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Screening of Blackgram (*Vigna mungo* (L.) Hepper) genotypes for salt tolerance

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Abstract
Black gram is an important grain legume grown for its high protein content. Salinity is the major abiotic stress that adversely affects the yield of black gram. Ability to withstand salt stress varies among different genotypes. In the present study, twelve black gram genotypes were screened in the laboratory under two different salt concentrations *viz.*,100 mM and 150 mM NaCl at the Department of Plant Breeding and Genetics, Agricultural College and Research Institute, Madurai, Tamil Nadu during 2022. Germination and seedling growth parameters such as germination percentage, shoot length, root length, fresh and dry weight of the seedlings, vigour index and salt tolerant index were studied on 7th day. Germination percentage was significantly reduced at 150 mM when compared to 100 mM and control. Seedling growth in terms of shoot and root length was significantly retarded with increase in salt concentration. The difference in shoot length in 150mM to control was 2.78 cm and the reduction in shoot length in higher concentration is half than that of control. The grand mean of root length was 2.19 cm at 100mM and this was shorter than control (2.93 cm) and the root length was drastically reduced at 150 mM (1.73cm). Vigour Index was significantly lower in 150mM (120.16) than 100 mM (249.26) and control (456.86). Salt Tolerance Index followed the same trend. Among the genotypes screened, VBN 11 and CO 5 were adjudged as salt tolerant genotypes in view of shoot length, fresh weight, dry weight and Vigour Index in the former and germination %, dry weight, Vigour Index and Salt Tolerant Index in the latter. Hence, VBN 11 and CO 5 could be utilized as donors in the evolution of salt tolerant black gram varieties.

Keywords: Black gram, salinity, screening, germination, salt tolerant index

Introduction

Blackgram (*Vigna mungo* (L.) Hepper) is an important grain legume, widely cultivated for its high protein content. Globally, India is the largest producer and consumer of blackgram accounting for 33 per cent of the world area and 70 per cent of the world production. In India, black gram is cultivated in an area of 4.2 million hectares with a production of 2.34 million tons and the average productivity of 557 kg / ha. Cultivation of Black gram in marginal environments under various stresses is the main cause for low productivity.

Pulses are highly sensitive to salt stress. Salt stress hampers germination and severely affects the growth and development of the crop plant leading to reduction in yield and productivity [1], [2], [3]. Globally more than 800 million ha of land is affected by salt. The area under salinity is increasing at the rate of 10 per cent annually. It has been estimated that by the year 2050, more than 50 per cent of the arable land would be brought under salinity stress [4]. In India, about 5.95 million ha area have been affected by salinity. The loss of productivity due to salinity has been reported as 6.2 million tons. In Tamil Nadu, 35.5 lakh ha is affected due to alkalinity and 13.2 thousand ha area is affected by coastal salinity [5].

Seed germination is the very sensitive stage to abiotic stresses such as drought and salinity. Salinity stress creates potential problems during the seed germination viz., reduction in germination per cent, seedling vigour affecting the survival of seedlings. This in turn severely affect the grain yield. To breed salt tolerant black gram varieties, identification of resistant donors for salinity is crucial. Hence, the present experiment was proposed to screen the black gram genotypes with salt tolerance at the germination and the seedling stages.

Materials and Methods

Twelve black gram genotypes comprising of six advanced breeding lines *viz*., KKB -15-052, VBG-18-043, VBN-17-021, VBN-18-052, COBG-18-005, VBG-19-033 and six popular varieties *viz*., CO 5, CO 7, MDU 1, VBN 4, VBN 8 and VBN 11 were screened *in vitro* under two different salt concentrations *viz*., 100 mM and 150 mM NaCl at the Department of Plant Breeding and Genetics, Agricultural College and Research Institute, Madurai during 2022.

Seeds were surface sterilized with 70% ethanol for 2 minutes and repeatedly washed with distilled water. Seed germination test were performed by evenly distributing the seeds on a sterilized petri dish with layers of germination paper.

Different concentrations of NaCl *viz*., 100mM and 150mM was prepared by preparing 1 M solution of Sodium Chloride, by taking 58g of NaCl (based on molecular weight) and dissolving it in distilled water to make up to a final volume of one litre. This was then diluted with required quantity of distilled water as given below to prepare 100mM and 150 mM solution. Distilled water was used as control. The experiment was carried out in Completely Randomized Design (CRD) replicated twice.

Molar concentration	Description
100mM	20 ml of 1 M solution + 180 ml of distilled water
150mM	30 ml of 1 M solution+ 170 ml of distilled water

On the 7th day, germination percentage, shoot length, root length, fresh weight, dry weight, vigour index and salt tolerant index were recorded and mean was worked out. Germination percentage, Vigour index and Salt tolerance index were calculated using the following formulae.

