Received: 15 Nov 2023 | Revised: 21 Dec 2023 | Accepted: 27 Dec 2023

Electronic Journal of Plant Breeding

Research Article

In vitro screening of pre-breeding lines for moisture stress tolerance in greengram

Azmeera Swetha Sahithi[1], P. Jayamani[2*], K. Anandhi[2], D. Vijayalakshmi[3] and E. Rajeswari[4]

[1]Department of Genetics and Plant breeding, Tamil Nadu Agricultural University, Coimbatore – 641003, Tamil Nadu, India
[2]Department of Pulses, Centre for Plant Breeding and Genetics Tamil Nadu Agricultural University, Coimbatore – 641003, Tamil Nadu, India
[3]Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore – 641003, Tamil Nadu, India
[4]Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore – 641003, Tamil Nadu, India
***E-Mail**: jayamani1108@gmail.com

Abstract
Greengram (*Vigna radiata* L. Wilczek) is the third most important pulse crop. One of the main factors limiting its production and productivity is drought, in different growth stages of the crop. Drought stress at the seedling stage of greengram influences their adaptation at the early crop establishment phase. In this study, 200 pre-breeding lines derived from VBN(Gg) 2 X *Vigna radiata* var.*sublobata/2* of greengram were evaluated for drought stress by *in vitro* screening, using Poly ethylene glycol (PEG 6000) at -0.5MPa stress level. Significant differences were observed among the genotypes, treatments and interactions for the evaluated seedling traits and stress indices, suggesting a high variability for drought tolerance in pre- breeding lines. A total of eleven tolerant and six susceptible lines were selected based on seedling vigour index, > 900.00 and < 220.00, respectively during initial screening and independent confirmation screening was carried out. The results of the present study revealed that the pre-breeding lines *viz.,* GGISC 45, GGISC 41, GGISC 132, GGISC 125, GGISC 116, GGISC 55, GGISC 147, GGISC 17, GGISC 73, GGISC 49 and GGISC 37 were recorded with high seedling vigour index along with significant stress indices and identified as tolerant for drought. These identified tolerant lines can be further evaluated under rain-out shelter to assess their ability to withstand drought. Subsequently, the promising lines can be used for the development of drought tolerant varieties in greengram.

Keywords: Drought, greengram, pre-breeding lines, PEG 6000

INTRODUCTION

Greengram (*Vigna radiata L.* Wilczek) is a important short duration leguminous crop with a broad range of adaptability and minimal input needs. In addition, it constitutes significant proportion of the Indian diet because of its low glycemic index with essential amino acids and easily digestible dietary proteins. India is the world's pioneer grower of greengram with production of 30.9 lakh tonnes cultivated under 51.3 lakh hectares of area and 601 kg/ha productivity (www.indiastat.com).

Abiotic stresses are the leading factors contributing to crop losses globally, which negatively impacts crop growth and productivity through morphological, physiological and biochemical changes (Baroowa *et al.*, 2016; Dharani *et al.,* 2023). Drought stands out as a primary abiotic stress factor that consistently lowers crop productivity. With the projected rise in instances of water shortages, the drought restricted zone is expanding, posing a threat to greengram cultivation. Notably, the water shortage at seedling stage, hinders the production of healthy greengram seedlings and diminishes overall productivity (Nair *et al.,* 2019). Consequently, the development and adoption of drought tolerant greengram varieties are important to maintain stable production during periods of water scarcity. However, the existing breeding population of greengram is limited in genetic diversity rendering it susceptible to various stresses. Therefore, the utilization of greengram wild relatives not only enhances the genetic diversity but also introduces beneficial traits into cultivated lines (Jiang *et al.,* 2015). With this backdrop, in the current study, pre-breeding lines developed through wide hybridisation between VBN(Gg) 2 X *Vigna radiata* var.*sublobata/2* have been systematically evaluated for drought tolerance ability at the seedling stage under *in vitro* condition using Poly ethylene glycol (PEG 6000).

PEG screening is an alternative approach to field experiments related to drought stress to induce moisture stress in an *in vitro* condition. It is a non-ionic polymer that is often used to induce drought stress in higher plants since it is soluble in water and does not easily penetrate plant tissues (Badiane *et al*., 2004; Surendhar *et al.,* 2020). Therefore, *in vitro* screening using PEG 6000 is a dependable approach for assessing the seedling stage drought tolerance of pre-breeding lines.

