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Evaluation of PGPR Isolated from *Sesuvium portulacastrum* on Crop Growth under Salinity

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***Abstract*:** Salinity is one of the major factors that adversely affect plant growth causing considerable loss in agricultural production. The halo-tolerant bacteria associated with halophyte rhizosphere can be used as a cost effective and economical tool for salinity tolerance and growth promotion in plants. The total of 8 independent isolates from the rhizosphere of *Sesuvium portulacastrum* from coastal soils were isolated for their plant growth promotion potential. All the isolates had at least one plant growth promoting property. In order to ascertain the true salinity tolerance levels of the isolates, a growth curve experiment with 0, 3, 5 and 7% NaCl was carried out. Among all the isolates RB_5 and RB_6 had high tolerance for salinity. The highest ammonia production was in RB_5 at 1.46 mg/L and IAA (Indole Acetic Acid) production was in RB_6 at 21.75 µg/ml. The bioinoculation of *Neobacillus niacini* increased seed germination (23.2%), shoot length (35.1%), root length (34.2%), and total dry matter (43.5%) even under high salinity (7.78 dS/m). These saline tolerant beneficial bacteria could serve as inoculant for non-host plant cultivation or for phytoremediation of saline soils.

***Keywords*:** Halophilic bacteria, Salinity tolerance, Plant growth promotion, Germination study

Salinity is a major problem causing substantial loss in agricultural production around many parts of the world leading to degradation of land. Soil salinization is a worldwide problem that could affect 1-10 billion hectares with a potential increase of around 15% per year. It is also estimated that up to 50% of the irrigated lands could be affected by salinity or sodicity (Rodríguez-llorente et al 2019). Saline soil is characterized by the presence of neutral soluble salts on the soil surface and root zones at higher concentration. It has an electrical conductivity of >4 dS m^{-1} (~20 mM NaCl) at 25°C and exchangeable sodium <15%. Reclamation of saline soil requires good quality irrigation water for leaching, infrastructure for drainage and amendments like gypsum. These processes are continuous in nature and is laborious and cost-intensive. An alternative approach to remediate saline soils is use of plants with its associated endophytes and rhizosphere microorganisms (Nath et al 2020). *Sesuvium portulacastrum* being a succulent halophyte somehow manage to uptake water from the soil with high salinity. The unique metabolic activities such as production of phytohormones and siderophores by the microorganisms associated with halophytic plants are responsible for the plant-microbe interactions at saline sites (Majeed et al 2015). Siderophore, a low molecular weight iron chelator is released by some microbes when plant is under salt stress (Beneduzi et al 2012). Similarly, phytohormones (auxins, gibberellins, abscisic acid, indole acetic acid) are growth regulators synthesized in defined organs of the plant and play a major role in the mitigation of abiotic stresses (Ahemad 2014).

To use PGPR as a saline soil remediation tool, more research of halophyte-associated rhizobacteria and interactions with halophytes and glycophytes' is needed to understand the processes of their survival and protection from salt stress and to design plant protection measures (Ruppel et al 2013, Miransari 2014). All bacteria require different environmental conditions for expression of genes and beneficial characters for plant growth especially halophilic microbes require saline environment (Oren 2008). Plant tolerance to salt is more effective in rhizobacteria isolated from a salty habitat than by PGPR isolated from non-saline habitats (Katoka et al 2017, Palacio-Rodríguez et al 2017, Etesami and Beattie 2018, Numan et al 2018). Black gram (*Vigna mungo* L.) belongs to the family of Fabaceae and is one of the most significant economical pulse crops used as a food, green manure and fodder. Being salt sensitive crop, high salinity inhibits photosynthetic activity, growth rate along with denaturation of membrane and chlorophyll (Munns et al 2008, Chaves et al 2009, Mittal et al 2012). Hence, the potential of halotolerant bacteria to improve the growth of black gram under saline condition was evaluated.

MATERIAL AND METHODS

Collection of *Sesuvium portulacastrum*: The halophyte, *S. portulacastrum* (L.) was collected from coastal region (11º29'40'' N, 79º46'2'' E) of Parangipettai, Cuddalore, India. Identification and authentication using the inflorescence was carried out with the help of Botanical Survey of India (BSI), Madras zone, Coimbatore. Even though *S. portulacastrum* could be propagated through vegetative methods and was collected along with rhizospheric soil for isolation of PGPR bacteria.

