Vigna radiata* (L.) Wilczek) commonly known as green gram is the most important pulse crop having economic and nutritional significance. It has tremendous health benefits due to presence of bioactive compounds. Short life cycle and nitrogen fixing ability of mung bean makes it compatible for major cropping system. However the production of mung bean is drastically affected by several biotic and abiotic stresses. Among the abiotic stresses, drought stress is major constrain in mung bean production. Seed germination and early seedling growth are considered as most critical phases for seed establishment, which determines successful crop production and gets reduced with the onset of drought. Occurrence of dry spell with varying magnitude affects number of physiological and metabolic process like germination, growth, photosynthesis, respiration, nutrient metabolism etc. during the crop growth period which ultimately lead to poor growth and yield [Durga *et al.* (2003)] [1]. Therefore it becomes necessary to develop drought tolerant genotype which could withstand limited soil moisture stress and give better yield. Selection of new variety is difficult due to the wide range of plant stress responses with overlapping functions between their components creating complex mechanisms of resistance [Kaur *et al*. (2017) [4]. It clearly implies that, for successful drought tolerance breeding, reliable methods for screening of desirable genotypes must be opted.
The use of osmotic substances having high molecular weight such as PEG is most common and reliable method to test the drought tolerance ability of crop plants during seed germination and seedling establishment [Turkan *et al.* (2005) [13] & Zarei *et al.* (2007)] [14]. Polyethylene glycol (PEG) leads to reduced water potential causing osmotic shock without any harmful toxic effect on plants. Varieties that can germinate under reduced water potential usually germinate and establishes into seedlings. In the present investigation attempt has been made to screen twenty five mung bean genotypes at three different concentrations (-0.3 MPa, -0.6 MPa and -0.9 MPa) of PEG besides control by evaluating their germination, root length, shoot length and vigour index under laboratory conditions to identify drought tolerant genotype.

Materials and Methods

Twenty five mungbean genotypes were screened for water stress tolerance using Poly Ethylene Glycol- 6000 at different concentrations (-0.3, -0.6 and -0.9 MPa) besides control (water) in CRD design with two replications at ICAR-RCAER Palandu, Ranchi. Twenty five seeds were taken from each genotype and was surface sterilized initially with 0.1% (w/v) Mercuric chloride for three minutes and then washed thoroughly with distilled water. Poly Ethylene Glycol (PEG) 6000 was used to prepare external water potentials of -0.3, -0.6 and -0.9 MPa as per method described by Swathi *et al.* (2017) [11]. The surface sterilized seeds were soaked in different concentrations of PEG solution as well as in distilled water (control) separately for five hours. Further the seeds were germinated on a paper towel placed in petridish which was completely wetted with water and different concentrations of PEG solutions respectively and were incubated for 10 days at room temperature. After ten days of incubation, seedlings were removed from petridishes and data was recorded for germination percentage, root length, shoot length and vigour index. The germination percentage was calculated by the formulae

$$\text{Germination (\%)} = \frac{\text{number of seed germinated}}{\text{total number of seed kept for germination}} \times 100$$

Root length was recorded in centimeters from base to tip of main root while, shoot length was recorded in centimeters from base to the tip of the leaf blade. Vigour index was calculated by the following formula.

Vigour index = (shoot length + root length) × germination percentage.

The data obtained was further subjected to ANOVA of CRD.

Results and Discussion

In the current scenario of climate change, the most important factor for crop production is water. Mung bean is an important legume cultivated throughout the country which can thrive well under drought prone condition. But the response of mung bean genotypes varies differentially under drought condition. The study of water potential enables to identify the genotype suitable for cultivation under moisture stress condition. Polyethylene glycol is the best osmoticum that induces significant water stress without causing any toxic effect. The analysis of variance for four characters was significant which clearly indicates that ample amount of variation was present for all the traits at different concentration of PEG (-0.3 MPa, -0.6 MPa and -0.9 MPa) besides control (table 1). Similar findings were observed by Mahapara *et al.* (2022) [5], Imtiyaz *et al.* (2020) [3], Kaur *et al.* (2017) [4], Swathi *et al.* (2017) [11] and Dutta and Bera (2008) [2].

