Chapter 4

Plant inoculation studies with PGPR consortia for salinity and biotic stress with *Cajanus cajan*

4.1. Introduction

PGPR employ a variety of mechanisms for encouraging plant growth constituting an excellent environment for them to flourish especially under the current paradigm of climatic changes. Among these PGPR traits - EPS, phytohormones production, nutrient assimilation and secondary metabolites such as siderophore production play a vital role in stressed conditions (Bhat et al., 2020; Salwan et al., 2019) for establishing ecological communities and consortium development as discussed in the previous Chapters. *Pseudomonas* strain having tolerance towards high NaCl (0.5M) concentration has been reported by Costa-Gutierrez et al. (2020), where least effect of the saline stress was observed on the PGPR traits (IAA, siderophore and phosphate solubilization) under salt stress, it showed significant improvement in plant growth under saline conditions compared to uninoculated plants. The study attributed increased efficiency to alleviate salt stress towards biofilm production which enhanced the fitness for competitive survival in the rhizosphere. A study reported by (Redondo-Gómez et al., 2021) showed five PGPR consortia with multiple PGPR traits when applied to eight different crop plants under saline (85mM NaCl) condition, six out of the total number showed enhanced biomass content than the uninoculated control, especially in strawberry which showed 35% shoot biomass and 80% root biomass enhancement.

The phytohormones stimulate plant growth regulating root and shoot hormone signalling in plants, EPS production helps via protection against direct interaction with the salt ions by trapping them outside, the volatile organic compounds (VOCs) secreted by the PGPR are also involved in the nutrient assimilatory activity which get trapped inside the biofilm matrix making them easily accessible for plant growth (Etesami & Maheshwari, 2018; Vaishnav et al., 2016a). Vaishnav et al. (2016) have shown enhanced germination in soybean seeds in presence of VOCs under salt stress (100mM NaCl).

Additionally, the PGPR also assist in stress alleviation via stimulating the accumulation of osmolytes and antioxidants production to counter the reactive oxygen species (ROS) (Etesami & Beattie, 2017). Parihar et al. (2020) have reported that the negative effects of salinity can be counteracted by improving the antioxidant system and ionic balance by the mycorrhizal fungi, which resulted in growth and yield enhancement in pea and the consortium was reported be more effective than individual inoculations for stress alleviation in the plants. Chu et al. (2019) have reported a *P. putida* strain which stimulated salt tolerance in *Arabidopsis* plants via up-regulation of LOX2 gene, an early salt stress

responsive gene, which led to the plant growth at 225mM NaCl stress where uninoculated plants were unable to survive.

Understanding the interaction between consortium of microbes and plant systems will pave way to harness more benefits from microbial inoculants for improving plant growth and yield (Aguilar-Paredes et al., 2020; Santoyo et al., 2021). Rhizobia are known to confer several other PGP benefits aside from nitrogen fixation (Jaiswal et al., 2021) and a multispecies consortium having PGPR with additional benefits such as biocontrol activity and/or the ability to confer stress tolerance in plants while also showing synergistic effects of multiple traits is an added privilege under stressed condition (Ha-tran et al., 2021; Santoyo et al., 2021). Similar results have been reported by Kumawat et al. (2021), where the PGPB consortium consisting of *Rhizobium* with a PGPR *Enterococcus mundtii*, was found to improve the plant growth under salt stress via significantly enhancing the symbiotic efficacy, stimulating antioxidant enzyme system, osmolyte accumulation and nutrient mobilization over individual strains.

Ansari & Ahmad (2019) have reported high biofilm activity in two PGPR strains (*Pseudomonas* and *B. licheniformis*) when in consortia, along with IAA, ammonia, siderophore production and phosphate solubilization, heavy colonization by both the strains over rhizoplane was observed by the SEM analysis while the consortia treatment led to enhanced vegetative growth and photosynthetic ability over individual strains. Another study reported by Molina-Romero et al. (2021), shows increased seed adhesion and rhizospheric colonization by four PGPR strains when in consortium (*Azospirilllium brasilense*, *Pseudomonas putida*, *Acinetobacter* sp. *Sphingomonas* sp.), over individual inoculations, which led to increased biomass accumulation and plant growth promotion as an alternative to application of chemical fertilizers.

Joshi et al. (2020) have reported enhanced PGP by the consortia (*Bacillus puralicheniformis, B. licheniformis, B. haynessi*) over individual strains in rice plants under abiotic stress, decreased antioxidant enzymes activity and enhanced plant biomass accumulation, the strains were selected for consortia preparation based on various PGPR abilities including EPS, IAA and siderophore production. Microbial strains (*Aneurinibacillus aneurinilyticus* and *Paenibacillus* sp.) with multiple PGPR trains were reported (Gupta & Pandey, 2019) to alleviate NaCl (6% w/v) and drought stress *Phaseolus vulgaris* however, the isolates when applied in the form of consortia, were able to alleviate ~60% stress by virtue of their combined ACCd activity and enhance several plant growth parameters in salinity stress.

Kapadia et al. (2021) have reported a multispecies consortium comprising four strains with multiple PGPR traits (*Bacillus* sp., *Delftia* sp., *Enterobacter* sp. and *Achromobacter* sp.) which alleviated the saline stress by regulating the ionic balance, enhanced nutrient uptake in plants irrigated with increasing concentration of salt water, thus leading to enhanced growth, chlorophyll content and mineral uptake in plants over individual strains.

Wilt disease caused by *Fusarium* sp. is a major constraint to pigeon production world-wide, which can be diagnosed by symptoms such as loss of turgidity, veinal chlorosis and purple bands extending upwards from the base (Pande et al., 2013). Several strains belonging to *P. fluorescence* have been identified for disease resistance against the *Fusarium* wilt (Arya et al., 2018), while (*P. fluorescence* in) combination with *Sinorhizobium fredii* strains have also been reported to reduce the disease incidence in pigeon pea crops (Kumar et al., 2010). In a study reported by Devi et al. (2018), it was observed that a multispecies consortium (*P. aeruginosa*, *S. marcescens*, *Alcaligenes faecalis*) showed inhibition of *Fusarium* infection in potato plants, which was attributed to the siderophore and IAA production showed by all the isolates, along with chitinolytic, HCN and antibiotic production activity.

In the present study, PGPR from three different genera as well as their consortia, which have been evaluated in Chapter 3, were used for the pot study experiments to assess their stress alleviation efficiency in-planta in the presence of biotic and abiotic stress factors.

4.2. Materials and Methods

4.2.1. Surface sterilization and seed germination

The present study was performed using Pigeon pea (*Cajanus cajan*) as the host plant and the seeds (var. BDN-2) were procured from Pulse research station, Anand Agricultural University (Model Farm), Alembic road, Vadodara for the pot study experiments. Healthy looking seeds were placed in a 250ml Erlenmeyer flask and washed with 1% Teepol solution for 5min and rinsed using sterile D/w until the foaming stopped. Inside the laminar hood, the seeds were further treated with sterile 0.1% mercury chloride solution and 70% methanol (v/v) for 1min each and in the same order. Each treatment followed by sterile D/w rinse three times, the seeds were finally soaked in sterile D/w for 30min. The seeds were then transferred to moist chamber plates (sterile petri plates with moist cotton bed) and kept in dark at 30°C for 2-3d until germination and the seedlings showed radical growth (1.5-2cm length). Seedling preparation was conducted in the same way for all the following experiments.

4.2.1.2. Effect of salt stress on seed germination

To test the effect of NaCl on seed germination, sterile water agar (0.8% agar) plates amended with salt in the concentrations 0.5%, 1.0% and 1.5% NaCl (w/v) were prepared and 10 seeds per plate in three independent sets of each were kept for seed germination. In another set, seed germination was carried out in 0.8% water agar petri plates amended with NaCl in concentrations 0.5%, 1.0% and 1.5% (w/v). The petri plates were prepared by fixing a cardboard bridge vertically in the centre before sterilization. On one side of the bridge, no salt agar was added while the other side had the salt amended agar medium, the bridges were removed aseptically after the agar solidified. Surface sterilized seeds were placed in the centre of the plates. All the plates were incubated in dark for 5d before.

4.2.2. Preparation of microbial inoculants for plant assays

4.2.2.1. Bacterial consortia for PGPR treatment: The bacterial strains (Table 2.1) were maintained as given in Section 2.2.1 while the bacterial inoculum was prepared as given in Section 3.2.1. For consortial studies, the 9 combinations from the Chapter3 were continued for the pot studies.

4.2.2.2. Fungal inoculum for Biotic stress: The pathogen challenge selected for biotic stress was *Fusarium oxysporum f. sp. udum*. The fungal cultures were regularly maintained on PDA at 4°C. The inoculum was prepared by raising the culture in PD broth (500ml Erlenmeyer flasks) on shaker at 30°C for 15d. The culture supernatant was aseptically transferred to centrifuge bottles and the thick mycelial biomass was separated by spinning down at 4000rpm / 5min. The spore count was estimated using Haemocytometer in 10 individual sets and the suspension was adjusted to spore count of ~1 x 10^6 spores/ml. The spore suspension was prepared freshly each time before an experiment.

4.2.3. Plant inoculation studies for alleviation of salt stress

4.2.3.1. Preparation of soil samples

The soil was procured from the agricultural farms of Pulse research station, Anand Agricultural University (Model Farm), Alembic road, Vadodara. The soil was tested for the physio-chemical analysis which was conducted at the Gujarat State Fertilizer and Chemicals Ltd (GFSC), Vadodara, Gujarat).

The soil aggregates were broken down and passed through sieve (2mm size) for obtaining fine particulates free of roots and other organic matter. The soil was mixed thoroughly and put into autoclavable bags and autoclaved at 15psi for 30min. This process was repeated

thrice each at 1d interval to allow the microbial spores (if present) to germinate during the night, which were eliminated during the following sterilization.

4.2.3.2. Seed inoculation and plant growth

Germinated seedlings were added to bacterial and/or consortia suspension and incubated in dark for 1h. The seedlings were transferred to sterile petri plates and air dried for 30min. The petri plates were sealed and the seeds shifted to green house where they were sown (4 seedlings per pot, three replicates, 12 seedlings per treatment) in the pots at a depth of 1cm and watered regularly at using sterile D/w. The pot arrangement was completely randomized until the end of incubation. The plants were grown in green house conditions (30°C,16h daylight /25°C, 8h dark) for a period of 45d.