Isolation of salt tolerant rhizospheric bacteria: For isolation of rhizospheric bacteria, soil from rhizosphere of halophyte plants (1 g) were mixed with 25 ml of sterile distilled water and were plated in nutrient agar (NA) (Anburaj et al 2012). After the appearance of colonies, individual colonies were picked up with sterilized loop, transferred to fresh NA slants and the pure cultures so obtained were stored in refrigerator at 4ºC (Haiyambo et al 2015). Subsequent sub-culturing was then made in NA media and Nutrient Broth for further biochemical and molecular analyses. All the cultures were tested for salt tolerance by culturing in Mannitol Salt Agar (MSA) containing 5% NaCl to confirm the salt tolerance ability (Shields and Tsang 2006). The development of halo zone indicates the salt tolerance ability. The halo zones were measured for each culture which is used to calculate the tolerance index (Equation 1).

$$\text{Salinity tolerance index} = \frac{\text{(Colony diameter + Halozone diameter)}}{\text{Colony diameter}} \quad (1)$$

Production of plant growth promoting substances: The production of ammonia by rhizobacteria was tested in 10 ml of peptone water. After 48 h of incubation at 30ºC, the Nessler's reagent (0.5 ml) was added to each tube (Bhavani and Kumari 2019). Development of brown to yellow color was quantified using spectrophotometer. Bacterial isolates were also screened to produce siderophores on the Chrome azurol S (CAS) agar medium (Schwyn and Neilands 1987) and SPI (Siderophore Production Index) was calculated (Equation 2). The production of IAA like compounds was detected from the culture supernatants of the bacterial isolates (Thakuria et al 2004). Pure colonies from a 24 h culture was inoculated into nutrient broth with 2 mg of tryptophan/g and in the absence of tryptophan, and were incubated at 28ºC for 48 h. Five ml culture was taken from each tube and centrifuged at 10,000 rpm for 15 min. Two milliliter aliquots of the supernatant were transferred to a fresh tube and washed with ethyl acetate to extract free IAA-like substance. The extracts were then treated with 4 ml Salkowski reagent and incubated at room temperature for 25 min. The absorbance of the solution (pink colour developed) was read at 530 nm. For the control experiment, sterile nutrient broth was used. The concentration of IAA in the culture supernatants was determined using a calibration curve with pure IAA as a standard (Shahzad et al 2017).

$$\text{Siderophore production index} = \frac{\text{Colony diameter + Orange zone diameter}}{\text{Colony diameter}} \quad (2)$$

Assessing salinity tolerance of isolates: The ability to grow in different concentration of NaCl was assessed by growth curve experiment. A 1ml inoculum from 48 hours broth was transferred to 100 ml NA broth supplemented with 0, 3, 5 and 7% NaCl and incubated at 30ºC. The OD 600 nm value was observed every 4 hours after inoculation. The absorbance is recorded for 72 hours or until the curve attains stationary phase (Ramadoss et al 2013, Nagaraju and Mahadevaswamy 2020).

Genetic identification and phylogenetic tree construction: The DNA was isolated from microbial samples and PCR amplification was done by adding 5 µL of isolated DNA in 25 µL of PCR reaction solution (1.5 µL of Forward Primer and Reverse Primer, 5 µL of deionized water, and 12 µL of Taq Master Mix). The forward Primer, 27F (5' AGAGTTTGATCTGGCTCAG 3') with 20 base pairs and Reverse primer, 1492R (5' TACGGTACCTTGTTACGACTT 3') with 20 base pairs was used to perform PCR. Then the DNA sequencing was performed using an ABI PRISM® Big Dye TM Terminator Cycle Sequencing Kits with AmpliTaq® DNA polymerase (FS enzyme) (Applied Biosystems). The 16s rRNA sequence was blasted using NCBI blast similarity search tool. The phylogeny analysis with the closely related sequence of blast results was performed by multiple sequence alignment. The MUSCLE 3.7 was used for multiple alignments of sequences (Edgar 2004). Poorly aligned positions and divergent regions were cured using the program G blocks 0.91b (Talavera and Castresana 2007). Finally, the program Tree Dyn 198.3 was used for tree rendering (Dereeper et al 2008).

Germination study using selected isolates as bioinoculum: The two most salt tolerant isolates were selected to study their effect on germination and growth attributes of black gram (*Vigna mung L.*) under salinity. The black gram genotype, VBN-8 seeds were used for the study after surface sterilization in 0.1% sodium hypochlorite for 3 mins and repeated washing with distilled water. After which the 28-hour old inoculum of isolate, I_1-RB5 and I_2-RB6 was used for seed priming and distilled water as control. The seeds were soaked in optimized microbial inoculum with OD of 1 at 600 nm for 3 hours. In initial screening, black gram seeds failed to germinate at 2 % NaCl. The seeds were grown in germination sheets at 3 levels of salinity (T_1 - 0, T_2 - 0.5 and

T_3 - 1 percent of NaCl). The EC of the 0, 0.5 and 1 percent of NaCl concentrations were 0, 4.28 and 7.78 dS/m, respectively. The germination percentage, root length, shoot length and total dry weight were calculated after 15 days.

RESULTS AND DISCUSSION

Microbial isolates and salt tolerance: The soil in coastal area was sandy in nature with low organic and nutrient content. The seasonal intrusion of sea water through backwaters results in high EC of 12 dS/min the soil. The total of 8 bacterial isolates (RB_1 to RB_8) were isolated from rhizosphere of *S. portulacastrum*, naturally established in the extreme saline conditions located in Parangipettai, India. These isolates were pure cultured and tested in mannitol agar with 5% NaCl (Kumar et al 2020). Among these, isolates RB_1, RB_4, RB_5, RB_6, RB_7 and RB_8 developed halo zone around the colonies proving their potential to tolerate salinity. Salinity tolerance index (STI) was in the order of $RB_5 > RB_6 > RB_1 > RB_8 > RB_7 > RB_4$ (Table 1). The isolate RB_5 had the highest STI of 3.69 followed by RB_6 (3.13). The isolates RB_2 and RB_3 failed to grow in the saline condition (5% NaCl) and lowest STI was in RB_4 at 2.56. The pure cultures were isolated from morphologically different colonies. The isolate RB_6 was very high in numbers during enumeration which could be due to high association with *S. portulacas*trum rhizosphere. Since plants release root exudates which plays a major role in the root microbiome.

