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Evaluation of black gram genotypes for saline tolerance at seedling stage

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ABSTRACT
Black gram [*Vigna mungo* (L.) Hepper] is the third most important pulse crop in India after chickpea and pigeon pea. Soil salinity is one of the major factor responsible for loss in agricultural production. Forty eight black gram genotypes were evaluated for salt tolerance with six different salinity levels *viz.*, EC_0, EC_4, EC_7, EC_{10}, EC_{13}, EC_{16} at early seedling stage. All the seedling parameters and salt tolerant index were investigated from four days old seedlings. From the results, it was found that there was a gradual decrease with increasing levels of salinity in all the genotypes studied. Seed germination and dry matter production showed more variations than other parameters studied for all the genotypes at different salinity levels. Based on the results, it may be concluded that the genotypes VNBG 017, AUB 3 AND AUB 20 were saline tolerant and VNBG 022, AUB 31 AND AUB 12 were susceptible to salinity.

Key words: Black gram, Saline tolerance, Seedling parameters.

INTRODUCTION
Black gram [*Vigna mungo* (L.) Hepper] (2n=2x=22) occupies third important place after chickpea and pigeon pea among the pulses. India is the largest producer of pulses in world (FAO STAT,2013) accounting for 22 and 33 percent of the world's area and production. The per capita availability of pulses is 42 g per day, even though the Recommended Dietary Allowances (RDA) for adult male and female is 60 g and 55 g per day respectively (Annual Report, DPD 2016-17). This situation warrants to produce threefold increase as that of the current pulse production. The main reason for such low productivity is that pulses are grown mostly in marginal and rainfed areas.

Seed germination is the most sensitive stage to abiotic stress (Patade *et al.*, 2011; Shahi-Gharahlar *et al.*, 2010;Khajeh- Hossaini *et al.*, 2003). Soil salinity is one of the major factors responsible for losses in agricultural production in most of the arid and semi arid regions in the world leading to loss in yield (Saha *etal.*,2010). Salt tolerance is a complex mechanism where numerous adaptations such as osmoregulation and osmotic adjustment, hormonal regulation, activation of the antioxidant defense system and ion homeostasis are involved. In grain legumes, tolerance to salt stress involves ion excretion (or) compartmentalization to other plant parts (Farooq *et al.*, 2017). Eventhough there are number of reports on saline tolerance in pulses, only limited works are reported at seedling stage saline tolerance in black gram. The present study has been carried out with 48 genotypes for screening under different salinity levels to evaluate the early seedling stage tolerance in black gram.

MATERIALS AND METHODS
The laboratory experiment was conducted in the Department of Genetics and Plant Breeding, Faculty of Agriculture, Annamalai University, Chidambaram, Tamilnadu during December 2016 - 2017. The seeds of 48 black gram genotypes (G_1- G_{48}) (*viz.*, VNBG 005, VNBG 001, VNBG 014, VNBG 015, VNBG 017, VNBG 018, VNBG 019, VNBG 021, VNBG 022, AUB 1, AUB 2, AUB 3, AUB 4, AUB 5, AUB 6, AUB 7, AUB 8, AUB 9, AUB 10, AUB 11, AUB 12, AUB 13, AUB 14, AUB 16, AUB 17, AUB 18, AUB 20, AUB 21, AUB 22, AUB 23, AUB 24, AUB 25, AUB 26, AUB 28, AUB 29, AUB 30, AUB 31, AUB 32, AUB 35, AUB 36, AUB 37, AUB 38, AUB 39, AUB 40, AUB 41, ADT 5, VBN 3 and VBN 5) collected from the germplasm collection which was maintained in the Department of Genetics and Plant Breeding were used for evaluation and the experiment was conducted in completely randomized design with three replications under controlled condition in petriplates with 6 different EC levels *viz.*, EC_0, EC_4, EC_7, EC_{10}, EC_{13} and EC_{16} along with a control. Saline treatment was imposed by using molecular grade sodium chloride (NaCl) (Hi-Media) and seedlings were evaluated after 4 days for seedling parameters *viz.*, germination percentage, shoot length, root length, total seedling length, dry matter production, seedling vigour index I and II and salt tolerant index. Six different salinity levels used were presented in Table1.

The seeds were surface sterilized with 70% ethanol for 2 min. and then repeatedly washed with distilled water. Then the seeds were allowed to germinate on germination paper in sterile petriplates (60× 15mm),with 5ml of distilled water

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Table 1: EC levels and concentrations of NaCl used to impose saline treatments.

