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Influence of Rhizobacterium Inoculation on NaCl Salinity Tolerance in Pusa Sukomal and RC101 Varieties of Cowpea (*Vigna unguiculata* L.)

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Influence of Rhizobacterium Inoculation on NaCl Salinity Tolerance in Pusa Sukomal and RC101 Varieties of Cowpea (*Vigna unguiculata* L.)

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Abstract
Soil salinity is one of the most severe factors limiting growth and physiological response in cowpea plants. In the present study, the effect of rhizobacterium strains BR2 and BR3 on the growth of cowpea (*Vigna unguiculata* L.) varieties—Pusa Sukomal and RC101—tolerance to 0, 25, 50, and 75 mM concentrations of NaCl salinity was evaluated. The rate of growth, in general, was high in plants irrigated with 25 mM NaCl saline water as compared to control, and thereafter, the growth reduced with increase in salinity concentrations. The results revealed that treating the seeds with rhizobacteria accompanied by NaCl salinity increased growth parameters of the cowpea plant as compared to the seeds irrigated with sodium chloride alone. Treatment with rhizobacteria mitigated the harmful effect of NaCl, and the growth was significantly better than the plants growing in saline water without rhizobacterium inoculation. The overall performance of Pusa Sukomal with BR3 strain was found to be better than the other combinations tested. Flowering in field plants started within 45 days of sowing, and the seeds in plants irrigated with saline water, in the presence of rhizobacterium, were found to be healthy as compared to control seeds. Seed protein profile was analyzed by SDS PAGE gel studies.

Keywords: NaCl salinity; Stress; Cowpea (*Vigna unguiculata*); Rhizobacteria.

1. INTRODUCTION

Salinity is one of the most severe abiotic problems in arid and semiarid regions of world agriculture [1]. Of approximately 900 million hectors of land worldwide, about 6% of the global land is estimated to be affected by salinity, and this is increasing every year across the globe [2, 3]. According to the data published by National Remote Sensing Agency, Hyderabad, about 78% of the soil (2,596,942 ha) in India is salt affected, out of which 22% (733,608 ha) land is in the central (Madhya Pradesh and Maharashtra) region [4].

Salinity affects the plants' growth in different ways such as osmotic stress effects, specific-ion toxicity and/or nutritional disorders [5]. When the soil salinity level is high, it confers high osmotic potential and specific-ion toxicity in plants, leading to significant inhibition in seed germination and growth; seedling growth is the most sensitive stage of the plant life cycle for salinity [6]. The evaluation of germination of salt treated and control seeds is being used as an indicator of the tolerance of some species and cultivars to salinity. These evaluations are important to estimate the performance potential of the seeds at the field in salt-stressing environments. Germination of seeds, growth parameters of seedlings, and weight are the most commonly used criteria for the selection of cultivars for salinity tolerance [7, 8].

Plant-growth-promoting rhizobacteria (PGPR) is a group of bacteria that actively colonizes plant root and increases plant growth [9-11]. Salt-tolerant plant-growth-promoting rhizobacteria reduced the impact of salinity on plant growth and improved productivity. Salt-tolerant rhizobacteria can play an important role in alleviating soil-salinity stress during plant growth [12].

Legumes are important agricultural crop plants and are rich sources of proteins. Rhizobacteria are well-known symbiotic nitrogen fixers in legumes. Their survival and distribution in soil and the rhizospheres of plants is greatly affected by salinity [13, 14], and strains visibly possess variations in their sensitivity to salinity [15-19].

Cow pea (*Vigna ungiculata* L. Walp) is a dicotyledonous legume annual crop, with strong taproot and many lateral roots in the surface soil. In Madhya Pradesh, it is cultivated between February and May. Cowpea with a high level of protein content (23-35%) makes it important, and its capability to fix atmospheric nitrogen to ammonia in the soil allows it to germinate well on poor soils and increase its fertility [20]. They provide a valuable source of protein and thereby sustain the nutritional balances of low-income populations [21]. Tolerance against salinity is important in the establishment of cowpea in saline soils.

Present study reports the performance of two cowpea varieties, namely, Pusa Sukomal and RC101, under different levels of NaCl saline water irrigation, with or without rhizobacterium inoculation at the stage of seed sowing. We have used BR2 and BR3 strains for the present work.

