5. DISCUSSION

Plant growth promoting rhizobacteria (PGPR) enhance plant growth directly or indirectly; directly by fixing nitrogen, ammonification, solubilizing phosphate, mineralizing essential minerals; producing plant hormones like IAA, gibberellins and cytokinines or indirectly by inhibiting plant pathogens. They stimulate plant growth through mobilizing nutrients in soils, producing numerous plant growth regulators, protecting plants from phytopathogens by controlling or inhibiting them, improving soil structure and bioremediating the polluted soils by sequestering toxic heavy metal species (Ahamed and Kibret 2013). Application of PGPRs has been reported to improve health and productivity of different plant species under both normal and stressed conditions.

Groundnut being a leguminous crop, fix atmosphere nitrogen by associating with rhizobia. However, a number of plant growth promoting rhizobacteria like Pseudomonas fluorescens PGPR1, P. fluorescens PGPR2 and P. fluorescens PGPR4 have been reported to enhance growth, yield and nutrient uptake of groundnut by exhibiting multiple plant growth promoting traits (Dey et al. 2004).

As indiscriminate use of pesticides and chemical fertilizers have caused wide spread pollution and the level of toxicity has gone up many folds, use of biofertilizer and biocontrol agents gained momentum to manage agricultural production in eco-friendly and sustainable manner. Of late, there has been worldwide efforts to explore a wide range of rhizobacteria possessing novel traits like heavy metal detoxifying potentials (Ma et al., 2011a; Wani and Khan, 2010), salinity tolerance (Tank and Saraf, 2010; Mayak et al., 2004), biological control of phytopathogens and insects (Hynes et al., 2008; Russo et al., 2008; Joo et al., 2005; Murphy et al., 2000) along with the normal plant growth promoting properties such as production of phytohormones (Ahamed and Khan, 2012c), siderophore (Jahanian et al., 2012), 1-aminocyclopropane- 1-carboxylate, hydrogen cyanate (HCN), and
ammonia production, nitrogenase activity (Glick, 2012; Khan, 2005) and phosphate solubilization, etc. Eventually, a number of rhizobacteria \((Pseudomonas fluorescens, Enterobacter cloacae, Pseudomonas putida, Bacillus spp., Burkholderia cepacia, Gluconobacter diazotrophicus, etc.)\) have been reported to enhance growth, yield and nutrient uptake in many a crop species including groundnut.

Groundnut is a major oilseeds crop in India and the yield of groundnut is affected by salinity and the crop is susceptible to salinity. The problem is further complicated due to use of saline irrigation water in some part of this country. As development of transgenics tolerant to salinity is a distant dream and varieties tolerant to salinity is not available, alternative strategies are required to manage salinity to enhance productivity under salinity stress. Application of salinity tolerant PGPR as biofertilizer can alleviate salinity stress in groundnut.

However, reports on the application of plant growth promoting rhizobacteria in alleviation of salinity stress in groundnut are scanty.

Therefore, the present investigation was undertaken to identify potential salinity tolerant PGPR strains for enhancing groundnut, yield and nutrient uptake in groundnut under salinity stress.

5.1 Collection and purification and identification of rhizobateria tolerant to salinity

A total of five isolates (two rhizobia and three pseudomonads) were selected from the culture collection of Directorate of Groundnut Research. These bacterial cultures were generated under AMAAS project. The isolates were characterized morphologically, physiologically, biochemically for different traits following Bergey’s Manual of Systematic Bacteriology and following the description of Bossiss et al. (2000). As per above results obtained AMAAS 57, AMAAS 357 and BM6 were identified as \(Pseudomonas fluorescense\) biovar V, \(Pseudomonas putida\) biovar A and \(Pseudomonas fluorescense\) biovar III, respectively. Similarly, R29 was tentatively identified as \(Enterobacter\) sp. and R5 was found to be \(Pantoea\) sp.
The identity of the cultures were further authenticated by 16S rRNA sequencing and aligning the sequences with the NCBI 16S ribosomal rRNA databases which confirmed the identity as *Pseudomonas aeruginosa* AMAAS57, *Pseudomonas* sp. AMAAS357, *Pseudomonas aeruginosa* BM6, *Enterobacter* sp. R29, *Pantoea dispersa* R5. The differences in the identity of the isolates might be due to less number of traits taken into consideration in biochemical traits. However, identity based on 16S rRNA would be more authentic because of the conserve nature of the 16S rRNA sequences among the domain of life of prokaryotes.

