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IMPROVEMENT OF GROWTH AND SALINITY TOLERANCE OF RICE (*Oryza sativa* L.) CV. NAVEEN AND LUNA SANKHI BIOPRIMED WITH OSMOTOLERANT PHYTONIC PLANT GROWTH PROMOTING BACTERIA

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ABSTRACT
Five osmotolerant plant growth promoting bacteria (PGPB) viz. *Bacillus* (SV4, W1), *Pseudomo*nas (NR4), *Enterobacter* (RP8) and *Salinicola* (S36) spp. were evaluated for enhancement of growth and osmotolerance of rice cv. Naveen (NV) and Luna Sankhi (LS) to select most prospective PGPB to enhance rice production. Biopriming of NV seeds with *Salinicola* and *Pseudomonas* spp. increased radical/plumule length, root no., seedling fr./dr. wt. (114.29 - 229.32%) more than three (SV4, W1, RP8) other bacteria (84.62 - 140.48%) and control (100%). Vegetative and reproductive growth of LS seedlings for NR4 (103.33-181.94%) or S36 (108.19-209.39%) and N:1/2P:K fertilizer co-treatments were either comparable or more than those of sole N:1/2P:K (103.58-195.75%) treatment. LS seedling (7d) growth declined for ≥ 0.21% NaCl (11.61 - 94.74%) and secondary root emergence for ≥ 0.43% NaCl (0%) in petriplate test but S36 (superior) and NR4 priming reduced stress effects, enhanced osmotolerance of seedlings from inherent 6-8 dS/m for 15-21d to 8 dS/m (0.43% NaCl) for 30d in submerged pot test, 12 dS/m (0.7% NaCl) for 30d by S36 treatment in salinity tank and growth components by 124.96-400% over control (12dS/m). Investigations identified S36 and NR4 as prospective osmotolerant PGPB for rice improvement under saline and normal conditions.

INTRODUCTION

Salinity (osmotic pressure ≥ 0.2 MPa, EC ≥ 4 dS/m, ≥ 40 mM NaCl) restricts nutrient availability, growth, development and productivity of rice and plants in general (Shrivastava and Kumar, 2015, Sampangi-Ramaiah *et al*., 2020). Rice (*Oryza sativa* L., Poaceae) is most sensitive (threshold 3.0 dS/m) cereal to salinity (Kalaiyarasi *et al*., 2019) which impairs tiller, panicle and spikelet production, floret fertility, grain size, delay heading etc. and seedling mortality at 50 mM level (Rad *et al*., 2012). However, rice production needs to be increased for food security in India from 94 to 130 mt and globally from 600 to 800 mt by 2025 (Zeigler and Adams, 2008). Thus, improvement of rice/crop production in about 6.5 mha (5700 sq. km) inland saline area in India (Maji *et al*., 2010) and ever-increasing global saline land is the primary target to achieve the projected food demand.

The ecto- and endophytic plant growth promoting microbes (PGPM) like *Azotobacter, Azospirillum, Bacillus, Pseudomonas, Burkholderia, Rhizobium, Pantoea, Herbaspirillum, Beauveria, Trichoderma* spp. *etc*. were recorded to enhance seed germination, root, shoot, flag leaf area, tiller, pollination, panicle number, grain wt./number, photosynthesis *etc*.; salinity (150-200 mM, ~12-15 dS/m NaCl), drought, metal, disease etc. resilience; endogenous hormone, macronutrient and osmolyte metabolism in different rice genotypes cv. Naveen, Swarna, Khandagiri, IR64 etc. and other crops (bean, wheat, tomato, chickpea, soybean *etc*.) although functionalities differed in intra-/intergenotypic phyto-microbiome (Bal *et al*., 2012, Sahoo *et al*., 2014, Pradhan and Mishra, 2015, Pahari *et al*., 2016, Dash and Dangar, 2019, Egamberdieva *et al*., 2019, del Carmen Orozco-Mosqueda *et al*., 2020). Furthermore, the salt tolerant plant growth promoting bacteria (PGPB) *i*.e. Salinicola spp., *Bacillus* spp.and *Pseudomonas* spp. were observed to promote growth and salt or metal tolerance of rice (0.3% NaCl, 2% As), wheat and other crops (Tiwari *et al*., 2011, Bal *et al*., 2012, Pradhan and Mishra, 2015, Pahari *et al*., 2016, Zhao *et al*., 2017, Egamberdieva *et al*., 2019, Mukherjee *et al*., 2019) through production of different plant growth regulatory substances, nutrients, antimicrobial substances, exo-polysaccharide (EPS) *etc*. (Pradhan and Mishra, 2015, Shukla *et al*., 2016). Thus, the resident PGPB (especially the phytonic osmotolerant colonizers) of paddy would be best suited for sustainable rice production/improvement under normal/salt stress conditions which is but underutilized to date.

Importance of PGPB on rice cultivation in saline soils led to evaluate five potent osmotolerant phytonic PGPB (*Bacillus* (SV4, W1), *Pseudomonas* (NR4), *Enterobacter* (RP8) and *Salinicola* (S36) spp.) of rice for promotion of growth/production of high yielding rice cv. Naveen (NV) and Luna Sankhi (LS), and salinity tolerance of LS to select most promising PGPB for

futuristic exploitation to improve/sustain rice cultivation under non-stress and osmotic stress conditions.

MATERIALS AND METHODS

Processing of soil, pot, glass vessel and tray for laboratory and net house experiments

The soil of rice field was collected up to 30 cm depth and mixed thoroughly, dried under sun, added with farm yard manure (FYM) ca. 5 t/ha, powdered, sieved to 200 mesh size, sterilized at 121ºC for 30 min for 3d consecutively and used for the experiments. Portions of rice field and FYM mixed soils were used for soil analysis. The plastic pots, glass vessels and trays were washed with 1% teepol followed by sterile water to remove traces of detergent, finally with 50% (w/v) bleaching powder and dried under sun. Inner wall of dried pots were washed with 70% formaldehyde, air dried and used for the experiments.

For evaluation of the bacteria in the net house under normal condition, the pots (20 cm top dia. x 20 cm h) were filled with 4 kg sterile soil keeping 5 cm top space free and filled with water. After 3d, 5 g soil sample from each pot was collected and the composite soil was used for physico-chemical analysis. The glass vessels (25 × 9 cm dia. x h) were filled with sterile soil keeping 5 cm top space free. For seedling production in net house, the trays (60 x 45 x 10 cm l x w x h) were filled with 5 cm deep layer of sterile soil and seeded the pre-soaked seeds.

Processing of perforated pots for salinity tolerance evaluation in the net house

The experiment was designed (Chattopadhyay *et al*., 2017, 2018) in perforated (1 mm dia. at 2 × 2 cm spacing) bottom plastic pots (12 cm top dia. x 12 cm h) internally lined with compact nylon mess (to prevent soil loss), 20% (v/v) bottom filled successively one layer each with 10-15, 6-8 and 2-3 mm dia. gravel followed by 1.5 kg sand and soil (containing ca. 5t/ha FYM) mixture (1:1 by wt.), autoclaved at 121ºC, 1h, 3d consecutively). The bottom of the pots were dipped in NaCl solution of desired salinity, allowed to percolate into pot soil and saturated to attain the desired EC at the top. A piezometer (finely porous tube) was fitted at one side of the pots to monitor pot salinity by handheld EC and pH meters.

Processing of salinity tank for salinity tolerance evaluation

Under the shed of transparent polycarbonate sheet (>80% light transmission), the concrete salinity tanks of 20 x 2 x 1 m lbh sizes fitted with PVC (poly vinyl chloride) delivery tubes along the bottom of the walls and cross connected with lateral perforated tubes (1 mm dia., 2 × 2 cm spacing) covered with compact nylon sleeves to avoid clogging of the holes were used for evaluation (Chattopadhyay *et al*., 2017). The tank bottom was filled (15-20 cm) with acid washed gravels (6-8 mm dia.) covering up to delivery tubes followed by acid washed coarse sand (10 cm) and 50 cm loose dry soil, sprinkled with water and compacted to ~1.25 Mg/m^3 average bulk density. The soil samples were collected from 3 tanks for physico-chemical analysis. Tank soil was salinized by releasing saline water of desired EC at 3-4d intervals from an overhead tank, allowed to saturate to obtain desired EC of top soil and sprinkled with water time to time to avoid evaporation loss.

Physico-chemical characters of soil of rice field, pot and salinity tank experiments

Physico-chemical characters of the rice field (without FYM), pot and salinity tank soils were analyzed following standard procedures (Gupta, 2004) through State Soil Testing Laboratory, Bhubaneswar, Odisha, India.

Processing of seeds for germination and seedling growth evaluation in petridish in laboratory and trays in net house

The rice cv. Naveen (NV) and Luna Sankhi (LS) seeds were suspended in distilled water; sedimented seeds (*i.e*. healthy filled grains) were collected, washed thoroughly in tap water followed by 3 times in sterile distilled water, surface sterilized for 5 min each in 70% ethanol followed by 0.2% mercuric chloride solution and washed 5 times with sterilized distilled water.

The seeds were soaked overnight in sterile distilled water for initiation of germination, spread in sterile (autoclaved) petridishes lined with a sterile water soaked filter paper, maintained in darkness for 2d for plume emergence followed by under two fluorescent tube lights of 2100 Lux light intensity for 12h photoperiod at 28 ± 2°C for 15d, periodically moistened the paper with sterile distilled water (Nakbanpote *et al*., 2014, Ng *et al*., 2012). For seedling production in net house, soil layered (5 cm) trays were moistened with sterile water with a hand sprayer, 12h pre-soaked seeds were broadcasted, covered with a thin layer of moist sterile soil and periodically watered by spraying sterile distilled water avoiding stagnation, grown for 15d under sun and used for evaluation.