2. MATERIALS AND METHODS

Two cowpea varieties—Pusa Sukomal, obtained from Indian Agriculture and Research Institute, New Delhi, and RC101, obtained from Agriculture College, Gwalior—and two strains of Rhizobacteria, BR2 and BR3, obtained from IARI, New Delhi. Cowpea seeds that were surface sterilized and sowed in the field of College of Life Sciences, Cancer Hospital, and Research Institute, Gwalior. The soil used for plantation was sandy loam in texture consisting of sand, 84.2%; silt, 12.9%; clay, 2.9%; pH, 7.8; EC, 0.5 dSm; and organic matter, 1.2%. For salinity studies, we used 0 (control), 25, 50, and 75 mM concentration NaCl saline water prepared in tap water for irrigation. Field was divided into six columns, three for each variety. Each column was further divided into four blocks, one for each saline treatment.

Strains of bacteria BR2 and BR3 were grown in YEM broth at 28 ± 2°C, 120 rpm for 14 days (incubator shaker) [22]. After 14 days, cultures of BR2 and BR3 were centrifuged, and the pellet was used for the coating the cowpea seeds.

Three sets of seeds (50 seed per treatment/set each) from both varieties of cowpea—Pusa Sukomal and RC101—were taken. One set was sown in the field without rhizobacterium inoculation and the other two were inoculated with BR2 and BR3 strain of rhizobacterium, respectively, and sown in the field in the month of February in the prepared soil beds at 1-2 cm depth and 4-5 inches gap.

The field was then irrigated immediately with saline water (0, 25, 50, and 75 mM NaCl), and thereafter, irrigation was applied with two days interval. Once in every 15 days, field was irrigated with tap water to avoid accumulation of salt.

Effect of salinity and influence of rhizobacterium application on salinity tolerance of cowpea crop was analyzed in 30-day plants by recording data on agronomic characters, such as plant shoot length, root length, fresh weight, dry weight, and nodules number from three plants randomly selected from the field per experiment per treatment.

Plants were collected carefully from the field on 30th day and the roots were washed with tap water to remove the soil and gently blotted by pressing in newspaper to remove water. The length of root and the shoot was measured from the crown region to the tip of the root and shoot. The number of nodules present was counted. Plant fresh weight was recorded immediately on top pan electric balance. The plants were left inside the oven at 60°C until the plants were dried completely, and the dry weight was recorded.

Each experiment was repeated, and the data obtained from each experiment were pooled, and mean and standard error were calculated.

Pods from each treatment were collected, and protein qualitative analysis was done by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed on 12.5% acrylamide slab gels according to the method described by Laemilli [23]. The gel was stained with coomassie blue and destained with water: Ethanol:acetic acid solution. When the bands appeared clearly, then the gel was photographed, and the number of bands per sample and their Rf values were calculated.

3. RESULTS

Rhizobacteria play an important role in plant physiology by a combination of direct and indirect mechanisms [24]. Rhizobia are able to cope with salinity more than their host legumes, but they show strain variability both in growth and in survival under saline conditions [25].

In the present study, the effect of different concentrations (0, 25, 50, and 75 mM NaCl) of salinity in the presence of rhizobacterium was evaluated by analyzing the growth parameters such as shoot length, root length, number of root nodules, fresh and dry weight in 30-day plants of cowpea varieties Pusa Sukomal and RC101, and the data are presented in Table 1.

3.1. Shoot Length and Root Length

Plants raised under salinity conditions were stunted as compared to controls (Figure 1a). The mean shoot length of 30-day-old Pusa Sukomal and RC101 control plants was 35.00 and 29.67 cm, whereas plants inoculated with rhizobacterium BR2 recorded 44.33 and 45.24 cm shoot length, and plants inoculated with BR3 recorded 47.33 and 47.33 cm shoot length, under nonsaline (0 mM) conditions in Pusa Sukomal and RC101, respectively. Similarly, the root length of Pusa Sukomal and RC101 plants was 10.00 cm under nonsaline conditions without inoculation of rhizobacteria. The BR2 inoculated plants recorded 17.00 and 11.33 cm root length and plants inoculated with BR3 recorded 15.67 and 12.33 cm root length, respectively (Table 1).

On exposure to salinity, the shoot length decreased from 38.33 to 15.00 cm and root length from 9.00 to 5.00 cm in Pusa Sukomal and in RC101 the shoot length decreased from 24.67 to 13.00 cm and root length decreased from 8.67 to 5.00 cm, respectively, with increase in salinity level from 25 to 75 mM (Table 1).