Evaluation of direct plant growth promotion mechanisms: All the eight isolates had the potential to produce ammonia, IAA and Siderophore (Table 1). The ammonia production by the bacteria promotes high growth and yield in crops along with various benefits like remediation of polluted environment (Bledsoe and Boopathy 2016, Raklami et al 2021), carbon sequestration (John and Lakshmanan 2018) and various ecosystem services (Ebadi et al 2018, Razzaghi et al 2019). Highest ammonia production was reported in the isolate RB_8 (2.40 mg/L) and lowest was in RB_6 (0.16 mg \/L). Ammonia production is associated with the presence of *nif* gene in diazotrophs which is to be verified with further study. Among the isolates, RB_8, RB_5, RB_4 and RB_2 recorded the highest ammonia production of 2.40, 1.46, 1.36 and 1.25. mg/L、 respectively. The ammonia and nitrogen producing bacteria isolated from rhizosphere ensures plants growth even in nitrogen deficit soils (Patrick et al 2018). The IAA production (µg/ml) was reported in all the isolates except RB_4 and ranged from 1.34 to 21.75 µg/ml (Table 1). Isolates RB_6, RB_5, RB_3 and RB_2 recorded highest IAA production of 21.75, 18.13, 11.26 and 10.81 µg/ml, respectively. This IAA production of microbes in the host plant rhizosphere increase the yield and stress tolerance in crops (Shahzad et al 2017). The PGPR bacteria application improved the crop stress tolerance and evaluated over recent years (Seema et al 2016, Bhavani and Kumari 2019, Goyal et al 2020). Upadhyay et al (2009) found that only 18% of strains isolated from wheat rhizosphere in soils of Varanasi, were tolerant to 8% of NaCl, while maintaining PGP activities. Siderophore production ensures the availability of nutrient through iron chelation hence it was qualitatively assessed through SPI (Siderophore production index) (Panda and Parida 2019). The siderophore production ranged from RB_6 (2.77) to RB_4 (1.92). The SPI was recorded in the order of $RB_6 > RB_1 > RB_8 > RB_7 > RB_5 > RB_4$. The isolates RB_1, RB_5, RB_6 and RB_8 had all the PGPR activity investigated in this study. These isolated strains were assessed for their salt tolerance and can be used as inoculum in the nutrient management practice after formulating the application strategies (Hameeda et al 2006, Rundan et al 2021).

Growth curve experiment to assess the salt tolerance: In the experiment to assess the growth potential of the isolated strains, strain RB_1 and RB_4 failed to grow in broth with 3 % NaCl. The growth curve showed that RB_5, RB_6, RB_7 and RB_8 reached stationary phase during different time (Fig. 1). In isolates RB_5, RB_6, RB_7 and RB_8 lag phase lasted upto 8, 8, 16 and 20 hours, respectively in control (0% NaCl). This is due to

Table 1. Salinity tolerance and PGPR (Ammonia, IAA and siderophore) production potentials of the microbial isolates

Isolates	Salinity tolerance index	Ammonia production (mg/L)	IAA production (µg/ml)	Siderophore production Index
RB_1	3.00	0.31	8.92	2.25
RB_2	0.00	1.25	10.81	0.00
RB_3	0.00	0.86	11.26	0.00
RB_4	2.56	1.36	0.00	1.92
RB_5	3.69	1.46	18.13	2.00
RB_6	3.13	0.16	21.75	2. 77
RB_7	2.76	0.00	5.71	2.07
RB_8	2.89	2.40	1.34	2.17

difference in the microbial growth kinetics among the isolates and phase at which the microbe prepares itself for the log phase (Rolfe et al 2012). The log phase lasted upto 32, 44, 64 and 48 hours in isolate RB_4, RB_5, RB_6 and RB_7, respectively after inoculation in control. The shorter log phase indicates the potential of microbe to grow at favourable environment. All the isolates showed growth in 3 % NaCl broth. However, the isolates RB_4 and RB_7showed low growth in 3 % NaCl than their respective control treatments (0% NaCl). The growth curve indicated that both isolates (RB_4 and RB_7) didn't generously grow at 3 % NaCl and stationary phase was achieved much earlier (12 and 24 hours in RB_4 and RB_7, respectively) than their respective controls. The long stationary phase could be due to the synthesis of protective factors and adaptation of current environmental conditions at higher NaCl concentrations (Finkel and Kolter 1999). But, isolates, RB_5 and RB_6 had similar growth pattern to control in NA with 3% NaCl but lag and log phase delayed slightly in both isolates. This change could be the time required to adapt the high salt content. In RB_5, lag phase was upto 28 hours and 32 hours in RB_6. The reasons for long delay in RB_5 are unclear. Isolates RB_4 and RB_7, didn't record any growth in NA broth with 5 and 7% NaCl indicating their low potential for salinity tolerance. But RB_5 and RB_6 had fair growth in 5 and 7% NaCl concentration. Isolate RB_5 had similar growth pattern at 5 and 7% NaCl, but the curve lacked distinctive lag, log and stationary phase. The OD 600 nm at 7% of NaCl was lower than 5%. The isolate RB_6 had distinctive lag, log, and stationary phase in 5% NaCl indicating high tolerance than RB_5. But the growth was less pronounced at 7% NaCl with same hours of lag, log and stationary phase. The microbial growth curve of RB_5 and RB_6 at different salt concentration were similar to the extreme halophytes isolated from saline lakes (Ramadoss et al 2013).

