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Effect of a number of rhizospheric bacteria on growth promotion of three Rice cultivars were tested. Results showed significant variations in plant growth according to the different PGPR treatments. The growth parameters such as plant height and number of leaves were observed at 20 days interval from the date of transplanting the seedlings to the experimental plot. Maximum enhancement of growth and dry biomass was observed in Rice plants treated with *Burkholderia symbiont*, *Bacillus altitudinis* and *Enterobacter cloacae*. In order to determine the potential of these bacteria on suppression of Brown spot disease of Rice, their antagonistic activities against *Drechslera oryzae* were tested *in vitro*. Seed bacterization as well as foliar application of *Bacillus altitudinis* (BRHS/ S 73) could reduce the natural occurrence of Brown spot disease markedly. Biochemical parameters such as total soluble protein, phenol, carbohydrate and chlorophyll content of leaf, activity of defense enzymes (chitinase and peroxidase) were also evaluated following treatment. HPLC analysis of treated Rice plants showed highest level of phytoalexin suggesting induction of resistance in Rice plants against Brown spot disease.

Key words: *Drechslera oryzae,* rice, PGPR , phytoalexin

INTRODUCTION

In modern agriculture where crop production has to be enhanced, preferably through the use of eco-friendly means specially by using biological fertilizers. Micro-organisms are important for agriculture in order to promote the circulation of plant nutrients and reduce the need for chemical fertilizers (Chakraborty *et al*, 2014). In this context, plant growth promoting rhizobacteria (PGPR) which are able to exert a beneficial effect upon plant growth, have been considered as an important strategy to increase production in sustained agricultural systems. Biological N fixation provides a major source of nitrogen for plants as a part of environmentally friendly agricultural practices. Research on Plant Growth-Promoting Rhizobacteria (PGPR) with non-legumes such as Rice have shown beneficial effects through biological nitrogen fixation and increased root growth as per (Mia *et al*, 2012) with plant growth enhancement stimulation by other beneficial bacteria and fungi according to (Saharan and Nehra, 2011). The beneficial effects of the selected rhizobial isolates could be due to their plant growth-promoting abilities namely biological Nitrogen fixation, phosphate solubilization and plant growth regulator or phytohormone similar to the known valuable effects of PGPR according to (Araujo *et al*, 2013). Elicitors have the property of inducing the production of phytoalexins in Rice plants, as well as to an agents for controlling rice diseases. Phytoalexins synthesized in the Rice plant bodies in response to the disease were extracted so as to check the level of the production

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of phytoalexins following the treatment . This study was undertaken in order to investigate the effectiveness of novel bacterial strains on Rice cultivars. The objectives of this study were to determine the effect of different PGPR strains on total protein, total phenol and total soluble sugar content of rice plants, and to evaluate the effects on defense enzymes, establishment of natural disease, phytoalexin production and plant growth parameters.

MATERIALS AND METHODS

Plant material

Seeds of three cultivars of Rice (*Oryza sativa* L.), Black nuniya, Brimful and Champasari obtained from Bijanbari were selected. These were surface sterilized with 0.1% $HgCl_2$, washed thrice with sterile distilled water and then sown as per experimental design.

PGPR

Ten previously isolated, characterized and sequenced PGPR strains were taken for the study. The bacterial strains with NBAIM Acc. No. and NCBI(Gen Bank) Acc. No. are as follows *Bacillus pumilus* (NAIMCC-B01483) (JF836847), *Bacillus pumilus* (NAIMCC-B01487) (JQ765579),*Bacillus pumilus* (NAIMCC-B01488)(JQ765580), *Burkholderiasymbiont* (NAIMCC-B01489) (JQ765578), *Bacillus aerophillus* (NAIMCC-B01490) (KC603894), *Paenibacillus polymyxa* (NAIMCC-B01491) (KC703775), *Bacillus methylotrophicus* (NAIMCC-B01492) (JQ765577), *Bacillus altitudinis* (NAIMCC-B01484) (HQ849482), *Bacillusaltitudinis* (NAIMCC-B01485) (JF899300), *Enterobacter cloacae* (NAIMCC-01486) (KC703974) which are coded as (BRHS/C1), (BRHS/T382), (BRHS/T384), (BRHS/P92), (BRHS/ B104), (BRHS/R72), (BRHS/P91), (BRHS/ P22), (BRHS/S73), (BRHS/R71) accordingly.