Characterisation of the isolates for tolerance of salinity revealed that whereas *Pseudomonas aeruginosa* AMAAS57 and *Pseudomonas aeruginosa* BM6 were tolerant to 5% of NaCl, *Pseudomonas* sp. AMAAS357, *Enterobacter* sp. R29 and *Pantoea dispersa* R5 were tolerate upto 4%, 10% and 8% of NaCl, respectively. The level of tolerance in the present lot of the cultures was consistent with earlier reports that rhizobacteria and Gram negative bacteria could tolerate moderate level of salinity (Chang 2007; Cheng et al. 2007; Mayak et al. 2004a; Mayak et al. 2004b; Nadeem et al. 2007; Saravanakumar and Samiyappan 2007).

5.2. Detection, characterization, and quantification of plant growth promoting traits of the rhizobacteria

The isolates were characterized for plant growth promoting traits like production of IAA like substances, phosphate solubilization, and production of siderophore and ammonia. In addition, isolates were also screened for production of ligninase and cellulase besides biocontrol activities like antifungal antibiotics, fluorescent pigments and production of HCN as described earlier (Pal et al. 2001; Dey et al. 2004).

Studies revealed that *Pseudomonas aeruginosa* AMAAS 57 and *Pseudomonas aeruginosa* BM6 were having multiple PGP traits i.e. production of IAA (29 ug/ml, and 15.53 ug/ml, respectively), solubilization of tri-calcium phosphate (38.40 and 33.67 mg of TCP/25 ml broth, respectively), HCN, ammonia, besides antifungal activity against soil-borne fungal pathogens of groundnut like *S. rolfsii*, *A. flavus*, and *A. niger*. 125
Plant growth promoting fluorescent pseudomonads have been reported to exhibit multiple plant growth promoting traits like phosphate solubilization, production of siderophore, HCN, antifungal metabolite, fluorescent pigments, IAA like substances, ammonia, etc. to enhance plant growth directly or indirectly upon inoculation (Glick et al. 1995; Glick et al. 2005; Pal et al. 2001, Dey et al. 2004, Ahemad et al. 2013, Chang 2007; Cheng et al. 2007; Mayak et al. 2004a; Mayak et al. 2004b; Nadeem et al. 2007; Saravanakumar and Samiyappan 2007; Jahanian et al., 2012).

The rhizobacteria of the present studies particularly *Pseudomonas aeruginosa* AMAAS 57 and *Pseudomonas* sp. AMAAS357 showed tolerance to heavy metals like Cd and Co.

The potential of rhizobacteria in alleviating heavy metal toxicity (Ma et al., 2011a; Wani and Khan, 2010), and biotic stresses like insects and diseases (Hynes et al., 2008; Russo et al., 2008; Joo et al., 2005; Murphy et al., 2000) and abiotic stresses like salinity (Tank and Saraf, 2010; Mayak et al., 2004) has also been reported. Thus, present observation was consistent with the reported earlier observations.

The nodulation and nitrogen fixation traits have been restricted to the genus *Rhizobium*, *Sinorhizobium*, *Mesorhizobium*, *Azorhizobium*, *Photorhizobium*, *Bradyrhizobium*, *Alchorhizobium* and few other related genera (Franche et al. 2009). However, due to horizontal and vertical flow of genes in nature, of late many other genus and species of rhizobacteria including pseudomonads and enterobacteria have acquired the traits of nodulation and nitrogen fixation in legume crops. However, there were no reports about *Enterobacter* sp. and *Pantoea dispersa* become nodulating and nitrogen fixing strains of groundnut. The presence of *nifH* and *nodBC* genes in these two rhizobacteria viz. *Enterobacter* sp. R29 and *Pantoea dispersa* R5 were confirmed by PCR amplification. For *nod* genes, expected amplicons of 660 bp was obtained. But in case of *nif* genes, instead of expected amplicons of 360 bp, the *nif* fragment of *Pantoea dispersa* R5 gave an amplicon size of 400 bp whereas it was about 415 bp in case of *Enterobacter* sp. R29. The differences in amplicon length might be due to insertion, duplication, rearrangement in the genetic loci of *nif* genes in these organisms during the
process of evolution and acclimatization. This is the first report that *Pantoea dispersa* can have both nodulation and nitrogen fixation traits.

