Original Research Article

Evaluation of blackgram genotypes for their droughttolerance at seedling and vegetative phase

ABSTRACT

Aim: To screen and identify blackgram genotypes for drought tolerance at seedlings and vegetative stage.
Study design: Completely randomized complete block design.
Place of Study: National Pulses Research Centre (NPRC), Tamil Nadu Agricultural University, Vamban, Pudukkottai District
Methodology: Laboratory experiments was conducted at NPRC, Vamban. Twenty-Five blackgram varieties were used for this study. For screening at seedling stage, the treatments were Control – Water (0 Mpa) and PEG 6000 induced drought stress (-0.5 MPa). Blackgram seeds were germinated in water and PEG 6000 solution (-0.5 MPa). On the eighth day various seedling growth indices were recorded. For pot culture experiments, the two treatments were imposed T_1 – Control and T_2 – Drought stress (50% Field capacity) after the appearance of two trifoliate leaves by dry down approach. The stress was imposed for five days. At the end of fifth day, growth parameters, relative water content and leaf chlorophyll content was recorded. Two-way ANOVA was used to analyze the data.

Results: In blackgram, drought stress at seedling stage affects the seedling germination, establishment and its growth indices. At vegetative stage, drought stress negatively affects the plant growth, leaf area, leaf relative water content and chlorophyll content. Among the blackgram genotypes screened, the genotypes VBG 11031 and VBG 1711 were found to be tolerant to PEG 6000 induced drought stress. At vegetative stage, the genotypes VBG 11062, VBG 11024 and VBG 1725 were tolerant to drought stress at vegetative stage.

Conclusion: Present study concluded that the blackgram genotypes VBG 11031, VBG 1711, VBG 11062, VBG 11024 and VBG 1725 were found to be tolerant against drought stress at seedling and vegetative stage.

Keywords: Blackgram; Drought; Seedling stage, Seedling vigour; Chlorophyll Stability Index; Relative Water Content, Stress Tolerance Index.

1. INTRODUCTION

Pulses are the chief source of protein in human diet, and they have a major role in ensuring nutritional security of the developing Countries. In human diet pulses have a vital Role as it is the chief source of vegetable protein; besides protein, it is also rich in vitamins, complex carbohydrates and minerals. Pulses cultivation also improves soil fertility by biological nitrogen fixation. India ranks first in world's pulses production. In recent years, there has been a constraint in pulse production due to climate change induced drought stress, which severely affect the growth and productivity of the pulse crop. Blackgram (*vigna mungo L*.; *Fabaceae*), one of the important pulse crops and widely cultivated across Tamil Nadu. Drought stress is one of the serious threats for blackgram cultivation, it causes multiple damaging effects in crops. The dehydration in leaves was increased during drought stress. It primarily disrupts the osmotic balance, affects the metabolic pathway and leads to physiological disorders [1].Drought stress at seedling establishment stage is detrimental to crop growth, establishment and ultimately affects its yield.

To develop crops which have better tolerance to drought stress, a basic understanding of physiological and biochemical networks involved in abiotic stress tolerance mechanism is essential. In blackgram, the basic understanding of the basic physiology and the parameters that contribute for its drought tolerance is very much essential to identify and evolve drought tolerant varieties. Therefore, the physiological screening and identification of tolerant genotypes of blackgram genotypes for their tolerance against drought stress is very much essential for development of climate resilient blackgram varieties that can survive and yield better under aberrant climatic conditions.

With this background, the current study was conducted to screen and identification of blackgram genotypes tolerant to drought stress at seedling and vegetative stage based on the morphological indices and physiological parameters that may pave the way for the identification of tolerant lines against drought stress.

2. MATERIAL AND METHODS

2.1 Study Location

The experiment was conducted at National Pulses Research Centre (NPRC), Tamil Nadu Agricultural University (TNAU), Vamban, (10°21''N 78°54'E), Pudukkottai District. The impact of osmotic stress on blackgram seed germination, seedling growth indices was conducted in the laboratory and the pot culture experiments was carried out under glass-house conditions at NPRC, Vamban.