Phylogenetic identification of 16S R DNA: The bacterial strains were identified by 16S rDNA sequencing and their sequences were submitted for comparison with the sequences in the NCBI database by BLAST search in order to find out the homologous sequences of related strains. The Blast results based on 16S rRNA, showed that the two isolates were 99 and 100% related to *Metabacillus indicus* and *Neobacillus niacini,* respectively (Fig. 2, 3). In Figure 2, the 0.01 shows the length of branch that represents an amount genetic change between the strains. The units of

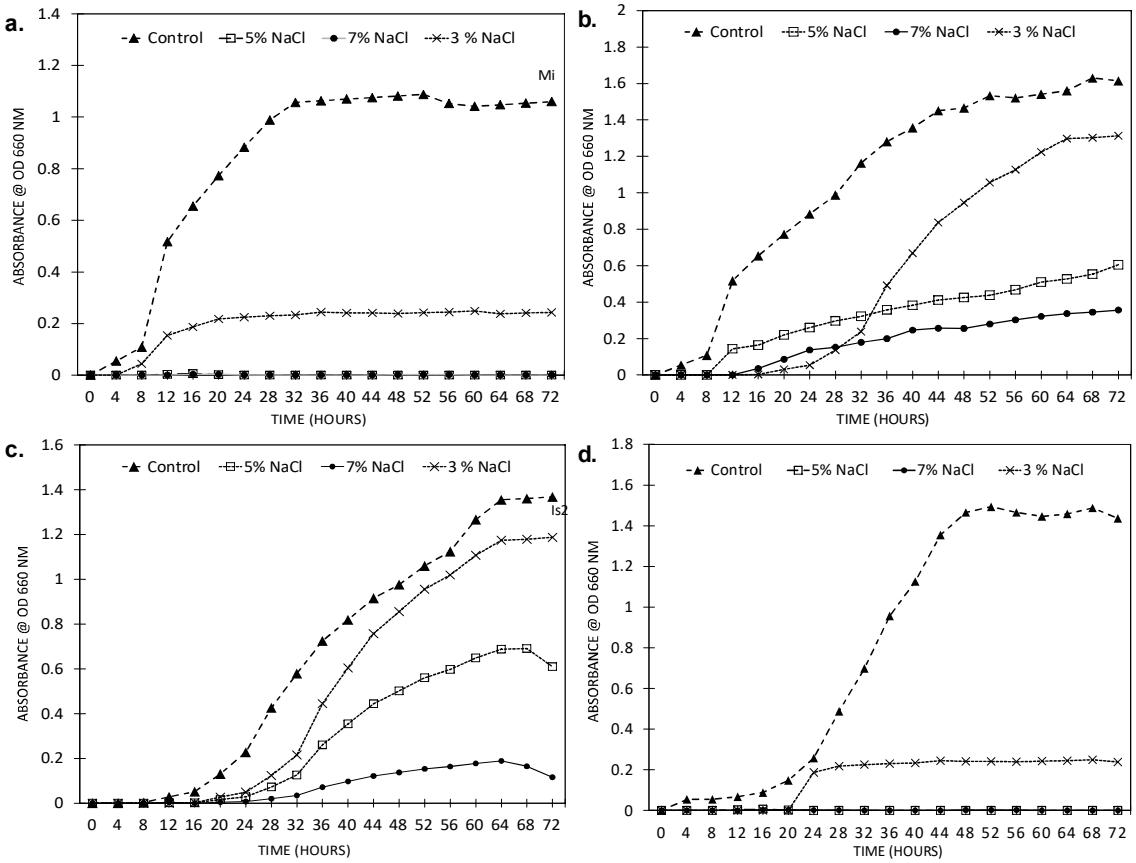

Fig. 1. Growth curve of isolates at 0%, 3%, 5% and 7% NaCl concentrations. (a. RB_4, b. RB_5-*Metabacillus indicus*, c. RB_6-*Neobacillus niacini*, d. RB_7)

branch length are usually nucleotide substitutions per site *i.e.,* the number of changes or 'substitutions' divided by the length of the sequence. Since the tree is developed by neighbour linking method the nearest resembling strain for RB_5 is *Metabacillus indicus*. The isolate comes under the genera of *Metabacillus* which is generally in the coastal saline environments. The strain *Paenibacillus endophyticus* is ascertained as the outward link to the isolate. In Figure 3, the 0.002 shows the length of branch that represents an amount genetic change between the strains and the nearest resembling strain for RB_6 is *Neobacillus niacini*. The isolate comes under the genera of *Neobacillus* which is a new sub-genus in the genera *Bacillus*. The strain *Neobacillus pocheonensis* is ascertained as the outward link to the phylogenetic tree. The organism before the node of the isolated is earlier in the evolution in this case the *Neobacillus pocheonensis* and *Neobacillus ginsengisoli.*

