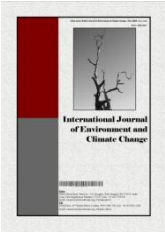

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Effect of PEG Induced Drought Stress on Seed Germination and Seedling Growth of Greengram Genotypes

A. Priya Dharshini[1], V. Babu Rajendra Prasad[1*], K. Vanitha[1] and N. Manivannan[2]

[1]Department of Crop Physiology, TamilNadu Agricultural University, Coimbatore, India.
[2]Department of Genetics and Plant Breeding, TamilNadu Agricultural University, Coimbatore, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Greengram (*Vigna radiata* L. Wilczek) is the third most important pulse crop and drought is the most severe constraint to greengram growth and productivity. The present study was conducted to identify the drought tolerant greengram genotypes. Four greengram varieties used for standarization of drought stress using Polyethyleneglycol (PEG) 6000. The effect of water stress caused by different concentration of PEG 6000 are control (0 MPa), -0.4MPa, -0.5 MPa, -0.6MPa and -0.7 MPa. Increasing PEG concentration decrease the germination percentage, root length, shoot length, fresh weigh and dry weight of seedlings. At -0.5 MPa shows 50% seedling mortality , So control and -0.5 MPa level of drought stress was used for screening the greengram genotypes. Under PEG induced drought situations, parameters such as germination percentage, growth indices and proline content were recorded in all greengram genotypes. Compared to control, PEG induced drought stress (-0.5MPa) decrease all these parameters studied, where as drought has increased the proline content in all greengram genotypes screened. Among the greengram genotypes VGG17019 and VGG17004 posses higher germination percentage, GSI and proline content indicates high level of tolerance to drought stress.

**Corresponding author: E-mail: prasadvenugopal@gmail.com;*

Keywords: Drough tolerance; Greengram; Germination stress index(GSI); Polyethylene glycol; Proline.

1. INTRODUCTION

Drought stress is one of the most important abiotic stresses constraining the crop productivity Worldwide. Due to climate change and increasing global temperature, many countries faces the adverse effect of drought and this limits the crop production in last few decades [1]. Greengram is the third most important pulse crop after chickpea and pigeon pea.It is a short duration crop with high nutritional value(24% protein) and mostly cultivated in rainfed condition. It also grown as intercrop and enhances the soil fertility by atmospheric nitrogen fixation [2- 3]. The growth and productivity of mungbean in arid and semi-arid regions is adversely affected by drought stress [4]. However, varieties respond differently to drought stress depending on the stress duration, crop growth stage and genetic potential, which might result in moderate to severe yield loss [5]. Germination is an important stage of plant life which is greatly influenced by drought and limited water supply during this stage inhibits seed germination and suppresses growth and development of seedlings [6]. So, Germination percentage is used as criteria for screening against moisture stress tolerance and to imitate drought stress responses PEG polymer has been utilized in plants with minimal metabolic intervention. Hence polyethylene-glycol (PEG)-based in vitro screening approach is used for selecting tolerant genotypes able to germinate under drought stress conditions [7]. In this present experiment was conducted to know the germination responses of selected greengram genotypes exposed to drought stress by using PEG and to identify the best tolerant greengram genotypes that can be grown successfully in drought prone areas.

2. MATERIALS & METHODS

The greengram varieties CO-8,VBN2,VBN3 and VBN4 were used for standardization of drought stress using PEG 6000.The greengram seeds were first sterilized with 0.1% mercury chloride for 2-3 mins and washed thoroughly with distilled water. Then 20 sterilized seeds were placed in petridish containing moistened blotting paper with various water potential viz., 0.0 (control), -0.4, -0.5, -0.6 and -0.7 MPa PEG 6000.Three replication were maintained for each treatment.Among five concentrations of PEG,0.5 MPa shows 50 % mortality rate in seedlings. So, control and 0.5 MPa further taken for screening of 11 greengram genotypes. The number of germinated seeds of each genotype was counted on alternative days from day 2 to day 8 to determine germination percentage. Then seedlings allowed grow for 8 days and growth parameters like root length and shoot length were recorded. After recording fresh weight of seedlings, they were dried at 70 º C for 48 h in an oven and their dry weight were estimated. From these data promptness index (PI),germination stress index (GSI), root and shoot length stress index (RLSI & SLSI) and seed vigour (SV) were calculated by using following formulas.