MATERIALS AND METHODS

In the present study, 200 pre-breeding lines (F_{10} generation) of greengram derived from VBN(Gg) 2 X *Vigna radiata* var.*sublobata/2* were used for *in vitro* drought screening using PEG 6000 at the Department of Pulses, Tamil Nadu Agricultural University, Coimbatore. Based on literature survey and also previous work done in the department (unpublished), it was found that under *in vitro* screening using -0.5MPa (PEG 6000), 50 per cent seedling mortality was observed in greengram (Jincy *et al*., 2021; Dharshini *et al*., 2021). Hence, in the present study, the PEG 6000 concentration of -0.5MPa was used for screening the pre-breeding lines. Ten healthy seeds of uniform size were surface sterilized with 1% sodium hypochlorite for 1 minute and then carefully rinsed with sterile water to remove any traces of sterilizing agent and were allowed to germinate in a petri dish containing germination paper moistened with distilled water (control) and -0.5Mpa of PEG 6000 solution (treatment). The experimental design adopted was factorial completely randomized design (FCRD) with two replications. The development of a 2 mm radicle was established as the germination standard (Kaur *et al*., 2017). To ascertain the germination percentage, the number of germinated seeds of each genotype was counted. Five seedlings were randomly selected from each replication for the measurement of root length (cm) and shoot length (cm) on 8th day after sowing. In addition, other derived indices *viz.,* promptness index (George *et al*., 1967) and seedling vigour index (Germination percentage × seedling length) were calculated.

Germination stress index, shoot length stress index and root length stress index were also calculated based on the formula described by Saima *et al*. (2018). Stress tolerance index based on seedling vigour was calculated using formula given by Dhopte and Livera (1989). The per cent reduction of shoot and root growth over control was calculated using the formula suggested by Senthil and Muthappa (2001).

Confirmation screening of the pre-breeding lines of greengram for drought tolerance at seedling stage: Based on seedling vigour index observed during initial screening, a set of 17 pre-breeding lines namely, eleven tolerant (>900.00) and six susceptible (<220.00) were selected for confirmation screening. Ten uniform seeds from each genotype were surface sterilized and equidistantly placed in petri dishes containing germination paper moistened with distilled water (control) and -0.5MPa of PEG 6000 solution (treatment). The experimental design adopted was completely randomized design with 2 factors (genotype and stress level) in two replications. Shoot length and root length were recorded on 8^{th} day in five randomly selected seedlings in each replication. All other derived observations *viz*., germination per cent, promptness index, germination stress index, shoot length stress index, root length stress index, stress tolerance index and per cent reduction in root and shoot growth were computed similar to that used in initial screening.

Statistical analysis: Statistical analysis was performed with the R software package (version 4.3.1). Screening data were subjected to analysis of variance (ANOVA) to determine statistically significant differences among genotypes, drought levels and their interaction levels. Least significant difference (LSD) was applied to compare treatment means using GRAPES software (version 1.0.0). Box and Whisker charts illustrating the variation of the seedling traits under control and drought stress conditions were constructed using Excel 2021 for Windows.

RESULTS AND DISCUSSION

Greengram is frequently exposed to drought stress as it is mostly grown in rainfed agricultural systems. In this context, it becomes crucial to identify varieties of greengram that exhibit tolerance to drought, especially in light of the evolving climatic conditions.

In the present study, ANOVA pointed out that the pre-breeding lines showed highly significant variation among the genotypes, treatments and interaction for all the seedling parameters and stress indices during initial screening (**Table 1**). On comparison with control, there was reduction in germination, shoot length, root length, seedling length and other indices except root-shoot length ratio under PEG induced stress condition in all the pre-breeding lines of greengram (**Table 2**). The results of reduction in seedling parameters were in accordance with Jincy *et al*. (2021) in greengram.

Table 1. ANOVA for different seedling parameters of pre-breeding lines of greengram during initial screening

Source	Df	Germination per cent	Promptness index	Shoot length	Root length	Seedling length	Root/ shoot length ratio	Seedling vigour index
Genotype	199	253.00***	29.00***	4.00***	6.80***	14.00***	37.00***	160485.00***
Treatment	1	49961.00***	5465.00***	32231.00***	1565.70***	48004.00***	15011.00***	531479636.00***
G × T	199	213.00***	9.00***	3.00***	2.90***	7.00***	37.00***	75267.00***
Error	400	52.00	3.00	1.00	0.60	2.00	8.00	18927.00

*** = highly significant (P≤0.001)

Table 2. Mean performance of seedling growth parameters of pre breeding lines of greengram during initial screening