2.2 Experiment details

2.2.1 Laboratory experiment

The blackgram genotypes (24 Numbers) were screened for their osmotic stress tolerance using PEG 6000. The blackgram 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 petri-dish containing moistened blotting paper with water (control) (or) PEG 6000 solution (-0.5 MPa). Three replications were maintained for each treatment. The number of germinated seeds of each genotype was counted on alternative days from day 2 to day 8 to determine germination percentage. Emergence of 2mm radicle was set as the criteria for germination [2].

Table1: Blackgram genotypes used in this experiment.

S. no.	Genotype	S. no.	Genotype	S. no.	Genotype	S. no.	Genotype	S. no.	Genotype
1.	VBN (Bg) 4	6.	ADT 5	11.	VBG11031	16.	VBG 1710	21.	VBG 1725
2.	VBN (Bg) 5	7.	MDU 1	12.	VBG 1612	17.	VBG 1711	22.	VBG 1727
3.	CO 6	8.	UTTRA	13.	VBG 1605	18.	VBG 1714	23.	VBG 1728
4.	VBN 6	9.	VBG11062	14.	VBG 1704	19.	VBG 1719	24.	VBG 1729
5.	VBN 8	10.	VBG11024	15.	VBG 1707	20.	VBG 1724	25.	VBG 1730

After eight days after germination, in randomly selected seedlings, radicle length and the following parameters were calculated.

2.2.2 Germination Percentage

It was calculated described by [3].

$$\text{Germination Percentage} = \frac{\text{Total no. of germinated blackgram seeds}}{} \times 100$$

Total seeds placed for germination

2.2.3 Promptness Index

Promptness Index (%) = nd2 (1.00) + nd4 (0.75) + nd6 (0.5) + nd8 (0.25)

Where, nd2, nd4, nd6and nd8 were seeds germinated on the 2^{nd}, 4^{th}, 6^{th}, 8^{th} day after sowing respectively [4].

2.2.4 Germination Stress Tolerance Index

GSTI calculated by determining Promptness Index [5].

$$GSTI = \frac{\text{Promptness Index of seeds exposed to osmotic stress}}{\text{Promptness Index of control seeds}} \times 100$$

2.2.5 Root Length Stress Index: [6].

$$RSTI = \frac{\text{Root length of the plant exposed to drought}}{\text{Root length of plant without stress}} \times 100$$

2.6 Shoot Length Stress Index: [6]

$$SLSI = \frac{\text{Shoot length of the plant exposed to drought}}{\text{Shoot length of plant without stress}} \times 100$$

2.7 Seedling Vigour

It was calculated described by [7].

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

2.2.8 Pot Culture experiment

The blackgram genotypes (Table 1) were sown in pot to study the influence of drought stress on vegetative phase of blackgram. The plants were imposed to drought (50% field capacity for 5 days) during vegetative Stage (20 Days after sowing) after the appearance of two trifoliate leaves. At the end of the stress period, leaf samples were collected for relative water content, chlorophyll Stability Index, shoot length, root length and leaf area were measured.

2.2.9 Measurement of relative water content

The physiologically functional leaf (third leaf from the top) samples were collected from blackgram genotypes, and 50 uniform leaf discs were taken. Fresh weight (FW) of the leaf discs were recorded and the leaf discs were soaked in water for three hour to attain turgid conditions, then the excess water droplets present on the leaf discs surface were removed and the turgid weight (TW) was recorded and then the leaf discs were transferred to a butter paper cover and kept in hot air oven at 80°C for 48 h, then the dry weight (DW) was recorded. Relative water content (RWC) of leaf was measured according to [8].

