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Research article

Supplementation of *Trichoderma* improves the alteration of nutrient allocation and transporter genes expression in rice under nutrient deficiencies

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ABSTRACT

Nutrients are the finite natural resources that are essential for productivity and development of rice and its deficiency causes compromised yield along with reduced immunity against several biotic and abiotic stresses. In this study, the potential of *Trichoderma reesei* has been investigated as a biofertilizer (BF) to ameliorate nutrient stress in different rice cultivars at physiological, biochemical and molecular levels. The results indicated that cultivar Heena is much more compatible with BF as compared to cultivar Kiran at 50% nutrient limiting condition. Enhancement in physiological attributes and photosynthetic pigments were observed in BF treated Heena seedlings. The localization of biofertilizer in treated roots was further validated by scanning electron micrographs. This result correlated well with the higher levels of Indole acetic acid and Gibberellic acid in biofertilizer treated rice. Similarly, the uptake of micro-nutrients such as Fe, Co, Cu and Mo was found to be 1.4–1.9 fold higher respectively in BF treated Heena seedlings under 50% nutrient deficient condition. Furthermore, different stress ameliorating enzymes Guaiacol peroxidase, Super oxide dismutase, Total Phenolic Content, Phenol Peroxidase, Phenylalanine ammonia lyase and Ascorbate peroxidase in Heena seedlings were also increased by 1.8, 1.4, 1.2, 2.4, 1.2, and 8.3-fold respectively, at 50% nutrient deficient condition. The up-regulation of different micro and macro-nutrients allocation and accumulation; metal tolerance related; auxin synthesis genes in BF treated Heena as compared to 50% nutrient deficient condition was further supported by our findings that the application of biofertilizer efficiently ameliorated the deficiency of nutrients in rice.

1. Introduction

Rice (*Oryza sativa*), a significant part of the staple diet for half of the world's population, is grown in every continent apart from Antarctica (Muthayya et al., 2014). The productivity of rice has been affected severely due to the gradual depletion in the availability of soil nutrients. To achieve higher production of rice, synthetic chemical fertilizers have been used as an additional inputs, but have encountered diminishing returns (Miransari and Mackenzie, 2011). Several issues, including excessive use of chemical fertilizers, less availability of soil nutrients, less mobility of nutrients in soil, poor solubility of nutrients makes the soil unproductive and nutrient deficient with unfavourable effects on environment including groundwater pollution and degradation of soil (Miransari and Mackenzie, 2011). Rice requires various macro and micro-nutrients for their growth and development. Some of these micro and macro-nutrients act as co-factors of various enzymes which are involved in photosynthesis, respiration and other developmental processes. The insufficient quantity of nutrients and their supply causes impaired growth which ultimately poses a harmful impact on the rice yield (Miller and Welch, 2013).

Plant growth promoting microbes are known to play a significant role in increasing plant growth and productivity (Dutta and Thakur, 2017; Singh and Gaur, 2016). They help in growth of the plant by mobilizing the soil nutrients (siderophore production and P-

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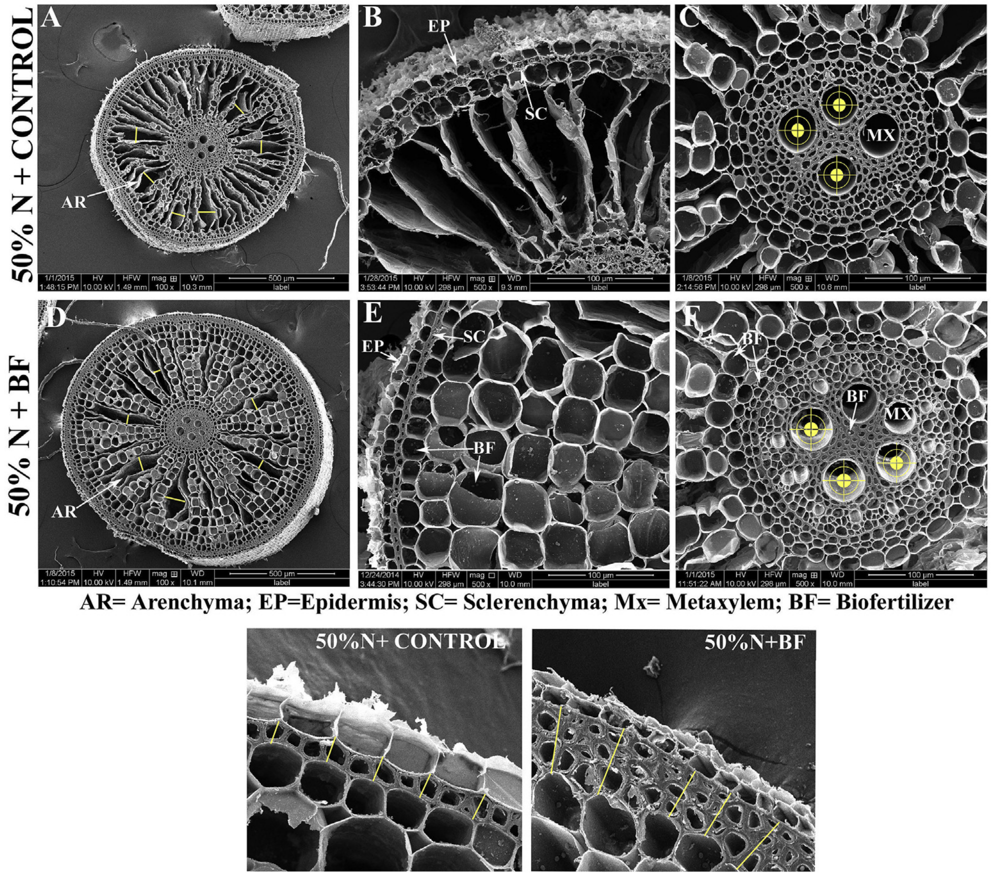

Fig. 1. Scanning electron micrographs of root tissues of drought resistant rice cultivar Heena in 50% control and 50%N treated with *Trichoderma reesei* (BF) treatments under hydroponic condition.

solubilization), synthesis of different plant growth promoting hormones (IAA, gibberellic acid production), along with protecting them against numerous phyto-pathogens (Dutta and Thakur, 2017; Singh and Gaur, 2016). In addition, various plant growth promoting fungi and bacteria (*Trichoderma* spp. and *Rhizobium* spp.) are also able to help the host plant to cope up against nutrient deficiencies such as, zinc (Zn) and iron deficiency (Fe) (Kamran et al., 2017; Mishra, 2017) through mineralization of soil organic matter, plays a pivotal role in nutrient cycling.

Species of genus *Trichoderma*, the most representative inhabitants of soil and plant roots, have been frequently used to reduce the adverse effects of varied stresses on the host plant. Concerning the root nutrient uptake, there are evidence showing that *Trichoderma* spp. affects the plant growth and nutrient uptake by stimulation of various plant growth promoting substances. *Trichoderma asperellum* produces siderophore to chelate the iron (Fe^{+++}) molecules and works as a Fe donor to ameliorate the iron deficiency in white lupin under hydroponic condition (De Santiago et al., 2009). Furthermore, some strains of *Trichoderma* have a greater ability to solubilize high amount of phosphate of soil as compared to bacteria due to overproduction of acids (Altomare et al., 1999). Some complex forms of nutrients (MNO_2; Zn and rock phosphate) were also reported to be solubilized by *Trichoderma harzianum* strain T-22 (Altomare et al., 1999). The association between host plant roots and *Trichoderma* spp. increases plant biomass by the secretion of auxin and their derivatives (Nieto-Jacobo et al., 2017). Similarly, at primary stage, the production of plant growth promoting hormones and other proteinaceous compounds is stimulated by *Trichoderma* for a host to accelerate the plant growth mechanisms (Garnica-Vergara et al., 2015) along with maintaining a healthy system for effective nutrient and water uptake strategies (Contreras-Cornejo et al., 2015). Findings of these studies have also elucidated the multifaceted roles of *Trichoderma* in the production of various secondary metabolites i.e. non-ribosomal peptides; terpenoids and pyrones (Contreras-Cornejo et al., 2016). Additionally, interactions of *Trichoderma* spp. with a host species may alter the ISR (Induce systemic Resistance), and enhance plant's defence against biotic and abiotic stresses (Shoresh et al., 2010). Besides this, *Trichoderma* spp. also able to penetrate the root and inhabit in the host plant which may lead to a molecular dialogue during the interaction (Hermosa et al., 2012). However, the relationship between *Trichoderma* and host plant for nutrient uptake management, along with the exchange of signaling molecules and molecular aspects behind this activity still needs further investigation. Nevertheless, several studies in the past explored the physio-chemical and molecular effect of a potential interaction between *Trichoderma* spp. and the host plant in nutrient deficient condition (Contreras-Cornejo et al., 2014). For example, *T. harzianum* induces significant alteration in shoot stress, defense, energy, protein synthesis and folding related proteome when they colonize within the root of corn and cucumber seedlings (Shoresh and Harman, 2008). The aim of the current study was to investigate the possible interaction of *Trichoderma* spp. with the two-rice cultivars named Heena (drought resistant) and Kiran (drought sensitive), in nutrient deficient conditions at morphological, bio-chemical, physiological and molecular levels.