The mean performance of twenty five genotypes of mungbean for four different characters at different concentration of PEG -6000 is presented as follows (table 2). The rate of germination varied in control and at various concentration of PEG (-0.3 MPa, -0.6 MPa and -0.9 MPa) due to decrease in water potential gradient resulting in limited water uptake by seed leading to reduced seed germination. Germination percentage ranged from 15-100% under control, 12.5 to 97.50% at -0.3 MPa, 10% to 92.86% at -0.6 MPa and 5% to 70% at -0.9 MPa. It was observed that, the genotypes KL 4, samrat, banka local, MH 921 HUM 16 and Pusa Vishal depicted higher germination percentage under control as well as different concentration of PEG (-0.3 MPa, -0.6 MPa and -0.9 MPa). Similar findings were reported by Kaur *et al.* (2017) [4], Mahapara *et al.* (2022) [5], Nanditha *et al.* (2022) [6], Osmani *et al.* (2021) [7], Rohit *et al.* (2020) [10], Rajput *et al.* (2022) [8] and Swathi *et al.* (2017) [11].

Shoot length decreased with increase in PEG concentration**.** Under control condition shoot length ranged from 0.62 cm to 8.01 cm with an average mean of 4.91 cm while it was 0.25 cm to 8.00 cm with an average of 3.94 cm at -0.3 MPa. At -0.6 MPa shoot length ranged from 0.40 cm to 5.57 cm with an average of 1.93 cm. while, at -0.9 MPa it was 0.14 cm to 2.27 cm with a mean of 0.85 cm. The genotype Samrat (2.27 cm), KL 4 (1.79 cm), Banka Local (1.51 cm) and Pusa Vishal (1.50 cm) recorded maximum shoot length. The studies of Kaur *et al.* (2017) [4], Mahapara *et al.* (2022) [5], Nanditha *et al.* (2022) [6], Osmani *et al.* (2021) [7], Rohit *et al.* (2020) [10], Reyes *et al.* (2023) [9] and Swathi *et al.* (2017) [11] reported the same.

Under reduced water potential, there was decrease in the root length, but those genotypes whose relative decrease in root length was less were considered as drought tolerant genotypes. The range of root length under control condition was 0.50 cm to 10.06 cm having mean of 5.49 cm while at -0.3 MPa it was 0.37 cm to 6.72 cm depicting average of 4.26 cm. At high water potential (-0.6 MPa), the range of root length was 0.21 cm to 4.67 cm with an average of 1.79 cm while it was 0.10 cm to 2.31 cm having mean of 0.83 cm at -0.9 MPa. The genotype Samrat (2.31 cm), KL 4 (2.28 cm), Banka Local (2.24 cm) and LGG 524 (1.69 cm) recorded maximum root length and were considered as drought tolerant genotype. Similar findings were reported by Mahapara *et al.* (2022) [5], Kaur *et al.* (2017) [4], Nanditha *et al.* (2022) [6], Osmani *et al.* (2021) [7], Rohit *et al.* (2020) [10], Reyes *et al.* (2023) [9] and Swathi *et al.* (2017) [11].

Reduced water potential inhibit rate of germination as well as suppress growth of seedling hence lead to reduction in vigor index. Under control condition vigour index ranged from 21.30 to 1502.60 with an average value of 947.59. At reduced water potential (-0.3 MPa) the vigour index ranged from 9.75 to 1438.65 with a mean value of 659.22. At high water potential (-0.6 MPa), the range of vigour index was 19.35 to 825.97 with an average of 216.34. At higher concentration of PEG (-0.9 MPa), vigour index ranged from 1.35 to 284.90. The genotype KL 4 (284.90), Samrat (256.30) and IPM 409-4 (124.16) recorded maximum vigour index. The results was in accordance with the study of Mahapara *et al.* (2022) [5], Kaur *et al.* (2017) [4] and Swathi *et al.* (2017) [11].