In the presence of BR2, under different saline concentrations, Pusa Sukomal recorded 44.67 cm shoot length and 16.00 cm root length at 25 mM, which reduced to 29.67 cm (shoot length) and 10.67 cm (root length) with increase in salinity to 75 mM. Similarly, under the identical conditions, RC101 recorded 34.00 cm and 11.33 cm shoot and root lengths at 25 mM concentration and 26.21 and 5.67 cm shoot and root lengths at 75 mM concentration (Table 1).

Table 1: Effect of 0, 25, 50, and 75 mM NaCl saline water irrigation on the growth of 30-day field plants of cowpea varieties—Pusa Sukomal and RC101—with or without Rhizobacterial (BR2, BR3) inoculation.

NaCl concentration	Pusa Sukomal					RC101				
	Plant height (cm)		Number of root nodules	Fresh weight (g)	Dry weight (g)	Plant height (cm)		Number of root nodules	Fresh weight (g)	Dry weight (g)
	Shoot length	Root length				Shoot length	Root length			
Control	30 days									
0 mM	35.00 ± 2.89	10.00 ± 1.45	9.67 ± 0.88	16.10 ± 0.91	2.73 ± 0.30	29.67 ± 3.18	10.00 ± 2.65	8.53 ± 1.73	13.52 ± 0.53	1.66 ± 0.18
25 mM	38.33 ± 2.03	9.00 ± 1.15	9.57 ± 0.88	17.75 ± 1.73	2.93 ± 0.52	24.67 ± 1.45	8.67 ± 0.33	4.27 ± 0.55	14.36 ± 0.46	1.97 ± 0.14
50 mM	30.00 ± 2.89	8.67 ± 0.33	9.33 ± 0.88	14.04 ± 0.58	1.94 ± 0.19	22.67 ± 4.06	5.33 ± 0.88	4.17 ± 0.57	11.18 ± 0.53	1.08 ± 0.03
75 mM	15.00 ± 1.00	5.00 ± 1.15	2.67 ± 0.67	9.29 ± 0.24	0.99 ± 0.19	13.00 ± 1.00	5.00 ± 0.58	2.47 ± 0.58	8.40 ± 0.49	0.93 ± 0.06
With rhizobacteria BR2										
0 mM	44.33 ± 2.60	17.00 ± 1.15	27.00 ± 1.15	16.71 ± 0.89	2.59 ± 0.30	45.24 ± 2.89	11.33 ± 1.86	17.33 ± 1.20	15.24 ± 0.94	2.29 ± 0.21
25 mM	44.67 ± 2.60	16.00 ± 1.15	8.67 ± 0.88	18.43 ± 0.49	2.99 ± 0.22	34.00 + 2.08	11.33 ± 0.88	15.84 ± 0.58	14.04 ± 0.45	1.87 ± 0.16
50 mM	34.00 ± 2.08	15.67 ± 0.67	16.67 ± 0.88	14.18 ± 0.60	1.24 ± 0.02	31.33 + 2.03	8.67 ± 1.20	9.16 ± 0.57	11.84 ± 0.86	1.24 ± 0.10
75 mM	29.67 ± 3.18	10.67 ± 0.88	4.00 ± 0.88	11.01 ± 0.57	0.91 ± 0.04	26.21 ± 1.73	5.67 ± 1.20	4.32 ± 0.58	9.12 ± 0.35	0.82 ± 0.09
With rhzobacteria BR3										
0 mM	47.33 ± 2.91	15.67 ± 0.67	22.33 ± 0.67	18.76 ± 0.87	2.71 ± 0.34	47.33 ± 2.35	12.33 ± 0.33	30.42 ± 2.89	15.29 ± 1.00	2.31 ± 0.18
25 mM	49.00 ± 3.60	18.33 ± 0.33	10.00 ± 1.53	20.04 ± 0.44	3.41 ± 0.30	42.67 ± 1.15	11.67 ± 1.20	25.67 ± 1.45	14.86 ± 0.62	1.99 ± 0.15
50 mM	34.08 ± 2.08	16.67 ± 0.88	10.00 ± 1.57	14.19 ± 0.39	1.96 ± 0.21	33.69 ± 2.03	11.00 ± 0.58	8.87 ± 0.58	12.29 ± 0.86	1.34 ± 0.13
75 mM	29.67 ± 2.91	10.67 ± 0.88	12.33 ± 0.67	12.55 ± 0.29	1.09 ± 0.07	26.54 ± 1.00	10.00 ± 1.15	3.73 ± 0.52	9.16 ± 0.26	0.87 ± 0.06

