International Journal of Ecology and Environmental Sciences
www.ecologyjournal.in
Online ISSN: 2664-7133; Print ISSN: 2664-7125; Impact Factor: RJIF 5.28
Received: 13-10-2020; Accepted: 28-10-2020; Published: 18-11-2020
Volume 2; Issue 4; 2020; Page No. 395-399

Effect of salt stress on blackgram during seedling stage under hydroponics

Donia A[1], Babu Rajendra Prasad V[2*], Manivannan N[3], Vijayalakshmi D[4]
[1, 2] Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India
[3] National Pulses Research Centre, Vamban, Pudukkottai, India
[4] Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Abstract
The effect of salt stress on four varieties of blackgram (ADT6, CO6, VBN6, VBN8) were studied under hydroponics during seedling stage. Results indicate that salt stress decreases shoot length and leaf area at seedlings stage. The effect of salt stress on root growth varies with intensity of the stress level. Proline content increases under salt stress in blackgram. This study will be used for identifying the varieties that confers tolerance during salt stress.

Keywords: salt tolerance, VBN 8, proline, blackgram

1. Introduction

Important source of proteins are Pulses. Blackgram and greengram are cultivated in 71 per cent land areas in Tamilnadu [1]. Blackgram is one of the important legume crops that are grown throughout India [2]. Blackgram contains protein (25%), carbohydrates (60%), fat (1.3%) and rich in phosphoric acid. It accounts for 13% total pulses area and 10% pulse production in India. It improves the soil fertility by fixing atmospheric nitrogen into soil. As a pulse crop, blackgram is grown in all types of soil ranging from sandy loam to heavy clay, except the alkaline and saline soils. Due to climate change, salinity is becoming a global issue. Salinity is a common abiotic stress that severely limits crop growth and development, productivity and causes the continuous loss of arable land, which results in desertification in arid and semi-arid regions of the world [3]. Salt stress leads to a series of morphological, physiological, biochemical and molecular changes that adversely affect plant growth and productivity. Salinity stress affects photosynthesis mainly by reducing the leaf area, chlorophyll content and stomatal conductance and to a lesser extent by decreasing the photosystem II efficiency [4]. In rice under hydroponics condition under salt stress there was a reduction in the shoot length, root length, total fresh and dry weight, shoot and root fresh weight, shoot and root dry weight and root- shoot ratio when compared to control [5]. In blackgram in two cultivars leaf proline content increased as a result of increase in salt concentration in rooting medium [6]. Together, these effects reduce plant growth, development and survival [7]. In blackgram yield loss due to salinity stress is upto 86.75% at 120mM NaCl [8]. Developing blackgram varieties that are tolerant against salinity stress is essential and selection of germplasms that adopt themselves to the abiotic stress is now having a greater importance due to the changes in environment that is caused by the change in climate [9]. In this experiment the effect of salinity stress on blackgram varieties was studied under hydroponics conditions. Hydroponics method of screening the cultivars was found to be the easy and rapid method. The majority of crops cannot be grown on saline soils, however genetic mechanism of salinity tolerance can be used towards the economic utilization of saline soils. The ability of plants to tolerate varying salt concentration in the substrate is characteristic of the species to which they belong. To increase the productivity and to stabilize production in the ever-changing environment, developing the genotypes that are capable to survive better under abiotic stresses is essential

2. Materials and Method

2.1. Plant material

Four blackgram varieties ADT6, CO6, VBN6, and VBN8 were taken for this study. Seeds were collected from National Pulses Research Institute, Vamban, Tamil Nadu, India. Characteristic of the varieties used were given in detail in Table 1.

Table 1. Characteristics of blackgram varieties used in this study.