2.1 Greengram Genotypes Used in this Experiment

S. no.	Genotypes
1.	CO-8
2.	COGG1332
3.	VBN3
4.	VBN4
5.	VGG15029
6.	VGG16069
7.	VGG17019
8.	VGG17036
9.	VGG17037
10.	VGG17003
11.	VGG17004.

2.2 Germination Percentage

It was calculated described by [8].

Germination% =Total no.of germinated seeds /Total seeds placed for germination ×100

2.3 Promptness Index

Promptness Index(%) = nd_2 (1.00) + nd_4 (0.75) + nd_6 (0.5) + nd_8 (0.25)

Where, $nd_2$$nd_4$, $nd_6$and nd_8 were seeds germinated on the 2nd, 4th, 6th, 8th day of sowing respectively [9].

2.4 Germination Stress Tolerance Index

GSI calculated by determining Promptness Index [10].

GSI(%) =Promptness Index of stressed seeds / Promptness Index of control seeds x 100

2.5 Root Length Stress Index: [11]

RLSI(%)= Root length of stressed plant / Root length of control plants x 100.

2.6 Shoot Length Stress Index: [11]

SLSI(%) = Shoot length of stressed plant / Shoot length of control plants x 100

2.7 Seed Vigour

It was calculated described by [12].

Seed Vigour (%) = Germination percentage × Seedling length.

2.8 Proline Content

Greengram seedlings of both control and PEG (-0.5 MPa) treatments were used to estimate the proline content according to Bates et al. [13]. Seedling tissues (500 mg) were homogenized in 10 ml of 3% sulfosalicylic acid and centrifuged (High Performance Centrifuge Machine) at 11,500 rpm for 10 min.Supernatant was mixed with acid ninhydrin, glacial acetic acid and phosphoric acid. The mixture was incubated at 100°C for 1 h and then cooled down. Toluene was added to the mixture in separating funnel and mixed thoroughly. Pink coloured upper layer was collected to determine the proline content, it was measured in spectrophotometer (Eppendorf BioSpectrometer kinetic) at 520 nm.

2.9 Statistical Analysis

The experimental design was factorial experiment under completely randomized design (FCRD) with three replications for standardization of drought stress and screening of 11greengram genotypes. All growth parameters were expressed as the means of three replicates and significant differences between treatments were analyzed using Analysis of Variance (ANOVA) following the method as described by [14].

3. RESULTS AND DISSCUSSION

3.1 Standarization of Drought Stress Using PEG 6000

A standardization experiment was conducted with greengram varieties CO 8, VBN2, VBN3 and, VBN4 to determine osmotic stress level. These varieties grown under different concentrations of PEG solutions *i.e.,* Control(0 MPa), -0.4 MPa, -0.5Mpa, -0.6M Pa and - 0.7 MPa.After 8 days of sowing (DAS) No.of germinated seeds, root length,shoot length,fresh weight and dryweight were recorded. And from this recorded data Germination percentage, Promptness Index, Germination stress index, Root length stress index, Shoot length stress index and Seed vigour like indices are calculated. Growth parameters results are means of three replicates and given in Tables 1-6. From Table 1, it is clear that germination percentage decreased with increasing osmotic stress. These results were also in accordance with the findings of Dutta et al. [15] and Basal et al. [16]. It could be seen that germination percentage reduce from 100%(control) to 5% (-0.7 M Pa) *i.e.,* 95% reduction was observed. And 50 % seedling mortality was observed under-0.5 MPa. Highest germination rate (80%) was recorded in VBN 4and lowest was recorded in VBN2 (40%). And root length, shoot length, fresh weight, dry weight, PI, GSI, RLSI, SLSI and Seed Vigour also decreasing with increasing PEG concentration (Tables 1-6). Increased moisture stress reduced shoot length and root length and negatively affect plant growth and development. Compared to root length, shoot length reduction was more at decreasing water potential. And increasing PEG concentration drastically reduced the fresh weight and dry weight of seedlings *i.e.,*90 % reduction compared to control. These results were similar to findings of Kaur et al. [6]. Therefore in greengram moisture limitation during seedling stage adversely affect plant growth and development.