$$RWC = \frac{(FW - DW)}{(TW - DW)} \times 100$$

2.2.10. Measurement of chlorophyll stability index (CSI)

The Chlorophyll stability index (CSI) was measured according to [9]. The blackgram leaf bits were taken in two test tubes, distilled water was added in control tubes and hot water in treatment test tubes. Then the test tubes with hot water were kept in water bath for 30 min followed by homogenization with 80% acetone and centrifuged at 3000 rpm for 10 min. Absorbance of the supernatant was measured at 652 nm in UV-VIS spectrophotometer (Eppendorf BioSpectrometer kinetic).

$$RWC = \frac{\text{Total Chlorophyll Content of leaves (from plants exposed to drought stress)}}{\text{Total Chlorophyll Content (from plants grown without drought stress)}} \times 100$$

3. RESULTS AND DISCUSSION

3.1 Impact of osmotic stress on blackgram seed germination and seedling growth parameters

A standardization experiment was conducted using four blackgram varieties to determine the drought stress level using PEG 6000 (0, -0.4, -0.5, -0.6 and -0.7 MPa) at which more than 50% inhibition rate was observed for germination and other seedling growth parameters. Observations indicated that a significant decrease in the germination percentage (> 50%) was observed at -0.5 MPa PEG 6000 concentration (Data not shown) and it was higher in other concentration. Hence a drought stress level of – 0.5 MPa was used for screening the blackgram genotypes in this study. Different blackgram genotypes seeds were exposed to drought stress at -0.5 MPa for eight days. On the 8th day, seedling growth characteristics such as germination percentage, PI, radicle length, RLSI, GSI and seed vigour were recorded.

The blackgram genotypes germination rate recorded at 0.0 (control) and -0.5 MPa was given in table 2. Among the genotypes evaluated, the highest germination percentage was recorded by VBG 11031 (76.33%) AND VBG 1612 (69.67%) followed by others. No germination was observed in the genotype VBG 1710, while the genotypes VBG 1714 (7.48%), VBG 1719 (7.69%), VBG 1724 (9.05%) and VBN (Bg) 5 (9.33%) recorded very low germination rates. Lower water potential drastically inhibits seed germination and thereby suppresses the growth and development of seedlings [2]. With respect to the seedling growth parameters, VBG 11301 and MDU 1 have recorded the highest promptness index (Table 2). Maximum radical length was noticed in the genotypeVBG 1727 (3.57 cm), and the least radicle length was observed in VBG 1724 (0.27 cm) (Table 2).

Table 2. Impact of PEG 6000 induced osmotic stress on blackgram genotypes germination percentage and different seedling vigour indices

S. No.	Blackgram Genotypes	Germination percentage (%)		Promptness index		Radicle length (cm)		Seedling vigour	
		Control	Drought Stress (- 0.5 MPa)	Control	Drought Stress (- 0.5 MPa)	Control	Drought Stress (- 0.5 MPa)	Control	Drought Stress (- 0.5 MPa)
1	VBN (Bg) 4	100	23.5	50.53	9.75	5.41	0.5	541	11.8
2	VBN (Bg) 5	100	9.33	44.03	4	4.21	0.87	421	8.1
3	CO 6	100	20.25	44.03	6.67	2.87	1	287	20.3
4	VBN 6	100	34.6	45.28	12.75	6.91	1.7	691	58.8
5	VBN 8	100	23.85	47.36	9.75	2.94	1.34	294	32.0
6	ADT 5	100	33.84	47.45	9.84	4.17	2.44	417	82.6
7	MDU 1	100	62.45	46.11	26.25	5.54	1.64	554	102.4
8	UTTRA	100	20.96	46.53	6.17	2.27	1.7	227	35.6
9	VBG11062	100	42.76	46.36	13.34	6.21	0.67	621	28.6
10	VBG110 24	100	45.24	41.7	19.25	6.24	2.17	624	98.2
11	VBG11031	100	76.33	45.78	31.17	6.01	2.3	601	175.6
12	VBG 1612	100	69.67	43.78	26	4.11	2.34	411	163.0
13	VBG 1605	100	21.84	45.2	6.92	4.34	1.04	434	22.7
14	VBG 1704	100	49.19	48.03	21.42	5.57	1.37	557	67.4
15	VBG 1707	100	39.06	44.28	16.59	5.74	1.64	574	64.1
16	VBG 1710	100	0	48.28	0	5.77	0	577	0