Processing of osmotolerant phytonic plant growth promoting bacteria for the experiments

Out of 59 ecto- and edophytic i.e. rhizospheric, rhizoplanic, endorhyzic, phyllospheric, phylloplanic, endophyllic and endocaulic PGPB of three rice (*Oryza spp*. L., Poaceae) cultivars *i.e. O. sativa* cv. Naveen (NV, salt sensitive), Swarna Sub-1 (SS, submergence tolerant, salt sensitive), Luna Sankhi (LS, moderate salt tolerant, 6-8 dS/m, 15-21d at seedling stage), and two wild rices *i.e. O. latifolia* Desv .(OL, salt tolerant, 17.5 dS/m, 26d seedling stage) and *O. alta* Swallen (OA, salt tolerant, 17.5 dS/m, 33d at seedling stage), selected 5 most prospective osmotolerant PGPB and evaluated for growth promotion of NV and LS without/with salinity stress in the laboratory, net house and salinity tanks. The prospective organisms *viz*. LS rhizoplanic *Salinicola* sp. (S36) possessing 12% NaCl and sea salt tolerance; PGP functions like 1-aminocyclopropane-1-carboxylate deaminase (ACCD), inorganic phosphate (IP, calcium phosphate), organic phosphate (OP, phytate), ammonia, IAA and siderophore metabolism under non-stress condition;IP/OP/IAA/siderophore/ACCD/ammonia metabolism under 6% NaCl stress; OP/IAA/ammonia/siderophore metabolism with CO_2 stress and biocidal lipase activity; LS rhizoplanic *Bacillus* sp. (SV4) having 14 and 16% NaCl and sea salt tolerance; IP/OP/ammonia/ACCD/siderophore metabolism under normal condition; siderophore (CO_2, 40ºC), ACCD/ammonia (6% NaCl) metabolism under stress and antimicrobial glycosidase, protease, pectinase, lipase, cellulase production; NV rhizoplanic *Pseudomonas* sp. (NR4) with 10 and 12% NaCl and sea salt tolerance; IP/OP/ammonia/ACCD/siderophore/IAA metabolis /N_2 fixation under normal condition; metabolism of IP at 40ºC, phytate under CO_2/40ºC

stress, IAA under NaCl (6%)/CO2/40°C challenge, ammonia with 6% NaCl/CO_2 stress and protease/cellulase production; SS phyllospheric *Bacillus* sp. (W1) with 14 and 16% NaCl and sea salt tolerance; IP/ammonia/ACCD/siderophore metabolism /N_2 fixation at stress-free situation; siderophore (CO_2) and ammonia (6% NaCl) metabolism under stress and biocidal glycosidase/protease/pectinase/lipase/ cellulase enzymes producers; and OL rhizoplanic *Enterobacter* sp. (RP8) having 10% NaCl/sea salt endurance; IP/OP/ammonia/ACCD/ siderophore/IAA metabolism at stress free condition; metabolism of IP (6% NaCl), IAA (6% NaCl, CO_2, 40ºC), siderophore (CO_2) under stress and biocidal lipase enzyme producers were used. The bacteria were grown in nutrient broth (NB, composing (g/l) peptone 5.0, beef extract 3.0, NaCl 3.0, pH 7.0) at 100 rpm in an environmental shaker for 48h at 28 ± 0.1ºC. The cultures were either used directly or centrifuged at 10000g for 5 min at 4 ± 0.1ºC, washed 3 times with sterile distilled water by centrifugation and the bacterial pellet was suspended in sterile distilled water. If required, the bacterial populations in NB or washed bacteria suspensions were adjusted with water to ~10^8 bacteria/ml (A600 0.5) for experimentation.

Assessment of 5 potent bacteria against rice cv. Naveen for seed germination and seedling growth in petriplate in laboratory

PGP effects of the bacteria were evaluated using pre-soaked seeds of cv. Naveen (NV) by seed germination (%) under normal conditions in petriplates in the laboratory. The seeds were inoculated by soaking separately with the five (S36, NR4, SV4, W1 and RP8) potent bacterial suspensions (10^8 cfu/ml as per Bureau of Indian Standards (BIS) recommendation) for 6h at 28 ± 2ºC and air-dried for 2h. Bacterized 10 seeds each (3 replications) were taken in separate petridishes lined with a sterile moist Whatman no.1 filter paper. The petridishes were incubated at 28 ± 2ºC for 5d but 2d under darkness and 3d under fluorescent light (mentioned elsewhere) with 12h light-dark cycle in the laboratory. Number of germinated seeds, and plumule and radical lengths were recorded after 5d. All seedlings were blotted to dryness within the filter papers and average fr. wt. (g/seedling) was recorded. The seedlings were air dried under shed for 12h followed by in an oven at 65 ± 0.2ºC to attain constant wt. and average dr. wt. (g/seedling) was noted (Nakbanpote *et al*., 2014) .

Evaluation of 5 potent bacteria for growth of rice cv. Naveen seedlings grown in glass vessel in the laboratory

The 5d old Naveen seedlings grown in trays in the net house were washed thoroughly with sterile distilled water, roots were separately dipped 6h in the potent bacterial (NR4, W1, SV4, S36 and RP8) suspensions (10^8 cfu/ml as per BIS), excess inoculum was drained off and dried under fan for 2h. In each vessel (25 × 9 cm h × dia.) one bacteria primed seedling was planted with 3 replications. The vessels were watered with sterile water keeping 5 cm water stand and maintained under fluorescent light (mentioned elsewhere) with 12h light-dark cycle. After 15d growth in vessel, growth parameters *viz*. shoot length, root length, fresh and dry wt. of shoot and roots were recorded and analyzed to reveal the effects of the bacteria on growth of the NV seedlings (Dash and Dangar, 2019).

Evaluation of S36 and NR4 for growth of rice cv. Luna Sankhi seedlings along with different fertilizer doses in pots in net house

Healthy, 15d old tray grown rice (cv. Luna Sankhi, LS) seedlings were dipped separately in bacterial suspensions (1.2 x 10^8 cells/ml) for 6h as per BIS, drained off excess inocula, air dried for 2h and transplanted 3 seedlings/pot (20 cm top dia. x 10 cm h) each with three replications *viz*. T_1: control without any fertilizer and seedlings soaked in sterile water, T_2: seedlings initially treated with sterile water and received recommended dose of fertilizers (RDF) (120:60:60 mg N:P:K/kg soil), T_3: seedlings received half P (as the bacteria were P solubilizers) and recommended doses of other fertilizers (N:1/2P:K 120:30:60 mg/kg soil), T_4: seedlings treated with 1.2 x 10^8 cells/ml *Salinicola* sp. (S36), T_5: seedlings treated with 1.2 x 10^8 cells/ml *Salinicola* sp. (S36) and N:1/2P:K fertilizers (120:30:60 mg/kg soil), T_6: seedlings treated with 1.2 x 10^8 cells/ml *Pseudomonas* sp. (NR4), and T_7: seedlings treated with 1.2 x 108 cells/ml *Pseudomonas* sp. (NR4) and N:1/2P:K fertilizers (120:30:60 mg/kg soil). The plants were grown in the net house under solar radiation with daily maximum photosynthetic photon flux density, air temperature and relative humidity (about 1660 μM (m^2/s), 32.6ºC and 70 to 75%, respectively). The pots were watered with autoclaved RO (reverse osmosis) water to maintain 3-5 cm of standing water up to harvest stage (Dash and Dangar, 2019).

Growth parameters *viz*. plant height (cm/plant) and tiller/hill (no./plant *i.e.* hill) were recorded at panicle initiation (PI) stage after 50d growth *i.e.* 30d after transplant (DAT); prior to harvest (110d) plant height (cm/plant), panicle length (cm/panicle) and leaf area (sq. cm/leaf) were measured and postharvest observations like shoot fr. wt. (g/plant) and dr. wt. (g/plant); root length (cm/plant), fr. wt. (g/plant) and dr. wt. (g/plant); total leaves (no./plant), panicle weight (g/panicle) and 100 grain wt. (g) were recorded after harvest (110d),and plant height (cm) was measured from the soil surface up to the tip of the top most shoot each of 3 sampling hills and averaged. Height (cm) at maturity was recorded from the soil surface up to the tip of the top most panicle. Total tillers (no.) in each pot were counted and average tillers per plant (hill) were recorded. Length of 10 panicles (arbitrarily selected) was measured (cm) from the neck node to the tip of the top most grain and averaged. Three plants from each treatment were uprooted by loosening the soil and without damaging the roots. The roots were washed thoroughly, length was measured (cm) and averaged, then the roots were kept in oven (65 ± 2ºC) to attain a constant dr. wt. (g). The leaves were separated, dried and leaf area were measured through Licor-300 leaf area meter and average leaf area (sq. cm) was noted. Gain yield (g) was recorded from the harvest of each pot, dried to 14% moisture content and obtained average of 100 seed weight (g).

Evaluation of the most promising bacteria S36 and NR4 for seed germination and seedling growth of rice cv. Luna Sankhi grown in petriplate in the laboratory under saline condition

The pre-soaked LS seeds were bacterized by dipping separately in each bacterial suspension (10^8 cfu/ml as per BIS) for 6h at 28 ± 2ºC. Batches of treated seeds (10 each) were incubated separately in sterile petridishes lined with a filter paper containing 10 ml sterile 0.21%, 0.43% and 0.87% (w/v) NaCl (4, 8, 16 dS/m EC *i.e.* Eh) along with sole S36 and NR4

challenged, and only pre-soaked seeds in sterile deionized water. The petriplates were arranged in a complete randomized block design with three replications, maintained 2d in darkness and 5d under fluorescent light (mentioned elsewhere). Number of germinated seeds was counted after 7d, germination rate (%), radical length (cm/seedling), plumule length (cm/seedling), fr. wt. (g/seedling), dr. wt. (g/seedling), lateral root (no./seedling) were recorded and analyzed (Nakbanpote *et al*., 2014).