5.3 Evaluation of the effect of rhizobacteria onto seedling vigour of groundnut in *in vitro* and potted conditions

Presence of excess amount of salt in the growth media adversely affects growth and development of plants. Process such as germination, seedling growth and vigour, vegetative and other stages of plant growth are affected due to the stress (Sairam and Tyagi 2004). Attempts have been made to understand whether salinity stress tolerant rhizobacteria can alleviate salinity stress in groundnut. In the present studies, all the five isolates were tested in vitro in Petridish bioassay with germinating seedlings to ascertain their role in enhancing the seedling vigour with cultivar GG2 in stressed conditions.

The results revealed that primary root length was improved significantly over uninoculated control when the seedlings were inoculated with *Enterobacter* sp. R29. However, treatment of seedlings with other rhizobacteria did not influence the primary root growth except with *Pseudomonas aeruginosa* AMAAS57 wherein the primary root growth was reduced significantly. The secondary root length was improved significantly when inoculated with *Pseudomonas* sp. AMAAS357, *Pseudomonas aeruginosa* BM6 and *Enterobacter* sp. R29 without salinity stress. However, the number of secondary root was improved significantly only when seedlings were treated with *Enterobacter* sp. R29 and fresh weight of the seedlings was improved significantly only when inoculated with *Pseudomonas* sp. AMAAS357.

Evaluation of the rhizobacteria in alleviating the salinity stress in in vitro conditions revealed that there was non-significant impact of inoculation of PGPR on the primary and secondary root length of the seedlings of groundnut cultivar GG2 but there was improvements in the length of shoot and primary root when inoculated with *Enterobacter* sp. R29. Application of the PGPR isolates like *Pseudomonas* sp. AMAAS357 and *Enterobacter* sp. R29 significantly improved the secondary root length at 4 dSm⁻¹ and 2 dSm⁻¹,
respectively as compared to uninoculated treatment and without application of NaCl. At ECe of 6 dSm⁻¹, germination percentage was increase with the application of Enterobacter sp. R29 as compared to uninoculated control. The number of secondary roots also increased appreciably when inoculated with Enterobacter sp. R29 upto 4 ECe and Pantoaea dispersa R5 at 4 and 6 ECe though the effect on fresh weight of the seedlings was non-significant.

To confirm the results obtained in vitro, experiments were conducted in potted conditions. Application of Pseudomonas aeruginosa BM6 significantly enhanced root length, pod yield, shoot dry weight and plant biomass as compared to uninoculated control without salinity. Significant improvement in the pod yield was also obtained with the inoculation of Enterobacter sp. R29.

When combined effect of application of rhizobacteria and salinity was evaluated, it was found that there was significant improvements in the shoot length and pod yield of groundnut, cultivar GG2 at different level of salinity and inoculation of rhizobacteria. Most pronounced impact was noticed when Pseudomonas aeruginosa BM6 was applied along with different level of saline water. It was found that application of BM6 significantly enhanced the shoot length at 0, 2, and 6 EC of saline irrigation water as compared to uninoculated control at the same level of salinities. In general, application of salinity tolerant Enterobacter sp. R29, Pseudomonas aeruginosa BM6 and Pantoaea dispersa R5 also enhanced the root length at 0, 2, and 4 EC of irrigation water as compared to their respective uninoculated controls. Application of all the salinity tolerant rhizobacteria enhanced the pod yield of groundnut upto 2 EC of irrigation water as compared to their respective uninoculated control. In addition, Pseudomonas aeruginosa BM6 also enhanced the pod yield of groundnut, cultivar GG2, at 4 EC of saline water as compared to corresponding uninoculated control.

Similar trend was also obtained in summer 2011 with few exceptions.