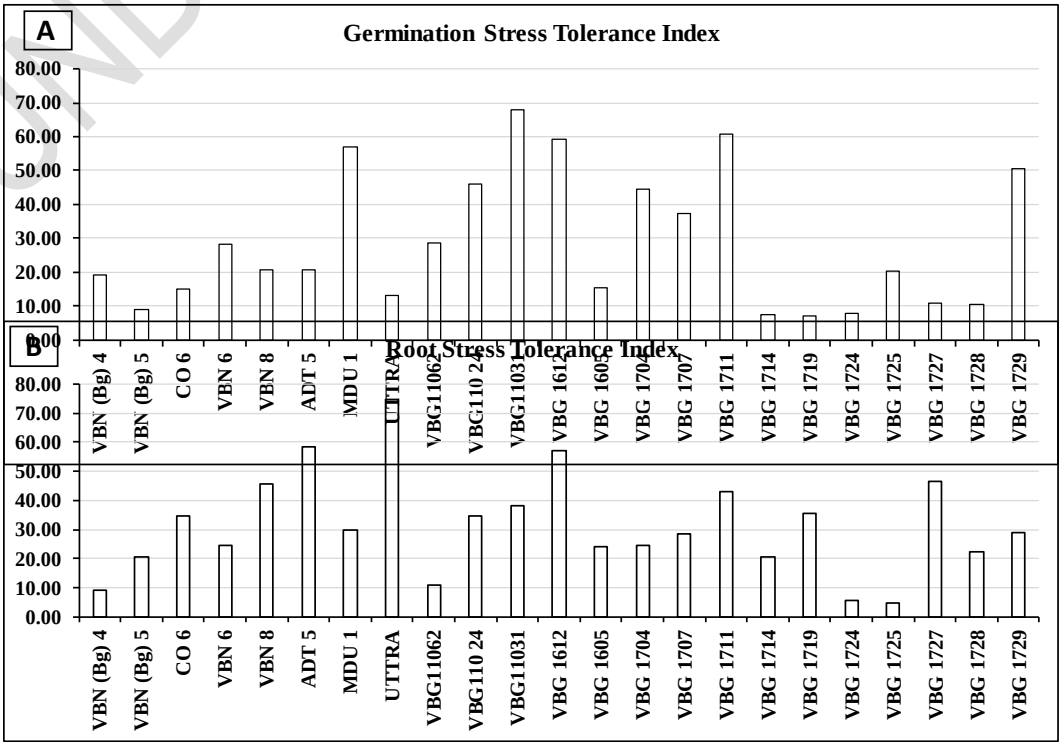
S. No.	Blackgram Genotypes	Germination percentage (%)		Promptness index		Radicle length (cm)		Seedling vigour	
		Control	Drought Stress (- 0.5 MPa)	Control	Drought Stress (- 0.5 MPa)	Control	Drought Stress (- 0.5 MPa)	Control	Drought Stress (- 0.5 MPa)
17	VBG 1711	100	61.74	45.7	27.84	7.47	3.2	747	197.6
18	VBG 1714	100	7.48	43.11	3.17	4.87	1	487	7.5
19	VBG 1719	100	7.69	44.11	3.17	3.94	1.4	394	10.8
20	VBG 1724	100	9.05	45.53	3.59	5.07	0.27	507	2.4
21	VBG 1725	100	24.5	43.7	8.84	7.71	0.37	771	9.1
22	VBG 1727	100	12.76	46.03	5.09	7.64	3.57	764	45.6
23	VBG 1728	100	20.05	44.78	4.75	4.21	0.94	421	18.8
24	VBG 1729	100	35.74	45.2	22.92	4.74	1.37	474	49.0
	Average	100.00	31.33	45.54	12.47	5.17	1.45	516.50	54.66
		SEd	CD (p = 0.05%)	SEd	CD (p = 0.05%)	SEd	CD (p = 0.05%)	SEd	CD (p = 0.05%)
T		0.234	2.973	0.342	4.346	0.040	0.508	3.800	48.284
V		0.811	1.678	1.184	2.449	0.137	0.283	13.164	27.232
T X V		1.146	2.371	1.674	3.463	0.194	0.401	18.616	38.510