Foliar Spray

The bacteria were grown in nutrient broth for 48 h at 28^0C and centrifuged at 12,000 rpm for 15 min. The pellet obtained was suspended in sterile distilled water. The optical density of the suspension was adjusted using UV-VIS spectrophotometer to obtain a final density of $3X10^6$ cfu ml^{-1}. The bacterial suspension after the addition of a few drops of Tween-20 was sprayed to the plants at the seedling stage of rice plants. Application was repeated four times at 15 days interval.

Biochemical analyses of leaves
Total Soluble Protein

Soluble proteins were estimated following the method as described by Lowry *et al*, (1951). To 1ml of protein sample 5ml of alkaline reagent (1ml of 1% $CuSO_4$ and 1ml of 2% sodium potassium tartarate, added to 100ml of 2% Na_2 CO_3 in 0.1 NaOH) was added. This was incubated for 15 min at room temperature and then 0.5ml of 1N Folin Ciocalteau reagent was added and again incubated for further 15 min following which optical density was measured at 720 nm. Quantity of protein was estimated from the standard curve made with bovine serum albumin (BSA).

Total Sugar

One gm of leaf tissue were weighed and crushed with 95% ethanol. The alcoholic fraction was evaporated off on a boiling water bath. The aqueous fraction was centrifuged at 10,000 rpm for 15 min and the supernatant was collected. Total sugar content was determined following the Anthrone's method as given by Plummer (1978).

Phenol

One gm of leaf tissue was cut into small pieces and immersed in boiling alcohol (100%) in water bath and heated for 5-10 mins. Tissue was crushed using 80% alcohol and filtered in Whatman no. 1 filter paper in dark and phenol content was determined following the method as described by Mahadevan and Sridhar (1982) using caffeic acid as standard.

Quantification of chlorophyll content in leaves

Extraction of chlorophyll from leaves was done according to the method of Harbone (1973). 1g of leaf sample was homogenized in 80% acetone and filtered through Whatman No. 1 filter paper in a dark chamber. Addition of 80 % acetone from the homogenized sample was done repeatedly.The filtrate was collected and the total volume was made up to 10 ml using 80% acetone. Estimation of chlorophyll was done by measuring the OD of the filtrate at 663 nm and 645nm respectively in a UV-VIS spectrophotometer (UV-VIS spectropho-

tometer 118 systronics) against a blank of 80% acetone and calculated using standard.

Assessment of defense enzymes in leaves
Peroxidase Extraction

Extraction of peroxidse enzyme from the leaves were done by homogenizing 1g of the sample leaf in 5ml of ice-cold 50mM sodium phosphate buffer, pH 6.8, containing 1%(w/v) polyvinylpolypyrrolidone using liquid nitrogen in a pre-chilled mortar and pestle. The homogenate was then centrifuged at 10,000 rpm for 20 min at -40^{0}C. The supernatant was taken out and used directly as crude extract for enzyme assays.

Estimation

Following the method of (Chakraborty *et al*, 1993) peroxidase (EC 1.11.17) activity was assayed spectrophotometrically at 465 nm by monitoring the oxidation of O-dianisidine in presence of H_2O_2.

Chitinase
Extraction

Enzymes were extracted from leaf tissues using suitable buffers and liquid nitrogen. 0.1M sodium acetate buffer, pH 5 was used as extraction buffer for extraction of chitinase.

Estimation

Chitinase (CHT- EC. 3.2.1.39) activity was assayed following the method described by Boller and Mauch (1998). The enzyme activity was expressed as mg N-acetyl glucosamine (GlcNAc) released/ min/ g fresh tissue.

Disease Assessment

Establishment of natural brown spot disease caused by *Drechslera oryzae* (Breda de Haan) was observed and disease severity was assessed in terms of lesion number per leaf and percent disease index (PDI) was calculated following the formula - [(class rating x class frequency)/(total no. of leaves x maximum rating)] x 100.

