Cowpea cultivation in saline soils- influence of Rhizobacterial application on growth of Cowpea (*Vigna-unguiculata* L. Walp)

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Abstract

Soil salinity is one of the most severe abiotic stress factors that limit the growth of crops. Cowpea (*Vigna-unguiculata* L.Walp) is one of the most commonly grown leguminous crop in arid and semiarid regions by low income people for food and fodder. Plant growth promoting Rhizobacteria (PGPRs) are known to enhance plant growth under saline conditions.

Symbiotic Rhizobacteria are known to confer tolerance to salinity in legumes, however plant– rhizobial genotype's selection is the major factor that influence plant tolerance to salinity.

In the present study, the effect of *Rhizobacterium* strains BR2 and BR3 on the growth of Cowpea varieties – Pusa Sukomal and RC101, irrigated with 0, 25, 50 and 75mM NaCl saline water. Influence of *Rhizobacterium* on Cowpea tolerance to salinity was studied by applying Rhizobacterial strains on seed before germination and physiological parameters- Shoot and root length, number of root nodules and fresh and dry weight were evaluated in 90 day old field plants.

The salinity has reduced the growth with respect to all growth and physiological parameters. The influence of salinity was highest at 75mM concentration of NaCl. Application of *Rhizobacterium* strains BR2 and BR3 on seeds before exposing to saline water has shown recovery in the growth of Cowpea varieties. The overall performance of seeds of Pusa Sukomal was found good and is noticed to be more tolerant to salt stress as compared to RC101variety. The Cowpea variety Pusa Sukomal with BR3 strain has performed better than the other combinations tested.

Keywords: Cowpea, *Rhizobcterium*, Salinity

Introduction

Salinity is one of the major environmental stress that severely affecting plant productivity worldwide (Bharti *et al.,* 2016). It is the most widespread problem in symbiotic nitrogen fixation and restricts the extension of legume cultivation (Faghire *et al.,* 2011; Farissi *et al.,* 2011, 2013).

Plant growth promoting rhizobacteria (PGPRs) are a group of microbes which colonize around the plant roots and improve the plant growth (Lugtenberg and Kamilova 2009). They directly and indirectly contribute to the plant growth which includes symbiotic and non-symbiotic rhizobacteria. Rhizobacteria have been well documented to promote plant growth as well as alleviate various abiotic stresses including salinity (Dimpka *et al.,* 2009).

The symbiotic association of leguminous plants and rhizobia has the greatest impact on the soil enrichment. It is estimated that the rhizobial symbiosis with the agriculturally important legumes contribute nearly half the annual quantity of Biological Nitrogen Fixation entering soil ecosystems (Tate, 1995). Marcar *et al.,* (1991) stated that the soil salinity specifically disturb the symbiotic interaction between legumes and rhizobia. Many studies have been shown that the soil salinity decreases rhizobial colonization and nitrogen fixation and nodulation (Singleton and Bohlool, 1984; Zahran and Sprent, 1986; Elsheikh and Wood, 1995; Zahran, 1999). Similarly, Saadallah *et al.,* (2001), Rao *et al.,* (2002) and Faghire *et al.,* (2011) reported that the initiation, development, and function of nodules are reported to be effected more than the symbiotic nitrogen fixation. Further, the infection process appears to be the most sensitive stage to salinity (Payakapong *et al.,* 2006). Hence, selection of host genotypes and Rhizobacterial strains that are tolerant to high-salt conditions and the selection of salt-tolerant plant-rhizobia combinations might improve plant productivity under saline conditions (Kenenil *et al.,* 2010).

Cowpea (*Vigna unguiculata* L. Walp) is one of the non-traditional legume crop grown under rain fed semi arid and arid regions by poor farmers for food and fodder (Singh *et al.,* 1997). Cowpea provides a valuable source of protein (23-35%) and its capability to fix atmospheric nitrogen to ammonia in the soil allows it to germinate well on poor soils and increase its fertility (Steele, 1972). Present study reports the effect of *Rhizobacterium* strains BR2 and BR3 inoculation on the tolerance of Cowpea varieties- Pusa Sukomal and RC101 to different levels of NaCl salinity in the field conditions..

Materials and Methods

In this study we have used 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).

Four concentrations of NaCl - 0 (control), 25, 50 and 75mM water was prepared in tap water and used for the irrigation. The field with sandy loam soil with sand 84.2%; silt 12.9%; clay 2.9%; pH 7.8; EC 0.5 dSm and organic matter 1.2% was prepared for the experiment. Field was divided into six columns, three for each variety (for non –inoculated control, inoculated with BR2, inoculated with BR3). Each column was further divided into four blocks, one for each saline (0, 25, 50 and 75mM NaCl) treatment.

