SUMMARY AND CONCLUSIONS

Plant growth promoting rhizobacteria (PGPR) play an important role in developing sustainable crop production systems. To obtain novel bacterial strains that could be used for improving the vigour of cocoa seedlings, *Bacillus* spp. and fluorescent *Pseudomonas* spp. were isolated from the rhizosphere soil and roots of cocoa growing in different agroclimatic regions in the southern states of India. Enumeration of PGPR populations in the rhizospheric soil and root samples of cocoa revealed the presence of PGPR in all the samples irrespective of the place or agroclimatic region. The population of *Bacillus* spp. was higher than that of fluorescent *Pseudomonas* spp. in all the locations. A total of 519 morphologically different *Bacillus* spp. and fluorescent *Pseudomonas* spp. were isolated using selective media from the soil and root samples. *In vitro* characterization revealed that isolates were functionally diverse and 93% isolates exhibited more than one plant growth promotion (PGP) trait. Higher percentage of fluorescent *Pseudomonas* spp. exhibited PGP traits such as production of IAA, ACC deaminase, siderophore and phosphate solubilization whereas higher number of *Bacillus* spp. showed PGP traits like ammonification and ability to grow on N-free medium. The rigorous screening of the 519 isolates for PGP attributes finally led to the selection of 104 promising bacterial isolates, having combination of PGP traits. *In vitro* (plant growth chamber assay/seedling bioassay) and *in vivo* (green house studies) tests were conducted for evaluating the PGP potential of these selected isolates on test crop, cowpea (short duration test crop for dicots). Eighty eight promising isolates (out of 104 isolates) from seedling bioassay were further screened under greenhouse conditions on cowpea seeds. Incremented growth influence of some isolates in test crop, cow pea under *in vitro* and *in vivo* conditions led to further testing of the isolates for the growth promotion ability in cocoa. Twenty one PGPR were selected based on their overall performance in the screening studies viz., *in vitro* plant growth promotion traits, seedling bioassay and green house studies. These 21 PGPR were identified up to species level by employing polyphasic methods (biochemical characterization, BIOLOG GEN III microbial identification system and 16S rRNA sequencing) and subjected for various analyses such as quantification of various PGP metabolites, abiotic stress tolerance studies and polybag studies on cocoa seedlings. Their phenotypic and RFLP patterns were also generated. Selected PGPR were evaluated
for growth promotion of cocoa seedlings (Forastero variety) in single as well as in
dual inoculations in polybags under field conditions. Bacterization with *B. cereus*
ASB3, *B. subtilis* VEB4, *B. licheniformis* KGEB16 and *P. putida* KDSF23 resulted in
maximum enhancement in growth parameters and NPK content in the rhizosphere
soil and leaves of cocoa seedlings in individual inoculation studies. Dual inoculation
of compatible combination of these four potent isolates revealed that application of
*B. subtilis* VEB4 with *B. licheniformis* KGEB16 on cocoa seedlings recorded the
maximum enhancement in the growth parameters compared to other treatments. In
both single and double inoculation experiments, the best treatments were observed
to produce significant positive influence on the population of beneficial
microorganisms such as *Bacillus* spp., P-solubilizers and nitrogen fixers in the
rhizosphere of the cocoa seedlings. In addition, it was observed that inoculation of
the promising PGPR isolate was effective to improve the nutrient uptake by the
cocoa seedlings as evidenced by the higher NPK contents in the leaves. Talc based
powder formulations of efficient PGPR were prepared and their shelf life studies
indicated that the recommended population level of $10^7$ cfu g$^{-1}$ as per Bureau of
Indian Standard (BIS) norms (2002) could be maintained even after 180 days of
storage under room temperature. However future studies are important to develop
liquid bioformulation, as the liquid formulation has several advantages including
high cell count, zero contamination, longer shelf life, greater protection against
environmental stresses and increased field efficacy. It can be surmised that
biopriming with these selected PGPR could help to increase the vigour of cocoa
seedlings and reduce the amount of fertilizer input by increasing the efficiency of
nutrient availability/uptake. Functionally diverse plant growth promoting bacteria,
isolated in the current study, can be used in organic farming as natural bioresources
which would not only enhance the agricultural productivity but also maintain soil
quality.