Antifungal test of PGPR

The bacteria were streaked on one side of the Petri plate and 4mm fungal pathogen block was placed at the other side of the plate, incubation was undertaken for 5-7 days at 28^0±2^{0}C and inhibition zone towards the fungal colony in individual plate was quantified. Results were expressed as mean of percentage of inhibition of the growth of the pathogen in presence of the bacterial isolates.

HPLC Analysis of phytoalexin

For phytocassanes extraction 2 g of Rice leaf sample was cut into small pieces and shaken with 20 ml of ethyl acetate and 20 ml. of Na_2CO_3 (pH 10.5) for 18 hour. After collecting the ethyl acetate fraction it was mixed with 0.02N HCl and centrifuged at 15,000 rpm for 30 min followed by evaporation in rotary evaporator. For HPLC analysis, the supernatant was collected ,loaded on a C-18 column and eluted with 45 % acetonitrile.(UV-VIS Detector and Liquid Chromatogram, SHIMADZU). Phytocassanes were monitored at 280 nm (Umemura *et al*, 2003).

RESULTS AND DISCUSSION

Effect of PGPR on growth of Rice plants

Plant growth in terms of height of plant was recorded at 20 days interval from the date of transferring seedlings to the experimental plot. Results revealed that growth was affected by the different bacterial treatments. Maximum growth was observed in plants treated with*Burkholderia symbiont* (BRHS/P 92) in variety Black nuniya, *Bacillus altitudinis* (BRHS/ S 73) in variety Champasari and in case of variety Brimful plants treated with *Bacillus altitudinis* (BRHS/P 22) and *Enterobacter cloacae* (BRHS/R 71) showed maximum growth (Figure 1). Similarly, dry biomass of root and shoot ratio of rice plants were also found to be enhanced by application of the PGPR treatments (Table 1). The present report is in agreement with the reports of Shirinzadeh *et al,* (2013) who found positive effect of seed priming with PGPR on agronomic traits and yield of Barley cultivars. Generation of salt tolerance Rice genotypes through the treatments of PGPR have been reported by some researchers (Adesemoye *et al,* 2013) which are in agreement with findings of current study that PGPR has a positive effect in development of plant health.

Effect of different treatments on biochemical components of Rice plants
Proteins, phenols, sugar and chlorophyll

Estimation of protein contents in all the Rice cultivars following various PGPR treatments revealed

Table 1 : Dry biomass of root and shoot of Rice plants per plot

Treatments		Root shoot ratio of dry biomass of rice plants*		
		Black Nuniya	Champasari	Brimful
Untreated Control		0.73	0.65	0.57
PGPR treated				
Bacillus pumilus	(BRHS/C1)	1.28	0.59	0.75
Bacillus altitudinis	(BRHS/P 22)	0.71	0.46	0.41
Bacillus altitudinis	(BRHS/ S 73)	0.47	0.54	0.42
Enterobacter cloacae	(BRHS/R 71)	0.80	0.57	0.54
Bacillus pumilus	(BRHS/T 382)	1.24	0.50	0.33
Bacillus pumilus	(BRHS/T 384)	1.13	0.73	0.57
Burkholderia symbiont	(BRHS/P 92)	0.72	0.33	1.56
Bacillus aerophilus	(BRHS/B 104)	0.49	0.40	0.74
Paenibacillus polymyxa	(BRHS/R 72)	0.50	0.46	0.53
Bacillus methylotrophicus	(BRHS/P -91)	0.73	0.61	0.69

*Average of ten plants

Table 2 : Protein content of Rice leaves following treatments with PGPR

Treatments		Protein content (mg/gm tissue)*		
		Black Nuniya	Champasari	Brimful
Control		23.90±0.34	37.25±0.93	31.19±0.67
Bacillus pumilus	(BRHS/C1)	45.50±0.67	53.86±0.29	50.17±0.54
Bacillus altitudinis	(BRHS/P 22)	55.25±0.27	50.53±0.54	46.41±0.96
Bacillus altitudinis	(BRHS/ S 73)	49.56±0.35	49.45±0.44	56.72±0.58
Enterobacter cloacae	(BRHS/R 71)	55.03±0.34	57.72±0.69	55.45±0.72
Bacillus pumilus	(BRHS/T 382)	66.77±0.56	57.22±0.82	59.42±0.60
Bacillus pumilus	(BRHS/T 384)	34.93±0.80	30.20±0.68	40.63±0.86
Burkholderia symbiont	(BRHS/P 92)	45.10±0.70	51.05±1.08	52.00±0.35
Bacillus aerophilus	(BRHS/B 104)	65.73±2.11	80.03±1.02	87.73±3.00
Paenibacillus polymyxa	(BRHS/R 72)	73.44±1.70	94.47±2.25	70.84±0.75
Bacillus methylotrophicus	(BRHS/P 91)	55.44±1.78	39.81±1.33	52.49±1.25