Seedlings inoculated with individual strains were grown as reference for comparison of the effect of consortia on stress alleviation while a set grown without any bacterization was used as negative control. For studying the effect of salinity, 0.1%, 0.2% and 0.3% concentrations of NaCl (w/w) was mixed with 1.2kg autoclaved soil in bulk respectively and distributed to 3 pots (400g) as replicates for each treatment, while a set without salt was kept as no salt control for each PGPR treatment. The pots were immediately shifted to green house and watered daily using sterile D/w. The plants were harvested gently 45 days after sowing (DAS), followed by the measurements of physical growth parameters, leaf samples were weighed out for respective chemical assays and shifted to 4°C till the analysis. The soil samples from rhizospheric region in each treatment was collected for electrical conductivity measurements.

4.2.4. Determination of plant growth parameters

The following physical and biochemical parameters were analysed for studying the effect of PGPR on stress alleviation in plants.

4.2.4.1. Physical parameters

Shoot length and root length: The effects of seed inoculation with bacteria on plant elongation parameters were studied in terms of measuring shoot and root length. The results are calculated as the percentage variation using no salt control as reference.

Leaf length: The leaf length was considered as an important parameter, the length of the mature leaves found in the lowermost region was measured from each plant per treatment.

Shoot and Root weight (fresh / dry): Leaf samples were collected for biochemical analysis, weighed and stored 4°C. The plants were cut into smaller pieces and the fresh weights were measured respectively for biomass estimation. The biomass content was measured as the total dry weight devoid of moisture content for the respective plant tissue.

The samples were stored into paper sachets and shifted to hot air oven at 70°C until the complete moisture evaporation (4d) and the dry weight was constant. The moisture content of shoot and root samples was calculated as given below:

$$\% \text{ Moisture content} = \frac{(\text{Fresh weight} - \text{Dry wieight}) \times 100}{\text{Fresh weight}} \qquad \textit{Eq. (5)}$$

The biomass content was measured as the total dry weight devoid of moisture content for the respective plant tissue.

The salt tolerance index (STI) and biomass allocation was determined according to Saghafi et al. (2019), which were calculated as given below. The dry weights obtained before were taken as the final biomass (Bm) content each respective tissue (shoot/root).

$$\text{Salt tolerance index (STI)} = \frac{Bm\ in\ salt\ stress}{Bm\ in\ no\ salt\ control} \qquad \textit{Eq. (6)}$$

$$\% \text{ Biomass allocation} = \frac{Dry\ weight\ (shoot\ or\ root) \times 100}{Total\ dry\ weig} \qquad \textit{Eq. (7)}$$

Electrical conductivity (EC) measurement: The EC measurement was carried out as per the soil quality analysis using 1:5 soil-water ratio (Corwin & Scudiero, 2019). The rhizospheric soil (2g) from each pot was collected in glass tubes and kept in hot air oven to remove all moisture. 10ml of sterile D/w was added to each tube and vortexed 3 times in sequence for salt dissolution. After keeping the tubes overnight on benchtop, the upper liquid phase was used for EC measurement using Thermo Scientific PCS Testr 35.

4.2.4.2. Plant Biochemical analysis: The leaf samples stored at 4°C were cut into small pieces and minced in the Bio-Rad tissue homogenizer using respective solvents for each biochemical assay as mentioned below:

Sample preparation from plant extract: From each treatment, 0.1 g of leaf samples were weighed and homogenized in 2ml of plant extraction buffer (PEB, Appendix I) at 4°C The resulting sample was centrifuged at 10k rpm / 10min at 4°C and the supernatant was collected in sterilized 2 ml Eppendorf tubes and stored in deep freezer (−20°C) for further use as crude

plant extract (CPE). This enzyme extract was used for assay of total soluble sugars and protein content.

Total soluble sugar: Anthrone's method as described by Prakash et al. (2017) was employed for sugar estimation with a few modifications. CPE (0.1ml) was diluted with 0.9ml PEB in glass tubes kept in ice-cold water. To this, 2.5ml freshly prepared anthrone's reagent (Appendix I) was added and the reaction was allowed to cool down to avoid charring. The tubes were then placed in boiling water bath (100°C) for 10min. The tubes were kept in ice-bath again before colorimetric estimation. D-Glucose was used as standard (Appendix II) and the colour change measured at 540nm using a spectrophotometer.

Protein content: Estimation of protein content was done by Bradford's method using bovine serum albumin as standard (Appendix II, adapted and modified from Bradford, 1976). To 0.5ml Bradford's reagent (Appendix I) 0.5ml CPE was added the reaction allowed to develop colour for 30min in dark. The protein content was measured at 595nm using spectrophotometer.

Chlorophyll (chl) content: Three leaves from each set was collected and 0.1g leaf sample was homogenised in 2ml of 80% acetone (v/v, aq.) at 4°C. The samples were centrifuged at 10k rpm / 10min and stored in dark for 6h. The supernatant was separated by centrifugation (10k rpm / 10min) at 4°C and used for measurement of absorbance at 645nm, 665nm and 470nm (Azarmi et al., 2016, modified and adapted from Arnon 1949).

The following equations were used for calculation of chlorophyll components:

$$\text{Chl a } (\mu g/ml) = [(12.25 \times A_{663.3}) - (2.55 \times A_{646.6})] \quad \textit{Eq. (8)}$$

$$\text{Chl b } (\mu g/ml) = [(12.25 \times A_{646.6}) - (2.55 \times A_{663.3})] \quad \textit{Eq. (9)}$$

$$\text{Total Carotenoids } (\mu g/ml) = [(1000 \times A_{470}) - 3.27 \times (\text{Chl a}) - 104 \times (\text{Chl b})] / 227 \quad \textit{Eq. (10)}$$

$$\text{Total Chlorophyll } (mg/g \text{ FW}) = 20.2 \times A_{645} + 8.02 \times A_{665} \quad \textit{Eq. (11)}$$

Leaf Proline estimation: For proline estimation, 0.2g leaf samples were homogenised in 4ml 3% sulfosalicylic acid (SSA) in D/w (w/v) and centrifuged at 10k rpm/ 10min. The supernatant was transferred to separate vials and stored at -20°C till further analysis. Proline estimation was done by the acid ninhydrin method (Wang et al., 2016). 2ml supernatant was mixed with 2ml each of glacial acetic acid and acid ninhydrin reagent (Appendix I). The mixture was kept in boiling water bath (100°C) for 1h to develop colour and transferred to

ice. To this, 4ml of toluene was mixed and the reaction vortexed vigorously for 30s before allowing it settle. The upper toluene phase retains the colour, which was measured spectrophotometrically at 520nm using toluene as blank while proline was used as the standard (Appendix II).

4.2.5. Plant inoculation studies for biotic stress alleviation

4.2.5.1. Soil preparation and seed inoculation

Plants were subjected to fungal stress using *Fusarium oxysporum f. sp. udum* as the pathogen challenge. 10ml of spore suspension (10^6 spores/ml) was mixed with each 1kg sterile soil. Each pot was filled up with 400g soil and incubated in moist condition for 5-7d for pathogen establishment before plant growth (Dukare & Paul, 2021). The germinated seedlings were inoculated with respective PGPR and their consortia and the bacterized seedlings were then sown in the pots containing pathogenic soil. The plants were kept isolated in conditions similar to green house and watered daily, just enough to maintain soil moisture, using sterile D/w (10ml/day).

4.2.6. Growth assessment for biotic stress tolerance in plants

Since the plants had been infected with pathogenic fungi they were not considered safe for the physicochemical analysis in the microbiology laboratory system and the soil used for the study was immediately transferred to incineration bags after use. The biotic stress alleviation in plants was assessed based on the number of plants survived at the end of 45d period. The disease incidence was calculated as per the following formulae (Sharma et al., 2016).

$$\% \text{ Disease incidence} = \frac{\text{Number of infected plants} \times 100}{\text{Total } no.of\ plants} \qquad \textit{Eq. (12)}$$

Where, Total number of plants = 15 / treatment

Fusarium wilt disease in each of the pot replicates was recorded via visual observations.

4.2.7. Statistical analysis

The significant differences among the growth attributes of control sets and the PGPR treated plants under no salt and salt stressed conditions were analysed by the One-way ANOVA ($P \leq 0.05$). The values were calculated as mean ± standard deviation of the triplicate sets. The growth parameters were assessed as percent variation from the no PGPR control set at the respective salt concentration, and since there were no plants survived at 0.3% the percentage variation was calculated based on those at 0.2% salinity.

4.3. Results

4.3.1. Pigeon pea seed growth under salt stress

It was observed that without any applied stress the germination vigour was 85% and the seedlings showed uniform radical growth by the end of 3 days indicating the healthy seeds were ready for plant growth. Whereas under salt stress, the rate of seed germination (percentage of seeds germinated out of total) decreased with increasing salinity (Table 4.1). The seeds were unable to germinate beyond 3% NaCl concentration, while the process of seed germination was delayed under the saline stress.

Table 4.1: Seed germination rate under salt stress

Salt (%)	Germination rate (%)
Control (0%)	85.0 ± 3.69
1	50.0 ± 10.80
2	14.3 ± 4.34
3	9.5 ± 4.20

In another set of experiments for seed germination under salt stress, similar results were observed where root development was stimulated towards control (0% salt) (Figure 4.1). The root growth towards salted agar was thicker with no lateral branching (see plate III, 0.5% NaCl), as well as the growth was seen to be stimulated towards unstressed medium. As the salt concentration increased, the root growth into the salt amended agar medium was stunted. All the seeds showed similar root elongation and lateral branching in the no salt agar section however, root length in salt amended agar decreased with increasing salinity (see plate III, 1% NaCl).