Evaluation of S36 (*Salinicola* sp.) and NR4 (*Pseudomonas* sp.) for growth of Luna Sankhi seedlings in perforated pots under salt stress condition in the net house

Pre-soaked LS (seedling tolerance 6-8 dS/m for 15-21d) seeds were dipped separately in bacterial suspensions (1.2 x 10^8 cells/ml as per BIS) for 6h, air dried for 2h under fan and sowed 10 seeds in each perforated bottom pots containing moist soil without any fertilizer and watered with sterile RO water. After 15d of germination, pots (3 replications) were placed on perforated plastic platforms kept in deep trays containing 4 lit (or as required) sterile distilled water (control) submerging up to 5 cm basal part and another set (3 replications) in 4 lit (or as required) sodium chloride solution (8 dS/m). Water level of the trays were maintained with sterile deionized distilled water and the pots were maintained under solar radiation (mentioned elsewhere) in net house. Growth parameters like tillers (no./plant), root length (cm/plant), leaves (no./plant) leaf area (cm^2/leaf measured by leaf area meter), fr. wt. of root (g/plant), dr. wt. root (g/plant), fr. wt. shoot (g/plant), dr. wt. shoot (g/plant) and plant height (cm/plant) were recorded after 30d (15 DAT) growth (Nakbanpote *et al*., 2014, Chattopadhyay *et al*., 2018).

Effect of the most potent bacteria S36 on growth of rice cv. Luna Sankhi seedlings under enhanced salinity (12 dS/m) in saline tanks

Pre-soaked LS seeds were bacterized in S36 bacterial suspensions (1.2 x 10^8 cells/ml as per BIS) for 6h along with control (water without bacteria), air dried for 2h and planted the bioprimed seeds with 15 × 10 cm (column × rows) spacing in the salinity test tanks containing moist soil. The established (15d) plants were allowed for salinization of desired EC (8dS/m) for 2d followed by 12dS/m from the reservoirs into the soil tank. On 30d growth parameters *viz*. plant height (cm/plant), root length (cm/plant), green leaves (no./plant), affected (dry) shoot (no./plant), shoot area (cm^2/shoot), no. of tiller/plant, fr. wt. of shoot (g/plant), dr. wt. of shoot (g/plant), fresh wt. of root (g/plant) and dr. wt. of root (g/plant) were recorded (Chattopadhyay *et al*., 2018).

Effects of S36 *ect*C gene cloned *E. coli* DH5α on seed germination and seedling growth of cv. Luna Sankhi in petriplates under 0.21% NaCl stress

The *ect*C gene was successfully cloned in *E. coli* DH5*a*; salt tolerance limit of the donor (S36), recipient (*E. coli* DH5*a*) and the clones was evaluated by growing them with different NaCl concentration (1-12% considering 12% tolerance of S36) in petridish in the laboratory and three positive clones (no. 3, 5, 7) with enhanced osmotolerance than the recipient mother bacteria were assessed. Effects of the clones on improvement of osmotolerance of Luna Sankhi were evaluated in petridish in the laboratory (Dash and Dangar, 2019). Pre-soaked Luna Sankhi seeds were bioprimed in S36, *E. coli* DH5*a* and 3 (no. 3, 5 and 7) cloned bacterial suspensions (1.12 x 10^8 cells/ml as per BIS) for 6h. In 0.21% NaCl (10 ml) containing filter paper lined petridishes, 10 bacteria-primed seeds were incubated along with 0.21% NaCl control (non-primed seed) and maintained for 2d in darkness and followed by 5d under fluorescent light (mentioned elsewhere). Seed germination, radical length (cm/seedling), shoot length (cm/seedling), secondary root (no./seedling), dr. wt. (g/seedling) and fr. wt. (g/seedling) were recorded and analyzed.

RESULTS AND DISCUSSION

Physico-chemical properties of soils used in the experiments

Physical properties *i.e.* pH and EC (Table 1) of the field, pot and salinity tank soils were comparable which suggest that FYM had no effect on them but customarily improved NPK levels in pot and tank soils compared to field soils (Table 1). However, as field soil was dry but pot/tank soils were collected

Table 1: Physicochemical properties of experimental field, pot and salinity tank soils

Soil parameters	Field soil	Pot soil	Salinity tank soil
Type and texture	Sandy-clayey loam	Sandy-clayey loam	Sandy-clayey loam
pH	6.03 ± 2.20	6.53 ± 2.20	5.90 ± 0.34
EC (dS/m)	0.46 ± 0.25	0.59 ± 0.34	0.64 ± 0.44
Total organic C (%)	1.56 ± 0.45	1.04 ± 0.43	1.18 ± 0.38
Available N (kg/ha)	314.85 ± 6.98	470 ± 0.02	560.72 ± 0.03
Available P (kg/ha)	3.00 ± 1.03	9.00 ± 2.24	6.00 ± 2.88
Available K (kg/ha)	129.06 ± 2.11	166.90 ± 2.26	138.30 ± 2.26

Results are mean of 3 replications ± SE prior to treatment imposition.

Figure 1A: Germination(5d) of rice cv.Naveen seed treated with potent isolates S36,NR 4,SV4,W1,RP8 in petriplate

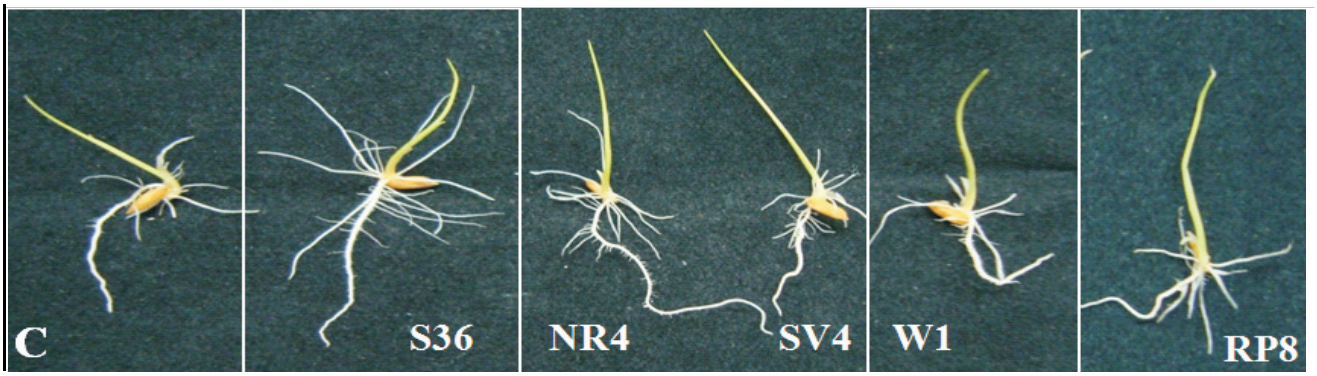

Figure 1B: Growth (5d) of isolates rice cv.Naveen seedings under control and treatment with potent isolates S36,NR4,SV4,W1,RP8

Table 2: Effect of potent bacteria on germination and growth of rice cv. Naveen seed treated in laboratory in petriplate

Growth	Bacterial treatment						LSD	CV (%)	P-value
parameter (5d)	Control	S36	NR4	SV4	W1	RP8	-5%		
Seed	30	30	30	30	30	30	0	0	0.0000***
germination (%)	(100)	(100)	(100)	(100)	(100)	(100)			
Radical length (cm/seedling)	4.2	7.21	8.58	4.2	4.03	5.9	1.16	5.5	0.0033**
	(100)	(171.67)	(204.29)	(100)	(95.95)	(140.48)			
Plumule length (cm/seedling)	3.81	5.4	5.52	4.61	4.12	4.6	0.18	4.6	0.0011***
	(100)	(141.73)	(144.88)	(121)	(108.14)	(120.73)			
Secondary root (no./seedling)	3.1	6.4	7.1	4.1	4.1	4.2	0.14	4.5	0.0201*
	(100)	(206.45)	(229.32)	(132.26)	(132.26)	135.48)			
Fresh wt. (g/seedling)	0.056	0.066	0.064	0.058	0.058	0.059	0.001	6.2	0.0001***
	(100)	(117.86)	(114.29)	(103.57)	(103.57)	(105.38)			
Dry wt. (g/seedling)	0.013	0.02	0.019	0.012	0.011	0.014	0.001	5.8	0.0001***
	(100)	(153.85)	(146.15)	(92.31)	(84.62)	(107.69)			

Total no. of seeds per test was 30. S36 = *Halomonas* sp., NR4 = *Pseudomonas* sp., W1 = *Bacillus* sp., SV4 = *Bacillus* sp., RP8 = *Enterobacter* sp. Values are means of three replications each of 15 seedlings. Data recorded after 5d. Data in parentheses are percent values. LSD = least significant difference, CV = coefficient of variance. Level of significance = *p < 0.05, **p < 0.01, ***p < 0.001.