2. Material and method

2.1. *Trichoderma reesei* inoculum and plant materials

Rice seeds of Heena (drought resistance) and Kiran (drought sensitive) varieties were used for axenic hydroponics cultures. Surface sterilization of seeds of both varieties was carried out with 2% NaOCl and 70% ethanol (for 2 min) followed by washing with sterile distilled water. Seeds were germinated in moist pre-sterilized blotting sheets in

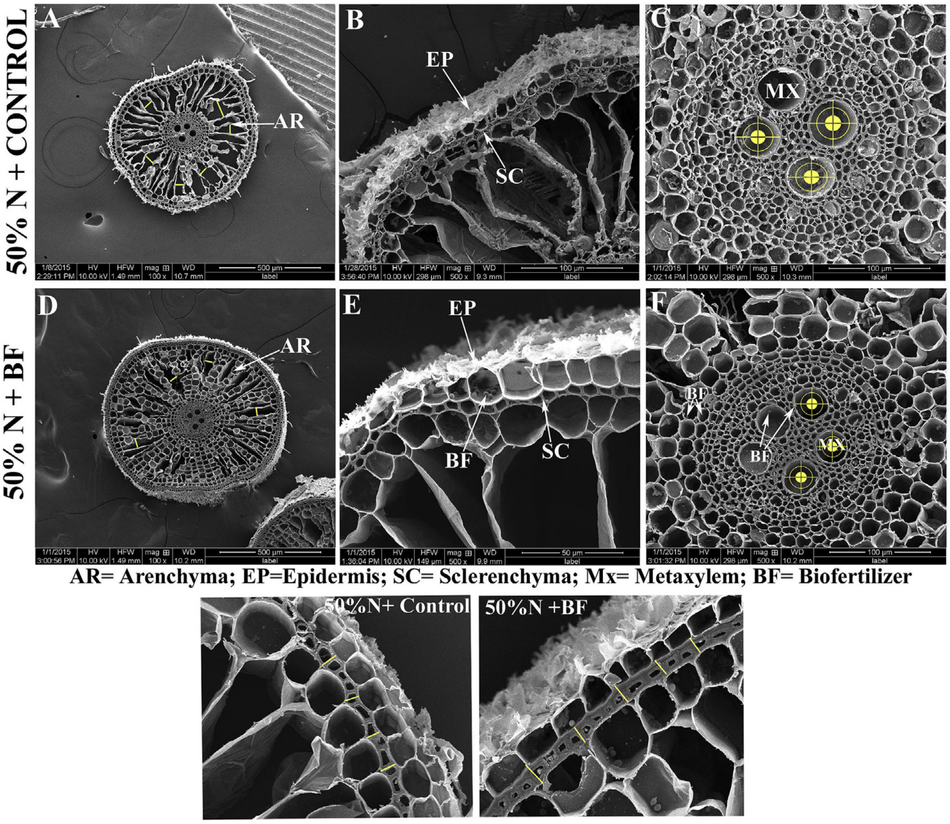

Fig. 2. Scanning electron micrographs of root tissues of drought sensitive rice cultivar Kiran in 50% control and 50%N treated with *Trichoderma reesei* (BF) treatments under hydroponic condition.

plastic trays aseptically and placed in germinating trays inside a growth chamber (65% relative humidity; PGR15, Growth Chamber, Conviron, Canada) at 37 °C for one week. After uniform growth, seedlings of both rice varieties were transplanted in nursery trays (containing 24 PVC cups in each tray) to provide a hydroponic condition (Figs. 1 and 2). Both varieties (Heena and Kiran) were transplanted in 18 trays individually. The trays of each treatment were equally divided into two groups. The first group of tray (in triplicate) contained 0%, 50% and 100% concentration of Hewitt medium (Hewitt and Smith, 1975) and considered as control whereas, the second group of tray contained 0%, 50% and 100% concentration of Hewitt medium along with the supplementation of *Trichoderma reesei* (1×10^8 spores ml^{-1}; MTCC5659; BF) as per the treatment.

For spore suspension, *T. reesei* (MTCC5659; BF) was grown in Potato Dextrose Broth (PDB) and incubated at 24 °C for 7–10 days. After incubation, broth was centrifuged at 12,000 × *g* for 5 min and the pellet was adjusted to concentration 1×10^8 spores ml^{-1} (in double sterile distilled water) and 300 ml (100 ml per liter of Hewitt medium) of suspension was poured in seedling containing trays to observe the effect of *Trichoderma reesei* with concentration 1×10^8 spores ml^{-1} at different level of nutrients in rice seedlings.

The treatments of nutrient and nutrient supplemented with *Trichoderma reesei* (1×10^8 spores ml^{-1}) were given individually for both the cultivars in following concentrations: (i-ii) 100% Nutrient control (100% N) and Treated with *Trichoderma reesei* (100% N + BF); (iii-iv) 50% Nutrient control (50%N) and Treated (50%N + BF); (v-vi) 0% Nutrient control (0%N) and Treated (0%N + BF). All the treatments were placed in a growth chamber at controlled condition (28/21 °C at a light intensity of 210 mmol cm^2 s^1; 16 h light/8 h dark) with relative humidity ~70% for one month (Figs. S1(A) and (B)). The seedlings were observed regularly, and the significant changes were recorded on the basis of physiological parameters, enzymatic activity and expression profiling of nutrient related genes with the final harvested samples at 30 days.

2.2. Scanning electron microscopy (SEM)

SEM analysis was performed to confirm the colonization of BF and its effect on rice root under nutrient deficient conditions. Transverse sections of roots were cut and transferred to small cap tube vials, fixed for 1 h in 2.5% (v/v) glutaraldehyde in 0.1 M sodium cacodylate buffer (Sigma). This was followed by 40 min treatment in 1% (w/v) osmium tetraoxide (OsO_4, Sigma). The root cells were dehydrated by sequential passage through increasing concentration of ethanol (30–100%) in 10% increments, dried in a critical point dryer (K850-Critical Point Dryer, Quorum Technologies), coated with gold-palladium for 60s in a Pelco 3 sputter coater (SC 7620, mini sputter coater, Quorum Technology Ltd., UK)., and visualized using a scanning electron microscope (Quanta 450FEG, FEI, The Netherlands) as described earlier (Mishra et al., 2018a).

2.3. HPLC analysis for plant hormones

Plant seedling's shoot tissues were collected aseptically for the estimation of endogenous phytohormones *viz*. gibberellic acid (GA), indole-3-acetic acid (IAA), and abscisic acid (ABA) by using the prescribed method of Pan et al. (2010). The estimation of phytohormones was performed on HPLC system (Waters 2475) by using reverse phase C-18 column (250 × 9.4 mm; 5 μm; Phenomenex Luna). The mobile phase of isocratic elution contained 30 mM orthophosphoric acid in HPLC-grade water (component A) and acetonitrile (component B) in 70:30 ratio. HPLC was run at 0.5 ml/min for 40 min at 280 nm wavelength. The different concentration of phytohormones was examined by using external standards (Sigma Aldrich, USA) and expressed as μg

mg^{-1} of dry weight of plant tissue.

2.4. Estimation of micro and macro-nutrients in rice

Micro-elemental depositions in *Trichoderma reesei* treated rice seedlings of both varieties were analyzed by Inductively Coupled Plasma Mass Spectrometry (Agilent 7500CX, Agilent Technologies, Palo Alto, CA, USA). Plant tissues (0.3 g) were digested with 3.0 ml of HNO_3 (Sigma) and 1.0 ml of H_2O_2 (Sigma). Reference standards of Manganese (Mn), Iron (Fe), Cobalt (Co), Nickel (Ni), Copper (Cu), Zink (Zn), and Molybdenum (Mo) were taken from E-Merck, Germany (Mishra et al., 2019) .

To the analysis of total nitrogen content, 0.5 g of leaf tissue was used for the digestion, distillation and back titration by Kjeldahl method (Nelson and Sommers, 1973). Total phosphorous content was estimated by the described protocol of Tsvetkova and Georgiev (2003). All experiments were performed in triplicate.

2.5. Enzyme extraction and biochemical assays

Leaf tissue of all treatments (both varieties) was sampled aseptically and stored in liquid N_2 for different enzymatic assays simultaneously. 1.0 g of plant tissues were homogenized in 10 mM sodium phosphate buffer (pH 6.0) containing 1% polyvinylpoly pyrrolidone (PVP) (w/v), 0.3 mM phenyl methyl sulfonyl fluoride (PMSF) and 1 mM EDTA at 4 °C and centrifuged at 12000 rpm for 10 min to eliminate the impurities and plant debris from the enzymatic extract. All enzymatic activities of treated plant tissues were assessed spectrophotometrically.

Guaiacol peroxidase (GPX) activity was determined according to the method of Hemeda and Klein (1990). The rate of fluctuations in absorbance at 470 nm was measured and expressed in U mg^{-1} protein. Superoxide dismutase (SOD) activity was evaluated by using riboflavin/methionine system (Fridovich, 1974) and the impact of enzymatic extract to reduce the photochemical oxidation of Nitroblue Tetrazolium (NBT) was recorded at 560 nm. Furthermore, Total Phenolic Content (TPC) was quantified according to Zheng and Shetty (2000) by using Folin–Ciocalteu regent. The obtained absorbance at 725 nm were quantified by calibration curve of Gallic acid and converted to mM Gallic acid equivalent (GAE) g^{-1} FW. Poly Phenoloxidase (PPO) activity was analyzed by adding the 0.1 ml of plant enzymatic extract into 0.01 M catechol and was observed at 495 nm (Mohammadi and Kazemi, 2002). Phenylalanine ammonia-lyase (PAL) activity of treated plant tissues were evaluated by using the calibrating curve of trans-cinnamic acid and the activity was considered in terms of μmol trans-cinnamic acid g^{-1} FW (Brueske, 1980). Ascorbate peroxidase (APX) activity was calculated according to Nakano and Asada (1981). The enzymatic extract (0.1 ml) was homogenized in 25 mM phosphate buffer (pH 7.0) containing 0.1 mM EDTA, 0.25 mM ascorbic acid and 1.0 mM H_2O_2. The absorbance was taken at 290 nm and was further converted into U mg^{-1} protein. The total protein content of enzymatic extract was evaluated according to Bradford method (Bradford, 1976) and was further calculated by using of calibration curve of Bovine Serum Albumin (BSA). All the enzymatic assays were performed in triplicate and documented as the average of three parallel measurements.