T = Control and drought stress treatment;V = Blackgram varieties; T × V = Interaction between drought treatment and blackgram varieties; SEd = Standard Error Difference; CD = Critical Difference

3.2 Effect of osmotic stress on blackgram growth indices

Among the genotypes evaluated, the highest seedling vigour was recorded in the genotype VBG 11031 (175.6%) followed by VBG 1612 (163%), while the least vigour was noticed in the genotype VBG 1724 (2.4%). Similar results were also reported in greengram and blackgram [2] & [10]. The GSTI was high in the genotype VBG 11031 (68.09%) and least in VBG 1719 (7.19%) respectively. With respect to RSTI, the genotype UTTRA has recorded the highest value of 74.89% while the genotype VBG 1724 has registered 5.33% among its counter parts (Table 2 and Figure 1). GSTI and RSTI are important criteria for identifying drought tolerant genotypes in sunflower and chickpea breeding programs [11] & [12].

Figure 1. Effect of PEG 6000 induced osmotic stress on Germination Stress Tolerance Index and Root Stress Tolerance Index in the blackgram genotypes



3.3. Drought stress alters the plant morphology

Drought stress at vegetative stage has a significant impact on plant growth and development. Experiment results indicated that drought stress has negative impact on shoot length, root length and leaf area. Among the genotypes studied, the percent reduction in plant height was least in the genotype VBG 1605 (13.3%), while the effect was very high in VBG 1711 (48.2%) (Table 3). Drought stress has significantly affected the root growth in majority of the blackgram genotypes under drought conditions. Least reduction in root growth was observed in the genotype's CO 6 (4.4%), UTTRA (7.3%) and VBG 1730 (7.7%), while highest reduction in root growth was observed in VBN (Bg) 5 (27%) and ADT 5 (25.7%) respectively (Table 3). Similar types of results were observed in blackgram, and garden pea plants grown under drought stress conditions [13] & [14].

Table 3. Effect of drought stress on leaf area, shoot and root length in the blackgram genotypes