The 14 day old culture of Rhizobacterial strains BR2 and BR3, grown on YEM broth (Aneja, 2003) at 28±2°C, 120 rpm for 14 days (incubator shaker) was used for the coating the Cowpea seeds before sowing on the field.

The influence of *Rhizobacterium* application on salinity tolerance of Cowpea crop was analyzed in 90 day old field plants by studying the influence on agronomic characters- shoot length, root length, number of nodules, fresh weight and dry weight. Plant shoot and root length were measured from the crown region to the tip. The numbers of nodules present were counted. Then the plant fresh weight was recorded on top pan electric balance. For dry weight the plants were dried inside the oven at 60ºC and the dry weight was recorded on top pan electric balance.

The data was recorded from 5 plants collected randomly from each group. The experiment was repeated and data collected from both replicates was polled. Mean and standard deviation were calculated.

Results

In the present study, the influence of *Rhizobacterium* (BR2 and BR3) on the growth of Cowpea varieties Pusa Sukomal and RC101 under different concentrations (0, 25, 50 and 75mM NaCl) of salinity was evaluated by analyzing the agronomic parameters such as plant height shoot, root length, number of root nodules, fresh and dry weight in 90 days old plants and the data is presented in the Table -1.

The 90 days old control (un inoculated) plants of Pusa Sukomal recorded 45.33cm and 23.67 cm of shoot and root length. The mean fresh and dry weight was 34.88g and 7.44g respectively. The mean number of root nodules was about 18.33.

The RC101 field plants recorded 36.67cm shoot and 12.33cm root length in control (un inoculated) plants with 26.19g fresh and 8.51g dry weight and 15.0 mean number of root nodules per plant (Table 1).

The plants without Rhizobacterial inoculation have shown increase in shoot length (57.33cm and 40.0 cms) on exposure to 25mM NaCl , while other agronomic parameters reduced on exposure to the NaCl salinity. The decrease in the parameters increased with increase in the level of NaCl salinity.

Salinity causes adverse effects on plant growth and seed germination percentage (Lobato *et al.*, 2009; Gulzar *et al.*, 2003; Kaymakanova, 2009), fresh and dry weight (Raptan *et al.*, 2001; Yupnis *et al.*, 2001; Ghoulam *et al.*, 2002) in Chick pea, Cowpea and lettuce (Murrillo-Amador and Troyo-Die Guez, 2000; Ashraf and Waheed, 1992; Barassi *et al.*, 2006).

Decrease in fresh weight of plants under high levels of salinity may be attributed to increase in osmotic pressure which causes a drop in plant water content and inhibit both meristematic activity and elongation of cells (Kinebery, 1994; Rawya, 2001). Salinity significantly decreases shoot and root dry weight, nodule dry weight and average individual nodule dry weight (Pilar et al., 1995).

In presence of BR2 Rhizobacterial strain-

The plants grown in 0mM NaCl gained 62.67cm and 19.33cm shoot and root length in varieties Pusa Sukomal. Similarly in RC101 the mean shoot and root length was 50.0cms and 13.67cms. The plants developed about 31.33 and 23.33 mean number of nodules per plant. The fresh and dry weight of these plants was 38.49g and 8.72 g in Pusa Sukomal and 40.74 g and 10.13 g in RC101 respectively. These results show improvement in all parameters tested in plants on inoculation of BR2 rhizobacterium.

In the presence of BR3 Rhizobacterial strain-

The shoot and root length of Pusa Sukomal was 64.33cms and 25.0cms, while of RC101 was 53.67cms and 13.67cms respectively. About 31.67 and 35.33 root nodules development was noticed in control plants which recorded 31.72g and 11.16g (in Pusa Sukomal) and 41.23g and 10.32g (in RC101) fresh and dry weight respectively.

Effect of NaCl salinity-

All the parameters in both Pusa Sukomal and RC101 reduced from 0mM concentration of NaCl salinity to 75mM NaCl salinity. Shoot length reduced from 65.00cm to 38.67cm, root length from 24.33cm to 15.0cm, mean root nodules from 26.33 to 9.0, fresh weight from 36.27g to 21.82g and dry weight from 8.35g to 3.42g respectively with increase in salinity from 25mM to 75mM in Pusa Sukomal plants, in presence of BR2 Rhizobacterial strain (table 1).

Similarly, in RC101 variety, the shoot length reduced from 47.00cm to 36.53cm, root length from 13.33cm to 11.67cm, mean root nodules from 21.33 to 6.0, fresh weight from 25.54g to 23.17g and dry weight from 7.81g to 2.97g respectively with increase on salinity from 25mM to 75mM in presence of BR2 Rhizobacterial strain (table 1) .