Table 1: Analysis of variance under control and different concentration of PEG in twenty five (25) mungbean genotypes for four parameters

	control				-0.3 MPa			
	G%	SL	RL	VI	G%	SL	RL	VI
Treatment (MS)	1448.72**	9.29**	13.02**	455171.25**	1208.97**	6.52**	6.13**	276189.53**
Error (MS)	44.62	0.25	0.52	17449.75	50.65	0.43	0.91	14735.04
CV%	8.19	10.25	13.12	13.94	9.93	16.55	22.39	18.41
SED	6.68	0.50	0.72	132.10	7.11	0.65	.95	121.39
CD (5%)	18.62	1.40	2.01	369.47	19.84	1.82	2.66	339.52
	-0.6 MPa				-0.9 MPa			
	G%	SL	RL	VI	G%	SL	RL	VI
Treatment (MS)	1153.99**	2.20*	2.64**	76495.45**	768.27**	0.64**	1.08**	11790.63**
Error (MS)	122.11	0.90	0.20	5651.33	61.96	0.08	0.03	692.28
CV%	22.61	49.21	25.13	34.74	23.17	33.52	21.33	36.47
SED	11.05	0.95	0.45	75.18	7.87	0.28	0.18	26.31
CD (5%)	30.80	1.96	1.25	210.26	21.94	0.79	0.49	73.59

G%- Germination percentage; SL- Shoot length; RL- Root length; VI- Vigour index, CV- Co-efficient of variation, SED- standard error of difference between two means, CD- critical differance

Table 2: Mean performance of twenty five (25) mungbean genotypes at control and various concentrations of PEG for four parameters