Effect of bio inoculum on black gram growth under salinity germination potential of seeds: The germination percentage decreased with the increase in salinity. However, the inoculation of isolates increased the germination potential of black gram (Table 2). The highest germination potential was recorded in T_1I_2 followed by T_1I_1. The germination percentage was 27% lower in T_3Cthan T_1C (non-inoculated). In comparison with T_1C, the germination percentage was only 24 and 13% lower in T_3I_1 than T_3I_2. In treatment T_2 (4.28 dS/m) the inoculation of I_2 recorded germination percentage same as T_1C, denoting the potential of the isolate to promote germination under salinity. The inoculation of *M. indicus* (I_1) and *N. niacini* (I_2) improved the germination percentage of the seeds even under high salinity (7.78 dS/m).

Growth attributes of seedlings: The highest shoot length was recorded in T_1I_1 (14.6 cm) followed by T_1I_2 (14.1 cm) (Table 2). In treatment T_2 (4.25 dS/m), $I_1$and I_2 recorded 12.5 and 18.8 % high shoot length than C (control). Similarly, in T_3 (7.78 dS/m) $I_1$and I_2 recorded 31 and 35.1 % high shoot length than C (control). The root length was also high in all

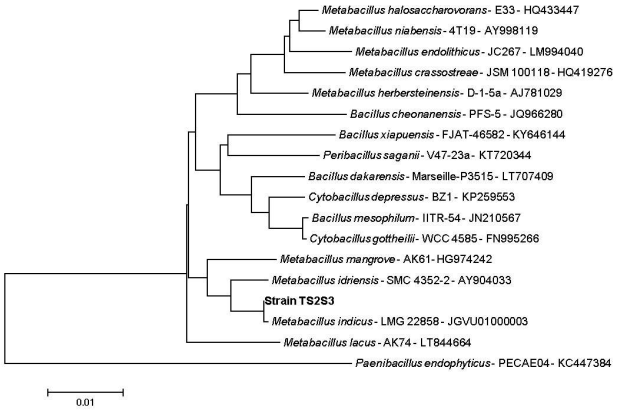

Fig. 2. Phylogenetic tree by neighbour joining method of salt tolerant rhizospheric bacteria RB_5 with unit branch length of 0.01

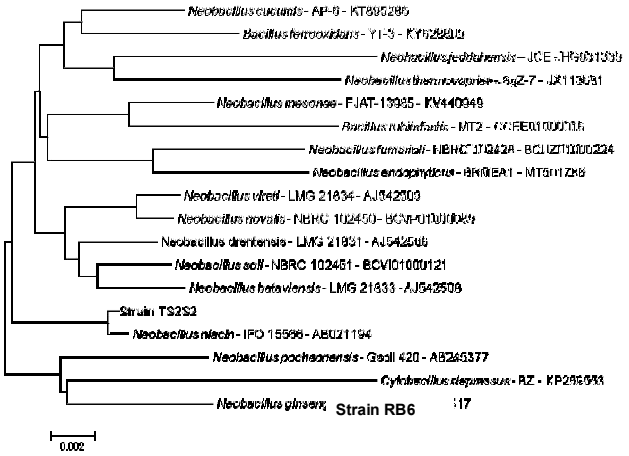

Fig. 3. Phylogenetic tree by neighbour joining method of salt tolerant rhizospheric bacteria RB_6 with unit branch length of 0.002

Table 2. Growth attributes of black gram (*Vigna mungo* L.) under different salinity levels

NaCl concentration (%)	Treatment	GP (%)	SL (cm)	RL (cm)	TDM (mg/plant)
T_1	C	93 (±3.8)	13.8 (±0.6)	5.9 (±0.2)	75.2 (±3.1)
	I_1	95 (±3.7)	14.6 (±0.8)	6.8 (±0.4)	110.4 (±6.3)
	I_2	96 (±1.5)	14.1 (±0.3)	7.4 (±0.2)	119.8 (±2.9)
T_2	C	90 (±6.4)	11.2 (±1.2)	5.3 (±0.6)	64.8 (±7.2)
	I_1	92 (±4.9)	12.6 (±0.7)	4.9 (±0.3)	75.2 (±4.0)
	I_2	96 (±3.8)	13.3 (±0.5)	6.8 (±0.3)	89.2 (±3.6)
T_3	C	75 (±2.8)	8.2 (±0.3	3.8 (±0.2)	54.0 (±2.2)
	I_1	79 (±4.2)	10.7 (±0.6)	4.3 (±0.2)	65.8 (±3.8)
	I_2	84 (±3.5)	11.1 (±0.5)	5.1(±0.2)	77.5 (±3.2)