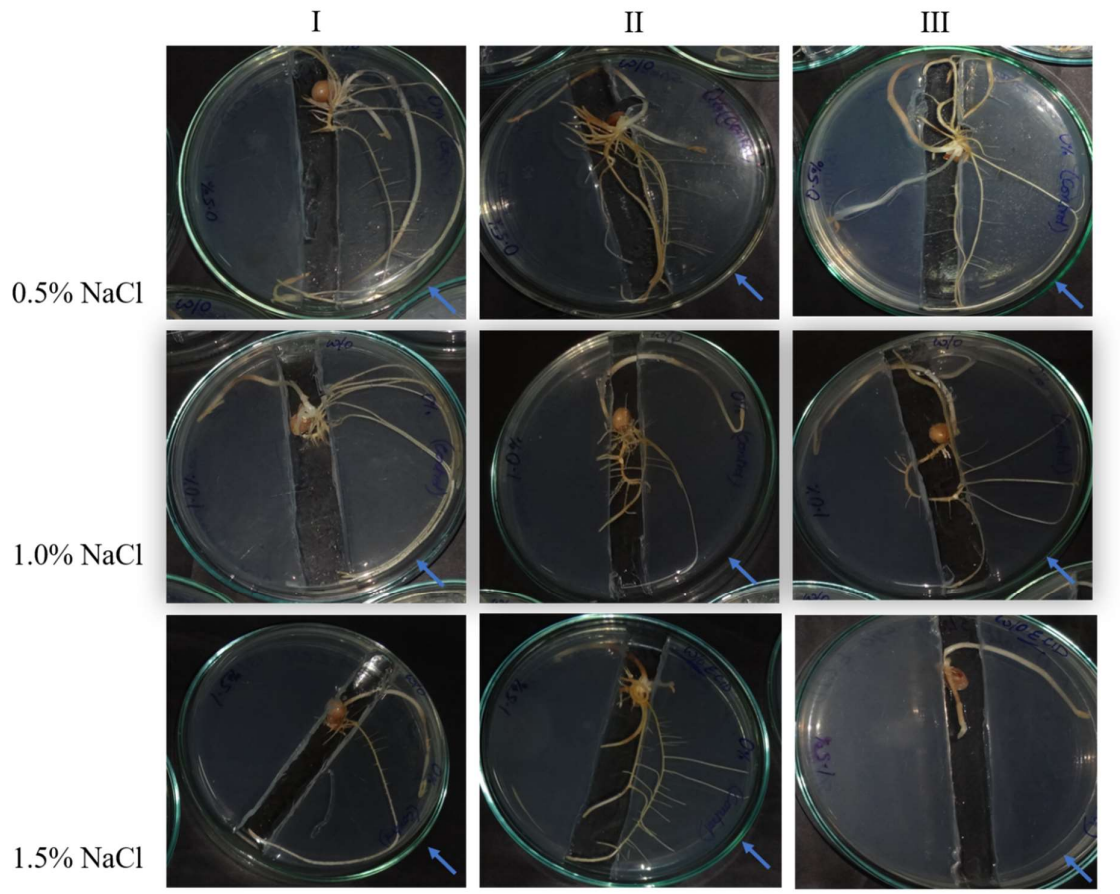


Figure 4.1: Development of seeds and radical growth in presence of control and salt stress. The blue arrows indicates part of the agar which did not receive any salt, the other side has salt amended agar. The vertical columns represent replicates.

4.3.2. Soil analysis

Soil analysis report as given in Table 4.2, confirmed the non-saline nature of the soil due to which it was deemed suitable for the pot studies in presence of salt stress. This soil was procured from agricultural lands used for cultivation of pigeon pea, high organic components along with potash and phosphorus were found which could due to the excessive application of chemical fertilizers in the field. The composition of micronutrients was also found to negligible or nil.

Table 4.2: Soil analysis report for nutrient composition, pH and electrical conductivity of the soil for pot inoculation studies.

Test	Result	Interpretation (optimal range)	Micronutrients	Quantity (ppm)
Organic Carbon (OC %)	0.73	High	Available Zinc (Zn)	--

Available phosphorus (P_2O_5 Kg/Ac)	28.0	High (4 – 14)	Available iron (Fe)	--
Available potash (K_2O Kg/Ac)	200.0	Very High (100 – 160)	Available Manganese (Mn)	--
pH (1:2)	7.10	Optimal (6.0 – 7.2)	Available copper (Cu)	--
Electrical conductivity (1:2, µS/cm)	0.26	Optimal (< 0.6)	Sulphur (S)	0.00

4.3.3. Effect of PGPR treatment on Abiotic stress in plants – Pot Studies

The PGPR effect on plant growth under salt stress was assessed using no salt control as the reference, while a set of uninoculated plants were taken as control for the effect salt stress in the absence of PGPR treatment. Plants were harvested 45 DAS and the following parameters were recorded.

4.3.3.1. Assessment of plant physical parameters

The number plants that survived the salt stress with or without PGPR treatment is indicated as given in the Figure 4.2. Overall, the PGPR strains EC1D, PG38, NGR234 and consortia C1S, C2S supported better survival of plants than the unbacterized counterparts.

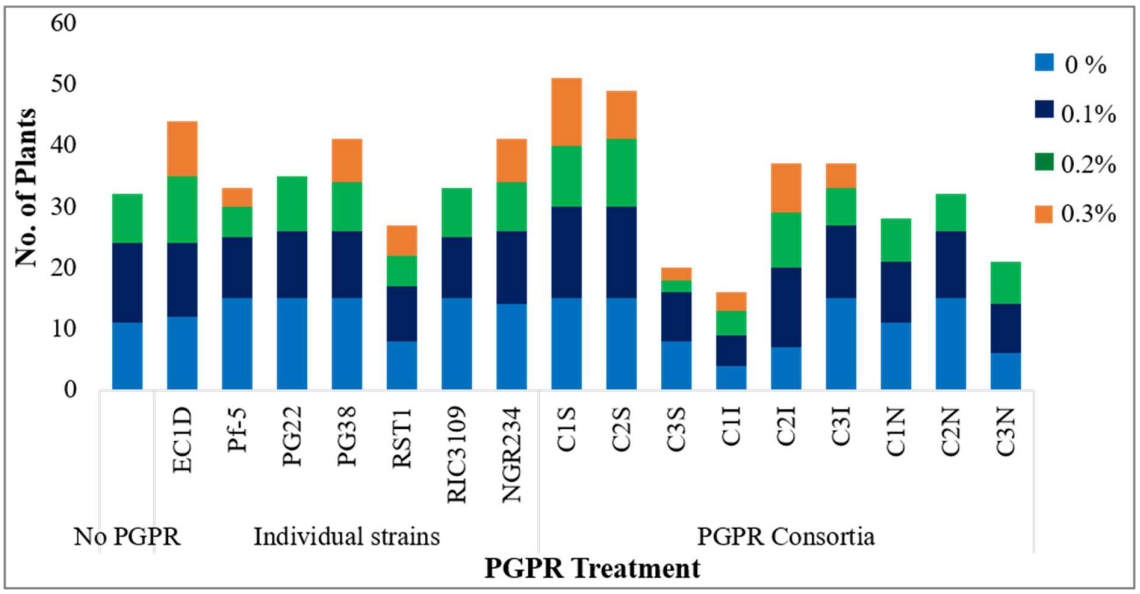


Figure 4.2: Number of plants surviving 45 DAS with or without PGPR treatment under different salt conditions.

4.3.3.1.1. Shoot and Root development

The shoot length in the no salt control was enhanced with Pf-5, C2I and C3I treatments compared to the uninoculated set (Figure 4.3). Under salt stress, unbacterized plant were not able to survive at 0.3% salt, however many individual strains (EC1D, Pf-5, PG38, RST1 and NGR234) and consortia (C1S, C2S, C3S etc.) supported plant survival and growth of roots and shoots.

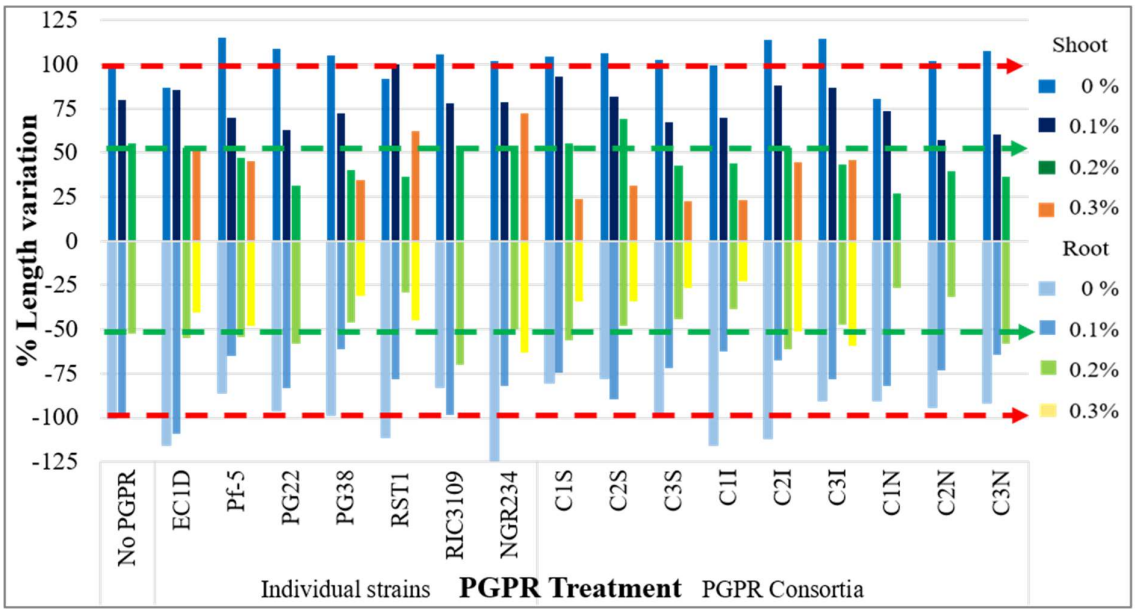


Figure 4.3: Percentage variation in shoot length (positive axis) and root length (negative axis) in plants treated with individual PGPR and consortia under increasing salt stress. The dotted lines in red indicate growth in uninoculated plants, red line: no salt no PGPR, green: 0.2% salt no PGPR - as the 100% reference.

4.3.3.1.2. Total biomass content

As observed in Figure 4.4, the plants showed enhanced root biomass under salt stress, (uninoculated control) which was ~1.8-folds, while the shoot biomass decreased due to salinity (0.2% salt stress). Strains Pf-5, PG38 and NGR234 and consortia C1S, C2S, C2I and C3I showed increase in the shoot biomass.