*Mean value of three replicates ± Standard error

Table 3 : Total phenol content of Rice leaves following treatments with PGPR

Treatments	Total phenol content(mg/gm tissue)		
	Black Nuniya	Champasari	Brimful
Control	2.71±0.08	3.50±0.20	3.60±0.23
Bacillus pumilus (BRHS/C1)	4.23±0.17	4.79±0.15	3.93±0.20
Bacillus altitudinis (BRHS/P 22)	4.70±0.07	4.83±0.27	5.06±0.12
Bacillus altitudinis (BRHS/ S 73)	4.93±0.20	6.22±0.15	5.76±0.08
Enterobacter cloacae (BRHS/R 71)	5.58±0.10	6.58±0.16	6.30±0.20
Bacillus pumilus (BRHS/T 382)	6.68±0.24	7.13±0.18	7.83±0.17
Bacillus pumilus (BRHS/T 384)	5.83±0.23	5.60±0.30	4.93±0.26
Burkholderia symbiont (BRHS/P 92)	7.10±0.15	6.80±0.20	6.72±0.13
Bacillus aerophilus (BRHS/B 104)	6.76±0.14	6.34±0.17	6.65±0.12
Paenibacillus polymyxa(BRHS/R 72)	8.26±0.14	7.06±0.12	7.33±0.21
Bacillus methylotrophicus (BRHS/P91)	5.86±0.26	5.63±0.31	5.96±0.14

Mean value of three replicates; ± Standard error

Table 4 : Total sugar content of Rice leaves following treatments with PGPR

Treatments		Total sugar content (mg/gm tissue)		
		Black Nuniya	Champasari	Brimful
Control		41.33±1.45	27.33±0.40	33.23±0.72
Bacillus pumilus	(BRHS/C1)	57.70±0.74	51.40±1.05	55.46±1.21
Bacillus altitudinis	(BRHS/P 22)	46.80±0.55	50.46±0.52	44.77±0.92
Bacillus altitudinis	(BRHS/ S 73)	46.39±0.48	46.83±0.60	40.54±0.89
Enterobacter cloacae	(BRHS/R 71)	57.65±0.70	59.42±0.67	57.53±0.29
Bacillus pumilus	(BRHS/T 382)	56.16±0.95	44.36±0.63	48.97±0.48
Bacillus pumilus	(BRHS/T 384)	34.68±0.15	38.13±0.85	35.80±0.33
Burkholderia symbiont	(BRHS/P 92)	47.63±0.20	42.20±0.49	41.00±3.01
Bacillus aerophilus	(BRHS/B 104)	64.48±1.05	47.33±0.48	59.49±0.44
Paenibacillus polymyxa	(BRHS/R 72)	56.30±0.45	49.20±0.41	51.73±0.93
Bacillus methylotrophicus	(BRHS/P-91)	59.86±0.75	58.47±0.86	62.87±0.73

Mean value of three replicates; ± Standard error

Table 5 : Total chlorophyll content of Rice leaves following treatments with PGPR