Table 3: Effect of the potent bacteria on growth of rice cv. Naveen seedling grown in glass vessel in the laboratory

Growth parameters	Control	S36	NR4	W1	SV4	RP8	LSD (5%)	CV (%)	P-value
				Bacterial treatment					
SL (cm/plant)	23.16	29.1	27.86	25.93	26.33	27.3	2.05	8.4	0.102
	(100)	(125.48)	(120.29)	(111.96)	(113.69)	(117.88)			
RL (cm/plant)	7.63	11.03	10.03	8.43	8.33	9.13	1.67	16.1	0.139
	(100)	(144.53)	(131.45)	(110.48)	(109.17)	(119.66)			
Fr. wt. of shoot (g/plant)	0.17	0.3	0.3	0.24	0.21	0.26	0.09	19.9	0.052*
	(100)	(176.47)	(176.47)	(141.18)	(123.53)	(152.94)			
Dr. wt. of shoot (g/plant)	0.02	0.09	0.07	0.03	0.05	0.05	0.01	15.1	0.013**
	(100)	(450)	(350)	(150)	(250)	(250)			
Fr. wt. of root (g/plant)	0.06	0.12	0.08	0.09	0.08	0.07	0.07	17.1	0.017*
	(100)	(200)	(133.33)	(150)	(133.33)	(116.67)			
Dr. wt. of root (g/plant)	0.01	0.03	0.02	0.03	0.02	0.02	0.01	12.9	0.013**
	(100)	(300)	(200)	(300)	(200)	(200)			

RL = Root length, SL = Shoot length, Values are means of three replications each of 3 seedlings after 15d growth. S36 = *Halomonas* sp., NR4 = *Pseudomonas* sp., W1 = *Bacillus* sp., SV4 = *Bacillus* sp., RP8 = *Enterobacter* sp. Data in parentheses are percent values. LSD = least significant difference. CV = coefficient of variance. Level of significance = *p < 0.05, **p < 0.01, ***p < 0.001.

after 3d watering more native microbial growth would utilize more available C than former and reduced C content in wet soil. However, salinity would inhibit growth of salt sensitive resident microbes, thereby, reduce C utilization which would be reasoned for nominally higher C levels in saline tank than pot soil. Available N/P/K in saline tank would be lesser than pot soil as salinity reduces nutrient availability in soil (Shrivastava and Kumar, 2015).

Manifestations of seed germination and seedling growth of Naveen bioprimed by the bacteria and grown in petriplate (5d) and glass vessel (15d) without stress in the laboratory

Effects of the 5 salt tolerant P mineralizing PGPB (S36, NR4, W1, SV4, RP8) on seed germination and initial seedling growth (5d) of Naveen (NV) in plate culture in the laboratory (Table 2, Fig. 1A,B) resulted in 100% seed germination for both bacterized and control treatments but the bacteria differentially improved (other than radical length for W1 and seedling dr. wt. for SV4 and W1) overall growth (radical length 95.95-204.29%, plumule length 108.14-144.88%, root no. 132.26-229.32%, seedling fr. wt. 103.57-117.86%, seedling dr. wt. 84.62-153.85%) of the seedlings over control seeds. Radical elongation (cm/seedling) was more for *Salinicola* sp. S36 (7.21), *Pseudomonas* sp. NR4 (8.58) and *Enterobacter* sp. RP8 (5.90) treatments over control (4.20) which was comparable to

Table 4: Effects of treatment of the potent bacteria S36 and NR4 on growth of rice cv. Luna Sankhi seedlings grown along with different fertilizer doses in pots in net house

Growth parameters	Treatment							LSD	CV	P-value
	T_1	T_2	T_3	T_4	T_5	T_6	T_7	(5%)	(%)	
Plant height at PI (cm/plant)	51.97	60.69	59.96	55.35	57.83	52.71	60.02	1.14	3.3	0.0000***
	(100)	(116.78)	(115.37)	(106.5)	(111.28)	(101.42)	(115.49)			
Tillers at PI (no./plant)	4.66	5.66	6	5	6.66	5	6.33	0.72	7.3	0.0005***
	(100)	(121.46)	(128.76)	(107.3)	(142.92)	(107.3)	(135.84)			
Plant height at harvest (cm/plant)	68	76.66	77.18	72.66	76	65.66	75.23	2.61	4.3	0.0039**
	(100)	(112.74)	(113.4)	(106.85)	(111.76)	(96.59)	(110.32)			
Fresh wt. of shoot at harvest (g/plant)	14.35	22.73	28.09	18.017	29.93	16	24.31	3.99	12.6	0.0001***
	(100)	(158.39)	(195.75)	(125.51)	(208.57)	(111.5)	(169.41)			
Dry wt. of shoot at harvest (g/plant)	4.79	8.33	8.71	5.64	10.03	5.34	8.22	1.89	14.6	0.0004***
	(100)	(173.9)	(181.84)	(117.75)	(209.39)	(111.48)	(171.61)			
Root length at harvest (cm/plant)	32.91	36.82	34.64	33.63	37.13	33.44	34.68	1.16	6	0.0061**
	(100)	(111.88)	(105.25)	(102.18)	(112.82)	(100.69)	(105.37)			
Fresh wt. of root (g/plant)	17.7	25.54	22.24	18.9	28.17	17.93	24.69	1.82	14.8	0.0106**
	(100)	(144.29)	(125.65)	(106.78)	(159.15)	(101.23)	(139.49)			
Dry wt. of root at harvest (g/plant)	4.54	8.3	6.05	5.11	7.32	5.24	8.26	1.01	23.8	0.0445*
	(100)	(183.45)	(133.26)	(112.56)	(161.23)	(115.41)	(181.94)			
Leaves at harvest (no./plant)	19	24.66	25.33	20.66	30	20.66	31.33	2.66	6.1	0.0000***
	(100)	(129.79)	(133.32)	(108.74)	(157.89)	(108.74)	(164.89)			
Leaf area at harvest (cm^2/leaf)	14.8	21.86	20.82	18.91	22.39	15.47	20.88	2.44	7.1	0.0001***
	(100)	(147.7)	(140.67)	(127.77)	(151.28)	(104.53)	(141.08)			
Panicle length at harvest (cm/panicle)	19.53	20.6	20.23	18.86	21.13	18.86	20.18	1.9	5.4	0.1536
	(100)	(105.48)	(103.58)	(96.57)	(108.19)	(96.57)	(103.33)			
Panicle wt. at harvest (g/plant)	5.47	8.47	7.78	6.62	9.38	6.24	8.45	1.59	12	0.0018***
	(100)	(154.45)	(142.23)	(121.02)	(171.48)	(114.08)	(154.48)			
100 grain wt. at harvest (g)	1.62	2.39	2.25	1.77	2.81	1.77	2.44	0.25	6.6	0.0000***
	(100)	(147.53)	(138.89)	(109.26)	(173.46)	(109.26)	(105.62)			

PI = Panicle initiation stage. T_1 = Control, T_2 = Recommended fertilizer dose (RFD) of N:P:K, T_3 = N:1/2P:K, T_4 = Sole S36, T_5 = S36 + N:1/2P:K, T_6 = Sole NR4, T_7 = NR4 + N:1/2P:K. Data in the parentheses are percent values. LSD = least significant difference. CV = coefficient of variance. Level of significance = *p < 0.05, **p < 0.01, ***p < 0.001.

Table 5: Effects of treatment of the potent bacteria S36 and NR4 on germination and growth of rice cv. Luna Sankhi seeds grown in petriplate in the laboratory under saline condition

Treatment (7d)	Seed germi nation (%)	Radical length (cm/ seedling)	Plumule length (cm/ seedling)	Fr. wt. (g/seedling)	Dr. wt. (g/seedling)	Lateral root (no./seedling)
Distilled water	30 (100.00)	5.08 (100)	3.82 (100)	0.069 (100)	0.019 (100)	4.00 (100)
NaCl (0.21%)	30 (100.00)	3.21 (63.19)	3.44 (90.05)	0.064 (92.75)	0.018 (94.74)	1.66 (41.50)
NaCl (0.43%)	24 (80.00)	1.94 (38.19)	2.67 (69.90)	0.05 (72.46)	0.016 (84.21)	0
NaCl (0.87%)	14 (46.66)	0.59 (11.61)	1.19 (31.15)	0.037 (53.62)	0.016 (84.21)	0
S36	30 (100.00)	5.20 (102.36)	4.32 (113.09)	0.094 (136.23)	0.042 (221.05)	9.33 (233.25)
S36 + NaCl (0.21%)	30 (100.00)	3.48 (68.50)	3.66 (95.81)	0.074 (107.25)	0.025 (131.58)	8.00 (200.00)
S36 + NaCl (0.43%)	30 (100.00)	2.03 (39.96)	3.36 (87.99)	0.057 (82.61)	0.019 (100.00)	3.33 (83.25)
S36 + NaCl (0.87%)	26 (86.66)	1.19 (23.43)	1.78 (4.60)	0.041 (59.42)	0.018 (94.74)	0
NR4	30 (100.00)	5.29 (104.13)	4.30 (112.57)	0.091 (131.88)	0.041 (215.89)	8.33 (208.25)
NR4 + NaCl (0.21%)	30 (100.00)	3.41 (67.13)	3.57 (93.46)	0.067 (97.10)	0.021 (110.53)	5.66 (141.50)
NR4 + NaCl (0.43%)	28 (93.33)	1.98 (38.98)	2.78 (72.77)	0.055 (79.71)	0.018 (94.74)	0
NR4 + NaCl (0.87%)	22 (73.33)	0.48 (9.45)	0.75 (19.63)	0.038 (55.07)	0.014 (73.68)	0
LSD	6.87	1.09	0.96	0.02	0.01	2.02
CV (%)	3.45	5.09	13.78	7.45	10.16	21.21
P-value	0.32	0.028*	0.023*	0.002**	0.001**	1.98

Total no. of seeds per test was 30. Values are means of three replications each of 15 seedlings. Data in parentheses are percent values. LSD = least significant difference. CV = coefficient of variance. Level of significance = *p < 0.05, **p < 0.01, ***p < 0.001

Bacillus spp. (SV4 = 4.20 and W1 = 4.03) treatments. Plumule growth (cm/seedling) for *Pseudomonas* sp. NR4 (5.52), *Salinicola* sp. S36 (5.40), *Enterobacter* sp. RP8 (4.60) and *Bacillus* sp. SV4 (4.61) and W1 (4.12) was more than control (3.81). Moreover, *Salinicola* sp. S36 (n = 6.40), *Pseudomonas* sp. NR4 (n = 7.10) and SV4/W1/RP8 (n = ~ 4) effected more secondary root emergence compared to control (n = 3.10). S36 (0.066) and RP8 (0.064) augmented fr. wt. (g/seedling) of rice seedlings better than other bacteria (~0.058) which was comparable to that of control (0.056). Dry wt. (g/seedling) was also more for S36 (0.020) and NR4 (0.019) treatments than other 3 isolates (0.011-0.014) which was comparable to control (0.013). Broadly, effects of S36 and NR4 on NV germination and growth did not differ significantly but NR4 was grossly more effective up to 5d seedling growth.