Application of PGPR have already been reported to alleviate salinity stress and promotes plant growth under saline conditions (Chang 2007; Cheng et al. 2007; Mayak et al. 2004a; Mayak et al. 2004b; Nadeem et al. 2007; Saravanakumar and Samiyappan 2007). Mayak et al. (2004a) reported that an ACC-deaminase-containing PGPR, Achromobacter piechaudii ARV8,
can significantly lowered ethylene production and increased biomass production of tomato plants grown in the presence of up to 172 mM NaCl. Recently, Nadeem et al. (2007) reported that several strains of ACC-deaminase-containing PGPR significantly increased plant height, root length, total biomass and grain yield in maize under salt stress. Saravanakumar and Samiyappan (2007) reported that ACC-deaminase containing *Pseudomonas fluorescens* strain TDK1 significantly promoted plant growth in groundnut seedlings under salt stress relative to the strains lacking ACC-deaminase and untreated control treatments. Cheng et al. (2007) also found that inoculation of *Pseudomonas putida* UW4 containing ACC-deaminase significantly improved shoot biomass of canola, whereas inoculation of the mutant strain of UW4 18 lacking ACC-deamminase activity (UW4/AcdS-) did not promote plant growth. That indicated the involvement of ACC deaminase in alleviation of salinity stress in plant. Application of salinity-resistant PGPR has been found to alleviate NaCl stress in tomato (Tank and Saraf 2010). However, it is reported here that besides organisms belonging to pseudomonads, other rhizobacteria like *Enterobacter* sp. and *Pantoea dispersa* can also alleviate salinity stress in groundnut by mechanisms other than ACC-deaminase.

The present investigation with five salt tolerant rhizobacteria viz. *Pseudomonas aeruginosa* AMAAS57, *Pseudomonas* sp. AMAAS357, *Pseudomonas aeruginosa* BM6, *Enterobacter* sp. R29 and *Pantoea dispersa* R5 confirmed the above observations. With few exceptions, application of *Pseudomonas aeruginosa* BM, *Pseudomonas aeruginosa* AMAAS57 and *Pantoea dispersa* R5 improved the pod yield of groundnut cultivar GG2 significantly at different levels of salinity as compared to their respective control. Besides alleviations of salinity stress, there might have been improvement in nutrient uptake and also in the production of plant growth hormones by PGPR strains.

### 5.4 Alleviation of salinity stress in groundnut by application of PGPR in potted conditions

The experiment conducted in potted conditions in two summer seasons of 2010 and 2011 with cultivar GG2 and four level of EC of irrigation water (0, 2, 4 and 6 dS/m) indicated that there was improvement in shoot and root
length, pod yield, nodule number and shoot dry weight with few exceptions when inoculated with PGPR isolates. However, significant improvement of root length, pod yield, shoot dry weight and plant biomass was achieved with inoculation with *Pseudomonas aeruginosa* BM6 as compared to uninoculated control without salinity. There was significant improvement in the pod yield due to inoculation of *Enterobacter* sp. R29. However, effect of inoculation of salinity tolerant rhizobacteria on nodule number and mass and root dry weight was non-significant. It was found that there was significant reduction in pod yield, nodule number and mass, shoot dry weight and plant biomass from without application of NaCl to the application of irrigation water with salinity level of 6 dSm\(^{-1}\) in groundnut, cultivar GG2. When combined effect of application of rhizobacteria and salinity was evaluated, it was found that there was significant improvements in the shoot length and pod yield of groundnut, cultivar GG2 at different level of salinity and inoculation of rhizobacteria. Inoculation of groundnut with *Enterobacter* sp. R29 at 2 and 4 EC of saline water significantly improved the shoot length at harvest over application of 0, 2 and 4 EC irrigation water alone without inoculation. Application of *Pseudomonas aeruginosa* BM6 significantly enhanced the shoot length at 0, 2, and 6 EC of saline irrigation water as compared to uninoculated control at the same level of salinities. In general, application of salinity tolerant *Enterobacter* sp. R29, *Pseudomonas aeruginosa* BM6 and *Pantoea dispersa* R5 also enhanced the root length, pod yield and biomass at 0, 2, and 4 EC of irrigation water as compared to their respective uninoculated controls. However, enhancement in pod yield was achieved upto 2 EC of irrigation water as compared to their respective uninoculated control. Enhancement in shoot and plant biomass was also noticed in inoculated rhizobacterial treatments as compared to their corresponding control.

Application of salt tolerant rhizobacteria *Pseudomonas*, *Flavobacterium*, and *Enterobacter* strain in maize significantly promoted the growth and yield of maize as compared to non-inoculated control. *Pseudomonas fluorescens* increased plant height, biomass, cob yield, grain yield, 1000 grain mass, and straw yield of maize up to 29%, 127%, 67%, 60%,
17%, and 166%, respectively, over the control (Nadeem et al. 2011). Under stress conditions, more N, P, and K uptake and high K+/Na+ ratios were recorded in inoculated plants compared with the control. ACC demainase activity of rhizobacteria has been assumed as the trait responsible for alleviation. However, present studies indicated that trait of rhizobacteria other than ACC deaminase might also be useful in alleviating salinity stress and enhance growth and yield.