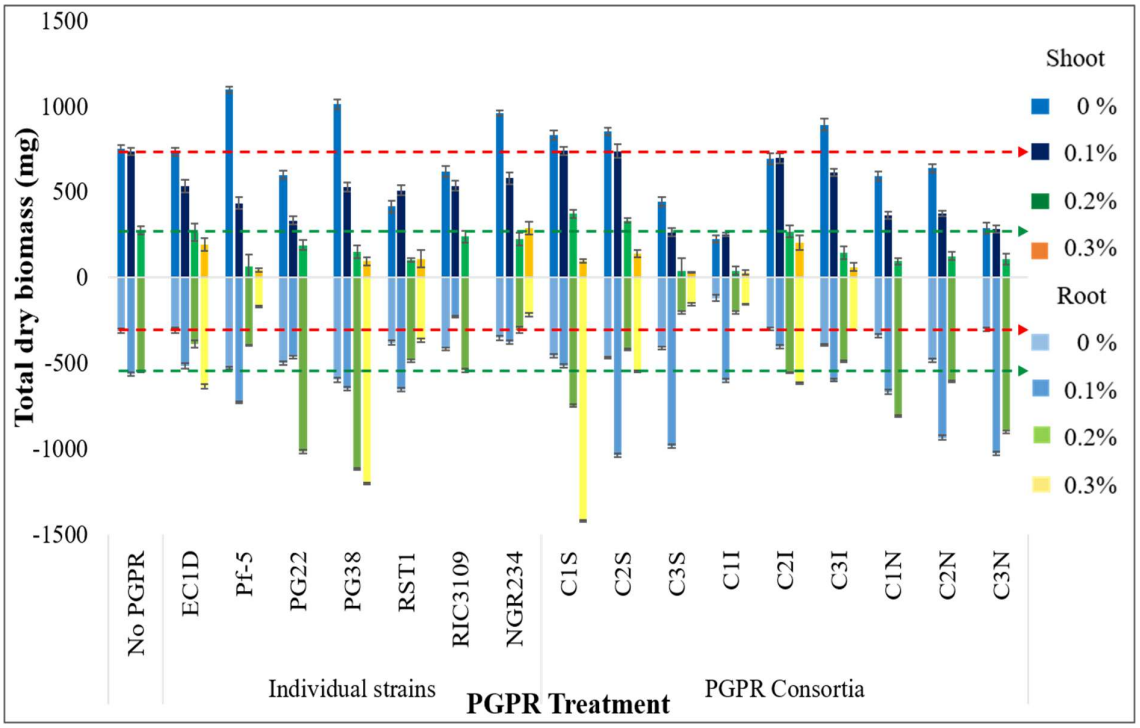


Figure 4.4: Total biomass content in shoot length (positive axis) and root length (negative axis) from all plants per treatment bacterized with individual PGPR and consortia under increasing salt stress.

Root biomass enhancement was observed in most instances under salt stress. Uninoculated plants showed enhancement in root biomass at 0.1% salinity which was the same at 0.2% salt stress well. PG22, PG38, the consortia having RST1 and NGR234 all showed root biomass enhancement under salt stress while the maximum biomass content was observed in C1S and PG38 at 0.3% salinity.

4.3.3.1.3. Salt tolerance index (STI)

The STI values close to 1 indicated equal biomass in salt treated as compared the untreated (without salt as well as PGPR) (Table 4.3). Clear indication of increase in root biomass is observed in case of individual PGPR e.g. G22 at 0.2% NaCl and EC1D at 0.3% NaCl. Many consortia (e.g. C1S, C2I, C1N and C3N) led to increase in STI.

Table 4.3: Salt tolerance index (STI) of plants treated with individual PGPR and consortia. The data in green colour represent values ≥ 1 while red colour is given to values showing >2-fold or higher STI.

Salt (%) →		Shoot			Root			Total		
		0.1	0.2	0.3	0.1	0.2	0.3	0.1	0.2	0.3
No PGPR		0.98	0.37	0.00	**1.82**	**1.76**	0.00	**1.22**	0.77	0.00
Individual Strains	EC1D	0.73	0.36	0.26	**1.68**	**1.26**	**2.07**	**1.01**	0.63	0.80
	Pf-5	0.40	0.06	0.04	**1.38**	0.75	0.32	0.71	0.28	0.13
	PG22	0.56	0.32	0.00	0.93	**2.04**	0.00	0.73	**1.10**	0.00
	PG38	0.52	0.15	0.09	**1.09**	**1.87**	**2.02**	0.73	0.79	0.81
	RST1	**1.23**	0.25	0.26	**1.72**	**1.27**	0.96	**1.46**	0.74	0.60
	RIC3109	0.87	0.39	0.00	0.55	**1.31**	0.00	0.74	0.76	0.00
	NGR234	0.61	0.23	0.30	**1.07**	0.87	0.62	0.73	0.40	0.39
PGPR Consortia	C1S	0.89	0.45	0.12	**1.13**	**1.64**	**3.12**	0.98	0.87	**1.18**
	C2S	0.86	0.39	0.16	**2.22**	0.90	**1.17**	**1.34**	0.57	0.52
	C3S	0.60	0.09	0.07	**2.39**	0.49	0.38	**1.46**	0.29	0.22
	C1I	**1.11**	0.18	0.13	**5.04**	**1.70**	**1.30**	**2.47**	0.71	0.54
	C2I	**1.01**	0.39	0.29	**1.35**	**1.86**	**2.07**	**1.11**	0.83	0.83
	C3I	0.69	0.16	0.07	**1.52**	**1.25**	0.78	0.94	0.49	0.29
	C1N	0.61	0.16	0.00	**1.96**	**2.37**	0.00	**1.11**	0.97	0.00
	C2N	0.59	0.19	0.00	**1.93**	**1.25**	0.00	**1.17**	0.65	0.00
	C3N	0.99	0.37	0.00	**3.40**	**2.98**	0.00	**2.22**	**1.71**	0.00

Here, it was observed that the consortia C1S and C2S led to a synergistic effect in the overall plant growth under salt stress and the STI was increased in comparison to individual strains.

4.3.3.1.4. Biomass allocation

The biomass allocation as the ratio of percent root biomass/shoot biomass (RSR ratio) in the plants is as given in Table 4.4. The uninoculated plants without salt stress showed ~30/70 ratio in the root/shoot biomass content. Salt stress led to increase in this ratio. Without NaCl, some of the PGPR treatments (PG22 and RST1) showed an increase in root biomass allocation implying root proliferation effect. Similar pattern was observed in case of consortia C3S and C3N. Under salt stress, the PGPR treatments which led to increased root biomass allocation included pseudomonads and the *Rhizobium* spp. All the consortia demonstrated high RSR ratio under salt stress.

Table 4.4: Biomass allocation as the ratio of % variation of biomass allocation in Root/Shoot tissue for individual PGPR and consortia.. The data in green indicate values ≥1, those in red indicate values ≥2

Salt (%) →		% Biomass allocation (Root/Shoot)			
		0	0.1	0.2	0.3
No PGPR		0.41	0.76	**1.97**	-
Individual strains	EC1D	0.42	0.96	**1.46**	**3.30**
	Pf-5	0.48	**1.67**	**6.08**	**3.91**
	PG22	0.83	**1.39**	**5.34**	-
	PG38	0.59	**1.23**	**7.42**	**12.54**
	RST1	0.91	**1.28**	**4.75**	**3.35**
	RIC3109	0.67	0.42	**2.26**	-
	NGR234	0.37	0.65	**1.36**	0.75
PGPR Consortia	C1S	0.55	0.69	**2.01**	**14.48**
	C2S	0.55	**1.41**	**1.26**	**3.90**
	C3S	0.93	**3.71**	**4.92**	**5.25**
	C1I	0.53	**2.40**	**4.92**	**5.25**
	C2I	0.43	0.58	**2.06**	**3.04**
	C3I	0.44	0.97	**3.41**	**4.96**
	C1N	0.57	**1.83**	**8.39**	-
	C2N	0.76	**2.49**	**4.86**	-
	C3N	**1.04**	**3.60**	**8.33**	-

4.3.3.1.5. Moisture content

The moisture content was analysed as the percentage variation, which was least affected in shoot and root biomass in most of the PGPR treatments and under salt stress and ranged within 60-80% in all plants (Figure 4.5). There was no significant difference in the moisture content among the different PGPR treatments.

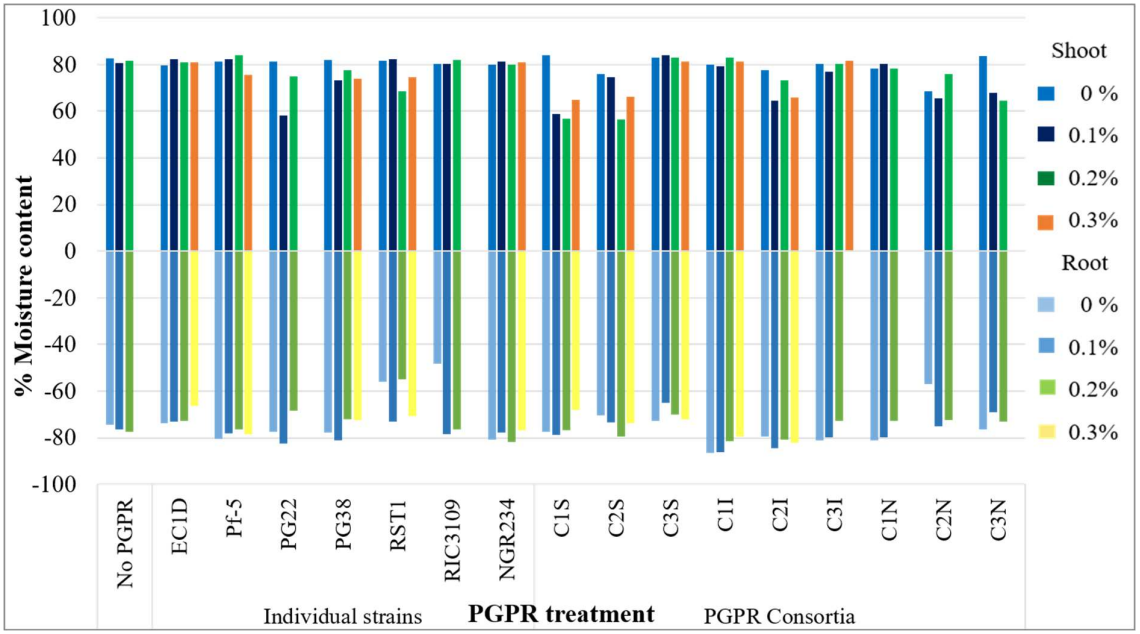


Figure 4.5: Percent moisture content in shoots (positive axis) and roots (negative axis) in plants given different PGPR treatments and increasing salt concentrations.

