ABSTRACT

The 20th century green revolution transformed agriculture with a massive gain in global production of food. A major effort of green revolutions comprised the use of new chemical fertilizers, including nitrogen (N) fertilizers. However, increase in agricultural production at the expense of application of higher quantities of N-fertilizers has been realized to cause enormous environmental hazards and thus imposing doubts on sustainability of food production. Hence, newer and sustainable agricultural approaches of enhanced food production with minimum use of chemical fertilizers are highly desired. In true sense, phytomicrobiome including PGPR, can play vital role in 21st century green revolution due to their key roles in nutrient acquisition and assimilation, improved soil texture and modulating extracellular molecules, like hormones and other signalling molecules, and improved stress tolerance, all leading to enhancement in plant growth. However, bacterial formulations with PGPR have not always the desired effectiveness. Keeping these in views, present investigation aimed to develop nutrient formulation (NF) containing PGPR with reduced N input for optimum plant growth promotion. For achieving the objective of the study, initially 82 bacterial strains isolated from rhizospheric soil. The isolates initially screened on the basis of their ability to fix atmospheric N. In total 27 isolates were found to fix nitrogen, which were further checked qualitatively and quantitatively for the other plant growth promoting trait. Among the N fixing isolates, 12 produced Indole acetic acid (IAA) (44%), 22 solubilised dicalcium phosphate (DCP) (81 %), 6 solubilised tricalcium phosphate (TCP) (22%), 16 produced phytase (59%), 14 and 16 isolates showed zone of solubilisation in media with zinc phosphate (52 %) and zinc carbonate (59 %), respectively, 11 isolates showed positive for ACC deaminase (40%). All the 27 isolates when checked for quantitative ammonia production in Asbhy's N free broth medium, the isolates RS3, RS23, RS26 and RS51 produced more than 6 µg mL^{-1} of ammonia with significantly higher production by RS3 (9.52 µg mL^{-1}) and RS26 (10.13 µg mL^{-1}). The liberation of Pi by RS3, RS23, RS26 and RS49 were recorded to be 49, 40, 26 and 30 µg ml^{-1}, respectively. Significantly higher phytase activity of RS3, RS23, RS26 and RS49 were recorded in the pH range 4.5 to 5.5. The isolates RS3, RS9 and RS26 showed Zinc solubilisation index (ZSI) on Zinc carbonate of 2.34, 2.06 and 2.07, respectively. Bacterial strains RS3 and RS26 produced significantly greater quantity of IAA and their respective production levels were 5.06 and 7.13 µg mL^{-1} in absence of tryptophan and 10.13 and 14.51 µg mL^{-1} in presence of tryptophan. RS2, RS3, RS6, RS10, RS16, RS26, RS31, RS53, RS59, RS60 and RS65 were ACC deaminase positive. Principal Component Analysis (PCA) of the PGPR traits of the isolates was used to select the potent PGPR strains for pot experiment. PCA extracted two components, PC1 and PC2 which explain 32.7 and 30.1 % variability, respectively. The microbial strains RS3 and RS26 contributed to PC1 with a correlation coefficient of 0.607 and 0.602, respectively. PCA thus suggested RS3 and RS26 as potent PGPR. The phylogenetic analysis based on 16S rRNA gene sequence identified isolate RS3 as *Cedacea davisae* RS3 (GenBank accession number KX101223) and the strain RS 26 as *Klebsiella pneumoniae* RS26 (Gene bank Accession number MH 819506.1). The

two isolates showed compatibility with each other and were negative in hemolysin production test in sheep blood agar medium.

Further, nutrient formulations containing PGPR (*Cedacea davisae* RS3 and *Klebsiella pneumoniae* RS26) and varying N levels were formulated by following general and central composite rotatable design (CCRD) based response surface methodology (RSM) approaches and their effects on plant growth and yield were compared. In general approach, mustard plants were grown in four different NF treatment regimens, namely, N-appropriate without microbes (N^+PGPR^-), N-appropriate with microbes (N^+PGPR^+), N-deficit without microbes (N^-PGPR^-), N-deficit with microbes (N^-PGPR^+) and their growth characteristics were compared. Plant under N^+PGPR^- NF showed the highest seed yield (5.76 g plant-1), protein content of root (378.67 mg g^{-1} FW) and shoot (675.89 mg g^{-1} FW), the carbohydrate content of root 6.76 (mg g^{-1} FW) and shoot (12.17 mg g^{-1} FW). The other parameter like number of siliqua per plant (39.40), number of seeds per siliqua (30.12), 100 seed weight (0.48 g) and seed yield (5.76 g $plant^{-1}$) at 75 DAT were also maximum in N^-PGPR^+ treatment group. In RSM based approach, the effect of three variables, N concentration (A), inoculum volume of strain1 i.e. *C. davisae* RS3 (B) and inoculum volume of strain 2 i.e. *K. pneumoniae* RS26 (C) of the NF, on plant growth (carbohydrate and protein content of shoot) and seed yield was investigated, using Central composite rotatable design (CCRD). The experimental runs suggested that the application of NF containing N at 0.5 mM and strain 1 and strain 2 at 50% v/v each to the cultivation of mustard plant, yielded actual response of shoot carbohydrate content (16 mg g^{-1}FW), shoot protein content (824 mg g^{-1}FW) and seed yield (8.10 g $plant^{-1}$), which are quite close to the model predicted response comprising shoot carbohydrate content of shoot (15.04 mg g^{-1} FW), shoot protein content (819.25 mg g^{-1} FW), seed yield (8.20 g $plant^{-1}$).

A comparison of the effect of general and RSM based approaches of plant NF treatments to the growth and yield of mustard plant indicated that carbohydrate and protein contents and seed yield, GS activity (211 µmole g^{-1} FW) and Chlorophyll a (2.7 mg g^{-1} FW) of RSM based treatment plants were significantly greater than that of the plant treated with general approach with respect to the carbohydrate content, protein contents, seed yield, GS activity (121 µmole g^{-1} FW) and Chlorophyll a content (2.3 mg g^{-1} FW). The results herein suggest that RSM based optimization of NF could be beneficial for enhanced plant growth and yield leading to agricultural sustainability.

To gain an insight into the role of NFs in plant growth, mustard plants treated with NFs showing highest yield (designated as 'treated') was subjected to transcriptomic based differential gene expression (DGE) analysis using plants treated with NF containing optimum level of inorganic N as control (designated as 'control'). The DGE analysis showed that 25,088 protein coding genes were expressed in both control and treated group of plant, 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 down regulated as compared to the control group. Gene ontology annotation associated with upregulated, downregulated, expressed both and exclusively expressed genes for the sample combination control vs treated were obtained. In case of treated plants, 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. Metabolic pathways related to plant hormone signalling and antenna protein mediated photosynthesis 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.

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 etc. Furthermore, several gene products which indirectly affect plant performances by giving resistance against biotic and abiotic stress were also upregulated, glutathione peroxidase, 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, transmembrane transporter and $NADP^+$ isocitrate dehydrogenase. Those genes whose downregulation have significant role in plant growth and yield found in this study were cell wall / vacuolar inhibitor of fructosidase 1, WRKY18 trancription factor, pyruvate decarboxylase, senescence-associated protein-related and glutamine dumper 1.

Thus, *Cedecea davisae* RS3 and *Klebsiella pneumoniae* RS26 proved to be potent PGPR and the RSM approach can be a promising tool for designing the nutrient formulations containing reduced N input with PGPR supplements for enhanced plant growth and yield. Such type of work, not only increase crop productivity without affecting the environment but is also cost effective. Hence, this effort can put a small building block in the development of sustainable agriculture.