5.5 Understanding the basis of alleviation of salinity stress in groundnut by application of salt tolerant rhizobacteria: Physiological and Biochemical traits

Plant growth promoting rhizobacteria have been reported to alleviate salinity stress by modulating the enzymatic pathways of ROS system, helping in accumulation of compatible solutes, regulation in stomatal opening and Na+/K+ ratio, modulating nutrient uptake and mobilization, regulation in photosynthesis, regulating root growth and development by producing IAA and other hormones and ACC demainase activity, production of antioxidants besides modulating the antioxidant pathways (Ahmad et al. 2013; Uma Maheshwari et al. 2013; Pedrosa et al. 2011; Hossain et al. 2011; Yao et al. 2009; Ahmad et al. 2008; Bacilio et al. 2004).

Inoculation of Pseudomonas putida Rs-198 in cotton enhance growth and provided protection against salt stress (Yao et al. 2009) as evident from increase the rate of germination and the healthy stand of cotton by 23.8% and 30.7%, respectively. Further analysis showed that Rs-198 could increase the absorption of the Mg$^{2+}$, K$^{+}$ and Ca$^{2+}$ and decrease the uptake of the Na$^{+}$ from the soil and also improve the production of endogenous indole acetic acid (IAA) content and reduce the abscisic acid (ABA) content of cotton seedling under salt stress.

In the present investigation, five plant growth promoting rhizobacteria viz. Pseudomonas aeruginosa AMAAS57, Pseudomonas sp. AMAAS357, Pseudomonas aeruginosa BM6, Enterobacter sp. R29 and Pantoea dispersa R5 were studied to evaluate whether there was any alleviation of salinity stress in groundnut, cultivar GG2, by imposing different levels of salinity as
compared to uninoculated control in potted conditions. The biochemical and physiological parameters like electrolyte leakage, relative water content, SLA, chlorophyll content, total carbohydrates, total phenol, OD phenol, free amino acids, proline and $H_2O_2$ contents, etc. were studied along with monitoring of enzyme activities of GR, APX, SOD, PPO, Catalase, peroxidase, etc. linked to salinity stress were monitored.

Results indicated that application of salinity tolerant rhizobacteria reduced the electrolyte leakage when groundnut was cultivated with 6 dSm$^{-1}$ saline water as compared to uninoculated control except *Enterobacter* sp. R29 which could reduced the electrolyte leakage upto 4 dSm$^{-1}$ salinity only. In case of RWC, inoculation of plant growth promoting rhizobacteria like *Pantoea dispersa* R5, the RWC increased with the increase in salt stress (69.30%, 72.44%, 81.77% and 83.67%, respectively at 0, 2, 4 and 6 EC of irrigation water) and with *Pseudomonas* sp. AMAAS57 and *Pseudomonas aeruginosa* BM6, RWC decreased in 2dSm$^{-1}$ (AMAAS57: 68.96% to 87.82% and BM6 87.82% to 81.21%) and then increased in 4 dSm$^{-1}$ (AMAAS57: 74.49% and BM6: 83.4%) and again decreased at 6 dSm$^{-1}$ salt stress (AMAAS57: 52.46% and BM6: 79.57%). In general, only nominal improvement in RWC was noticed upto 2 EC of saline water application when inoculated with *Enterobacter* sp. R29, *Pantoea dispersa* R5 and *Pseudomonas aeruginosa* BM6. Evaluation of specific leaf area (SLA) revealed that with increase in salinity, there was drastic decrease in SLA in uninoculated control. However, there was enhancement in the SLA when inoculated with all the plant growth promoting rhizobacteria upto 6 EC of saline water application except *Enterobacter* sp. R29 which improved the SLA upto 4 EC. When inoculated with *Pseudomonas aeruginosa* AMAAS57, the leaf area gradually decreased upto 4 dSm$^{-1}$ ($5.61 \text{ cm}^2$, $4.37 \text{ cm}^2$ and $3.62 \text{ cm}^2$) and then increased in 6dSm$^{-1}$ dose ($4.56 \text{ cm}^2$). Estimation of chlorophyll a and chlorophyll b and total chlorophyll were gradually decreased in uninoculated control and increased with inoculation of *Pseudomonas aeruginosa* AMAAS57, *Pseudomonas aeruginosa* BM6 and *Pseudomonas* sp. AMAAS357 at 4 EC and 6 EC of irrigation water.
Evaluation of total carbohydrates in the leaf of groundnut, cultivar GG2 indicated that there was enhancement in the contents of total carbohydrates when inoculated with all the five rhizobacteria as compared to uninoculated control but without the presence of salinity. But with increase in salinity, the contents of total carbohydrates improved at 2 EC of saline water application as compared to corresponding uninoculated control when inoculated with AMAAS57, AMAAS357 and BM6 isolates only.