EC (dS m^{-1})	Weight of NaCl (g)
0	Control (Distilled water)
4	2.56 gm l^{-1}
7	4.38 gm l^{-1}
10	6.40 gm l^{-1}
13	8.32 gm l^{-1}
16	10.24 gm l^{-1}

for control and respective test solutions for inducing salt stress. The experiment was carried out with mean temperature of 28±2°C and relative humidity of 65°C maintained with 16 hours of photoperiod and 8 hours of darkness to minimize the changes of salt concentration and to prevent the evaporation of water, petriplates were sealed tightly with parafilm. The experiment was maintained for 4 days and all the observations from the seedlings namely germination percentage, shoot length, root length, dry matter production, vigour index I and vigour index II were recorded from each replicate and mean was worked out. Ten normal seedlings were taken randomly at the end of the germination test and the length from the collar region to tip of the primary root was measured and the mean value was expressed in centimeter for root length. The length between the collar region to tip of the primary shoot was measured and the mean value was expressed in centimeter for shoot length.

Germination percentage was calculated using this formula.

Germination percentage =

$$\frac{\text{No of seeds germinated}}{\text{Total no. of seeds taken up for sowing}} \times 100$$

Ten normal seedlings used for growth measurements were placed in a butter paper cover and dried under shade for 24 hrs. Then kept in a hot air oven maintained at 60°C for 24 hrs. The dried seedlings were cooled in a desiccator for 30 minutes and then dry weight was recorded for 10 seedlings and expressed in grams. Vigour index values were computed using the formula suggested by Abdul-Baki and Anderson (1973) and expressed in whole number.

Vigour index I = Germination percentage × total seedling length in cm.

Vigour index II = Germination percentage × Seedling dry weight in g.

Salt tolerance index: Ten normal seedlings were taken for measuring salt tolerance index.

Salt tolerance index (STI) was calculated on dry weight basis using the following formula (Zeng *et al.*, 2002).

STI =

$$\frac{\text{Total dry weight of seedlings (salt stress)}}{\text{Total dry weight of seedlings (control)}} \times 100$$

The mean values were computed for each genotype over three replications and data were subjected to statistical analysis as per the procedure of Panse and Sukhatme (1985).

RESULTS AND DISCUSSION

Lack of suitable varieties and genotypes which adapt to saline conditions is one of the reasons affecting the production. Among all the stages, germination and seedling stages are very crucial under stress conditions, particularly in salinity and the seedlings which survive under seedling stage can thrive well in natural conditions. Hence the seedling parameters *viz.*, germination percentage, shoot length, root length, total seedling length, dry matter production, vigour index I, vigour index II and salt tolerance index were studied to identify saline tolerant genotypes. All the 48 black gram genotypes were screened *in vitro* with NaCl at six different levels of concentrations *viz.*, EC_0, EC_4, EC_{7}, EC_{10}, EC_{13} and EC_{16}. The use of NaCl for experimental study have been proved to be very effective method for studying the effect of salinity on seed germination and seedling growth characters.

Germination percentage: Among the genotypes studied, VNBG 017, AUB 3, AUB 20 recorded highest germination of 100, 100 and 95%, respectively under normal condition and 95% under EC_4 saline condition. The genotypes such as VNBG 022, AUB 41 and AUB 12 recorded lower germination % of 80, 90 and 90 under normal condition and 77, 80 and 80 under EC_4 saline condition (Fig. 1a).

Shoot length: The shoot length under normal condition ranged from 7.20-0.92 cm with the maximum shoot length observed in VNBG 017, AUB 3 and AUB 20 genotypes with 7.20, 5.54 and 4.54 cm, respectively under normal and 5.33, 4.42 and 3.77 cm under EC_4 saline condition, respectively. Similarly, the minimum shoot length was recorded in VNBG 022, AUB 41 and AUB 12 with 0.92 cm in all the three genotypes under normal and 0.72, 0.73 and 0.73 cm under EC_4 saline condition, respectively (Fig. 1b).

Root length: The maximum root length was observed in VNBG 017, AUB 3 and AUB 20 with 13.81, 12.21 and 11.86 cm, respectively under unstressed condition and with 11.36, 10.42 and 9.66 cm under EC_4, respectively. The minimum root length under EC_4 was observed in VNBG 022, AUB 41, and AUB 12 with 1.72, 2.14 and 2.16 cm, respectively (Fig. 1c).