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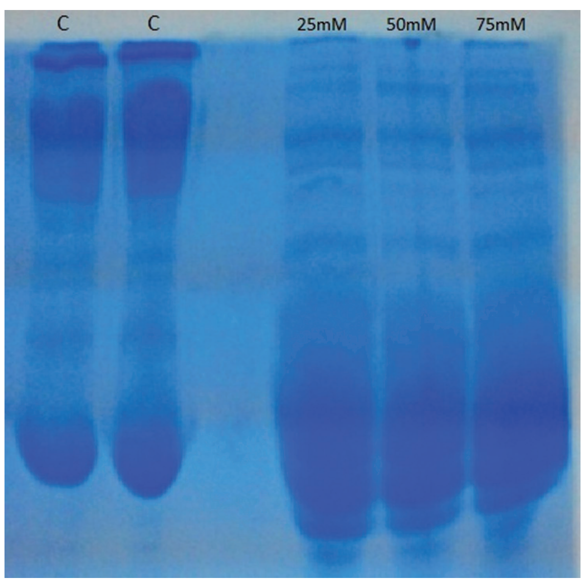
Figure 1a: 30-day-old cowpea plants: effect of salinity on growth under—(a) 0 mM and (b) 75 mM NaCl stress conditions (i) with out and with rhizobacteria (ii) BR2 and (iii) BR3 inoculation.

Figure 1b: Cow pea plants showing root nodules development under NaCl saline water irrigation conditions.

Figure 1c: Pods of seeds harvested from plants irrigated with (i) 75 mM, (ii) 50 mM (iii) 25 mM NaCl water.

Figure 1d: SDS PAGE showing seed protein-banding pattern in cowpea plants grown under 0, 25, 50, and 75 mM NaCl salinity conditions.



When BR3 was applied, the Pusa Sukomal plants recorded 49.00 cm shoot and 18.33 cm root length in 25 mM salinity level, and this reduced to 29.67 and 10.67 cm, respectively, as the salinity level increased to 75 mM. The variety RC101 recorded similar reduction in shoot length (42.67-26.54 cm) and root length (11.67-10.00 cm), as the salinity level increased from 25 to 50 mM (Table 1).

3.2. Nodulation

Nodules in plants grown under salinity stress were relatively small and were black to brown in color (Figure 1b). The average number of nodules in control plants without rhizobacterium inoculation varied from 8.53 ± 1.73 to 9.67 ± 0.88 in both varieties of cow pea. This number decreased to 9.57 ± 0.88, 9.33 ± 0.88 and 2.67 ± 0.67 in Pusa Sukomal and 4.27 ± 0.55, 4.17 ± 0.67 and 2.47 ± 0.58 in RC101 plants grown under 25, 50 and 75 mM salinity stress conditions, respectively (Table 1). With BR2 and BR3 strain of rhizobacterium inoculation, the number of nodules in these varieties decreased from 27.00 ± 1.15 to 4.00 ± 0.88 on BR2 strain application and from 22.33 ± 0.67 to 4.32 ± 0.58 on BR3 strain application in 30 days Pusa Sukomal plants (Table 1). While in RC101, the number decreased from 17.33 ± 0.20 (0 mM) to 4.32 ± 0.58 (75 mM) on BR2 strains inoculation and from 30.42 (0 mM) to 3.73 ± 0.52 (75 mM) on BR3 strain inoculation in 30-day-old field plants (Table 1).

3.3. Fresh Weight and Dry Weight

Cowpea varieties Pusa Sukomal plants and RC101 plants attained 16.10 and 13.52 g fresh weight and 2.33 and 1.66 g dry weight in 30 days under nonsaline conditions without inoculation of rhizobacteria. When the plants were irrigated with saline water, fresh weight and dry weight of the Pusa Sukomal plants measured 17.75 and 2.93 g, respectively, at 25 mM NaCl concentration that further decreased to 9.29 and 0.99 g, respectively, at 75 mM NaCl concentration. RC101 variety recorded a similar pattern as that of Pusa Sukomal. With increase in salinity levels, this variety recorded 14.36 and 1.97 g fresh and dry weight at 25 mM and 8.40 and 0.93 g fresh and dry weight at 75 mM concentrations, respectively (Table 1).

