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Screening of Ricebean (*Vigna umbellata* (Thumb.) Ohwi and Ohashi) Accessions at Early Seedling Stage for NaCl Tolerance under Controlled Condition

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Authors' contributions

This work was carried out in collaboration between both authors. Author BCN designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author DKD edited, read and approved the final manuscript.

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Short Communication

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ABSTRACT

More salt reduces the growth and causes leaf damage, necrosis, and eventually subjected to death of the crop. Ricebean, belonging to family *Fabaceae* is a fast growing summer legume crop can produce high green fodder with high content of protein, calcium, phosphorous, tryptophan and also starch content. Approximately, 21.5 million hectares of cultivatable land in Asia are affected due to salinity. An experiment was set up in Completely Randomized Block Design (CRBD) along with three replications in a growth room of the Department of Plant Breeding, Faculty of Agriculture, Mohanpur, Nadia, West Bengal, India to study the potentiality of Ricebean to withstand against imposed salinity. Data were collected on different seedling growth parameters for screening of available 30 Ricebean genotypes at a 120 mM NaCl salinity level which was identified as standardize salt concentration for screening Ricebean genotypes after standardizing the protocol.

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Six genotypes viz., KRB-77 (81.58 and 0.41), KRB-273 (79.97 and 0.44) KRB-81 (79.63 and 0.44), KRB-10 (79.17 and 0.60), KRB-95 (76.48 and 0.51) and KRB-271 (71.31 and 0.54) were exhibiting more tolerance to imposed salinity level while genotypes viz., KRB-44 (19.48 and 1.73), KRB-66 (21.38 and 1.69), KRB-115 (22.25 and 1.26) KRB-56 (32.00 and 1.45) and KRB-211 (29.07 and 1.42) KRB-73 (33.10 and 1.43) were identified as susceptible to salinity, based on the study emphasized on germination percentage, relative reduction of dry weights, tolerance index (TI) and salinity susceptibility index (SSI) of different seedling traits.

Keywords: Ricebean; screening; NaCl; tolerance index; SSI.

1. INTRODUCTION

Salinity is one of the major environmental stresses responsible for the inhibition of seed germination either by creating osmotic stress that prevents water uptake or by causing specific ion toxicity that inhibits the processes of division and expansion of cells, as well as alternation in the activity of some important enzymes like α-amylase, that consequently reduces the utilization of seed reserves and cause a significant delaying of the germination time in glycophytes [1,2]. Salinity stress refers to the building-up of soluble salts, which makes soil saline [3] as a result limits the expansion of arable land as well as crop production. This problem occurs particularly in the arid and semi-arid regions due to insufficient rainfall leading to leaching of the accumulated salt and also it affects many irrigated areas, mainly due to the use of underground water. Under these conditions, drought and salinity are the major abiotic stresses that severely inhibit germination, seedling establishment and plant growth; consequently, seed yield reduces significantly [4]. Excessive salt reduces growth and induces leaf damage, necrosis, and eventual death of the crop. Approximately 21.5 million hectares of arable land in Asia are prone to salinity and the estimated crop loss will be up to 50% of fertile land by the 21st midcentury [5,6].

The response to salt stress by plant is highly complex and involves diverse mechanisms aimed at minimizing the salinity-induced cellular damages including membrane stability, neutralization of ROS. During the last two decades, various mechanisms and characterization of genes involved in combating salinity have been revealed [6]. For best utilization of such information in crop improvement, it is important to know the exact mechanisms that are predominant or lacking in a particular crop. Therefore, identification of germplasm through screening and further experimental analysis appears most crucial. The main approach of screening cultivars for salinity tolerance is growing them on the salt affected soils. However, selection based on germination and seedling growth under controlled conditions is simple, quick, precise and time saving [7].