The 5 bacteria treated Naveen seedlings (15d old) grown in glass vessels (15 DAT) in the laboratory (Table 3, Fig. 2A,B) improved shoot and root lengths (cm/plant) *viz*. *Salinicola* sp. S36 (29.1, 11.03), *Pseudomonas* sp. NR4 (27.86, 10.03), Bacillus sp. W1 (25.93, 8.43), *Bacillus* sp. SV4 (26.33, 8.33) and *Enterobacter* sp. RP8 (27.3, 9.13) more than control (23.16, 7.63). Enhancement of fr. (0.21-0.30 g/plant) and dr.

Table 6: Effects of treatment of the potent bacteria S36 and NR4 on growth of rice cv. Luna Sankhi grown in perforated pots under NaCl (8 dS/m) osmoticum

Growth parameters after	Treatment						LSD	CV (%)	p-value
15d shock	T_1	T_2	T_3	T_4	T_5	T_6	(5%)		
Plant height (cm/plant)	40.03	37.35	44.85	41.34	45.26	42.14	1.64	2.2	0.0000***
	(100)	(93.33)	(112.04)	(103.27)	(113.07)	(105.27)			
Root length (cm/plant)	18.05	15.97	20.79	18.35	22.4	19.78	1.34	3.9	0.0000***
	(100)	(88.48)	(115.18)	(101.66)	(124.1)	(109.58)			
Leaves (no./plant)	7.33	5.66	9.33	7.66	10.33	8.33	1.08	7.4	0.0001***
	(100)	(77.22)	(127.29)	(104.5)	(140.92)	(113.64)			
Leaf area (cm^2/leaf)	18.89	17.73	23.21	18.9	22.46	20.36	2.48	6.8	0.0037**
	(100)	(93.86)	(122.87)	(100)	(118.92)	(107.78)			
Fresh wt. of root (g/plant)	2.03	1.5	2.68	2.53	3.94	3.45	0.68	14.2	0.0002***
	(100)	(73.89)	(132.02)	(124.63)	(194.09)	(169.95)			
Dry wt. of root (g/plant)	0.2	0.18	0.33	0.31	0.44	0.37	0.05	17.1	0.0010***
	(100)	(90)	(165)	(155)	(220)	(185)			
Fresh wt. of shoot (g/plant)	2.47	2.1	3.75	3.23	4.94	4.11	0.61	10.2	0.0004***
	(100)	(85.02)	(151.82)	(130.77)	(200)	(166.4)			
Dry wt. of shoot (g/plant)	0.52	0.48	0.85	0.74	1.41	0.8	0.02	14.3	0.0000***
	(100)	(92.31)	(163.46)	(142.31)	(271.15)	(153.85)			
Tillers (No./plant)	2.66	2.66	4.33	4	4.33	4.33	1.1	19.4	0.0313*
	(100)	(100)	(162.78)	(150.37)	(162.78)	(162.78)			

T_1 = Control (distilled water), T_2 = NaCl 8dS/m, T_3 = NR4 without stress, T_4 = NR4 with NaCl 8dS/m, T_5 = S36 without stress, T_6 = S36 with NaCl 8dS/m. Data in parentheses are percentage (%) over control. Values are means of three replications. LSD = least significant difference, CV = coefficient of variance. Level of significance = *p < 0.05, **p < 0.01, ***p < 0.001.

Table 7: Effect of treatment of the potent bacterium S36 on growth of rice cv. Luna Sankhi grown in salinity (12 dS/m) tank

Growth parameters treatment	T_1	T_2	LSD (5%)	CV (%)	P-value
Plant height after 30d (cm/plant)	33.77 (100)	42.20 (124.96)	4.17	3.2	0.0102**
Root Length (cm/plant)	9.28 (100)	16.61 (178.99)	1.82	4.1	0.0023**
Green leaves (no./plant)	6.66 (100)	9.33 (140.09)	1.41	5.1	0.0120*
Affected (dry) leaf (No./plant)	4.33 (100)	2.66 (61.43)	1.74	23.9	0.2002
Leaf area (cm^2/leaf)	13.13 (100)	20.8 (158.42)	6.86	11.7	0.0388*
Tiller (no./plant)	2.33 (100)	4.33 (185.84)	0.44	21.2	0.0727
Fresh wt. of shoot (g/tiller)	1.35 (100)	3.11 (230.37)	0.39	4.5	0.0010***
Dry wt. of shoot (g/tiller)	0.26 (100)	0.71 (273.08)	0.12	7.3	0.0027**
Fresh wt. of root (g/tiller)	0.34 (100)	1.36 (400.00)	0.18	13	0.0054**
Dry wt. of root (g/tiller)	0.17 (100)	0.34 (200.00)	0.04	8.4	0.0082**

T_1 = Control (12 dS/m NaCl), T_2 = S36 (12 dS/m NaCl). Data in parentheses are percentage (%) over control. Values are means of three replications and data calculated per plant. LSD = least significant difference. CV = coefficient of variance. Level of significance *p < 0.05, **p < 0.01, ***p < 0.001

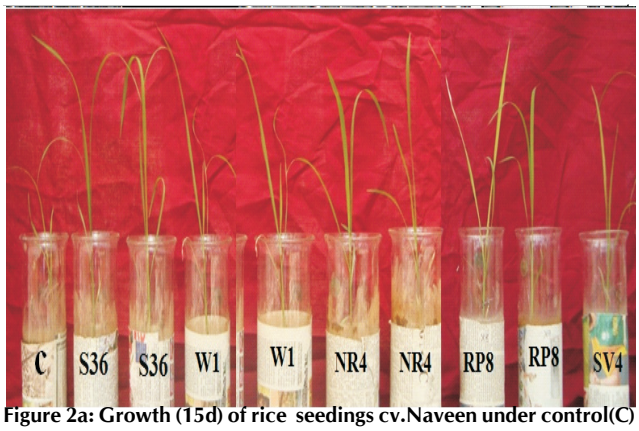

Figure 2a: Growth (15d) of rice seedings cv.Naveen under control(C) and treatment with S36,NR4,W1,RP8 and SV4 in glass vessel culture in laboratory

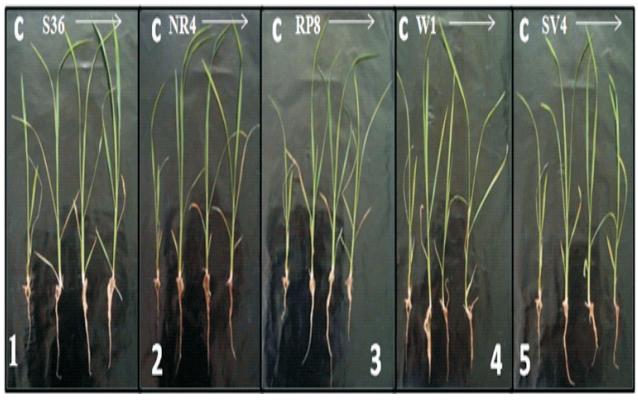

Figure 2B: comparative growth of cv.Naveen seedlings under1. control(C) and S36;2. Control (C) and NR4; 3. Control(C) and RP8;4.Control(c) and W1;5.Control(C) and SV4 and SV4 in glass vessel culture in laboratory

wt. (0.03-0.09 g/plant) of shoot were significantly more for bacteria treatment over bacteria-free plants (0.17 and 0.02 g/plant) but impacts on root growth were insignificant. Unlike plate culture (5d), seedling growth (15d) was better (but insignificant) for S36 than NR4.

Naveen seed germination was not affected by the 5 ecto-/endo-PGPB (Table 2, Fig. 1A,B) which contradicted more (87-93%) rice seed germination by P and IAA metabolizing rice rhizospheric *E. gergoviae* and *C. agropyri* (Ng *et al.*, 2012). However, enhancement of initial (5d) seedling growth by the PGPB over control; superiority of *Salinicola* sp. (S36) and *Pseudomonas* sp. (NR4) than other bacteria or control in

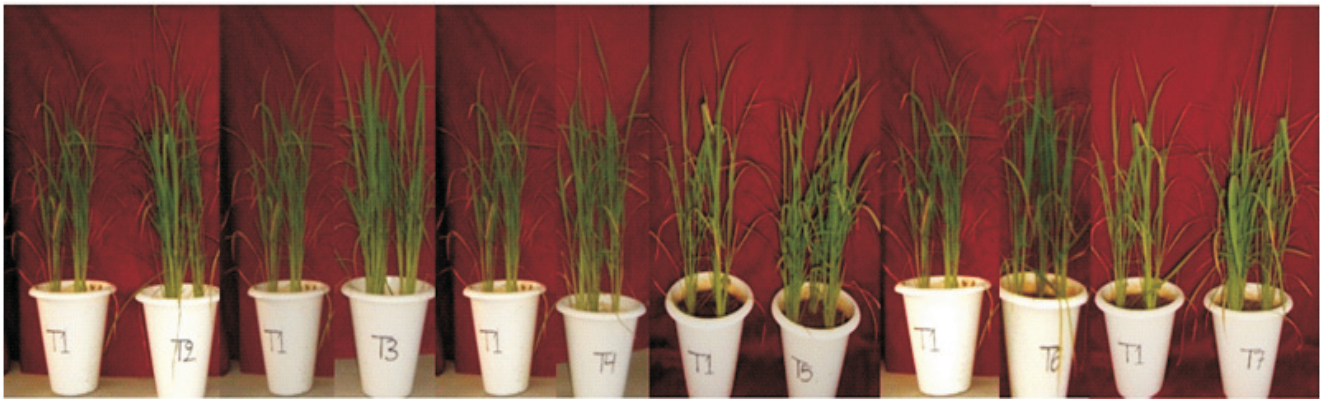

Figure3A: Vegetative growth of Luna Sankhi plant control and bacteria treatment along with different fertilizer doses.Each treatment effec t is compares with control(T1)