4.3.3.1.6. Soil Electrical conductivity (EC)

The electrical conductivity of rhizospheric soil collected after harvesting the plants was as shown Figure 4.6. Only the plants treated with consortia C2S led to decreased EC value under 0.1% salinity while most conditions the EC value remained same as the control. Surprisingly some treatments have resulted in increased EC value.

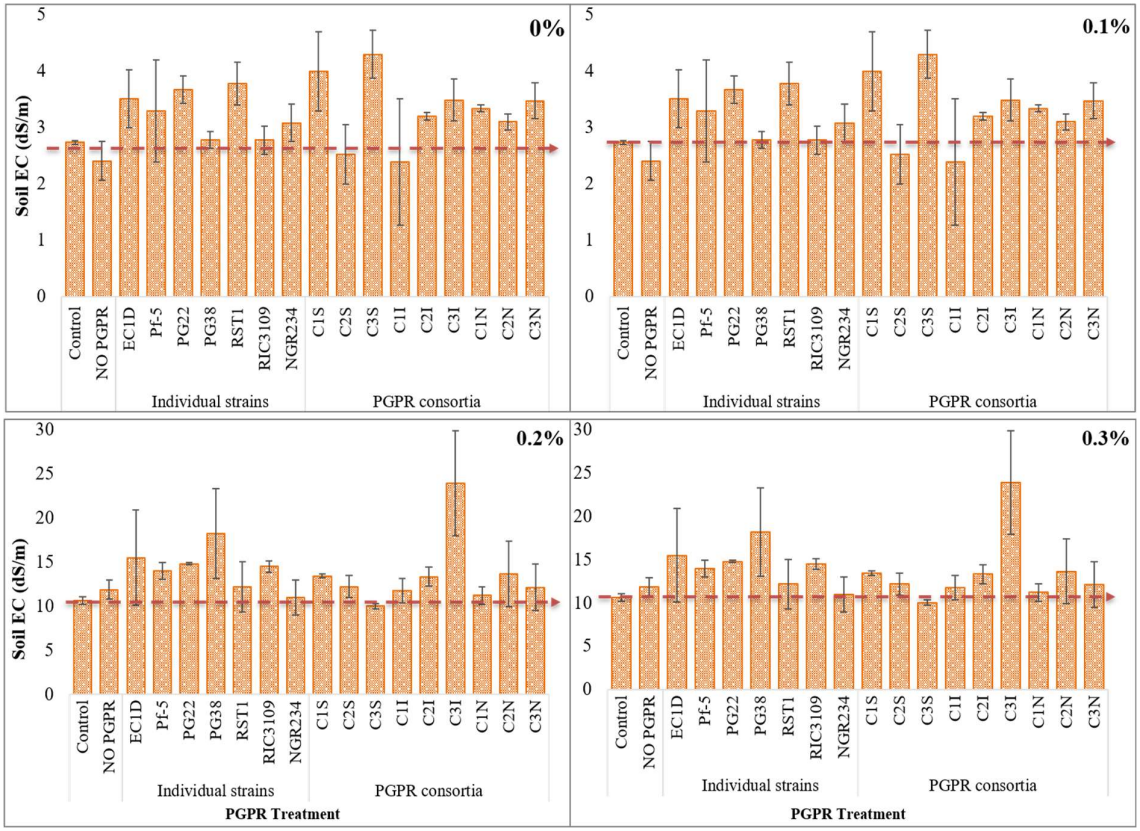


Figure 4.6: Soil electrical conductivity (EC) after plant growth at different salt concentrations, indicated in the top right corner, with individual PGPR and consortia. The dotted line indicates the EC content of soil before plant growth.

4.3.3.1.7. Leaf growth estimation

Leaves are the last sink for salt accumulation in cell vacuoles. It is the most sensitive part being in direct contact with atmosphere. For shoot development, leaf counts per plant and average leaf length of the oldest leaves were also measured (Table 4.5). The average number of leaves was 6 – 8 per plant 45 DAS, the highest number was observed in Pf-5 treatment. It decreased with increasing salinity in most of the PGPR treatments. The plants treated with EC1D, Pf-5 and NGR234 least detrimental effect of salt on leaves, while consortia e.g. C3S, C1I, and C3I showed good leaf counts at high salt concentrations.

Table 4.5: Mean leaf length and leaf numbers in plants treated with individual PGPR and consortia under increasing salt stress.

		Mean Leaf length				No. of leaves per plant			
	Salt (%)	0	0.1	0.2	0.3	0	0.1	0.2	0.3
No PGPR		6.9± 0.5	6.2± 0.7	4.7± 0.7	0.0± 0.0	7.3± 1.7	5.8± 1.8	4.6± 1.1	0.0± 0.0
Individual strains	EC1D	6.3± 1.0	6.2± 0.7	4.0± 1.5	4.0± 1.1	5.3± 2.1	5.5± 2.4	4.7± 2.3	4.3± 2.3
	Pf-5	6.3± 0.6	5.4± 0.4	3.8± 0.9	4.0± 0.0	8.5± 2.2	5.4± 2.2	5.0± 3.0	4.3± 0.6
	PG22	5.6± 0.5	4.4± 0.7	1.7± 0.8	0.0± 0.0	6.6± 1.9	4.5± 1.2	2.5± 0.6	0.0± 0.0
	PG38	6.6± 0.5	6.3± 0.5	3.1± 1.0	2.0± 0.0	6.8± 1.9	4.8± 2.3	3.4± 1.6	2.0± 0.0
	RST1	5.6± 1.2	5.4± 1.0	2.0± 0.6	3.4± 1.3	5.6± 2.3	6.3± 1.3	2.2± 0.4	4.6± 2.3
	RIC3109	5.9± 0.8	5.0± 0.8	4.1± 1.3	0.0± 0.0	6.4± 2.1	6.2± 2.1	4.1± 1.6	0.0± 0.0
	NGR234	6.5± 0.7	5.3± 0.8	4.2± 1.1	4.8± 1.1	6.0± 1.4	5.8± 2.1	4.0± 2.1	4.7± 1.4
PGPR Consortia	C1S	7.3± 0.7	5.5± 0.4	4.0± 0.0	1.3± 0.6	5.8± 1.6	4.9± 2.0	2.5± 1.0	2.0± 0.0
	C2S	6.7± 0.4	5.9± 1.0	3.7± 0.4	3.1± 1.0	5.7± 1.5	4.4± 2.1	4.3± 1.3	2.8± 1.4
	C3S	6.0± 1.0	5.5± 0.8	4.0± 0.0	2.3± 0.4	7.4± 1.2	4.7± 2.1	4.0± 5.7	4.0± 1.4
	C1I	6.2± 0.6	5.5± 0.8	4.0± 0.5	2.3± 0.7	7.5± 2.4	4.7± 1.0	4.0± 2.1	4.0± 1.5
	C2I	6.8± 0.5	6.0± 0.0	4.8± 0.8	3.1± 0.6	6.9± 1.2	5.8± 1.8	4.0± 1.7	2.9± 0.9
	C3I	6.0± 0.7	5.5± 0.5	2.0± 0.0	2.3± 0.6	5.8± 1.8	6.0± 2.1	4.0± 1.5	4.5± 1.7
	C1N	6.7± 1.5	6.0± 0.0	1.8± 0.0	0.0± 0.0	5.5± 0.6	5.0± 0.4	2.0± 0.3	0.0± 0.0
	C2N	6.2± 0.5	4.3± 0.7	2.0± 0.0	0.0± 0.0	7.0± 2.2	5.6± 0.9	4.0± 1.5	0.0± 0.0
	C3N	7.2± 0.3	4.6± 0.8	3.3± 0.4	0.0± 0.0	6.7± 1.5	4.3± 1.4	2.7± 1.2	0.0± 0.0

4.3.3.2. Biochemical parameters for plant growth and stress alleviation

4.3.3.2.1. Total proteins

The protein content was found to decrease in the salt stress in uninoculated plants. The individual strains Pf-5, PG22 and RST1 and consortia C1I, C2I and consortia with NGR234 enhanced protein content of the plant leaves (Figure 4.7).

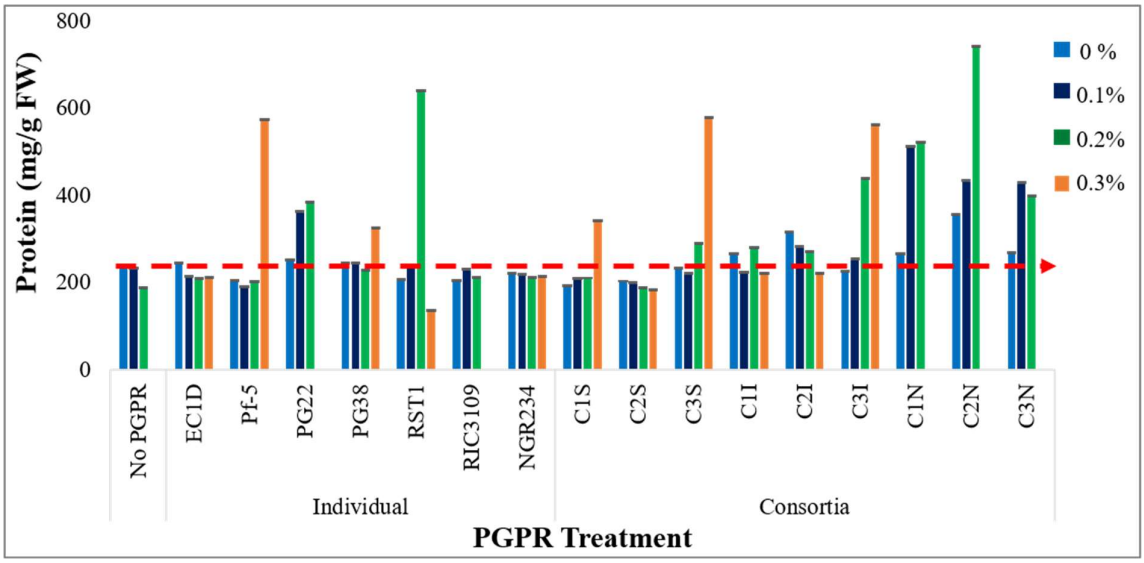


Figure 4.7: Total protein content from leaf tissue in plants treated with individual PGPR and consortia under increasing salt stress. The dotted line indicates maximum protein content in no PGPR control set.