Ricebean, (*Vigna umbellata* (Thumb.) Ohwi and Ohashi) belonging to family *Fabaceae* with somatic chromosome number 2n=2x=22, is a versatile fast growing summer legume crop with high protein, calcium, phosphorous, tryptophan and starch content [8,9], and is considered as underutilized [10]. Salt tolerance of this plant was confirmed in different studies [11]. Besides an excellent fodder crop, *Ricebean* can also be gown as green manure and cover crop in North Eastern hilly regions of India. Therefore, there is a dire need to identify salt tolerant varieties and introgression of their salt tolerant characteristics into high yielding cultivars to utilize salt affected lands for sustainable crop growth. Available literature reveals that no such definitive work has been reported on screening with NaCl salt for Ricebean in India. Thus, the objective of this study was to screen available 30 *Ricebean* accessions in identified and standardize does of 120 mM of NaCl salt concentration for their salinity tolerance during early seedling stage to identify tolerant and susceptible genotypes. Because, some legume crops including cowpea are salinity tolerant at germination, but sensitive at the seedling and early vegetative growth stages, but again become tolerant at maturity [12].

2. MATERIALS AND METHODS

2.1 Plant Materials

The seeds for the present experiment were obtained from All India Coordinated Research Project (AICRP) on Forage Crops of Indian Council of Agricultural Research, Kalyani Centre, Bidhan Chandra Krishi Viswavidyalaya, West Bengal, India.

Table 1. List of the genotypes used in the present experiment

Sr. No	Collector No	IC No/ Status	Sr. No	Collector No	IC No/ Status
1	KRB-10	IC 433978	16	KRB-115	IC 552997
2	KRB-44	Local collection.	17	KRB-116	IC 552999
3	KRB-39	Local collection.	18	KRB-126	IC 564832
4	KRB-56	Local collection.	19	KRB-128	IC 564834
5	KRB-66	IC 545609	20	KRB-179	IC 564882
6	KRB-70	IC 545613	21	KRB-189	Local collection.
7	KRB-73	IC 545616	22	KRB-211	Local collection.
8	KRB-77	IC 545620	23	KRB-227	Local collection.
9	KRB-81	IC 552964	24	KRB-263	IC 573526
10	KRB-90	IC 552973	25	KRB-271	Local collection.
11	KRB-95	IC 552978	26	KRB-272	IC 573553
12	KRB-100	IC 552983	27	KRB-273	Local collection.
13	KRB-101	IC 552984	28	KRB-274	Local collection.
14	KRB-102	IC 552985	29	BIDHAN-1	Adopted Variety
15	KRB-104	IC 552987	30	BIDHAN-2	Adopted Variety

IC- Indigenous Collection number given by NBPGR, New Delhi, India

2.2 Screening for Salinity Tolerance at Seedling Stage

The experiment was set up in Completely Randomized Block design (CRBD) design with three replications in a growth room of the Department of Plant Breeding, Faculty of Agriculture, BCKV, Mohanpur, West Bengal, India. The suitable concentration of salt (120 mM) and setting up of the experiment were as per Nandeshwar, et al*.* [13]. Thirty six viable and healthy seeds of each thirty *Ricebean* genotype were surface sterilized with 0.1 $HgCl_2$ solutions for two minutes followed by thorough washing in distilled water. Then twelve seeds of each of the genotype were arranged in row over a glass plate (20 x 30 cm) lined with the saline solution soaked blotting paper. The whole set was then placed in transparent polythene bag. This set was replicated thrice. Then the seeds were allowed to germinate in the plates containing saline solution absorbed filter paper in the laboratory in presence of sufficient light and air in growth room. In the treatment plates, salt solution of desired salinity level of 120 mM salinity dose was used as germinating medium. Control sets were maintained in which pure distilled water was used for the purpose. The seedlings were allowed to grow for 10 days under laboratory conditions under sufficient light, 70-80% relative humidity (RH) and at temperature range of 25-30^0 C. Three replications were maintained for all the treatments including the respective control. Germination percentage was calculated from final count on 5th day following setup of experiment. Data for different seedling traits were recorded from 10 day old seedlings through destructive sampling method for all 30 genotypes of Ricebean.

Following parameters were analyzed;

i) Final Germination Percentage.
ii) Length of roots (cm).
iii) Length of shoot (cm).
iv) Total length of seedling (cm).
v) Fresh weight of Root (mg).
vi) Fresh weight of shoot (mg).
vii) Fresh weight of leaf (mg).
viii) Total fresh weight (mg).
ix) Dry weight of root (mg).
x) Dry weight of shoot (mg).
xi) Dry weight of leaf (mg).
xii) Total dry weight (mg).
xiii) Tolerance index (TI).
xiv) Salinity susceptibility index (SSI).