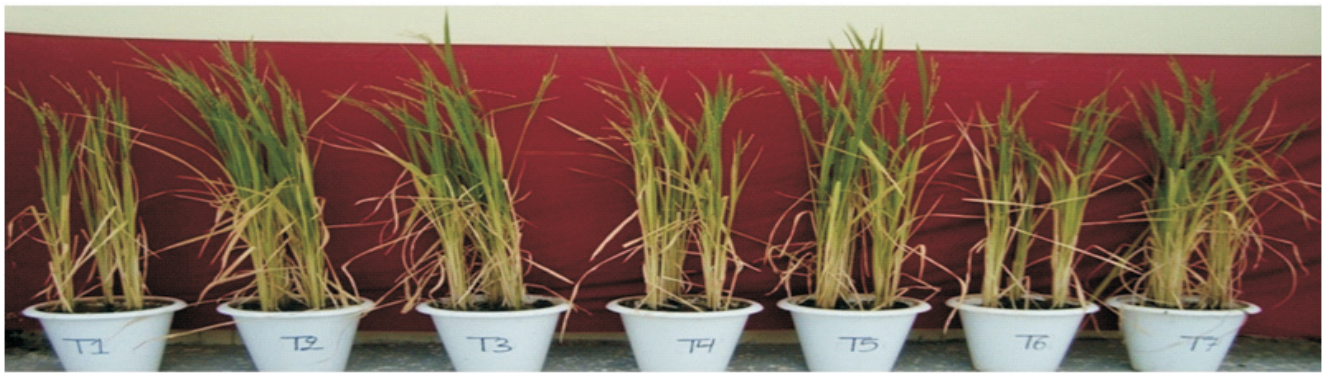

Figure3B: Panicle initiation stages of Luna Sankhi plant under control and bacteria treatment along with different fertilizer doses

Table 8: Validation of salt tolerance of S36 *ect*C clones of *E. coli* DH5α on NA containing NaCl

Organism	NaCl (%) tolerance
S36	12
E. coli DH5*á*	5
E. coli DH5*á* C3	7
E. coli DH5*á* C5	7
E. coli DH5*á* C7	6.5
CD, P = 0.05	0.09

petridish (Table 2, Fig. 1A,B), as well as, support of S36 and NR4 for more growth of the seedlings on 15 DAT (30d growth) in glass vessels (Table 3, Fig. 2A,B) proved that NR4 and S36 were superior PGPB of NV. Plant hormone (IAA, zeatin) and P metabolism by the bacteria (cf. materials and methods *i.e.* MM) might favour root/shoot growth and root formation of NV as recorded from other wild/cultivar/salt tolerant/sensitive/ drought tolerant/sensitive rice genotypes or plants in general by different ecto-/endo-PGPB (Ng *et al*., 2012, Pradhan and Mishra, 2015, Pahari *et al*., 2016, Walitang *et al*., 2017, Girsowicz *et al*., 2019, del Carmen Orozco-Mosqueda *et al*., 2020). Alike the 5 PGPB of present investigation, relative to control more growth of NV seedlings (5d) in petridish (radical length 207.69%, root no. 700%, plumule length 270%) and for 15d growth in glass vessel (root length 161%, shoot length 130.6%, root fr. wt. 250%, root dr. wt. 500%, shoot fr. wt. 164.71%, shoot dr. wt. 300%) by P metabolizing *Enterobacter* sp. were observed which were, however, better than those of the 5 PGPB of the present investigation (Dash and Dangar, 2019), However, S36 and NR4 were more effective for shoot and root growth (15d) of NV grown in glass vessel soil in the laboratory than E. gergoviae (42.43, 20.32%) and C. agropyri (28.47, 17.11%) (Ng *et al*., 2012). As NR4 was isolated from NV but S36 from LS, therefore, NR4 may have some co-evolutionary relation and might support better (although insignificant) plumule/radical growth (5d) of NV initially (Table 2, Fig. 1A,B) but S36 being superior PGPB it would surpass subsequent growth (15d) of the NV seedlings (Table 3, Fig. 2A,B). Thus, the results imply that the 5 bacteria, especially S36 and NR4 are promising PGPB for rice cultivation.

Improvement of growth of Luna Sankhi seedlings treated with S36 (*Salinicola* sp.) and NR4 (*Pseudomonas* sp.) along with different fertilizer doses in pot culture in the net house

The 2 most promising osmotolerant P solubilizing PGPB *viz*. S36 and NR4 (cf. materials and methods) combined with different fertilizer doses were assessed through growth and production of Luna Sankhi (LS, moderate salt tolerant) in pot culture in the net house (Table 4, Fig. 3A, B). Control plants without fertilizer (T_1) were most poor performers and sole S36 (T_4) or NR4 (T_6) treatments were better (although insignificant) than control but inferior to the remainder treatments. The recommended fertilizer doses (RFD) (T_2) supported better

Table 9 : Effects of *ect*C cloned E. coli DH5α on germination and growth of Luna Sankhi seedlings in 0.21% NaCl in petriplates

Growth parameter (5d)	Bacterial treatment along with 0.21% NaCl						LSD	CV (%)	P-value
	Control (0.21% NaCl)	S36	*E. coli* DH5α	*E. coli* DH5α Clone 3	*E. coli* DH5α Clone 5	*E. coli* DH5α Clone7			
Seed germination (%)	30 (100)	30 (100)	30 (100)	30 (100)	30 (100)	30 (100)	0 0	0.001**	
Root length (cm/seedling)	3.19 (100)	3.49 (109.4)	3.21 (100.63)	3.22 (100.94)	3.23 (101.25)	3.25 (101.88)	0.21	4.1	0.010***
Shoot length (cm/seedling)	3.4 (100)	3.64 (107.06)	3.58 (105.29)	3.6 (105.88)	3.88 (114.12)	4.05 (119.12)	0.23	4.4	0.011*
Secondary root (no./seedling)	1.63 (100)	7.92 (485.9)	1.74 (106.75)	2.09 (128.22)	2 (122.7)	1.9 (116.56)	0.23	6.5	0.020*
Fresh wt. (g/seedling)	0.061 (100)	0.072 (118.03)	0.06 (98.36)	0.063 (103.28)	0.066 (108.2)	0.065 (106.57)	0.021	5.6	0.003**
Dry wt. (g/seedling)	0.016 (100)	0.025 (156.25)	0.016 (100)	0.017 (107.25)	0.018 (112.5)	0.017 (107.25)	0.003	9	0.002**

Results are means of data of 10 seedlings. Data in parentheses are percentage (%) over control. LSD = least significant difference. CV = coefficient of variance. Level of significance *p < 0.05, **p < 0.01, ***p < 0.001.

(mostly) growth of LS seedlings than N:1/2P:K (T_3) and S36 + N:1/2P:K (T5) or NR4 + N:1/2P:K (T7) surpassed growth (except for plant height for S36; fr./dr. wt. of shoot, grain wt. for NR4) over N:1/2P:K (T3). Evidently, the results (%) of plant height 111.28 - 116.78 and tiller no. 121.46 - 142.92 at PI stage (30 DAT); root length 105.25 - 112.82, plant height 110.32 - 134.40, root fr. wt. 125.65 - 159.15, root dr. wt. 83.45 - 181.94, shoot fr. wt. 158.39 - 208.57, shoot dr. wt. 171.61- 209.39, leaf no. 129.79 - 164.89, leaf area 140.67 - 151.28, panicle length 103.58 - 108.19, panicle weight 142.23 - 171.48 and 100 grain wt. 105.62 - 173.46 for T_2, T_3, T_5 and T_7 were more than respective control (T_1) (100%). Nevertheless, gross results proved that S36 combination with N:1/2P:K (T5) treatment bettered most of the growth parameters (%) *viz*. root length (112.28), tiller no. (142.92), root fr. wt. (159.15), root dr. wt. (161.23), shoot fr. wt. (208.57), shoot dr. wt. (209.39), leaf no. (157.89), leaf area (151.28), panicle length (108.19), panicle wt. (171.48) and 100 grain wt. (173.46) than other treatments.

Similar to S36 and NR4, more improvement (%) of shoot (102.65-110.39)/root (106.82-134.09) elongation, root fr. (115.60-133.68)/dr. wt. (114.90-141.14), shoot fr. wt. (109.98-133.91), dr. wt. (114.71-138.04), tillering (80-140), leaf no. (110-130)/area (102-89-108.99), panicle length (109-136.36)/ wt. (103.33-110.83) and 100 grain wt. (100-101.55) for RFD, N:1/2P:K, sole *Enterobacter* sp., N:1/2P:K + *Enterobacter* sp. treatments of NV seedlings in the net house in pot culture were recorded by Dash and Dangar (2019). However, root growth results for S36 (T_5) and NR4 (T_7) were lower than those observed by Khan *et al*. (2017) who recorded 5% more rice root length for *Burkholderia* sp. BRRh-5 with N:1/2P:K compared RFD fertilized plants. S36 and NR4 with (N:1/2P:K) (T_5, T_7) produced 21.46% and 14.38% more tillers, respectively than RFD treatment (121.46) (T_2) which corroborated 17% more tillers by *Burkholderia* sp. BRRh-4 with half fertilizer treated plants compared to un-bacterized RFD supplemented plants (Khan *et al*., 2017). The results implicated that bacteria with 1/2P:N:K would be better for rice growth than bacteria alone and can reduce at least about half of the P requirement for rice cultivation (Table 4, Fig. 3A,B). As S36 and NR4 were effective phosphate and IAA metabolizers than other bacteria (cf. materials and methods) they would affect better growth of the rice cv. Luna Sankhi in non-saline pot culture (Table 4, Fig. 3A,B). Furthermore, S36 being a LS colonizers would have co-evolutionary implication and would better support LS growth. Moreover, the results proved that S36 is most promising PGPB for improvement of rice production.