4.3.3.2.2. Proline Content

The leaf proline content was enhanced under salt stress up to 10-folds at 0.2% salinity in uninoculated plants, however in presence of PGPR proline content was enhanced to much higher values. As seen in the Figure 4.8, pseudomonads and RST1 treatment led to high proline accumulation under salt stress, the maximum being in PG22.

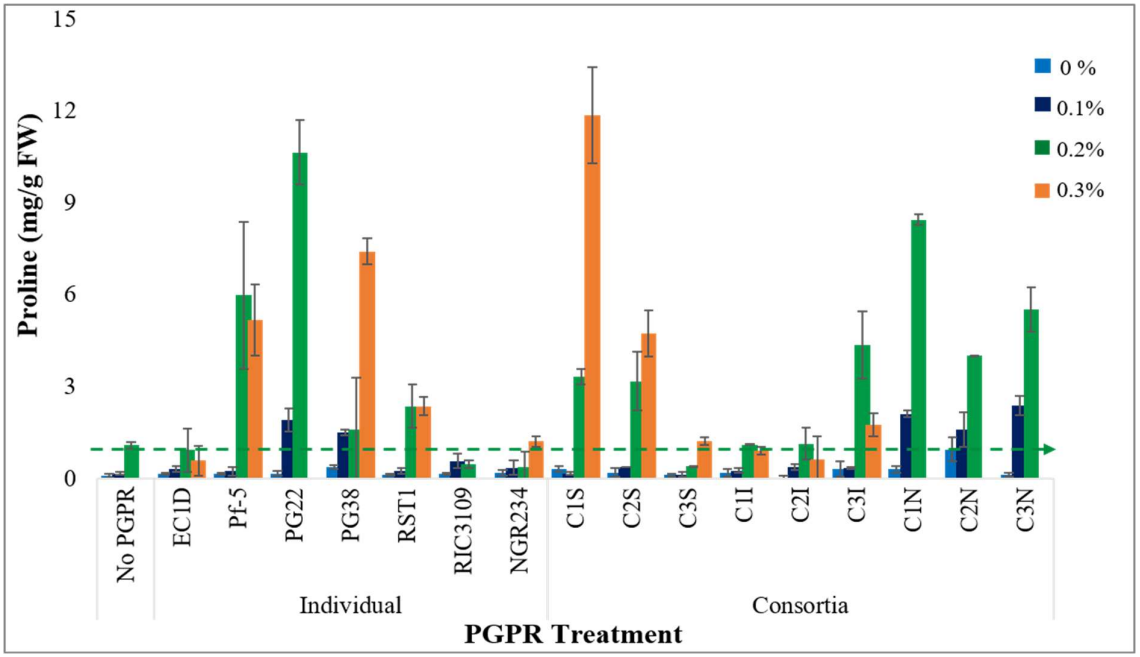


Figure 4.8: Total leaf proline content in plants treated with individual PGPR and consortia under increasing salt stress.

Among consortia, the highest proline content was observed in C1S treated plants at 0.3% salinity while C3I, C1N, C2N and C3N under 0.2% salt stress.

4.3.3.2.3 Total soluble sugars (TSS)

Total sugar content in uninoculated plants was found to decrease with increasing salinity (Figure 4.9). An increase in TSS was observed under salt stress in PG22 (2-folds) and several consortia.

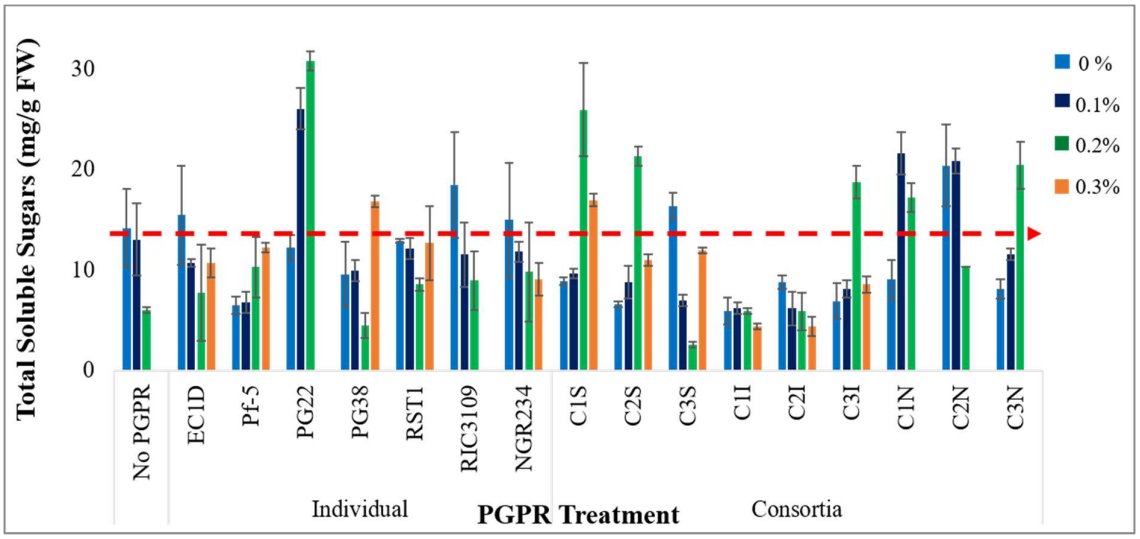


Figure 4.9: Total soluble sugar content from leaves in plants treated with individual PGPR and consortia under increasing salt stress. The dotted line indicates maximum sugar content in no PGPR control set.

4.3.3.2.4. Carotenoids

Carotenoids play an important role for oxygen diffusion within the leaf tissue during the process of photosynthesis and are also antioxidant agents that protect the plants against ROS. Its production increased naturally in the plants under stress condition without any PGPR (Figure 4.10). However, application of PGPR treatment led to higher carotenoid production in plants without stressed condition with the exception of EC1D. Plants treated with C3I and C3N showed increase in carotenoid production at all salt concentrations when compared to no PGPR control sets.

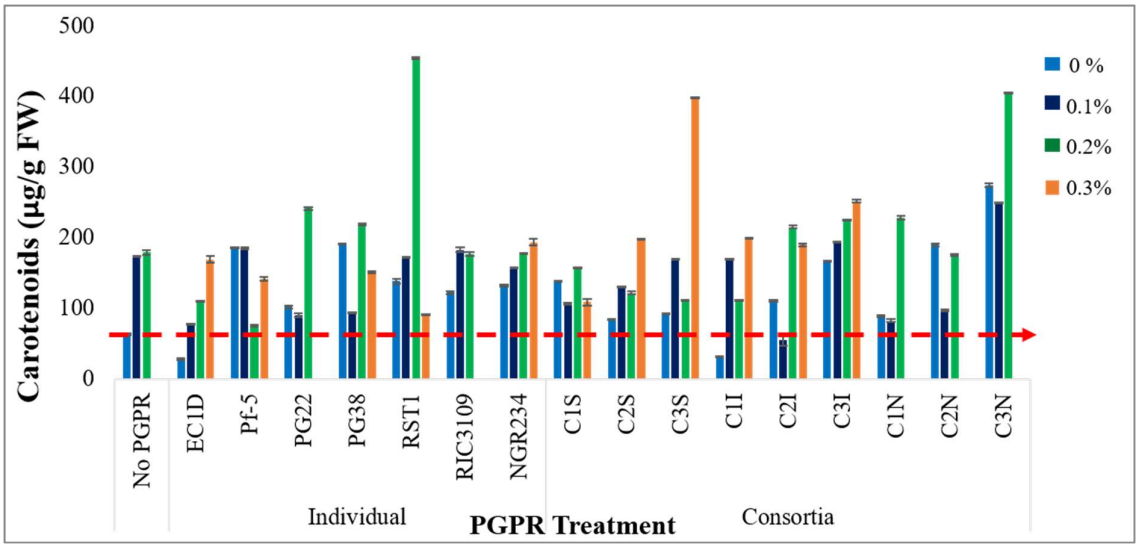


Figure 4.10: Leaf carotenoid content in plants treated with individual PGPR and consortia under increasing salt stress. The dotted line indicates maximum carotenoid content in no PGPR control set.

4.3.3.2.5. Chlorophyll content

The concentration of chl a was least affected among most of the plants under control and salt stressed condition (data not shown). The concentration of chl b hugely varied based on salt stress and the PGPR treatment. The chl components were analysed as the ratio of chl a/b which increased under saline stress in uninoculated plants. When compared to no PGPR control plants, this ratio was reduced in the plants treated with EC1D (Figure 4.11). Pf-5, C2N and C3N treated plants showed increase in the chl b content with increasing salinity.

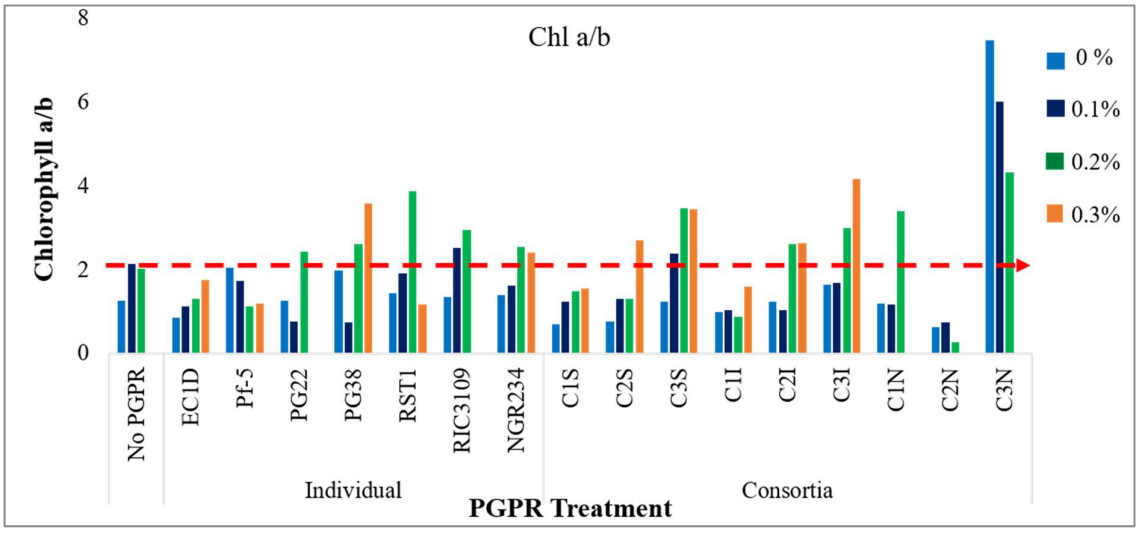


Figure 4.11: Chlorophyll a/b ratio in plants treated with individual PGPR and consortia under increasing salt stress. The dotted line indicates maximum value in no PGPR control set.