2.6. Quantitative real time PCR analysis of nutrient inducible genes

All the experiments were performed under RNase-free conditions. Total RNA was extracted from the flag leaf of rice tissue using TRIzol reagent (TRI reagent, Sigma, USA) according to the manufacturer's protocol. The concentration of RNA was measured by Nano Drop (Thermo Fisher Scientific, USA). Rice tissue cDNA was synthesized using 1 μg RNA and the Super Script III First-strand synthesis system (Thermo Fisher Scientific, USA) as per manufacturer's instructions. Quantitative RT-PCR was carried out on a Stratagene Mx3005P instrument (Agilent Technologies, USA). Rice cDNA was diluted in the ratio of 1:10 with nuclease-free water. cDNA (1 μl) was mixed with 5.0 μl of Brilliant III Ultra-Fast Cyber Green QPCR master Mix (Agilent Technologies, USA) and 1.0 μl of primer (10 pmol forward/reverse). The final volume was adjusted to 10 μl with nuclease-free water. Nucleotide sequences of all the 26 sets of genes (except *Actin*) were retrieved from National Centre for Biotechnology (NCBI) database, while PRIMER 3 online software program (frodo.wi.mit.edu/) was used to design the primers. *Actin* gene was considered as a housekeeping gene. The primers used for qRT-PCR are listed as supplementary information S1. Thermal cycling was carried out as follows: The first segment of the amplification cycle consisted of denaturation at 95 °C for 10 min; the second segment consisted of denaturation (15 s at 95 °C), primer annealing (30 s at 50–58 °C) and extension (30 s at 72 °C) for 40 cycles. The third segment consisted of melting curve programme (95 °C for 5s, 58 °C for 15s). The final segment consisted of cooling to 4 °C. The threshold cycle (C_T) was used to represent the relative mRNA amounts. All samples were run and analyzed in triplicate using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001) and heat map was constructed by using MEV software.

2.7. Statistical analysis

All the observations were statistically analyzed by using SPSS package (SPSS V16.0, SPSS Inc., Chicago, USA). All the experimental observations were the mean of three technical replicates and significant level (at $P \geq 0.05$) between the treatments was assessed on the basis of Tukey's multiple range test. The Hierarchical clustering and Principle component analysis (PCA) was performed by using PAST3 software. The significance level of HPLC data was assessed by using *t*-test between control and BF treated rice.

3. Results

3.1. Plant biomass and yield of two rice cultivars

The effect of *T. reesei* as a biofertilizer was evaluated under different nutrient stress conditions (Supplementary Figs. 1a and 1b). Root length, shoot length, fresh weight and dry weight were analyzed after the 30 days of post treatment. Results indicated an increase in all the above parameters with the supplementation of *T. reesei* in all the three treatments (100%N + BF; 50%N + BF and 0%N + BF) comparative to their respective control. A significant fold increment was observed in the 100% N + BF which was followed by 50%N + BF and 0%N + BF respectively in comparison to their controls of both Heena and Kiran (Table 1). Further, the level of different photosynthetic pigments *viz.* Chl A, Chl B, total chlorophyll and carotenoids were evaluated. An enhancement in the pigments was witnessed in the *T. reesei* treated rice in all the nutrient conditions; however, highest fold increment was recorded in 50%N + BF treatment (1.28, 1.51, 1.33 and 1.59-fold for Heena; 1.24, 1.49, 1.31 and 1.56-fold for Kiran respectively) in comparison to their respective controls (50%N Control in both cultivars) (Table 1). It is noteworthy that Heena showed much progressive response to the BF treatments as compared to the Kiran.

3.2. Scanning electron microscopy

Scanning electron microscopy was performed to confirm the colonization of BF and its effect on rice root morphology at 50% nutrient deficient condition (Figs. 1 and 2). The localization of BF spores was clearly evident in the SEM micrographs of both varieties (Fig. 1 E, F and Fig. 2 E, F). Detailed analysis of SEM images of Heena revealed irregular aerenchymatous cells in the control (Fig. 1A) whereas this irregularity was reversed with the BF treatment which is evident as well arranged aerenchymatous cells under the same condition (Fig. 1D). Another important observation from the SEM micrographs was an increase in the number of metaxylems along with its diameter in the BF treated

Table 1

Effect of *Trichoderma reesei* (BF) on yielding attributes and physiological performance of two rice cultivars, Heena and Kiran, grown at different nutrient concentrations under hydroponic condition.

Heena	Root length (cm)	Shoot length (cm)	Fresh Weight	Dry weight	Chl.-A	Chl.-B	Total Chl.	Carotenoid
100% N	11.83 ± 0.18b	14.2 ± 0.17b	298.67 ± 5.93c	18.73 ± 1.24b	2.75 ± 0.73b	1.09 ± 0.052a	3.83 ± 0.33b	0.21 ± 0.014b
100% N + BF	14.07 ± .09a	17.1 ± 0.15a	401.67 ± 4.28a	28.2 ± 0.83a	3.31 ± 0.18a	1.19 ± 0.02a	4.49 ± 1.29a	0.27 ± 0.04a
50% N	11.53 ± 0.53b	12.4 ± 0.12c	298.67 ± 5.4c	17.8 ± 2.8b	1.96 ± 0.36e	0.59 ± 0.03cd	2.54 ± 1.52f	0.17 ± 0.03bc
50% N + BF	13.43 ± 0.2a	16.3 ± 0.26a	355.67 ± 4.64b	27.03 ± 0.98a	2.5 ± 0.28c	0.89 ± 0.024b	3.38 ± 1.12c	0.27 ± 0.06a
0%N	5.87 ± 0.2d	8.17 ± 0.4d	78.33 ± 8.76e	13.67 ± 0.12c	2.03 ± 0.37e	0.7 ± 0.13d	2.72 ± 1.3e	0.11 ± 0.003d
0% N + BF	8.27 ± 0.35c	11.43 ± 0.29c	102 ± 5.53d	18.3 ± 0.3b	2.18 ± 0.04d	0.82 ± 0.09bc	3 ± 0.89d	0.16 ± 0.005bcd
Kiran								
100% N	12.33 ± 0.23b	15.067 ± 0.47b	342 ± 6.53b	24.33 ± 0.17b	2.88 ± 0.84b	1.08 ± 0.23b	3.95 ± 0.89b	0.19 ± 0.023b
100% N + BF	15.43 ± 0.27a	17.5 ± 0.36a	384 ± 5.69a	30.17 ± 1.3a	3.55 ± 0.2a	1.47 ± 0.42a	5.01 ± 0.93a	0.25 ± 0.01a
50% N	6.97 ± 0.79c	12.2 ± 0.68cd	190.67 ± 2.4d	19.13 ± 0.7c	2.68 ± 0.4c	1.02 ± 0.047b	3.7 ± 0.85c	0.16 ± 0.013c
50% N + BF	13.7 ± 0.59 ab	13.63 ± 0.22bc	241 ± 5.08c	22.43 ± 0.48b	3.33 ± 0.25a	1.52 ± 0.053a	4.84 ± 0.72a	0.25 ± 0.009a
0%N	8.83 ± 0.23c	10.43 ± 0.48e	81.33 ± 1.2f	12.33 ± 0.3d	1.83 ± 0.24e	0.6 ± 0.02c	2.43 ± 1.04e	0.12 ± 0.038d
0% N + BF	12.23 ± 0.58b	12.03 ± 0.66cd	140.67 ± 3.21e	14.83 ± 0.15d	2.29 ± 0.17d	0.83 ± 0.06bc	3.11 ± 0.23d	0.14 ± 0.024c

Values are mean of three replicates with ± standard error (SE) are indicated. Means followed by the same letter(s) within the column are not significantly different according to Tukey's multiple comparison test ($P < 0.05$). In table, 100%N = 100% concentration of Nutrient; 100%N + BF = 100% concentration of Nutrient with Biofertilizer; 50%N = 50% concentration of Nutrient; 50%N + BF = 50% concentration of Nutrient with Biofertilizer; 0%N = without any Nutrient; 0% N + BF= Biofertilizer supplementation without any Nutrient.

roots (97.3 ± 2.35 μm in Heena and 79.6 ± 4.32 μm in Kiran) as compare to 50%N control of Heena (71.6 ± 3.4 μm) as well as Kiran (67.8 ± 2.9 μm) (Fig. 1F). Similarly, thin sclerenchymatous cells were observed in the 50% nutrient deficient condition (23.315 ± 0.77 in Heena) (Fig. 1B) which was rather thicker and rigid in the BF treated roots (47.122 ± 2.415 in Heena) (Fig. 1E). The same pattern was also exhibited by Kiran (17.627 ± 0.328 in 50%N condition whereas, 21.99 ± 0.190 in 50%N + BF), also distorted morphology of aerenchymatous cells was evident (Fig. 2A) which later normalized to its shape with the help of BF (Fig. 2D). A number of metaxylem also increased in the drought sensitive Kiran treated with BF (Fig. 2F).