3.2 Screening of Greengram Genotypes under PEG Induced Drought Stress Condition

Based on the standardization, osmotic stress level of -0.5 MPa was selected and was used to screen eleven greengram genotypes at seedling stage. At the end of stress period (8 days after sowing) root length, shoot length, fresh weight and dry weight were recorded (Table 7). Observations indicate that moisture stress has significantly reduced the growth parameters of the greengram genotypes at seedling stage.

3.2.1 Germination percentage and promptness index

Germination is one of the most important stages in plant life cycle. From Fig. 1 & Fig. 2, it is evident that drought stress(-0.5MPa) decreased

Table 1. Effect of different PEG 6000 concentration on germination percentage of greengram varieties

Greengram varieties	No.of Germinated seeds(8DAS)					Germination Percentage(%)				
	Control	-0.4 MPa	-0.5MPa	-0.6 MPa	-0.7 MPa	Control	-0.4 MPa	-0.5M Pa	-0.6 MPa	-0.7 MPa
CO 8	20	14.00	10.00	3.00	2.00	100	70.00	50	15	10
VBN2	20	8.00	4.00	2.00	0.00	100	40.00	20	10	0
VBN3	20	16.00	8.00	5.00	0.00	100	79.86	40	25	0
VBN4	20	16.00	11.00	9.00	2.00	100	80.00	55	45	10
Mean	**20**	**13.50**	**8.25**	**4.75**	**2.00**	**100**	**67.47**	**41.25**	**23.75**	**5.00**
	G**	**T****		**G×T****		**G****	**T****		**G×T****	
SED	0.18	0.2		0.4		0.83	0.93		1.86	
CD(P=0.05)	0.36	0.41		0.82		1.68	1.88		3.77	
CD(P=0.01)	0.49	0.55		1.09		2.25	2.52		5.05	

*DAS – Days After Sowing; G- Genotype; T-Treatment; Significance differences are indicated ** at 0.05 and 0.01 level*

Table 2. Effect of different PEG 6000 concentration on root length and shoot length of greengram varieties

Greengram Varieties	Root length (cm)					Shoot Length (cm)				
	Control	-0.4 MPa	-0.5MPa	-0.6 MPa	-0.7 MPa	Control	-0.4 MPa	-0.5M Pa	-0.6 MPa	-0.7 MPa
CO 8	2.52	1.00	0.60	0.50	0.40	4.40	0.50	0.40	0.35	0.30
VBN2	2.50	0.90	0.70	0.50	0.00	4.10	0.60	0.50	0.40	0.00
VBN3	2.45	1.10	0.80	0.40	0.00	3.50	0.60	0.50	0.40	0.00
VBN4	2.00	0.70	0.50	0.30	0.20	4.10	0.50	0.41	0.30	0.20
Mean	**2.37**	**0.93**	**0.65**	**0.43**	**0.15**	**4.03**	**0.55**	**0.45**	**0.36**	**0.13**
	G **	**T ****		**G×T ****		**G ****	**T ****		**G×T ****	
SED	0.02	0.03		0.05		0.02	0.03		0.06	
CD(P=0.05)	0.04	0.05		0.11		0.05	0.06		0.13	
CD(P=0.01)	0.06	0.07		0.14		0.07	0.08		0.17	

*G- Genotype; T-Treatment; Significance differences are indicated ** at 0.05 and 0.01 level.*

Table 3. Effect of different PEG 6000 concentration on freshweight and dry weight of greengram varieties

Greengram Varieties	Fresh weight (g)					Dryweight (g)				
	Control	**-0.4 MPa**	**-0.5MPa**	**-0.6 MPa**	**-0.7 MPa**	**Control**	**-0.4 MPa**	**-0.5M Pa**	**-0.6 MPa**	**-0.7 MPa**
CO 8	3.10	1.60	1.10	0.80	0.50	2.30	1.00	0.70	0.60	0.30
VBN2	2.30	1.80	1.00	0.60	0.00	1.20	0.90	0.60	0.40	0.00
VBN3	2.40	1.50	1.17	0.55	0.00	2.10	1.10	0.90	0.50	0.00
VBN4	2.30	1.40	0.90	0.70	0.40	1.40	0.70	0.50	0.40	0.20
Mean	**2.53**	**1.57**	**1.04**	**0.66**	**0.23**	**1.75**	**0.93**	**0.68**	**0.48**	**0.13**
	G **		**T ****		**G×T ****	**G ****		**T ****		**G×T ****
SED	0.02		0.03		0.06	0.01		0.02		0.03
CD(P=0.05)	0.05		0.06		0.12	0.03		0.04		0.07
CD(P=0.01)	0.07		0.08		0.17	0.04		0.05		0.11