Germination Percentage (GP)

$$\text{Germination percentage} = \frac{\text{Seeds germinated}}{\text{Total seeds}} \times 100$$

Vigour Index (VI)

Vigour Index = Germination % x Total seedling length (cm)

Salt tolerance index (STI)

$$\text{STI} = \frac{\text{Total dry weight of seedling (salt stress)}}{\text{Total dry weight of seedling (normal)}}$$

Results and Discussion

Blackgram (*Vigna mungo* L.) (2n=2x=22) is an important grain legume widely cultivated in India for its high nutritional profile. Being a legume, it enriches soil health through biological N fixation with rhizobia. It is unfortunate that, as other legumes, black gram tends to grow in marginal environments under various biotic and abiotic stresses. Salinity is an important abiotic stress that causes morphological, physiological and biochemical changes and hampers the growth and development of the plant, further leads to drastic reduction in the yield and productivity. Evolving salt tolerant black gram varieties is essential to cope with the current climatic changes.

Ample seed germination is the deciding factor for the successful establishment of the crop under saline condition. High germination percentage and early vigour under saline condition has been used as a criterion for selecting salt-tolerant genotypes. Hence, the present study aims to assess the response of twelve black gram genotypes to salinity stress on the basis of germination percentage and the seedling parameters under laboratory conditions. The research on black gram for salt tolerance is scanty and the present study may help in identifying the salt tolerant genotype for the saline areas.

Twelve black gram genotypes were subjected for screening at two different salt concentration level (100 mM and 150 mM) along with control and response of genotypes for seven different parameters (germination percentage, shoot length, root length, fresh weight, dry matter weight, vigour index and salt tolerance index) were recorded.

The mean germination percentage of all the twelve genotypes showed that increasing salinity level affects the germination percentage, and at 150mM level the germination was significantly (56%) affected when compared to 100mM (76.15%) and control (93 %). The results revealed that as salt concentration level increases, germination was severely affected due to osmotic pressure which prevents the water uptake during seed germination. Among the 12 genotypes, CO 5 and VBN -17-021 had scored highest germination percentage (80 %) followed by KKB-15-002 and VBN-18-052 (65%), while the genotype VBN-18-043 had lower germination percentage (30%) at 150mM (Table 1). Significant reduction in germination percentage with increasing salinity concentration is in agreement with the findings of [3] and [6] in black gram;

Seedling growth in terms of shoot and root length was significantly retarded with increase in salt concentration. Shoot length ranged from 1.98 to 4.6 cm at 100 mM and 1.18 to 2.93 cm at 150 mM. Grand mean for shoot length was 4.91 cm, 3.25 cm and 2.13 cm in control, 100mM and 150mM respectively. The difference in shoot length in 150mM to control was 2.78 cm and the reduction in shoot length in higher concentration is half than that of control. Root length of 12 genotypes ranged from 0.54 to 3.35 cm and 0.32 to 2.74 at 100 mM and 150 mM respectively (Table 1). The grand mean of root length was 2.19 cm at 100mM and this was shorter than control (2.93 cm) and the root length was drastically reduced at 150 mM (1.73cm). Reduction in shoot and root length with response to higher salt concentration is in agreement with the findings of [7], [8] and [9]. Reduction in shoot and root length can be attributed to an increase in salt concentration, wherein ions get accumulated affecting the elongation of cells. Salt stress affects the germination, shoot and root growth through ionic stress, which disrupts enzyme function, metabolism, hormonal signaling and energy reserve utilization. Shoot length is more affected with ion toxicity than root length. At 150mM, maximum root length was recorded by CO 7 (2.93 cm) followed by CO 5 (2.72 cm).

The grand mean value of fresh weight was 0.814 g at 150 mM whereas in control it was 0.860g (Table 4). VBN 11 (0.967g), MDU 1 (0.920g) and VBN 18 -052 (0.920g) recorded higher fresh weight even in 150mM. Similarly the dry weight in higher concentration was maximum in CO 5 (0.161) followed by CO 7 (0.150g). Salt stress drastically reduces the vigour index of blackgram (Table 1). At 150 mM, the genotypes showed significantly lower Vigour Index (120.16) than 100 mM (249.26) and control (456.86). The maximum Vigour Index was recorded by VBN-17-021 followed by VBN-18- 052 for 100 mM and CO 5 followed by KKB-15-052 for 150 mM (Fig. 1). The seedling Vigour Index increased when the NaCl concentration decreased, which shows that increased NaCl concentration caused a harmful effect in seedling. Similar trends were reported by [10].

The Salt Tolerance Index based on dry weight greatly varied under salt stress. Salt Tolerance Index ranged from 0.37 to 0.74 at 100 mM and at 150 mM it ranged from 0.16 to 0.44. VBN-18-

052 and CO 5 registered maximum salt tolerant index of 0.74 and 0.44 in 100 mM and 150 mM respectively (Fig. 2). Increasing salt stress progressively reduced the Salt Tolerance Index values.