Tolerance index was calculated according to Garg and Singla [14].

$$\mathrm{TI} = \frac{\text{Dry weight of seedling of a genotype grown in saline condition}}{\text{Dry weight of same genotype seedling growing in non} - \text{saline condition (Control condition)}}$$

For calculating salinity susceptibility index (SSI) the formula of Fisher and Maurer [15] was used:

$$\mathrm{SSI} = 1 - X_{SS} / X_{NS}$$

Where, X_{SS} and X_{NS} are the mean dry weight of the seedlings of all the genotypes under study in salinity-stressed and non-stressed conditions respectively.

The extent of influence of salt solution on seedling growth was reflected in the amount of reduction in growth with compared to the untreated controls. In the present study the percentage of relative reduction (RR %) was estimated for different seedling characters. Relative reduction was calculated as below.

RR% = [1 – (Mean performance as measured for a character under salinity / the same under control)] X 100.

In the present experiment the data obtained with respect to the seedling characters of the above 30 genotypes and their mean values were subjected to statistical analysis using MSTAT-C program (Michigan State University). The differences between the means were tested by the Cortical Differences at $P < 0.05$ level.

3. RESULTS AND DISCUSSION

Plants differ in their tolerance potential to the different concentrations of salts in the affected soil and it may vary with their growth stages [16]. Saline soil and water shortage highly restricts the productivity of forage crops and pastures in semiarid and arid environments. Some grains and legume crops including cowpea are tolerant to salinity at germination and at maturity, but sensitive at the seedling and early vegetative growth stages [12]. The response of plants to excess sodium chloride (NaCl) is complex and involves changes in their morphology, physiology and metabolism [17], The results obtained in the present experiment on *Ricebean* germplasms to identify tolerant and susceptible genotypes for biomass production at early vegetative growth stage with respect to, with respect to germination and seedling growth parameter, salinity tolerance index (TI) and salinity susceptibility index (SSI) (Table 2).

All the genotypes were affected due to salinity as revealed by relative reduction of different parameters under the study. However, there was differential response of different genotypes due to treatments which might have been due to their differential genetic makeup. The reduction in germination percentage (Ger %) varied from 47.22 - 100.00 percentages. Lower levels of salinity delayed germination while higher levels in addition, reduced the final percentage of seed germination [18,19,20]. Similar, variation in germination percentage and other traits has earlier been reported by Pal et al. [21] in case of rice and by Chauhan et al. [22] and [23] in *Sorghum bicolor*. With increasing salt concentration germination percentage in sorghum decreased and degree of reduction varied with the salinity levels and genotypes of sorghum. Generally, RR-RL was more affected than RR-SL in all the genotypes except in case of KRB-39, KRB-56, KRB-66, KRB-100 KRB-101, KRB-211, KRB-263 and Bidhan-1. Rahman [24] opined that root length of all the cultivars of rice in seedling stage were remarkably suppressed over shoot length in all the imposed concentrations with exception at 0.01 % of NaCl. The RR-SL of 14 genotypes was significantly higher while RR-SL of 13 genotypes were lower. Considering RR-RL, 16 genotypes had higher values and only 9 genotypes had lower values. In case of RR-TL however, 15 genotypes exhibited significantly high reduction values and 13 genotypes reported non-significantly minimum values. Importantly, there were 10 genotypes (KRB-39, KRB-56, KRB-66, KRB-90, KRB-101, KRB-211, KRB-263, KRB-272, KRB-274 and Bidhan-2) in which all the three parameters (RR-SL, RR-RL and RR-TL) exhibited significantly higher values. The present findings corroborate the earlier findings of Khan et al. [25] in wheat. Patel et al. [26] reported the impact of NaCl salt stress in cowpea genotypes and observed the germination and total seedling length to be affected. Two major factors might be involved in soil-water salinity which inhibits plant growth and development. Firstly, salt particle reduce the capacity of water potential in the cell sap and this might slower the growth and development. Secondly, salt concentration inside the plant cell may cause toxicity that retards plant growth. Plants initially adjust to saline condition by decreasing tissue water content through osmotic adjustment [27]. Therefore, water status is highly sensitive to salinity and is dominant in determining plant responses to stress [28].