Among all the treatments, Pf-5 and PG22 caused an increase in the total chl content with increasing salinity, the highest being at 0.3% salt stress in Pf-5 (Figure 4.12).

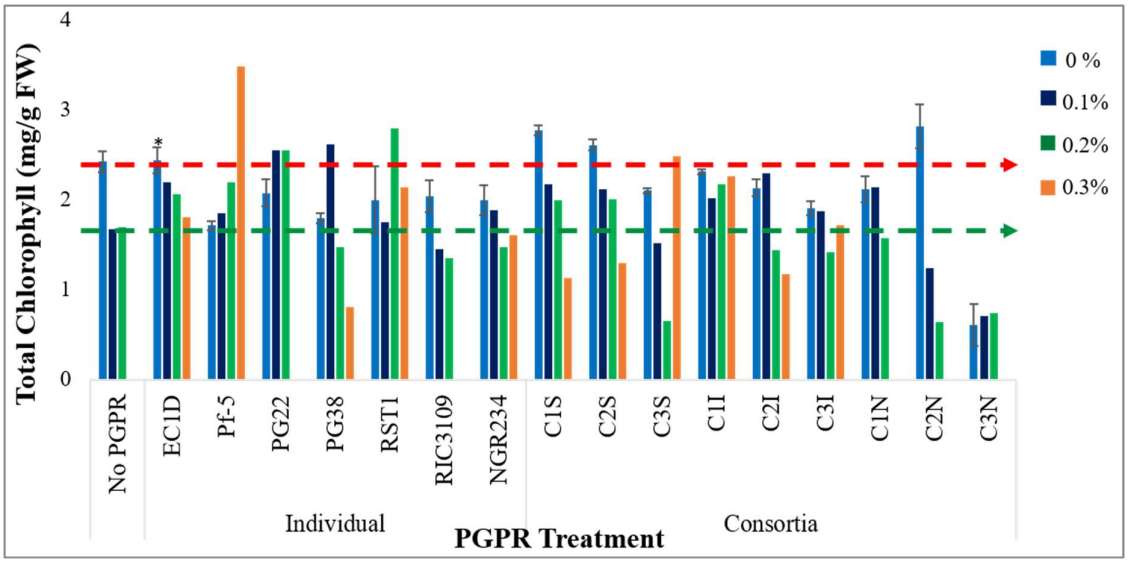


Figure 4.12: Total chlorophyll content in in plants treated with individual PGPR and consortia under increasing salt stress. The dotted line indicates maximum protein content in no PGPR control set.

4.3.4. Tolerance towards Biotic stress due to PGPR treatment

Although the fungus was pre-mixed in soil before sowing, the symptoms of *F. udum* infection started appearing after two weeks of plant growth; most of the survived plants showed healthy growth up till then. The first of the symptoms observed was the loss of shoot turgidity followed by wilting and chlorosis. As observed in Figure 4.13, the plant growth did not corelate to disease incidence at this stage of growth, and there were no significant differences observed.

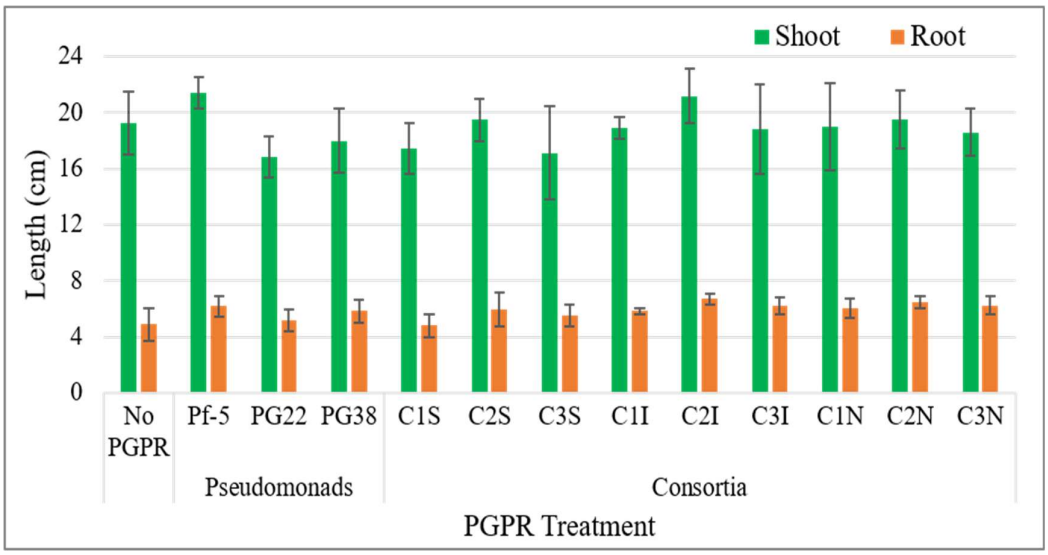


Figure 4.13: Physical growth parameters for plant treated with individual PGPR and consortia in presence of F. udum.

The disease incidence was calculated based on the number of infected plants or those showing either wilting or chlorosis.

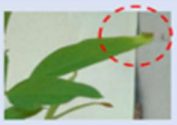
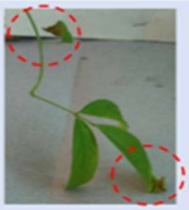
The plants without PGPR treatments showed highest disease incidence with most of the plants showing infection symptoms as shown in the Table 4.6. Among the individual strains, PG22 inoculated plants appeared healthiest with least wilted leaves and chlorosis. Plants inoculated with Pf-5 and PG38 showed wilting along with 2-3 spots of chlorosis on the leaves while PG38 treated plants showed least effect on stem turgidity.

Table 4.6: Biotic stress of F. udum in plants in no PGPR control and plants inoculated with individual PGPR strains. The portions encircled in red show the infection in plants.

PGPR Treatment	Shoot Turgidity	Wilting	Veinal Chlorosis	Disease incidence
No PGPR (Control)				83.33
Pf-5				33.33
PG22		--		14.29
PG38				33.33

As seen in Table 4.7, the plants treated with consortia C3S, C3I and C1N showed improvement in growth and reduced disease symptoms and were more effective in reducing disease incidence as compared to individual PGPR strains.

Table 4.7: Biotic stress of F. udum in plants inoculated with PGPR consortia. The portions encircled in red show the infection in plants.

PGPR Treatment	Shoot Turgidity	Wilting	Veinal Chlorosis	Disease incidence
C1S				33.33
C2S				57.14
C3S				16.67
C1I				50.00
C2I		--		28.57
C3I		--	--	14.29
C1N		--		14.29
C2N				42.86
C3N				28.57

4.4. Discussion

An effective microbial inoculant should have the ability to alleviate multiple stress factors including biotic and abiotic stresses which can be achieved by application of a multispecies consortium. A multispecies consortium comprising rhizobia with non-rhizobial strains such as *Pseudomonas* sp. (H. Kumar et al., 2010; Molina-Romero et al., 2021) and/or *Enterobacter* sp. (A. Kumar et al., 2017) as a consortium have been reported previously for providing multiple PGP benefits (Menéndez & Paço, 2020), and stress tolerance against multiple factors (Barra et al., 2016; Egamberdieva et al., 2017; Joshi et al., 2020). Several microbes in consortium with rhizobia have been reported to exert PGP effect in non-legume crops as well (Behera et al., 2021). Rhizobial co-inoculation with other PGP strains for the synergistic effects needs a careful evaluation as these combinations can improve not only growth and yield but also prove to be cost effective and highly efficient (Jaiswal et al., 2021).

In the present study, seven bacterial strains and their consortia (nine combinations), which have been previously evaluated for several PGP traits under different salt concentrations, were further assessed for the PGP activity against salt stress and fungal stress in pigeon pea plants as pot inoculation study. Plant growth assessment was performed based on the plant growth parameters (Appendix III) **shoot/root length** along with **biomass** content and moisture percentage. In control conditions the plants treated with the pseudomonads showed the highest **shoot length** percentage followed by NGR234. These PGPR as well as all the consortia, also led to **highest biomass** content.

Shu et al., (2017) have reported the negative impact of exogenous NaCl (150mM or 0.9% w/v) treatment which reduced 2-3 folds seed (soybean) germination which was in line with the present study. Bertrand et al. (2015), showed that salt stress caused decline in shoot and root biomass in alfalfa cultivars with increasing salt stress. Pre-treatment of seeds with different PGPR has been reported to promote seed (*Glycine max*.) germination and seedling growth (Bakhshandeh et al., 2020). Safari et al. (2018) have shown improved seed vigour and seedling emergence on pre-treatment of the seeds (wheat variety) with *P. fluorescence* strains, showing IAA and ACCd activity under salt stress (up to 0.36% NaCl or EC: 6dS/m). Here we observed that PG22 and PG38 treated plants showed ~2-fold increase in the **biomass content** as well as the **root moisture** content. Both of these strains have shown high amounts of **biofilm** production under salt stress. It was observed that the consortia prepared using Pf-5, RST1 and NGR234 showed **highest stress alleviation** in all the aspects of physical growth compared to no PGPR control under salt stress.

The **STI** provides represents the most reliable method for assessment of stress alleviation in the plants (Saghafi et al., 2019). As observed from the results, maximum effect of stress alleviation was in **root development**, among the plants treated with consortia, while the strains EC1D, PG22, PG38 and consortia C1S, C2I, C3N showed stress alleviation at higher salt concentrations.