In experiments, where BR2 was inoculated, Pusa Sukomal recorded 18.76, 20.04, 14.18 and 12.55 g fresh weight and 2.71, 3.41, 1.96 and 1.09 g dry weight at 0, 25, 50, and 75 mM NaCl salinity conditions. Similarly, the variety RC101 recorded 15.24, 14.86, 12.29, 9.12 g fresh weight and 2.29, 1.99, 1.34 and 0.87 g dry weight under 0, 25, 50, and 75 mM NaCl saline-water-irrigation conditions.

On application of BR3, Pusa Sukomal recorded 16.71, 18.43, 14.19 and 11.01 g, and RC101 variety recorded 15.29, 14.04, 11.84 and 9.16 g fresh weight under 0, 25, 50, and 75 mM levels of salinity conditions. These plants' dry weight was 2.59, 2.99, 1.24 and 0.82 g (in Pusa Sukomal) and 2.31, 1.87, 1.24 and 0.91 g (in RC101) under 0, 25, 50, and 75 mM concentrations of NaCl salinity-stress conditions (Table 1).

Flowering in the field plants started within 45 days after sowing and plants irrigated with saline water recorded early flowering. Pods in plants inoculated with rhizobacterium under saline conditions were healthy (Figure 1c) and matured early as compared to the controls.

SDS PAGE analysis of seed proteins of Pusa Sukomal plants grown under 0, 25, 50, 75 mM NaCl salinity conditions is given in the Figure 1d and shows no significant qualitative difference in protein profile between control (0 mM), 25, 50, and 75 mM plant seeds.

Present results clearly show the application of BR2 and BR3 strains of rhizobacterium has enhanced the saline toxicity tolerance in cowpea varieties Pusa Sukomal and RC101, and overall performance of Pusa Sukomal with BR3 was better than the other treatments used, indicating that correct combination of plant variety and salt-tolerant rhizobacterium strain can be of better choice in cultivating cowpea in saline soils, with prior inoculation of rhizobacterium on seeds.

4. DISCUSSION

Salinity causes adverse effects on plant growth [26] and fresh and dry weight [27-29]. Treatment with rhizobacteria increases germination percentage, root and shoot growth, total biomass of the plant and seed weight, and yield under salinity stress [30-32]. Ertan *et al.* [33] in 2008 reported that rhizobacteria significantly increased the growth parameters of radish plant both under saline and nonsaline conditions. Our results are in accordance with these results. Similar results were reported in egg plant [34], beans [35], lettuce [36], squash [37], and tomato [38].

Salt stress inhibits nodule formation by inhibiting the initial steps of rhizobia-legume symbiosis [39]. Yousef and Sprent [40], 1983 showed that NaCl affects nodulation of *V. faba*. In the present work, we also found that saline-water irrigation has decreased the number of root nodules in both varieties of cowpea irrespective of without or with rhizobacterium inoculation. Further, this pattern was common for both the strains. However, the number of nodules significantly increases on application of rhizobacteria on seeds before sowing (Table 1).

Rhizobia can grow under saline conditions by making ionic adjustment. They are capable of coping with salinity more efficiently than their host legumes; however, their growth and survival under saline conditions vary with the strain used [41]. In the present study, on an average, a maximum of 27 nodules per plant in Pusa Sukomal with BR2 strain inoculation and about 30 nodules per plant in RC 101 with BR3 strain inoculation were noticed (Table 1).

In conclusion, compared to control (nonsaline water irrigation), plants irrigated with 50 and 75 mM NaCl saline water showed decrease in plant height, number of root nodules, and in fresh and dry weight. Further, the application of rhizobacterium before sowing increased the plant tolerance to NaCl salinity as compared to the control. Present results also clearly indicate that the application of BR2 and BR3 strains of rhizobacterium enhanced the saline-toxicity tolerance in cowpea varieties Pusa Sukomal and RC101, and overall performance of Pusa Sukomal with BR3 was better than the other treatments used.

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Author Contributions
Sadhna Chaturvedi has done all the experimental work while Dr. Tejovathi Gudipati and Dr. Archana Shrivastava advised her and provided their research facilities to carry out this work.

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Conflict of Interest
None.

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