3.3. Hormone profiling

The differential accumulation of GA3, IAA and ABA were observed after treating the rice varieties with *T. reesei*. The significant 2.4-fold and 1.16-fold increment of IAA was found in treatment 50%N + BF of Heena and Kiran respectively as compared to the control (Fig. 3A–B). The lower concentration of GA3 implies reduction in the length of internode, further leading to reduced shoot length (Claeys et al., 2014) which is well corroborated with our findings of reduced shoot length of 50%N + BF (Table 1) plants than 100%N + BF ones (Fig. 3C–D).

3.4. Mineral element accumulation in rice plant

Micro elemental investigation of rice plants revealed the highest fold change in 50%N + BF compared to non-treated controls. Among the elements Mn, Fe, Co, Cu, Zn, Mo were (1.2, 1.5, 1.8, 1.4, 1.6 and 1.7-fold respectively) higher in BF treated Heena, whereas Fe, Co, and Cu were found higher in BF treated Kiran as compared to 50%N control of both cultivars (1.4, 1.6, and 1.3-fold respectively; Table 2). The least fold increment was found in 0%N + Control (both varieties). Furthermore, the hierarchical clustering and Principle Component Analysis (PCA) was also assessed to analyze the efficacy of BF treatment on the elemental deposition. The hierarchical clustering based dendrogram, bifurcated the population into three in Heena (Fig. 4A) and two groups in Kiran (Fig. 4B) respectively. In Heena, among the three major clusters, supplementation of BF under 50% nutrient deficient condition (50%N + BF) showed significantly higher nutrient deposition as it clustered separately (Fig. 4A) whereas, 100%N + BF was clustered with 100%N and 50%N. The supplementation of BF under 0% nutrient deficient condition (0%N + BF) did not affect the nutritional value of Heena and clustered with 0% nutrient control (0%N). On other hand, the application of BF was unable to enhance the nutritional value of Kiran grown under 50% nutrient deficient condition (50%N + BF) and consequently clustered with 0%N and 0%N + BF (Fig. 4B). The supplementation of BF under nutrient deficient condition was further validated by PCA where the elemental deposition was significantly affected by the supplementation of BF under 50%N deficient condition and clustered separately in both varieties (Fig. 4C and 4D).

Furthermore, the level of macro-nutrients i.e. nitrogen and phosphorous in different treatments were also assessed to investigate the impact of BF treatment on rice under nutrient limiting conditions. The level of nitrogen and phosphorous resulted higher in Heena (1.18–2.23% and 0.87–0.92 μg gm^{-1}) as compared to the Kiran (0.32–0.69% and 0.50–0.87 μg gm-1; Table 2). The amount of nitrogen deposition was non-significantly differed in BF treated Heena (1.72%) and Kiran (0.43%) tissues and as compared to their respective controls at 50% N condition (1.66% and 0.53%). Similarly, the phosphorous content was also revealed non-significant difference between BF treated Heena (0.859 μg gm^{-1}) and Kiran (0.783 μg gm^{-1}) with non-treated 50%N plants (0.880 and 0.826 μg gm^{-1}) respectively. The content of nitrogen in 100% N and 0% N Heena variety were varied with above investigations as the level of respective controls was higher than BF treated tissues with an exemption of 0% N + BF (Table 2). Although, the higher amount of phosphorous was deposited in Kiran variety after the supplementation of BF at 100% (0.741 μg gm^{-1}) and 0% N (0.871 μg gm^{-1}) condition respectively. Whereas, the phosphorous content in Heena under nutrient deficient condition was not greatly affected (Table 2).

3.5. Analysis of stress ameliorating enzymes

To observe the effect of BF treatment in rice, various plant stress ameliorating enzymes were also examined under different nutrient conditions. The highest increment of GPX was obtained in Heena with 0.0089 U mg^{-1} protein followed by 0.006 U mg^{-1} protein in 50% and 100% N under treatment (Fig. 5A). A similar pattern was also observed in Kiran under 50%N condition (Fig. 5B). The level of SOD in Heena was found to be highest in 50%N + BF (61.07 U mg^{-1} protein), while recorded least in 100%N + BF (25.97 U mg^{-1} protein) (Fig. 5C) whereas, in Kiran variety the level of SOD did not shows any significant difference after the treatment of BF at 50%N (Fig. 5D). The amount of TPC was also found higher in Heena at 50%N + BF (55.5 mg gallic acid g^{-1}) which was followed by 50%N + BF (48.5 mg gallic acid g^{-1}) in Kiran (Fig. 5E–F).

Higher generation of PPO in Heena was also recorded in 50% N + BF (5.4 U mg^{-1} protein) as compared to Kiran at 50% N + BF

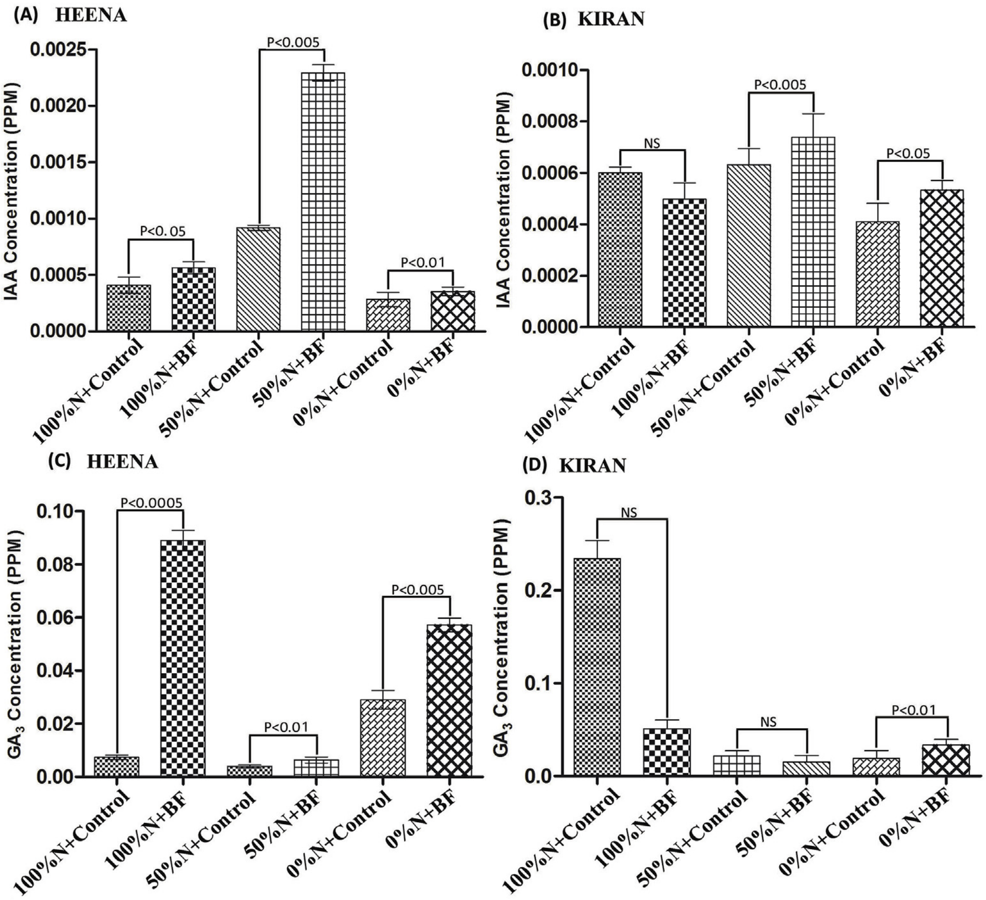

Fig. 3. Plant hormones i.e. Indole acetic acid (IAA) and Gibberellic acid (GA3) profiling of drought resistant and sensitive rice cultivars Heena and Kiran after the supplementation of *Trichoderma reesei* (BF) at different nutrient conditions. Fig. 3(A) indicates the IAA concentration in Heena at different nutrient condition in controlled and treated conditions. Fig. 3(B) indicates the IAA concentration in Kiran variety at different nutrient condition in controlled and treated conditions. Fig. 3(C) indicates the GA3 concentration in Heena variety at different nutrient condition in controlled and treated conditions. Fig. 3(D) indicates the GA3 concentration in Kiran variety at different nutrient condition in controlled and treated conditions.

(3.60 U mg^{-1} protein) (Fig. 6A–B). Furthermore, the PAL activity was found to be higher in Heena and Kiran variety under 50%N condition (24.72 and 22.7μmole $TCAg^{-1}$ FW respectively) after the treatment of BF (Fig. 6C–D). Additionally, a higher level of APX was observed in Heena after the treatment of BF (0.32 U mg^{-1} protein) under 50%N condition which was higher as compared to Kiran (0.098 U mg^{-1} protein) (Fig. 6E–F).