Varieties	Parentage	Year of release	Duration (days)	Grain yield (kg/ha)		Special features
				Rainfed	Irrigated	
ADT 6	Vamban 1 X VBG 04-2006	2017	65 - 70	741 (Rice Fallow)	-	Highyield; moderately resistant to Mungbean Yellow Mosaic Virus (MYMV), leaf curl virus and powdery mildew; suitable for rice fallow
CO 6	DU 2 x VB 20	2010	60 - 65	-	877	It is moderately resistant to MYMV, stem necrosis and root rot diseases
VBN 6	VBN 1 x *Vigna mungo var. silvestris*	2011	65 - 70	850	890	High yield and resistant to MYMV disease in all seasons
VBN 8	VBN 3 X VBG 04-008	2016	65 - 70	870	990	High yield and resistant to MYMV disease and moderately resistant to powdery mildew.

2.2. Seed Treatment and seedling growth conditions:

Blackgram seeds were surface sterilized with 1% mercuric chloride solution for 2 min. and washed 3 to 4 times thoroughly with sterile distilled water. The excess water was removed and grown by roll towel method. On fourth day the blackgram seedlings were placed in plastic trays containing Yoshida nutrient solution and grown under hydroponic condition. The composition of Yoshida Nutrient solution is given in Table 2. After the development of true leaves (three leaf stagei.e., after 22 days) the blackgram seedlings was exposed to different levels (EC: 8, 9, 10, 11, 12 and 13dS/m) of salt stress, by dissolving NaCl in the Yoshida solution. Plain Yoshida nutrient solution was used as control, which has the Electric Conductivity 1.8 dS/m. The pH and EC of the Yoshida nutrient medium was checked on daily basis and maintained at their respective levels.

Table 2: Composition of nutrient solution

Stock No	Chemical	Quantity (g/L)	Volume of stock required perlitre of Yoshida Nutrient solution (ml)
I	NH_4NO_3	91.4	1.25
II	$NaH_2PO_4.2H_2O$	35.6	1.25
III	K_2SO_4	71.4	1.25
IV	$CaCl_2.2H_2O$	117.35	1.25
V	$MgSO_4.7H_2O$	324	1.25
VI	a. $MnCl_2.2H_2O$	1.5	1.25
	b. $(NH_4)_6Mo_7O_{24}.4H_2O$	0.074	
	c. $ZnSO_4.7H_2O$	0.035	
	d. H_3BO_3	0.934	
	e. $CuSO_4.5H_2O$	0.031	
	f. $FeCl_3.6H_2O$	7.7	
	g. $C_6H_6O_7.H_2O$	11.9	

2.3. Nutrient solution

Yoshida Nutrient solution used for growing the blackgram seedlings under hydroponics condition. The composition of Yoshida nutrient solution is given in the Table 2. All the Macro nutrients (five nutrients) are prepared and maintained as separate stocks and the micronutrients were dissolved together and maintained as single stock.

2.4. Plant Analysis

Six days after the imposition of salt stress treatment, shoot length, root length andbiomass, was measured as per the procedure of Kargbo *et al*. [10]. Proline content was estimated as per Bates *et al*. [11]. Leaf area was measured using leaf area meter.

3. Result

3.1. Effect of salt stress on seedling count

Table 3: Survival percentage (%) of genotypes under different level of salt stress

Treatment	Varieties			
	ADT6	CO6	VBN6	VBN8
T_1	100	100	100	100
T_2	100	100	93.3	100
T_3	80	73.3	73.3	86.6
T_4	80	66.6	53.3	73.3
T_5	73.3	73.3	60	80
T_6	60	60	46.6	66.6
T_7	53.3	53.3	33.3	60

T_1- Control, T_2- 8 dS/m, T_3- 9 dS/m, T_4- 10 dS/m, T_5- 11 dS/m, T_6- 12 dS/m, T_7- 13 dS/m

3.2. Effect of salt stress on shoot growth

In all the four blackgram varieties studied there was significant reduction in shoot length was observed with increase in salt concentration, and the reduction in shoot length varies with varieties studied (fig 1). Among the four blackgram varieties the reduction in shoot length was lower in blackgram variety VBN 8 (27.4cm, 26.6 cm, 25.8cm, 25.1cm, 24.9 cm, 22.1 cm and 24.5cm in control, EC 8 dS/m, EC 9dS/m, EC 10 dS/m, EC 11 dS/m, EC 12 dS/m and EC 13 dS/m respectively). On the other hand, the reduction was higher in blackgram variety VBN6 (32.1cm, 26.7cm, 25.7cm, 24.4cm, 23.9cm, and 21.5 and 20.9 in control, EC 8 dS/m, EC 9 dS/m, EC 10 dS/m, EC 11dS/m, EC 12 dS/m and EC 13 dS/m respectively).