*G- Genotype; T-Treatment; Significance differences are indicated ** at 0.05 and 0.01 level.*

Table 4. Effect of different PEG 6000 concentration on promptness index and germination stress index of greengram varieties

Greengram Varieties	Promptness index (%)					Germination stress Index (%)			
	Control	**-0.4 MPa**	**-0.5MPa**	**-0.6 MPa**	**-0.7 MPa**	**-0.4 MPa**	**-0.5M Pa**	**-0.6 MPa**	**-0.7 MPa**
CO 8	55	32.50	19.70	5.50	4.50	59.00	35.90	9.83	8.10
VBN2	45	14.75	8.25	4.50	0	32.77	18.33	10.00	0
VBN3	55	37.00	11.75	7.00	0	53.67	21.30	12.72	0
VBN4	50.75	30.50	24.23	18.79	3.73	60.00	47.78	38.80	8.40
Mean	**51.44**	**28.69**	**15.98**	**8.95**	**2.06**	**51.36**	**30.83**	**17.84**	**4.12**
	G **		**T ****		**G×T ****	**G ****		**T ****	**G×T****
SED	0.58		0.64		1.29	0.46		0.52	1.05
CD(P=0.05)	1.17		1.31		2.62	0.94		1.06	2.12
CD(P=0.01)	1.56		1.75		3.51	1.27		1.42	2.84

*G- Genotype; T-Treatment; Significance differences are indicated ** at 0.05 and 0.01 level*

Table 5. Effect of different PEG 6000 concentration on root length stress index and shoot length stress index of greengram varieties

Greengram Varieties	Root length stress index (%)				Shoot length stress Index (%)			
	-0.4 MPa	-0.5MPa	-0.6 MPa	-0.7 MPa	-0.4 MPa	-0.5M Pa	-0.6 MPa	-0.7 MPa
CO 8	44.00	24.00	20.00	16.00	12.50	10.00	9.20	7.50
VBN2	40.00	26.00	20.00	0.00	14.60	12.19	9.70	0.00
VBN3	41.67	32.00	16.00	0.00	17.14	14.28	11.42	0.00
VBN4	39.33	25.00	15.00	10.00	11.00	8.00	6.66	4.40
Mean	**41.25**	**26.75**	**17.75**	**6.50**	**13.81**	**11.12**	**9.25**	**2.97**
	G	**T**	**G×T**		**G**	**T**		**G×T**
	**	**	**		**	**		**
SED	0.64	0.72	1.44		0.18	0.2		0.41
CD(P=0.05)	1.31	1.45	2.91		0.37	0.42		0.84
CD(P=0.01)	1.77	1.95	3.9		0.5	0.56		1.12

*G- Genotype; T-Treatment; Significance differences are indicated ** at 0.05 and 0.01 level*

Table 6. Effect of different PEG 6000 concentration on seed vigour of greengram varieties

Greengram Varieties	Seed vigour (%)				
	Control	-0.4 MPa	-0.5MPa	-0.6 MPa	-0.7 MPa
CO 8	650.00	112.00	50.00	6.00	3.00
VBN2	626.67	84.00	24.00	4.00	0.00
VBN3	612.00	124.10	53.67	10.00	0.00
VBN4	630.00	104.00	49.63	15.00	2.33
Mean	**629.67**	**106.03**	**44.33**	**8.75**	**1.33**
	G**	**T ****		**G×T ****	
SED	1.69	1.89		3.78	
CD(P=0.05)	3.42	3.82		7.65	
CD(P=0.01)	4.58	5.12		10.24	

*G- Genotype; T-Treatment; Significance differences are indicated ** at 0.05 and 0.01 level.*

the germination rate and promptness index of the greengram genotypes compared to control. But there were variations in the magnitude of the reduction among 11 greengram genotypes studied. At -0.5 MPa level of osmotic stress, highest germination percentage and promptness index was observed in the greengram genotype VGG17019 (70%, 26.75%) followed by VGG17004 (67%,26.5%) and lowest germination percentage and promptness index was observed in the genotype VBN3 (35%,16.5%) followed by CO 8 (35%,15.5%).Thus, the osmotic stress might reduce germination rate by decreasing the metabolic and enzymes activity and consequently, reducing meristem development [16]. These results were similar with findings of Kaur et al. [6], Musculo et al. [7], Dutta et al. [15] and Imitiaz et al. [17]. Thus the difference in germination rate among the greengram genotypes under moisture limited (or) stress condition would be helpful to identify the tolerant genotypes against drought condition.