Treatments	Total chlorophyll content(mg/g tissue)		
	Black Nuniya	Champasari	Brimful
Control	12.17±0.10	11.35±0.06	12.93±0.23
Bacillus pumilus (BRHS/C1)	14.67±0.22	14.50±0.15	16.08±0.31
Bacillus altitudinis (BRHS/P 22)	12.81±0.21	13.60±0.07	13.50±0.11
Bacillus altitudinis (BRHS/ S 73)	12.84±0.07	11.98±0.17	12.60±0.18
Enterobacter cloacae (BRHS/R 71)	10.50±0.02	11.37±0.06	12.71±0.11
Bacillus pumilus (BRHS/T 382)	12.78±0.34	12.44±0.06	13.54±0.18
Bacillus pumilus (BRHS/T 384)	14.68±0.04	14.73±0.08	14.82±0.26
Burkholderia symbiont (BRHS/P 92)	14.87±0.17	15.64±0.23	15.80±0.10
Bacillus aerophilus (BRHS/B 104)	15.11±0.11	15.24±0.14	14.55±0.52
Paenibacillus polymyxa (BRHS/R 72)	11.69±0.45	13.07±0.19	12.68±0.43
Bacillus methylotrophicus (BRHS/P-91)	12.69±0.09	14.47±0.09	14.62±0.93

Mean value of three replicates; ± Standard error

Table 6 : Evaluation of Disease index for Brown spot in Rice plants following treatments with PGPR

Treatments	Black Nuniya		Champasari		Brimful	
	PDI(%)	Mean diameter of lesion(mm.)	PDI(%)	Mean diameter of lesion(mm.)	PDI(%)	Mean diameter of lesion(mm.)
Control	76.19	2.1	31.08	1.6	69.33	2.0
Bacillus pumilus (BRHS/C1)	26.18	1.7	28.80	0.6	13.33	2.1
Bacillus altitudinis (BRHS/P 22)	09.83	2.0	48.19	3.0	32.67	0.3
Bacillus altitudinis (BRHS/ S 73)	22.54	1.5	41.17	1.9	62.50	1.4
Enterobacter cloacae (BRHS/R 71)	16.92	0.6	34.54	2.2	16.54	1.6
Bacillus pumilus (BRHS/T 382)	19.73	1.8	36.17	0.9	24.50	1.5
Bacillus pumilus (BRHS/T 384)	32.94	0.8	44.14	0.5	47.05	1.0
Burkholderia symbiont (BRHS/P 92)	28.40	1.5	44.79	1.5	44.79	1.6
Bacillus aerophilus (BRHS/B 104)	54.42	2.0	46.30	1.0	25.8	1.8
Paenibacillus polymyxa (BRHS/R 72)	38.45	0.8	52.80	1.5	30.56	0.6
Bacillus methylotrophicus (BRHS/P-91)	37.95	0.5	61.12	1.5	14.47	0.4

PDI- Percentage of Disease Index

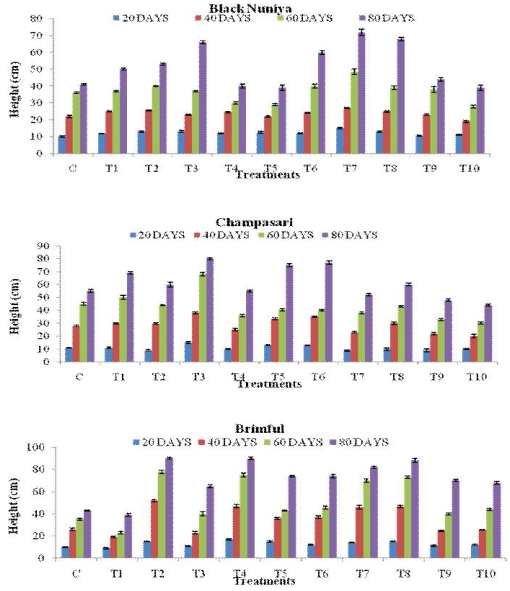

Fig.1: Increase in the height of Rice cultivars following different treatments at specific time intervals
[C-control,T1-*Bacillus pumilus* (BRHS/C1), T2-*Bacillus altitudinis* (BRHS/P 22), T3-*Bacillus altitudinis* (BRHS/ S 73), T4-*Enterobacter cloacae* (BRHS/R 71), T5-*Bacillus pumilus* (BRHS/T 382), T6-*Bacillus pumilus* (BRHS/T 384),T7- *Burkholderia symbiont* (BRHS/ P 92), T8-*Bacillus aerophilus* (BRHS/B 104), T9- *Paenibacillus polymyxa* (BRHS/R 72), T10-*Bacillus methylotrophicus* (BRHS/ P-91)]