Total seedling length: Among the genotypes, VNBG 017 (13.81 cm), AUB 3 (12.21 cm) and AUB 20 (11.86 cm) recorded maximum length and VNBG 022 (1.97 cm), AUB 41 (3.65 cm) and AUB12 (3.83 cm) recorded minimum seedling length under unstressed conditions whereas under EC_4, VNBG 017 (16.69 cm), AUB 3 (14.84 cm) and AUB 20 (13.43 cm) recorded maximum length (Fig. 1d).

Seedling drymatter: The maximum seedling dry matter of 0.2414 g was recorded in VNBG 017, followed by 0.1146 g in AUB 3 and the minimum dry matter production of 0.0093 g was recorded in VNBG 022 . Under moderate saline conditions of under EC_4, the maximum dry matter

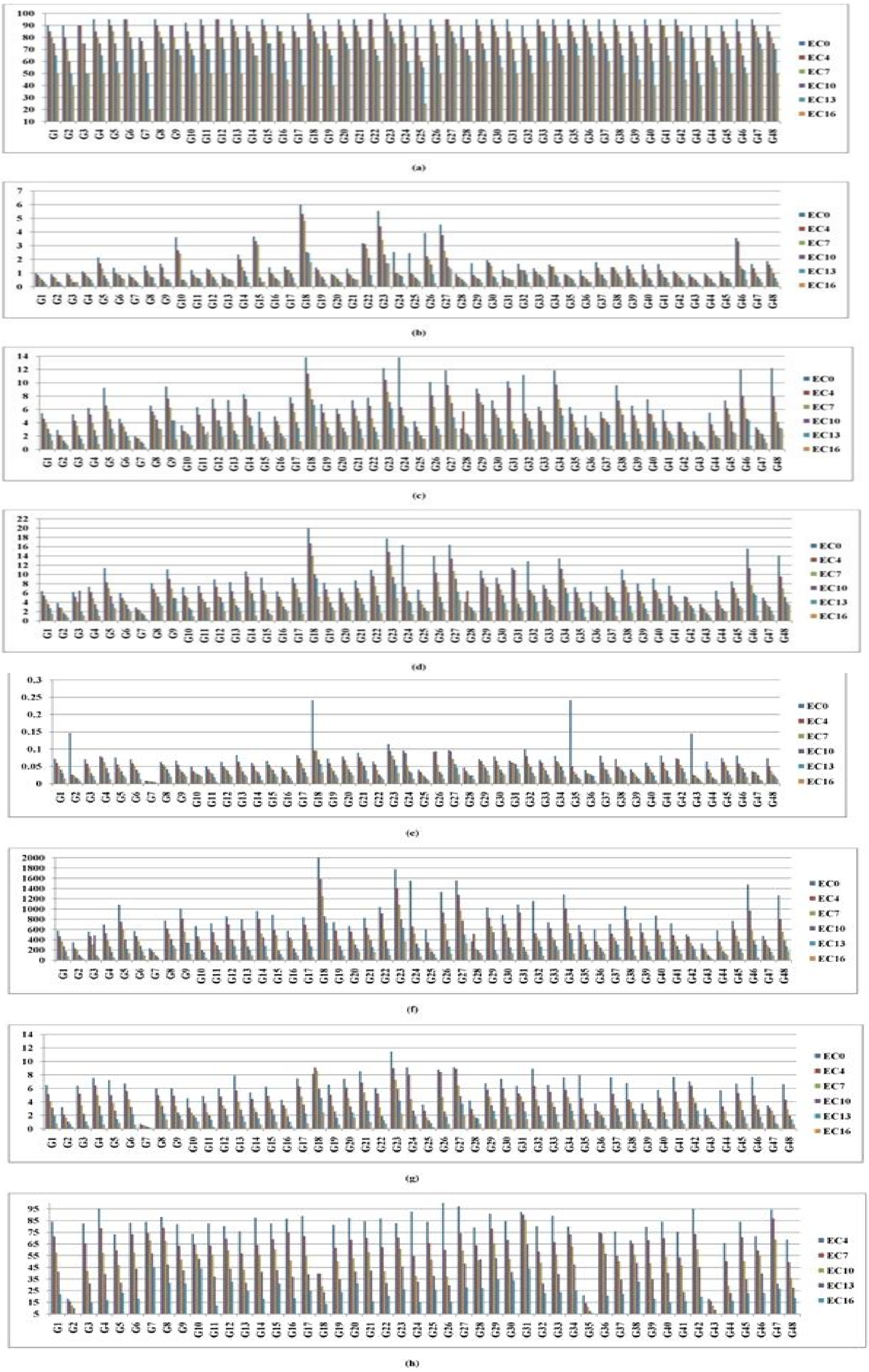

Fig 1: Response of blackgram genotypes at different salinity levels.
(a). Germination percentage, (b). Shoot length, (c). Root length and (d). Total seedling length (e). Dry matter production, (f). Vigour index I, (g). Vigour index II and (h). Salt tolerance index

production were recorded as 0.0958 g (VNBG 017), 0.0948 g (AUB 3) and 0.0936 g (AUB 20) and minimum dry matter production as 0.0078 g (VNBG 022), 0.0259 g (AUB 41) and 0.0265 g (AUB 12) (Fig. 1e).