Note: C – control, I_1 – Bioinoculum of *Metabacillus indicus*, I_2 - Bioinoculum of *Neobacillus niacini;* T_1 – 0 % NaCl, T_2 – 0.5 % NaCl, T_3 - 1 % NaCl; GP – Germination percentage, SL – Shoot Length, RL - Root Length, TDM – Total Dry Matter.
The values represent the mean of three replications, and the figures in parenthesis are the standard deviation

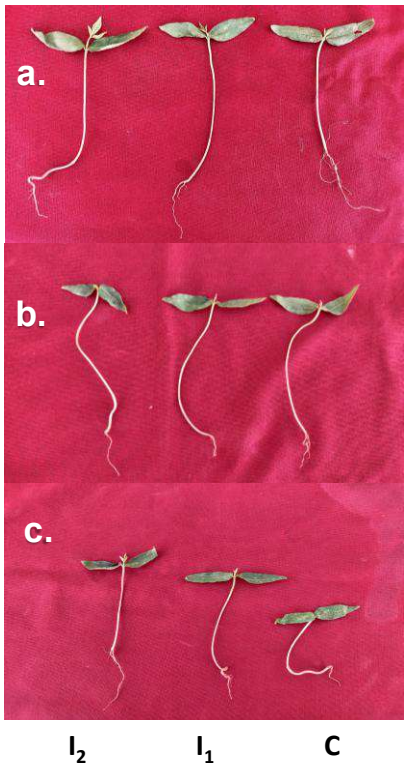

Fig. 4. Effect of bioinoculants on black gram (*Vigna mungo* L.) under salinity levels (a. 0 %, b. 0.5 %, c. 1 % of NaCl concentration and I_1 - inoculation of RB_5, I_2 - inoculation of RB_6, C- uninoculated)

treatments with bioinoculant I_2 (Fig. 4). Apart from root length the number root nodes were high in treatments with both I_1 and I_2. The increase in total dry matter (TDM) was significant with application of bioinoculants (Hassan et al 2020). The application of bioinoculant I_1 and I_2 increased the TDM by 46.9 and 59.4 %, respectively in T_1. The inoculation of these saline tolerant isolates as bioinoculants improved the seed germination and growth attributes significantly even under high EC (7.78 dS/m). These results were in line with the earlier studies (Etesami and Maheshwari 2018, Hassan et al 2018, Priyadharshini et al 2019).

CONCLUSION

The potential of rhizobacteria to stimulate plant growth in poor quality soil is an important component that is required to be addressed for sustainable future. Hence, the isolation and characterization of rhizospheric soil bacteria from saline environments was carried out. In summary, isolated plant growth promoting bacterial species associated with rhizosphere of *Sesuvium portulacastrum* growing in highly saline coastal soil and evaluated salinity tolerance of two promising isolates (*Metabacillus indicus* and *Neobacillus niacini*). Subsequently, their potential to promote black gram growth was assessed by germination study. These isolates have high potential to survive under saline conditions (EC > 4 dS/m) and even promote plant growth. High siderophore production index , ammonia and IAA production were also reported in the two isolates. The seed primming of black gram with *Metabacillus indicus* and *Neobacillus niacin*) resulted in significant improvement in crop growth attributes under high saline conditions (7.78 ds/m). The promising results from this study warrant further, in-depth analysis of plant growth promotion by these and other halophilic bacterial species isolated from non-target halophilic crops. This research will have a significant impact on efforts to identify bacteria that stimulate growth of crop under high saline conditions.

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REFERENCES

Ahemad M 2014. Mechanisms and applications of plant growth promoting rhizobacteria : Current perspective. *Journal of King Saud University Science* **26**(1): 1-20.

Anburaj R, Nabeel MA, Sivakumar T and Kathiresan K 2012. The role of Rhizobacteria in salinity effects on biochemical constituents of the halophyte *Sesuvium portulacastrum*. *Russian Journal of Plant Physiology* **59**(1): 115-119.

Beneduzi A, Ambrosini A and Passaglia LMP 2012. Plant growth-promoting rhizobacteria (PGPR): Their potential as antagonists and biocontrol agents. *Genetics and molecular biology* **35**: 1044-1051.

Bhavani DG and Kumari AM 2019. Chick pea root endophytic bacteria and their plant growth promoting traits. *Indian Journal of Ecology* **46**(7): 72-76.

Bledsoe R and Boopathy R 2016. Bioaugmentation of microbes to restore coastal wetland plants to protect land from coastal erosion. *International Biodeterioration & Biodegradation* **113:** 155-160.

Chaves MM, Flexas J and Pinheiro C 2009. Photosynthesis under drought and salt stress: Regulation mechanisms from whole plant to cell. *Annals of Botany* **103**(4): 551-560.

Dereeper A, Guignon V, Blanc G, Audic S, Buffet S, Chevenet F, Dufayard JF, Guindon S, Lefort V, Lescot M, Claverie JM and Gascuel O 2008. Phylogeny.fr: robust phylogenetic analysis for the non-specialist. *Nucleic Acids Research* **36**(Web Server Issue): W465-W469.