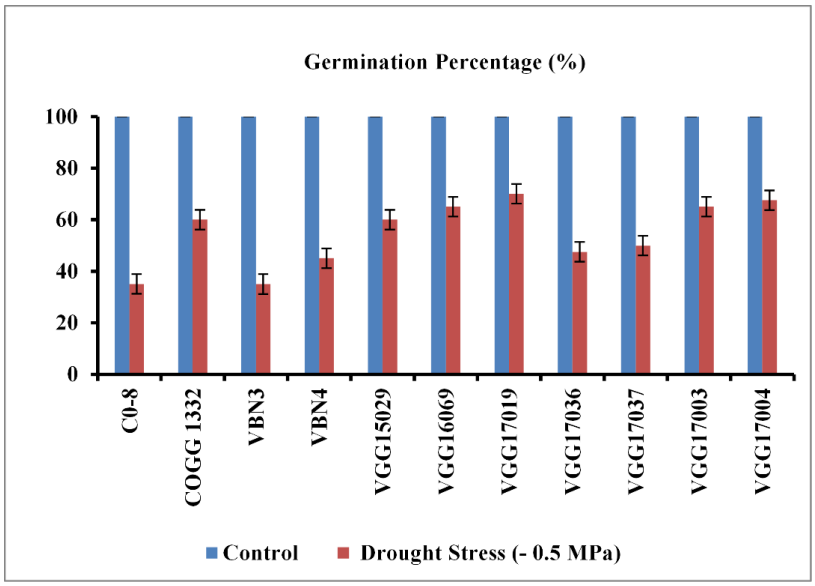

Fig. 1. Effect of PEG induced drought stress (-0.5MPa) on germination percentage of greengram genotypes. Bars represent the standard errors of mean values

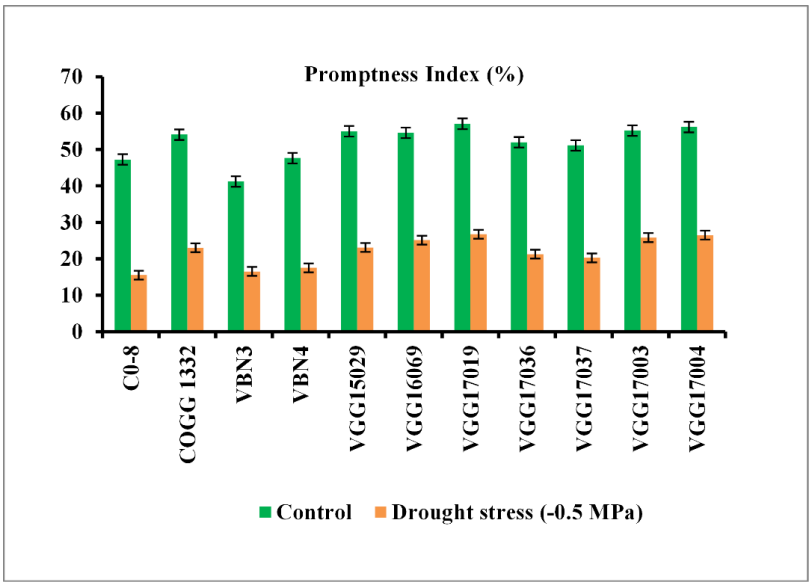

Fig. 2. Effect of PEG induced drought stress (-0.5MPa) on promptness index of greengram genotypes. Bars represent the standard errors of mean values

3.2.2 Root length and shoot length

Data represented in Table 7 shows that osmotic stress -0.5 MPa cause significant reduction in root length and shoot length compared to control.The reduction in root and shoot length of the greengram seedlings under drought stress may be attributed to a reduced cellular division and elongation during germination [7]. Greengram genotypes showed varied response at low water potential and Among them, VGG17019 and VGG17004 shows highest root length and shoot length compared to other genotypes. Greater percent reduction in shoot length (94 % reduction) was noticed in comparison to root length (60 % reduction) under water stress .These results were similar with the findings of Kaur et al. [6], Basal et al. [16], Dutta and Berra [18].