Screening with NaCl is an effective method for studying the effect of salt on germination and seedling growth. The germination and seedling parameters studied showed negative effect on increasing concentration of salt which corroborated with the findings of [11]. Among the genotypes screened, VBN 11 and CO 5 were adjudged as salt tolerant varieties in view of shoot length, fresh weight, dry weight and Vigour Index in VBN 11 and germination %, dry weight, Vigour Index and Salt Tolerant Index in CO 5. Hence, VBN 11 and CO 5 could be utilized as donors in the breeding program of developing salt tolerant black gram varieties.

Table 1: Germination and seedling parameters of blackgram genotypes under different salt concentrations

S. No	Genotypes	Germination %			Root length (cm)			Shoot length (cm)			Mean Initial weight (g)			Mean Dry weight (g)		
		Control	100 mM	150 mM	Control	100 mM	150 mM	Control	100 mM	150 mM	Control	100 mM	150 mM	Control	100 mM	150 mM
1	CO 7	100	85	50	4.12	1.63	1.12	4.84	3.93	2.93	0.912	0.871	0.759	0.203	0.169	0.150
2	KKB-15-052	85	75	65	2.67	3.35	2.74	5.70	3.68	2.68	0.902	0.900	0.876	0.140	0.125	0.096
3	VBG-18-043	70	65	30	1.25	0.82	0.75	4.55	2.95	2.05	0.677	0.421	0.423	0.098	0.082	0.075
4	VBN-17-021	100	90	80	3.13	2.61	1.60	5.30	3.89	1.35	0.719	0.437	0.853	0.186	0.153	0.132
5	VBN-18-052	80	70	50	1.28	0.54	0.32	5.43	4.60	2.60	1.123	1.063	0.920	0.199	0.165	0.055
6	CO BG 18 005	100	80	65	3.09	1.87	1.08	3.80	2.05	1.53	0.890	0.782	0.785	0.114	0.095	0.073
7	VBG 19 033	100	65	40	3.33	2.05	1.76	3.83	2.25	1.65	0.892	0.788	0.852	0.200	0.112	0.050
8	VBN 4	90	75	55	2.52	1.96	1.85	4.47	1.98	1.18	0.741	0.787	0.689	0.252	0.163	0.068
9	VBN 8	95	85	55	3.36	2.14	2.09	5.63	3.40	2.43	0.686	0.835	0.829	0.194	0.185	0.104
10	VBN 11	100	80	55	3.56	3.33	2.65	6.40	4.32	2.72	0.752	0.750	0.967	0.202	0.149	0.131
11	CO 5	100	80	80	2.73	2.75	2.40	4.50	3.18	2.45	0.976	0.951	0.809	0.186	0.174	0.161
12	MDU 1	100	70	50	3.75	2.43	1.92	5.25	3.05	1.85	0.990	0.981	0.920	0.164	0.100	0.050
	Grand Mean	93.1	76.2	56.5	2.93	2.19	1.73	4.91	3.25	2.13	0.861	0.806	0.815	0.178	0.139	0.091
	Maximum	100	90	80	4.12	3.35	2.74	6.4	4.6	2.93	1.123	1.063	0.967	0.252	0.185	0.161
	Minimum	70	65	30	1.25	0.54	0.32	3.8	1.98	1.18	0.677	0.421	0.423	0.098	0.082	0.50

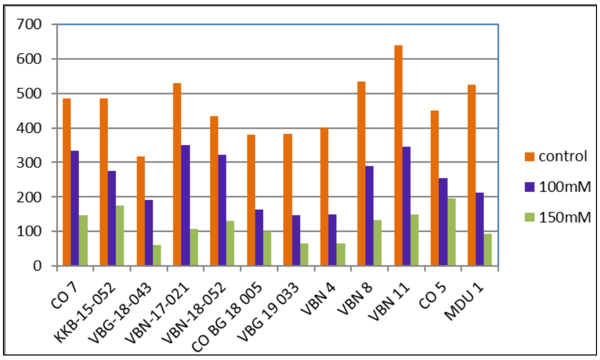

Fig 1: Vigour index of black gram genotypes under varied salt concentration

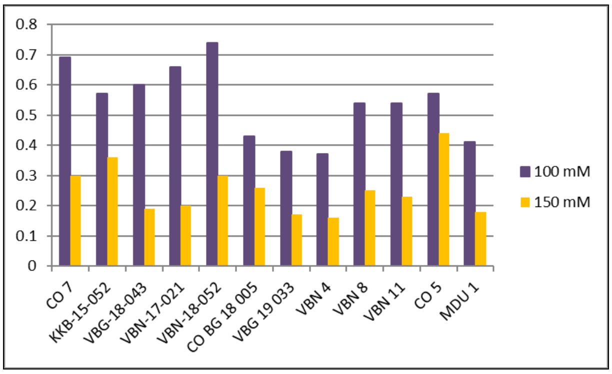

Fig 2: Salt Tolerancece Index of black gram genotypes under varied salt concentration

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