Considering fresh weight, relative reduction for four different seedling traits (RR-SFW, RR-RFW, RR-LFW and RR-TFW) exhibited similar trend of results as observed in case of seedling length. Fifteen genotypes each for RR-SFW and RR-RFW; 17 genotypes for RR-LFW and 16 genotypes for RR-TFW were reported significant results. Generally, the genotypes that exhibited significant relative reduction for length of different characters indicated significant relative reduction for weight also. The present findings showed that

Table 2. Germination percentage (Ger %), tolerance index (TI), salinity susceptibility index (SSI) and relative reduction (RR %) in different seedling characters of 30 genotypes of Ricebean at 120 mM of NaCl salinity level

Sl No	Genotypes	Ger %	RR-SL	RR-RL	RR-TL	RR-SFW	RR-RFW	RR-LFW	RR-TFW	RR-SDW	RR-RDW	RR-LDW	RR-TDW	TI	SSI
1	KRB-10	86.25	14.84	43.33	32.42	30.20	1.23	48.30	14.38	34.02	26.18	14.46	18.51	79.17	0.60
2	KRB-39	86.21	75.37	73.20	79.34	62.08	78.66	88.94	71.35	40.83	62.08	75.90	59.12	36.48	1.29
3	KRB-44	50.00	48.65	79.50	67.82	80.43	79.64	91.27	82.92	73.72	77.64	90.92	80.38	19.45	1.73
4	KRB-56	55.55	85.42	76.59	75.84	73.19	74.24	98.20	77.47	77.55	56.91	90.11	67.31	32.00	1.45
5	KRB-66	49.89	81.53	78.70	79.18	96.18	81.01	97.43	94.17	77.66	93.40	91.01	78.40	21.38	1.69
6	KRB-70	91.66	15.77	58.83	46.53	18.36	29.34	64.42	27.35	3.37	35.28	34.86	20.94	79.16	0.45
7	KRB-73	86.11	54.90	72.40	62.92	67.32	71.05	87.11	62.81	66.93	32.20	73.02	66.49	33.10	1.43
8	KRB-77	94.44	24.75	47.35	40.81	16.32	5.41	55.43	27.25	7.61	10.47	46.63	18.83	81.58	0.41
9	KRB-81	91.66	30.62	35.29	31.49	23.71	21.59	43.54	25.26	16.56	5.73	38.22	20.59	79.83	0.44
10	KRB-90	47.22	70.83	75.90	73.10	82.99	62.56	68.39	79.59	58.89	32.42	38.35	51.25	48.93	0.84
11	KRB-95	100.00	25.52	46.13	49.33	32.75	33.62	65.61	40.23	5.45	19.22	41.97	23.65	76.48	0.51
12	KRB-100	63.99	78.75	62.42	69.33	83.73	62.53	90.08	80.62	54.23	11.10	90.11	47.97	41.32	1.20
13	KRB-101	72.23	81.07	78.42	79.74	71.43	54.28	89.84	79.59	51.94	12.68	88.31	48.31	51.69	0.98
14	KRB-102	72.22	69.16	71.04	66.03	67.81	71.40	75.21	77.20	55.56	47.02	84.78	64.69	29.58	1.44
15	KRB-104	69.44	49.71	81.01	67.58	57.02	84.23	88.49	78.35	48.42	68.54	75.72	63.93	36.06	1.31
16	KRB-115	80.57	31.38	61.96	44.19	52.34	20.51	33.48	30.91	88.03	16.67	24.09	77.75	22.25	1.26
17	KRB-116	80.55	10.05	75.51	44.99	40.24	15.68	7.70	31.52	40.23	52.81	51.97	48.33	51.60	0.91
18	KRB-126	77.78	49.20	62.32	56.59	27.62	29.49	76.38	41.39	9.20	55.23	72.74	37.79	62.18	0.76
19	KRB-128	72.15	37.00	66.39	52.58	42.60	44.47	74.35	48.38	21.04	55.01	51.83	39.37	60.51	0.86
20	KRB-179	69.22	18.35	74.55	47.95	28.09	36.43	29.11	30.22	9.29	13.26	38.22	47.63	51.18	0.96
21	KRB-189	72.17	33.70	73.26	53.50	20.06	36.43	67.78	34.24	30.69	14.93	24.88	25.95	74.09	0.53
22	KRB-211	72.11	75.56	72.88	75.70	76.97	58.09	84.72	76.44	75.04	58.24	80.01	70.93	29.07	1.42
23	KRB-227	80.55	25.39	69.59	53.26	41.98	59.06	81.91	58.19	15.00	54.11	62.51	44.91	55.08	0.86
24	KRB-263	72.09	74.00	72.77	70.72	50.24	81.96	84.14	63.79	18.96	26.11	82.01	46.71	53.49	0.92
25	KRB-271	86.00	10.92	66.64	42.91	4.59	11.23	56.99	20.62	33.23	34.45	11.81	27.69	71.31	0.54
26	KRB-272	80.61	80.38	86.39	85.30	90.07	94.09	94.61	93.07	90.21	93.90	91.49	65.85	35.50	1.32
27	KRB-273	80.51	13.57	69.08	44.19	29.01	49.68	81.41	40.88	20.63	22.06	14.43	20.03	79.97	0.44
28	KRB-274	86.17	58.26	81.00	69.32	61.10	84.61	89.38	79.41	65.64	71.40	79.54	72.89	27.11	1.46