S.No.	Traits	Control		Treatment at -0.5MPa		Per cent reduction over control	Number of lines lying over treatment mean value
		Mean	Range	Mean	Range		
1	Germination (%)	98.90	90.00-100.00	83.03	50.00-100.00	16.05	115
2	Promptness index	23.03	12.00-25.00	17.77	6.75-25.00	22.82	109
3	Shoot length (cm)	13.64	8.16-19.80	0.94	0.15-3.90	93.11	83
4	Root length (cm)	8.43	4.30-13.12	5.63	2.25-10.10	33.16	98
5	Seedling length (cm)	22.06	13.93-31.07	6.57	2.40-12.91	70.22	98
6	Root/Shoot ratio	0.63	0.34-1.18	9.29	1.92-34.35	-	74
7	Seedling vigour index	2183.16	1281.50-3106.67	552.36	120.00-1133.33	74.70	99

(-): Not worked out since root-shoot length ratio is higher in stressed conditions (-0.5MPa)

The results of initial screening of 200 pre-breeding lines were presented in the **table 2 and fig. 1**. During the initial screening, the germination per cent ranged from 90.00 to 100.00 in control with the mean of 98.90 per cent and from 50.00 to 100.00 at -0.5MPa with the mean of 83.03 per cent. Out of 200 pre-breeding lines, 115 lines recorded higher mean values than the treatment mean for germination per cent. Promptness index in the control ranged from 12.00 to 25.00 with mean of 23.03 and at -0.5MPa ranged from 6.75 to 25.00 with the mean of 17.77. Mean of shoot length, root length and seedling length in all the pre-breeding lines were found to be declined in response to moisture stress at -0.5MPa studied (**Table 2**). The shoot length varied from 8.16 to 19.80 cm in control and 0.15 to 3.90 cm at -0.5MPa. The root length varied from 4.30 to 13.12 cm in control and 2.25 to 10.10 cm at -0.5MPa. The mean shoot length and root length was recorded as 13.64 and 8.43 cm (control) and 0.94 and 5.63 cm (-0.5MPa), respectively. Reduction in shoot length was found to be higher in comparison to root length under moisture stress condition, this result is in accordance with Dutta and Bera, (2008) in greengram. The root-shoot length ratio ranged from 0.34 to 1.18 in control with the mean of 0.63 and from 1.92 to 34.35 at -0.5MPa with the mean of 9.29. The seedling vigour index was significantly reduced in all the pre-breeding lines at -0.5MPa as compared with control. The seedling vigour index varied between 1281.50 and 3106.67 in control with the mean of 2183.16 and between 120.00 and 1133.33 at -0.5MPa with the mean of 552.36. Among the 200 pre-breeding lines, 99 lines recorded higher mean values than the treatment mean.

According to International Seed Testing Association (ISTA), seedling vigour encompasses all the characteristics of a seed that collectively influence its performance and effectiveness in diverse environmental conditions. High germination rate along with better seedling growth under stress conditions can be considered as a vital trait for identifying tolerant genotypes against drought (Nivethitha *et al*., 2020). Hence, selection of genotypes with high seedling vigour index under stress condition will be rewarding. Therefore, 17 pre-breeding lines of greengram comprising eleven highly tolerant and six highly susceptible lines were selected based on seedling vigour index (SVI) (> 900.00 for tolerant lines and < 220.00 for susceptible lines) recorded during initial screening. In addition to higher SVI, the identified tolerant lines were characterized by notably significant values in terms of germination stress index, shoot length stress index, root

