SUMMARY & CONCLUSION

The research concluded with the following outcomes:

1. Among the 82 rhizospheric isolates altogether, twenty-seven isolates showed positive growth on Asbhy's N-free medium, a differential media for screening PGPR with potential biological N_2-fixation ability.
2. Further, N_2-fixer isolates were screened qualitatively and quantitatively for other PGP traits and all of them exhibited PGP traits viz. ammonia production, inorganic phosphate solubilisation, organic phosphate solubilisation, zinc solubilisation, IAA production, ACC deaminase production.
3. Majority of PGPR belonged to the class gamma-proteobacteria and few were members of class betaproteobacteria and phylum Firmicutes.
4. Two bacterial isolates (RS3 and RS26) showed significantly higher production of ammonia, auxin and ACC deaminase; and solubilisation of Zinc and phosphate. They were selected as the two most potent PGPR strains using principal component analysis (PCA) of their qualitative PGP traits.
5. These two strains RS3 and RS26 were negative for hemolysin production in sheep blood agar medium. The strains didn't show antagonistic effect indicating their compatibility with each other and hence, selected for further study.
6. The phylogenetic analysis based on 16S rRNA gene sequence analysis identified the bacterial isolates RS3 and RS26 as *C. davisae and K. pneumoniae* and therefore, names as *C. davisae* RS3 and *K. pneumoniae* RS26, respectively.
7. PGPR strains RS3 and RS26 are used for designing nutrient formulations (NF) by general and statistical (RSM) method, for application to mustard plants.
8. In general method, the mustard plants were divided into four different treatment regimens based on NF treatments, namely, N-appropriate without microbes, N-appropriate with microbes, N-deficit without microbes and N-deficit with microbes. Plants were analysed for morphological, biochemical and physiological parameters at 15, 45 and 75 days after treatment (DAT). Among the various NF treatments, plant under N^-PGPR^+ treatment regimen showed significantly higher plant growth and seed yield parameters. For example, seed yield of N^+PGPR^-, N^+PGPR^+, N^-PGPR^-, N^-PGPR^+ plants were 3.98, 4.08, 3.22, and 5.76 g $plant^{-1}$, respectively.
9. Plants treated with RSM optimised NF with conditions 0.50 mM N, 50% v/v of Strain 1(RS3) and 50% v/v of strain 2 (RS26) were found to have better growth performance and productivity than N^-PGPR^+ plants. Consequently, at 75 DAT, N^-PGPR^+ and RSM optimized NF treated plants showed carbohydrate content, protein content and seed yield of 12.17 mg g^{-1} FW, 675.89 mg g^{-1} FW, 5.76 g $plant^{-1}$, and 16.12 mg g^{-1} FW, 924.76 mg g^{-1} FW, 8.10 g $plant^{-1}$, respectively.
10. Mustard plants treated with RSM optimized NFs showing highest yield was subjected to differential transcriptomic analysis (DGE) in order to gain an insight into the role of

NF in plant growth promotion. Mustard plant treated with N appropriate (5 mM N) NF without PGPR served as control.

11. The DGE results showed that 25,088 protein coding genes were expressed in both control and treated group of plants, where as 357 genes were exclusively expressed in treated group only, 351 genes were exclusively expressed in control group of plants only. The expression level of total 556 genes were found to be up regulated and 690 genes were found to be downregulated as compared to the control group
12. It has been found that more than 10% of the genes such as genes from organelle, cell, cellular part, membrane assembly were down regulated and designated as a part of cellular component, whereas genes involved with catalytic activity, protein binding, transcriptional regulator activity, antioxidant activity, metabolic processes were downregulated as a part of molecular function and gene cluster of biological process such as cellular component organization or biogenesis, biological regulation, multi organism process, positive and negative regulation of biological process, signalling were found to be down regulated significantly in PGPR treated plants.
13. The genes that were downregulated and upregulated in the treated group with respect to control group are shown in the Fig 3.15 and Fig 3.16 respectively. Among the cellular components and biological processes, the expression of genes related to organelle, extracellular region part, localization, cell proliferation, detoxification, carbon utilization was increased, whereas genes encoding proteins for nucleiod and rhythmic processes were found to be downregulated. The results of transcriptomics analysis showed enhanced expression of several genes directly and indirectly associated with improved plant growth and development in the roots of treated group of plants, like genes for ammonium, nitrate and amino acid transporters, ammonium and amino acid biosynthesis. biotic and abiotic stress tolerance, auxin signaling, light reaction of photosynthesis, antioxidant pathways, promotion of flowering, lipid storage proteins, resistance to pathogens etc. Expressions of mRNA of some genes related to copper uptake transmembrane transporter, ABA responses, senescence associated proteins, accumulation of free amino acids etc. were downregulated.
14. The functional annotations of the genes were carried out against the curated KEGG gene data base using KAAS (KEGG automatic annotation server). The KEGG ontology (KO) database of plants was used as the reference for pathway mapping. Two pathways related to plant hormone signalling and antenna protein mediated photosynthesis were generated which potrayed the expression of the genes related to phytohormone signaling and light harvesting genes were also upregulated. Five genes associated with the phytohormone signal transduction pathway were found to be elevated. They encoded Auxin-responsive GH3 family protein (GH3), SAUR-like auxin-responsive protein family, ABA-responsive element binding factor (ABF), Sucrose non-fermenting 1 (SNF1)-related protein kinase (SnRK2), and basic-leucine zipper (bZIP) transcription factor family protein and three genes of antenna protein mediated photosynthesis namely Lhca1 (Chlorophyll a/b binding protein 6), Lhcb1 (PSII Light harvesting complex protein 1), Lhcb2 (PSII Light harvesting complex protein 2) were upregulated.
15. Application of RSM optimized NF to the plant resulted in upregulation of several genes encoding proteins or enzymes to be directly associated with plant productivity, like

nitrate reductase, ammonia transporter, amino acid transporter family protein, inorganic phosphate transmembrane transporter, flowering promoting factor 1, seed storage/lipid Transfer Protein (LTP) family protein, mitochondrial phosphate transporter. Furthermore, several gene products which indirectly affect plant performances by giving resistance against biotic and abiotic stress were also upregulated, Lys/His transporter 7, UDP-glucosyl transferase family protein, glutathione S-transferase F3, mildew resistance locus O12, INH3, monooxygenase, disease resistance response and pleiotropic drug resistance 7, phenylalanine ammonia-lyase, WRKY35 transcription factor, NADP-dependent oxidoreductase, chorismate mutase 1, CTP synthase, growth regulating factor 2, 1, glutathione peroxidase, transmembrane transporter and $NADP^+$ isocitrate dehydrogenase etc.(Table 3.18).

16. RSM approach can be a promising tool for designing the nutrient formulations containing potent PGPR *Cedecea davisae* RS3 and *Klebsiella pneumoniae* RS26 and reduced inorganic fertilizer levels for improved plant growth and yield in a cost effective manner, and thus can put a brick in the development of sustainable agriculture.