S. No.	Blackgram genotypes	Shoot length (cm)		Percent reduction over control	Shoot Length Stress Index	Root length (cm)		Percent change over the control	Root Length Stress Index	Leaf area (cm^2)		Percent reduction over control
		Control	Drought			Control	Drought			Control	Drought	
1	VBN(Bg) 4	13.7	8	-41.6	58.4	9.2	7.5	-18.5	81.5	12.3	9	-26.8
2	VBN(Bg) 5	10.5	8.8	-16.2	83.8	10	7.3	-27.0	73.0	12	7	-41.7
3	CO 6	13.2	8.5	-35.6	64.4	9	8.6	-4.4	95.6	13.4	9	-32.8
4	VBN 6	13.3	8.9	-33.1	66.9	11.5	9.8	-14.8	85.2	11.8	10.8	-8.5
5	VBN 8	13.1	7.2	-45.0	55.0	11.2	8.5	-24.1	75.9	11.4	6.9	-39.5
6	ADT 5	12.1	7.1	-41.3	58.7	10.9	8.1	-25.7	74.3	13.9	7	-49.6
7	MDU 1	13.5	8.5	-37.0	63.0	6.9	5.2	-24.6	75.4	11.9	8.6	-27.7
8	UTTRA	11	8.5	-22.7	77.3	11	10.2	-7.3	92.7	11.1	6.6	-40.5
9	VBG11062	9.7	7.3	-24.7	75.3	10.7	8.8	-17.8	82.2	10.1	9.9	-2.0
10	VBG11024	9.8	7.8	-20.4	79.6	9.5	7.6	-20.0	80.0	9	7.7	-14.4
11	VBG11031	11	8.5	-22.7	77.3	12.7	10.7	-15.7	84.3	12.2	8	-34.4
12	VBG 1612	13.1	9.8	-25.2	74.8	13.5	10.3	-23.7	76.3	12.9	7	-45.7
13	VBG 1605	9	7.8	-13.3	86.7	14.2	11.6	-18.3	81.7	10.8	6.1	-43.5
14	VBG 1704	10.3	6.3	-38.8	61.2	14.9	12.5	-16.1	83.9	12.4	8.2	-33.9
15	VBG 1707	10.5	6.3	-40.0	60.0	15	13	-13.3	86.7	12.8	8.5	-33.6
16	VBG 1710	11.7	6.4	-45.3	54.7	12.8	10.4	-18.8	81.3	12.3	7.9	-35.8
17	VBG 1711	13.9	7.2	-48.2	51.8	10.5	9.2	-12.4	87.6	11.4	8.8	-22.8
18	VBG 1714	12.7	9.3	-26.8	73.2	13.7	11	-19.7	80.3	13.4	7.9	-41.0
19	VBG 1719	11.7	7	-40.2	59.8	12.7	11.3	-11.0	89.0	12.8	5.6	-56.3
20	VBG 1724	12.5	6.8	-45.6	54.4	13.5	10.3	-23.7	76.3	12.2	6	-50.8
21	VBG 1725	12.8	6.8	-46.9	53.1	12.6	10	-20.6	79.4	11.4	5.1	-55.3

22	VBG 1727	11.9	8.5	-28.6	71.4	11.6	9.2	-20.7	79.3	12	5.2	-56.7
23	VBG 1728	11.5	7.8	-32.2	67.8	12.2	11	-9.8	90.2	11.7	6.8	-41.9
24	VBG 1729	12.6	8.5	-32.5	67.5	13.3	11.4	-14.3	85.7	12.8	6.4	-50.0
		SEd	CD (p = 0.05%)			SEd	CD (P= 0.05%)			SEd	CD (p=0.05%)	
	T	0.09	0.19			0.1	0.2			0.09	0.19	
	V	0.34	0.68			0.36	0.72			0.34	0.67	
	T xV	0.48	0.96			0.51	1.02			0.48	0.95	

T = Control and drought stress treatment;V = Blackgram varieties; T × V = Interaction between drought treatment and blackgram varieties; SEd = Standard Error Difference; CD = Critical Difference

3.4. Drought stress at vegetative stage reduces the leaf area in blackgram

In the present study, drought stress has reduced the leaf area significantly as compared to the blackgram plants not exposed to drought stress conditions. Among the genotypes evaluated more reduction in leaf area was observed in the genotype VBG 1727 (-56.7%) followed by VBG 1719 (-56.3%) and the genotypes VBG 11062 and VBN 6 has recorded the least reduction leaf area viz., -2.0% and -8.5% respectively (Table 3). This observation was similar to that ofchickpea [15] and greengram [16] under drought conditions.

3.5 Impact of drought stress on leaf relative water content

Drought stress adversely affect the soil plant water relation and thereby affects the plant growth and productivity [17]. Reduction in leaf water status in plants is one of the important effects under drought stress conditions[18]. The loss of turgidity due to reduction in RWC leads to closure of stomata this in turn reduced the photosynthetic rate. The drought stress significantly reduced the relative water content in the blackgram genotypes. The decrease in RWC might be induced by drought stress induced water deficit condition in the soil because of water lost in plants through the stomata [19]. Among the blackgram genotypes screened, least reduction in relative water status was observed in the genotype VBG 1729 (- 6.8%) followed by VBG 1727 (-6.9%), while highest reduction was recorded in VBG 1714 (-26.6%) followed by VBG 1724 (-22.9). Similar observations were recorded in maize [20] and Tomato [21] under drought stress conditions.