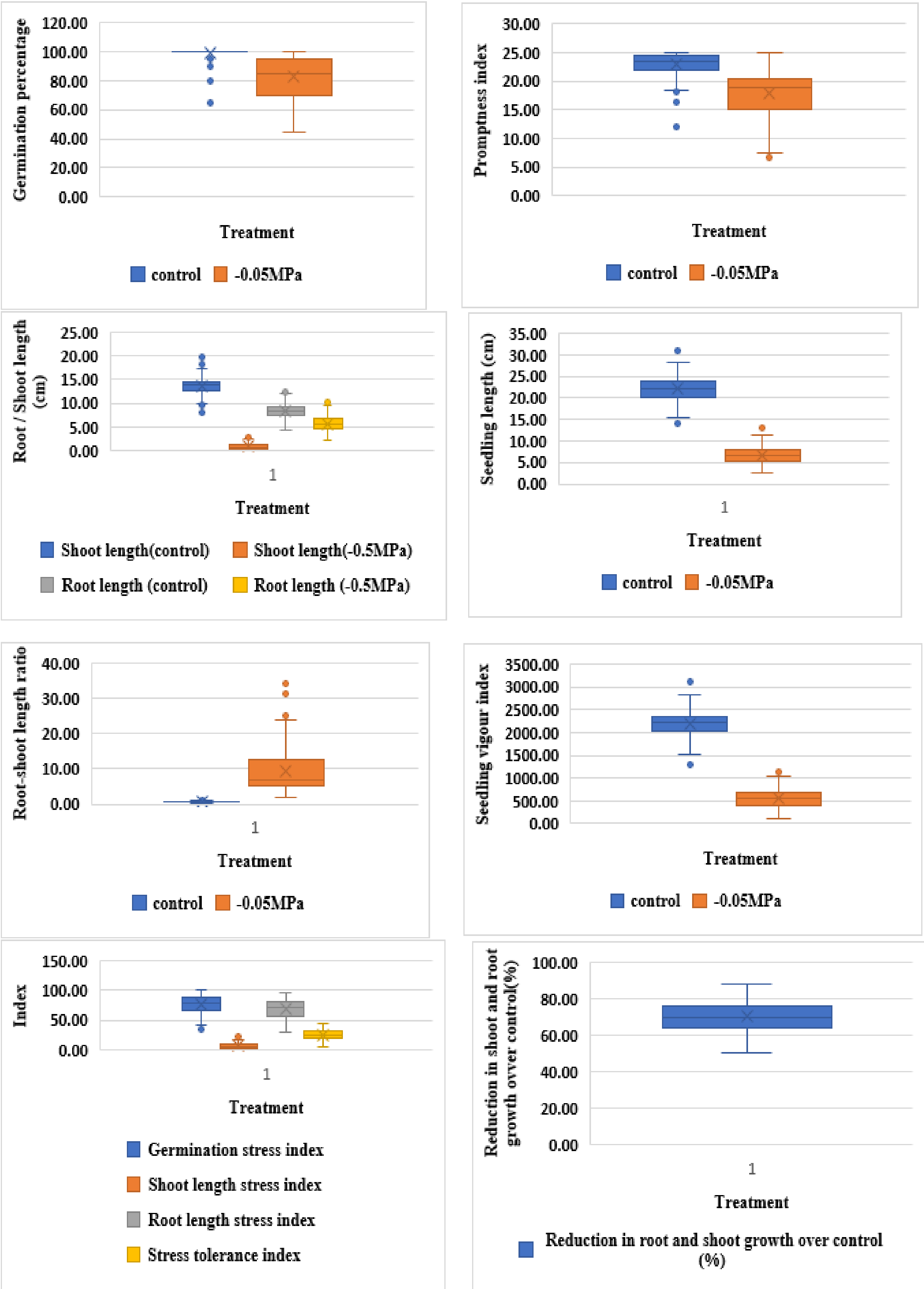

Fig. 1. Comparative response of pre breeding lines of greengram for different seedling traits and indices during initial screening

https://doi.org/10.37992/2023.1404.172

Table 3. Effect of PEG 6000 induced drought stress on seedling growth parameters of 17 pre-breeding lines of greengram during confirmation screening