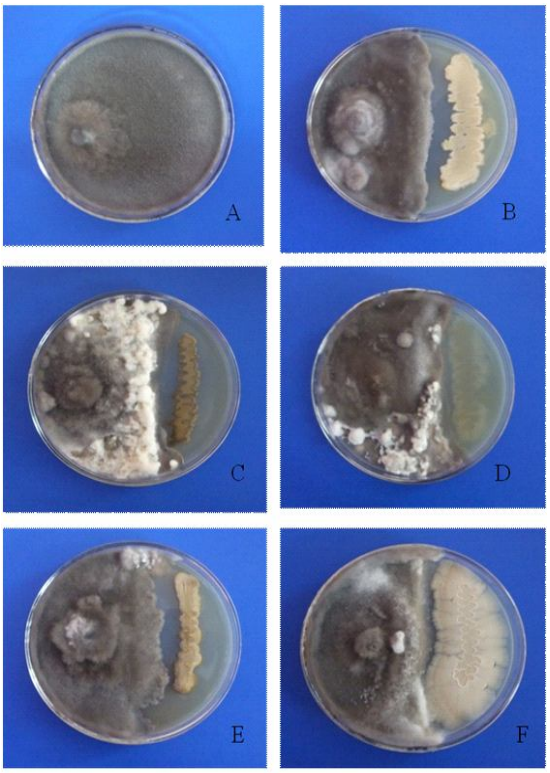

Fig.3: *In vitro* antifungal activities of PGPR against *Drechslera oryzae*
[Inhibition of *Drechslera oryzae* in dual plate culture assay by BRHS/ S 73 (B),BRHS/C 1 (C),BRHS/R 71 (D), BRHS/T 384 (E),BRHS/P 92 (F). Control (A)]

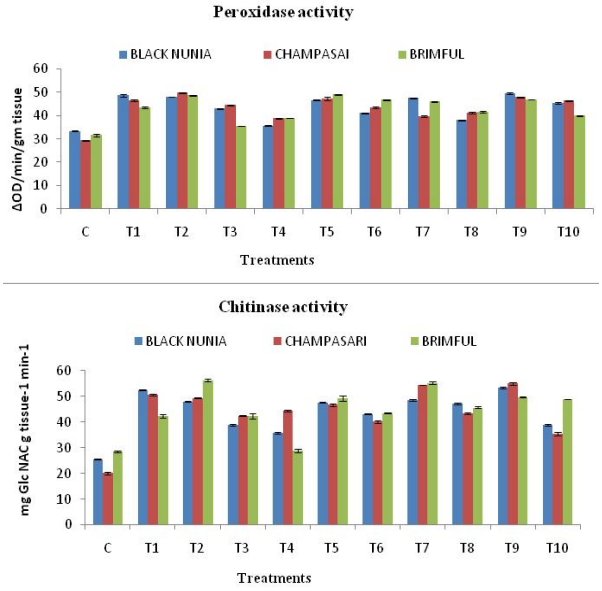

Fig.2: Activity of defense enzymes (Peroxidase and Chitinase) in Rice cultivars following different treatments
[C-control,T1-*Bacillus pumilus* (BRHS/C1), T2-*Bacillus altitudinis* (BRHS/P 22), T3-*Bacillus altitudinis* (BRHS/ S 73), T4-*Enterobacter cloacae* (BRHS/R 71), T5-*Bacillus pumilus* (BRHS/T 382), T6-*Bacillus pumilus* (BRHS/T 384),T7- *Burkholderia symbiont* (BRHS/ P 92), T8-*Bacillus aerophilus* (BRHS/B 104), T9- *Paenibacillus polymyxa* (BRHS/R 72), T10-*Bacillus methylotrophicus* (BRHS/ P-91)]

enhancement in protein content of which highest accumulation was obtained in treatment containing *Bacillus altitudinis* (BRHS/P 22). Maximum protein content in all the cultivars ranged between 71-95 mg/gm tissue (Table 2). Total phenols showed variations according to the treatments. Highest amount of total phenol was obtained in plants treated with *Bacillus altitudinis* (BRHS/P 22) in all the three cultivars. Total phenol content ranged between 7-8 mg/gm tissue (Table 3). In case of total sugar and chlorophyll content, results revealed that here also maximum accumulation occurred in treatment with *Bacillus methylotrophicus* (BRHS/P-91) and *Bacillus pumilus* (BRHS/C-1) (Table 4, 5). Total soluble protein, total phenol, total sugar and total chlorophyll content when estimated were found in increased amount in treated plant in comparison to control set of plant. Similar result was found in study of previous worker in the experiment on plant growth promoting rhizobacteria mediated improvement of health status of tea plants (Chakraborty *et al,* 2013).