3.2.3 Fresh weight and dry weight

Observations (Table 7) reveals that PEG 6000 induced reduction in water potential has caused a remarkable reduction in greengram seedlings fresh weight (80% reduction) and dry weight(60 % reduction). It may due to lower dry matter partitioning between cotyledons and embryonic axis under water stress during seedling development [15]. At-0.5 MPa level of osmotic stress, greengram genotypes VGG 17019 and VGG 17004 shows higher fresh weight (1.08& 0.8g) and dry weight(0.8 &0.6 g)and CO 8, VBN-4, VGG17036 and VGG17037recorded the lowest fresh weight(<0.60 g) and dry weight (<0.35g)as compared to other greengram genotypes (Table 7).

3.2.4 Seedling vigour

Seedling vigour has significantly decreased in all the greengram genotypes at -0.5 MPa level of osmotic stress as compared to that of control. Different greengram genotypes significantly varied in vigour index and among the genotypes VGG 17019(125.5%) and VGG17004 (121.5%) showed higher seedling vigour and CO 8 (59.5 %) and VBN 3 (60%) showed lowest seedling vigour compared with other genotypes (Table 7). This vigour index results shows that percent germination was reduced with increasing moisture stress, but the level of reduction was not same for all greengram genotypes [17].

3.2.5 Stress indices: GSI, RLSI& SLSI

Germination Stress Index (GSI) is indicative of the speed of germination and quick establishment under reduced water potential conditions. Higher GSI indicates the quicker the establishment capacity of a particular genotype [6]. From Fig. 3 it is evident that greengram genotypes VGG17019 (48.4%) and VGG17004 (48%) posses high GSI followed by VGG17069 (47.3%) & VGG17003 (47%).High GSI indicate higher level of drought tolerance. So, GSI can be used as criteria for screening drought stress tolerance [19]. And also higher RLSI and SLSI was recorded in the greengram genotype VGG17019(47% & 7%) and VGG 17004 (44% & 7%) (Fig. 4 & Fig. 5).

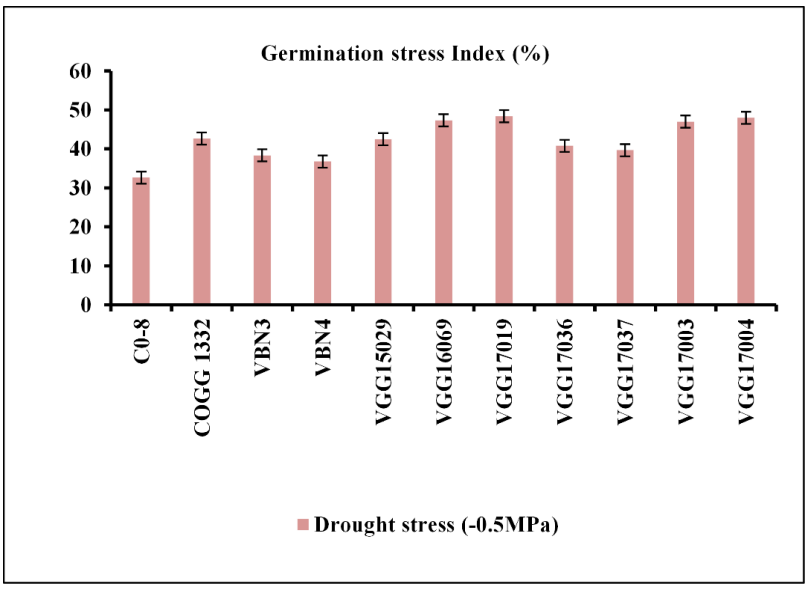

Fig. 3. Germination Stress Index of greengram genotypes. Bars represent the standard errors of mean values

Table 7. Effect of PEG 6000 induced drought stress on seedling growth parameters of 11 greengram genotypes