S.No	Genotypes	Germination (%)		Promptness index		Shoot length (cm)		Root length (cm)		Seedling length (cm)		Root-Shoot length ratio		Seedling vigour index	
		Control	-0.5MPa	Control	-0.5MPa	Control	-0.5MPa	Control	-0.5MPa	Control	-0.5MPa	Control	-0.5MPa	Control	-0.5MPa
	Tolerant lines														
1	GGISC 45	100.00	100.00 ^{a}	21.50	19.88 ^{de}	19.20	1.34 ^{a}	11.05	10.10 ^{a}	30.25	11.44 ^{a}	0.58	7.60 ^{de}	3025.00	1144.00 ^{a}
2	GGISC 41	100.00	95.00 ^{a}	22.00	17.63 ^{ef}	14.51	2.22 ^{bc}	9.97	8.75 ^{b}	24.47	10.97 ^{bc}	0.69	3.94 ^{ghi}	2447.17	1044.33 ^{bc}
3	GGISC 132	100.00	100.00 ^{a}	21.88	20.05 ^{cde}	15.62	2.34 ^{bc}	8.83	7.96 ^{c}	24.45	10.30 ^{bc}	0.56	3.41 ^{hi}	2444.50	1030.05 ^{bc}
4	GGISC 125	100.00	95.00 ^{a}	25.00	22.88 ^{a}	11.70	1.80 ^{cd}	11.52	9.01 ^{a}	23.22	10.81 ^{cd}	0.98	5.00 ^{fgh}	2322.00	1028.20 ^{bcd}
5	GGISC 116	100.00	100.00 ^{a}	24.50	21.88 ^{ab}	13.10	2.08 ^{cd}	10.74	7.93 ^{b}	23.84	10.01 ^{cd}	0.82	3.83 ^{ghi}	2384.00	1001.00 ^{bcd}
6	GGISC 55	100.00	95.00 ^{a}	24.00	20.38 ^{bcd}	13.73	2.20 ^{de}	9.13	8.18 ^{bc}	22.86	10.38 ^{cd}	0.67	3.74 ^{hi}	2285.50	988.60 ^{cde}
7	GGISC 147	100.00	100.00 ^{a}	25.00	19.88 ^{abc}	16.07	1.81 ^{cd}	8.73	8.01 ^{c}	24.80	9.82 ^{bc}	0.55	4.46 ^{fgh}	2479.50	982.00 ^{bc}
8	GGISC 17	100.00	95.00 ^{a}	24.00	20.88 ^{abc}	12.52	1.88 ^{def}	9.36	8.22 ^{bc}	21.88	10.09 ^{de}	0.76	4.38 ^{fgh}	2187.50	956.80 ^{de}
9	GGISC 73	100.00	100.00 ^{a}	24.50	22.25 ^{ab}	13.80	2.96 ^{efg}	7.19	6.37 ^{d}	20.99	9.33 ^{e}	0.52	2.16 ^{i}	2099.00	932.50 ^{e}
10	GGISC 49	100.00	95.00 ^{a}	22.50	19.88 ^{cde}	17.47	2.05 ^{ab}	9.45	7.72 ^{bc}	26.92	9.77 ^{b}	0.54	3.76 ^{hi}	2691.67	929.68 ^{b}
11	GGISC 37	95.00	95.00 ^{a}	23.25	20.50 ^{bcd}	12.82	1.45 ^{def}	9.48	8.06 ^{bc}	22.30	9.51 ^{de}	0.75	5.95 ^{efg}	2126.45	903.45 ^{e}
	Mean	99.55	97.27	23.47	20.55	14.59	2.00	9.58	8.21	24.18	10.21	0.67	4.39	2408.39	993.72
	Susceptible lines														
12	GGISC 60	100.00	50.00 ^{b}	19.00	11.00 ^{h}	13.27	0.29 ^{fgh}	7.27	4.03 ^{ef}	20.54	4.32 ^{f}	0.55	13.82 ^{a}	2053.50	215.75 ^{fg}
13	GGISC 195	95.00	55.00 ^{b}	21.00	11.00 ^{gh}	14.22	0.51 ^{gh}	5.74	3.36 ^{gh}	19.96	3.87 ^{fg}	0.40	6.72 ^{ef}	1897.80	212.10 ^{fg}
14	GGISC 175	100.00	55.00 ^{b}	22.75	11.43 ^{g}	14.97	0.28 ^{efgh}	7.02	3.27 ^{fg}	21.99	3.55 ^{f}	0.47	11.93 ^{ab}	2199.00	195.10 ^{f}
15	GGISC 100	100.00	50.00 ^{b}	20.50	12.38 ^{gh}	12.66	0.38 ^{efgh}	8.93	3.41 ^{d}e	21.59	3.79 ^{f}	0.71	9.02 ^{cd}	2158.50	189.50 ^{f}
16	GGISC 178	100.00	55.00 ^{b}	25.00	12.88 ^{f}	12.20	0.34 ^{h}	6.40	3.09 ^{gh}	18.60	3.42 ^{g}	0.53	9.20 ^{cd}	1860.00	189.15 ^{g}
17	GGISC 159	100.00	60.00 ^{b}	22.25	11.00 ^{g}	16.74	0.27 ^{fgh}	5.67	2.65 ^{h}	22.41	2.92 ^{f}	0.34	10.02 ^{bc}	2241.00	175.20 ^{f}
	Mean	99.17	54.17	21.75	11.61	14.01	0.34	6.84	3.30	20.85	3.64	0.50	10.12	2068.30	196.13

	Germination (%)			Promptness index			Shoot length (cm)			Root length (cm)			Seedling length (cm)			Root-Shoot length ratio			Seedling vigour index		
	G	T	GxT	G	T	GxT	G	T	GxT	G	T	GxT	G	T	GxT	G	T	GxT	G	T	GxT
SED	2.97	1.02	4.2	0.76	0.26	1.08	0.34	0.16	0.67	0.38	0.13	0.54	0.58	0.2	0.81	0.52	0.18	0.74	76.27	26.16	107.8
CD(P=0.05)	6.03	2.07	8.54	1.55	0.53	2.2	0.47	0.33	1.36	0.78	0.26	1.1	1.17	0.4	1.65	1.06	0.37	1.5	155	53.16	219.2

G- Genotype; T- Treatment

length stress index, stress tolerance index and minimal reduction in root and shoot length over control. These lines were further subjected to confirmation screening.