Activity of defense enzymes in Rice plants

Defense enzymes activity when tested showed sig-

Table 7 : *In vitro* pairing of PGPR isolates with *Drechslera oryzae*

Interacting microorganisms		Diameter of fungal colony (cm)	% of inhibition
Drechslera oryzae		9.50±0.15	-
D. oryzae + Bacillus altitudinis	(BRHS/S73)	1.50±0.14	84±1.73
D. oryzae + Bacillus pumilus	(BRHS/C1)	1.98±0.21	79±1.63
D. oryzae + Enterobactor cloacae	(BRHS/R71)	2.10±0.23	77±1.73
D. oryzae + Bacillus pumilus	(BRHS/T384)	2.21±0.27	76±1.62
D. oryzae + Burkholderia symbiont	(BHRS/P92)	2.46±0.24	74±1.54
D. oryzae + Bacillus altitudinus	(BRHS/P22)	2.51±0.22	73±1.52
D. oryzae + Bacillus pumilus	(BRHS/T 382	2.52± 0.20	72±1.46
D. oryzae + Bacillus aerophilus	(BRHS/B 104)	2.53±0.23	72±1.45
D. oryzae + Paenibacillus polymyxa	(BRHS/R 72)	2.59± 0.25	71±1.43
D. oryzae + Bacillus methylotrophicus	(BRHS/P-91)	2.60±0.22	70±1.42

Mean value of three replicates; ± Standard error; Diameter of fungal colony after 7 days growth (cm)

nificant variation according to the treatment and higher activity was observed in treated rice plants rather than control set of plants. More enzymatic activity were found in plants treated with *Bacillus altitudinis*(BRHS/P 22), *Burkholderia symbiont* (BRHS/P 92), R72- *Paenibacillus polymyxa* (BRHS/R 72) (Figure 2). The results of our study agreed with the previous findings (Jha *et al,* 2013) where similar results were obtained on paddy plants inoculated with PGPR show better growth physiology and nutrient content under saline conditions.

Influence of PGPR on natural disease and antagonism

Rice plants were under observation from seedling stage to mature stage and data was collected for the establishment of natural disease caused by *Drechslera oryzae* under natural condition and disease index were prepared accordingly which showed higher amount of PDI percentage in control set of plant (76.19%) in comparison with the plants treated with PGPR (9.83%) (Table 6). *In vitro* pairing of PGPR isolates with *Drechslera oryzae* was also conducted as a result *Bacillus altitudinus* (BRHS/S73) showed the maximum percentage of inhibition followed by *Bacillus pumilus* (BRHS/C1), *Enterobacter cloacae* (BRHS/ R71), *Bacillus pumilus* (BRHS/T384) and *Burkholderia symbiont* (BRHS/ P92) (Table 7)(Figure 3).

HPLC analysis

HPLC analysis was done for detecting the phytoalexin namely Phytocassanes with the leaves of Rice cultivar Black nuniya in untreated inoculated and PGPR (*Bacillus altitudinus*) treated and inoculated plants. Treated plants had exhibited lowest PDI percentage. A total of 10 peaks were clearly visible in healthy as well as treated plants inoculated with the pathogen. However the compounds increased markedly in treated inoculated plants as evident in peak heights of nos. 2, 3, 5, 6 and 10 (Figure 4).