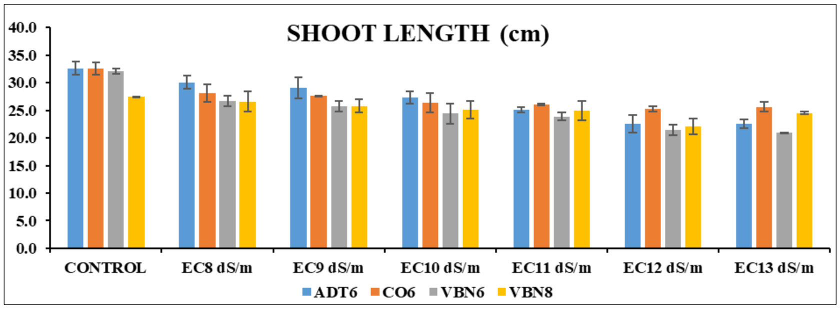

Fig 1: Effect of different salinity level on shoot length of blackgram

3.3. Effect of salt stress in root growth

Root length of the blackgram decreases with increase in salt concentration (fig 2). All the varieties showed a same degree of decrease in root length when the salt concentration increases.

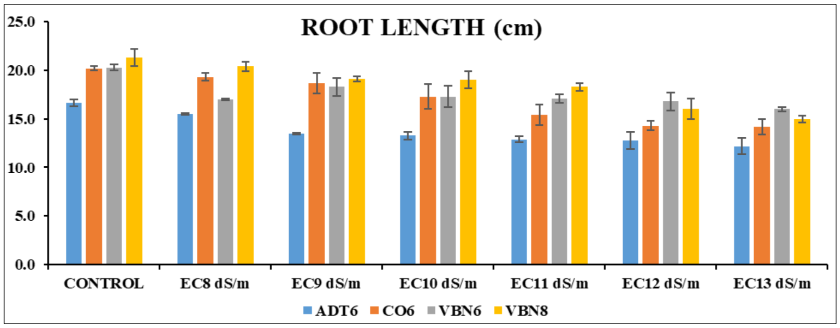

Fig 2: Effect of different level of salinity on root length of blackgram varieties

3.4. Effect of salt stress on leaf area

Leaf area decreases with increase in salt concentration (fig 3). Compared to control, salt stress induced blackgram plants have shown a drastic decrease in the leaf area and as the intensity of salt stress increases, there was a corresponding reduction in the leaf area was observed. As the salt stress increases there was a decrease in the leaf area.

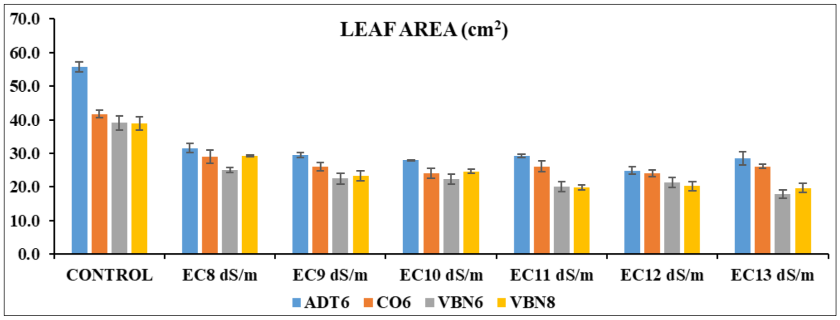

Fig 3: Effect of different level of salinity on leaf area of blackgram varieties

3.5. Effect of salt stress on proline content

The proline content was higher in all the varieties under EC 9 dS/m in all the varieties, while the proline content reduced under higher concentration of salt stress, but higher than the control (fig. 4). Among all the varieties tested, blackgram VBN8 has the higher proline content (198.3 µg/g of leaf sample) in EC 9 dS/m. In all the different salt stress, VBN8 has showed higher proline content when compared to all varieties studied (27.4 µg/g, 100.6 µg/g, 198.3 µg/g, 112.2 µg/g, 92.5 µg/g, 80.2 µg/g, 81.2 µg/g in control, EC 8 dS/m, EC 9 dS/m, EC 10 dS/m, EC 11dS/m, EC 12 dS/m and EC 13 dS/m respectively).