In confirmation screening, 17 pre-breeding lines exhibited a remarkably high level of significant variation for all the seedling parameters and stress indices. At -0.5MPa, GGISC 45 (100%), GGISC 132 (100%), GGISC 125 (100%), GGISC 116 (100%) and GGISC 73 (100%) were recorded with highest germination and least germination per cent was observed in GGISC 60 (50%) and GGISC 100 (50%). Earlier reports in greengram have indicated a decline in germination as a result of decreased water potential (Dutta and Bera, 2008; Kaur *et al*., 2017). The decline in germination could be attributed to a reduced water uptake in the seeds induced by PEG, which in turn leads to a decline in the functioning of hydrolytic enzymes and the transport of stored nutrients essential for the growth of seedlings during germination (Bukhari *et al*., 2021). Under stressed conditions, promptness index was maximum of 22.88 (GGISC 125) and minimum of 11.00 (GGISC 60, GGISC 195, GGISC 159); shoot length recorded was maximum in GGISC 73 (2.96 cm) and minimum in GGISC 159 (0.27 cm); root length was maximum in GGISC 45 (10.10 cm) and minimum in GGISC 159 (2.65 cm). Maximum root-shoot length ratio was observed for GGISC 125 (0.98) in control and for GGISC 60 (13.82) at -0.5MPa. Root-shoot length ratio indicates developmental status of seedling. Root-shoot length ratio was significantly high in stressed condition(-0.5MPa) compared to control. It was observed that, in stressed conditions root-shoot length ratio was higher when compared to control. It could be due to higher root growth than shoot growth was observed under stress condition as the seedling encourages root cell elongation more than shoot cell elongation (Jincy *et al*., 2019). The seedling vigour index varied between 1281.50 (GGISC 195) and 3106.67 (GGISC 45) in control and between 175.20 (GGISC 159) and 1144.00 (GGISC 45) at -0.5MPa. Under stress condition, seedling vigour index of the selected tolerant lines were in the range of 893.75 to 1144.00. The observed range of seedling vigour index for tolerant lines aligns with that of the SVI values recorded during initial screening. Since, seedling vigour index serves as a unifying metric encompassing multiple traits essential for determining the quality and emergence potential of seedlings, pre-breeding lines exhibiting higher seedling vigour index under stress conditions may be extrapolated to exhibit favourable field performance. The higher germination stress index (GSI) of the tolerant lines ranging from 79.50 (GGISC 147) to 92.49 (GGISC 45) indicated rapid germination and development of seedlings at reduced water potential (**Table 4**). Numerous research investigations have indicated that the GSI can also serve as a screening factor for drought tolerance in pulses (Ahmad *et al.,* 2009; Dhopte and Livera, 1989). In addition, the stress tolerance index of the tolerant lines was higher (Vijay *et al.,* 2018) with minimal reduction in root and shoot growth compared to susceptible lines. Therefore, eleven pre-breeding lines *viz.,* GGISC 45, GGISC 41, GGISC 132, GGISC 125, GGISC 116, GGISC 55, GGISC 147, GGISC 17, GGISC 73, GGISC 49 and GGISC 37 were confirmed to be drought tolerant under *in vitro* conditions.

The tolerant pre-breeding lines of greengram *viz*., GGISC 45, GGISC 41, GGISC 132, GGISC 125, GGISC 116, GGISC 55, GGISC 147, GGISC 17, GGISC 73, GGISC 49 and GGISC 37 with higher SVI under stress conditions possessed an inherent potential to survive under high osmotic potential. Subsequently, these pre-breeding lines could undergo field screening under rain-out shelter to