Vigour index I &II: The maximum vigour index I was recorded in VNBG 017 (2101.00) followed by AUB 3 (1775.00) and AUB 20 (1558.00) and the minimum was observed in VNBG 022 (231.20) followed by AUB 411 (328.50) and AUB 12 (344.70) genotypes, respectively under normal situations whereas maximum vigour index I was recorded in VNBG 017 (1585.55) and the minimum was recorded in VNBG 022 (187.88) under EC_4 saline condition. A similar trend was followed in vigour index II also. For all the seedling characters in EC_7, EC_{10}, EC_{13} and $EC_{16,}$ similar trend was obtained as that of in EC_4 *i.e.,* with decreasing level of salinity a decreasing trend was observed for all the characters (Fig. 1f and 1g).

Salt tolerance index: Under EC_4 saline condition, the genotypes VNBG 017 (39.69), AUB 3 (82.72) and AUB 20 (97.19) recorded higher salt tolerant index whereas the lower values were recorded by VNBG 022 (83.87), AUB 41 (75.52) and AUB 12 (18.05) (Fig. 1h).

The improvement in field emergence could be attributed to activation of cells, which results in the enhancement of mitochondrial activity leading to the formation of more high energy compounds and vital biomolecules, which are made available during the early phase of germination (Ananthi *et al*., 2015).There are many reports that salinity has delayed germination (Ayers and Westcot, 1985 and Mensah and Ihenyen, 2009).Variation in seed germination due to change in NaCl osmotic potential indicated that NaCl has direct harmful effects on common bean seed germination(A. Cokkizgin, 2012). Al-Mutawa (2003) reported that increased salinity leads to decreased root length in chickpea.

The 48 black gram genotypes were screened *in vitro* for evaluating their seedling traits under salinity using molecular grade NaCl. The use of NaCl for experimental study have been proved to be very effective method for studying the effect of salinity on seed germination and seedling growth characters (Kumawat *et al.,* 2017). In the present study, all the genotypes screened under saline conditions showed a negative effect on germination percentage, shoot length, root length and dry matter production which corroborated with the findings of Wani *et al.* (2013) in *Brassica juncea*.

A similar observation was made by Kandil *et al.* (2012) in mung bean and reported that germination efficiency gradually decreased with increase in salinity concentrations at different levels. Kumawat *et al.* (2017) studied the effect of different salinity levels (0, 20, 40 and 60 mM NaCl) on germination and seedling and characters with 10 lentil genotypes and found mean values were found decreased for all the seedling parameters at higher salinity levels as compared with lower salinity level.

Decrease in seed vigour index is noticed with increase in salt concentration with maximum decrease at highest salinity level. Both the high vigour index I and II and, low vigour index I and II were observed for tolerant and susceptible genotypes of black gram as reported by Nasim *et al.* (2008). Reduction in salt tolerant index values were observed with increase in salinity levels which was also reported by Carpici *et al.* (2009). Among the varieties studied VNBG 017, AUB 3 and AUB 20 reported highest salt tolerance index followed by other varieties at all salinity levels. Similar observations were made by Tsegay and Gebreslassie (2014) and Hadush and Gebreslassie (2012). The selected genotypes showed reduction in mean values for germination percentage, shoot length, root length, seedling dry weight and seedling vigour indices. A significant variation in salt tolerance index was observed among all the genotypes studied.

Thus screening of 48 black gram genotypes for tolerance to salinity at seedling stage using various concentrations of NaCl resulted in identification of six genotypes *viz.,* VNBG 017, AUB 3, AUB 20, VNBG 022, AUB 41 and AUB 12. Direct or indirect exploitation of these genotypes through hybridization is recommended for breeding of salinity tolerant genotypes. It would be ideal if studies on effect of salinity on germination and seedling characters be done at some higher concentrations of salts to identify salt tolerance genotypes for further breeding programmes (Kumawat *et al.,* 2017).

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