Ebadi A, Azam N, Sima K, Olamaee M and Hashemi M 2018. Remediation of saline soils contaminated with crude oil using the halophyte Salicornia persica in conjunction with hydrocarbon-degrading bacteria. *Journal of Environmental Management* **219**: 260-268.

Edgar RC 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* **32** (5): 1792-1797.

Etesami H and Beattie GA 2018. Mining halophytes for plant growth-promoting halotolerant bacteria to enhance the salinity tolerance of non-halophytic crops. *Frontiers in Microbiology* **9**: 148.

Etesami H and Maheshwari DK 2018. Ecotoxicology and Environmental Safety Use of plant growth promoting rhizobacteria (PGPRs) with multiple plant growth promoting

traits in stress agriculture : Action mechanisms and future prospects. *Ecotoxicology and Environmental Safety* **156** (October 2017): 225-246.

Finkel SE and Kolter R 1999. Evolution of microbial diversity during prolonged starvation. *Proceedings of the National Academy of Sciences* **96**(7): 4023-4027.

Goyal RK, Sindhu SS and Godara AK 2020. Effect of rhizobacterium on growth, yield and quality of Strawberry. *Indian Journal of Ecology* **47**(1): 92-95.

Haiyambo DH, Chimwamurombe PM and Reinhold-/hurek B 2015. Isolation and screening of rhizosphere bacteria from grasses in East Kavango Region of Namibia for plant growth promoting characteristics. *Current Microbiology* **71**(5): 566-571.

Hameeda B, Rupela OP, Reddy G and Satyavani K 2006. Application of plant growth-promoting bacteria associated with composts and macrofauna for growth promotion of Pearl millet (*Pennisetum glaucum* L.). *Biology and Fertility of Soils* **43**(2): 221-227.

Hassan MJ, Raza MA, Khan I, Meraj TA, Ahmed M, Shah GA, Ansar M, Awan SA, Khan N, Iqbal N, Peng Y and Li Z 2020. Selenium and salt interactions in black gram (*Vigna mungo* L.): Ion uptake, antioxidant defense system, and photochemistry efficiency. *Plants* **9**(4) : 467-483.

Hassan N, Kamrul Hasan M, Obaidullah Shaddam M, Sohidul Islam M, Barutçular C and Sabagh AEL 2018. Responses of maize varieties to salt stress in relation to germination and seedling growth. *International Letters of Natural Sciences* **69**: 1-11.

John JE and Lakshmanan A 2018. Carbon sequestration as biomass carbon and mineral carbonates by cyanobacterial systems in rice soil. *Trends in Biosciences* **11**(27): 3478-3484.

Katoka R, Guneri E, Turgay OC, Yaprak AE, Sevilir B and Baskose İ 2017. Sodium-resistant plant growth-promoting rhizobacteria isolated from a halophyte, Salsola grandis, in saline-alkaline soils of Turkey. *Eurasian Journal of Soil Science* **6**(3): 216-225.

Kumar N, Fatima T, Mishra J, Mishra I, Verma S and Verma R 2020. Halo-tolerant plant growth promoting rhizobacteria for improving productivity and remediation of saline soils. *Journal of Advanced Research* **26**: 69-82.

Majeed A, Kaleem Abbasi M, Hameed S, Imran A and Rahim N 2015. Isolation and characterization of plant growth-promoting rhizobacteria from wheat rhizosphere and their effect on plant growth promotion. *Frontiers in Microbiology* **6**: 198.

Mapelli F, Marasco R, Rolli E, Barbato M, Cherif H, Guesmi A, Ouzari I, Daffonchio D and Borin S 2013. Potential for plant growth promotion of rhizobacteria associated with *Salicornia* growing in Tunisian hypersaline soils. *BioMed Research International* **2013:** 1-13.

Miransari M 2014. *Use of microbes for the alleviation of soil stresses*. Vol. 1. In M. Miransari (eds.), : 1-162. Springer New York

Mittal S, Kumari N and Sharma V 2012. Differential response of salt stress on *Brassica juncea*: Photosynthetic performance, pigment, proline, D1 and antioxidant enzymes. *Plant Physiology and Biochemistry* **54**: 17-26.

Munns R and Tester M 2008. Mechanisms of salinity tolerance. *Annual Review of Plant Biology* **59:** 651-681.

Nagaraju Y and Mahadevaswamy RCG 2020. Mining Saline soils to manifest plant stress - alleviating halophilic bacteria. *Current Microbiology* **77**: 2265-2278.

Nath A, Kaur T, Kour D, Lata K, Yadav N and Asghar A 2020. Saline microbiome : Biodiversity, ecological significance, and potential role in amelioration of salt stress. *In*: *New and Future Developments in Microbial Biotechnology and Bioengineering*, 283–309. Elsevier Inc.

Numan M, Bashir S, Khan Y, Mumtaz R, Shinwari ZK, Khan AL, Khan A and AL-Harrasi A 2018. Plant growth promoting bacteria as an alternative strategy for salt tolerance in plants: A review. *Microbiological Research* **209**: 21-32.

Oren A 2008. Microbial life at high salt concentrations: Phylogenetic and metabolic diversity. *Saline Systems* **4** (1): 2.