Sl. No.	Name of genotype	Germination %				Shoot length				Root length				Vigour index			
		Control	-0.3 MPa	-0.6 MPa	-0.9 MPa	control	-0.3 MPa	-0.6 MPa	-0.9 MPa	control	-0.3 MPa	-0.6 MPa	-0.9 MPa	control	-0.3 MPa	-0.6 MPa	-0.9 MPa
1	IPM 205-7	99.00	91.46	92.86	57.28	5.96	5.34	5.57	0.50	7.23	5.77	3.33	0.16	1305.48	1016.35	825.96	37.61
2	IPM 409-4	86.94	65.00	47.50	62.15	7.06	4.55	1.63	1.14	7.70	4.72	0.87	0.86	1283.15	604.00	115.30	124.15
3	IPM 2-3	15.00	25.00	27.50	10.00	0.62	1.02	1.04	0.14	0.50	0.37	0.87	0.12	21.30	36.20	62.90	2.60
4	IPM 2-14	90.00	63.75	36.34	32.50	6.26	4.07	1.61	0.87	9.08	4.91	1.75	0.16	1389.60	573.20	95.38	34.05
5	IPM 430-1	95.00	75.00	25.00	12.50	4.76	3.68	1.67	0.17	3.89	2.89	0.21	0.27	818.40	493.40	40.80	5.92
6	IC 39403	82.50	80.00	67.50	52.50	5.70	3.09	2.65	0.56	6.73	5.18	3.56	0.73	1027.10	662.00	418.25	67.85
7	IC 314326	99.00	90.00	52.50	10.00	6.37	5.14	2.57	0.41	5.56	4.52	1.98	0.13	1180.68	868.95	241.45	5.40
8	HUM 12	92.50	90.00	72.50	47.50	7.58	4.71	3.03	0.85	8.65	6.65	4.67	0.22	1502.60	1022.40	561.65	53.00
9	HUM 16	100.00	92.50	90.00	52.50	6.83	3.72	2.32	0.65	7.01	4.62	1.05	0.42	1384.00	773.67	303.30	56.25
10	PM 5	92.50	90.00	60.00	45.00	6.01	6.48	2.77	1.30	10.05	5.87	1.77	0.72	1487.23	1111.5	272.40	91.50
11	Pusa 1081	80.00	65.00	30.00	25.00	4.08	3.11	1.61	0.54	3.41	3.64	0.82	0.63	598.80	449.15	72.90	31.30
12	MH 921	100.00	92.50	75.00	45.00	5.93	5.76	1.90	1.30	5.20	5.82	1.44	1.50	1113.00	1066.53	249.90	123.40
13	MH 96-1	90.00	50.00	10.00	15.00	8.01	2.63	1.14	0.18	7.24	3.33	0.80	0.59	1378.40	295.40	19.35	12.00
14	MH 565	95.00	85.00	35.00	20.00	4.82	3.92	1.27	1.28	6.46	5.48	1.37	1.22	1073.00	806.60	87.70	50.40
15	MH 805	82.50	47.50	17.50	15.00	2.71	3.75	0.40	0.34	2.67	4.30	1.01	0.10	449.32	388.15	23.40	5.40
16	ML 818	99.00	85.00	57.50	20.00	5.92	6.34	1.21	0.65	6.01	5.78	2.57	0.29	1180.46	1037.80	216.9	20.20
17	KL 4	100.00	85.00	75.00	70.00	5.86	3.62	2.70	1.79	7.14	3.21	3.13	2.28	1299.50	579.40	437.00	284.90
18	LGG 524	40.00	30.00	20.00	25.00	1.07	2.04	0.97	0.80	1.67	1.93	1.44	1.69	114.15	113.00	55.10	69.30
19	GM 99-125	75.00	72.50	45.00	42.50	3.92	3.41	1.70	1.17	5.24	5.14	0.46	1.31	689.05	616.42	92.50	104.08
20	Samrat	95.00	92.50	65.00	57.50	5.72	5.00	1.68	2.27	5.74	4.05	2.24	2.31	1082.10	837.10	256.30	265.25
21	Pant mung	15.00	12.50	17.50	5.00	0.73	0.25	0.98	0.17	0.95	0.52	0.30	0.10	24.60	9.75	23.65	1.35
22	Pusa baishakhi	25.00	37.50	22.50	7.50	1.03	0.71	0.95	0.23	1.32	1.34	0.99	0.17	57.35	76.98	42.30	3.25
23	Banka local mung	95.00	97.50	45.00	40.00	4.29	8.00	3.08	1.51	5.39	6.72	2.56	2.24	917.30	1438.65	258.90	152.30
24	Meha	95.00	80.00	65.00	27.50	6.73	3.42	1.50	0.73	6.29	4.86	3.21	1.23	1233.9	662.40	309.10	53.95
25	Pusa vishal	99.00	97.50	70.00	52.50	4.69	4.70	2.32	1.50	6.22	4.97	2.31	1.33	1079.44	941.40	326.00	148.40
	Mean	81.52	71.71	48.87	33.98	4.91	3.94	1.93	0.84	5.49	4.26	1.79	0.83	947.59	659.22	216.34	72.15

Fig 1: *In vitro* screening of mungbean genotypes at control and different concentrations of PEG

Conclusion

In vitro screening utilizing PEG- 6000 is very useful and economical way to screen genotypes that can perform well under reduced water potential or water stress / drought condition as it saves time and also provides uniform drought/ water deficit conditions which becomes very hard to obtain at field. Germination percentage, root length, shoot length and vigour index showed significant reduction with increase in water stress induced by increase in PEG concentration. From the present investigation, genotype like Samrat, KL 4, Banka Local, LGG 524, MH 921, HUM 16 and Pusa Vishal were considered as drought tolerant genotype as they showed very less relative reduction in the mentioned traits under water stress condition. These genotype could be further utilized as parents in hybridization programme associated with drought tolerance breeding. But before recommending any genotype to the growers of drought prone areas, it should undergo conformational studies apart from the result obtained with PEG screening.

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Authors Contribution

Conceptualization and designing of research work (Dr. Amitava Paul & Dr. R. S. Pan)

Execution of field/lab experiment and data collection (Shreya Sen)

Analysis of data & interpretation (Shreya Sen, Dr. Amitava Paul & Dr. R. S. Pan)

Preparation of manuscript (Shreya Sen, Dr. B, N. Saha)

Declaration

The authors declare that they do not have any conflict of interest.

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