Greengram Genotypes	Root Length (cm)			Shoot length (cm)			Fresh weight (g)			Dry weight (g)			Seed Vigour (%)		
	Control	-0.5MPa		Control	-0.5MPa		Control	-0.5MPa		Control	-0.5MPa		Control	-0.5MPa	
CO-8	4.51	1.51		9.02	0.50		4.17	0.58		1.25	0.27		1301.88	59.50	
COGG 1332	4.90	2.06		9.25	0.61		4.37	0.62		1.53	0.46		1350.33	114.00	
VBN3	3.48	1.05		8.83	0.44		3.57	0.55		1.07	0.26		1222.67	60.00	
VBN4	4.26	1.32		8.91	0.50		3.36	0.59		1.18	0.33		1286.67	81.00	
VGG15029	4.91	1.91		9.24	0.60		4.22	0.60		1.51	0.43		1347.05	112.00	
VGG16069	5.01	2.06		9.16	0.63		4.25	0.63		1.55	0.50		1356.67	120.00	
VGG17019	5.22	2.50		9.34	0.70		4.25	1.08		1.80	0.80		1400.47	125.50	
VGG17036	3.44	1.17		8.21	0.50		4.11	0.55		1.40	0.33		1200.00	92.17	
VGG17037	3.54	1.28		8.00	0.51		4.01	0.50		1.30	0.30		1205.00	84.33	
VGG17003	5.18	2.17		9.31	0.60		4.16	0.79		1.68	0.60		1359.67	117.00	
VGG17004	5.18	2.36		9.59	0.70		4.55	0.81		1.91	0.65		1394.67	121.50	
Mean	**4.51**	**1.76**		**8.99**	**0.57**		**4.09**	**0.66**		**1.47**	**0.45**		**1311.37**	**98.82**	
	G	**T**	**G×T**	**G**	**T**	**G×T**	**G**	**T**	**G×T**	**G**	**T**	**G×T**	**G**	**T**	**G×T**
	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**
SED	0.09	0.03	0.12	0.06	0.02	0.09	0.07	0.03	0.11	0.03	0.01	0.04	11.5	4.89	16.2
CD(P=0.05)	0.18	0.07	0.26	0.13	0.05	0.19	0.15	0.06	0.21	0.06	0.02	0.09	23.1	9.86	32.7
CD(P=0.01)	0.24	0.11	0.35	0.18	0.07	0.25	0.21	0.08	0.29	0.08	0.03	0.12	31.0	13.2	43.6

*G- Genotype; T-Treatment; Significance differences are indicated ** at 0.05 and 0.01 level.*

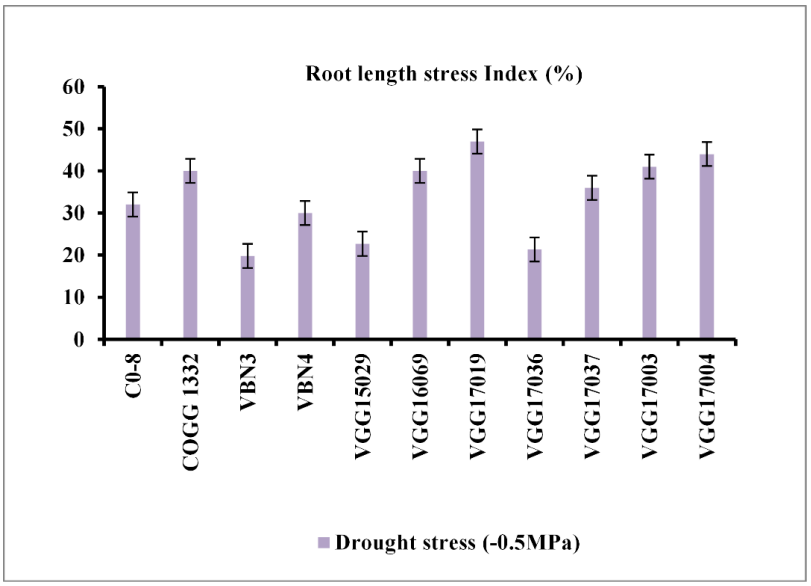

Fig. 4. Root length Stress Index of greengram genotypes. Bars represent the standard errors of mean values