Manifestation of seed germination, seedling growth and osmotolerance of Luna Sankhi bioprimed by S36 (*Salinicola* sp.) and NR4 (*Pseudomonas* sp.) grown in petriplate under osmotic stress condition

Impact of S36 and NR4 on seed germination and preliminary seedling growth of Luna Sankhi (moderate salt tolerant) under control (0, distilled water), 0.21, 0.43 and 0.87% (or 4, 8, 16 dS/m or 35.93, 73.58, 148.87 mM) NaCl stress; sole S36 and NR4; and S36 or NR4 combined with 0.21%, 0.43% and 0.87% NaCl stress conditions were evaluated in petriplates in the laboratory (Table 5, Fig. 4, 5A,B). Seed germinated was 100% in control and 0.21% NaCl but reduced to 80 and 46.66% in 0.43 and 0.87% NaCl stress, respectively. Furthermore, 100% seeds germinated in both sole S36 and NR4, NR4/S36 + 0.21% NaCl and S36 + 0.43% NaCl treatments but 93.33% seeds germinated in NR4 + 0.43% NaCl, whereas, S36 + 0.87% NaCl allowed more (86.66%) seed germination than NR4 + 0.87% NaCl (73.33%). The trends of the effects of the treatments on radical and plumule length, fr. and dr. wt. of seedlings, and lateral root emergence were similar to seed germination. In control (100%) perspective,

Figure 4: Growth(7d) of rice cv.Luna Sankhi seedling under control(C) and S36 and NR4 treatment in petriplate

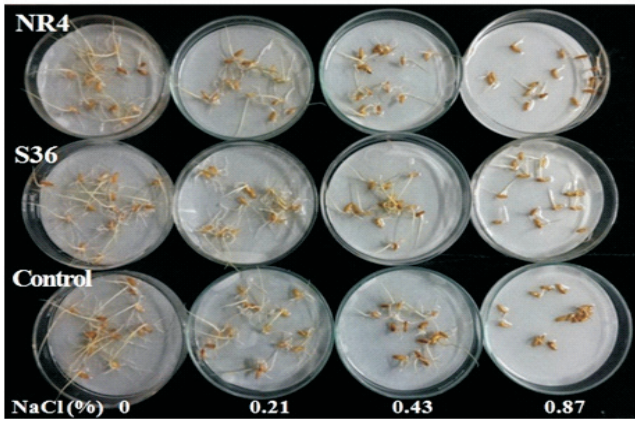

Figure5A: Luna Sankhi seed germination treated with S36 and NR4 under NaCl(0,0.21,0.43,0.87%) stress condition in petriplate(7d)

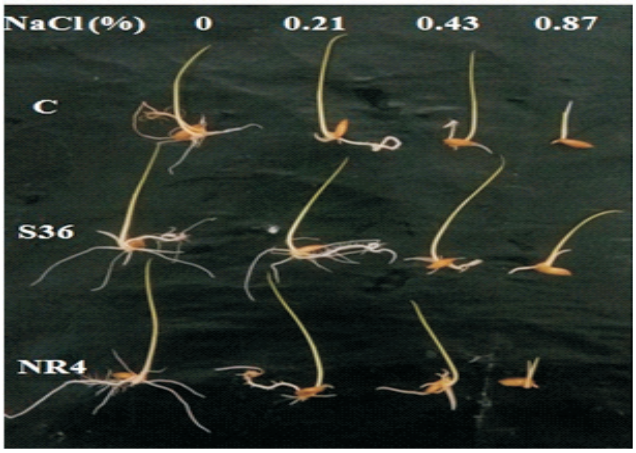

Figure 5B: Luna Sankhi germinated seeds treated with S36 and NR4 under NaCl (0.21,0.43,0.87%) stress condition in petriplates

results (%) of radical (63.19-11.61)/plumule (90.05-31.15) length, fr. wt. (92.75-53.62)/dr. wt. (94.74-84.21) and lateral roots (41.50-0) dropped progressively with increasing NaCl concentrations (0.21- 0.87%). Sole S36 and NR4 improved all seedling growth parameters over control, co-inoculation of the bacteria with various osmoticum levels bettered all growth parameters than respective sole salt concentrations and treatments of S36 + NaCl stresses had superior positive effect compared to equivalent osmoticum with NR4. The results proved that S36 and NR4 could counter the stress effects on seed germination and seedling growth but the former is a superior stress alleviator than the latter.

Although lower salt levels (0.21% NaCl) did not alter seed germination of Luna Sankhi but negative effects *i.e.* 80.00% (20% reduction) and 46.66% (<50% reduction) on germination for 0.43% and 0.87% osmoticum indicated that 0.43% (8 dS/m) salt had moderate stress effect and inhibition of germination by higher salt concentrations would be due to ion toxicity (Ali *et al*., 2014). Significant improvement of seed germination in NaCl (0.43 and 0.87%) co-inoculated with the bacteria S36 followed by NR4 in comparison to corresponding sole NaCl stresses suggest that they would alleviate osmotic stress of the rice genotype. Similar results on seed germination and seedling growth *i.e.* root, shoot and dry matter reduction due to NaCl stress were recorded in rice, cabbage etc. (Razzaque *et al*., 2009, Xu *et al*., 2011, Nakbanpote *et al*., 2014). Enhancement of growth traits by S36 and NR4 over control, counter action of osmotic shock by the bacterized LS seedlings conformed to enhancement (over control) of seed germination, growth, yield of rice and alleviation of inhibition of higher salinity (10 dS/m) co-inoculated with osmotolerant B. megaterium A12ag (Sujunya *et al*., 2009). More root and shoot length, lateral root emergence and fr./dr. wt. of seedling by S36/NR4 under NaCl (0.21, 0.43 and 0.87%) corroborated to those of rice and bean by salt tolerant PGPB, and ACCD producing rice PGPR viz. Bacillus, Microbacterium, Methylophaga, Agromyces and Paenibacillus spp. bacterized Naveen seeds with 150 mM (~0.8%, ~16 dS/m) NaCl (Bal *et al*., 2012) stress.

Promotion of growth and salinity endurance of Luna Sankhi seedlings treated with S36 (*Salinicola* sp.) and NR4 (*Pseudomonas* sp.) grown in perforated pots under saline stress (8 dS/m) condition in the net house

Effect of the two promising PGPB NR4 and S36 on growth (15d after treatment) of Luna Sankhi seedlings (tolerance 6-8 dS/m for 15-21d) was evaluated under moderate stress *i.e.* 8dS/m NaCl (0.43%) compared to stress relaxed conditions (Table 6, Fig. 6, 7, 8). All growth parameters (but tiller no.) *viz*. plant height (40.0 cm/plant), root length (18.05 cm/plant), no. of leaves (7.33 cm/tiller), shoot area (18.89 cm^2/shoot), root fr. wt. (2.03 g/tiller), root dr. wt. (0.20 g/tiller), shoot fr. wt. (2.47 g/tiller), shoot dr. wt. (0.52 g/tiller) and tillers no. (2.66/hill) of control (T_1) were impeded under 8 dS/m salt stress (T_2) to 93.33, 88.48, 77.22, 93.86, 73.89, 90.00 85.02, 92.31 and 100% (tillers unaffected), respectively. Relative to control (T_1) and osmotic stress (T_2), NR4 treatments (T_3, T_4) augmented 112.04% (plant height) to 165.00% (root dr. wt.) the growth parameters by (T_3) and 100% (leaf area) to 155% (root dr. wt.) (T_4) but improvement levels for S36 (T_5, T_6) were 113.07% (plant height) to 271.15% (shoot dr. wt.) (T_5) and 105.27% (plant height) to 185.00% (root dr. wt.) (T6). The results proved that both bacteria augmented osmotolerance (8 to 12 dS/m) of the LS seedlings but S36 superseded NR4 both under stress free and stress challenged conditions.

Negative effect of 8 dS/m NaCl on growth of LS seedlings (T_2) in comparison to control (T_1) is a universal stress induced growth inhibition effect on plants in general including rice (Nakbanpote *et al*., 2014, Mukherjee *et al*., 2019). More root/shoot length, leaf no./area, fr./dr. wt. of root/shoot and tiller production for NR4 (T_3) and S36 (T_5) than control (T_1) and 8dS/m NaCl stress (T_2), besides, better effect of S36 (T_5, T_6) than NR4 (T_3, T_4) proved osmoprotection function of both PGPBs but superiority of S36. Improvement of salt/metal tolerance and growth promotion of paddy by phytonic PGPB e.g. *Bacillus, Pseudomonas, Halomonas, Corynebacterium, Enterobacter, Corynebacterium, Bacillus* spp. *etc*. also supported overall growth and salt (up to 200 mM NaCl)/drought/metal endurance of different rice cultivars (Naveen, IR64, moderate osmotolerant Jarava *etc*.) through osmolyte, osmozyme, IAA, GA3, zeatin; nutrients (N, P, K, S, Ca, Mg) etc. metabolism (Jha *et al*., 2011, Ng *et al*., 2012, Jha and Subramanian, 2013, Pradhan *et al*., 2018, Mukherjee *et al*., 2019). As S36 was Luna Sankhi rhizoplanic but NR4 was Naveen rhizoplanic colonizer, so more favour of NR4 for