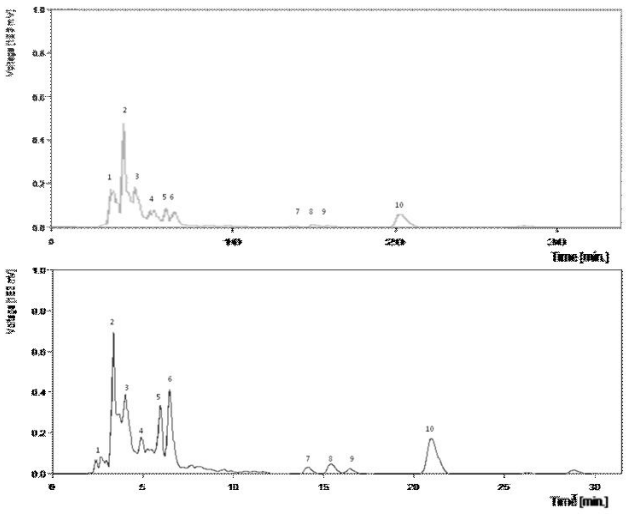

Fig. 4: HPLC analysis of Phytocassanes in Rice plant (cultivar Black nuniya) following treatment with *Bacillus altitudinus* (BRHS/ S73) [A. Treated healthy B. Treated infected]

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REFERENCES

Adesemoye A. O. and Egamberdieva D. 2013. Beneficial Effects of Plant Growth-Promoting Rhizobacteria on Improved Crop Production: Prospects for Developing Economies. Springer-Verlag Berlin Heidelberg .*Bacteria in Agrobiology*: *Crop. Prodct.*45-46.

Araujo A.E.D.S., Baldani V.L.D., Galisa P.D.S., Pereira J.A. and Baldani J.I. 2013. Response of traditional upland rice varieties to inoculation with selected diazotrophic bacteria isolated from rice cropped at the Northeast region of Brazil. *Applied Soil Ecol.* **64,** 49-55.

Boller T. and Mauch F.1998. Colorimetric assay for chitinase. *Methods . Enzymol.* **161,** 430-435.

Chakraborty, B.N.,Chakraborty, U., Sunar, K. and Dey, P.L.2014. *Harnessing beneficial microbial resources for crop improvement.* In : D.P.Singh and H.B.Singh (eds.) Trends in Soil Microbial Ecology, Studium Press, USA,pp.175-201.

Chakraborty U., Chakraborty B.N. and Kapoor M. 1993. Changes in the levels of peroxidase and phenyl alanine ammonia lyase in *Brassica napus* cultivars showing variable resistance to *Leptosphaeria maculans*. *Folia. Microbiol.* **38,** 491-496.

Chakraborty U., Chakraborty B.N., Chakraborty A.P., Sunar K. and Dey P.L. 2013. Plant growth promoting rhizobacteria mediated improvement of health status of tea plants. *Ind. J. Biotech.* **12,** 20-31.

Harborne J.B. 1973. *Phytochemical Methods*, Chapman and Hall, Ltd., London, pp. 49-188.

Jha, Y. and Subramanian, R.B. 2013. Paddy plants inoculated with PGPR show better growth physiology and nutrient content under saline conditions. *Chil. J. of Agri. Res.* **73:,** 213-219.

Lowry O.H., Rosebrough N.J, Farr A.L. and Randall R. J.1951. Protein measurement with folin phenol reagent. *J. Bio. Chem*. **193,** 265-275.

Mahadevan A. and Sridhar R.1982. *Methods in physiological plant pathology*. 2nd Ed. Sivakami Pub. India.

Mia, M.A.B., Shamsuddin Z.H. and Mahmood M., 2012. Effects of rhizobia and plant growth promoting bacteria inoculation on germination and seedling vigor of lowland rice. *African J. Biotechnol.* **11,** 3758-3765.

Plummer D. 1978. *An introduction to plant biochemistry*. Tata Mc Graw Hill Pub., New Delhi. 326.

Saharan B.S. and Nehra V.2011. Plant growth promoting rhizobacteria: A critical review. *Life Sci. Med. Res*., **21,** 1-29.

Shirinzadeh A., Soleimanzadeh H. and Shirinzadeh Z. 2013. Effect of Seed Priming with Plant Growth Promoting Rhizobacteria(PGPR) on Agronomic Traits and Yield of Barley Cultivars. *W. App. Sci. J.* **21:** 727-731.

Umemura K.,Ogawa N.,Shimura M.,Koga J.,Usami H. and Kono T. 2003. Possible Role of Phytocassane, Rice Phytoalexin, in Disease Resistance of Rice against the Blast Fungus *Magnaporthe grisea*.*Biochem.***67,**899-902.