3.6 Impact of drought stress on chlorophyll Stability Index

The Chlorophyll Stability Index (CSI) is also one of the important parameters that reflects the ability of plants to sustain photosynthesis under stress conditions[22]. Under drought conditions, the genotypes VBG 11024 and VBG 11031 were able to record lowest reduction in CSI (Figure 2 and Table 4), while the genotypes VBG 1730 and VBG 1725 has recorded highest reduction in CSI under similar situations. In wheat [23] and greengram [24] drought at seedling stage reduces the chlorophyll stability index.

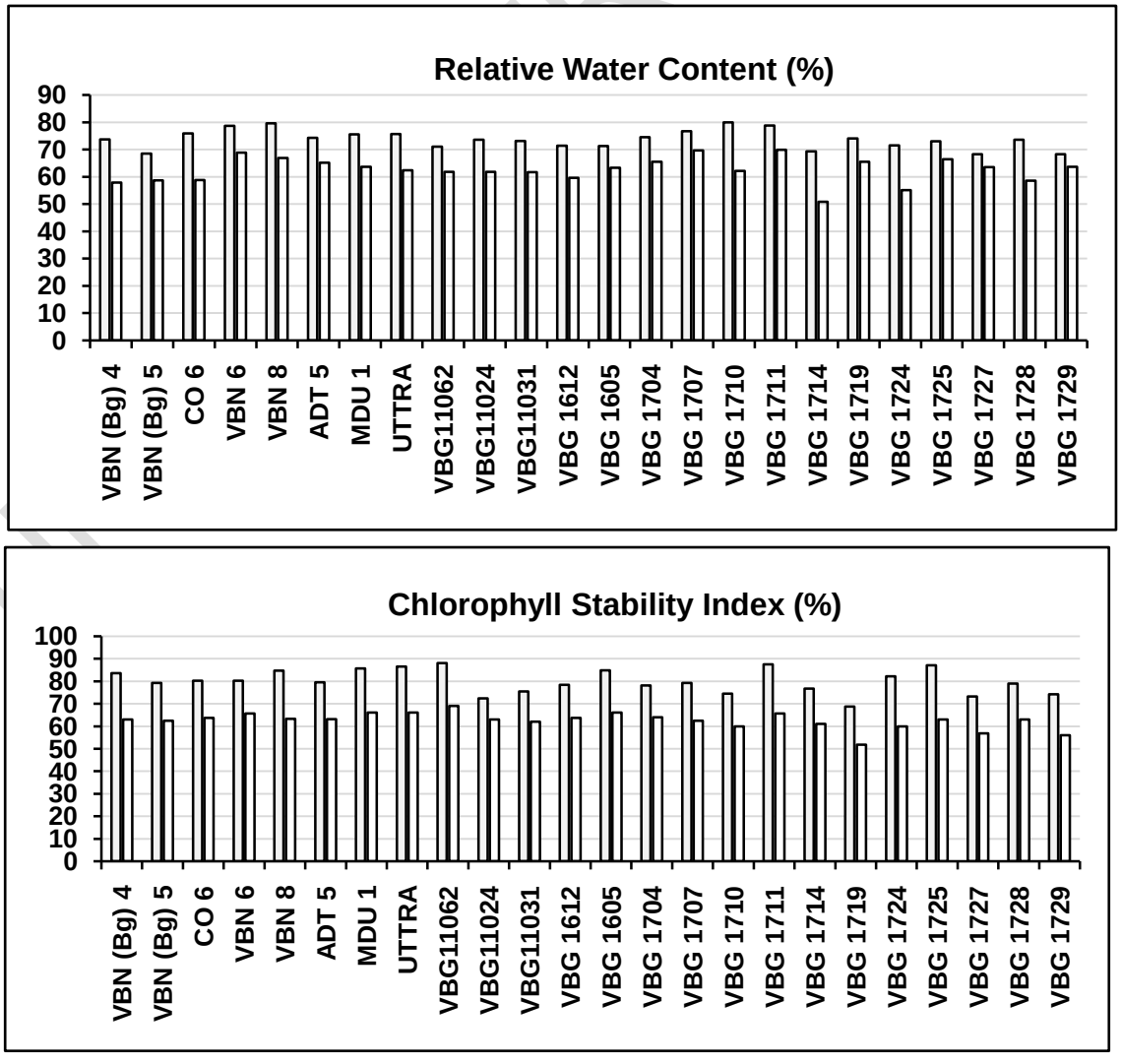
Table 4. Effect of drought stress on Relative Water Content (RWC) and Chlorophyll Stability Index in the blackgram genotypes