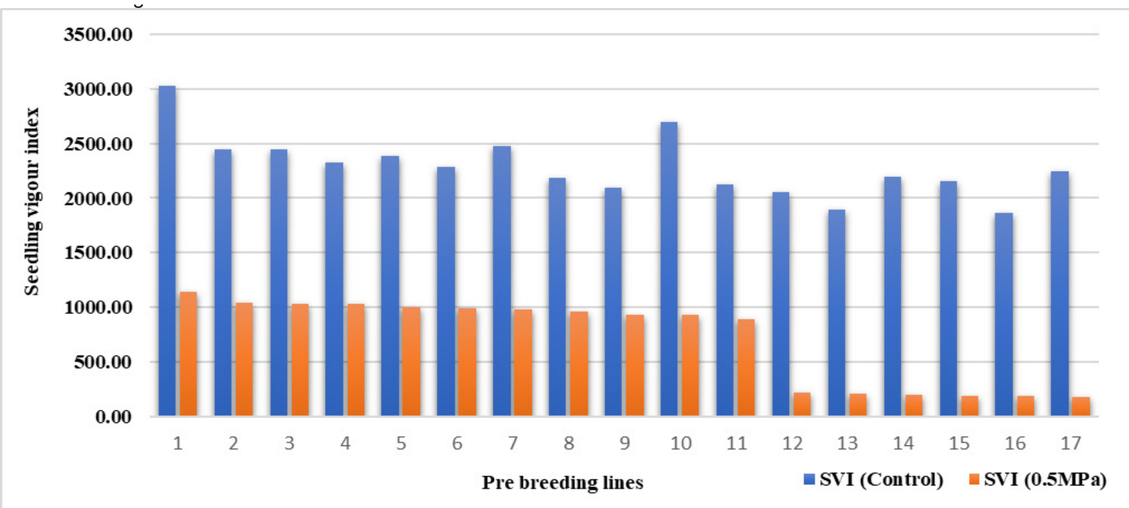

Fig. 2. Effect of drought on seedling vigour index of pre-breeding lines of greengram during confirmation screening

Note: For name of pre-breeding lines, refer Table 3

Table 4. Effect of PEG 6000 induced drought stress on stress tolerance indices in pre-breeding lines of greengram during confirmation screening

S.No.	Genotypes	Germination stress index	Shoot length stress index	Root length stress index	Stress tolerance index	Reduction in root and shoot growth over control (%)
		-0.5MPa	-0.5MPa	-0.5MPa	-0.5MPa	-0.5MPa
			Tolerant lines			
1	GGISC 45	92.49 a	7.02 d	91.74 a	37.81 ab	62.19 cd
2	GGISC 41	80.01 a	15.30 b	87.86 ab	42.57 a	55.16 ef
3	GGISC 132	91.76 a	14.98 b	90.43 ab	42.16 b	57.84 def
4	GGISC 125	91.50 a	15.39 b	78.22 bc	44.23 b	53.45 f
5	GGISC 116	89.35 a	15.86 c	74.63 ab	42.07 b	57.93 def
6	GGISC 55	84.90 a	16.04 b	89.74 ab	43.23 b	54.60 f
7	GGISC 147	79.50 a	11.36 c	91.75 a	39.67 c	60.33 cde
8	GGISC 17	86.98 a	15.16 b	87.81 ab	43.77 b	53.75 f
9	GGISC 73	90.77 a	21.47 a	88.75 ab	44.50 a	55.50 ef
10	GGISC 49	88.33 a	11.76 c	81.59 abc	34.62 c	63.68 c
11	GGISC 37	88.67 a	10.67 c	85.05 abc	42.94 c	57.57 def
	Mean	87.66	14.09	86.14	41.60	57.45
			Susceptible lines			
12	GGISC 60	57.85 b	2.19 e	55.29 d	10.47 c	79.06 b
13	GGISC 195	52.41 b	3.55 e	58.56 d	11.28 c	80.64 b
14	GGISC 175	50.47 b	1.86 e	46.59 de	8.90 c	83.85 ab
15	GGISC 100	60.90 b	3.01 e	38.34 e	8.80 c	82.40 ab
16	GGISC 178	51.50 b	2.75 e	48.22 de	10.17 c	81.61 ab
17	GGISC 159	49.41 b	1.61 e	46.95 de	7.82 c	86.96 a
		G	G	G	G	G
	Mean	53.76	2.50	48.99	9.57	82.42
	SED	6.3	1.22	5.83	3.75	2.58
	CD(P=0.05)	8.32	12.26	8.0	12.37	3.9

G- Genotype

validate their potential for drought tolerance. In conclusion, the pre-breeding lines identified as drought-tolerant in this study could be exploited to develop drought tolerant varieties in greengram.

ACKNOWLEDGEMENT

The first author acknowledge the Indian council of agricultural research (ICAR) for providing a postgraduate scholarship.

REFERENCES

Ahmad, Shamim, Rashid Ahmad, Muhammad Yasin Ashraf, M., Ashraf and Ejaz Ahmad Waraich. 2009. Sunflower (*Helianthus annuus* L.) response to drought stress at germination and seedling growth stages. *Pakisthan Journal of Botany,* **41**(2): 647-654.