Sl No	Genotypes	Ger %	RR-SL	RR-RL	RR-TL	RR-SFW	RR-RFW	RR-LFW	RR-TFW	RR-SDW	RR-RDW	RR-LDW	RR-TDW	TI	SSI
29	BIDHAN-1	80.53	79.36	52.00	65.15	83.63	54.33	80.33	77.70	75.17	46.03	94.90	72.88	27.12	1.47
30	BIDHAN-2	86.19	62.63	79.01	72.46	63.75	81.90	92.29	81.50	94.61	59.85	76.45	61.94	38.06	1.24
	Mean	76.45	48.89	68.12	60.01	52.53	52.29	72.89	57.56	45.32	42.16	61.04	49.70	50.38	1.01
	C.V.	7.57	3.81	2.77	3.57	3.57	3.65	2.92	3.50	4.23	4.43	3.70	3.44	4.20	2.66
	S.E.	3.34	1.08	1.09	1.24	1.08	1.10	1.23	1.16	1.11	1.08	1.30	0.99	1.22	0.02
	C.D. 5%	9.45	3.04	3.08	3.50	3.06	3.12	3.48	3.29	3.13	3.05	3.69	2.80	3.46	0.04
	Range Lowest	47.22	10.05	35.29	31.49	4.59	1.23	7.70	14.38	3.37	5.73	11.81	18.51	19.45	0.40
	Range Highest	100.00	85.42	86.39	85.30	96.18	94.09	98.20	94.17	94.61	93.90	94.90	80.38	81.58	1.73

under salt stress, fresh weights of shoots and roots decreased. This reduction in weights with increasing salinity may be due to limited supply of metabolites to growing tissues and also due to distraction of metabolic production at high salt stress either as consequence of low water uptake or toxic effect of NaCl [29].

Relative reduction for dry weight revealed that 14 genotypes for shoot, 15 genotypes for root, 17 genotypes for leaf and 13 genotypes for total dry weight exhibited high relative reduction. In case of rice, Rahman [24] found that total dry matter accumulation was significantly suppressed by 0.3% NaCl and by higher level in all the fourteen cultivars. Generally, the genotypes that exhibited significant relative reduction for one character had produced similar result for the other character also. Verma [30] found that germination, plant height, fresh and dry weights of shoot and root decreased tremendously under salt stress. Poonia and Jhorer [31] found that increased concentration of solutes like Na^+ decreased dry weight of shoots and roots in case of wheat. Similar such effects might have caused reduction of dry weight of the treated seedlings in the present experiment.