S. No	Blackgram genotypes	Relative water content (%)		Percent reduction over control	Chlorophyll Stability Index (%)		Percent reduction over control
		Control	Drought		Control	Drought	
1	VBN (Bg) 4	73.7	57.86	-21.5	83.6	63.02	-24.6
2	VBN (Bg) 5	68.5	58.66	-14.4	79.3	62.42	-21.3
3	CO 6	75.9	58.86	-22.5	80.2	63.72	-20.5
4	VBN 6	78.7	68.86	-12.5	80.3	65.62	-18.3
5	VBN 8	79.6	66.86	-16.0	84.7	63.32	-25.2
6	ADT 5	74.3	65.16	-12.3	79.6	63.12	-20.7
7	MDU 1	75.6	63.66	-15.8	85.7	66.12	-22.8
8	UTTRA	75.7	62.36	-17.6	86.6	66.12	-23.6
9	VBG11062	71	61.76	-13.0	88.1	69.02	-21.7

10	VBG11024	73.6	61.86	-16.0	72.4	63.02	-13.0
11	VBG11031	73.1	61.66	-15.6	75.5	62.02	-17.9
12	VBG 1612	71.4	59.66	-16.4	78.5	63.72	-18.8
13	VBG 1605	71.3	63.26	-11.3	84.9	66.12	-22.1
14	VBG 1704	74.5	65.56	-12.0	78.2	64.02	-18.1
15	VBG 1707	76.7	69.66	-9.2	79.3	62.42	-21.3
16	VBG 1710	79.9	62.16	-22.2	74.5	59.92	-19.6
17	VBG 1711	78.8	69.86	-11.3	87.6	65.62	-25.1
18	VBG 1714	69.3	50.86	-26.6	76.7	61.12	-20.3
19	VBG 1719	74	65.56	-11.4	68.8	51.82	-24.7
20	VBG 1724	71.5	55.16	-22.9	82.2	59.92	-27.1
21	VBG 1725	73	66.46	-9.0	87.1	63.02	-27.6
22	VBG 1727	68.3	63.56	-6.9	73.3	56.82	-22.5
23	VBG 1728	73.6	58.56	-20.4	79	63.02	-20.2
24	VBG 1729	68.3	63.66	-6.8	74.2	56.02	-24.5
		SEd	CD (p=0.05%)		SEd	CD (p=0.05%)	
	T	0.68	1.35		0.76	NS	
	V	2.4	4.77		2.69	5.35	
	T xV	3.4	NS		3.81	NS	

T = Control and drought stress treatment;V = Blackgram varieties; T × V = Interaction between drought treatment and blackgram varieties; SEd = Standard Error Difference; CD = Critical Difference

Figure 2. Effect of drought stress on Relative Water Content (RWC) and Chlorophyll Stability Index in the blackgram genotypes



4. CONCLUSION

Observations from this study indicate that in blackgram, osmotic stress at seedling stage affects the seedling germination, establishment and its growth indices. At vegetative stage, drought stress negatively affects the plant growth, leaf area, leaf relative water content and chlorophyll content. Among the blackgram genotypes screened, the genotypes VBVG 11031 and VBG 1711 were found to be tolerant to PEG 6000 induced osmotic stress. At vegetative stage, the genotypes VBG 11062, VBG 11024 and VBG 1725 were tolerant to drought stress at vegetative stage.

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