3.6. Expression profiling of stress and nutrient inducible genes

In the presence of BF, with an increase in nutrient concentrations (50%) the expression of iron regulating genes *ysl15* (5.9 fold), *iro2* (5.8 fold), *irt1* (3.3 fold), and *nramp* (7.49 fold) (Figs. S3, S4, S6, S14); sulphate transporter genes *sult1* (4.1 fold), *sult2* (3.3 fold), and *sult3* (4.8 fold) (Figs. S22, S23, S24); nitrate transporter gene *nrt1.1A* (3.34 fold), *nrt2* (3.6 fold), *nrt2.3a* (3.64 fold), *nia1* (3.89 fold) and *nir1* (7.8 fold) (Figs. S7, S8, S9, S10, S11); phosphatase regulatory genes *pht1* (5.5 fold), *pht2* (4.46 fold), *pt8* (5.5 fold), *pt9* (4.7 fold), *pt10* (5.39 fold) (Figs. S16, S17, S18, S19, S20); sucrose transporter gene *sut2* (2.12 fold); Fig. S15) and ammonium transporter genes *amt1* (3.84 fold), *amt1-1* (3.24 fold), *amt1-2* (3.61 fold) and *amt1-3* (3.33 fold) (Figs. S25, S26, S27, S28); were significantly up-regulated in Heena as compared to their respective control (Fig. 7A). This up-regulation of genes gradually decreases under 100% nutrient concentration in *iro2* (4.8 fold), *nrt1.1A* (2.13 fold), *nrt2.3a* (3.3 fold), *nir1* (4.3 fold), *nia1* (3.5 fold), *nramp* (4.7 fold), *pt8* (4.7 fold), *pht1* (5.4 fold), *pt10* (3.0 fold), *iaa 13* (5.54 fold), *sult3* (4.0 fold), and *amt1-1* (1.96 fold) (Fig. 7A; Figs. S4, S7, S9, S10, S11, S13, S14, S16, S18, S20, S24, S26). In Kiran, there is no significant increment was observed at 50% nutrient concentration as compared to respective control. Whereas, less fold expression were recorded in 100% nutrient concentration with an exception in *ysl15* (3.9 fold), *nir1* (4.42 fold), *sut2* (2.79 fold), *pht2* (3.1 fold), *pt8* (2.8 fold), *sult1* (2.2 fold), *sult2* (4.9 fold), *amt1* (2.10 fold), *amt1-1* (2.48 fold) and *amt1-2* (2.3 fold) where it was significantly increased (Fig. 7B; Figs. S3–S28).

Among the hormones stimulating genes *iaa13* (6.9 fold), *arf12* (4.8 fold), a significant up-regulation was observed at 50% nutrient concentration in Heena (Fig. 7A; Figs. S12 and S13). The up-regulation was maintained at 100% concentration with an exception in *IAA13* (5.54 fold) where it was down regulated (Fig. S13). In Kiran, the expression of *arf12* (1.36 fold) in treatment at 50% nutrient concentration was down regulated, whereas, at 100% (2.5 fold) concentration it was significantly up-regulated (Fig. S12). On the other hand, the expression level of *IAA13* (4.6 fold) was significantly up-regulated at 50% nutrient concentration but down regulated when the concentration was raised to 100% (2.3 fold) (Fig. 7B; Fig. S13).

Further the expression of cadmium tolerance gene *ala* was evaluated and observed a down regulation (1.46 fold) at 50% nutrient concentration in Heena (Fig. 7A; Fig. S21). This expression level was up-regulated at 100% nutrient condition; however, Kiran showed a reverse pattern of expression (Fig. 7A–B; Fig. S21). Similarly, drought and salt tolerant gene (*nac6*) was significantly up-regulated (4.2 fold) with BF treatment in Heena as well as in Kiran in comparison to their controls respectively. This expression was down regulated at 100% nutrient concentration in both varieties. Overall, Heena showed an enhanced level of fold expression when compared to Kiran (Fig. 7A–B; Fig. S5). The expression level of all nutrient related genes were assessed as compared to their respective controls.

4. Discussion

Improving the productivity of edible crops is a vital approach to

Table 2

Impact of *Trichoderma reesei* (BF) on Macro & Micro-elemental accumulation in the tissue of two rice cultivars, Heena and Kiran, grown under different nutrient conditions.

	Macro-nutrients		Micro-nutrients						
Heena	N (%)	P (μg gm^{-1})	Mn	Fe	Co	Ni	Cu	Zn	Mo
100% N	1.405 ± 0.03c	0.924 ± 0.01a	95.82 ± 1.4b	622.25 ± 3.7b	2.10 ± .09b	5.78 ± .15b	21.98 ± 1.43b	23.33 ± 1.2b	4.6 ± .2b
100% N + BF	1.185 ± 0.02d	0.875 ± 0.03b	113.75 ± .25a	690.15 ± 2.3b	3.66 ± .10a	7.54 ± .036a	28.28 ± .93a	28.62 ± 1.2a	5.52 ± .23a
50% N	1.665 ± 0.01b	0.880 ± 0.03 ab	82.2 ± 1.2bc	572.56 ± 3.06bc	1.96 ± .25b	4.87 ± .087bc	17.06 ± .82c	16.88 ± .11c	2.44 ± .04c
50% N + BF	1.725 ± 0.01b	0.859 ± 0.01b	95.63 ± 1.1b	841.65 ± 3a	3.53 ± .03a	7.75 ± .01a	23.02 ± .07b	27.55 ± .46a	4.12 ± .03b
0%N	2.235 ± 0.03a	0.888 ± 0.01 ab	59.61 ± 1.6d	380.35 ± 2.8d	1.61 ± .03d	1.62 ± .03d	10.38 ± .4e	12.03 ± .72d	1.61 ± .02d
0% N + BF	1.26 ± 0.07d	0.898 ± 0.03 ab	71.09 ± .39c	486.8 ± 3.9c	1.72 ± .04c	3.59 ± .16c	12.59 ± .66d	15.85 ± .8c	2.08 ± .05cd
Kiran	**N (%)**	**P (μg/gm)**	**Mn**	**Fe**	**Co**	**Ni**	**Cu**	**Zn**	**Mo**
100% N	0.325 ± 0.01c	0.614 ± 0.05c	84.41 ± .99b	637.95 ± 2.45b	2.60 ± .092bc	6.57 ± .04b	22.42 ± .962 ab	19.34 ± .16b	2.77 ± .06c
100% N + BF	0.164 ± 0.01d	0.741 ± 0.02b	96.88 ± 1.3a	679.6 ± 3.4a	3.66 ± .094a	7.48 ± .303a	24.98 ± .14a	26.3 ± .51a	5.58 ± .09a
50% N	0.535 ± 0.01b	0.826 ± 0.01 ab	79.38 ± .52bc	531.5 ± 3.9c	1.78 ± .082c	4.51 ± .12c	15.41 ± .47c	15.95 ± .91c	2.14 ± .071d
50% N + BF	0.43 ± 0.01bc	0.783 ± 0.03 ab	85.35 ± 3.05b	753.7 ± 12.73a	2.77 ± .011b	6.84 ± .165b	19.98 ± .075b	22.67 ± .8 ab	3.55 ± .028b
0%N	0.5 ± 0.01b	0.502 ± .01c	52.25 ± 1.6d	392.45 ± 2.15d	1.27 ± .031d	1.40 ± .023d	8.72 ± .027d	6.51 ± .12d	1.33 ± .027e
0% N + BF	0.69 ± 0.08a	0.871 ± 0.04a	72.15 ± .35c	461.85 ± 3.15c	1.67 ± .025c	4.09 ± .02c	10.35 ± .26d	14.77 ± .59cd	2.05 ± .015d

Values are mean of three replicates with ± standard error (SE) are indicated. Means followed by the same letter(s) within the column are not significantly different according to Tukey's multiple comparison test ($P < 0.05$). In table, 100%N = 100% concentration of Nutrient; 100%N + BF = 100% concentration of Nutrient with Biofertilizer; 50%N = 50% concentration of Nutrient; 50%N + BF = 50% concentration of Nutrient with Biofertilizer; 0%N = without any Nutrient; 0%N + BF= Biofertilizer supplementation without any Nutrient.

fulfill the rapid demand of food for the ever-increasing population of the world. Among the edible crops, rice is the major crop which is consumed by nearly 90% of global population (Muthayya et al., 2014). The growth and development of rice require a larger quantity of micronutrients, which in turn depends upon the various physiological and chemical constituents of the soil. Some of these constituents are available freely in soil, while remaining is present in their complex forms. It has been reported that micro-organisms facilitate the availability of different nutrients which are required for the proper development of plants. Whereas, the role of micro-organisms to ameliorate the nutrient deficient conditions has not much studied (Altomare et al., 1999; Shoresh et al., 2010). In this study, we explored one of our lab repository strain *T. reesei* (MTCC5659) in ameliorating nutrient stress in drought tolerant and sensitive varieties of rice.

It has been widely reported that *Trichoderma* efficiently helps in the amelioration of biotic and abiotic stress tolerance activity of plants (Shoresh et al., 2010). Moreover, several other applications of *Trichoderma* were also observed in addition to its plant protection abilities such as enhancing the plant root growth and development followed by other plant growth promoting strategies *i.e.* siderophore production, P-solubilization, IAA production and GA3 production to improve the growth of the plants (Lei and Ya-qing, 2015). The improvement in growth and development was witnessed by several alterations in plants such as enhancement of plant root, shoot and other yielding attributes (Contreras-Cornejo et al., 2009). The current observations showed that treatment of *T. reesei* as a biofertilizer has a great potential to colonize plant roots and ameliorate the various growth regulating parameters in both drought resistance and sensitive rice varieties, even in the case of 50% nutrient deficient condition. The present findings are well corroborated with previous reports where *Trichoderma* spp. are able to colonize inside the treated plant tissue to establish a symbiotic relationship with host plants for plant growth and development in biotic (Shoresh et al., 2010), as well as abiotic stress condition (Tripathi et al., 2017). Furthermore, it has been reported that *Trichoderma* is also involved in the synthesis of volatile organic compounds (VOCs) and microbial auxin which also helps to improve the growth of plants (Nieto-Jacobo et al., 2017).