There was enhancement in the content of phenol when inoculated with rhizobacteria as compared to corresponding control except inoculation of *Pseudomonas aeruginosa* BM6 application of upto 4 EC of saline water. However, inoculation of Enterobacter sp. R29 also enhanced the phenol content when 6 EC irrigation water was applied as compared to corresponding control (Figure 12). In general, the phenol concentration increased with the increase in salt stress in control (0.3 μg/mg to 0.37 μg/mg).

Application of *Pseudomonas aeruginosa* AMAAS57 and *Pseudomonas* sp. AMAAS357 enhanced the content of free amino acids at 2 and 4 EC of saline water application as compared to corresponding uninoculated controls. The content of proline was enhanced in the leaf of groundnut, cultivar GG2 when inoculated with *Pseudomonas aeruginosa* AMAAS57, *Pseudomonas* sp. AMAAS357 and *Pseudomonas aeruginosa* BM6 when irrigation water of 2 EC was used as compared to corresponding uninoculated control. However, application of *Pantoea dispersa* R5 and *Pseudomonas aeruginosa* AMAAS57 also enhanced the proline content but without salinity.

The content of total protein gradually decreased with increase in salt stress (4.12μg/mg to 3.21μg/mg) except 4 dSm⁻¹, in uninoculated control. Application of Enterobacter sp. R29 enhanced the protein concentration at 2 dSm⁻¹ (3.9μg to 4.66μg/mg) but in the other two doses i.e. 4 dSm⁻¹ (3.8μg/mg) and 6dSm⁻¹ (4.09μg/mg), the concentration was more or less same as obtained without application of saline water. Application of *Pantoea dispersa* R5, increased the protein content with increasing in salt concentration (2.88μg/mg to 4.95μg/mg), and at 2 dSm⁻¹ (5.8 μg/mg), the protein concentration was higher than the other three doses. However, application of
*Pseudomonas aeruginosa* BM6, increased the concentration of protein at 2 dSm⁻¹ and then decreased in other salt doses.

Overall assessment of physiological and biochemical parameters revealed that there was drastic decrease in the SLA, RWC, chlorophyll, total protein, free amino acids and increase in proline, electrolyte leakage, phenol and OD phenol content with increase in salinity imposed to groundnut crop. However, with few exceptions, application of plant growth promoting rhizobacteria particularly, *Pseudomonas aeruginosa* BM6, *Pseudomonas aeruginosa* AMAAS57 and *Pantoea dispersa* R5 significantly enhanced SLA, RWC, total protein, chlorophyll, free amino acids, accumulation of prolines, total proteins, total carbohydrates and also enhanced the content of phenol and OD phenol in groundnut, cultivar GG2 and alleviated the stress of salinity in groundnut which was also reflected from the enhancement of growth and yield parameters of groundnut.

Experiments in groundnut also revealed that imposition of salinity triggered proline synthesis in leaves (Hossain et al., 2011) along with reduction in shoot dry matter, relative water content, chlorophyll and K⁺ with increasing salinity. In contrast, Na⁺, hydrogen peroxide and proline contents increased with increasing salinity level. Ahmad et al. (2008) reported the possible involvement of activated oxygen species as the mechanism of damage by NaCl stress in pea. The imposition of salt significantly increased the activities of the antioxidant enzymes like SOD and APX, GR and DHAR.

