Chapter VI

Summary and Conclusion
It can be concluded from the above discussion that Plant growth promoting rhizobacteria are increasingly used for crop improvement and protection. In the same context, present study was focused for the isolation and characterization of PGPR from rhizosphere of the Dhaincha plant (*Sesbania bispinosa*) and there application for improvement of germination, plant growth and yield of chickpea (*Cicer arietinum* L.).

PGPR interacts with plants either directly or indirectly. The direct effects includes- enhancing Nitrogen supply to the host through Nitrogen fixation, enhancing supply of some major plant nutrients (e.g. P, S, Fe), suppressing soil borne pathogens by the production of hydrogen cyanide, siderophores, antibiotics, enzymes like chitinase and/or competition for nutrients, producing of phytohormones such as indole-3-acetic acid (IAA). The indirect effects of PGPR are mainly antagonismto phytopathogens by antibiosis, production of inhibitory enzymes etc.

In this study the main aim were –

(i) To explore the phytohormone producing, phosphate solubilizing PGPR colonizing at the rhizosphere of *Sesbania bispinosa*.

(ii) Selection, characterization and identification of the best PGPRs.

(iii) Application of the selected PGPRs in growth promotion of plant in the field.

Major laboratory works were performed in the Environmental Physiology laboratory, Department of Physiology, University of Kalyani, and Microbiology Laboratory, Department of Botany, University of Kalyani. Works on molecular biology were performed by Chromous biotech Pvt Ltd lab. The Pot experiment was done in a net house of Department of Botany, University of Kalyani. The Field experiment was carried out in the nearby village of Iswaripur.

In the present experiment, rhizospheric soil of *Sesbania bispinosa* was collected from four different spots both from local farmer’s field (around Kalyani area of Nadia district) and research plots of Horticultural faculty of Bidhan Chandra Krishi Viswavidyalay (State Agricultural University).
Bacterial strains from collected soil samples were isolated by soil dilution plate count technique and were characterized by their morphological, cultural and staining properties. Different characteristics of colonies such as shape, size, elevation, surface, margin, colour, etc were recorded. Gram nature of each isolates was initially determined by Gram Staining. Different plant growth promoting activities of the bacterial isolates viz. Phosphate solubilization, Indole acetic acid production, Siderophore production, Hydrogen cyanide production and ammonia production was considered for the present study. Isolates with good plant growth promoting potentialities were analysed and the best three efficient isolate among them were selected according to their multiple positive PGPR activities.

From this study, it is clear that several naturally occurring isolates from the rhizosphere of dhaincha are capable of solubilizing inorganic phosphates and producing a significant amount of IAA. IAA production was increased when the cultural conditions were optimized. The significance of the study could be stated as the potential of these IAA producing and phosphorus solubilizing isolates and optimization of the parameters for IAA production. It was found that maximum IAA production was recorded at pH 7, 37º C temperature and 1gm/Lit L-tryptophan concentration. This huge amount of IAA production will flourish the growth of the plant in the field condition.

To confirm the identity of the best three PGPR isolates, the strains were included in the biochemical and molecular characterization. Different biochemical tests like Indole production, Methyl red, V-P, Citrate, Catalase, Gelatin hydrolysis, H₂S production, Urease and Starch hydrolysis were performed. Molecular characterization was done on the basis of 16S rDNA sequence analysis.

The morphological, cultural, biochemical and molecular characterization of these three efficient isolates revealed that the most efficient PGPR inhabiting in Dhaincha rhizosphere were Escherichia coli DACG2, Pseudomonas fluorescens strain DACG3 and Burkholderia sp. DACG1. The 16S rRNA sequences obtained were submitted to NCBI GenBank databases under the accession number of JN858966, KP641168 and JN639877 respectively.
The results were promising for design of potentially active plant growth promoting PGPR strain based formulation which would be beneficial for crop improvement. The potential of these strains were investigated in detail and field application was studied. The growth nature and performance in seed germination were studied initially. In further study, the performances of the three PGPRs were studied either alone and in all possible combinations on chickpea plant in pots as well as field to verify their role on growth enhancement.

The study revealed that, inoculation of PGPR strains increased all parameters determined in-pot experiment as well as in field. Again, Mixed Inoculants showed the highest results, which indicate that mixed inoculants of PGPRs are more effective than single inoculation. The present experiment the mixed inoculants with all the three showed the best results in all the test parameters like plant height, number of leaves per plant, number of pod bearing branches per plant, number of pods per plant, number of nodules per plant and hundred seeds weight.

So, finally it can be said that the three Plant growth promoting rhizobacteria (viz. \textit{Escherichia coli} DACG2, \textit{Pseudomonas fluorescence} DACG3, \textit{Burkholderia sp.} DACG1) isolated from the rhizosphere of \textit{Sesbania bispinosa} significantly enhanced growth and biomass production of chickpea. Therefore it is suggested that the use of PGPR isolates of as effective biofertilizers might be beneficial for chickpea cultivation and prevent environmental pollution by avoiding excessive applications of industrially produced fertilizers to cultivated fields. Further studies are required to use these PSB isolates as a bioinoculum for the better productivity of crops which are non-hazardous to the environment as well a population who has threatened.