Palacio-Rodríguez R, Coria-Arellano JL, López-Bucio J, Sánchez-Salas J, Muro-Pérez G, Castañeda-Gaytán G and Sáenz-Mata J 2017. Halophilic rhizobacteria from Distichlis spicata promote growth and improve salt tolerance in heterologous plant hosts. *Symbiosis* **73**(3): 179-189.

Panda A and Parida AK 2019. Development of Salt Tolerance in Crops Employing Halotolerant Plant Growth – Promoting Rhizobacteria Associated with Halophytic Rhizosphere Soils. *In*: Kumar M, Etesami H and Kumar V (eds.), *Saline Soil-Based Agriculture by Halotolerant Microorganisms*, 75-101. Springer, Singapore.

Patrick OR, Abimbola OA and Adeniyi AO 2018. Screening of bacteria isolated from the rhizosphere of maize plant (*Zea mays* L.) for ammonia production and nitrogen fixation. *African Journal of Microbiology Research* **12** (34): 829-834.

Priyadharshini B, Vignesh M, Prakash M and Anandan R 2019. Evaluation of black gram genotypes for saline tolerance at seedling stage. *Indian Journal of Agricultural Research* **53**(1): 83-87.

Rajput L, Imran A, Mubeen F and Hafeez FY 2013. Salt-tolerant PGPR strain Planococcus rifietoensis promotes the growth and yield of wheat (*Triticum aestivum* L.) cultivated in saline soil. *Pakistan Journal of Botany* **45**(6): 1955-1962.

Raklami A, Tahiri A ilah, Bechtaoui N, Abdelhay EG, Pajuelo E, Baslam M, Meddich A and Oufdou K 2021. Restoring the plant productivity of heavy metal-contaminated soil using phosphate sludge, marble waste, and beneficial microorganisms. *Journal of Environmental Sciences* **99**: 210-221.

Ramadoss D, Lakkineni VK, Bose P, Ali S and Annapurna K 2013. Mitigation of salt stress in wheat seedlings by halotolerant bacteria isolated from saline habitats. *SpringerPlus* **2**(1): 1-7.

Razzaghi B, Ali H and Etesami H 2019. Improved growth and salinity tolerance of the halophyte Salicornia sp . by co-inoculation with endophytic and rhizosphere bacteria. *Applied Soil Ecology* **138**: 160-170.

Rodríguez-llorente ID, Pajuelo E, Navarro- S, Mesa-marín J and Caviedes MA 2019. Bacterial Endophytes from Halophytes: How Do They Help Plants to Alleviate Salt Stress? In: Kumar M (eds.), *Saline Soil-Based Agriculture by Halotolerant Microorganisms*, 147–160. Springer Nature Singapore.

Rolfe MD, Rice CJ, Lucchini S, Pin C, Thompson A, Cameron ADS, Alston M, Stringer MF, Betts RP, Baranyi J, Peck MW and Hinton JCD 2012. Lag phase is a distinct growth phase that prepares bacteria for exponential growth and involves transient metal accumulation. *Journal of Bacteriology* **194**(3): 686-701.

Rundan V, Singh M, Kumar S and Meena BL 2021. Assessment of nutrient management practices on productivity and profitability of fodder maize+ricebean intercropping under irrigated condition impact of organic nutrient management on crop quality, yield and soil health: A Review view project improvement. *Indian Journal of Ecology* **48**(5): 1397-1402.

Ruppel S, Franken P, Witzel K, Ruppel S, Franken P and Witzel K 2013. Properties of the halophyte microbiome and their implications for plant salt tolerance. *Functional Plant Biology* **40**(9): 940-951.

Schwyn B and Neilands JB 1987. Universal chemical assay for the detection and determination of siderophores. *Analytical Biochemistry* **160**(1): 47-56.

Seema K, Mehta K and Chawla W 2016. Influence of plant growth promoting rhizobacteria (PGPR) on growth and yield of strawberry (Fragaria × ananassa Duch.) cv. Chandler. *Indian Journal of Ecology* **43**(1): 262-265.

Shahzad R, Waqas M, Latif Khan A, Al-Hosni K, Kang S-M, Seo C-W and Lee I-J 2017. Indoleacetic acid production and plant growth promoting potential of bacterial endophytes isolated from rice (*Oryza sativa* L.) Seeds. *Acta Biologica Hungarica* **68**(2): 175-186.

Shields P and Tsang AY 2006. Mannitol Salt Agar Plates Protocols. *ASM Conference for Undergraduate Educators*: 1-6.

Talavera G and Castresana J 2007. Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. *Systematic biology* **56**(4): 564-577.

Thakuria D, Talukdar NC, Goswami C, Hazarika S, Boro RC and Khan MR 2004. Characterization and screening of bacteria from rhizosphere of rice grown in acidic soils of Assam. *Current Science* **86**(7): 978-985.

Upadhyay SK, Singh DP and Saikia R 2009. Genetic diversity of plant growth promoting rhizobacteria isolated from rhizospheric soil of wheat under saline condition. *Current Microbiology* **59**(5): 489-496.

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