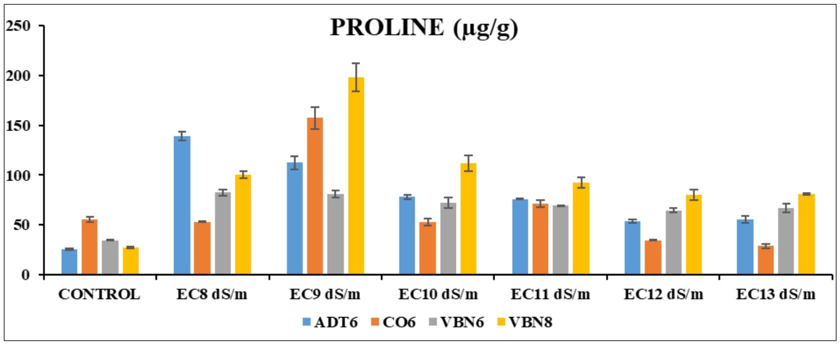

Fig 4: Effect of different level of salinity on proline content of blackgram varities

4. Discussion

Seedlings alive rate

All the blackgram seedlings were alive at EC 8 dS/m, EC 9 dS/m and EC 10 dS/m. Under EC 11 dS/m and EC 12 dS/m, some of the seedlings died due to the salt stress. Hence under hydroponics salinity stress level of above EC 13 dS/m can be used for blackgram screening. Among four blackgram varieties tested for their salt stress tolerance, VBN 8 showed higher survival percent even under high stress level, hence we can conclude that among the four blackgram varieties VBN 8 is more tolerant to salt stress level of 13 EC dS/m.

Root length

Root length was decreased under salt stress. In this study all the four varieties showed decrease in root length with increase in salt concentration. The same reduction in the root length with the increase in salt stress was observed by Hasan *et al* in three cultivars of blackgram [8].Cell cycles in the root meristem are regulated by protein called cyclins which is highly suppressed by salt stress which may be the reason for the reduction in the root growth [12].

Shoot length

Shoot length decreased with increase in salt concentration. All the blackgram varieties studied showed a decrease in shoot length with an increase in the intensity of salt stress. Shoot length decreases with the induction of salt stress in three blackgram cultivars was observed by Hasan *et al* [8]. Hence salt stress reduces the plant height was reconfirmed. Comparing the shoot length of different varieties blackgram variety VBN 8 has the higher shoot length even under higher salt concentration. This also shows that VBN 8 is more tolerant to stress among the varieties used.

Proline

Proline is a stress hormone. Proline content increases in plants during stress (drought and salinity) which confers tolerance to the pants. Here also proline content in the leaf was higher than control in all the different salt concentration. Ashraf noticed the same that there was an increase in proline content due to salt stress in two cultivars of blackgram [6]. Among all the varieties used proline content was higher in VBN 8. This may be reason for the VBN 8 to withstand salt stress.

Leaf area

In all the four varieties used leaf area drastically decreased with increase in salt concentration. This shows that salt stress highly affects the leaf area. All the varieties used are decreased to the same extent under salt stress. Hence, leaf area could not be used as a factor for determining the salt tolerance. Beisan and Camen studied that there was a reduction in the leaf area under salt stress in four local beans which was due to the reduction in photosynthetic activity [13]. Plant growth is usually reduced under saline conditions by reducing the rate of leaf elongation, enlargement and cell division in these leaves. On the other hand, it is suggested that the plant attempts to cope with the situation by reducing its leaf area under abiotic stress like salinity and therefore enables energy conservation [14].