Plant photosynthetic pigments are one of the unique tools to measure the stress tolerance in plants. Similarly, deficiencies of micro and macro-nutrients such as Fe, Cu, Mg, Mn, P and S in medium (i.e. nutrient stress condition) negatively affect the synthesis of chlorophyll and finally plant photosynthesis and other fundamental processes (Frydenvang et al., 2015). In the present study, we observed that the treatment of *Trichoderma* as biofertilizer (BF) reduces the instability of plant photosynthetic pigments under different nutrient deficient conditions. This is consistent with previous study (Nieto-Jacobo et al., 2017) where enhancement of chlorophyll was positively correlated with the amendment of *Trichoderma* as a biofertilizer which ameliorated the photosynthetic pigments by different plant growth promoting traits restoring the nutrient deficiencies under hydroponic condition (De Santiago et al., 2009) as well as in the field condition (Altomare et al., 1999).

Scanning electron microscopic analysis of *Trichoderma* treated Heena and Kiran varieties under 50% nutrient deficient condition revealed intact sections whereas distorted morphology of sections were obtained in 50% N Control plants. Furthermore, Heena root micrographs showed more compatibility with *Trichoderma* treatment as compared to Kiran variety. The change in the morphology of the rice stem may be because of the higher nutrient absorption in plants treated with *T. reesei*. The concentrations of appropriate solutes that could preserve membrane were not sufficient to maintain plant plasticity for plant tissue development and plants were unable to adjust osmotically in nutrient deficient condition. Additionally, the number of metaxylems also increased from four to five in BF treatments. Our results are in accordance with earlier finding where number of metaxylem was increased (108%; as compare to control) when the plants are treated with

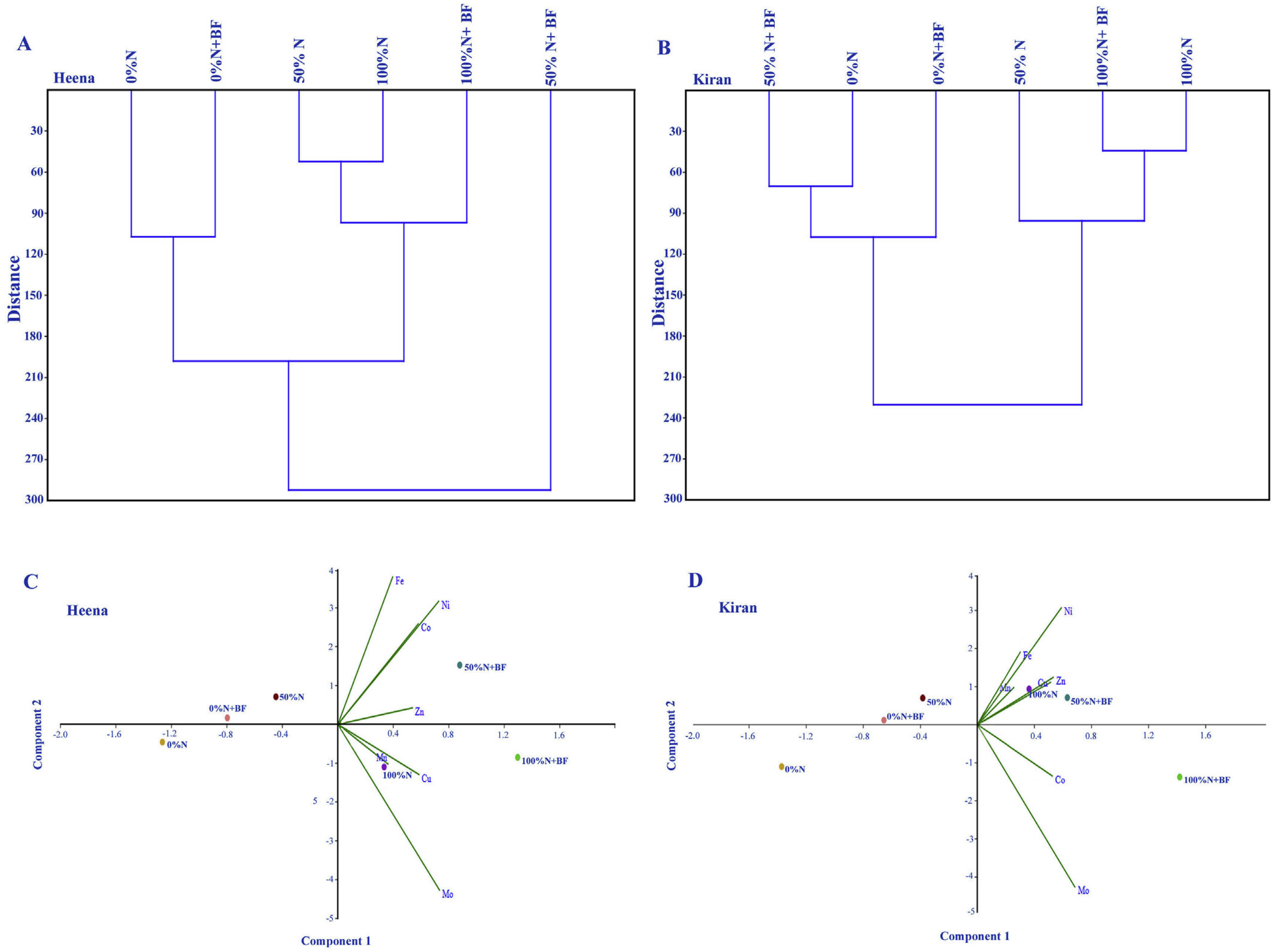

Fig. 4. Hierarchical clustering and Principal component analysis (PCA) of Micro-elemental accumulation in the tissue of *Trichoderma reesei* (BF) treated Heena (6A & 6C) and Kiran (6B & 6D) varieties grown under different nutrient conditions. In Figure, 100%N = 100% concentration of Nutrient; 100%N + BF = 100% concentration of Nutrient with Biofertilizer; 50%N = 50% concentration of Nutrient; 50%N + BF = 50% concentration of Nutrient with Biofertilizer; 0%N = without any Nutrient; 0%N + BF= Biofertilizer supplementation without any Nutrient.

the consortium of *Trichoderma asperellum, Burkholderia pyrrocinia* + *Pseudomonas fluorescens* (Rêgo et al., 2014).

The HPLC analysis of phytohormones exhibited the potential role of *Trichoderma* in nutrient deficient conditions in different treatments of Heena and Kiran rice variety. Microbes are well known for phytohormone production during stress conditions in plant (Egamberdieva et al., 2017; Datta and Basu, 2000). The physiological function of IAA is to enhance the formation of lateral roots and growth of shoot apices (Contreras-Cornejo et al., 2009). Our results showed the increased levels of IAA in *Trichoderma* treated Heena and Kiran seedlings as compare to their respective controls at 50% nutrient condition which signifies that IAA helped the plants in nutrient uptake *via* lateral root formation which improve the nutrient uptake management of plants during nutrient deficiency. Gibberlic acid (GA3) are known for the regulation of various metabolic pathways, growth promotion or suppression needed during the abiotic stress, photosynthesis as well as nutrient use efficiency of plants (Khan et al., 2007). The HPLC results of GA in Heena and Kiran treatment, revealed that during 50% nutrient condition plants adapted for its normal growth and development than in 0% and 100% nutrient condition. Also, the reduced shoot length of 50% N + BF (Table 1) plants as compared to 100%N + BF is corroborated with the findings of lower concentration of GA3 because it is generally related with an increase in the length of internode causing shoot elongation (Claeys et al., 2014). ABA is an internal signal inducer for the plants during adverse environmental stress situations for the regulation of water status (Keskin et al., 2010). Since, the experiment was conducted in the hydroponics, did not get any significant change in levels of ABA in both the rice varieties.