To understand the alleviation of the salinity stress by application of plant growth promoting rhizobacteria, the activity of polyphenol oxidase (PPO), glutathione reductase (GR), catalases, peroxidases, etc. in the leaves of groundnut, cultivar GG2 after imposition of salinity stress.

The activity of polyphenol oxidase enhanced in all inoculated treatments as compared to their corresponding uninoculated control when 6 EC of irrigation water was used in irrigation. However, application of *Pantoea dispersa* R5, *Pseudomonas* sp. AMAAS357 and *Pseudomonas aeruginosa* BM6 significantly enhanced the activity of PPO when 2 EC of irrigation water
was used as compared to their corresponding uninoculated controls. Application of rhizobacteria also enhanced the level of H₂O₂ as compared to corresponding uninoculated control in absence of salinity stress. However, when there was increase in salinity from 0 to 2 EC, application of Pantoea dispersa R5, Pseudomonas sp. AMAAS357 and Pseudomonas aeruginosa BM6 enhanced the level of H₂O₂ in leaf of groundnut, cultivar GG2, as compared to inoculation of other cultures and uninoculated control which indicated the level of detoxification of superoxide radicals produced in response to increase in salinity.

Further evaluation of the activity of glutathione reductase in the leaf of groundnut, cultivar GG2 revealed that inoculation of PGPRs like Enterobacter sp. R29, Pantoea dispersa R5 and Pseudomonas aeruginosa AMAAS57 enhanced the activity of the enzyme as compared to corresponding uninoculated control and other bacteria like Pseudomonas sp. AMAAS357 and Pseudomonas aeruginosa BM6 at 6 EC of application of saline water. So far as activity of peroxidaes are concerned, there was sharp decrease in the activity of peroxidase with increase in salinity (5U/min/gm to 1.2U/min/gm). But inoculation of plant growth promoting rhizobacteria like Enterobacter sp. R29, the activity was higher in 0 dSm⁻¹ then the activity decreased upto 4 dSm⁻¹ (8.1U/min/gm to 0.7U/min/gm) and again it was increased at 6 dSm⁻¹ (5.9U/min/gm) of irrigation water. Inoculation with Pseudomonas aeruginosa BM6 resulted in sudden increase in peroxidase activity at 2 dSm⁻¹ (from 9.2U/min/gm to 15.23U/min/gm) and then decreased at 4 dSm⁻¹ (5.4U/min/gm) and 6 dSm⁻¹ (5.6U/min/gm) but higher than the respective uninoculated control.

As speculated earlier about the enhancement in nutrient uptake, further analyses revealed that inoculated with Enterobacter sp. R29, Pantoea dispersa R5 and Pseudomonas sp. AMAAS357 at 4 EC as compared to inoculation of other isolates and corresponding uninoculated control. This was in addition to the alleviation of salinity stress by above described mechanisms like modulation in ROS system, enhancement in the SLA, RWC, conents of phenol, free amino acids, proline, etc. These observations confirms the earlier results obtained with other crops.
Similarly, mitigation of salt stress in wheat seedlings by a gfp-tagged *Azospirillum lipoferum* (Bacilio et al. 2004) indicated that colonization of wheat roots under 80 and 160 mM NaCl stress was similar to root colonization with this bacterial species under nonsaline conditions. Reduced plant growth (height and dry weight of leaves and roots) under continuous irrigation with 160 mM NaCl was ameliorated by bacterial inoculation with gfp-*A. lipoferum* JA4::ngfp15.

Application of plant growth promoting rhizobacteria (PGPR) influenced the antioxidant status, photosynthesis, mineral content and growth of lettuce (*Lactuca sativa* L.) (Han and Lee, 2005a) under different soil salinity conditions. Reduction in plant growth, photosynthesis, stomatal conductance, chlorophyll content, and mineral uptake compared to soil without salinity was noticed. But Inoculation with two PGPR strains, *Serratia* sp. and *Rhizobium* sp., into saline soils alleviated the salinity effects on the antioxidant enzymes ascorbate peroxidase (APX) and glutathione reductase (GR), along with those on photosynthesis, mineral content and growth.

It is also believed that salt stress elicit an effect on pigments, total soluble carbohydrates, free amino acids as well as total soluble proteins. Simultaneously, the levels of antioxidant compounds (glutathione, ascorbic acid, carotenoids, phenols, proline and glycine betaine) were changed in response to salt stress (Kattab, 2007) along with the increase in the level of antioxidant substances and by enhancing the activities of antioxidant enzymes (superoxide dismutase, SOD, phenol peroxidase and oxidase, GPX and POX as well as ascorbate peroxidase and oxidase, AXP and ASO).