5. Conclusion

In conclusion, we have observed that among the four blackgram varieties screened for salinity stress tolerance, variety VBN8 was found to be comparatively tolerant to salt stress and showed better growth in terms of shoot length, percentage of survival rate and high proline accumulation as compared to the other three varieties. Due to salt stress, there is a reduction in the shoot length, root length, leaf area while there is an increase in the proline content and for screening the blackgram germplasms for salt stress tolerance, EC 13 dS/m could be used.

6. Acknowledgements

I whole heartedly thank my chairman Dr. V. Babu Rajendra Prasad, Assistant professor, Department of Crop Physiology, TNAU, Coimbatore for his guidance, implementing his knowledge and great support to conduct this experiment. I would like to thank my Advisory committee members, Department faculties for their support while conducting the experiment.

7. References

1. Vasanthakumar J. Constraints to productivity of blackgram (*Vigna Mungo* L.) and greengram (*Vigna radiata* L.) in Tamil Nadu. Indian Journal of Natural Sciences. 2016; 7(38):976-999.
2. Mir AH, Lal SB, Salmani M, Abid M, Khan I. Growth, yield and nutrient content of blackgram (*Vigna mungo*) as influenced by levels of phosphorus, sulphur and phosphorus solubilizing bacteria. Saarc J Agri. 2013; 11(1):1-6.
3. Pons R, Cornejo MJ, Sanz A. Differential salinity-induced variations in the activity of H+-Pumps and Na+/H+ antiporters that are involved in cytoplasm ion homeostasis as a function of genotype and tolerance level in rice cell lines. Plant Physiology and Biochemistry. 2011; 49(12):1399-1409.

4. Netondo GW, Onyango JC, Beck E. Sorghum and Salinity: II. Gas exchange and Chlorophyll fluorescence of sorghum under salt stress. Crop Science. 2004; 44(3):806-811.
5. Lakshmi S, Ravichandran V, Arul L, Surendar K. Physiological evaluation of different rice genotypes to two salinity level during seedling stage under hydroponic system. International Journal of Plant & Soil Science. 2020; 32(8):42-56.
6. Ashraf M. The effect of Nacl on water relations, chlorophyll, and protein and proline contents of two cultivars of blackgram (*Vigna Mungo* L.). Plant and Soil. 1989; 119:205-210.
7. Hameed A, Dilfuza E, Hashem EA, Kumar A, Ahmad P. Use of microbes for alleviation of salt stress. Springer New York, 2014, 139-159.
8. Hasan MK, Islam MS, Islam MR, Ismaan HN, Sabagh AE. Salinity tolerance of black gram cultivars during germination and early seedling growth. Cercetari Agronomice in Moldova. 2018; 2(174):51-68.
9. Arteaga S, Hassan MA, Wijesinghe M, Bandara C, Yabor L, Llinares V, *et al*. Screening for salt tolerance in four local varieties of *Phaseolus lunatus* From Spain. *Agriculture*. 2018; 8(12):201.
10. Kargbo SS, Showemimo FA, Porbeni JBO, Akintokun P O. Response of rice genotypes to salinity under hydroponic Conditions. Agro-Science Journal of Tropical Agriculture, Food, Environment and Extension. 2019; 18(3):11-18.
11. Bates LS, Waldren RP, Teare ID. Rapid determination of free proline for water-stress studies. Plant and Soil. 1973; 39:205-207.
12. Duan L, Sebastian J, Dinneny JR. Salt-Stress regulation of root system growth and architecture in Arabidopsis Seedlings. Springer Science + Business Media New York, 2015, 108.
13. Beisan C, Camen D. Study concerning salt stress effect on leaf area dynamics and chlorophyll content in four bean local landraces from Banat area. Vegetable Growing, 2009, 416-419.
14. Aldesuquy H, Baka Z, Mickky B. Kinetin and spermine mediated induction of salt tolerance in wheat plants: Leaf area, photosynthesis and chloroplast ultrastructure of flag leaf at ear emergence. Egyptian Journal of Basic and Applied Sciences, 2014, 1-11.