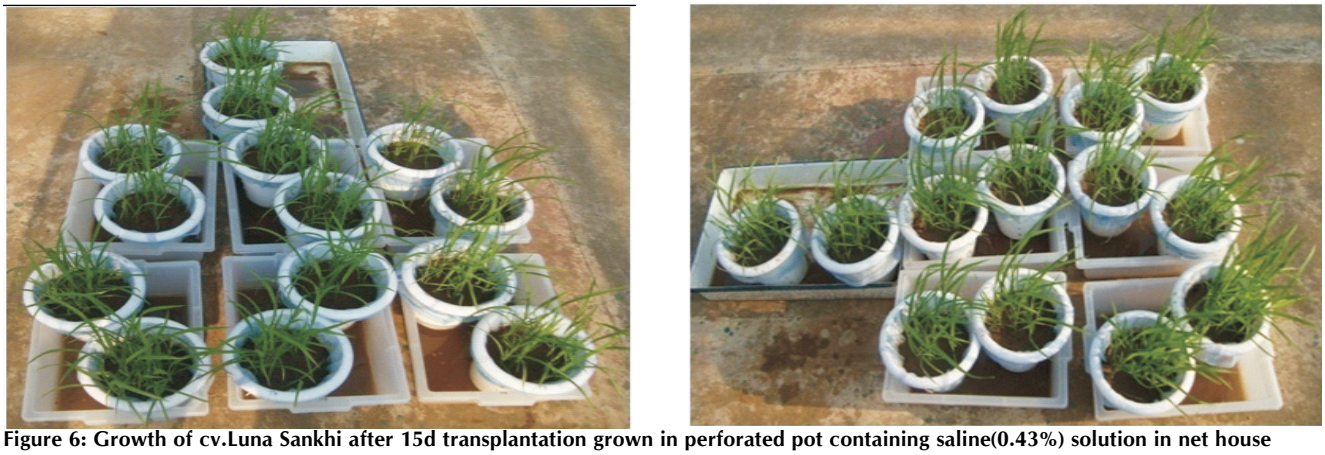

Figure 6: Growth of cv.Luna Sankhi after 15d transplantation grown in perforated pot containing saline(0.43%) solution in net house

Naveen and S36 for LS would be expected and S36/NR4 may have evolutionary significance with respective host. Nevertheless, superiority of S36 for both NV and LS proved that NR4 might be more host specific than S36.

Promotion of growth and salinity endurance of Luna Sankhi seedlings treated with S36 (*Salinicola* sp.) grown under salinity stress (12 dS/m) in salinity tanks

Growth and salinity resilience of Luna Sankhi seedlings (tolerance 6-8 dS/m for 15-21d) treated with the most promising S36 under 12 ds/m NaCl (0.70%) stress in salinity tanks up to 30d were assessed (Table 7, Fig. 9). In control (saline condition) (T1) growth results of plant height (33.77 cm/plant), root length (9.28 cm/plant), green leaves (6.66/plant), dry leaf (4.33/tiller), leaf area (13.13 cm^2/leaf), tiller no. (2.33/hill), shoot fr. wt. (1.35 g/tiller), shoot dr. wt. (0.26 g/tiller), root fr. wt. (0.34 g/tiller) and root dr. wt. (0.17 g/tiller) were recorded but S36 treated seedlings grown under salt stress (T_2) corresponding results were 124.96, 178.99, 140.09, 61.43 (lesser than control *i.e.* improved), 158.42, 185.84, 230.37, 273.08, 400.00 and 200.00%, respectively, on 30d growth in 12 dS/m (0.70%) NaCl osmoticum.

Significant growth improvement of LS seedlings by *Salinicola* sp. (S36) proved that the bacterium not only enhanced growth of LS but improved osmotolerance from 6-8 to 12dS/m and 15-21 to 30d (the experiment could not be continued further). As rice is the most salinity susceptible (threshold 3.0 dS/m) but an important global cereal crop (Kalaiyarasi *et al.*, 2019), enhancement of growth and production at higher salinity would achieve (at least partially) world food security. Higher NaCl and sea salt (12%) tolerance and PGP functions (including plant hormone and ACCD metabolism, retention of more PGP traits (cf. MM) of S36 would be responsible for all round development of the rice genotype Luna Sankhi beyond its natural osmotolerance limit and conformed to significant increase of growth of rice (IR36, Jarava, Naveen *etc.*) and other plants under salt stress by salt tolerant PGPB (Egamberdieva, 2014, Orhan, 2016, Mukherjee *et al.*, 2019, del Carmen Orozco-Mosqueda *et al.*, 2020). The results also proved that S36 can be exploited both in saline and non-saline conditions for rice improvement.

Salt tolerance of *ect*C cloned *E. coli* DH5α clones and their effect on seed germination and seedling growth of cv. Luna Sankhi grown under salt stress (4 dS/m) in petridish in the laboratory

NaCl tolerance of the clones (C3, C5, C7) with *ect*C genes of Salinicola sp. S36 on NA plates showed that the clones tolerated more NaCl (6.5-7%) than the recipient *E. coli* DH5*a* (5%) but lower than the donor S36 (12%) (Table 8). The result proved functional *ect*C gene of the mother bacterium could be cloned to develop desired osmotolerant PGPB. However, as the donor possessed several other stress resilience traits *i.e.* osmolytes/osmozymes (cf. materials and methods) it would be better stress tolerant than the single gene cloned *E. coli* DH5*a.*

Germination and growth of Luna Sankhi seeds bioprimed with the *ect*C clones and donor PGPB (S36) were checked after 5d of germination under 0.21% NaCl stress (Table 9). Seed germination was unaffected in all treatments but the seedlings challenged with the E. coli clones C3 (3.22 cm), C5 (3.23 cm) and C7 (3.25 cm) had longer roots compared to control (3.19 cm) and DH5*a* (3.21 cm) but shorter than S36 (3.49 cm). Similarly, shoot length (105.88-119.12%), root no. (116.56-128.22%), seedling fr. wt. (103.28-108.20%) and seedling dr. wt. (107.25-112.50%) increased over control but lower than the S36 primed seeds. Furthermore, although insignificant, *E. coli* DH5*a* was superior than the control and impact of all clones were not identical.

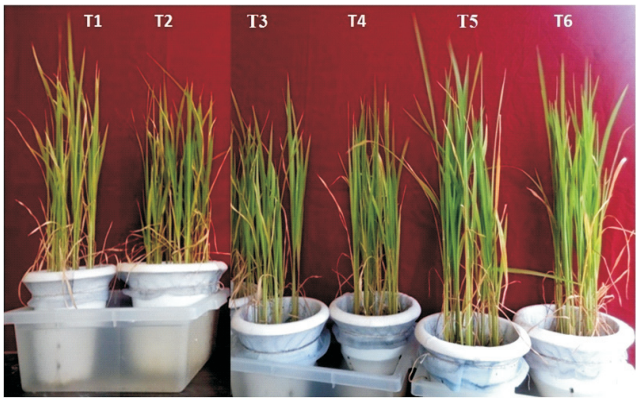

Figure7: Evaluation T4(NR4) *Pseudomona*s sp: and T6(S36,*Salinicola* sp: on growth of Luna Sankhi in perforated pots under saline stress condition in the net houses.T1 = Control(distilled water),T2 = Control (NaCl 8ds/m)T3 = Only NR4,T4 = NR4 + NaCl 8ds/m,T5 = Only S36,T6 = S36 + NaCl 8ds/m

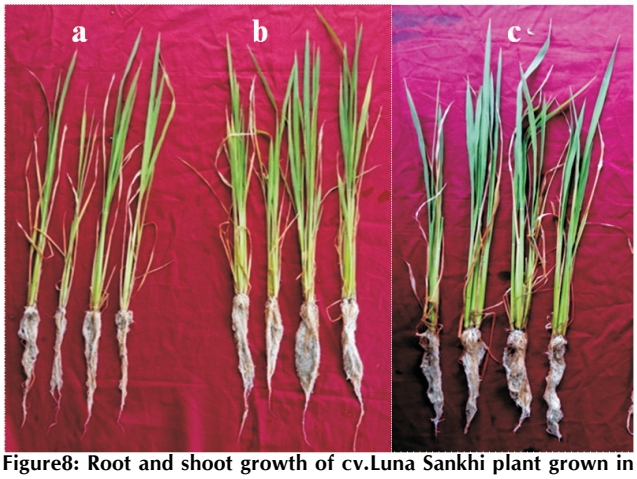

Figure8: Root and shoot growth of cv.Luna Sankhi plant grown in perforated pots in saline solution a = control plant root,b = NR4 treated root, c = S36 treated root

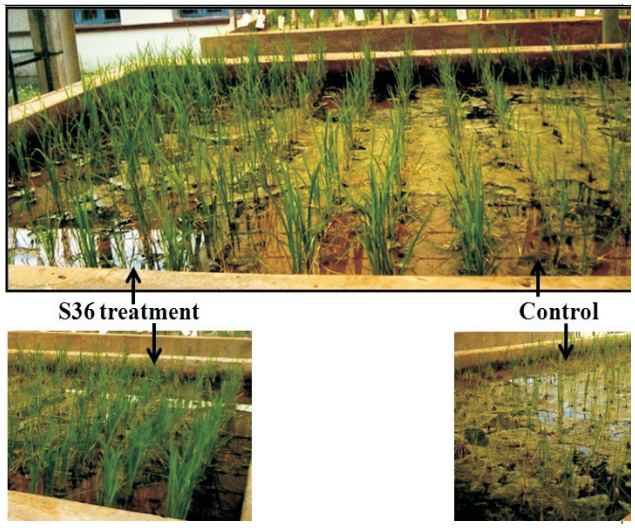

Figure 9: Growth after 30d transplant of Luna Sankhi seedling inoculated with S36 and grown in salinity tank with 12ds/m NaCl stress

The results clarified superiority of the *ect*C parent S36 for growth promotion of LS compared to clones which would be the result of cumulative actions of multiple anti-stress functionalities *viz*. osmolyte and osmozyme along with the PGP traits of S36 that would support all round promotion compared to only salt tolerance support by the clones. However, differential expression or even silent clone emergence is a common microbial phenomenon. Nominal positive effect of *E. coli* DH5*a* over control could be attributed to the PGP characters *viz*. P metabolism (unpublished information) and IAA metabolism of the *E. coli* clones (Nautiyal *et al*., 2010).

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