The highest relative reduction for shoot length could be recorded in case of the genotype KRB-56 but KRB-116 revealed to be least affected. In case of root length however, the highest relative reduction could be recorded from the genotype KRB-272 and the lowest from the genotype KRB-81. Interestingly, these latter two genotypes revealed similar results in case of relative reduction for total length also. This indicates the influence of root length on total length of seedling. But there are genotypes where in spite of insignificant relative reduction of root length the RR-TL was observed significant. Similar, results were also found by Mehmet et al. [7] in linseed seedling study. Such instances can be also seen from KRB-100 and Bidhan-1 genotypes. In case of fresh weight, the least relative reduction for shoot could be recorded from the genotype KRB-271 and the highest from the genotype KRB-66; for root they were KRB-10 and KRB-272 while for leaf they were KRB-116 and KRB-56 respectively. Under salinity stress conditions, nutrient and water absorption by roots and shoots are reduced [32] which might have resulted in such reduction of growth.

Considering dry weight however, the least relative reduction for shoot could be recorded from the genotype KRB-70 (3.37 g) and the highest from the genotype Bidhan-2 (94.61 g); for root the least was recorded for KRB-81 (5.73 g) and highest for KRB-272 ((93.90 g) while for leaf they were KRB-271(11.81 g) and Bidhan-1(94.30 g) respectively. Similarly, considering the total dry weight, the least relative reduction was noticed from the genotype KRB-10 (18.51 g) and highest from the genotype KRB-66 (78.40 g).

Tolerance index (TI) and salinity susceptibility index (SSI) are the two most important parameters for evaluating genotypes for tolerance to salinity. The highest value for the latter and lowest value for the former could be recorded from the same genotype *i.e.,* KRB-44 and the *vice versa* from the genotype KRB-77. Such differential response of different genotypes to salinity has earlier been reported by Win et al. [33] in rice. However, on the basis of above two impotent parameters for tolerance to salinity the genotypes *viz*., KRB-77 (81.58 and 0.41), KRB-273 (79.97 and 0.44) KRB-81 (79.63 and 0.44), KRB-10 (79.17 and 0.60), KRB-95 (76.48 and 0.51) and KRB-271 (71.31 and 0.54) exhibited more tolerance and genotypes *viz.,* KRB-44(19.48 and 1.73), KRB-66 (21.38 and 1.69), KRB-115 (22.25 and 1.26) KRB-56 (32.00 and 1.45) and KRB-211 (29.07 and 1.42) KRB-73 (33.10 and 1.43) exhibited greater susceptibility to the imposed 120 mM of salinity level. Chauhan et al*.* [22] stated that the lowest value of salinity susceptibility index (SSI) implies the greater tolerance against salinity.

4. CONCLUSION

Ricebean, a versatile fast growing summer legume that produces high yield, along with high content of protein, calcium, phosphorous, tryptophan and starch is still considered as underutilized crop. Besides being considered as an excellent fodder crop, it can also be grown as green manure and cover crop in North Eastern hilly regions of India. So far no definitive work has been reported on its tolerance to salinity in terms of NaCl tolerance. In this experiment mostly on the basis of relative reduction on total dry weight, tolerance index and salinity susceptibility index, genotypes *viz*., KRB-77 (81.58 and 0.41), KRB-273 (79.97 and 0.44) KRB-81 (79.63 and 0.44), KRB-10 (79.17 and 0.60), KRB-95 (76.48 and 0.51) and KRB-271 (71.31 and 0.54) appeared more tolerant and genotypes *viz.,* KRB-44(19.48 and 1.73), KRB-66 (21.38 and 1.69), KRB-115 (22.25 and 1.26) KRB-56 (32.00 and 1.45) and KRB-211 (29.07 and 1.42) KRB-73 (33.10 and 1.43) appeared susceptible to the imposed salinity level. The above screened genotypes may be recommended for direct cultivation in saline soil tract based on agronomic characters. Further the screened tolerant genotypes may be utilized as parents in salinity resistance breeding programme.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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