Badiane, François Abaye, Diaga Diouf, Djibril Sané, Omar Diouf, Venceslas Goudiaby, and Nicolas Diallo. 2004. Screening cowpea [*Vigna unguiculata* (L.) Walp.] varieties by inducing water deficit and RAPD analyses. *African Journal of Biotechnology,* **3**(3): 174-178. [Cross Ref]

Baroowa, B., Gogoi, N. and Muhammad Farooq. 2016. Changes in physiological, biochemical and antioxidant enzyme activities of green gram (*Vigna radiata* L.) genotypes under drought. *Acta physiologiae plantarum*, **38**: 1-10. [Cross Ref]

Bukhari, Muhammad Adnan, Adnan Noor Shah, Shah Fahad, Javaid Iqbal, Fahim Nawaz, Abdul Manan, and Mohammad Safdar Baloch. 2021. Screening of wheat (*Triticum aestivum* L.) genotypes for drought tolerance using polyethylene glycol. *Arabian Journal of Geosciences,* **14**(24): 2808. [Cross Ref]

Dharani, P., Nivethitha, T., Manju Devi, S., Selsiya, B., Indhu, S. M., Sritharan, D., Manonmani, S., Sudhakar, D. and John Joel, A. 2023. Unearthing the genetic potential of drought resilient rice (*Oryza sativa* L.) landraces through multipronged approaches. *Electronic Journal of Plant Breeding,* **14**(3): 1097-1104. [Cross Ref]

Dharshini, A., Priya, Babu Rajendra Prasad, V., Vanitha, K. and Manivannan, N. 2021. Effect of PEG induced drought stress on seed germination and seedling growth of green gram genotypes. *International Journal of Environment and Climate Change,* **11**(10): 79-90. [Cross Ref]

Dhopte, A. M. and Livera-Munoz, M. 1989. Measuring water potential and water flow in plants. *Useful techniques for plant sciences*: Akola, India, pp:116-154.

Dutta, Puspendu, and Bera, A.K. 2008. Screening of mungbean genotypes for drought tolerance. *Legume Research-An International Journal*, **31**(2): 145-148.

George, Donald, W. 1967. High temperature seed dormancy in wheat (*Triticum aestivum* L.) 1. *Crop Science,* **7**(3): 249-253. [Cross Ref]

Indiastat 2021, https://www.indiastat.com/data/agriculture/total-pulses.

Jiang, Yunfei, Rachid Lahlali, Chithra Karunakaran, Saroj Kumar, Arthur R. Davis, and Rosalind A. Bueckert. 2015. Seed set, pollen morphology and pollen surface composition response to heat stress in field pea. *Plant, cell & environment*, **38**(11): 2387-2397. [Cross Ref]

Jincy, M., Prasad, V., Jeyakumar, P., Senthil, A. and Manivannan, N. 2021. Evaluation of green gram genotypes for drought tolerance by PEG (polyethylene glycol) induced drought stress at seedling stage. *Legume Research-An International Journal,* **44(**6): 684-691.

Kaur, Rajwinder, Jagmeet Kaur and Bains, T.S. 2017. Screening of mungbean genotypes for drought tolerance using different water potential levels. *Journal of advanced agricultural technologies*, **4**(2): 159-164. [Cross Ref]

Nair, Ramakrishnan, M., Abhay, K., Pandey, Abdul, R., War, Bindumadhava Hanumantharao, Tun Shwe, Alam, A., K., M., M. and Aditya Pratap. 2019. Biotic and abiotic constraints in mungbean production progress in genetic improvement. *Frontiers in Plant Science,* **10**: 1340. [Cross Ref]

Nivethitha, T., Ravikesavan, R., KumariVinodhana, N. and Senthil. N. 2020. Deciphering drought tolerance potential of sweet corn genotypes through polyethylene glycol induced drought stress. *Electronic Journal of Plant Breeding,* **11**(1): 217-223. [Cross Ref]

Saima, Shehzadi, Guishuang Li, and Guang Wu. 2018. Effects of drought stress on hybrids of Vigna radiata at germination stage. *Acta Biologica Hungarica,* **69**: 481-492. [Cross Ref]

Senthil-Kumar and Muthappa. 2001. Development and characterization of thermotolerant sunflower (*Helianthus annuus* L.) hybrid: An approach based on temperature induction response (TIR) and molecular analysis. PhD diss., University of Agricultural Sciences, Bangalore.

Surendhar, A., Iyanar, K., Ravikesavan, R. and Ravichandran, V. 2020. Identification of Drought Tolerant Inbreds and Hybrids Through PEG-6000 Mediated Osmotic Stress In Pearl Millet [*Pennisetum glaucum* (L.) R. Br.]. *Multilogic in Science*, **10**(33): 706-712.

Vijay, R., Ravichandran, V. and Boominathan, P. 2018. Assessment of soybean genotypes for PEG induced drought tolerance at germination and seedling level. *Madras Agricultural Journal,* **105**(1-3): 1-6. [Cross Ref]