Biomass allocation is an indication for the strategies undertaken to endure habitat stress (Li et al., 2021). Under control conditions the biomass allocation was highest in shoot tissue, but as the salt stress increased the ratio was completely reversed. The results were in line with the study reported by Ma et al. (2017), which showed increased exposure to saline conditions led to increased biomass allocation towards the root tissue which served as survival strategy in the plants against salinity. Similar results were observed in the study reported by Saghafi et al. (2019), which showed the PGP effect of *Rhizobium* inoculation on plant growth by **promoting biomass allocation wards root tissues**. PGPR strains PG22, PG38 and consortia C3S, C3N reduced this ratio in control conditions which indicated that these PGPR enhanced both shoot and root development in control conditions as well. EC1D and NGR234 were the only strains which helped reducing this ratio to the minimum by enhancing shoot growth under saline stress.

As reported by Wang et al. (2018), decreased irrigational practices in saline soil led to decreased leaf area index, while Benbrik et al. (2021) showed that deficient soils amended with PGPR consortium led to increased leaf number along with other growth parameters. In the present study, there was a significant decrease in the leaf numbers due to increasing salinity while the leaf size decreased significantly at 0.2% salinity. Similarly, Li et al., (2021) have also reported significant decreases in leaf area and thickness due to salinity effect in the regions with less water supply. The strains EC1D, Pf-5, NGR234 and consortia C3I decreased the effect of salt stress, where the leaf growth was least affected by salt stress.

The **biochemical** parameters assessed for stress alleviation included proline, carotenoid and sugar accumulation while protein and chlorophyll contents were analysed for growth assessment due to PGPR effect of the consortia treatment under salt stress. (Dong et al., 2017) have reported a 10-fold increase in proline concentration under salt stress due to *Bradyrhizobium* treatment; whereas Nawaz et al., (2020) have reported highest proline concentration under salt stress when treated with the PGPR in consortium (*Pseudomonas fluorescence*, *Bacillus pumilus* and *Exiguobacterium aurantiacum*) as compared to *Bacillus* individually (upto ~300%, as compared to 138%). This also corelates with our findings: The

pseudomonads, RST1 and all the consortia showed enhanced (~10-fold) proline concentration in the stressed conditions. The increase in proline content in the presence of PGPR treatment under salt stress indicates that the PGPR stimulated the plants to adopt 'compatible solute strategy', so as to avoid intracellular accumulation of toxic ions (Sultana et al., 2020). Mahmood et al., 2017; and Pan et al., 2019 have reported that the salt tolerance was achieved by decrease in proline content while the carotenoids increased under salt stress when inoculated with PGPR, which led to increased biomass content, nutrient acquisition and increase in the TSS.

Antioxidant and osmolyte accumulation are sensitive markers for abiotic stress in plants. Increased **carotenoid** production is associated with osmoregulation as well as it also enhances photosynthetic ability under salt stress (Ben-abdallah, 2018; Ke et al., 2019). (Khalifa et al., 2021) have shown that significant increase in the carotenoid and chlorophyll content along with inhibition of proline, which was observed as an important approach for stress alleviation in maize plants. This corelates with the present study, where enhanced carotenoid production by PGPR under salt stress, while the consortia showed maximum carotenoid production under salt stress.

The soluble **sugars** have been reported to increase in PGPR treated plants under salt stress (Mahmood et al., 2017). Azarmi et al. (2016) reported an increase in TSS content in the pistachio seedlings under salt stress, while PGPR treatment led to ~25% increase in its concentration. Pandey & Gupta (2020) showed that *Pseudomonas* treatment led to improved growth characteristics due to accumulation of osmolytes such as soluble sugar and proline which led to biotic and abiotic stress alleviation in plants. This is in line with the current results, where highest amount of TSS accumulation was observed in PG22. This, along with PG38 exhibited high salt tolerance along with multiple PGPR traits including siderophore, HCN and phosphate solubilization. Wang et al., (2016) have reported 2-fold increase in the sugar concentration in plants (*Lycopersicon esculentum*) treated with three strain consortia to alleviate abiotic stress.

Increased **protein** concentration under unstressed condition, too is an indication of PGPR effect correlating with increased crop yield. Wu et al. (2019) reported an increased concentration of soluble proteins and sugar in broccoli and cauliflower when subjected to salt stress (100 – 200mM; ~1% NaCl). Sultana et al. (2020) too have shown, enhanced protein synthesis in plants inoculated with the PGPR was associated with the IAA production ability of the strain, which also led to enhanced biomass production in plants. Our results are in line

with these findings, where the PGPR treatment showed increased protein content and TSS accumulation under salt condition, consortia C2I and C2N showed significantly high amount of protein synthesis in the plants under the stressed condition.

Salinity decreases the **photosynthetic** activity, which can be attributed to feed-back mechanism from the unused carbohydrates, chlorophyll degradation due to ROS accumulation, breakdown of chloroplast ultrastructure (Ilangumaran & Smith, 2017; Lan et al., 2019; Yao et al., 2021). Cheng et al. (2012) reported that chl content tended to decrease with increase in salt stress. They showed that the chlorophyll content was enhanced upto 1.5-folds when inoculated with *P. putida* strain as compared to the uninoculated plants under salt stress (250mM NaCl in hydroponics system).

Under stressed condition, plants signal the conversion of the chl b to chl a molecule which is an important indication of environmental stress in plants (Kumawat et al., 2021). Zhu et al. (2019) have reported reduced photosynthetic rate and degradation of chlorophyll components in plants under salt stress. They have suggested that the salt stress accelerated transformation of chl b to chl a which could help increase the photosynthetic efficiency. This corelates with the present study, where an increase in the chl a/b ratio negatively corelated with total chl content as the salinity increased. The plants treated with consortia showed ~2-fold increase in the chl a/b ratio.

Change in soil electrical conductivity after PGPR treatment, can be attributed to various mechanisms of salt stress alleviation by the PGPR. As suggested by (Arora et al., 2020) PGPR mediated osmotic stress tolerance involves modulation of salt tolerance genes involved in establishing the ionic equilibrium in plants which helps avoid the desiccation and flaccidity caused by the salt stress in plant cells. Hassan & Bano (2019) have reported that application of a *Pseudomonas* strain led to decreased soil EC and pH by 6% in presence of tryptophan while the sodium absorption rate of soil (SAR) decreased by 10%, it also increased the absorption of various ions such as potassium (K) and nitrates, while increasing the plant height, chlorophyll content and leaf proline as well. It corelated to the present study where C2S treatment led to 10% decline in the soil EC at 0.1% salinity while enhancing plant biomass content. Therefore, PGPR treatment can lead to decreased soil EC value or decrease its absorption into the plant cells by ion exclusion principle all of which lead to alleviation of salt stress, while the major goal remains to be supporting the plant growth by stimulating biomass and osmolyte production.

Kim et al. (2014) reported an *Enterobacter* isolate with ACCd and IAA traits that caused PGP effect in the tomato plants by enhancing biomass accumulation (~50%) at 200mM salt (1.15% NaCl) stress in soil. The *Enterobacter* strain **EC1D** has been previously reported for heavy metal resistance in plants via ACC deaminase (ACCd) activity (Subrahmanyam et al., 2018). In the present study, plants treated with EC1D individually showed growth improvement only under salt stress.

Orozco-Mosqueda et al. (2019) showed at 0.2M salt (1.15% NaCl) stress ***Pseudomonas*** treatment significantly enhanced the physical growth attributes and chlorophyll content compared to control set under salt stress. Wang et al. (2016) have reported that the rhizobium symbiosis can improve the salt tolerance (200mM or 1.15% NaCl) in *Medicago sativa* based on survival rate, short term effects showing heightened increase in antioxidant activities were observed in the plants immediately followed by the shock treatment of salt stress.

Redondo-Gómez et al. (2021) showed that out of 5 different consortia, those with high **IAA and biofilm** producing capabilities demonstrated the maximum effect on growth of plants under salt stress. Egamberdieva et al. (2013) reported enhanced **biomass** content in *G. officinalis* under salt (50mM and 75mM NaCl) stress when inoculated with ***Rhizobia*** **in combination with** ***Pseudomonas*** sp. (~50% and 230% resp.) compared to only rhizobia treatment. Nawaz et al. (2020), reported salt sensitive variety of wheat plants inoculated with ***Pseudomonas*** **consortia** showed **increase in root length (~72%), proline content (~300%)**, The plants were subjected to salt stress (EC: 13.4 dS/m ≈ 0.3% soil salinity in the present study). Free **proline** was highest in consortium treatment than pseudomonas.

Dukare & Paul (2021), have reported alleviation of *Fusarium* infection in pigeon pea plants when inoculated with *Pseudomonas* strain, which possessed several biocidal compounds including antifungal metabolites, siderophore and HCN. Devi et al. (2018) have also reported suppression of *Fusarium* wilt in plants when treated with consortia having *Pseudomonas* which possessed IAA, siderophore, HCN along with several antimicrobial enzymatic characteristics. The pseudomonas under study were also found to demonstrate siderophore, HCN and IAA production along with phosphate solubilizing abilities under salt stress as well as in consortium with other PGPR strains. These strains also showed antifungal effect *in vitro* studies. In the pot studies, the pseudomonad PG22 and consortia C3I and C1N showed maximum alleviation of the biotic stress in pigeon pea plants.

Combining strains with superior salt tolerance is an effective strategy to improve crop productivity in salt affected regions. Several studies have extensively exploited and reported PGPR belonging to the genera *Pseudomonas, Rhizobium* and *Enterobacter* as potential candidates for the formulation of an effective biofertilizer as an alternative approach towards sustainable agriculture in saline environments (Egamberdieva et al., 2019).

In conclusion, the present study represents a systematic analysis of different PGPR strains as individual treatments or in different multispecies consortia for alleviation of salt stress on the salt sensitive legume, pigeon pea. Although the consortia having NGR234 could not promote plant growth at 0.3% salinity, the growth parameters were enhanced to the maximum in these. Consortia having RST1 also showed high PGP effect along with the pseudomonad strains. PG22 was good as a PGPR strain but not all the consortia having PG22 showed growth enhancement as compared to other consortia. Rhizobia RST1 and NGR234 showed stress alleviation as individual strains and as consortia also. Salt stress induced accumulation of high amount of proline and carotenoids which were accentuated to several folds in the PGPR treated plants. TSS accumulation was observed only at higher salt concentrations while proline content was enhanced in almost all the PGPR treatments including the no salt control sets. The strains PG22, PG38, RST1 and NGR234 in consortium showed most promising results have maximum potential to be utilized for the microbial inoculant preparation for stress alleviation in pigeon pea plants.