Macro and micronutrients are an essential part of the medium and its absorption in the plants are necessary for the development and growth of the plant (Qi et al., 2012). However, the deficiency of the nutrients in plant growth medium directly affects the different fundamental processes of plant which finally results in the reduction of yield. In the present investigation, we recorded the enhancement of micronutrients in plant tissues by the treatment of *Trichoderma* under nutrient deficient conditions. Our findings are well supported by previous research where association of *Trichoderma* with host plant significantly enhanced the absorption of essential macro and micro-nutrients in plant tissues (Huang et al., 2011). The possible reason for enhanced absorption of micro-nutrients in plant tissues by the treatment of *Trichoderma* may be attributed to the fact that *Trichoderma* possesses a cysteine rich cell wall protein that has a major role in lateral root growth along with hair formation and elongation which results in the enhancement of total absorptive surface. This enhancement resulted in higher uptake and translocation of nutrients in the host plant (Samolski et al., 2012). Similarly, microbial siderophore (Fe^{+++} ion chelating agent) binding sites which is prominently present in *Trichoderma* not only competes for Fe ions, but it was also able to bind other cations such as divalent *i.e.*

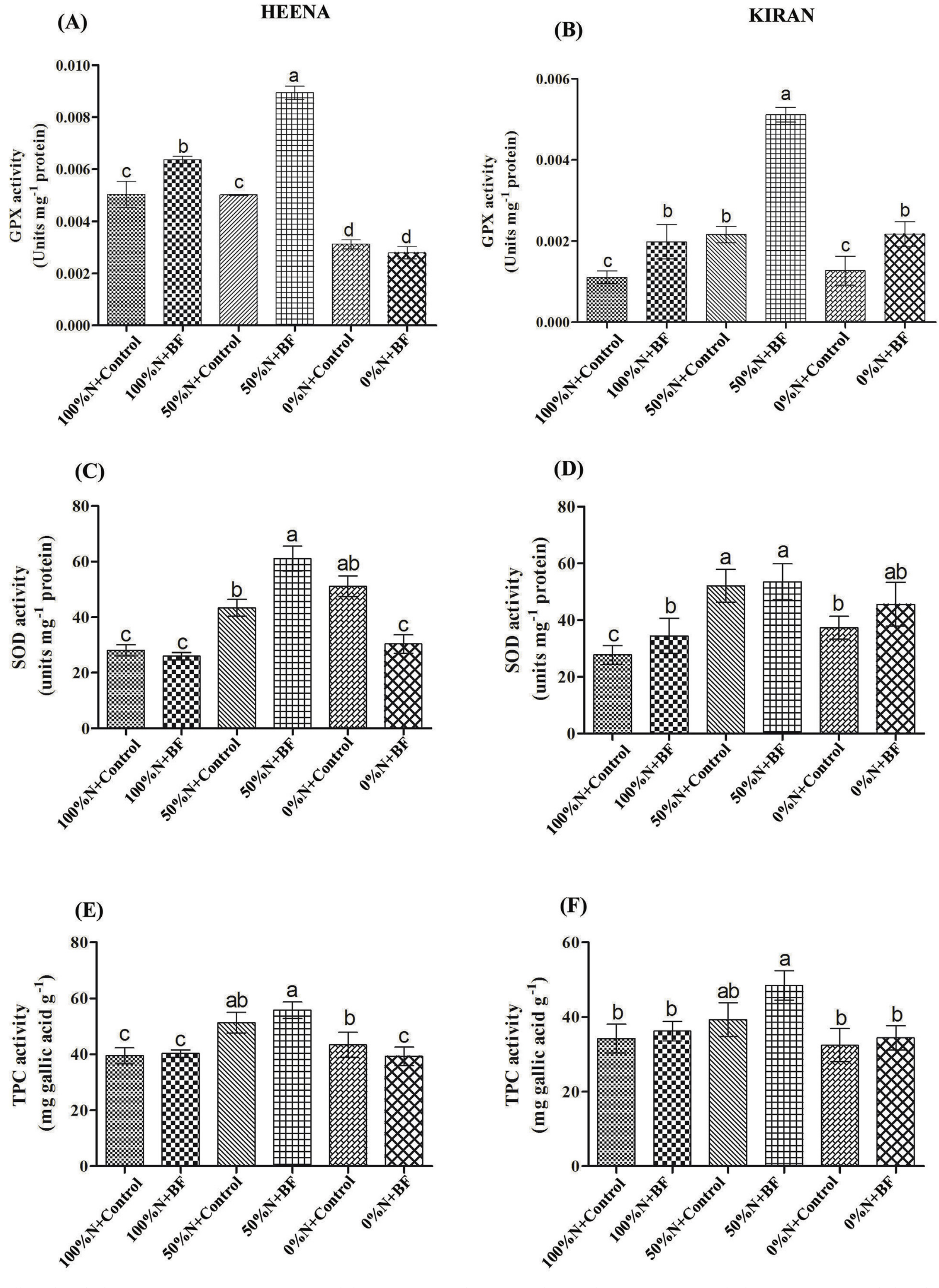

Fig. 5. Effect of *Trichoderma reesei* (BF) on stress responsive defence enzymes such as guaiacol peroxidase (GPX), superoxide dismutase (SOD), total phenolic content (TPC) in Heena and Kiran rice varieties at different nutrient concentrations under hydroponic condition. Results are mean of three replicates with ± standard error (SE). Different letters on vertical bars indicate significant differences among treatment according to Tukey's multiple comparison test at $P < 0.05$. In Figure, 100% N = 100% concentration of Nutrient; 100%N + BF = 100% concentration of Nutrient with Biofertilizer; 50%N = 50% concentration of Nutrient; 50% N + BF = 50% concentration of Nutrient with Biofertilizer; 0%N = without any Nutrient; 0%N + BF = Biofertilizer supplementation without any Nutrient.

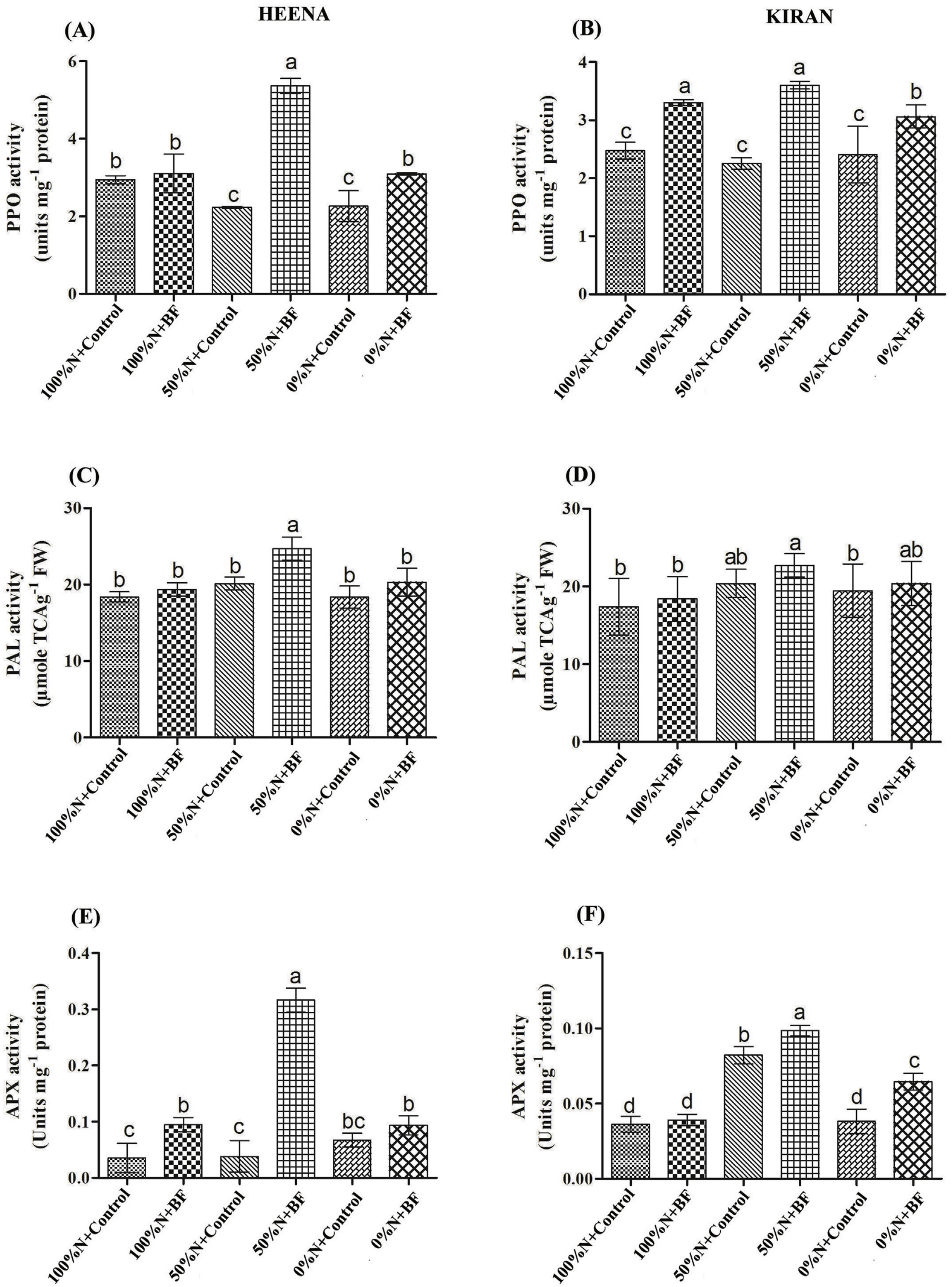

Fig. 6. Effect of *Trichoderma reesei* (BF) on stress responsive defence enzymes such as polyphenol oxidase (PPO), phenylalanine ammonia-lyase (PAL), ascorbate peroxidase (APX) in Heena and Kiran rice varieties at different nutrient concentrations under hydroponic condition. Results are mean of three replicates with ± standard error (SE). Different letters on vertical bars indicate significant differences among treatment according to Tukey's multiple comparison test at $P < 0.05$. In Figure, 100%N = 100% concentration of Nutrient; 100%N + BF = 100% concentration of utrient with Biofertilizer; 50%N = 50% concentration of Nutrient; 50% N + BF = 50% concentration of Nutrient with Biofertilizer; 0%N = without any Nutrient; 0%N + BF = Biofertilizer supplementation without any Nutrient.