**5.6 studying the population dynamics and establishment using molecular markers**

Molecular markers are widely used to study the establishment of inoculants strains in the rhizosphere of many crops species (Pal et al. 2000) as intrinsic antibiotic resistance patterns are not that authentic in counting the population. The molecular markers include Tn5:*lacZ*, Tn5:*gusA*, Tn5:*lacABC*, Tn5:*gfp*, Tn5:*rfp*, etc. (Pal et al. 2000).
To monitor the population of the inoculants strains and their rhizosphere competence and nodule occupancy, four each of the isogenic Tn5::gusA mutants of Enterobacter sp. R29 and Pseudomonas aeruginosa AMAAS57 were evaluated at two levels of salinity (0 and 2 EC of irrigation water). Evaluation of the nodule occupancy of Enterobacter sp. R29 by molecular marker like Tn5::gusA in groundnut at 45 DAS and at harvest indicated that at 45 DAS, the nodule occupancy varied from 49% (wild type) - 92% (mutants) at without application of salinity as compared to 21% (wild type) – 90% (mutants) when salinity was increased to 2 EC. This indicated that with increase in salinity there was decrease in nodule occupancy by R29. However, mutation improved the nodule occupancy at elevated salinity of 2EC as compared to wild type as evident from higher level of nodulation in mutants as compared to wild type Enterobacter sp. R29 at 45 DAS. However, evaluation at harvest revealed that there was 85-96% nodulation by wild type as compared to 100% in mutants. This might be due to polar effect on the regulatory elements (repressor) on nod functions due to insertion of Tn5::gusA.

Monitoring the population of the wild type and Tn5::gusA mutants of Pseudomonas aeruginosa AMAAS57 and Enterobacter sp. R29 and their mutants in the rhizosphere of groundnut, cultivar GG2 at 45 DAS and at harvest at 0 and 2 EC of salinity revealed that the population of wild type was much lower as compared to mutants without salinity in the rhizosphere at 45 DAS. However, with increase in salinity from 0 to 2 EC, the population of the wild type was better. However, there was reduction in the population of mutants. Population of Enterobacter sp. R29 and its mutants were much better in the rhizoplane across salinity and 45 and 90 DAS.

There were wide variations in the population of the mutants of Pseudomonas aeruginosa AMAAS57 both in the rhizosphere and rhizoplane across salinity and maturity of the plant.

The variation of the population of the mutants might be due to alternation in the genes responsible for colonization and also in the genes
responsible for salinity tolerance in Enterobacter sp. R29 due to insertion of Tn5::gusA.

Transposable genetic elements carrying antibiotic resistance genes have proven to be extremely useful tools in bacterial genetics (Kleckner et al. 1977). The transposition of a Tn element into a gene generally inactivates it and mutations are usually non leaky and stable. Such insertions also exert polar effects on downstream genes of the operons (De Bruijn and Lupski, 1984).

Vincent et al. (1991) reported the use of site directed Tn5 mutagenesis to assess the role of DAPG (2, 4 - diacetylphloroglucinol) produced by P. aureofaciens Q 2-87 to suppress G. graminis var. tritici. They could isolate two mutants of Q 2-87 with altered antifungal activity. One mutant Q 2-87 :: Tn5-1, did not inhibit G. graminis var. tritici in vitro and did not produce DAPG. Complementation analysis with two cosmids, isolated from the genomic library, coordinately restored the antifungal activity and DAPG production in vitro. Subcloning and deletion analysis of these cosmids identified a 4.8 kb genomic region responsible for DAPG production and antifungal activity. Fenton et al. (1992) used Tn5 mutagen to derive DAPG deficient mutant (F113 G22) from Pseudomonas sp. strain F113.

Inactivation of repressor could give overproducing strains having enhanced biocontrol ability.

O'Sullivan and O'Gara (1990) could inactivate the Fur-like repressor of a fluorescent pseudomonad which has been shown to improve the inhibitory characteristics of the strains under high-iron conditions. Therefore, the improvement in the nodulation in the mutants of Enterobacter sp. R29 might be due to inactivation of repressor proteins regulating the nodulation process.