On inoculation of BR3 strain Pusa Sukomal 90 days old field plants recorded reduction in shoot length from 60.67 cm to 37.33cm, root length from 22.67 cm to 15.67cm, root nodules number from 18.33 to 17.00, fresh weight from 49.16g to 23.16g and dry weight from 10.52g to 4.77g with salinity of NaCl increased from 25mM to 75mM (table-1). While the variety RC101 recorded reduction in shoot length from 52.67 cm to 38.67cm, root length from 15.67 cm to 10.33cm, root nodules number from 16.00 to 8.67, fresh weight from 36.96g to 21.87g and dry weight from 8.59g to 3.47g with salinity of NaCl increased from 25mM to 75mM (table-1).

Rhizobacteria are known to ameliorate the effect of salt stress on germination and growth (Habib *et al.*, 2016). Rhiozbacteria inoculation has been reported to have positive influence on plant growth under saline conditions and result in significant increase in the plant biomass (fresh and dry weight) (Bharti *et al.*, 2014, 2016).

In the present study, on application of *Rhizobacterium* strains BR2 and BR3 on the seeds of Cowpea varieties- Pusa Sukomal and RC101 before sowing has increased the plant tolerance to NaCl salinity as compared to the control plants. Overall the Cowpea variety Pusa Sukomal performed better than variety RC101 in all growth parameters. In conclusion, application of rhizobacterium culture on seeds has conferred tolerance to salinity in

Cowpea plants. The combination of genotypes of Pusa Sukomal with BR3 Rhizobacterium strain has performed better than other combinations, indicating the combination of plant rhizobacterial genotypes play a major role in tolerance of crops to salinity.

Table-1

NaCl sconce—tration	Pusa sucomal					RC101				
	Plant height		Number of root nodules	Fresh weight	Dry weight	Plant height		Number of root nodules	Fresh weight	Dry weight
	shoot length	Root length				Shoot length	Root length			
Control	90 days									
0mM	45.33±2.03	24.67+2.60	18.33±1.67	34.83±1.36	7.44±0.42	36.67±0.88	12.33+1.45	15.00±2.89	26.19±0.72	8.57±0.29
25mM	57.33±2.03	21.67+1.76	16.67±1.67	31.92±2.70	6.81±0.76	40.00±2.89	9.67+0.88	9.67±0.88	20.37±1.71	6.28±0.50
50mM	42.67±1.20	14.67+1.76	14.67±1.76	27.26±1.09	7.29±0.51	34.87±2.61	12.00+1.15	9.00 ±0.00	22.61±2.51	5.17±0.68
75mM	25.00±2.31	09.33+0.88	8.67±0.33	19.55±1.07	3.07±0.04	20.00±2.89	11.00+1.15	6.00±1.15	17.92±0.75	2.77±0.35
With *Rhizobacterium* BR2										
0mM	62.67±2.60	19.33+1.45	31.33±2.03	38.49±2.38	8.72±0.60	50.00±2.89	13.67+1.45	23.33±2.03	40.74±1.55	10.13±0.82
25mM	65.00±2.89	24.33+2.03	26.33±1.76	36.27±0.44	8.35±0.28	47.00±1.15	13.33+1.45	21.33±2.03	25.54±0.93	7.81±0.53
50mM	44.33±2.60	16.33+1.20	19.67±1.20	35.24±1.80	7.29±0.48	38.43±1.71	12.00+0.58	13.67±3.48	24.01±1.37	7.63±0.40
75mM	38.67±1.76	15.00+0.58	9.00±1.00	21.82±1.67	3.42±0.46	36.53±1.29	11.67+0.88	6.00±1.15	23.17±1.53	2.97±0.45
With *Rhizobacterium* BR3										
0mM	64.33±2.40	25.00+1.15	31.67±2.03	51.72±1.07	11.16±0.72	53.67±2.73	13.67+1.45	35.33±2.33	41.23±1.18	10.32±0.67
25mM	60.67±2.33	22.67+1.45	18.33±1.67	49.16±1.20	10.52±0.74	52.67±3.18	15.67+1.45	16.00±2.89	36.96±1.45	8.59±0.90
50mM	38.00±1.73	17.33+1.45	16.33±1.76	33.85±1.36	7.29±0.48	47.33±2.33	15.17+1.17	15.67±2.03	30.96±1.45	8.67±0.42
75mM	37.33±2.33	15.67+0.88	17.00±0.88	23.16±0.26	4.77±0.653	38.67±2.03	10.33+0.88	8.67±0.88	21.87±1.38	3.47±1.08

Table – Effect of 0, 25, 50 and 75mM NaCl saline water irrigation on the growth of 90 days field plants of Cowpea varieties-Pusa Sukomal and RC101 with or without Rhizobacterial (BR2, BR3) inoculation.

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