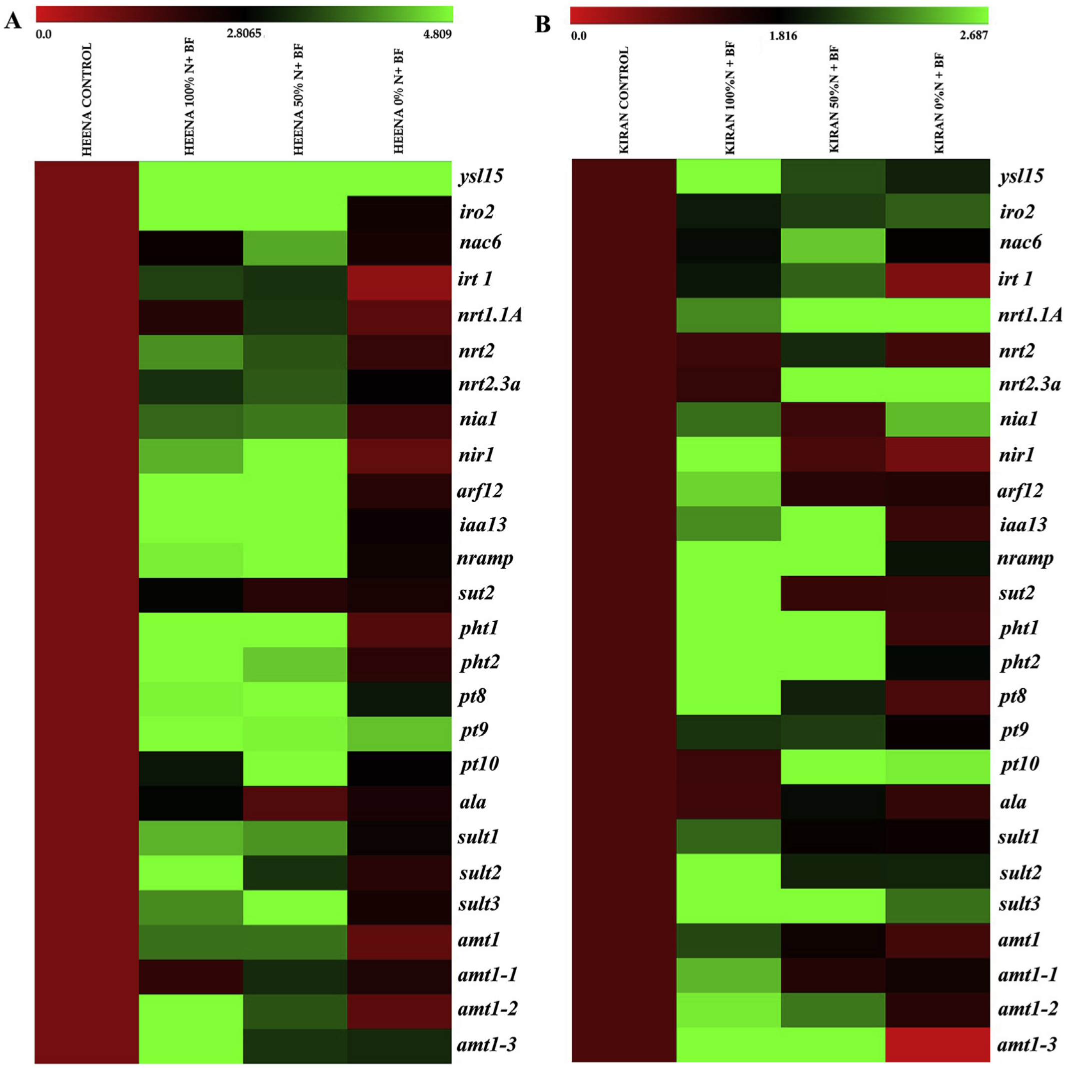

Fig. 7. Differential expression of nutrient related genes in drought resistant rice cultivar Heena (7A) and drought sensitive rice cultivar Kiran (7B) inoculated with *Trichoderma reesei* (BF) at different nutrient concentrations under hydroponic condition. The heat map has been generated based on the fold-change values in the treated samples compared with control plant. The colour scale for fold-change values is shown at the top. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Cd^{2+}, Cu^{2+}, Ni^{2+}, Pb^{2+} and Zn^{2+}; trivalent *i.e.* Mn^{3+}, Co^{3+} and Al^{3+} and others *i.e.* Th^{4+}, U^{4+} and Pu^{4+} (Peterson et al., 2004). This could also be the probable reason for finding higher concentration of various micronutrients in *Trichoderma* treated rice under nutrient deficient condition.

As similar to the micronutrients, several essential macro-nutrients such as phosphorous, nitrogen etc. are also contributes in the development and growth of plants which perform a decisive role in the cellular macromolecules, energy transfer reactions, cellular metabolism and physiological responses (Vinod and Heuer, 2012). The present investigation reflected the elevated or non-significant difference of phosphorous content in BF treated tissues as compared to their respective controls under different nutrient deficient conditions. The findings are supports the better uptake, allocation and transportation of phosphorous which might be responsible for the acceleration of the fundamental process of BF treated plants.

Furthermore, the level of nitrogen in the respective controls was found to be higher or non-significantly differs with BF treated rice plants. In-spite of this, the physiological observations and SEM micrographs recorded the least growth and development at cellular level in non-treated rice plants. Moreover, the higher expression level of nitrate transporter genes was supported about the maximum transportation of nitrogen in BF treated plants. The nitrogen uptake efficiency and their utilization by the plant for several fundamental activities is an interdependent process (Duan et al., 2007). Although, few studies highlight that under stress condition the utilization efficiency of nitrogen in different metabolic activities of plant was diminished even the uptake efficiency of nitrogen in plant was high (Ding et al., 2018; Perchlik and Tegeder, 2017; Vinod and Heuer, 2012; Duan et al., 2007). This might be the possible reason for the higher immobilization of nitrogen content in non-treated rice plants. Whereas, the supplementation of BF might create a homeostasis condition between uptake and utilization efficiency of nitrogen thus plant can easily expense it in the different fundamental processes.

Under several biotic and abiotic stress conditions, the excessive generation of Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) initiates nitro-oxidative burst causing substantive harm at cellular level (Mishra et al., 2018b), protein, lipid, molecular level (Georgiadou et al., 2018; Singh et al., 2016) as well as physiological level (Mishra et al., 2018a) in plants. In response, innate immunity of plant opposes or neutralizes the nitro-oxidative burst by the synthesis of different ROS and RNS scavenging molecules *i.e.* SOD, APX, GPX, PPO etc. (Singh and Gaur, 2017). Although, it has been reported that the deficiency of different micronutrients in plants affects the generation of various scavenging molecules, deficiency of Fe affects the synthesis of SOD (Tripathi et al., 2017), whereas Se deficiency reduces the level of ascorbate and glutathione (Hasanuzzaman and Fujita, 2011). Moreover, the deficiency of micro-nutrients also induces the reprogramming of several pathways including phenylpropanoid along with shikimate pathway which leads the synthesis of phenolic compounds, flavonoids and other antioxidants (Li and Lan, 2017). In the present study the diminished level of PAL, TPC, PPO, SOD, APX and GPX has been observed under 50% nutrient deficient condition in control plants which was further ameliorated by the treatment of *Trichoderma* as a biofertilizer. The above findings are well corroborated with the previous report where the treatment of *Trichoderma* elevates the mobility of micronutrients in plant tissues under As stress condition (Tripathi et al., 2017).

We further examined the effect of *Trichoderma* on the expression level of nutrient regulating, hormones stimulating, and stress tolerant genes under nutrient deficient conditions and observed an up-regulation in of most of the genes. It was previously observed that the increased up-regulation of plant nutrient transporter, hormones signalling and abiotic stress tolerance related genes was attributed to the treatment of *Trichoderma* which was possibly able to ameliorate the plant growth promotion as well as nutrient deposition in plant tissues. Our results are in accordance with earlier reports where an enhanced deposition of phosphate and micronutrient in tomato plants treated with plant growth promoting *Trichoderma harzianum* (Singh et al., 2014).

5. Conclusion

The present study emphasizes the beneficial role of *Trichoderma* in rice cultivars under nutrient deficient conditions by enhancing the plant physiological attributes and photosynthetic pigments, improving the activity of antioxidant enzymes under stress condition, elevating the absorption of micro-nutrients by structural alteration in root and through up-regulation of nutrient transporter and stress tolerance genes. The improvement in plant phytohormones levels in treated plant tissues further validated the efficacy of *Trichoderma* under nutrient stress condition. This study also constitutes a strong tool for establishing site-specific application of *Trichoderma* under nutrient limiting conditions. Additionally, our findings indicated that the plant growth promoting microbial treatments are beneficial for nutrient deficient plants and hence, future studies are needed to characterize the unexplored microbial communities for targeted applications.

Conflicts of interest

The authors have declared no potential conflicts of interest among them.

Availability of supporting data

The data set supporting the result of this article is (are) included with in the article (and it's additional files).

Contribution

C.S.N and A.M conceived and designed the experiment. S.P.S, S.P and N.M performed the experiments. A.B performed ICP-MS analysis for micronutrient accumulation. S.P.S, S.M, S.P, V.P.G and M.K compiled and analyzed the data. S.P.S, S.P and N.M wrote the manuscript, whereas S.M, A.M, V.P.G, A.B and P.C edited the manuscript. P.V analyzed N & P content in rice. All the authors contributed, read the final manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plaphy.2019.09.015.

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