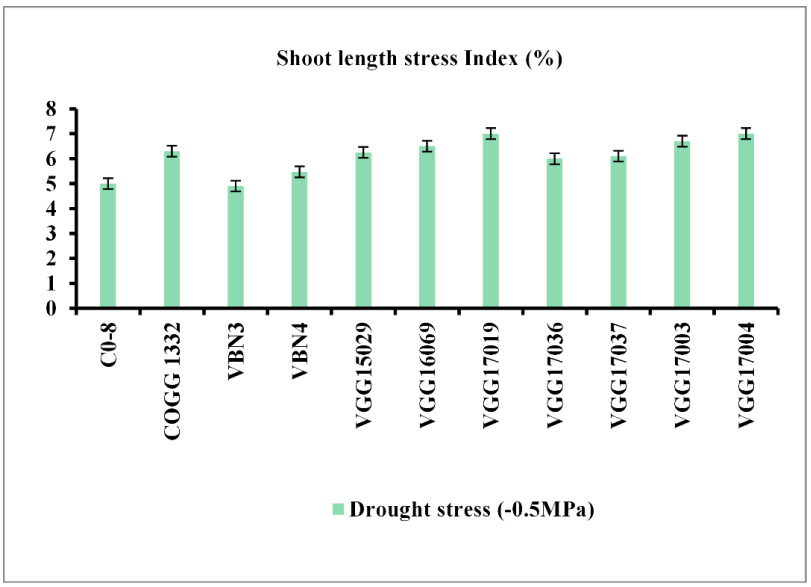

Fig. 5. Shoot length Stress Index of greengram genotypes. Bars represent the standard errors of mean values

3.2.6 Proline content

Proline accumulation is the important physiological indicator in crop plants in response to drought stress [20]. And compared to control, PEG induced osmotic stress has significantly increased the proline content in all greengram genotypes studied (Fig. 6). Among the genotypes, VGG17019 (3.8 µmolg^{-1} FW) followed by VGG17004 (3.76 8 µmolg^{-1} FW) recorded higher proline content and the genotypes CO 8 (2.5µmolg^{-1} FW) recorded lowest proline accumulation under moisture stress situations. Increase in proline accumulation under stress condition is an osmotic adjustment strategy, and therefore proline accumulation is considered as an important indicator for the onset of drought tolerant mechanism as observed at higher level in stress-tolerant genotypes as compared to susceptible ones. Similiar type of observations was reported in greengram by Muscolo et al. [7], Dutta et al. [15], Naidu et al. [21] and Saima et al. [22].

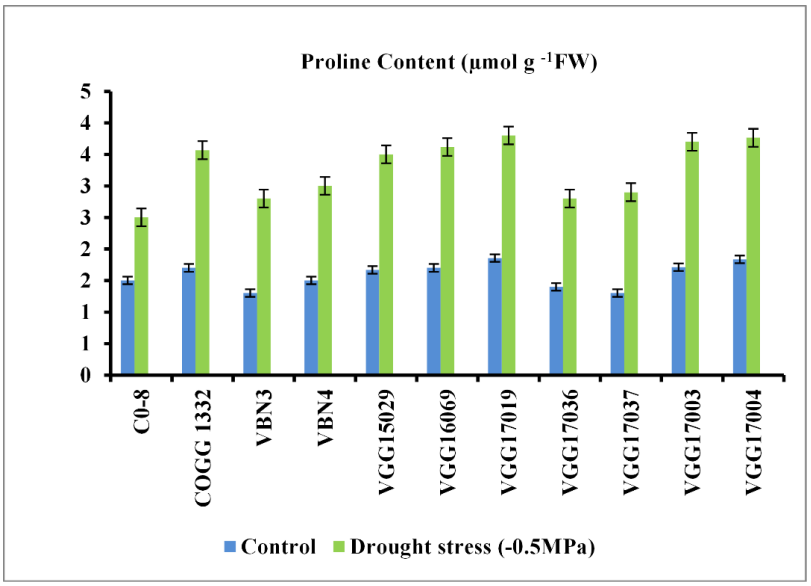

Fig. 6. Effect of PEG induced drought stress (-0.5MPa) on proline content of greengram genotypes. Bars represent the standard errors of mean values

4. CONCLUSION

The present study was conducted to screen and identify greengram genotypes on the basis of various growth indices such as germination percentage, germination stress index, vigour index and proline content under osmotic stress conditions. Among the eleven greengram genotypes evaluated, VGG17019 &VGG17004 posses osmotic stress tolerance traits like higher germination percentage(>65%), higher GSI and higher proline content followed by VGG17003, VGG16069,VGG15029,COGG1332. These identified greengram genotypes can be used for evolving drought tolerant greengram varieties in the pulses breeding programs which will be very much useful in the drought prone areas.

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COMPETING INTEREST

The authors declare that there is no competing interest exist.

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